STUDIES ON THE POSTIRRADIATION OXYGEN EFFECT IN BACTERIAL SPORES

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RECENT work involving exposure of dried bacterial spores to ionising radiations shows that the presence of oxygen after irradiation increases lethal damage caused by energy absorption (Powers, Webb and Kaleta, 1960; Tallentire and Davies, 1961). It is of particular interest in the application of "radiation sterilisation" that the lethal efficiency of a dose of radiation can depend on the storage conditions of the irradiated material. Preliminary experiments described are designed to elucidate the mechanisms of these postirradiation effects.

Kaolin powder contaminated with spores of *Bacillus subtilis* (NCTC 3610) (Tallentire and Davies, 1961) was used for the experiments. Weighed samples were dried at less than 10^{-5} mm. of mercury for 6 hr., refrigerated traps being incorporated in the drying train. Samples were then sealed under vacuum and treated at 22° with different doses of gamma-radiation from a cobalt-60 source. After irradiation, samples were stored in controlled gaseous atmospheres at 25°. Control samples were treated in an identical manner omitting only the irradiation step. The criterion of lethal damage chosen was the inability of the spore to give rise to a colony on incubation in nutrient agar. From colony counts of samples yielding surviving fractions less than 0·5, exponential dose/survival curves were constructed and the slopes of these estimated using the expression

$$\text{Surviving fraction} = e^{-kD}$$

where k is the slope and D the dose in Krad. The slope is used as a measure of the lethal efficiency of the radiation, higher values of k indicating greater efficiency.

The highest level of lethal efficiency shown in Fig. 1 is that resulting from postirradiation storage of spores in oxygen for 48 hr. (k = 0·045 $Krad^{-1}$). The lowest efficiency is that for identical oxygen treatment preceded by exposure to nitric oxide for 15 min. (k = 0·010 $Krad^{-1}$). This prevention of the postirradiation oxygen effect by nitric oxide confirms the previous work of Powers, Webb and Kaleta (1960) who concluded that such treatment with nitric oxide removes radiation-induced free radicals which on exposure to oxygen combine to produce damage lethal to the spore.

An intermediate value of k = 0·023 $Krad^{-1}$ results from exposure to oxygen for 30 min., and the same slope is obtained when spores are stored in oxygen for 30 min. then in a vacuum of less than 10^{-5} mm. of mercury for 47·5 hr. When the oxygen pressure is restored to 760 mm.

so that the total storage period in oxygen is 48 hr., k is increased to 0·046 Krad^{-1}, a value almost identical with that obtained from uninterrupted postirradiation exposure to oxygen for 48 hr. (0·045 Krad^{-1}, see Fig. 1). Clearly, the development of the postirradiation oxygen effect can be arrested by removing the oxygen and can be restarted by re-admitting oxygen to the dried spore system. Treatment with nitric oxide between exposures to oxygen, however, prevents further development of the oxygen effect. Thus exposure of irradiated spores in turn to oxygen for 30 min., to nitric oxide for 15 min., and then to oxygen up to 48 hr. gives a value for k of only 0·026 Krad^{-1}.

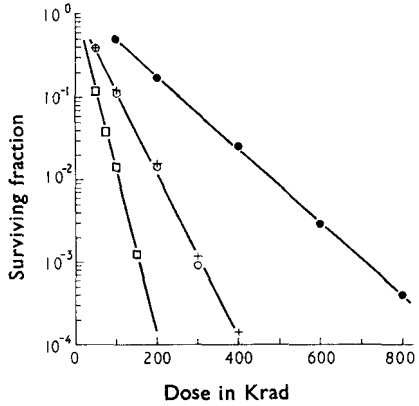

FIG. 1. Gamma irradiation of secondary dried *B. subtilis* spores in vacuum with different postirradiation storage treatments at 25°.

□ ——— □ 760 mm. O_2 for 48 hr. Slope k = 0·045
\+ ——— + 760 mm. O_2 for 30 min. Slope k = 0·23
○ ——— ○ 760 mm. O_2 for 30 min. and then vacuum (less than 10^{-5} mm. of mercury) up to 48 hr. Slope k = 0·23
●· ——— ● 90 mm. NO for 15 min. and then 760 mm. O_2 up to 48 hr. Slope k = 0·10

From these preliminary results and accepting the views of Powers, Webb and Kaleta (1960) on the scavenging role of nitric oxide, we infer that with samples dried as described above, the postirradiation oxygen effect in spores results from an association of free radicals with oxygen. In addition, we show that potentially harmful radicals can be removed even after exposure to oxygen and after partial development of the oxygen effect. Since these radicals are also harmless when the oxygen pressure is reduced, we propose that gaseous oxygen maintains the existence of an unstable oxygen-radical complex which itself is responsible for the postirradiation oxygen-dependent lethal effect.

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