EFFECTS ON GROWTH OF E.coli AFTER EXPOSURE TO X-RAYS AND TREATMENT WITH PENICILLIN: *Model for Screening radiation chemical modifiers in radiation oncology*

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ABSTRACT

Cells of E.coli B/r were x-irradiated (330Gy) in the presence of oxygen and immediately incubated in a non-lethal concentration of penicillin (10u/ml).This caused enhanced growth of the cell into filaments which later disintegrated (ghosts) and failed to form colonies. Irradiation (950Gy) of these cells under anoxic conditions and incubation in penicillin did not cause enhanced killing or growth into filaments of the cells.

These observations suggest that the combined action of x-irradiation followed by incubation in penicillin caused more cells to grow into non-viable filaments. The model has been successfully used to screen new hypoxic cell radiosensitizers and radioprotectectors by comparing their radiosensitization properties. Similar application of the method to test the efficacy of other drugs including natural herbs in our environment as radiation modifiers in radiation oncology is in progress.

Key words: E.coli. filaments. penicillin, natural herbs. aerobic, anaerobic.

INTRODUCTION.

The growth of certain strains of E.coli into non-septate filaments after exposure to radiation or other damaging agents is a well known phenomenon[1] .E.coli B/r, which is a radioresistant mutant strain does not form filaments after irradiation[2,3]. Penicillin is known to inhibit the synthesis of the bacterial cell wall and thus cause distinct morphological changes[4]. Low concentrations of the antibiotic affect the formation of the septum across the cell which results in elongation of the cell (filamentation)[5].

We have shown[6], that there is enhanced killing by non lethal concentration of penicillin in E.coli B/r cells provided the bacteria are x-irradiated in the presence of oxygen. This study attempts to establish a correlation between structural changes in the growth pattern of this strain as seen under the light microscope, with the observed enhanced killing brought about by penicillin under a similar condition.

MATERIALS AND METHODS.

The strain of E.coli B/r was used. The cells were prepared for irradiation and exposed to x-rays as described by Gillies, Obioha 1979[6]. In each instance, sufficient irradiation was given under either aerobic or anaerobic conditions, to reduce the surviving fraction to 10% as determined by counting viable colonies on plates of Oxoid Blood Agar Base (BAB).

For microscopic examination of the bacteria, the method used by Brown and Gillies[1] was used. Bacteria were grown on small pieces of cellophane lying on the surface of BAB. After the required incubation period, at 37^0c, duplicate pieces of cellophane were removed, fixed to a microscope slide, stained and the cells examined with a light microscope under oil immersion objective at a magnification of 2 x 800.When the bacteria were to be treated with penicillin, the antibiotic was incorporated into the BAB. The concentration of penicillin used was 10 units/ml. Details of the technique using cellophane and the classification of the bacteria as single cells, colonies, filaments and ghosts are given in Brown and Gillies,(1972)[2]

RESULTS.

Figure 1 (a, b, c) shows the morphological shapes of bacteria recorded.

Cells greater than three times the length of a

normal cell were defined as filaments. Figure 2, shows that about 5% of un-irradiated single cells grew into filaments which eventually divided to form colonies. The concentration of penicillin (10u/ml) used in these experiments was obtained as described by Gillies et al 1979[6] and was one which was insufficient to cause killing during a period of 4hours of incubation. The effect of this concentration of penicillin is shown in Figure 3.

After x-irradiation with 330 Gy under aerobic and 950 Gy under anaerobic conditions, it is seen in Figure 4 that about 15% of the cells grew into filaments by 4h after irradiation in each case. Figure 5 shows that following incubation with penicillin (after aerobic irradiation) for 4h, the number of filaments/ghosts was increased from 15% to 75% while irradiation of the cells under anoxia and subsequent incubation in penicillin did not cause significant increase in number of filaments (15%) over the same 4h period.

DISCUSSION.

It has been shown[1] that during normal growth, E.coli can vary in length from
1.5 to 5.5µm (i.e. equivalent to 3 4 normal length of a bacterium). Therefore cells were classified as filaments only when they had exceeded three times the length of the minimum sized cells seen under the microscope. Even then some normal cells may grow to lengths greater than three times the minimum before dividing. This may account partially for the finding that about 5% of un-irradiated cells and as many as 15% of irradiated cells of this strain, which is described as non-filamenting, apparently grew into filaments.

Penicillin on its own causes filamentation, but at the concentration used, in the present study, this effect was transitory and did not reduce the viability of the cells. As penicillin inhibits the formation of peptide cross-links in bacterial cell envelop, it is likely that filamentation is the cause of increased cell death. Thus when cells that had been reduced to 10% survival by aerobic x-irradiation were incubated with penicillin, a further 10 fold decrease in survival occurred and at the same time the total fraction of cells that filamented and/or subsequently disintegrated into 'ghosts' increased from 15% to 75%.

Several works had been done to characterize this lesion. Obioha et al[7] characterized and quantified the mechanisms of this damage in relation to DNA damage using known oxygen equation constants for radiation damage. Parallel studies by Gillies et al[8] supported this finding by measuring the synthesis of membrane macromolecules using labeled precursors. Another study by Obioha[9] has shown that other membrane-active antibiotics can demonstrate this lesion but not as efficiently and same magnitude as penicillin.

Gillies and Obioha 1982[10] had shown that this x-ray lesion can be demonstrated by substituting hypoxic cell radiosensitizers in place of oxygen. In the same study, the model was successfully used to screen new drugs: hypoxic cell radiosensitizers/protectors; 2-nitroimidazole (Ro-07-0582 & Ro-07-0554), 5-nitroimidazoles (metronidazole &Ro-11-3696) and misonidozoles by comparing their radio-sensitization properties

With this background, the author is tempted to suggest that in our environment where there are claims about the efficacy of various drugs and herbal preparations in the treatment of various diseases, including cancers, this model, can be used to test the cytotoxic properties of the drugs. The combined action of radiation and the drugs may also bring about reduction in patients' dose in radiation oncology. Work in this direction is in progress.

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LEGEND TO FIGURES.

Figure 1

a) Single bacteria and a filament .
b) Large micro-colony containing normal and filamentous forms.
c) Disintegrating filament or 'ghost'

Figure 2. Growth and division of un-irradiated cells on BAB.

○ Cells
● Colonies
▲ Filaments

Figure 3. Growth and division of un-irradiated cells incubated on BAB containing 10 units/ml of penicillin for 4hours.

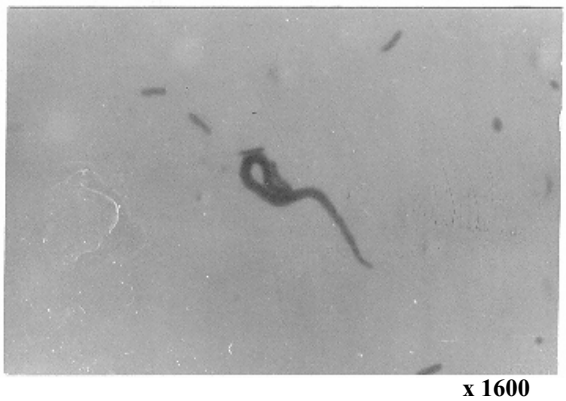
○Cells.
●Colonies
▲ Filaments
△ Colonies (data from fig.2)

Figure 4. Growth and division of cells x-irradiated under aerobic or anerobic conditions to 10% survival levels and subsequently incubated on BAB for 4hours.

Aerobic Irradiation
○ Cells ● Colonies ▲ Filaments
Anaerobic Irradiation
○ Cells ● Colonies ▲ Filaments

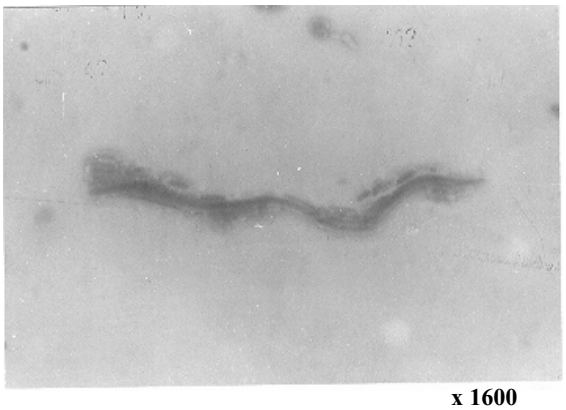
Figure 5. Growth into filaments of cells x-irradiated under aerobic and anaerobic conditions to 10% survival levels and subsequently incubated on BAB containing 10u/ml of penicillin for 4hours.

Aerobic Irradiation
○ Cells ▲ Colonies △ Filaments ● Ghosts
Anaerobic Irradiation
○ Cells ▲ Colonies △ Filaments



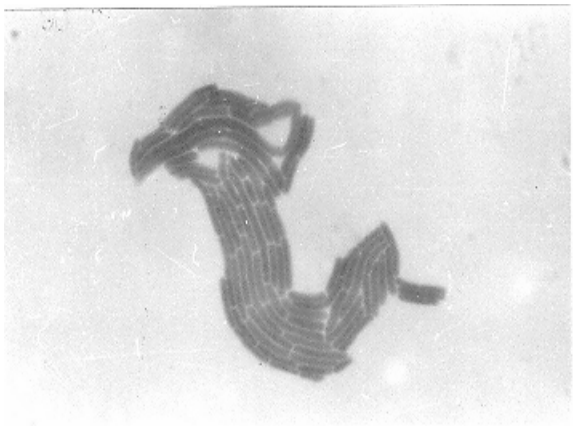
x 1600

Figure 1a. Single bacteria and a filament



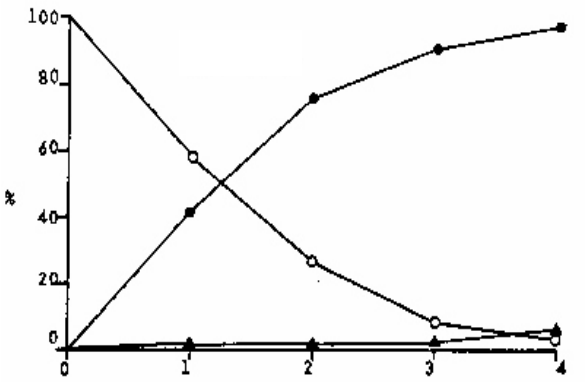
x 1600

Figure. 1c. Disintegrating filament or 'ghost'



x 1600

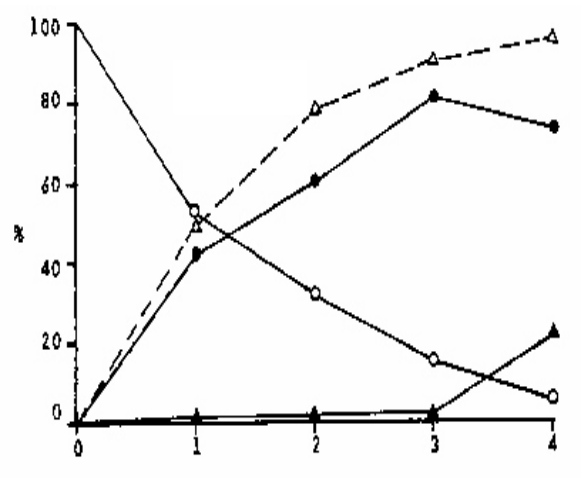
Figure 1b. Large micro-colony containing normal and filamentous cells.



Incubation Time on BAB (Hr)

Fig 2: Growth and division of un-irradiated cells on BAB

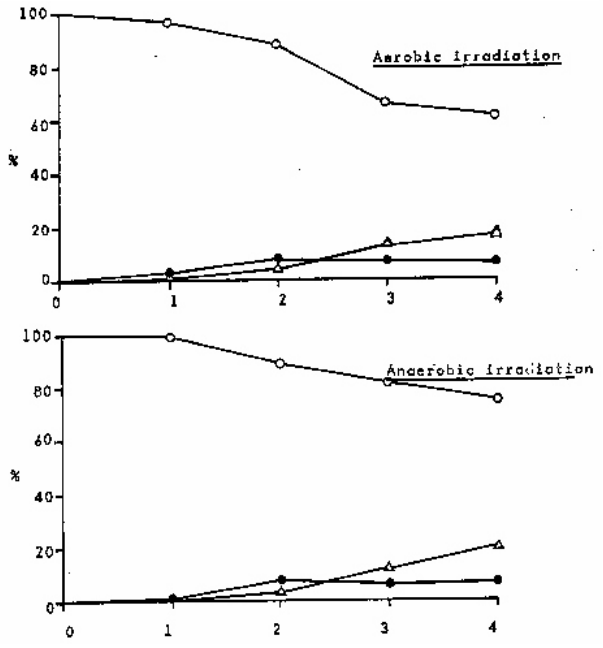
Cells
Colonies
Filaments



Incubation Time with Penicillin (Hr)

Fig 3: Growth and division of un-irradiated cells incubated on BAB containing 10 units /ml of penicillin for 4 hours

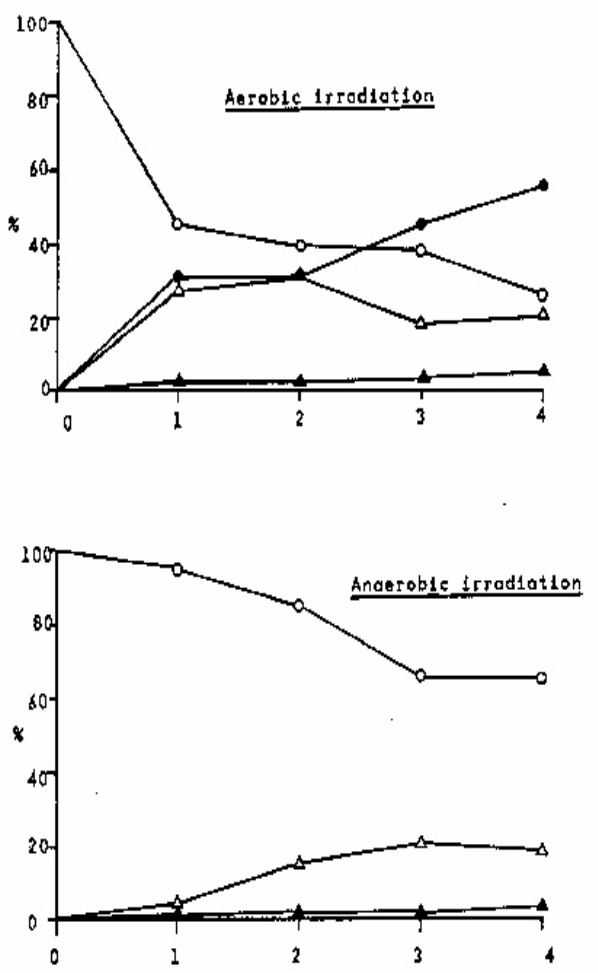
Cells **Filaments**
▲ Colonies **△ Colonies (data from figure 2)**



Incubation Time on BAB (Hr)

Fig. 4: Growth and division cells X-irradiated under aerobic or anaerobic conditions to 10% survival levels and subsequently incubated on BAB for 4 hours.

Aerobic Irradiation
○ Cells ● Colonies ▲ Filaments
Anaerobic Irradiation
○ Cells ● Colonies ▲ Filaments



Incubation Time with Penicillin (Hr)

Figure 5. Growth and division of cells, X-irradiated under aerobic or anaerobic conditions, to 10% survival levels and immediately incubated on BAB containing 10 units/ml of penicillin for 4 hours

Aerobic irradiation
○ Cells; ▲ Colonies △ Filaments; ● Ghosts
Anaerobic irradiation
○ Cells; ▲ Colonies; △ Filaments.

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