I. INTRODUCTION

The liver is known to respond to the presence of certain types of materials by an action known as enzyme induction. In this process there is a proliferation of the smooth endothelial reticulium resulting in an increased level of various enzymes commonly involved in metabolism. Although the liver demonstrates the greatest effect of enzyme induction, many other organs, tissues and cells respond in a similar manner to some of the inducing agents.

The process of enzyme induction is important because of the biological effects it produces coupled with the large number of inducers to which the population is exposed. Among the more common enzyme inducers one finds cigarette smoke (1), chlorinated pesticides such as DDT (2), a large variety of drugs (3), and enzyme inducing factors in foods such as cabbage (4).

Once induced, higher enzyme levels metabolize many substrates at a faster than normal rate. This effect can be beneficial if the substrate is toxic, carcinogenic, or mutagenic but it can be detrimental if the substrate is a substance such as vitamin D, a sex hormone, a drug needed for medical purposes or a carcinogen precursor.

For example, the metabolism of steroid compounds can be induced. Most contraceptive pills are combinations of an estrogen and a progestogen. The metabolism of both of these substances can be stimulated by enzyme-inducing agents so that their circulating concentrations may be reduced to ineffective levels. This has been known to occur in epileptics given anticonvulsant drugs, most of which are potent enzyme inducers.

Induction of drug-metabolizing enzymes is an important cause of drug interactions. An enzyme inducing drug can induce the metabolism of itself, similar drugs, or unrelated drugs given for general

medical purposes. When a liver enzyme-inducing drug is given chronically the stimulation of its own metabolism (auto-induction) can be a major factor in drug tolerance where continually higher doses of drug are needed to generate the same medical effect. In cases where the rate of metabolism of a drug is critical, such as drugs which alter blood coagulation, the unknown induction of liver enzymes may cause life threatening situations.

On the other hand, the deliberate induction of liver enzymes has been postulated to have a beneficial effect in the treatment of cancer (5) and atherosclerosis (6) as well as its more obvious role in detoxification. In addition, the use of rat liver enzymes which have been induced is now part of a routine procedure for *in vitro* metabolic activation of materials to be tested as suspected mutagens or carcinogens by bacterial assay systems such as the salmonella or e. coli test.

There are several aspects of enzyme induction which we feel merit investigation. A summary of some of these potential research projects follows.

II. CHARACTERIZATION AND OPTIMIZATION OF ENZYME INDUCTION FOR MUTAGEN TESTING (e.g., Ames Test)

It has been demonstrated that precarcinogens or premutagens can be metabolized *in vitro* to carcinogens or mutagens. The most effective fraction of cell homogenate for *in vitro* metabolism is the liver microsomal fraction with cytosol (the S-9 fraction). Even better metabolism can be obtained if the microsomes are obtained from the livers of animals (usually rats) which have been previously challanged with a microsomal enzyme inducing agent. Although phenobarbitol and 3-methylcholanthrene have been extensively used for inducing enzyme activity, Aroclor® 1254 has been shown to induce enzymes which metabolize a wider range of chemicals and is now being used in most tier 1 bacterial reversion tests for *in vitro* activation of mutagens (7, 8).

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A. ISOMERS OF POLYCHLORINATED BIPHENYLS

Aroclor 1254 is a mixture of chlorinated biphenyls with about 54% chlorine and is one of a family of polychlorinated biphenyls with varying degrees of chlorination. Several studies have been conducted on the extent of chlorination versus the enzyme induction produced (9). These studies generally indicate greater chlorination leads to enhanced enzyme induction with a maximum possibly occurring with the penta- and hexa-chlorobiphenyls. The relative inducing effect of only a few of the many isomers of the Aroclors has been ascertained (10). Preliminary indications are that chlorination in the 3 and 4 positions on the biphenyl rings is important in inducing liver enzymes for di- and tetra-chlorobiphenyls. In many drug systems, such as the tetrachloro-*p*-dioxins, the specific location of the chlorine atoms has a substantial effect on the biological activity of the molecules. We propose to study the biological activity of some of the isomers of polychlorinated biphenyl with emphasis on the isomers present in Aroclor 1254 which is presently used for enzyme induction in mutagen testing.

Monsanto Research Corporation has a long and extensive background in all aspects of polychlorinated biphenyls and is now operating a laboratory devoted to bacterial mutagen testing. We have also developed expertise in the assays for enzymatic determinations of markers of liver enzyme induction (6). These capabilities along with our associations with the University of Dayton for animal facilities make us ideally suited to conducting this investigation.

B. AMES TEST IMPROVEMENT

Presently the bacterial reversion tests using salmonella typhimurium or e. coli utilize the S-9 microsomal fraction of Aroclor 1254 induced rat livers. A standard quantity of the microsomes is generally used without regard to the activity of each batch of microsomes. The relative activity of the microsomes combined with

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bacterial viability is then determined by including known mutagens in control plates run simultaneously with the samples.

We propose to further characterize the microsomes used in these tests. Several different enzyme assays will be compared to determine which assay or assays correlate with microsomal activity on known mutagens. In doing this we hope to eliminate one of the variables and thus improve the reproducibility of these tests.

C. MICROSOMAL ACTIVATION FOR CELL CULTURE TESTS

Although microsomes have been used to activate mutagens both simultaneously with cell culture application and before addition to the cells (11, 12), most cell culture mutagen/carcinogen tests do not presently call for microsomal activation. We propose to conduct cell culture toxicology and/or mutagen testing utilizing induced microsomes in conjunction with the bacterial reversion tests cited in the previous section. The aim of this research is again to further define and optimize test conditions in cell culture testing.

The Toxic Substance Act is going to require extensive testing of many materials. Any means which can be further developed to give rapid indications of problem areas will be a definite aid. We believe the further development and understanding of toxicology and metabolism at the cellular level will be beneficial. The characterization and optimization of *in vitro* metabolic activation of toxicants/mutagens/carcinogens by microsomal preparations for use in cell culture will increase our knowledge in a needed field.

III. ENZYME INDUCTION AND ATHEROSCLEROSIS

Current research indicates high density lipoproteins (HDL) may be beneficial and aid in the prevention of atherosclerosis. Monsanto Research Corporation has participated with personnel at Cincinnati General Hospital to demonstrate a link between certain chlorinated hydrocarbons and elevated HDL (6). The chlorinated hydrocarbons which were most effective in elevating serum HDL were also found to be most effective in inducing liver enzymes More research needs to be conducted in determining the possible link between such enzyme inducers as the polychlorinated biphenyls and elevated serum HDL.

Potential research in this field could include the use of minipigs to demonstrate this effect in a system which more closely mimics human lipoprotein compositions. Also, patients receiving medical drugs which are known to be potent enzyme inducers (e.g., phenobarbitol, anticonvulsant drugs, etc.) could be tested for their level of serum HDL. As these experiments progress, studies could be defined to determine how the serum level of HDL is linked to chlorinated hydrocarbon administration or enzyme induction. Monsanto Research Corporation personnel have worked successfully with personnel at Cincinnati General Hospital in this field and with appropriate funding could continue to conduct research in this field together.

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