

THE EFFECTS OF HEAVY IONS ON DNA IN THE SALMONELLA MUTAGENICITY TEST^G

A.M.Kellerer, M.Lassmann, H.Roos, W.-H.Thomas, A.Wenning · Inst.für Med.Strahlenkunde, Universität Würzburg

The Ames test, i.e. the test for the reversion of histidine deficient (his⁻) auxotrophic Salmonella typhimurium strains to histidine prototrophy, is the most widely used bacterial test for the identification of mutagenic chemicals. In spite of the desirability of a comparison of chemically induced and radiation induced damage to DNA and the interest in assessing the interaction of the two types of damage, there have been comparatively few studies with ionizing radiations on this experimental system.

Although Ames had noted in 1972 that x-rays and fission neutrons induce reversions in a number of his⁻ tester strains, later authors came to the conclusion that the Ames test fails to respond to ionizing radiations. However, recent reports have established the mutagenic response of various Ames tester strains to x-rays and γ-rays. The new salmonella strain TA2638 has been found to be particularly sensitive to ionizing radiations. Extending previous work at the UNILAC on the phage PM2 we have, therefore, initiated experimental studies of the relative mutagenic efficiency of heavy ions in Ames tester strains.

The experimental procedure of the Ames test requires the plating of the bacteria on top of a layer of agar in the presence of a limited amount of histidine which supports initial growth of the bacteria until reversions have taken place which permit unlimited growth of the reverted bacteria even in the absence of histidine. In the usual procedure the bacteria are distributed within a 0.3mm layer of top agar; this is sufficiently close to the surface to permit exposure to the heavy ions at energies of 20 MeV/amu.

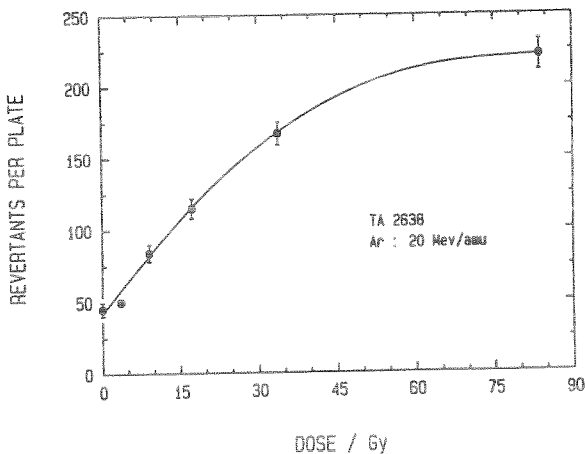
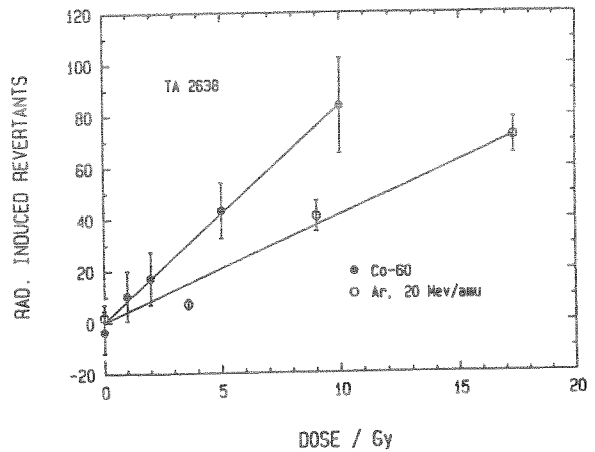


Fig.1 gives the dose dependence of the yield of revertants per plate (5×10^8 bacteria plated) for argon ions of 20 MeV/amu. The initial part of the dependence is linear. The deviation from linearity at higher doses reflects cell killing.



In Fig.2 the initial part of the dose-effect relation for Argon ions is compared to the dose-effect relation for Co-γ-rays. The relative biological effectiveness of the Argon ions is approximately 0.5.

More sophisticated procedures for the determination of the mutation frequencies are presently being developed. We have shown, in preparatory studies with γ-rays, that a delayed irradiation after an initial incubation period of 6 hours yields a substantially increased ratio of radiation induced to spontaneous revertants. The differences are due to the fact that the delayed irradiation affects a larger number of bacteria on the plates. We have also shown that the delayed irradiation has the additional advantage that the yield of revertants is largely insensitive to the number of bacteria initially plated. To apply the technique of the delayed irradiation with heavy ions, further studies will be required to ascertain that there can be a storage period of several hours at suitably low temperature before irradiation, without appreciable change of the yield of revertants. Such storage periods are unavoidable, as one can not commit the experiments to a fixed time schedule for the exposure at the UNILAC.

A further current effort is aimed at the development of procedures to decrease further the thickness of the top agar that contains the bacteria. The objective is an experimental technique that permits the utilization of heavy ions of lower energies.