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In Vitro Toxicity of Eight Mutagens/Carcinogens for Three Fish Cell Lines

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Recent interest in and concern for the quality of the environment has prompted a great deal of research into methods of measuring and assessing changes in it. One problem of major interest is that of increasing amounts of mutagenic/carcinogenic chemicals generated by modern society and released into marine and freshwater ecosystems (KRAYBILL et al. 1977). Numerous techniques involving whole animal testing for cancer, cell culture for mutation and cytogenetic changes and bacterial mutagenicity have been devised to assay specific chemicals and inspect unknown mixtures (KILBEY et al. 1977). Little, however, has been done to determine the effects of foreign chemicals on aquatic organisms (DIAMOND AND CLARK 1970).

Aquatic animals present unique problems for husbandry, which are not encountered when raising standard laboratory animals. Filtration, aeration, circulation and temperature must be carefully regulated when working with such species, and marine systems add even more complications. For these reasons, aquatic animals have seldom been selected for mutagenesis studies in medical laboratories.

To circumvent some of these problems and still determine whether various chemical agents affect aquatic animals, we have explored the use of fish cell culture for initial screening. The methodology is the same as that for mammalian systems (WOLF AND QUIMBY 1973a) and perhaps even somewhat less complex (WOLF AND QUIMBY 1973b). A variety of fish and other lower vertebrate cells can be successfully grown and are readily available. By using well-established *in vitro* mammalian tests we can determine toxicity, mutagenicity and metabolic alterations in aquatic animal cells exposed to known chemicals and extracts of material from suspect aquatic systems (KRAYBILL et al. 1977).

MATERIALS AND METHODS

Cells: Established fibroblast cell lines from three species of fish were used for the toxicity studies. RTG-2 (rainbow trout gonad), BF-2 (bluegill fry) and STE (steelhead embryo) were all grown in Eagle's

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minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS). These were maintained at 18°C (RTG-2 and STE) and 25°C (BF-2) in a 5% CO_2 atmosphere. Positive controls were cultures of human foreskin fibroblast (FS-13 grown under identical conditions, except at 37°C).

Duplicate cultures for each species were seeded at 2.5×10^4 cells $cm/^2$ in 35 mm tissue culture dishes and allowed to settle overnight in MEM+10% FCS. The cells were then incubated in the presence of mutagen for 72 hours, after which they were removed with EDTA (0.02%) -trypsin (0.5%) and counted in a counting chamber. Figure 1 a-b shows the relative toxicity of eight mutagens and promutagens for the three fish and one human cell line. Controls were cells treated with DMSO only and counted after three days incubation. The 100% level, therefore, represents a 40-90% cell increase, due to cell multiplication, over the initial seeding concentration.

Mutagens: Mutagens (except EMS, a liquid) were dissolved in DMSO at 1 to 5 mg/ml and added to the culture media in the concentrations given in Figure 1. The maximum DMSO per ml media never exceeded 5 ul or 0.05%. The following mutagens/promutagens were used:

1. EMS (Methanesulfonic acid othyl ester (ethyl methanesulfonate)) Sigma
2. MNNG (N-methyl-N^1-nitro-N-nitroso-soguanidine) Sigma
3. B(a)P (Benzo(a)pyrene) Eastman Kodak Co.
4. 3OH-B(a)P (3-hydroxy benzo(a)-pyrene) Gift
5. MCA (3-methylcholanthrene) Eastman Kodak Co.
6. AA (9-aminoacridine) grade II, Sigma
7. AFF (2-acetamidofluorene) Aldrich Chemical Co., Inc.
8. AF (2-Aminofluorene) Aldrich Chemical Co., Inc.

All of these agents have been shown to be toxic, mutagenic or carcinogenic in a variety of bacterial and mammalian systems (MCCANN AND AMES 1976). MCA, B(a)P, AFF, and AF all require microsomal activation from their promutagen state to the active mutagenic form. The remainder of the chemicals are all direct mutagens except 3 OH-B(a)P which acts both as a promutagen and a direct mutagen.

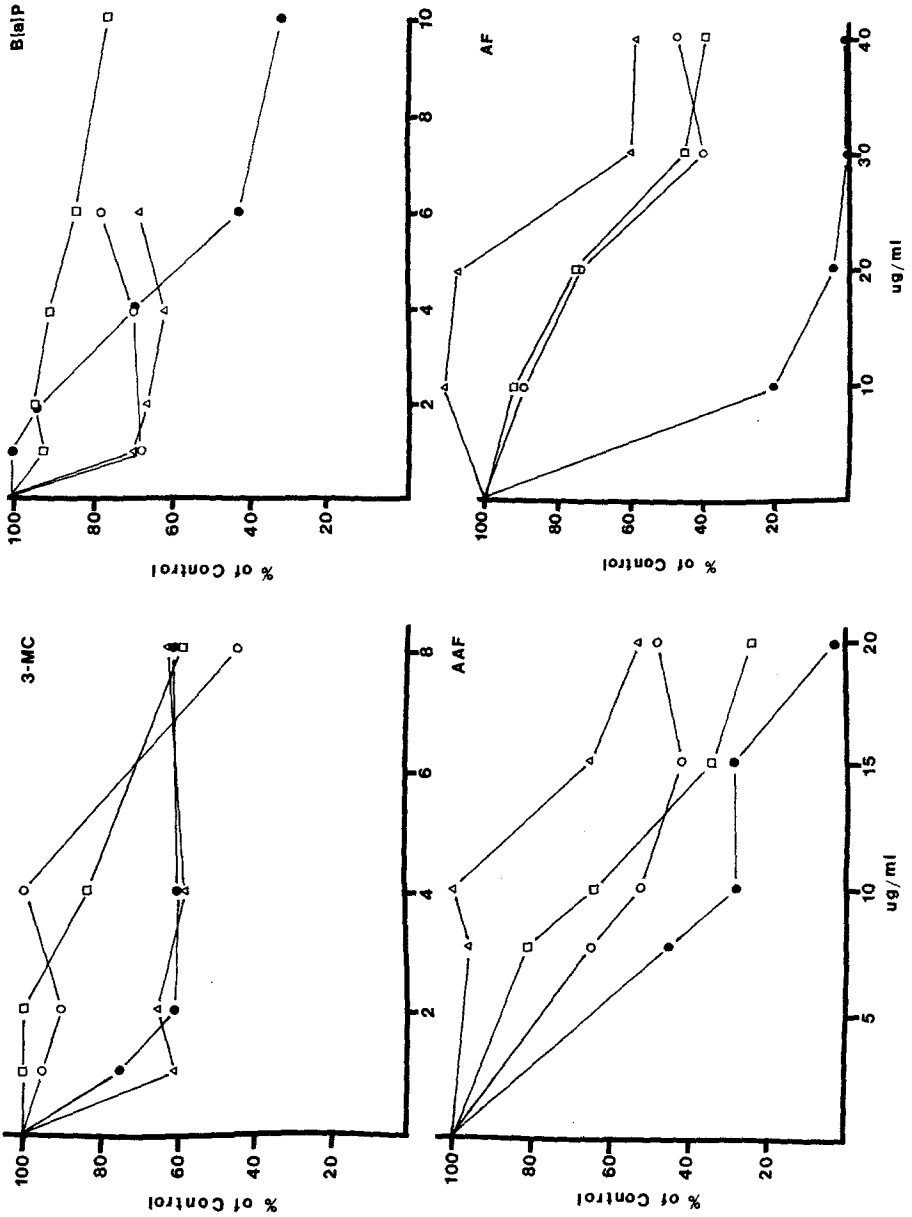

Figure 1a. In vitro toxicity of four promutagens for four established fibroblast cell lines. ○ Human foreskin (FS-13), ● Bluegill fry (BF-2), □ Steel head trout embryo (STE), △ Rainbow trout gonad (RTG-2).

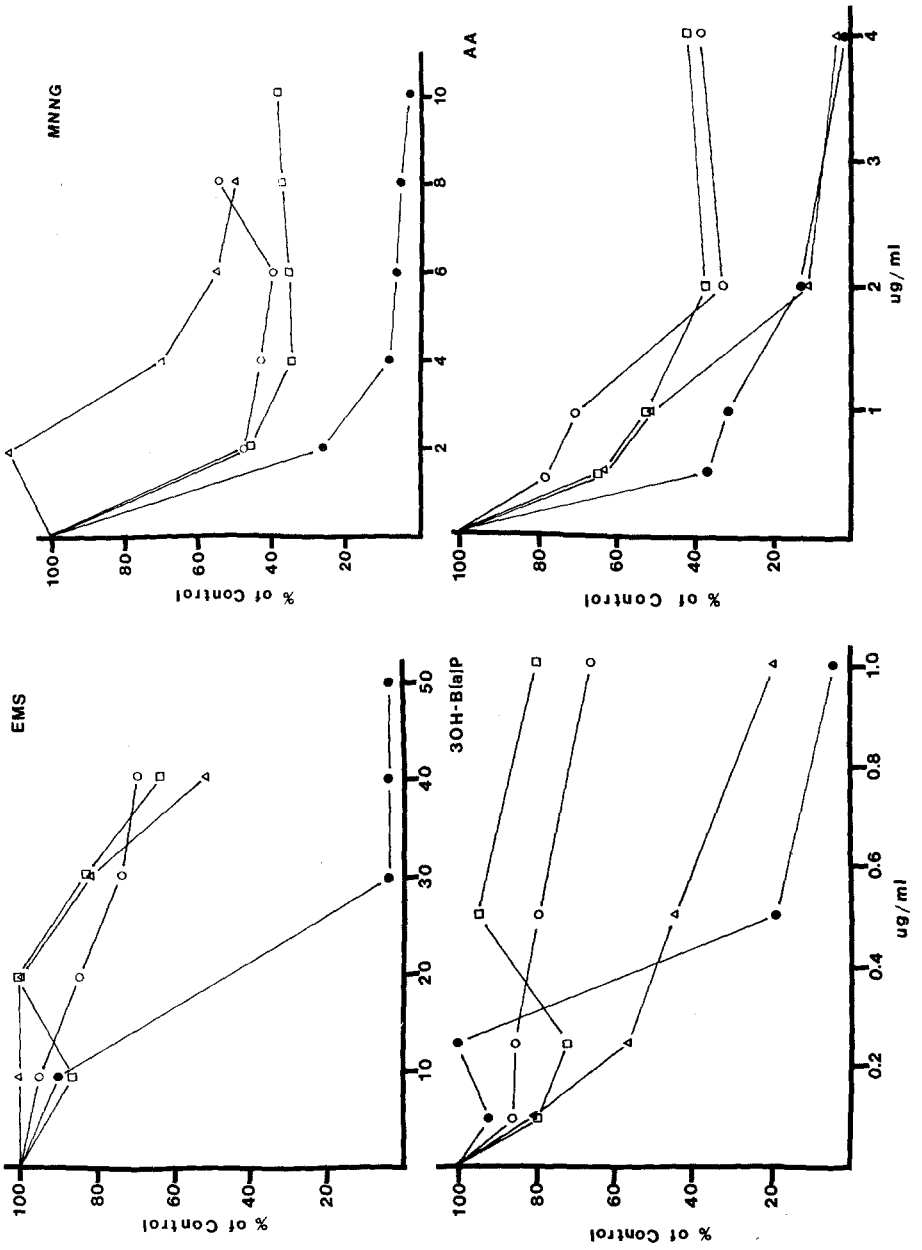

Figure 1b. In vitro toxicity of four direct mutagens for four established fibroblast cell lines. ○ Human foreskin (FS-13), ● Bluegill fry (BF-2), □ Steelhead trout embryo (STE), △ Rainbow trout gonad (RTG-2).

RESULTS

Figure 1 summarizes the data and shows the two distinct responses to the mutagens which were observed. In one, the number of cells continued to decline in direct proportion to the amount of chemical in the media. This represents increasing toxicity as the concentration of mutagen increases. The second response was one of an initial reduction in cell number below the controls but no further decrease in cell number as the mutagen concentrations increased. This response was the result of treated cells not dividing, while the untreated controls continued to replicate. These cells did, however, manage to survive in the presence of the mutagens.

In general the fish cells responded similarly to human fibroblasts. The bluegill fry (BF-2) cells appeared to be most susceptible to the toxic effects of seven of the eight agents used.

DISCUSSION

The response of three different fish cell lines to the toxic effects of eight mutagens and promutagens appears to be similar to that observed for early passage human foreskin fibroblasts. The different temperatures required for growth by the fish and human cells do not appear to influence the level of toxicity for the cells.

BF-2 and RTG-2 cells are both capable of metabolizing some of the promutagens to mutagens thus indicating that they possess the appropriate microsomal enzymes.

The greater sensitivity of BF-2 cells to all but one of the agents tested could be the result of a highly active aryl hydrocarbon hydroxylase system or a higher metabolic rate.

Since these cells respond similarly to mammalian cells (JACOBS AND DEMARS 1978) it should be possible to produce an increase in the number of mutant cells by exposing them to various mutagenic agents. It should also be possible to produce transformed cells, similar to those produced in rodents (BEWALD AND SACHS 1967, and HUBERMAN et al. 1972). These cells, transformed *in vitro*, could then be used to produce tumors in appropriate host fish, which would add a new dimension to the study of tumor growth.

Unlike normal mammalian cells which exhibit a finite life span *in vitro*, fish cells spontaneously transform *in vitro* to become immortal (PAUL 1973). This difference, along with their similar reaction to exposure to mutagens, may make these cells unique for

studying mutagenesis and malignant transformation *in vitro*.

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