PHYSICAL DETERMINANTS OF RADIATION SENSITIVITY IN BACTERIAL SPORES

APPENDIX I

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ABSTRACT

Several factors modifying radiation sensitivity in dry bacterial spores are described and discussed. Vacuum inducing the loss of critical structural water, very low dose rates of radiation from which the cell may recover, radiations of high linear energy transfer, and the action of temperature over long periods of time on previously irradiated cells are recognized from extensive laboratory work as important in determining survival of spores exposed to low radiation doses at low temperatures for long periods of time. Some extensions of laboratory work are proposed.

INTRODUCTION

The bacterial spore, if it did not already exist, would have to be invented to provide science with a proper test object that could be treated with the severity and harshness required of physico-chemical tests of the nature of very early processes responsible for biological injury after absorption of radiation energy by the cell. Certainly it is the biochemical inertness of the spore that allows it to survive a very large variety of unphysiological conditions. Enzymes--the critical, sensitive links among the many highly intertwined and interdependent elements of the cellular biochemical circuses--are in very small number, are largely non-functional, and appear to be unnecessary for the survival of the spore. Enzyme systems are synthesized and operate only after restoration of the spore to biological ambience; and it is at this time that the question of survival, or nonsurvival, is answered. Has the central element in the spore--most probably DNA--retained its ability to direct successfully the enormously complex processes of germination, outgrowth and colony formation? What physicochemical inferences can be made concerning the interaction between radiation and cellular DNA after the variety of treatments given the spore before, during, and after radiation?

Many experiments in recent years have demonstrated that, indeed, basic information of a physico-chemical nature can be gained by applying techniques of the radiation physical chemist. And it is coincidentally fortunate that some of this information is useful in considering whether these organisms have useful expectation of survival in space environments for any length of time.

In "empty" space itself two important environmental factors can militate against the survival of the bacterial spore: vacuum itself and radiation.

VACUUM

Vacuum *per se* is a determinant of radiation sensitivity because the spore in space is in a non-equilibrium condition with respect to water vapor and faces a constant (although perhaps slow) loss of important structural water as the consequence of thermal accidents. It must be noted that a change from ordinary "earth" vacuum ($<10^{-7}$ torr) to "space" vacuum ($<10^{-12}$ torr) cannot conceivably increase the rate of loss of water from the spore, but it drastically affects the chance of reentry of a compensating or substituting water molecule. The mean free path of a gas molecule at 10^{-12} torr is measured in thousands of miles. And the fact that the loss may be slow is, perhaps, of no consequence when put against the enormous times required for interstellar travel; i.e., however slow the loss, there is plenty of time.

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This structural water, called by some "water of specific hydration", is the water not easily removed by pumping techniques, and its importance for the continued "survival" of biologically important macromolecules of the cell is under examination and debate by only a few investigators. The particular problem of the bacterial spore is special because of the unique packaging of its DNA [1], and because of the indirect evidence from radiation studies that this DNA might be considered "dry" in comparison with others [2]. In these studies spores were irradiated with short pulses of high energy electrons and then at various short times afterwards were exposed to pulses of oxygen to measure the lifetimes of radicals that become lethal when complexed with oxygen. In the wet spore there are two lifetimes--seconds in length--that are intermediate in length between the very long-lived radicals seen in dry spores (hours), and the very short life-times seen in vegetative cells (milliseconds), leading the author to propose that spore DNA is in intermediate state of wetness.

If this is supported, then the prospects for survival after long-term drying might change. In addition to this evidence, however, much more is required and extensive special studies on the loss of structural water and its effect on survival over long periods of time should be begun forthwith.

RADIATION

The spore is sensitive to radiation, high and low energy, ionizing and non-ionizing. Fortunately there are available the two kinds of information required for making approximate predictions of survival in some regions of space over periods of time: 1) the nature of and approximate intensities of radiation in our solar system (not necessarily the same as that in others or that between systems); and 2) the response of the spore to radiation of various kinds under many different environmental conditions. The main purpose of this presentation is to review briefly some of the evidence obtained on the radiation sensitivity of the bacterial spore in environments similar to those of space, and with radiations that might be encountered therein.

There are many studies on the effectiveness of high-energy radiations in inducing lethality in spores. Among the experimental variables that have been examined, the following are important enough to list here.

Dose Rate. Surprisingly to some of us who expected differently because of the first order inactivation kinetics shown by spores, there is a dependence upon the rate at which the radiation energy is delivered to the spores [3]. Dose rate changes from 300 r/min up to about 65,000 r/min cause an increase of about 30% in radiation sensitivity (Figure 1).

Fig. 1 Survival of spores of *Bacillus megaterium* irradiated with 50 kVp X-rays at various dose rates [3].

The study of dose rate dependence at high dose rates has been extended considerably by Tallentire and colleagues and is treated separately in this volume [4]. But the problem of very low dose rates over long periods of time remains. No results of experiments designed to test this have been published, the kind requiring one or two years or longer exposure to radiation fields of low intensity. There is information on the reversibility of the O_2 dependent free radical component of damage in dried spores that bears on this question (below), but it has not been investigated at very low dose rates. In my opinion there is a need for this information, for these are the long-term conditions encountered in space.

Linear Energy Transfer. For photons there is a dependence of radiation sensitivity on their

energy. Deliberate attempts to compare radiations of different energy in the same laboratory [3] have shown that for radiations of 47.5 mm Al HVL to 0.065 mm Al HVL the inactivation rate constant varies from $12.5 \times 10^{-3} \text{kr}^{-1}$ to $30 \times 10^{-3} \text{kr}^{-1}$ (Figure 2) for spores of *Bacillus megaterium*.

Fig. 2 Survival of spores irradiated at a constant dose rate with high energy photons of varying energies [3].

Fig. 3 Changes in magnitude of the two oxygen effects and the anoxic component of damage in spores as Z of the accelerated charged particles is changed [5].

cles, this does not prove anything concerning sensitivity of spores to these radiations when the "water of specific hydration" referred to above is removed--if indeed it can be removed. Certainly in these experiments in which the lowest pressure was 10^{-7} torr and the longest time held at that pressure at room temperature was 10 to 12 hours, the structural water problem is not addressed at all. This question is entirely open, and it must be answered.

Temperature and Time. One of the remarkable features of the radiation biology of the spore is that in the dry state ionizing radiations produce free radicals with long life-times that become lethal to the spore if they are allowed to react with oxygen. Removal of them can be accomplished in various

density of ionization goes down in value (i.e. the energy of the photons increases) the radiation sensitivity decreases. That this is so (and it has been observed by several sets of investigators) is an as yet unexplained phenomenon, since it is the exact opposite of expectation on the basis of regular target theory. Whatever the explanation, LET is a parameter of importance as experiments with high energy charged particles support.

One set of experiments with charged particles from D^{1+} through Ne^{10+} at 8 MeV/nucleon shows a change in total radiation sensitivity in O_2 from $20 \times 10^{-3} \text{krad}^{-1}$ to $52 \times 10^{-3} \text{krad}^{-1}$ as LET goes from D^{1+} to C^{6+} at 200 keV/ μm [5] (Figure 3). This increase is due entirely to an increase in the oxygen independent part of the total damage (k_I), with the two oxygen components remaining the same (k_{II} and k_{III}). Beyond C^{6+} to Ne^{10+} there is a decrease in both O_2 -dependent and O_2 -independent damage, with the former going to zero; that is, at 500 keV/ μm there is no oxygen effect. Several sets of workers have begun investigations of very high Z charged particles on cells, particles of the kind found in space environments. The behavior of the various components of damage as described fully for the spores of *Bacillus megaterium* [5] must be accounted for in these studies.

In a later set of experiments we have shown [6] that water content at moderately high LET ceases to be an important variable, at least for the O_2 independent part of damage. With Ne^{10+} at 8 MeV/nucleon spores equilibrated with partial pressures of water from 2×10^{-6} torr to 14.5 torr show no difference in response (Figure 4). But while this indicates, perhaps, independence of water content at the LET produced by charged parti-

Fig. 4 Response of spores of varying water contents to irradiation by Ne^{10+} particles [6].

Fig. 5 The temperature dependence of the reversal by heat annealment of the oxygen-free radical effect in spores presented in the Arrhenius form [8].

Fig. 6 The temperature dependence of radiation sensitivity of the spore in the Arrhenius form [9].

ways: addition of water, or the free radical scavengers H_2S and NO , and the exposure of the spores to heat for periods of time inversely proportional to the temperature [7]--i.e., at higher temperature removal of this free radical component is completed in times short compared to those at lower temperatures [8]. The reduction in sensitivity by irradiating at higher than ambient temperatures is appreciable: the sensitivity is reduced to 1/3 the value seen at lower temperatures.

In the temperature range tested, the removal of the free radical component by heat follows an exact rule as shown by the Arrhenius relation in Figure 5, and it might be expected to operate at extrapolated low temperatures, though at correspondingly low rates.

But while thermal annealment of this free-radical portion of radiation-induced damage with time means that some part of the radiation damage disappears, a problem arises when we try to extend the thinking beyond the range of temperature at which the measurements were made. Our studies at very low temperature [9] reveal that the oxygen-independent part of damage is temperature-independent from very low ($6^\circ K$) temperatures to $125^\circ K$, and then shows a small temperature dependence ($\Delta E = 0.110$ kcal). Figure 6 is the Arrhenius plot of k_1 in this region. This means that thermal dependence is not observed for the spore below $125^\circ K$; and we can ask then, why believe that thermal annealment can occur at these low temperatures? This requires investigation.

Does annealment, obviously thermally dependent, operate at very low temperatures where other processes, thermally dependent at higher temperatures, are temperature independent?

QUESTIONS

These brief considerations raise a number of questions that bear on the expectation of survival of spores in the space environment.

1. What is the effect on viability of the removal over very long periods of time of that water not removed from macromolecules in ordinary low temperature laboratory experiments? Is "structural" water (or the "water of specific hydration") truly necessary for the survival of the spore?

2. What are the consequences concerning radiation sensitivity of the removal of this structural water component? There is evidence that the isotopic composition of water in this water compartment of the cell influences radiation sensitivity [10], so it appears likely that its removal in some way would also affect radiation sensitivity, perhaps by making impossible the transfer processes required for anoxic fixation of radiation injury, and the time dependent annealment by heat of toxic free radicals.

3. What is the efficiency of ionizing radiation delivered to the spore at extremely low dose rates over very long periods of time, time periods that allow experimental assessment of the various competing post-radiation processes observed experimentally in the dry spore? Are there low dose rates at which the spore is not sensitive to radiation?

4. How important is the "magic" temperature of $125^\circ K$ to the spore in space? Does the absence of temperature dependence below this mean that molecular movements and energy transfer are inhibited to the degree that post-radiation development or erasure of damage is inhibited or prevented?

5. How do the conceivable interferences with energy transfer processes by very low temperature on the one hand, and by removal of structural water on the other, add together, or synergistically interact, when they are acting on the spore simultaneously?

6. And how do all the experimental variables above operate when charged particles of high Z impinge on the spore?

The questions suggest several kinds of experiments that can be done beginning now on spores and model molecules that would make more secure our thinking of the spore in space, and the likelihood of its surviving. And the information will be important not only for that thinking, but it will be significant for our understanding as to how this unique cell is constructed, and how that structure relates to living processes.

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