SYNERGISM BETWEEN GAMMA AND ULTRASONIC IRRADIATION OF THE BACTERIUM E. COLI B

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1 INTRODUCTION

Using simple and conventional culture techniques for the bacterium E. Coli B synergism between independently lethal doses of ultrasonic and cobalt-60 gamma irradiation is established by comparing the surviving fraction for gamma irradiations for samples with and without a preceding exposure to ultrasonics.

The results reported here are those of a pilot study where the main aim of the work was to establish whether conditions could be found in which synergism could be demonstrated. As a result of the necessary flexibility of technique for such a project the experimental procedures lack a number of refinements which will be introduced in later work. While this lack of refinement may affect the sensitivity of the tests to determine whether synergism exists as indicated in the text such an effect can clearly not invalidate any positive conclusions reached from these tests.

2 EXPERIMENTAL METHOD

Irradiation cultures were produced by 100: 1 dilution into 0.85% (w/v) saline of a stationery phase culture, in 'Oxoid' nutrient broth, of the bacterium E. Coli B. This procedure leaves traces of the nutrient media in the samples which could be utilised by the repair mechanism known to operate at room temperatures (1). To avoid complications due to this repair mechanism samples were slowly cooled to around $4^{\circ}C$ prior to irradiation and maintained as close to this temperature as possible at all stages of the experiment up to final plating.

Sonication was achieved by lowering the sterilised probe of a 13 kHz ultrasonic cleaning unit into the sample. After a series of trial experiments to investigate the effect of variation of sample size and irradiation geometry upon the reproducibility of readings and the heating effects of sonication the following conditions were selected. The irradiation sample consisted of 80ml of culture in a 100ml beaker which was accurately located at the centre of a 1 litre beaker of crushed ice to limit the heating effect. Despite this precaution a limited temperature excursion did occur during sonication but was limited to a rise of some $10^{\circ}C$ after 30 mins exposure and samples returned to the original temperature very rapidly after sonication ceased.

The probe itself was immersed to a depth of 4mm in the culture; a cathetometer being used to ensure accurate relocation. This emphasis on standardised geometry during sonication ensured reasonable reproducability of dose under conditions where interface reflections were important.

The ultrasonic dose rate was obtained by noting the initial rate of

Ultrasonics		Gamma Exposure Times - (Mins) 2	9	19
Exp. (mins)	SF	SF	SF	SF
0	1	0.62 (0.48)	0.078 (-2.47)	0.0072 (-4.94)
4	0.54	0.34 (-0.67)	0.038 (-3.23)	0.0023 (-6.07)
17	0.005	0.39 (-0.47)	0.052 (-2.90)	0.0022 (-6.12)
30	0.014	0.30 (-0.85)	0.021 (-3.82)	0.001 (-6.91)

Table 1. Surviving Fractions for First Series of Experiments (Numbers in brackets are logits used in 't' test).

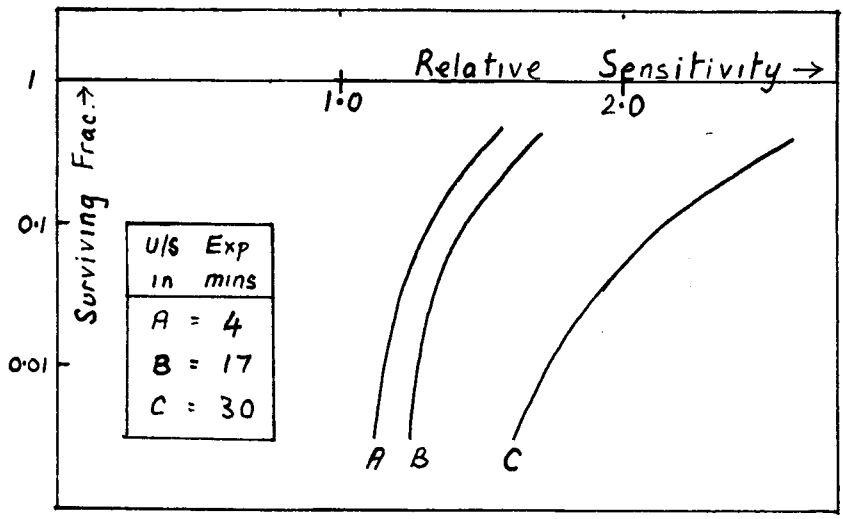

Fig.1 Second Series of Experiments. Relative Sensitivity to gamma irradiation as a function of survival.

increase of temperature of the sample and hence computing the rate of energy deposition as 64.5±1.3 W kg^{-1}.

The gamma irradiation of the samples was achieved by exposure of the 80ml sample in a kilo Curie cobalt-60 facility at a dose rate of 1.95 krad min^{-1} as measured by the Fricke method. The sample was contained in a boiling tube which was accurately located in a container of crushed ice at a fixed point in the radiation field. The use of such a large sample permitted small aliquots to be taken without serious perturbation of the irradiation geometry and eliminated the possibility of different samples experiencing different dose rates.

The large sample size clearly involves, however, a considerable variation in dose rate across samples placed close to a large gamma source. Measured plate counts thus yield the average response to a range of doses but this is not important when comparing samples provided the dose distribution function is the same for both. Although the normal smoothing effect of such an averaging process may result in a reduction of the sensitivity with which synergism can be detected this cannot affect the validity of the conclusions reached below.

A standard time interval of 10 min was allowed between sonication and the subsequent exposure to gamma irradiation. Aliquots taken before and after sonication allow evaluation of the sonic surviving fraction. Aliquots taken after the gamma irradiation allow evaluation of the gamma surviving fraction.

Following appropriate dilution of the aliquots obtained, surface plating on to prepared Petri dishes containing 'Oxoid" nutrient agar and incubation for 24 hours at 37°C visible colonies were counted and used to calculate the various surviving fractions.

3 RESULTS

From trial experiments ultrasonic exposures of 4, 17 and 30 mins were selected to provide a reasonable spread of sonic surviving fractions. These exposure times were used in presonication of samples in both series of experiments.

3.1 First Series of Experiments

From trials gamma ray exposures of 2, 9 and 19 mins were selected.

For each combination of ultrasonic and gamma ray exposures the surviving fraction following gamma irradiation was obtained for samples with and without presonication. These surviving fractions are presented in Table 1.

Since data in the form of proportions does not readily lend itself to standard statistical procedures the surviving fractions were converted into logits using

$$\text{logit, Q (SF)} = \ln (SF/(1-SF))$$

The Student 't' test as applied to matched pair data was then used to examine the difference in each pair of logits. The 't' value of 8.04 obtained for the nine pairs, and hence eight degrees of freedom, indicates quite clearly that the observed difference in logits, and hence surviving fractions, is most unlikely to have occurred as a

result of random error.

3.2 Second Series of Experiments

The differences in surviving fractions discussed in the previous section possibly indicate a dependence upon the magnitude of the ultrasonic and gamma ray exposures. For each value of presonication a gamma ray survival curve over 3 decades was obtained for samples with and without presonication.

Each survival curve was fitted by computer to the simple quadratic formula

$$\ln (SF) = -\alpha D - \beta D^2$$

and the relative sensitivity, (R.S. = dose without sonication to produce given survival divided by the dose with sonication to produce the same survival), obtained for different levels of survival, fig 1.

The curves in fig 1 indicate that the synergistic effect increases with increasing ultrasonic dose. With increasing gamma ray doses for a given presonication the synergistic effect decreases indicating that shouldered survival curves become less shouldered.

4 CONCLUSION & COMMENT

Both series of experiments indicate quite clearly that the gamma ray sensitivity increases following presonication. The question is whether this is a genuine synergism or is a result of the heating effect described by Clarke Hill (2) which may be used to explain a number of results for a simultaneous non-lethal sonication and x-irradiation.

Although a temperature excursion did occur during sonication this was eliminited before gamma irradiation commenced and it seems unlikely that the biological 'memory' of such a small excursion could produce such noticeable results. The author's believe, therefore, that these results represent a genuine synergism.

(1) Stapleton, G.E, et al., J. Cell Comp. Physiol, 41 (1953) 345

(2) Clarke, P.R, Hill, C.R Br. J. Radiol. 43 (1970) 97