CHANGES IN SENSITIVITY TO MUTAGENS AND A TUMOUR PROMOTER IN BACTERIA GROWN IN CONTINUOUS CULTURE

A.M. Anstice and C.J. Soper. School of Pharmacy and Pharmacology, University of Bath, BA2 7AY, U.K.

In a previous study the tumour promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was shown to increase the frequency of chemically induced mutagenesis in specially constructed strains of S.typhimurium, (Soper and Evans 1977). Enhancement of mutagenesis was also observed in strains lacking excision repair. Construction of other Salmonella strains suitable for studying the influence of DNA repair systems on TPA activity proved impracticable and so the tryptophan-dependent E.coli WP2 and a series of DNA repair deficient derivatives has been used in subsequent studies. E.coli grown in batch culture proved to be insensitive to the effects of TPA and we report here our attempts to enhance the TPA sensitivity of E.coli WP2 and WP2 uvrA by growing them in continuous culture under conditions of carbon limitation.

Organisms were grown in the dark in a 50 ml. chemostat at 37°C in Davis-Mingioli salts (DM) supplemented with 25 $\mu g\ ml^{-1}$ L-tryptophan and carbon limited by addition of 200 $\mu g\ ml^{-1}$ glucose. A dilution rate of 0.3 $hr.^{-1}$ was used giving a working viable count of 3 x 10^8/ml. Organisms were grown in the presence and absence of 1 $\mu g\ ml^{-1}$ TPA. Cells were diluted in DM and their sensitivity to mutagenesis by 2.5 $\mu g\ ml^{-1}$ ethylmethane sulphonate (EMS) and 0.5 $\mu g\ ml^{-1}$ methylmethane sulphonate (MMS) assessed using the 'microtitre' fluctuation test (Gatehouse 1978).

The sensitivity of E.coli WP2 to EMS and MMS was found to increase with the duration of continuous culture over a 10 day period. The excision repair deficient derivative WP2 uvrA also showed a culture time dependent increase in sensitivity to the test mutagens. When grown in the presence of TPA both WP2 and WP2 uvrA showed increased sensitivity to MMS, but their sensitivity to EMS was markedly reduced. This anti-mutagenic activity of TPA is in contrast to the co-mutagenic activity observed in S.typhimurium and is apparently independent of excision repair.

Inhibition of chemically induced mutagenesis by TPA was also observed when the test mutagen was added to the culture media in the chemostat and the appearance of tryptophan independent revertants determined over a 10 day period. The presence of the TPA and/or mutagens did not affect cell viability. With both test strains the appearance of EMS induced revertants was significantly delayed when TPA was present. In contrast, with MMS, the presence of TPA resulted in an initial delay in the appearance of induced revertants, but after culture periods in excess of 6 days enhanced mutagenesis was observed. These anti-mutagenic and co-mutagenic effects were observed in both test strains.

The data suggest that TPA is capable of both enhancing and inhibiting chemically induced mutagenesis in E.coli. Activity would appear to be independent of excision repair, but influenced by the nature of the mutagen and the cultural history of the organism.

Soper, C.J. and Evans, F.J. (1977), Cancer Res. 37, 2487-2491.

Gatehouse, D. (1978), Mutat. Res. 53, 289-296.