The Sensitivity of *Mycoplasma mycoides* var. *capri* Cells to γ -Radiation after Growth in a Medium Containing the Thymine Analogue 5-Vinyluracil

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Cells of the bacterium *Mycoplasma mycoides* var. *capri* grown in a medium containing the potential radiation-sensitive thymine analogue 5-vinyluracil show a 3-fold increase in sensitivity towards irradiation of a dose of 15 krad of γ -rays.

We have been interested for some time in the synthesis of potential radiation-sensitive thymine analogues, which we have then attempted to incorporate into the DNA of *Escherichia coli* T⁻, phage T3 and *Mycoplasma mycoides* var. *capri* (Chelton *et al.*, 1973; Jones & Walker, 1975). It was hoped that such an incorporation would confer sensitivity to γ -radiation on the organism so that incorporation of such analogues into cancer cells might lead to the possibility of tumours being made more sensitive to irradiation. We have reported the synthesis of two such analogues, 5-vinyluracil (Jones *et al.*, 1974) and 5-ethynyluracil (Barr *et al.*, 1976), the deoxynucleosides of both of which have structures very similar to that of thymidine (Hamor *et al.*, 1978; Barr *et al.*, 1978) and have been shown to be quite potent inhibitors of herpes virus (Walker *et al.*, 1978; De Clercq *et al.*, 1979). The indirect action of γ -radiation on DNA is mediated by radicals formed in water or by other organic radicals (Blok & Loman, 1973). These are likely to attack the unsaturated side chain of the analogue, causing the production of a molecule incompatible in structure with its position in double-helical DNA, or to produce radicals that may induce other lethal consequences. 5-Ethynyluracil, however, is quite toxic to cells, and we have evidence that its incorporation into the DNA of micro-organisms is extremely low, if any takes place at all, so for irradiation studies we have concentrated on 5-vinyluracil, which is relatively non-toxic (> 500 μ g/ml is not toxic to *Mycoplasma* cells) and which we have been able to incorporate into the DNA of *E. coli* (10% replacement of thymine) and *Mycoplasma* (17% replacement of thymine) (Chelton *et al.*, 1973; Jones & Walker, 1975). Attempts to use *E. coli*, however, for radiation-sensitivity experiments were abandoned when it was found that in order to achieve a sufficiently high incorporation of analogue into the cells, the concentration of analogue required in the medium was close to its toxic limit and it was difficult to interpret the results. With *Mycoplasma*, however, despite the difficulty in growing the cells

and obtaining viable counts, 5-vinyluracil is not toxic, and for irradiation purposes the organism has an advantage in that it possesses no cell wall and also that its DNA normally contains as much as 37.5% of its bases as thymine (Jones & Walker, 1963).

To our knowledge, no experiments on the sensitivity of any *Mycoplasma* species to γ -radiation have previously been reported, although γ -irradiation of mammalian cells (Cerutti, 1974) has been extensively studied and is the subject of many reviews (e.g. Blok & Loman, 1973). It is also known that incorporation of the thymine analogue 5-bromouracil into the DNA of *E. coli* (Bonara & Smith, 1977) and phage T4 (Myers *et al.*, 1977) causes an increase in the sensitivity of the cells to γ -radiation. Here we report that cells of a *Mycoplasma* species grown in the presence of 5-bromouracil become sensitive to γ -irradiation and that when grown in the presence of 5-vinyluracil, the incorporation into the DNA of which, unlike 5-bromouracil, causes no increase in u.v.-radiation sensitivity (E. T. J. Chelton, A. S. Jones & R. T. Walker, unpublished work), show a 3-fold increase in sensitivity to γ -radiation of a dose of 15 krad.

Materials and Methods

5-Vinyluracil was prepared as previously described (Jones *et al.*, 1974).

Organism

The strain *Mycoplasma mycoides* var. *capri* P.G.3 was obtained from Dr. D. G. ff Edward in 1961 and has subsequently been checked by Dr. R. M. Leach (Mycoplasma Reference Laboratory, Norwich, Norfolk, U.K.) for identity and purity by the appropriate cultural and serological tests.

Medium

The medium was the 'normal medium' previously described (Jones *et al.*, 1965), which contains Oxoid

brain/heart infusion (3.7%, v/v), horse serum (Wellcome; 1%, v/v) adjusted to pH 7.4, with penicillin (100 units/ml) and thallium(I) acetate (0.01%) added. All batches of media were incubated for several days before inoculation to check for freedom from contamination. The medium used for dilution when determining viable counts was the 'dilute medium' previously described (Jones *et al.*, 1965), which contains Oxoid brain/heart infusion (0.45%), Oxoid peptone (1%), NaCl (0.5%), NaH_2PO_4 (0.25%) and glucose (0.2%).

Conditions of growth

The organism was subcultured at weekly intervals with the normal medium. The inoculum was prepared by adding 0.02 ml of the latest subculture to 1 ml of normal medium and incubating at 37°C for 24 h. This culture (0.5 ml) was used to inoculate the medium (9 ml), which was then incubated at 37°C in a 20 ml screw-capped bottle without aeration for 16 h. An A_{750} of 0.25–0.35 was routinely obtained. If grown in the presence of analogue, 1 ml of a sterile aqueous solution of the analogue at the appropriate concentration was added at the time of inoculation. The control sample had 1 ml of sterile water added. Analogue solutions were sterilized by means of a Hemming filter.

Conditions of radiation

γ -Radiation from a ^{60}Co source was used. The source was regularly calibrated, and exposure of the cultures was performed at a constant distance from the source (10 cm) with the time being varied to enable a radiation dose of 15 or 30 krad (~6 or 12 min) to be delivered. A standard volume of 1 ml was exposed in a flat-bottomed glass tube of circular cross section. Portions of each culture used were removed before exposure to radiation and were left at room temperature while irradiation of the other portion took place. Results are quoted as the percentage of cells remaining viable in the irradiated portion compared with the

non-irradiated portion. It was found that the time taken to perform the irradiation procedure (45 min) caused no significant difference in the viable count of the non-irradiated portion. To investigate the effect of the presence of the analogue in the medium on the effect of radiation on the organism rather than the effect of incorporation of the analogue into the nucleic acid, further controls were performed in which the analogue was added to the cell culture immediately before irradiation. Water of equivalent volume was added simultaneously to the control culture medium.

Viable counts

Serial 10-fold dilutions of the cultures to be assayed were prepared with the 'dilute medium' and plated on agar (0.8%) made from normal medium containing 10% (v/v) horse serum and no penicillin or thallium(I) acetate by using the technique of Miles & Misra (1938). The plates had to be incubated at 37°C for 72 h and it is convenient to do this in a sealed can containing damp paper to prevent the plates from drying out. Under the conditions used, no significant toxicity due to the analogue could be seen; the viable counts only varied depending on the exact age of the culture used, from 1×10^8 to 5×10^8 viable cells/ml.

Results and Discussion

The results of irradiating cultures of *Mycoplasma mycoides* var. *capri* cells are given in Table 1.

It is known from our previous results (Jones & Walker, 1975) that 5-bromouracil and 5-vinyluracil can be incorporated into the DNA of *M. mycoides* var. *capri*, presumably replacing thymine residues. The present results show that incorporation of 5-bromouracil into DNA of a *Mycoplasma* species is accompanied by an increase in the sensitivity of the organism to γ -radiation, as is found generally with other organisms. These preliminary results convinced us that the incorporation of a potential

Table 1. γ -Irradiation of *Mycoplasma mycoides* var. *capri* cells

Cultures of *Mycoplasma* cells were grown in the presence of the analogue indicated, subjected to γ -radiation and the number of viable cells remaining were counted and expressed as a percentage of cells from the same culture that had not been irradiated. Each experimental value is the result of counting and averaging the number of cell colonies produced in six 0.02 ml drops on each of two plates. The number of independent experiments is given in parentheses.

Analogue (concn. in $\mu\text{g/ml}$)	Radiation dose (krads)	Cells remaining viable (%)	
		30	15
Control (no analogue added)	...	31 ± 5.5 (10)	71.5 ± 3.5 (6)
5-Bromouracil (10)		2 ± 1.0 (2)	—
5-Vinyluracil (100)		7 ± 1.5 (5)	28.5 ± 3.0 (4)
5-Vinyluracil (100)*		19 ± 4.0 (3)	74.0 ± 3.5 (2)

* Analogue added only immediately before irradiation.

radiation-sensitive base analogue into *Mycoplasma* DNA would be detected by using this technique, and the results shown that *M. mycoides* var. *capri* when grown in the presence of the analogue 5-vinyluracil shows a 3-fold increase in sensitivity to γ -irradiation at 15 krad. The dose-response curve (results not shown) of the control cells is essentially linear over a dose range of 0-40 krad, the cell viability decreasing by 20% for each 10 krad of irradiation used. At 30 krad, the results are somewhat more complex, as although a similar increase in sensitivity was observed, some of this increased sensitivity was apparently due to the presence of the analogue in the medium during the irradiation; presumably reaction products of the analogue reacted with the cells to cause non-viability. This feature was not present when cultures were irradiated at 15 krad. Thus we have demonstrated that the thymine analogue 5-vinyluracil, which has previously been incorporated into the DNA of *Escherichia coli* and a *Mycoplasma* species, causes, as predicted, an increase in the sensitivity of the latter organism to γ -irradiation.

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