CORRELATION OF BACTERIAL RADIOSENSITIVITY AND DNA BASE COMPOSITION*

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The incorporation of certain purine and pyrimidine base analogs into deoxyribonucleic acid (DNA) of bacterial and mammalian cells has been shown to augment their sensitivity to the lethal effects of ultraviolet and ionizing radiations (Greer, 1960; Djordjevic and Szybalski, 1960; Kaplan, Smith, and Tomlin, 1961, 1962). The fact that alteration (by the analogs) of DNA base composition can influence radiation response suggested that the radiosensitivity of DNA might be a function of natural base composition, despite the fact that X-irradiation of the free bases or of DNA in solution reveals only small differences in the extent of inactivation of the individual bases (Hems, 1960; Scholes, Ward, and Weiss, 1958). It has long been known that there are significant and characteristic differences in the average base composition of DNA from different bacterial species (see references in Belozersky and Spirin, 1960). These differences in base composition are reflected in the physical properties, such as melting temperature (Marmur and Doty, 1959) and density gradient equilibrium (Sueoka, Marmur, and Doty, 1959), of DNA from different species.

Accordingly, several different bacterial species known to exhibit a wide range of DNA base composition have been studied with respect to X-ray sensitivity. Limited UV studies have also been carried out on some of these species, but could not be extended to the larger bacteria because of dosimetric technical problems. Escherichia coli, strain B, was obtained from the Department of

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Genetics; Pseudomonas aeruginosa and Micrococcus pyogenes, var. aureus, from the Infectious Disease Laboratory, Department of Medicine; Bacillus subtilis (strain D-39), Bacillus cereus (strain D-73), and Serratia marcescens (strain Z-4) from the Department of Medical Microbiology. All were grown on a gyrotory shaker overnight at 37°C in brain-heart infusion medium (Baltimore Biol. Lab. No. 01-.239), harvested by centrifugation, washed once, and resuspended in glucose-free modified Davis mineral medium (Kaplan, Smith, and Tomlin, 1962). Irradiations were carried out by the techniques previously described (Gunter and Kohn, 1956). Those bacterial species which were able to tolerate anoxic conditions were also irradiated in an atmosphere of nitrogen by a technique previously described (Kaplan, Zavarine, and Earle, 1962).

The irradiated cells were then diluted appropriately, plated on yeast extract agar, and colonies counted after overnight incubation at 37°C. Survival was calculated from the colony counts at each dose level as a fraction of the colony counts of an unirradiated aliquot.

Under such closely controlled pre- and postirradiation culture conditions, reproducible dose-log survival curves were obtainable from each of these bacterial species, and a family of such curves, based on two or more experiments for each organism, is seen in Fig. 1. The slope of the exponential portion of each dose-log survival curve, expressed as the D_{10}, or dose associated with 10 per cent survival in this part of the curve, may be taken as a convenient index of radiosensitivity. When the D_{10} is plotted against the published guanine-cytosine (GC) content of each species (Belozersky and Spirin, 1960), a linear relationship is observed (Fig. 2), which is also fitted by data for two other organisms, Pseudomonas fluorescens and Azotobacter agile (Gunter and Kohn, 1956). Irradiation under nitrogen changed the slopes of the dose-log survival curves about equally for Escherichia coli B, Pseudomonas aeruginosa, Serratia marcescens, and Micrococcus pyogenes ($D_{10}N_2/D_{10}O_2$ = 2.65; 2.72; 2.78; and 2.56, respectively). It is concluded that a correlation exists between X-ray sensitivity and DNA base composition of these

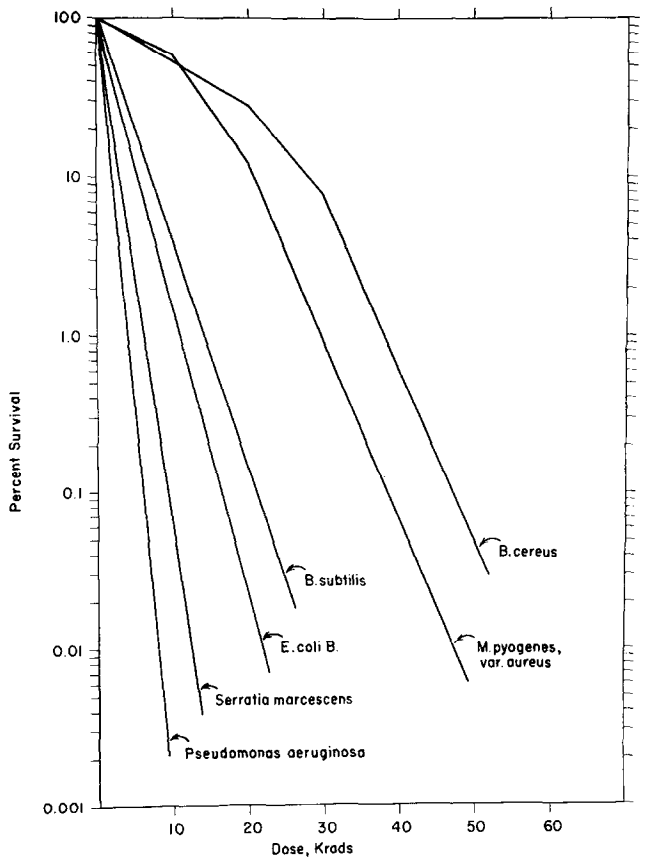

Figure 1. Percent Survival as a Function of X-ray Dose.

bacterial species under aerobic and anaerobic conditions. Preliminary UV data indicate a distinctly different, and possibly inverse, relationship for three species; the dose-log survival curve of Micrococcus pyogenes exhibited a steeper slope than that of either Serratia marcescens or Escherichia coli, which were very similar.

Although it seems most probable that the greater X-ray sensitivity of GC-rich species is attributable to radiobiochemical lesions produced in DNA, the possibility cannot be excluded from these data alone that radiosensitivity and GC content are related through some other common denominator. For example, it might be argued that the primary effect of irradiation is on messenger RNA, since the base composition of DNA probably determines the base composition of

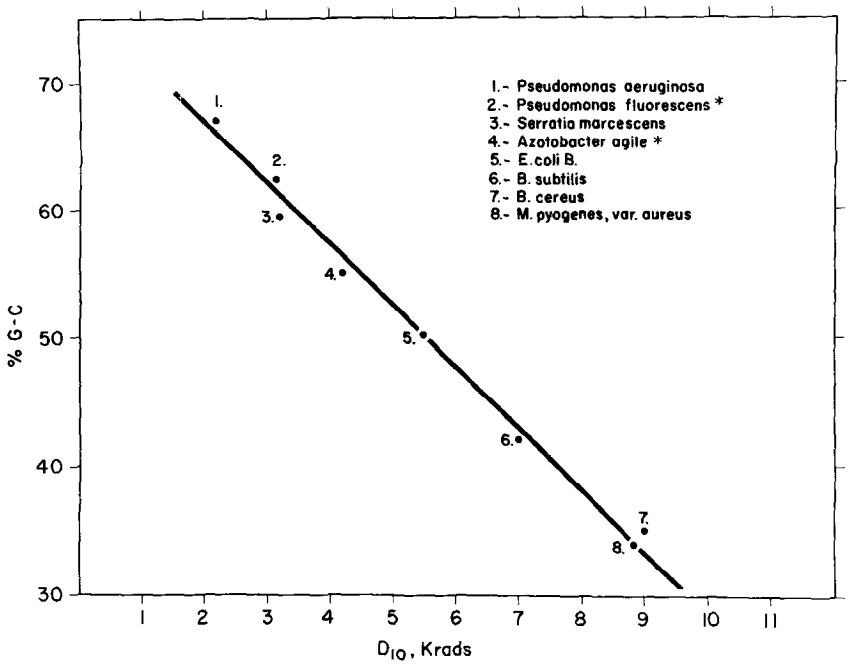

Figure 2. Relation between the GC Content of the DNA of Different Bacteria and the D_{10} in Krads, where D_{10} is the Dose which Reduces the Surviving Fraction to 10 Per Cent on the Exponential Part of the Survival Curve. *Data from Gunter and Kohn (1956).

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messenger RNA (Brenner, Jacob, and Meselson, 1961; Astrachan and Volkin, 1958). Moreover, even if the primary effect of irradiation were on DNA, it does not necessarily follow that GC-rich DNA is inherently more sensitive than AT-rich DNA. There is increasing indication that intracellular mechanisms exist for the repair of biochemical damage inflicted by ionizing radiation (Howard-Flanders, 1961). It might well be that radiation damages AT and GC equally, but that the former is preferentially acted upon by these repair mechanisms. If the difference in response is, in fact, due to a greater inherent sensitivity of the GC-rich segments in the DNA, the maintenance of the same relative relationship under anoxic conditions would suggest that the principal radiobiochemical effect is exerted on cytosine, rather than on guanine, in view of the differences in response to anoxia exhibited by purine and pyrimidine analogs in bacterial DNA (Kaplan, Zavarine, and Earle, 1962). These problems remain to be resolved.

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