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THE RELEVANCE OF PLASMID-DETERMINED MUTAGENICITY IN THE AMES TEST TO MAMMALIAN GENOTOXICITY

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The use of the Ames bacterial mutagenicity assay as a predictive test for carcinogens is dependent on the universality of DNA. However, some compounds are only mutagenic if plasmid pKM101 is present in the bacterial strains (McCann et al 1975). Since pKM101 also increases spontaneous mutagenesis, a positive result detected only in the presence of the plasmid may be a uniquely bacterial phenomenon not relevant to mammalian genotoxicity. This needed to be examined to prevent the rejection of valuable drugs on the basis of such mutagenicity data.
Compounds were tested as described by Maron and Ames (1983). 4-Acetoxy-3-acetoxymethyl-acetophenone (AAMAP)(Table), 1-(4'-hydroxy-3'-hydroxymethylphenyl)-2-(benzyl-t-butylamino) ethanone hydrochloride (compound I) (data not shown) and 1-(4'-hydroxy-3'-hydroxymethylphenyl)-2-(benzyl-t-butylamino) ethanol (compound II) (data not shown) all gave positive dose-related results ($p < 0.05$: Dunnetts statistic, Dunnett (1955)) for mutagenicity in Salmonella typhimurium strain TA100, which detects base change mutations and carries plasmid pKM101. None was mutagenic in strain TA1535, which is the plasmid-less derivative of strain TA100. No compound was mutagenic in strain TA1538, which detects frame-shift mutations, while in strain TA98, which is strain TA1538 carrying plasmid pKM101, only AAMAP produced a positive response at its highest dose (Table 1). The same compounds were also tested for the induction of sister chromatid exchanges (SCE) in Chinese hamster (CH) cell line V79a following the method described by Perry and Evans (1975). AAMAP (Table) and compound I (data not shown), which were the more potent mutagens in the Ames test, gave significant dose-related increases in SCE over controls ($p < 0.05$: test of Amphlett and Delow (1984)) whereas the weak mutagen, compound II, did not increase SCE.

Table. Summary of data for AAMAP

Ames Test (mutant colonies per plate[b])					SCE Analysis	
Dose	Strain of S. typhimurium				Dose	Mean
µg/ml	TA1538	TA98	TA1535	TA100	µg/ml	SCE[c]
0	10.6	28.1	19.9	71	0	5.9
25	10.8	30.6	19.2	116*	3.0	5.6
50	9.1	32.1	22.8	158*	7.5	8.3*
100	10.7	30.7	21.2	272*	15.0	11.6*
200[a]	12.6	40.4*	17.9	424*	30.0[a]	15.9*

*Result significantly different from control ($p<0.05$)
a Highest concentration before toxicity became evident.
b Average of 5 plates. c Average of 50 metaphase cells.

Plasmid pKM101-determined mutagenicity is not therefore a false positive indication of genotoxicity since AAMAP and compound I, which show as positive mutagens in S.typhimurium strain TA100, are also positive in the CH SCE test. Indeed, since compound II does not give positive SCE, but is a significant mutagen in strain TA100, it may be argued that Ames strains of S.typhimurium containing plasmid pKM101 are extremely sensitive detectors of genotoxicity.

Amphlett, G.E., Delow, G.F. (1984) Mutat. Res. 128: 161-166
Dunnett, C.W. (1955) J. Amer. Statist. Ass. 50: 1096-1121
McCann, J. et al (1975) P.N.A.S. 72: 979-983
Maron, D.M., Ames, B.N. (1983) Mutat. Res. 113: 173-215
Perry, P., Evans, H.J. (1975) Nature 258: 121-125