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Activation of Polycyclic Aromatic Hydrocarbons by Hepatic S-9 from a Marine Fish

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Considerable recent interest has been directed toward the possible hazards to aquatic species and to associated human populations posed by polycyclic aromatic hydrocarbons (PAH's) in aquatic ecosystems (Neff 1979; Richards and Jackson 1982; Stegemen 1982). We are examining the possiblities for monitoring aquatic systems for genotoxic hazards, including PAH's, with cytogenetic assays of feral fish and are thus interested in the extent to which possible differences in metabolism by fish and mammalian systems or by different fish systems can influence the genetic effects of metabolism-dependent genotoxic chemicals, such as the PAH's. This paper reports our initial efforts to characterize the in vitro metabolism of several weakly carcinogenic and non-carcinogenic (in mammals) PAH's by hepatic S-9 preparations (the supernatant suspension of microsomes in cytosol after centrifugation of liver homogenates at 9000 x g) from the oyster toadfish (Opsanus tau) to Salmonella mutagens. Our objective was to identify PAH's, if any, which were substantially more mutagenic with fish than with mammalian S-9. However, the results of these studies supported our previous experience and that of others (Balk et al. 1982; de Flora et al. 1982; Protic-Sabljic and Kurelec 1982) that the mutagenicities of metabolism-dependent genotoxic chemicals are similar with fish and mammalian activation systems.

MATERIALS AND METHODS

The toadfish used in this study were collected in crab traps in the Charleston, SC area from estuaries that were relatively free from sources of contamination. The fish were maintained in the laboratory for 1-2 weeks without feeding until sufficient numbers were accumulated for S-9 preparation. Toadfish from 200-400 g body weight were pretreated by injection of 20 mg/kg 3-methylcholanthrene (MC) in corn oil 5,3 and 1 day before S-9 preparation (MC S-9). Other toadfish were used for S-9 preparation (UI S-9) without treatment. Livers from 4-6 toadfish were pooled according to pretreatment but without regard to sex

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in 2 volumes of 0.15 M KCl and homogenized with a Brinkmann polytron. Liver homogenates were centrifuged for 10 minutes at 10,000 x g and the resultant supernate (S-9) was stored at -80°C. Protein concentrations were determined according to the method of Lowry et al. (1951) and diluted to desired concentrations during preparation of the S-9 mix according to the method of Ames et al. (1975).

Plate assays were conducted by the method of Ames et al. (1975) with tester strains kindly provided by Dr. Ames. Preincubation assays were performed by addition of test chemical in 25 µl DMSO, α-naphthoflavone (ANF) in 25 µl DMSO, 0.5 ml S-9 mix and 0.1 ml of a 16-hour static culture of bacteria, in that order. After a 20-minute preincubation at room temperature 2 ml molten soft agar with trace amounts of histidine and biotin were added and plates poured. Plates prepared by the plate assay or preincubation procedures were incubated for 12 hours at room temperature and then for 48 hours at 37°C. Test chemicals included the carcinogenic PAH's chrysene (CHR) and benz(a)anthracene (BAA), the very weakly carcinogenic PAH benzo(e)pyrene (BEP) and the non-carcinogenic PAH phenanthrene (PHE). CHR, BAA and BEP are mutagenic in the standard Salmonella plate assay (McCann et al. 1975).

RESULTS AND DISCUSSION

In this study two experiments were performed. The first experiment was designed to provide information on the mutagenicities in plate assays of single concentrations of four PAH's to Salmonella strains TA1537, TA1538, TA98 and TA100 with increasing concentrations of S-9 from untreated and MC-pretreated toadfish. The concentration chosen for each test chemical was based on published results with mammalian S-9 and was within the range yielding positive responses or was comparable to the highest concentrations tested for chemicals that did not yield a positive response (McCann et al. 1975). Since CHR was negative with strains TA1537 and TA1538 at 1000 µg/plate and positive with stains TA98 and TA100 at lower concentrations (Simon 1979), an intermediate concentration of 250 µg/plate was selected. Although the data reported for these chemicals were from assays with S-9 from Aroclor 1254-pretreated rats (McCann et al. 1975), we pretreated toadfish with 3-methylcholanthrene. Aroclor 1254 has been shown not to substantially alter the ability of toadfish S-9 to activate benzo(a)pyrene or 2-aminoanthracene (unpublished observations). The results of this experiment are presented in Figure 1. All test chemicals were negative for all tester strains when UI S-9 was used. When MC S-9 was used, increases in the levels of revertants were apparent for BAA with all strains and for CHR with strains TA98 and TA100. Optimal levels of revertants were obtained for BAA (10 µg/plate) with 2 and 5 mg S-9 protein/plate and for CHR (250 µg/plate) with 9 mg S-9 protein/ plate. BEP (25 µg/plate) also appeared to increase the level of TA1538 revertants with

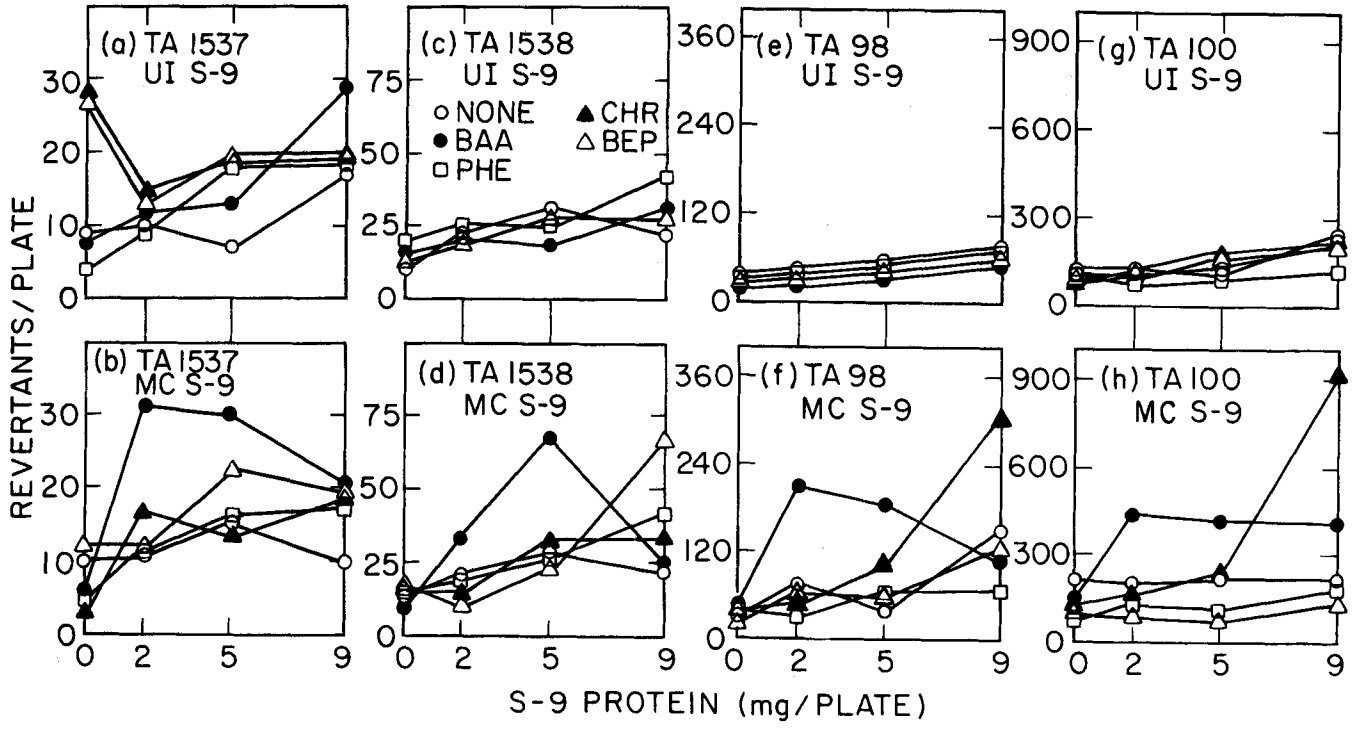

Figure 1. Mean levels of Salmonella (TA1537, TA1538, TA98, TA100) revertants obtained with duplicate plate assays of PHE (100 µg/plate), BEP (25 µg/plate), CHR (500 µg/plate), BAA (10 µg/plate) and no test chemical (50 µl DMSO) in the presence of increasing levels of S-9 from untreated (UI S-9) and MC-pretreated (MC S-9) toadfish.

9 mg S-9 protein/plate. In the absence of any test chemical the level of revertants obtained with 9 mg/plate MC S-9 was slightly higher than was obtained with lower levels of MC S-9 or with UI S-9. No increase in revertants was obtained with PHE for any of the combinations tested. The second experiment was designed to examine the possible effects of the cytochrome P-450 effector α-naphthoflavone (ANF) on the levels of TA98 revertants obtained with each of these test chemicals and 4 mg S-9 protein/plate. The results are presented in Figure 2. In the absence of any test chemical the presence of ANF (25 μM in 0.7 ml of preincubation mixture) had no effect on the levels of revertants obtained. With PHE and BEP revertants remained at spontaneous levels in the presence of ANF. However, with CHR (the concentration was reduced to 125 μg/plate for this experiment) and BAA the presence of ANF reduced substantially the revertant levels obtained in the absence of ANF.

Comparison of the results of this study with toadfish S-9 and available data for mammalian S-9 does not suggest substantial differences in the ability to activate the PAH's tested. PHE and BEP did not increase revertant levels in the presence of S-9 from untreated or MC-pretreated toadfish with the possible exception that BEP increased the level of TA1538 revertants with 9 mg protein/plate S-9 from toadfish. BEP is very weakly mutagenic to TA100 with standard mammalian S-9 (from Aroclor-pretreated rats). Increasing the levels of toadfish MC S-9 did not result in the activation of PHE as has been reported to occur with S-9 from Aroclor-pretreated rats, but not with S-9 from Aroclor-pretreated mice (Oesch et al. 1981). Although ANF altered the mutagenicity of BEP to TA100 with S-9 from untreated hamsters (Thakker et al. 1981), ANF did not alter the negative results obtained with TA98 and MC S-9 from toadfish. As with S-9 from Aroclor-pretreated rats, CHR and BAA increased the levels of TA98 and TA100 revertants with S-9 from MC-pretreated toadfish. The levels of TA1537 and TA1538 were also increased by BAA with toadfish MC S-9. The decrease of revertant levels in the presence of ANF in this study suggest that activation of CHR and BAA by MC S-9 from toadfish is cytochrome P-450-dependent.

The purpose of the present study was to provide a preliminary indication of the ability of toadfish S-9 to activate a series of weakly carcinogenic and mutagenic PAH's in the Salmonella assay. Additional studies will be necessary to adequately determine the mutagenic potencies of these test chemicals mediated by toadfish S-9. In addition systematic investigation of the relationships between test chemical concentration, S-9 concentration and mutagenicity may be of interest to establish whether optimal S-9 concentrations are a property of the test chemical (rank order of optimal concentrations of S-9 for different test chemicals is the same for all species), of the concentration of test chemical or of the particular activation system (different rank orders for different species). In the

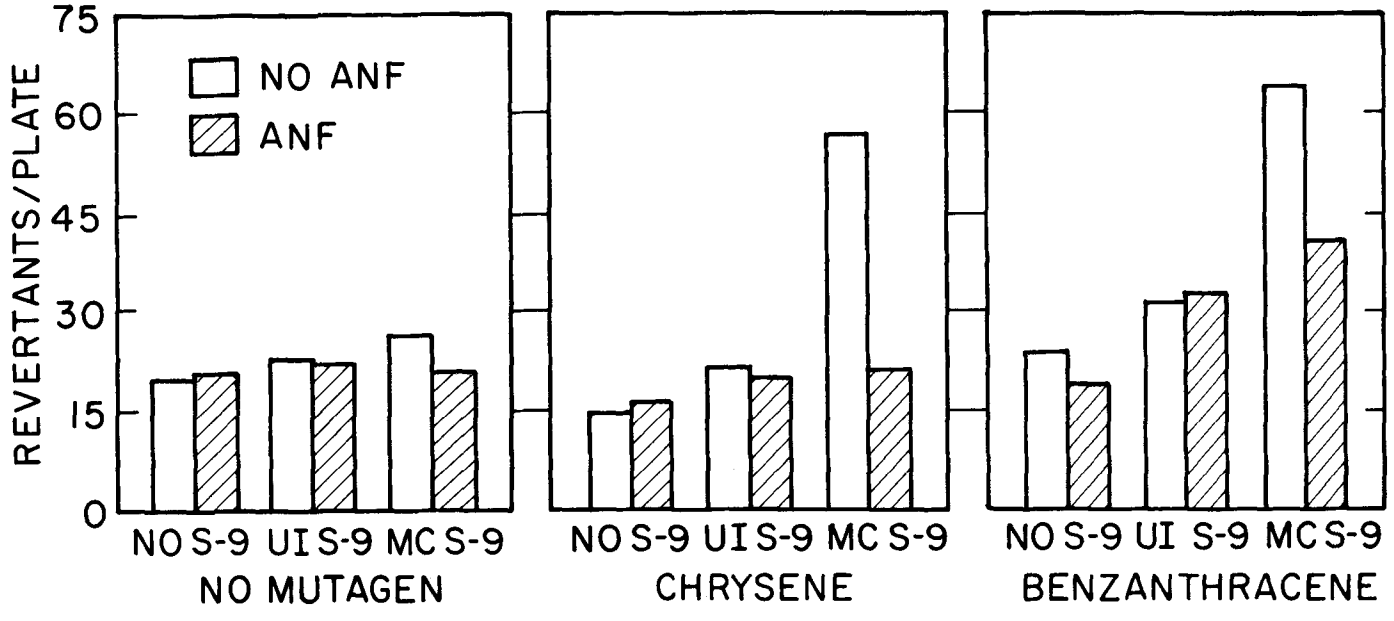

Figure 2. Mean levels of Salmonella (TA98) revertants obtained in duplicate preincubation assays of CHR (125 µg/plate), BAA (10 µg/plate) and no test chemical (25µl DMSO) with 4 mg protein/plate UI S-9, MC S-9 and no S-9 (0.5 ml cofactor mix) and in the presence or absence of 25 µM ANF.

present study CHR required a higher concentration of S-9 than BAA to achieve optimal mutagenicity. This difference may be related to the balance of activation and detoxication pathways for each chemical in toadfish MC S-9. In a previous study we have interpreted decreases in mutagenicity with supraoptimal increases in the concentration of S-9 like that which occurred with BAA to indicate increasing competition by detoxication pathways for limited test chemical (unpublished data). In the present study the ranking of PAH's according to the number of TA100 revertants/μmol at optimal concentrations of toadfish MC S-9 was BAA > CHR > BEP = PHE = 0, whereas, with the standard assay the ranking was CHR > BAA > BEP > PHE = 0. The potency ranking for these PAH's with respect to the induction of sister chromatid exchanges in hamsters in vivo was BAA > BEP = PHE > CHR (Rosinsky-Kocher et al.1979). The mutagenicity of BEP was greater with S-9 from untreated hamsters than with S-9 from untreated mammals of other species in the Salmonella assay (Thakker et al 1981).

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