AN ABSTRACT OF THE THESIS OF

WILLIAM THOMAS STOTT (Name) for the DOCTOR OF PHILOSOPHY (Degree)

in Food Science and Technology (Major) presented on April 28, 1978 (Date)

Title: Dietary Protein and Aflatoxin B_1 Metabolism in Rainbow Trout (Salmo gairdneri)

Abstract approved: Russell O. Sinnhuber

Dietary protein intake has been observed to influence the induction of cancer in animals exposed to carcinogens, presumably by affecting the activities of enzyme systems which metabolize them. One such carcinogen is aflatoxin B_1 (AFB_1) a mold metabolite and frequent contaminant of foodstuffs. Variations in the levels of dietary proteins fed rats and rainbow trout (Salmo gairdneri) influence the induction of hepatocellular carcinoma by AFB_1 in these animals. The following work was undertaken to provide an explanation of these results in terms of AFB_1 in vitro metabolism.

The effect of dietary casein and fish protein concentrate (FPC) levels upon the activities of potential AFB_1 metabolizing enzyme systems were studied in the rainbow trout (Salmo gairdneri). Hepatic subcellular protein and cytochrome P-450 contents, and the activities of glutathione-S-epoxide transferase (GTr) epoxide hydrase (EH) cytochrome c reductase and aldrin epoxidase (AE) enzymes were determined in trout fed varying levels of casein or FPC. In addition, the reduction of AFB_1

to aflatoxicol (AFL) and conversion to an active mutagen for Salmonella typhimurium TA 98 were examined. A prerequisite for this study was the adaptation of the Ames mutagen assay system for use with fish hepatic enzymes.

Lowered hepatic GTr and AE activities and increased conversion of AFB_1 to AFL and to a microbial mutagen were noted in trout fed high protein diets relative to those fed low protein diets. Cytochrome P-450 content was observed to decrease with increasing dietary casein and increase with increasing dietary FPC. Hepatic EH activity remained constant and decreased in trout fed increasing levels of casein or FPC respectively. These changes in enzyme activity are discussed in relation to dietary influences upon AFB_1 carcinogenesis in the rainbow trout.

Dietary Protein and Aflatoxin B_1 Metabolism in Rainbow Trout (*Salmo gairdneri*)

by

William T. Stott

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1978

APPROVED:

Professor of Food Science and Technology
in charge of major

Head of Department of Food Science and Technology

Dean of Graduate School

Date thesis is presented April 28, 1978

Typed by Sue Hecht for William Thomas Stott

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to his advisor, Professor Russell O. Sinnhuber, for making the rewarding experience at Oregon State University possible and for guidance and assistance in his research.

An expression of gratitude is given to the members of the graduate committee, Dr. Joseph Nixon, Dr. Lavern Weber, Dr. Robert Larson and Dr. Howard Wilson, and all the faculty and graduate students of the Department of Food Science and Technology for their interest and assistance in this study. A special thanks is extended to Ted Will, George Putnam, June Hunter, Dr. Jerry Hendricks and Dick Foster for their help with fish handling.

A sincere and deep appreciation is extended to my parents, Edna and Robert R. Stott, for their encouragement, inspiration and guidance throughout my education.

Finally, I would like to thank my companion and wife, Robbin, for her patience during my graduate work and emotional and intellectual involvement in my education.

This thesis is dedicated to Edna Knapp Stott, Robert R. Stott and Robbin Rosen Stott.

TABLE OF CONTENTS

Biological Activity and Metabolism of Aflatoxin B_1 in Rainbow Trout (*Salmo gairdneri*): A Review

W. T. Stott

R. O. Sinnhuber

Department of Food Science and Technology

Oregon State University

Corvallis, Oregon 97331

Between 1950 and 1962 several outbreaks of hepatocellular carcinoma occurred in hatchery raised trout in the U.S., and in turkeys (turkey X disease) in Great Britain. In both cases the causative agent was found to be a mycotoxin, aflatoxin, present in moldy feed components. The potent carcinogenic nature of aflatoxins and widespread contamination of foodstuffs has stimulated an extensive amount of research into its biological effects and metabolism. Fish, originally thought to be incapable of xenobiotic biotransformations, are extremely sensitive to the carcinogenic effects of aflatoxin and readily metabolize it. This review will examine the toxicity and metabolism of aflatoxin B_1 in fish, and the effects of diet and other xenobiotics.

Xenobiotic metabolism in fish

Aquatic animals, both wild and hatchery bred, are being exposed to more and more stable, lipophilic xenobiotics through their aquatic environment and/or food sources. Industrial and agricultural pollution of their water and the contamination of manufactured feeds challenge fish with a wide variety of xenobiotics, which were originally believed to be simply disposed of by passive diffusion through their gills (Brodie and Maickel, 1962; Maren et al., 1966; 1968). Also, it was believed that once into a fish, the xenobiotic remained unchanged as fish were unable to metabolize foreign chemicals (Brodie and Maickel, 1962; Goldstein et al., 1974; Hattula and Karlog, 1973). However, Adamson (1967) concluded that the gill membranes of the dogfish

and lemon shark are relatively impermeable to some xenobiotics, and conjugation reactions were demonstrated to occur in several species of fish (Dutton and Montgomery, 1958; Huang and Collins, 1962). Since then a great amount of information regarding xenobiotic metabolism in fishes have been reported, as reviewed by Adamson (1967), Bend et al. (1977), Malins (1977), Dewaide (1971), Schoenhard (1974), and Chambers and Yarbrough (1976). An extensive summary of metabolic biotransformation reactions in fishes has been compiled by Ahokas (1977) which includes glucuronide, glycine, sulphate and taurine conjugation reactions; acetylation and methylation reactions; azo and nitro reductase activity; N-demethylation; O-dealkylation; expoxidation; hydroxylations and aryl hydrocarbon hydroxylase activity. In addition, the epoxide metabolizing enzyme systems epoxide hydrase and glutathione epoxide transferase have been noted to occur in several marine fishes by James et al. (1976) and Bend et al. (1977), and in rainbow trout (Salmo gairdneri) (Stott and Sinnhuber, 1978a;b). In general, fish enzymes catalyzing these reactions have a lower activity and temperature optima (25°-30°C) than in the rat.

Fish mixed function oxidase

The presence of a cytochrome P-450 containing mixed function oxidase enzyme system (MFO) in fish was first detected by Garfinkel (1963) in carp (Carpoides thompson) liver and intestinal mucosa. A primary component of MFO enzyme systems, cytochrome P-450 with a lipid factor, NADPH cytochrome P-450 reductase and possibly cytochrome b_5 (NADH reduced) is involved in various hydroxylations, N-, S-, and O-demethylations, epoxidation, dealkylations, deaminations, and desulfuration reactions (Kappas and Alvares, 1975). Levels of cytochrome P-450 and its reducing

enzyme, NADPH cytochrome c reductase, are generally lower in fish than in rats or man (Ahokas, et al. 1976; Bend et al., 1974; Chan et al., 1967; Eisele et al., 1978; Lidman et al., 1976; Pohl et al., 1974) though up to 0.4 nm cytochrome P-450/mg protein have been observed in large skate (Raja ocellata) and trout (Salmo trutta lacustris) (Ahokas et al., 1977a;b; Pohl et al., 1974).

Fish cytochrome P-450 appears to be somewhat unique when compared to that from other animals. Compounds which produce type I spectral changes in rat cytochrome P-450 (hexobarbitone, 17-OH-progesterone, SKF 525A bromobenzene, DDT, piperonyl butoxide) fail to produce this change, or produce unclassified spectral changes in trout (S. trutta lacustris) cytochrome P-450 (Ahokas et al., 1977a;b). Benzo(a)pyrene however, causes a typical type I spectral change, and n-butanol a reversed type I spectral change (Ahokas et al., 1977a;b). Type II spectral changes in trout cytochrome P-450 have been noted with aniline, n-octylamine and cyanide (Ahokas et al. 1977a;b). The similarities of the trout cytochrome P-450 with rat cytochrome P-448 relative to selective cytochrome P-450 MFO inhibitors and high benzo(a)pyrene hydroxylase activity has been noted (Ahokas et al., 1975a). Yet in fish, only cytochrome P-450 is observed when reduced and bound to CO.

The induction of cytochrome P-450 MFO activity in fish by certain xenobiotics has been observed by several authors. A classical mammalian cytochrome P-450 MFO inducer, phenobarbital, does not appear to induce fish MFO activity (Bend et al., 1973) while 3-methylcholanthrene, an inducer of mammalian cytochrome P-448 MFO activity (eg. benzo(a)pyrene metabolism), does without the appearance of cytochrome P448 (Bend et al., 1973; 1974; 1977; Chevion et al., 1977; Pederson et al., 1974;

Stegeman, 1977). Polychlorinated biphenyls, phenylbutazone, crude oil, 1,2,3,4-dibenzanthracene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin have also been noted to induce MFO activity in winter flounder (Pseudopleuronectes americanus), little skate (Raja erinacea), rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), scup (Stenotomus versicolor, mummichog (Fundulus heteroclitus), coho salmon (Onchorhynehus kisutch), brown trout (S. trutta), cunner (Tantogolabrus adspersus), and capelin (Mallotus villosus) (Ahokas et al., 1975b; Bend et al., 1973; 1974; Burns, 1976; Chevion et al., 1977; Gruger et al., 1977; Hill et al., 1976; Lidman et al., 1976; Payne, 1977; Payne and Penrose, 1975; Pohl et al., 1975). The observed induction of MFO activities in fish living in polluted waters (Ahokas et al., 1975a; Chambers and Yarbrough, 1976; Dewaide and Henderson, 1970; Payne and Penrose, 1975; Wells et al., 1973) has been suggested as a useful index of environmental contamination.

Dietary protein influences upon rainbow trout (S. gairdneri) MFO activity have also been observed. Mehrle et al. (1974), found that trout fed a 32% casein diet were more susceptible to the MFO activated organochloride insecticide chlordane, than fish fed a 45% casein diet. Large variations in chlordane toxicity between trout fed different commercial fish diets was also noted by these authors. In trout fed varying levels of casein or fish protein concentrate (FPC) an inverse relationship existed between aldrin epoxidation activity and protein intake (Stott and Sinnhuber, 1978a;b). Cytochrome P-450 levels responded similarly in casein fed fish while they increased with increasing FPC in the diet. In rainbow trout fed 300 ppm cyclopropene fatty acids for

two months a decrease was noted in cytochrome P-450 content (60%) while an increase occurred in benzo(a)pyrene hydroxylase activity (40%) over that observed in control fish (Eisele et al., 1978).

Fish sensitivity to carcinogens

Fish have been observed to be sensitive to several mammalian carcinogens as shown in Table 1. Many of the these compounds are procarcinogens, requiring metabolic activation by the fish to their carcinogenic molecular state. For example, Lotlikar et al. (1967) have described the metabolism of the potent mammalian carcinogen 2-acetoaminofluorene to which fish appear less susceptible. It was found that 2-acetoaminofluorene was not metabolized to the N-hydroxylated intermediate, a highly carcinogenic metabolite to rats (Miller et al., L961). Ahokas et al. (1975a; 1977b;c;d) and Pedersen et al. (1974) have also characterized benzo(a)pyrene metabolism in trout (S. trutta lacustris and S. gairdneri), finding it to be similar to rats, both in its activation and production of hydroxylated products.

Hatchery raised fish have frequently been exposed to a chemical carcinogen of natural origin in their diets with subsequent hepatocellular carcinoma formation, often of epizootic proportions (Halver, 1969; Heuper and Payne, 1961; Rucker et al., 1961; Sinnhuber et al., 1977; Wales and Sinnhuber, 1966; Wood and Larson, 1961). Dry pelleted trout feeds containing cottonseed meal used during these outbreaks were observed to be carcinogenic to rainbow trout (Ashley and Halver, 1961; Sinnhuber, et al., 1968; Wolf and Jackson, 1963). It was found that the causative agent was due to a group of mold metabolites called aflatoxins

(Figure 1) present as contaminants of the cottonseed meal (Engebrecht et al., 1965; Jackson et al., 1968). Aflatoxins, and especially aflatoxin B_1 (AFB_1), are extremely carcinogenic to trout, with as little as 4 ppb in their diet causing a high incidence of liver tumors (Ashley et al., 1964; 1965; Sinnhuber et al., 1965; 1966).

Aflatoxin toxicity and carcinogenicity

Numerous workers have examined the toxicity and carcinogenicity of aflatoxins in a wide variety of animals and several comprehensive reviews of this subject have been published (Butler, 1969; Ciegler, 1975; Detroy et al., 1971; Wogan, 1966; 1973; Wogan and Pong, 1970). As shown in Table 2, rainbow trout are among the most sensitive of animals tested to the acute toxic effects of a single oral dose of AFB_1. The oral LD_{50} value of rainbow trout (0.5 mg AFB_1/Kg) (Halver, 1967) is much smaller than the oral LD_{50} values noted for male and female rats, 6.0mg AFB_1 and 19.7 mg AFB_1/Kg respectively. Oral dosages of 1-3 mg AFB_1/Kg observed to produce lower mortalities than LD_{50} dosage levels in trout by Halver (1967), may be explained by the trouts habit of regurgitating its stomach contents upon irritation and thus eliminating unabsorbed AFB_1 (Bauer et al., 1969). Silver salmon (Oncorhynchus kisutch) and channel catfish (Ictalurus punctatus) appear to be 10 - 30 times more resistant than the rainbow trout to acute aflatoxicosis when dosed daily over a five day period. Oral LD_{50} values of 5 - 10 mg AFB_1/Kg for silver salmon and 10 - 15mg AFB_1/Kg for channel catfish were reported by Halver et al., (1966a;b).

AFB_1 also appears to be a very potent carcinogen in many animal species (Wogan, 1973) and has been implicated in human primary liver

carcinoma (Peers and Linsell, 1973; Shank et al., 1972). As shown in Table 3, AFB_1 is a potent inducer of cancer in the rainbow trout with only 4 ppb AFB_1 in the diet producing a 50% tumor incidence after one year (Hendricks et al., 1978b). Jackson et al., (1968) have noted a 20 month tumor incidence of 20% following ingestion of only 0.06 µg AFB_1 over 9 months, representing a feed contamination level of only 0.4 ppb AFB_1. Trout eggs have also been shown to respond to very low levels of AFB_1 when exposed to AFB_1 contaminated water (Sinnhuber and Wales, 1974). When 15 day old trout embryos were exposed to an aqueous solution of 0.5 ppm AFB_1 for 1 hr, 40% of the fish developed tumors within a year.

AFB_1 metabolism

In early animal AFB_1 exposure trials it was noted that very little of the administered AFB_1 was recovered unchanged or even as fluorescent metabolites (Dann et al., 1972; Patterson and Allcroft, 1970; Shank and Wogan, 1965; Wogan et al., 1967). A study by Ayres (1969) regarding the fate of ^{14}C-AFB_1 *in vivo* in rainbow trout (*S. gairdneri*) revealed the production of a variety of AFB_1 metabolites excreted into water (Table 4). Fifty percent of the administered ^{14}C-AFB_1 was excreted in the urine within 12 hours, with half of this as unchanged AFB_1, while the rest passed through the liver to the bile and into the lower gut. At this time only 1.1% of the total 16% ^{14}C present in the liver was AFB_1. The subcellular distribution of aflatoxin in the livers of rainbow trout and rats relative to time is shown in Table 5. Similar *in vivo* hepatic subcellular distribution patterns were obtained except that trout appear

to concentrate more of the ^{14}C-aflatoxin (metabolites) in the nuclei and cell debris fraction and rats retain more in the soluble and microsomal fractions (Ayres, 1969; Wogan, et al., 1967). It was estimated that 44-49% and 68% of ^{14}C in the trout liver nuclei and microsomes respectively, was bound to nucleic acids at 12 hr. post ^{14}C-AFB_1 administration (Ayres, 1969). This finding was interpreted to indicate a preferential finding of aflatoxin metabolites to nucleic acids.

Several *in vitro* and *in vivo* comparative studies of AFB_1 metabolism in a variety of animal species have also been undertaken. Their findings are summarized in Table 6. It may be noticed that aflatoxicol (AFL), a reduced form of AFB_1 (Figure 2) is produced by many AFB_1 sensitive species. It has been suggested that AFL production represents the formation of an "aflatoxin reservoir" (Patterson, 1973; Patterson and Roberts, 1972) which can be reconverted back to AFB_1 in many animals (Salhab and Edwards, 1977), including rainbow trout (Loveland et al., 1977; 1978). The major fluorescent metabolites produced *in vitro* by trout post mitochondrial fraction have been AFL (16.2%) (Loveland et al., 1978; Schoenhard et al., 1976) and AFM_1 (1.3%) Loveland et al., 1978). Extensive reviews on AFB_1 metabolites have been written Campbell and Hayes, 1976; Patterson, 1973; Schoenhard, 1974) and a summary of known and theorized AFB_1 metabolic pathways are shown in Figure 2.

Comparisons of AFB_1 metabolites formed by AFB_1 sensitive versus more resistent animal species have led some authors to relate the *in vitro* conversion of AFB_1 to AFL, relative to the reverse reaction, to the AFB_1 sensitivity of the species (Edwards et al., 1975; Salhab and

Edwards, 1977). Increases in *in vitro* AFL formation has correlated with increased protein intake and increased AFB_1 induced tumor formation in rainbow trout (*S. gairdneri*) (Lee et al., 1978; Stott and Sinnhuber, 1978a;b). However, Roebuck and Wogan (1977), in a recent species comparison study of AFB_1 metabolism, could find no correlation between the production of any known or unidentified metabolites and species sensitivity to AFB_1. Thus, the use of *in vitro* AFB_1 metabolic patterns as an indicator of species sensitivity to AFB_1 remains questionable.

Active AFB_1 metabolite

The rapid conversion of AFB_1 *in vitro* to various metabolites by sensitive species (Patterson, 1973; and Patterson and Allcroft, 1970) and the requirement for enzymes, NADPH and O_2 for AFB_1 to be toxic and mutagenic to bacteria (Garner et al., 1971; 1972; Garner and Wright, 1973) and to bind to macromolecules (Garner, 1973; Gurtoo and Bejba, 1974; Gurtoo and Dave, 1973) has suggested the possibility that an MFO metabolite of AFB_1 is the true toxic molecular species. Indeed, the administration of an inhibitor, SKF 525A, to rainbow trout resulted in a decreased AFB_1 induced carcinogenic response (Scarpelli, 1976) and piperonyl butoxide decreased the mortality of a DNA repair deficient mutant *Bacillus subtilis* GSY 1057 when incubated with rainbow trout enzymes and AFB_1 (Schoenhard, 1974). The latter observation was relevant as evidence that a good correlation between mutagenicity and carcinogenicity exists, especially for aflatoxins (Ames et al., 1975; Wong and Hsieh, 1976).

It has been noted that the presence of the 2,3-unsaturation in the furofuran segment of the aflatoxin molecule is required for maximum

toxicity (Wogan et al. 1971) and carcinogenicity (Ayres et al., 1971) (Table 3). As reviewed by Campbell and Hayes (1976), the hemiacetal AFB_{2a}, whose production depends upon a 2,3-unsaturation, was extensively studied as a possible toxic form of AFB_1. AFB_{2a} was noted to bind covalently to proteins by formation of a Schiff base (Patterson and Roberts, 1970, Patterson, et al., 1969; Patterson and Allcroft, 1970), presumably by the opening of the hemiacetal at physiological pH to form a phenolate ion (Pohland et al., 1968), which in turn reacted with free amino groups in the protein. The observed lack of AFB_{2a} toxicity to AFB_1 sensitive ducklings (Patterson and Allcroft, 1970) has been attributed to its probable reaction with proteins prior to its ever reaching critical molecules within the hepatocyte (Gurtoo and Campbell, 1974; Patterson and Roberts, 1970). This protein reactivity may also account for the fact that no AFB_{2a} has been recovered from *in vitro* incubations of the sensitive rainbow enzymes with AFB_1, as it would have been precipitated out with proteins upon organic solvent extraction.

It was suggested that like several K-region containing polycyclic aromatic hydrocarbon carcinogens, AFB_1 may undergo an epoxidation reaction at the 2,3-unsaturated position of the molecule (Figure 2) (Schoental, 1970). Though the 2,3-epoxide of AFB_1 has not been isolated, much indirect evidence for its existence has been accumulated. Formation of 3-meta-chloroperbenzoic acid-2-hydroxy AFB_1 adducts and of water soluble 2,3-dihydro-2,3-dihydroxy AFB_1-like metabolites in hamster hepatic enzyme - AFB_1 incubation mixtures, led Garner (1973) to suggest the formation of a 2,3-epoxy AFB_1 precursor as the active species of AFB_1. The 2,3-dihydro-2,3-dihydroxy form of AFB_1 has also been recovered by Swenson and coworkers from *in vitro* and *in vivo* formed RNA and

DNA-AFB_1 adducts by rat, hamster and human hepatic enzymes (Swenson et al., 1973; 1974; 1977). Swenson et al., (1975) have prepared an AFB-2,3-dichloride compound and used it as a model for the hypothesized 2,3-epoxy AFB_1 reactions. The 2,3-dichloro AFB_1 was observed to covalently bind DNA and RNA, and was mutagenic to S. typhimurium TA98 and TA100 without prior incubation with enzymes. This compound also caused sarcomas upon s.c. injection in rats more readily than AFB_1. Evidence for the formation of a reactive 2,3-epoxy AFB_1 metabolite in vitro by rainbow trout hepatic enzymes has also been provided by Schoenhard (1974), who reported a decreased AFB_1 induced mortality of B. subtilis GSY 1057 when the electrophile "traps" cysteine and cytosine were added to incubation mixtures.

Inducer effects upon AFB_1 activity

Several xenobiotics have repeatedly been noted to affect the biological activities of toxicants and carcinogens, presumably by altering activities of enzyme systems involved in their metabolism (Conney et al., 1971; 1977; Conney and Burns, 1972; McLean, 1977). Administration of phenobarbital (PB) to rats prior to AFB_1 exposure led to decreases in the acute toxicity (Garner, Miller and Miller, 1975, unpublished data; Mgbodile et al., 1975), in tumor incidence (McLean and Marshall, 1971; Swenson et al., 1975; 1977) and in in vivo binding of AFB_1 to macromolecules (Garner, 1975; Swenson et al., 1977). However, PB pretreatment also resulted in the increased production of an AFB_1 lethal factor by hepatic enzymes for DNA repair deficient strains of Escherichia coli (Garner and Wright, 1973) and S. typhimurium (Garner et al., 1971, 1972), and in increased in vitro AFB_1 binding of RNA (Gurtoo and Dave, 1973; and Swenson et al. 1973).

Hendricks et al. (1977) have noted a decreased tumor incidence in rainbow trout which were fed a diet containing 6 ppb AFB_1 with 100 ppm Arochlor 1254, a mix of PCB's which contain an average of 54% chlorine. After 12 months a 30.4% tumor incidence occurred in the AFB_1 plus PCB treated fish while a 70.3% incidence was noted in trout dosed with only AFB_1. It was theorized that induction of AFB_1 detoxifying enzymes had occurred in the former group. Supportive of these findings was the report by Stott and Sinnhuber (1978c) that decreased mutagenic response of S. typhimurium TA 1538 to trout enzyme activated AFB_1 was obtained when rainbow trout were pretreated with several PCB's. The original method of Ames et al., (1975) utilized a pretreatment of rats with Arochlor 1254 to increase mutagenic responses to AFB_1. This laboratory has also found that inclusion of DDE into AFB_1 containing rainbow trout diets resulted in only slight decreases, while dieldrin caused increases (ca. 8%) in the 12 month tumor incidence of these fish (Hendricks, unpublished data).

Nutritional effects

Nutritional effects upon AFB_1 toxicity and carcinogenicity in animals are well documented as reviewed by Clayson (1975), McLean (1977), and Newberne and Rogers (1970). The biological activity of AFB_1 may be affected by the quality and quantity of protein as shown in Table 7. Newberne and coworkers (Newberne and Rogers, 1970) have observed that in rats fed diets deficient in lipotropes, an increased sensitivity to acute aflatoxicosis and AFB_1 induced hepatic carcinoma occurred. However, marginally lipotrope deficient AFB_1 containing diets, caused a higher incidence of tumors than control or severely lipotrope

deficient AFB_1 containing diets fed to rats. These animals were also resistant to the toxicity of single doses of AFB_1, but were much more sensitive than controls when challenged with repeated smaller AFB_1 dosages (Newberne and Rogers, 1970; Butler and Neal, 1973). The addition of arginine, lysine and methionine to the diets of ducklings may also influence the toxicity of AFB_1 (Newberne et al., 1966). Protein deficient diets can result in an increased sensitivity of rats, monkeys and swine to acute aflatoxicosis (Madhavan et al., 1965; Madhavan and Gopalan, 1965; Sisk and Carlton, 1972; Todd et al., 1968) while protecting rats from AFB_1 induced carcinoma (Madhavan and Gopalan, 1968).

Rainbow trout appear to respond as do rats to the carcinogenic activity of AFB_1 when fed diets of varying protein content (Table 7) (Lee et al., 1978). After 12 months of feeding on a 49.5% fish protein concentrate (FPC) diet containing AFB_1 (6ppb), trout had a 45% tumor incidence compared to a 3% incidence in those fed a 32% FPC diet. Casein diets, with AFB_1 fed fish at these same levels resulted in about a 16% tumor incidence in both diets. Increased conversion of AFB_1 to a lethal factor for B. subtilis GSY 1057 (Schoenhard, 1974) and to an active S. typhimurium TA 98 mutagen (Stott and Sinnhuber, 1978a; Stott, unpublished data) by enzymes from rainbow trout fed increasing levels of protein have also been observed. A recent study using the rainbow trout egg model (Sinnhuber et al., 1977) substantiated these findings (Table 7). Upon exposure as 15 day old embryos to a 0.5 ppm aqueous AFB_1 solution for 1 hr, trout were fed diets varying only in the levels of casein and FPC for 12 months. A direct correlation between protein intake and AFB_1 induced carcinoma was observed, with FPC supporting a greater tumor production than casein (Hendricks, unpublished data).

It was noted during early AFB_1 feeding trials in our laboratory that AFB_1 induced tumor incidences in rainbow trout obtained with diets containing cottonseed meal were always greater than with cottonseed free diets. Eventually a naturally occurring lipid component of cottonseed oil, cyclopropenoid fatty acids (CPFA), was found to be cocarcinogenic when fed to trout along with AFB_1. As summarized in Table 8, feeding of a few ppb AFB_1 with CPFA can result in a high incidence of hepatocellular carcinoma in as little as six months. Even previously AFB_1 resistent sockeye salmon (O. nerka), when placed on a diet containing 12 ppb AFB_1 with 50 ppm CPFA for 20 months, developed a 50% level of liver tumors (Wales and Sinnhuber, 1972). By comparison, only a slight increase in tumor incidence was noted in rats exposed to dietary AFB_1 and CPFA (Lee et al., 1969). Though CPFA by themselves are carcinogenic to rainbow trout at levels used (Sinnhuber et al., 1976), a definite cocarcinogenic activity with AFB_1 exists.

Rainbow trout (S. gairdneri) fed CPFA, metabolize AFB_1 at a slower rate than control fish, producing significantly lower levels of AFL and AFM_1 metabolites (Loveland et al., 1978). Lower levels of hepatic cytochrome P-450, cytochrome c reductase and enhanced benzo(a)pyrene hydroxylase activity have also been observed in these fish as noted above (Eisele et al., 1978). It was suggested (Eisele et al. 1978; Loveland et al., 1978) that the decreased trout MFO activity and the decreased production of hydroxylated AFB_1 metabolites was responsible for CPFA cocarcinogenic activity with AFB_1 in this animal.

In summary, fish are capable of metabolizing many xenobiotics including several mammalian carcinogens. Their MFO enzyme system, containing a unique form of cytochrome P-450, is quite active in the

conversion of several procarcinogens to their active metabolites. In the case of AFB_1 this has resulted in several epizootics in hatchery bred trout. Indeed, trout are extremely sensitive to AFB_1. Exposure of embyros to water containing a half ppm of AFB_1 for one hour results in a substantial amount of hepatocellular carcinoma formation. AFB_1 metabolism in rainbow trout proceeds much as it does in mammals with the production of AFL and AFM_1. Studies have demonstrated the influence of chemical inducers, dietary proteins and CPFA upon AFB_1 metabolism and AFB_1 induced carcinogensis in rainbow trout.

REFERENCES

Adamson, R.H. (1967) Drug metabolism in marine vertebrates Fed. Proc. 26, 1047-1054.

Ahokas, J.T. (1977) Ph.D. Thesis, University of Oulu.

Ahokas, J.T., Pelkonen, O., and Kärki, N.T. (1975a) Metabolism of polycyclic hydrocarbons by a highly active aryl hydrocarbon hydroxylase system in the liver of trout species. Biochem. Biophys. Res. Com. 63, 635-641.

Ahokas, J.T., Kärki, N.T., Oikari, A., and Soivio, A. (1975b) Mixed function monoxoygenase of fish as an indicator of pollution of aquatic environment by industrial effluent. Bull. Environ. Contam. Toxicol. 16, 270-274.

Ahokas, J.T., Pelkonen, O., and Kärki, N.T. (1976) Cytochrome P-450 and drug-induced spectral interactions in the hepatic microsomes of trout, Salmo trutta lacustris. Acta. Pharmacol. Toxicol. 38, 440-449.

Ahokas, J.T., Pelkonen, O., and Kärki, N.T. (1977a) Xenobiotic metabolism in fish. Xenobiotica 7, 105.

Ahokas, J.T., Pelkonen, O., and Kärki, N.T. (1977b) Characterization of benzo(a)pyrene hydroxylase of trout liver. Cancer Res. 37, 3737-3743.

Ahokas, J.T., Pelkonen, O., and Kärki, N.T. (1977c) The possible role of trout liver aryl hydrocarbon hydroxylase in activating aromatic polycyclic carcinogens. In Biological Reactive Intermediates, Plenum Press, New York, pp. 162-166.

Ahokas, J.T., Rääkkonen, R., Ronnholm, K., Raunio, V., Kärki, N., and Pelkonen, O. (1977d) Oxidative metabolism of carcinogens by trout liver resulting in protein binding and mutagenicity. In Microsomes and Drug Oxidations, Pergamon Press, Oxford and New York, pp. 435-441.

Ames, B.N., McCann, J., and Yamasaki, E. (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mut. Res. 31, 347-364.

Armbrecht, B.H., Shalkop, W.T., Rollins, L.D., Pohland, A.E., and Stoloff, L. (1970) Acute Toxicity of aflatoxin B_1 in wethers. Nature 225, 1062-1063.

Ashley, L.M., and Halver, J.E. (1961) Hepatomagenesis in Rainbow Trout. Fed. Proc. 20, 290.

Ashley, L.M., Halver, J.E., and Wogan, G.N. (1964) Hepatoma and aflatoxicosis in trout. Fed. Proc. 23, 25.

Ashley, L.M., Halver, J.E., Gardner, W.K., Jr., and Wogan, G.N. (1965) Crystalline aflatoxins cause trout hepatoma. Fed. Proc. 24, 627.

Ayres, J.L. (1969) Ph.D. Thesis, Oregon State University.

Ayres, J.L., Lee, D.J., Wales, J.H., and Sinnhuber, R.O. (1971) Aflatoxin structure and hepatocarcinogenicity in rainbow trout (Salmo gairdneri). J. Nat. Cancer Inst. 46, 561-564.

Bauer, D.H., Lee, D.J., and Sinnhuber, R.O. (1969) Acute toxicity of aflatoxins B_1 and G_1 in the rainbow trout (Salmo gairdneri). Toxicol. Appl. Pharmacol. 15, 415-419.

Bend, J.R., Pohl, R.J., and Fouts, J.R. (1973) Further studies of the microsomal mixed-function oxidase system of the little skate, Raja erinacea, including its response to some xenobiotics. Bull. Mt. Desert Island Biol. Lab. 12, 9-13.

Bend, J.R., Pohl, R.J., Davidson, N.P., and Fouts, J.R. (1974) Response of hepatic and renal microsomal mixed-function oxidases in the little skate, Raja erinacea, to pretreatment with 3-methylcholanthrene or TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). Bull. Mt. Desert Island Biol. Lab. 14, 7-12.

Bend, J.R., James, M.O., and Dansette, P.M. (1977) In vitro metabolism of xenobiotics in some marine animals. Ann. N.Y. Acad. Sci. 298, 505-521.

Brodie, B.B., and Maickel, R.P. (1962) Comparative biochemistry of drug metabolism. Proc. Intern. Pharm. Meeting 6, 299-324.

Burns, K.A. (1976) Microsomal mixed function oxidases in an estuarine fish, Fundulus heteroclitus, and their induction as a result of environmental contamination. Comp. Biochem. Physiol. 53B, 443-446.

Butler, W.H. (1969) Aflatoxicosis in laboratory animals. In Aflatoxin. Academic Press, New York and London, pp. 223-236.

Butler, W.H. and Neal, G.E. (1973) The effect of aflatoxin B_1 on the hepatic structure and RNA synthesis in rats fed a diet marginally deficient in choline. Cancer Res. 33, 2878-2885.

Campbell, T.C. and Hayes, J.R. (1976) The role of aflatoxin metabolism in its toxic lesion. Toxicol. Appl. Pharmacol. 35, 199-222.

Carnaghan, R.B.A., Hartley, R.D., and O'Kelley, J. (1963) Toxicity and fluorescence properties of the aflatoxins. Nature 200, 1101.

Chambers, J.E. and Yarbrough, J.D. (1976) Xenobiotic biotransformation systems in fishes. Comp. Biochem. Physiol. 55C, 77-84.

Chan, T.M., Gillett, J.W., and Terriere, L.C. (1967) Interaction between microsomal electron transport systems of trout and male rat in cyclodiene expoxidation. Comp. Biochem. Physiol. 20, 731-742.

Chevion, M., Stegeman, J.J., Peisach, J., and Blumberg, W.E. (1977) Electron paramagnetic resonance studies on hepatic microsomal cytochrome P-450 from a marine teleost fish. Life Sci. 20, 895-900.

Ciegler, A. (1975) Mycotoxins: Occurrence, chemistry, biological activity. Lloydia. 38, 21-35.

Clayson, D.B. (1975) Nutrition and experimental carcinogenesis: A review. Cancer Res. 35, 3292-3300.

Conney, A.H. and Burns, J.J. (1972) Metabolic interactions among environmental chemicals and drugs. Science 178, 576-586.

Conney, A.H., Welch, R., Kuntzman, R., Chang, R., Jacobson, M., Munro-Faure, A.D., Peck, A.W., Bye, A., Pohland, A., Poppers, P.J., Finster, M., and Wolff, J.A. (1971) Effects of environmental chemicals on the metabolism of drugs, carcinogens, and normal body constituents in man. Ann. N.Y. Acad. Sci. 179, 155-172.

Conney, A.H., Pantuck, E.J., Hsiao, K.C., Kuntzman, R., Alvares, A.P., and Kappas, A. (1977) Regulation of drug metabolism in man by environmental chemicals and diet. Fed. Proc. 36, 1647-1651.

Dann, R.E., Mitscher, L.A., and Couri, D. (1972) The *in vivo* metabolism of ^{14}C-labeled aflatoxins B_1, B_2, G_1 in rats. Res. Comm. Chem. Pathol. Parmacol. 3, 667-675.

Detroy, R.W., Lillehoj, E.B., and Ciegler, A. (1971) Aflatoxin and related compounds. In Microbial Toxins, Academic Press, New York and London, pp. 4-150.

Dewaide, J.H. (1971) Ph.D. Thesis, University of Nijmegen.

Dewaide, J.H. and Henderson, P.T. (1970) Seasonal variation of hepatic drug metabolism in the roach, *Leuciscus rutilus* L. Comp. Biochem. Physiol. 32, 489-497.

Dutton, G.J. and Montgomery, J.P. (1958) Glucuronide synthesis in fish and the influence of temperature. Biochem. J. 70, 17p.

Edwards, G.S., Rintel, T.D., and Parker, C.M. (1975) Aflatoxicol as a possible predictor for species sensitivity to aflatoxin B_1 (AFB_1). Proc. Amer. Assoc. Cancer Res. 1975, 133.

Eisele, T.A., Nixon, J.E., Pawlowski, N.E., and Sinnhuber, R.O. (1978) Effects of dietary cyclopropene fatty acids on the mixed function oxidase system of the rainbow trout (*Salmo gairdneri*). J. Toxicol. Environ. Health, In Press.

Engebrecht, R.H., Ayres, J.L., and Sinnhuber, R.O. (1965) Isolation and determination of aflatoxin B_1 in cottonseed meals. J. Ass. Offic. Anal. Chem. 48, 815-818.

Ermer, M. (1970) Versuche mit carcinogen Mitteln bei Kurzlebigen Fischarten. Zool. Anz. 184, 175-193.

Garfinkel, D. (1963) A comparative study of electron transport in microsomes. Comp. Biochem. Physiol. 8, 367-379.

Garner, R.C. (1973) Chemical evidence for the formation of a reactive aflatoxin B_1 metabolite, by hamster liver microsomes. FEBS Lett. 36, 261-264.

Garner, R.C. (1975) Reduction in binding of [^{14}C] aflatoxin B_1 to rat liver macromolecules by phenobarbitone pretreatment. Biochem. Pharmacol. 24, 1553-1556.

Garner, R.C. and Wright, C.M. (1973) Induction of mutations in DNA-repair deficient bacteria by a liver microsomal metabolite of aflatoxin B_1. Br. J. Cancer 28, 544-554.

Garner, R.C., Miller, E.C., and Miller, J.A. (1972) Liver microsomal metabolism of aflatoxin B_1 to a reactive derivative toxic to Salmonella typhimurium TA 1530. Cancer Res. 32, 2058-2066.

Garner, R.C., Miller, E.C., Miller, J.A., Garner, J.V., and Hanson, R.S. (1971) Formation of a factor lethal for S. typhimurium TA 1530 and TA 1531 on incubation of aflatoxin B_1 with rat liver microsomes. Biochem. Biophy. Res. Com. 45, 774-780.

Goldstein, A., Aronow, L., and Kalman, S.M. (1974) Principles of Drug Action: The Basis of Pharmacology, pp. 283-284. John Wiley and Sons, New York, pp. 283-284.

Grieco, M.P., Hendricks, J.D., Scanlan, R.A., Sinnhuber, R.O., and Pierce, D.A. (1978) Carcinogenicity and acute toxicity of dimethylnitrosamine in rainbow trout (Salmo gairdneri). J. Nat. Cancer Inst., In Press.

Gruger, E.H., Wekell, M.M., Numoto, P.T., and Craddock, D.R. (1977) Induction of hepatic aryl hydrocarbon hydroxylase in salmon exposed to petroleum dissolved in seawater and to petroleum and polychlorinated biphenyls, seperate and together, in food. Bull. Environ. Contam. Toxicol. 17, 512-520.

Gurtoo, H.L. and Bejba, N. (1974) Hepatic microsomal mixed function oxygenase: Enzyme multiplicity for the metabolism of carcinogens to DNA-binding metabolites. Biochem. Biophy. Res. Com. 61, 685-692.

Gurtoo, H.L. and Campbell, T.C. (1974) Metabolism of aflatoxin B_1 and its metabolism-dependent and independent binding to rat hepatic microsomes. Mol. Pharmacol. 10, 776-789.

Gurtoo, H.L. and Dave, C. (1973) Interaction of aflatoxin B_1 metabolite with rat liver RNA. Res. Com. Chem. Pathol. Pharmacol. 5, 635-645.

Halver, J.E. (1962) U.S. Fish and Wildlife Service Bull. 160, 22-56.

Halver, J.E. (1967) Crystalline aflatoxin and other factors for trout hepatoma. In Trout Hepatoma Research Conference Papers, pp. 78-102. Bureau of Sport Fisheries and Wildlife, Washington, D.C.

Halver, J.E. (1969) Aflatoxicosis and trout hepatoma. In Aflatoxin. pp. 265-306. Academic Press, New York and London, pp. 265-306.

Halver, J.E., Ashley, L.M., and Wogan, G.N. (1966a) Acute and chronic aflatoxicosis in fish. Proc. 7th Inter. Cong. Nutr. Vol. 4. Hamburg, West Germany.

Halver, J.E., Ashley, L.M., and Wogan, G.N. (1966b) Acute aflatoxicosis in rainbow trout and coho salmon. Fed. Proc. 25, 662.

Hattula, M.L. and Karlog, O. (1973) Absorption and elimination of polychlorinated biphenyls (PCB) in goldfish. Acta. Pharmacol. Toxicol. 32, 237-245.

Hendricks, J.D., Putnam, T.P., Bills, D.D., and Sinnhuber, R.O. (1977) Inhibitory effect of a polychlorinated biphenyl (Aroclor 1254) on aflatoxin B_1 carcinogenesis in rainbow trout (Salmo gairdneri). J. Nat. Cancer Inst. 59, 1545-1551.

Hendricks, J.D., Sinnhuber, R.O., Wales, J.H., and Stack, M.E. (1978a) The hepatocarcinogenicity of sterigmatocystin to rainbow trout embryos. J. Nat. Cancer Inst., In Press.

Hendricks, J.D., Sinnhuber, R.O., Nixon, J.E., Wales, J.H., Putnam, G.B., Loveland, P.M., Masri, M.S., and Hsieh, D.P.H. (1978b) Carcinogenicity of aflatoxin Q_1 to rainbow trout and its potentiation by cyclopropene fatty acids. Fed. Proc. 37, 451.

Heuper, W.C. and Payne, W.W. (1961) Observations on the occurrence of hepatomas in rainbow trout. J. Nat. Cancer Inst. 27, 1123-1135.

Hill, D.W., Hejtmancik, E., and Camp, B.J. (1976) Induction of hepatic microsomal enzymes by Aroclor 1254 in Ictalurus punctatus (Channel Catfish). Bull. Environ. Contam. Toxicol. 16, 495-502.

Huang, K.C. and Collins, S.F. (1962) Conjugation and excretion of aminobenzoic acid isomers in marine fishes. J. Cell. Comp. Physiol. 60, 49-52.

Jackson, E.W., Wolf, H., and Sinnhuber, R.O. (1968) The relationship of hepatoma in rainbow trout to aflatoxin contamination and cottonseed meal. Cancer Res. 28, 987-991.

James, M.O., Van Stee, E.W., and Bend, J.R. (1976) In vitro metabolism of epoxides in marine species. Fed. Proc. 35, 244.

Kappas, A. and Alvares, A. (1975) How the liver metabolizes foreign substances. Sci. Am. 232, 22-31.

Khudoley, V.V. (1971) The induction of hepatic tumors by nitrosamines in aquarium fish (Lebistes reticulatus). Vop. Onkol. 17, 67-72.

Khudoley, V.V. (1973) Morphological changes in the liver of fish (Lebistes reticulatus) under the action of diethyl- and dimethyl-nitrosamines. Vop. Onkol. 19, 88-94.

Kimura, M. and Kubota, S.S. (1972) Effects of carcinogens on guppy. Proc. Jap. Soc. Sci. Fish. P. 40.

Lee, D.J., Wales, J.H., and Sinnhuber, R.O. (1971) Promotion of aflatoxin induced hepatoma growth in trout by methyl malvalate and sterculate. Cancer Res. 31, 960-963.

Lee, D.J., Sinnhuber, R.O., Wales, J.H., and Putnam, G.B. (1978) Effect of dietary protein on the response of rainbow trout (Salmo gairdneri) to aflatoxin B_1. J. Nat. Cancer Inst. 60, 317-320.

Lee, D.J., Wales, J.H., Ayres, J.L., and Sinnhuber, R.O. (1968) Synergism between cyclopropenoid fatty acids and chemical carcinogens in rainbow trout (Salmo gairdneri). Cancer Res. 28, 2312-2318.

Lee, D.J., Wales, J.H., and Sinnhuber, R.O. (1969) Hepatoma and renal tubule adenoma in rats fed aflatoxin and cyclopropenoid fatty acids. J. Nat. Cancer Inst. 43, 1037-1044.

Lidman, U., Förlin, L., Molander, O., and Axelson, G. (1976) Induction of drug metabolizing system in rainbow trout (Salmo gairdneri) liver by polychlorinated biphenyls (PCBs). Acta. Pharmacol. Toxicol. 39, 262-272.

Lotlikar, P.D., Miller, E.C., Miller, J.A., and Halver, J.E. (1967) Metabolism of the carcinogen 2-acetylaminofluorene by rainbow trout. Proc. Soc. Exptl. Biol. Med. 124, 160-163.

Loveland, P.M., Nixon, J.E., Pawlowski, N.E., Eisele, T.A., Libbey, L.M., and Sinnhuber, R.O. (1978) Aflatoxin B_1 and aflatoxicol metabolism in rainbow trout (Salmo gairdneri) and the effects of dietary cyclopropene. J. Nat. Cancer Inst., Submitted.

Loveland, P.M., Sinnhuber, R.O., Berggren, K.E., Libbey, L.M., Nixon, J.E., and Pawlowski, N.E. (1977) Formation of aflatoxin B_1 from aflatoxicol by rainbow trout (Salmo gairdneri) liver in vitro. Res. Com. Chem. Pathol. Pharmacol. 16, 167-170.

Madhavan, T.V. and Gopalan, C. (1965) Effect of dietary protein on aflatoxin liver injury in weanling rats. Arch. Pathol. 80, 123-126.

Madhavan, T.V. and Gopalan, C. (1968) The effect of dietary protein on carcinogenesis of aflatoxin. Arch. Pathol. 85, 133-137.

Madhavan, T.V., Suryanarayana Rao K., and Tulpule, P.G. (1965) Effect of dietary protein level on susceptibility of monkeys to aflatoxin liver injury. Indian J. Med. Res. 53, 984-989.

Malins, D.C. (1977) Metabolism of aromatic hydrocarbons in marine organisms. Ann. N.Y. Acad. Sci. 298, 482-496.

Maren, T.H., Embry, R., and Broder, L.E. (1966) Excretion of drugs across the gill of the dogfish, S. acanthias. Bull. Mt. Desert Island Biol. Lab. 6, 25.

Maren, T.H., Embry, R., and Broder, L.E. (1968) The excretion of drugs across the gill of the dogfish, Squalus acanthias. Comp. Biochem. Physiol. 26, 853-864.

Matsushima, T. and Sugimura, T. (1976) Experimental carcinogenesis in small aquarium fishes. Prog. Exp. Tumor Res. 20, 367-379.

Matsushima, T., Sato, S., Hara, K., Sugimura, T., and Takashima, F. (1971) Bioassay of environmental carcinogens with the guppy, Lebistes reticulatas. Mut. Res. 31, 265.

McLean, A.E.M. (1977) Diet, DDT, and the toxicity of drugs and chemicals. Fed. Proc. 36, 1688-1691.

McLean, A.E.M. and Marshall, A. (1971) Reduced carcinogenic effects of aflatoxin in rats given phenobarbitone. Br. J. Exp. Path. 52, 322-329.

Mehrle, P.M., Johnson, W.W., and Mayer, F.L. (1974) Nutritional effects on chlordane toxicity in rainbow trout. Bull. Environ. Contam. Toxicol. 12, 513-517.

Mgbodile, M.U.K., Holscher, M., and Neal, R.A. (1975) A possible protective role for reduced glutathione in aflatoxin B_1 toxicity: Effect of pretreatment of rats with phenobarbital and 3-methylcholanthrene on aflatoxin toxicity. Toxicol. Appl. Pharmacol. 34, 128-142.

Miller, E.C., Miller, J.A., and Hartmann, H.A. (1961) N-hydroxy-2-acetylaminofluorene: A metabolite of 2-acetylaminofluorene with increased carcinogenic activity in the rat. Cancer Res. 21, 815-824.

Newberne, P.M. and Butler, W.H. (1969) Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals. Cancer Res. 29, 236-250.

Newberne, P.M. and Rogers, A.E. (1970) Aflatoxin carcinogenesis in rats: Dietary effects. In Mycotoxins In Human Health, I.F.H. Purchase (Ed.), Symposium Proc., Pretoria, S. Africa.

Newberne, P.M., Wogan, G.N., and Hall, A. (1966) Effects of dietary modifications on response of the duckling to aflatoxin. J. Nutr. 90, 123-130.

Patterson, D.S.P. (1973) Metabolism as a factor in determining the toxic action of the aflatoxins in different animal species. Fd. Cosmet. Toxicol. 11, 287-294.

Patterson, D.S.P. and Allcroft, R. (1970) Metabolism of aflatoxin in susceptible and resistant animal species. Fd. Cosmet. Toxicol. 8, 43-53.

Patterson, D.S.P. and Roberts, B.A. (1970) The formation of aflatoxins B_{2a} and G_{2a} and their degradation products during the *in vitro* detoxification of aflatoxin by livers of certain avian and mammalian species. Fd. Cosmet. Toxicol. 8, 527-538.

Patterson, D.S.P. and Roberts, B.A. (1972) Aflatoxin metabolism in duck liver homogenates: The relative importance of reversible cyclopentenone reduction and hemiacetal formation. Fd. Cosmet. Toxicol. 10, 501-512.

Patterson, D.S.P., Roberts, B.A., and Allcroft, R. (1969) Aflatoxin metabolism. Fd. Cosmet. Toxicol. 7, 277-278.

Payne, J.F. (1977) Mixed function oxidases in marine organizms in relation to petroleum hydrocarbon metabolism and detection. Marine Pollution Bull. 8, 112-116.

Payne, J.F. and Penrose, W.R. (1975) Induction of aryl hydrocarbon (benzo(a)pyrene) hydroxylase in fish by petroleum. Bull. Environ. Contam. Toxicol. 14, 112-116.

Pedersen, M.G., Hershberger, W.K., and Juchau, M.R. (1974) Metabolism of 3,4-Benzpyrene in rainbow trout (*Salmo gairdneri*). Bull. Environ. Contam. Toxicol. 12, 481-486.

Peers, F.G. and Linsell, C.A. (1973) Dietary aflatoxins and liver cancer--a population based study in Kenya. Brit. J. Cancer 27, 473-484.

Pliss, G.B. and Khudoley, V.V. (1975) Tumor induction by carcinogenic agents in aquarium fish. J. Nat. Cancer Inst. 55, 129-136.

Pohl, R.J., Bend, J.R., Guarino, A.M., and Fouts, J.R. (1974) Hepatic microsomal mixed-function oxidase activity of several marine species from coastal Maine. Drug Metab. Disp. 2, 545-555.

Pohl, R.J., Fouts, J.R., and Bond, J.R. (1975) Response of hepatic mixed-function oxidases in the little skate, Raja erinacea, and the winter flounder Pseudopleuronectes americanus to pretreatment with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) or DBA (1,2,3,4-dibenzanthracene). Bull. Mt. Desert Island Biol. Lab. 15, No. 37.

Pohland, A.E., Cushmac, M.E., and Andrellos, P.J. (1968) Aflatoxin B_1 hemiacetal. J. Ass. Offic. Anal. Chem. 51, 907-910.

Rao, K.S. and Gehring, P.J. (1971) Acute Toxicity of aflatoxin B_1 in monkeys. Toxicol. Appl. Pharmacol. 19, 169-175.

Roebuck, B.D. and Wogan, G.N. (1977) Species comparison of in vitro metabolism of aflatoxin B_1. Cancer Res. 37, 1649-1656.

Rucker, R.R., Yasutake, W.T., and Wolf, H. (1961) Trout hepatoma: A preliminary report. Prog. Fish-Culturist 23, 3-7.

Salhab, A.S. and Edwards, G.S. (1977) Comparative in vitro metabolism of aflatoxicol by liver preparations from animals and humans. Cancer Res. 37, 1016-1021.

Sato, S., Matsushima, T., Tanaka, N., Sugimura, T., and Takashima, F. (1973) Hepatic tumors in the guppy Lebistes reticulatus induced by aflatoxin B_1, dimethylnitrosamine, and 2-acetylaminofluorene. J. Nat. Cancer Inst. 50, 767-778.

Scarpelli, D.G. (1976) Drug metabolism and aflatoxin-induced hepatoma in rainbow trout (Salmo gairdneri). Prog. Expt. Tumor Res. 20, 339-350.

Schoenhard, G.L. (1974) Ph.D. Thesis, Oregon State University.

Schoenhard, G.L., Lee, D.J., Howell, S.E., Pawlowski, N.E., Libbey, L.M., and Sinnhuber, R.O. (1976) Aflatoxin B_1 metabolism to aflatoxicol and derivatives lethal to Bacillus subtilis GSY 1057 by rainbow trout (Salmo gairdneri) liver. Cancer Res. 36, 2040-2045.

Schoenhard, G.L., Lee, D.J., Wales, J.H., Sinnhuber, R.O., and Pawlowski, N.E. (1978) Aflatoxicol-induced carcinoma in rainbow trout (Salmo gairdneri). Cancer Res., Submitted.

Schoental, R. (1970) Hepatotoxic activity of retrorsine, senkirkine and hydroxysenkirkine in newborn rats, and the role of epoxides in carcinogenesis by pyrrolizidine alkaloids and aflatoxins. Nature 227, 401-402.

Shank, R.C. and Wogan, G.N. (1965) Distribution and excretion of C^{14} labeled aflatoxin B_1 in the rat. Fed. Proc. 24, 627.

Shank, R.C., Bhamarapravati, N., Gordon, J.E., and Wogan, G.N. (1972) Dietary aflatoxins and human liver cancer. IV. Incidence of primary liver cancer in two municipal populations of Thailand. Fd. Cosmet. Toxicol. 10, 171-179.

Sinnhuber, R.O. and Wales, J.H. (1974) Aflatoxin B_1 hepatocarcinogenicity in rainbow trout embryos. Fed. Proc. 33, 247.

Sinnhuber, R.O., Wales, J.H., Engebrecht, R.H., Amend, D.F., Kray, W.D., Ayres, J.L., and Ashton, W.E. (1965) Aflatoxins in cottonseed meal and hepatoma in rainbow trout. Fed. Proc. 24, 627.

Sinnhuber, R.O., Wales, J.H., and Lee, D.J. (1966) Cyclopropenoids, cocarcinogens for aflatoxin-induced hepatoma in trout. Fed. Proc. 25, 555.

Sinnhuber, R.O., Wales, J.H., Ayres, J.L., Engebrecht, R.H., and Amend, D.L. (1968) Dietary factors and hepatoma in rainbow trout (Salmo gairdneri). I. Aflatoxins in vegetable protein feedstuffs. J. Nat. Cancer Inst. 41, 711-718.

Sinnhuber, R.O., Lee, D.J., Wales, J.H., Landers, M.K., and Keyl, A.C. (1974) Hepatic carcinogenesis of aflatoxin M_1 in rainbow trout (Salmo gairdneri) and its enchanement by cyclopropene fatty acids. J. Nat. Cancer Inst. 53, 1285-1288.

Sinnhuber, R.O., Hendricks, J.D., Putnam, G.B., Wales, J.H., Pawlowski, N.E., Nixon, J.E., and Lee, D.J. (1976) Sterculic acid, a naturally occurring cyclopropene fatty acid, a liver carcinogen to rainbow trout. Fed. Proc. 35, 505.

Sinnhuber, R.O., Hendricks, J.D., Wales, J.H., and Putnam, G.B. (1977) Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogenesis. Ann. N.Y. Acad. Sci. 298, 389-408.

Sisk, D.B. and Carlton, W.W. (1972) Effect of dietary protein concentration on response of miniature swine to aflatoxins. Am. J. Vet. Res. 33, 107-114.

Smith, J.W. and Hamilton, P.B. (1970) Aflatoxicosis in the broiler chicken. Poul. Sci. 49, 207-215.

Stanton, M.F. (1965) Diethylnitrosamine-induced hepatic degeneration and neoplasia in the aquarium fish, Brachydanio rerio. J. Nat. Cancer Inst. 34, 117-130.

Stegeman, J.J. (1977) Fate and effects of oil in marine animals. Oceanus 19, 59-66.

Stott, W.T. and Sinnhuber, R.O. (1978a) Dietary casein levels and aflatoxin B_1 metabolism in rainbow trout (Salmo gairdneri). Submitted, Amer. Chem. Soc. Symposium, Miami Beach, Florida.

Stott, W.T. and Sinnhuber, R.O. (1978b) Dietary protein levels and aflatoxin B_1 metabolism in rainbow trout (Salmo gairdneri) J. Environ. Pathol. Toxicol., Submitted.

Stott, W.T. and Sinnhuber, R.O. (1978c) Trout hepatic enzyme activation of aflatoxin B_1 in a mutagen assay system and the inhibitory effect of PCBs. Bull. Environ. Contam. Toxicol. 19, 35-41.

Swenson, D.H., Miller, J.A. and Miller, E.C. (1973) 2,3-Dihydro-2,3-dihydroxyaflatoxin B_1: An acid hydrolysis product of an RNA-aflatoxin B_1 adduct formed by hamster and rat liver microsomes in vitro. Biochem. Biophy. Res. Com. 53, 1260-1267.

Swenson, D.H., Miller, E.C., and Miller, J.A. (1974) Aflatoxin B_1-2,3-oxide: Evidence for its formation in rat liver in vivo and by human liver microsomes in vitro. Biochem. Biophy. Res. Com. 60, 1036-1043.

Swenson, D.H., Miller, J.A., and Miller, E.C. (1975) The reactivity and carcinogenicity of aflatoxin B_1-2,3-dichloride, a model for the putative 2,3-oxide metabolite of aflatoxin B_1. Cancer Res. 35, 3811-3823.

Swenson, D.H., Lin, J.-K., Miller, E.C., and Miller, J.A. (1977) Aflatoxin B_1-2,3-oxide as a probable intermediate in the covalent binding of aflatoxin B_1 and B_2 to rat liver DNA and ribosomal RNA in vivo. Cancer Res. 37, 172-181.

Todd, G.C., Shalkop, W.T., Dooley, K.L., and Wiseman, H.G. (1968) Effects of ration modifications on aflatoxicosis in the rat. Am. J. Vet. Res. 29, 1855-1861.

Wales, J.H. and Sinnhuber, R.O. (1966) An early hepatoma epizootic in rainbow trout, Salmo gairdneri. Calif. Fish Game 52, 85-91.

Wales, J.H. and Sinnhuber, R.O. (1972) Hepatomas induced by aflatoxin in the Sockeye salmon (Oncorhynchus nerka). J. Nat. Cancer Inst. 48, 1529-1530.

Wells, M.R., Ludke, J.L., and Yarbrough, J.D. (1973) Epoxidation and fate of [^{14}C] aldrin in insecticide-resistant and susceptible populations of mosquitofish (Gambusia affinis). J. Agr. Fd. Chem. 21, 428-429.

Wolf, H. and Jackson, E.W. (1963) Hepatomas in rainbow trout. Descriptive and experimental epidemiology. Science 142, 676-678.

Wogan, G.N. (1966) Chemical nature and biological effects of the aflatoxins. Bacteriol. Rev. 30, 460-470.

Wogan, G.N. (1973) Aflatoxin carcinogenesis. In Methods In Cancer Research Vol. VII, 309-345.

Wogan, G.N. and Newberne, P.M. (1967) Dose-response characteristics of aflatoxin B_1 carcinogenesis in the rat. Cancer Res. 27, 2370-2376.

Wogan, G.N. and Pong, R.S. (1970) Aflatoxins. Ann. N.Y. Acad. Sci. 174, 623-635.

Wogan, G.N., Edwards, G.S., and Shank, R.C. (1967). Excretion and tissue distribution of radioactivity from aflatoxin B_1-^{14}C in rats. Cancer Res. 27, 1729-1736.

Wogan, G.N., Edwards, G.S., and Newberne, P.M. (1971) Structure-activity relationships in toxicity and carcinogenicity of aflatoxins and analogs. Cancer Res. 31, 1936-1942.

Wong, J.J. and Hsieh, D.P.H. (1976) Mutagenicity of aflatoxins related to their metabolism and carcinogenic potential. Proc. Natl. Acad. Sci. USA 73, 2241-2244.

Wood, E.M. and Larson, C.P. (1961) Hepatic carcinoma in rainbow trout. Arch. Pathol. 71, 471-479.

Table 1. Summary of carcinogen exposure trials with fish[a].

Compound[b]	Test Fish	Dose and Route	Exposure Time	Reference
DMN	Lebister reticulatus	0.48% in Diet	13 mon.	Sato et al. (1973)
	L. reticulatus	100 ppm in Water	56 days	Khudoley (1971; 1973) Pliss and Khudoley (1975)
	Salmo gairdneri	3-800 ppm in Diet	12 mon	Grieco et al. (1978)
	S. gairdneri	4800 ppm in Diet	20 mon	Halver (1962)
	S. gairdneri (embyro)	500 ppm in Water	24 hr	Hendricks (unpublished data)
DEN	Brachydanio rerio	10-100 ppm in Water	8 wk	Stanton (1965)
	L. reticulatus	13-100 ppm in Water	10-64 days	Khudoley (1971; 1973) Pliss and Khudoley (1975)
MNG	S. gairdneri (embryo)	100 ppm in Water	1 hr	Hendricks (unpublished data)
NM	L. reticulatus	75-320 ppm in Water	28 wk	Pliss and Khundoley (1975)
	B. rerio	75-320 ppm in Water	28 wk	Pliss and Khundoley (1975)
AAF	L. reticulatus	0.03% in Diet	11-13 mon	Sato et al. (1973)
	L. reticulatus	.03-.12% in Diet	48 wk	Pliss and Khudoley (1975)
	S. gairdneri	300 ppm in Diet	20 mon	Halver (1962)
DMAB	L. reticulatus	1 ml saturated ethanol solution/l water	40-90 days	Kimura and Kubota (1972)
	L. reticulatus	.03-.12% in Diet	56 wk	Pliss and Khudoley (1975)
	S. gairdneri	300 ppm in Diet	20 mon	Halver (1962)
AAT	L. reticulatus	0.0-10 ppm in Water	4090 days	Kimura and Kubota (1972)
	L. reticulatus	.03-.12% in Diet	56 wk	Pliss and Khudoley (1975)
	S. gairdneri	1200 ppm in Diet	20 mon	Halver (1962)
3-MC	Gasterosteus aculetus	0.5 mg Painted (twice/wk)	3-6 mon	Ermer (1970)
	Rhodeus amarus	0.5 mg Painted (twice/wk)	3-6 mon	Ermer (1970)
BP	G. aculetus	0.5 mg Painted (twice/wk)	3-7 mon	Ermer (1970)
	R. amarus	0.5 mg Painted (twice/wk)	3-7 mon	Ermer (1970)
SMC	L. reticulatus	20 ppm in Diet	57 days	Matsushima et al. (1971)
	S. gairdneri (embryo)	0.5 ppm in Water	1 hr	Hendricks et al. (1978a)
DDT	S. gairdneri	80 ppm in Diet	20 mon	Halver (1962)
TU	S. gairdneri	4800 ppm in Diet	20 mon	Halver (1962)
TA	S. gairdneri	1200 ppm in Diet	20 mon	Halver (1962)
U	S. gairdneri	4800 ppm in Diet	20 mon	Halver (1962)
VC	S. gairdneri (embryo)	5 ppm in Water	1 hr	Hendricks (unpublished data)

[a]Adapted from Matsushima and Sugimura (1976).

[b]DMN = Dimethylnitrosamine; DEN = Diethylnitrosamine; AAF = N-2-acetyaminofluorene; DMAB = 4-dimethylaminoazobenzene; AAT = Aminoazotoluene; 3-MC = 3-methylcholanthrene; BP = Benzo(a)pyrene; SMC = Sterigmatocystin; NM = Nitrosomorpholine; DDT = Dichlorodiphenyltrichloroethane; TU = thiourea; TA = tannic acid; U = Urethane; VC = versicolorin; MNG = N-Me-N-Nitrosoguanidine

Table 2. Oral LD_{50} values for aflatoxin B_1.

Species	LD_{50} (mg/Kg body weight)	References
Rabbit	0.3-0.5	Newberne and Butler (1969)
Duckling	.3-.6	Ciegler (1975); Carnaghan et al. (1963)
Pig	0.6	Ciegler (1975)
Rainbow trout	0.5	Halver (1967)
	0.8 (i.p.)	Bauer et al. (1969)
Guinea Pig	1.4-2.0	Rao and Gehring (1971); Newberne and Butler (1969)
Sheep	2.0	Armbrecht et al. (1970)
Monkey	2.2	Rao and Gehring (1971)
Chicken	6.5-16.5	Smith and Hamilton (1970)
Mouse	9.0	Ciegler (1975)
Rat	5.5	Wogan (1966); Wogan and Newberne (1967)
Hamster	10.2	Wogan (1966)

Table 3. Aflatoxin carcinogenicity to rainbow trout (Salmo gairdneri).

	Dose/Route	Exposure	Duration	No. fish with tumor / total no.	References
Aflatoxin B_1 (embryo)	0.5 ppm in Water	1-2 hr	321 day	20/50	Sinnhuber and Wales (1974)
Aflatoxin B_1 (embryo)	0.5 ppm in Water	1 hr	296 day	18/50	Sinnhuber and Wales (1974)
Aflatoxin B_1	4 ppb in Diet	12 mon	12 mon	55/114	Hendricks et al. (1978b)
Aflatoxin Q_1	100 ppb in Diet			12/113	
Aflatoxin M_1	4-64 ppb in Diet	12 mon	12 mon	1-31/100-110	Sinnhuber et al.(1974)
Aflatoxin L	20 ppb in Diet	8 mon	8 mon	21/80	Schoenhard et al. (1978)
Aflatoxin B_1	4 ppb in Diet	12 mon	12 mon	10/40	Ayres et al. (1971)
Aflatoxin B_1	8 ppb in Diet			40/57	
Aflatoxin B_1	20 ppb in Diet			62/80	
Aflatoxin B_2	20 ppb in Diet			1/20	
Aflatoxin G_1	20 ppb in Diet			1/20	
Aflatoxin G_2	20 ppb in Diet			0/20	

Table 4. ^{14}C distribution in environmental water 12 hours after i.p. injection of ^{14}C-AFB_1 into rainbow trout (S. gairdneri).[a]

Fraction	% total ^{14}C in the water (50± 1.6% of total ^{14}C injected)
Chloroform-soluble ^{14}C	44±2.5%
Low R_f compounds	7.0%
Compound X	7.9%
AFB_1	24%
Non-fluorescent	4.8%
Chloroform-insoluble ^{14}C	3.7±0.7%

[a]Adapted from Ayres (1969)

Table 5. Subcellular distribution of ^{14}C in rats and rainbow trout dosed i.p. with ^{14}C-AFB_1[a].

Fraction	Animal	% ^{14}C of total liver			
		0.5 hr.	4 hr.	12 hr.	24 hr.
Cell Debris and Nuclei	Trout	20	42	42	44
	Rat	9	9	11	16
Mitochondria	Trout	11	9	8	8
	Rat	12	16	14	12
Microsomal	Trout	15	30	35	19
	Rat	20	42	45	48
Soluble	Trout	43	20	14	16
	Rat	60	31	28	25

[a]Values represent approximate % adapted from figures of Ayres (1969) and Wogan et al. (1967).

Table 6. Species comparison of aflatoxin B_1 metabolites[a].

Species	AFM_1	AFB_{2a}	AFL	AFP_1	AFQ_1	AFH_1	$AFLM_1$
Rat							
in vivo	+	+					
in vitro	+	+		+	+		
Mouse							
in vivo	+						
in vitro	+	+		+	+		
Monkey							
in vivo	+			+			
in vitro	+		+	+	+	+	
Human							
in vivo	+			+			
in vitro	+	+	+	+	+	+	+
Hamster							
in vitro	+						
Guinea Pig							
in vivo	+						
in vitro	+	+					
Rabbit							
in vitro	+	+	+	+			+
Dog							
in vitro	+		+				+
Chick							
in vitro	+	+	+				
Duckling							
in vitro	+	+	+				
Trout							
in vivo	+						
in vitro	+		+				

[a]Adapted from Schoenhard (1974).

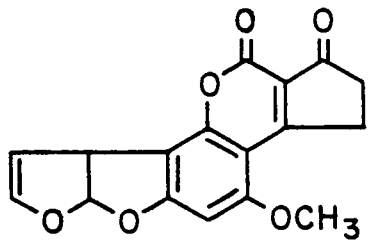
Table 7. Dietary protein effects upon aflatoxin B_1 carcinogenicity.

Animal	Diet and Treatment	Exposure Time	Duration	No. Animals with Cancer/ Total No.	Reference
Rainbow Trout	32% casein + 0.2% tryptophan + 6 ppb AFB_1	12 mon	12 mon	10/60	Lee et al. (1978)
	49.5% casein + 6 ppb AFB_1			9/60	
	32% fish protein concentrate			2/60	
	49.5% fish protein concentrate			27/60	
Rainbow Trout (Embryo)	Exposed to 0.5 ppm AFB_1 in water.				Hendricks (unpublished data)
	32% casein + 0.2% tryptophan	1 hr	12 mon	8/34	
	32% casein + 0.3% tryptophan + 0.6% arginine + 0.2% cysteine + 0.4% methionine			5/44	
	42% casein			33/118	
	52% casein			50/115	
	62% casein			85/114	
	32% fish protein concentrate			23/107	
	42% fish protein concentrate			48/106	
	52% fish protein concentrate			40/61	
	62% fish protein concentrate			33/49	
Rat	5% casein + 20-100 ugAFB_1/ day	10-47 days	12 mon	0/12	Madhavan and Gopalan (1968)
	20% casein + 20-100 ugAFB_1/ day			15/30	

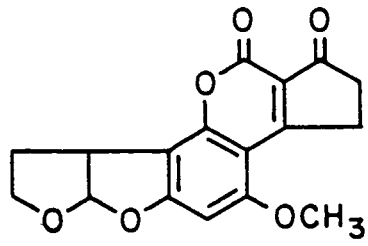
Table 8. Effect of cyclopropenoid fatty acid on aflatoxin B_1 carcinogenesis.

Animal	Treatment	Exposure Time	Duration	% Cancer Postive	References
Rainbow Trout	20 ppm Methyl Sterculate + 20 ppb AFB_1	1 mon	12 mon	65%	Lee et al. (1971)
	50 ppm Methyl Sterculate + 20 ppb AFB_1			80%	
	100 ppm Methyl Sterculate + 20 ppb AFB_1			95%	
	20 ppb AFB_1			37%	
	200 ppm CPFA + 4 ppb AFB_1	6 mon	6 mon	83%	Sinnhuber et al. (1966)
	4 ppb AFB_1			0%	
	112 ppm CPFA + 4 ppb AFB_1	12 mon	12 mon	98%	Lee et al. (1968)
	4 ppb AFB_1			50%	
	112 ppm CPFA			10%	
Sockeye Salmon	50 ppm CPFA + 12 ppb AFB_1	20 mon	20 mon	40-50%	Wales & Sinnhuber (1972)
	12 ppb AFB_1			0%	
Rats	220 ppm CPFA + 10-300 ppb AFB_1 + 8-252 ppb AFG_1	18 mon	18 mon	70%	Lee et al. (1969)
	10-300 ppb AFB_1 + 8-252 ppb AFG_1			59%	

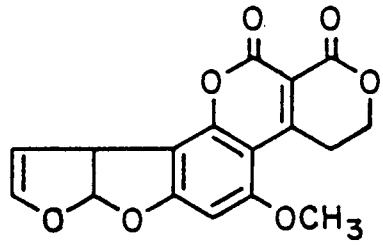
Fig. 1. Chemical structures of the four major aflatoxin mold metabolites.



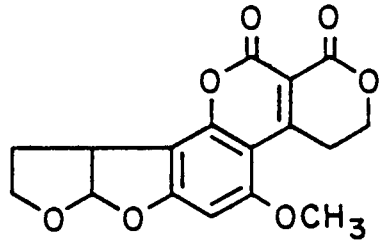
AFB_1



AFB_2



AFG_1



AFG_2

Figure 1.

Fig. 2. Summary of known and theorized AFB_1 *in vitro* metabolic pathways.

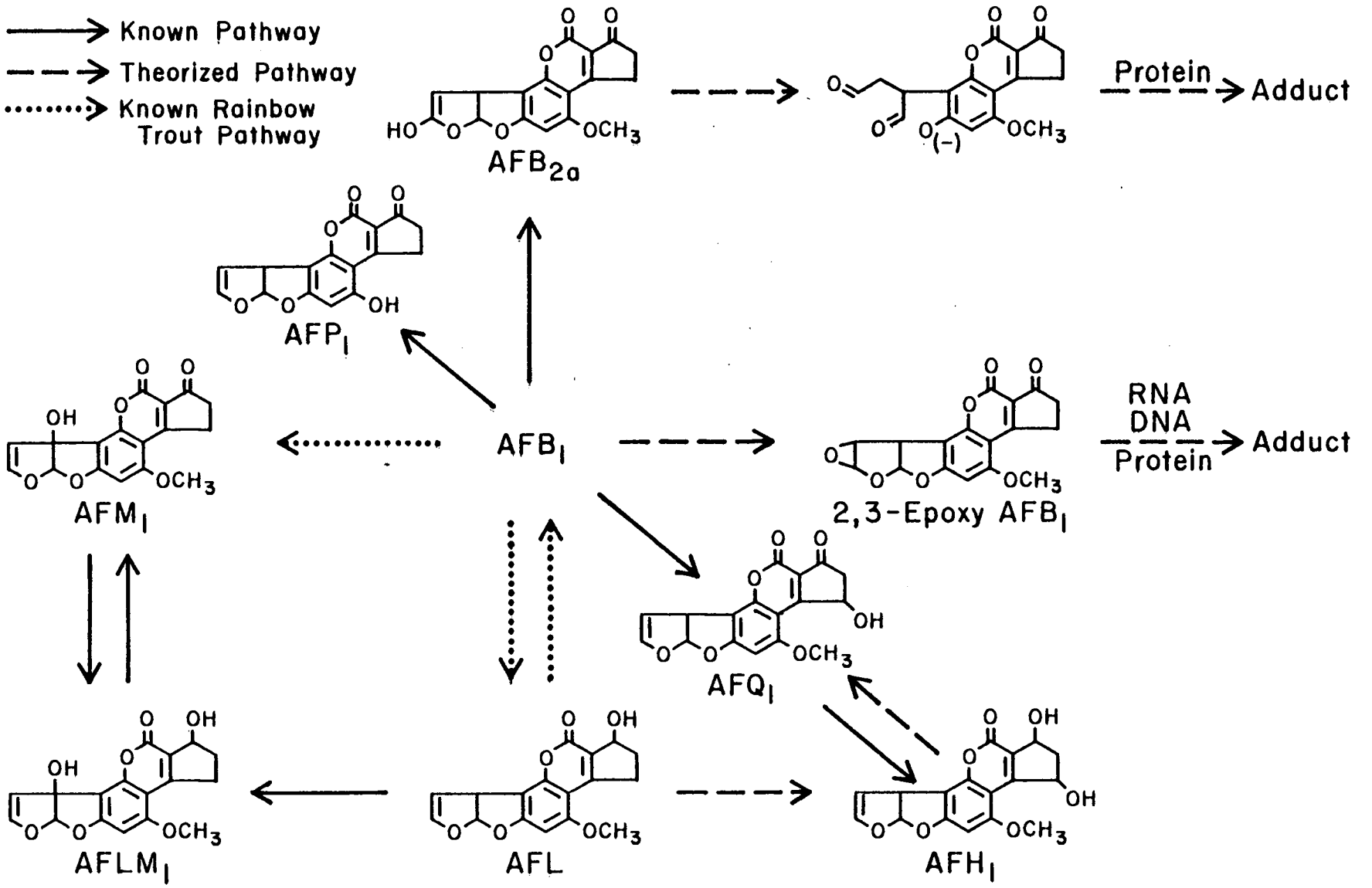

Known Pathway
Theorized Pathway
Known Rainbow Trout Pathway
AFB2a
Protein
Adduct
AFP1
AFM1
AFB1
RNA
DNA
Protein
Adduct
2,3-Epoxy AFB1
AFQ1
AFLM1
AFL
AFH1

Figure 2.

Trout Hepatic Enzyme Activation of Aflatoxin B_1 in a Mutagen Assay System and the Inhibitory Effect of PCBs

W. T. Stott

R. O. Sinnhuber

Department of Food Science and Technology

Oregon State University

Corvallis, Oregon 97331

INTRODUCTION

Bacterial mutagen assay systems have frequently been suggested as preliminary screening systems of potentially carcinogenic compounds. One such method, gaining wide acceptance, developed by Ames and co-workers (AMES et al. 1973; 1975), utilizes several histidine mutants of Salmonella typhimurium to detect metabolically activated mutagens. This method usually employs the submitochondrial fraction of rat liver to activate the test compound in a semisolid incubation mixture, but other mammalian tissues have also been utilized (AMES et al. 1975). To the authors' knowledge, nonmammalian tissues, such as fish, have never been used in this mutagen assay system.

It was originally believed that fish lacked the enzymes necessary for xenobiotic metabolism (BRODIE & MAICKEL, 1962), however, numerous studies have since shown a similarity with mammalian mixed function oxidase systems (MFO) (POTTER & O'BRIEN, 1964; CREAVEN et al. 1965; 1967; CHAN et al. 1967; LOTLIKAR et al. 1967; BAKER et al. 1963). Fish MFO appear to be capable of catalyzing many of the same drug metabolic reactions observed in mammalian MFO (BUHLER & RASMUSSON 1968; DEWAIDE 1971; LIDMAN et al. 1976; AHOKAS et al. 1976; BEND et al. 1973). The fish MFO enzymes appear to be more labile than mammalian MFO and have a lower optimum temperature.

Fish MFO drug metabolism has also been shown to be inducible by polychlorinated biphenyl compounds (PCBs) (LIDMAN et al. 1976; HILL et al. 1976; AHOKAS et al. 1976), much as these compounds induce mammalian

MFO activity (ALVARES et al. 1973; KONAT & CLAUSEN, 1973; OBERG & LUNDBERG, 1974; TURNER & GREEN, 1974; IVERSON et al. 1975; LITTERST et al. 1972; 1974). The degree of inducing activity in a series of PCBs, Aroclors, has been shown to be dependent upon the degree of chlorination, the higher chlorinated PCBs having greater activity (SCHMOLDT et al. 1974; ECOBICHON & COMEAU, 1974; OBERG, 1976; BICKERS et al. 1972). One of these Aroclor 1254, a 54% chlorinated biphenyl, has been recommended as a pretreatment (i.p. 500 mg/kg) of rats utilized in the Ames mutagen assay (AMES et al. 1975).

The present work was undertaken to define the optimal conditions for use of the Ames assay with rainbow trout (Salmo gairdneri) liver postmitochondrial fraction. In addition, the effect of several PCBs of varying chlorine contents upon the response of the assay to a metabolically activated carcinogen was investigated. Aflatoxin B_1 (AFB), a potent carcinogen in rainbow trout (SINNHUBER et al. 1968) and a potent mutagen in the Ames assay (AMES et al. 1973; 1975; WONG & HSIEH, 1976) when activated by rat liver postmitochondrial fraction, was utilized as the test compound.

METHODS

Rainbow trout (S. gairdneri), Mt. Shasta strain, average weight ca. 500 g, were used in all experiments. Fish were fed a casein-gelatin based semipurified diet to satiety prior to sacrifice or injection. All fish were held in circular tanks supplied with 3 gallons of well water/min. at 12°C. Fish were stunned by a cranial blow and their livers aseptically removed. Upon recording of liver weight they were perfused with ice-cold Krebs-Ringer solution adjusted for fish (SCHOENHARD et al. 1976) and homogenized in 2 volumes phosphate buffer (pH 7.4) using 4 passes of

a pestle in a Potter-Elvejhem apparatus. Liver homogenates were then centrifuged at 20,000 xg for 10 min. and the postmitochondrial fraction (PMF) collected, frozen in dry ice and held at 40°C for no longer than 12 hrs. All operations were carried out at 4°C and 3 replicates of 3 pooled livers were used. Protein content of the PMFs was determined by the Lowry method (LOWRY et al. 1951). PCB experiments were done utilizing fish from the same lot and conditions as outlined above. Anesthetized fish (Tricaine Methane Sulfonate) were injected i.p. with 500 mg/kg of either Aroclor* 1221, 1242, 1254 or 1260 in DMSO. Control fish received 0.3 ml i.p. DMSO. Fish were then fasted and sacrificed 5 days later. Two replicates of 3 pooled fish each for every PCB tested were done.

Microbial mutagen assays were carried out, in general, after the method of Ames et al. (1973; 1975) using the AFB sensitive tester strain S. typhimurium TA 1538**. Twelve hr. Trypticase-Soy Yeast Extract broth cultures were used in the experiments giving an inoculation level of ca. 10^8 cells/assay. Krebs-Ringer solution adjusted for fish was used as the incubation mixture salts solution. Fish PMF equivalent to 50 mg. of wet weight liver and 0.25µg AFB in 2.5µl ethanol were also added unless otherwise noted. The G-6-P, NADP(+), PMF, salts, bacteria and toxin were all mixed with top agar and poured upon preset Minimal Davis (Difco) with 2% glucose. Plates were incubated at 25°C for 9 hrs., then transfered to 37°C for 48 hrs. before counting of the

---

*Aroclor's were provided by Monsanto Chemical Co., St. Louis, MO.

**Culture was a gift of B.N. Ames, Biochemistry Dept., University of California, Berkeley, California.

colonies. Each assay was plated in duplicate for the parameters study and in triplicate for the PCB work.

Experiments were run to determine optimal assay conditions with fish PMF. Optimal metabolic incubation time at 25°C for the conversion of AFB to an active mutagen was determined by holding plates for 0, 3, 6, 9, 12, 24 and 48 hrs. prior to 37°C incubation. Varying levels of the PMF were added to each plate to define optimal protein concentration. Two, 4, 6, 8, 10 and 12 mg PMF protein/plate were used. Likewise, AFB concentrations, 0.05, 0.075, 0.1, 0.25, 0.5 and 0.75µg AFB /plate were employed to define optimal toxin concentration. Results were plotted on linear graph paper and analyzed by linear regression analysis and Students T-test for line-fit and significance.

The effects of pretreatment of fish with different Aroclors upon mutagen assay response to AFB were determined by varying the amounts of PMF used/assay and comparing the different curves obtained. Results were statistically analyzed as before.

RESULTS AND DISCUSSION

The results of this study clearly indicate the ability of fish liver drug metabolizing enzymes to function in the Ames mutagen assay. By utilizing a different salt solution and incubation regimen, rainbow trout (S. gairdneri) PMF enzymes were able to metabolize AFB to an active mutagen to the test microorganism. An average of 5.2×10^4 revertant bacteria/µg AFB/g wet liver were detected in plates pre-incubated at 25°C for 9 hrs. prior to transfer to 37°C. This pre-incubation allowed the trout MFO biotransformation of AFB to its active mutagenic form to occur before raising the temperature for rapid bacterial growth.

Optimal mutagen assay conditions were found to be obtained with 6.4 mg PMF protein and 160 ng AFB/incubation mixture and a metabolic incubation temperature of 25°C. As shown in figure 1, a significant ($P<.10$) increase in the number of revertants was observed when metabolic incubations were carried out at 25°C for 9 hrs. although higher, nonsignificant, responses were obtained at 12, 24 and 48 hours.

An attempt to determine the effects of a series of chlorinated biphenyls, known inducing agents of fish enzymes (AHOKAS et al. 1976; HILL et al. 1976; LIDMAN et al. 1976), upon the mutagen assay response was also made. As shown in figure 2, the result was a significant ($P<.05$) reduction in response versus control response when fish were pretreated with Aroclor 1242, 1254 and 1260. This result is in contrast with results of assays using rat PMF (AMES et al. 1973; 1975). A pattern of decreasing response with increasing chlorination number was observed, except with Aroclor 1260. Protein levels were not significantly different from control values in agreement with LIDMAN's et al. (1976) finding of nonsignificant changes in rainbow trout (S. gairdneri) PMF up to 21 days post oral dosage of 1000 mg PCB/kg. However, these authors reported significant increases in cytochrome P-450 and several drug metabolizing enzyme activities at 3 and 7 days post PCB exposure.

A possible explanation of the apparent conflict between reported PCB induction of trout MFO and of decreased mutagen assay responses in PCB treated fish may be that mutagen detoxifying enzyme systems were induced to a greater extent than the AFB activating MFO enzyme system. It has been reported that one such potential enzyme, epoxide hydrase, is inducible in the rat (BELLWARD et al. 1975; LU et al. 1975). The

decreasing assay response with increasing chlorination of the PCB may reflect the PCBs ability as an inducer of these enzyme systems in the rainbow trout. This finding appears to substantiate recent findings in rainbow trout feeding trials of Aroclor 1254 and AFB in which PCB treated fish had a lower AFB induced incidence of cancer (HENDRICKS, communication, our laboratory).

In summary, it was demonstrated that the trout PMF may be successfully employed in the Ames mutagen assay method by utilization of proper salts solution and a metabolic preincubation period at 25°C. It has also been observed that, unlike the rat, pretreatment of rainbow trout with various PCBs decrease the mutagen assay response to AFB.

REFERENCES

AHOKAS, J. T., O. PELKONEN, and N. T. KARKI: Acta Pharmacol. Toxicol. 38, 440 (1976).

ALVARES, A. P., D. R. BICKERS, and A. KAPPAS: Proc. Nat. Acad. Sci. USA 70, 1321 (1973).

AMES, B.N., W. E. DURSTON, E. YAMASAKI, and F. D. LEE: Proc. Nat. Acad. Sci. USA 70, 2281 (1973).

AMES, B.N., J. McCANN, and E. YAMASAKI: Mut. Res. 31, 347 (1975).

BAKER, J. R., A STRUEMPLER, and S. CHAYKIN: Biochem. Biophys. Acta 71, 58 (1963). BELLWARD, G. D., R. DAWSON, and M. OTTEN: Res. Commun. Chem. P

BELLWARD, G. D., R. DAWSON, and M. OTTEN: Res. Commun. Chem. Pathol. Pharmacol. 12, 669 (1975).

BEND, J. R., R. J. POHL, and J. R. FOUTS: Bull. Mt. Desert Island Biol. Lab. 12, 9 (1973).

BICKERS, D. R., L. C. HARBER, A. KAPPAS, and A. P. ALVARES: Res. Commun. Chem. Pathol. Pharmacol. 3, 505 (1972).

BRODIE, B. B., and R. P. MAICKEL: Proc. Intern. Pharm. Meeting 6, 299 (1962).

BUHLER, D. R., and M. E. RASMUSSON: Comp. Biochem. Physiol. 25, 233 (1968).

CHAN, T. M., J. W. GILLETT, and L. C. TERRIERE: Comp. Biochem. Physiol. 20, 731 (1967).

CREAVEN, P. J., W. H. DAVIES, and R. T. WILLIAMS: Life Sci. 6, 105 (1967).

CREAVEN, P. J., D. V. PARKE, and R. T. WILLIAMS: Biochem. J. 96, 879 (1965).

DEWAIDE, J. H.: Metabolism of xenobiotics, comparative and kinetic studies as a basis for environmental pharmacology. Nijnegen Drukkerij Leijn 1971.

ECOBICHON, D. J., and A. M. COMEAU: Chem.-Biol. Interactions 9, 341 (1974).

HILL, D. W., E. HEJTMANCIK, and B. J. CAMP: Bull. Environ. Contam. Toxicol. 16, 495 (1976).

IVERSON, F., D. C. VILLENEUVE, D. L. GRANT, and G. V. HATINA: Bull. Environ. Contam. Toxicol. 13, 456 (1975).

KONAT, G., and J. CLAUSEN: Environ. Physiol. 3, 139 (1973).

LIDMAN, U., L. FORLIN, O. MOLANDER, and G. AXELSON: Acta Pharmacol. Toxicol. 39, 262 (1976).

LITTERST, C. L., T. M. FARBER, A. M. BAKER, and E. J. VAN LOON: Toxicol. Appl. Pharmacol. 23, 112 (1972).

LITTERST, C. L., and E. J. VAN LOON: Bull. Environ. Contam. Toxicol. 11, 206 (1974).

LOTLIKAR, P. D., E. C. MILLER, J. A. MILLER, and J. E. HALVER: Proc. Soc. Exptl. Biol. Med. 124, 160 (1967).

LOWRY, O. H., N. J. ROSENBROUGH, A. L. FARR, and R. J. RANDALL: J. Biol. Chem. 193, 265 (1951).

LU, A. Y. H., D. RYAN, D. M. JERINA, J. W. DALY, and W. LEVIN: J. Biol. Chem. 250, 8283 (1975).

ORBERG, J.: Acta Pharmacol. Toxicol. 39, 145 (1976).

ORBERG, J., and C. LUNDBERG: Environ. Physiol. Biochem. 4, 116 (1974).

POTTER, J. L., AND R. D. O'BRIEN: Sci. N.Y. 144, 55 (1964).

SCHOENHARD, G. L., D. J. LEE, S. E. HOWELL, N. E. PAWLOWSKI, L. M. LIBBEY, and R. O. SINNHUBER: Cancer Res. 36, 2040 (1976).

SCHMOLDT, A., H. F. BENTHE, and R. FRUHLING: Arch. Toxicol. 32, 69 (1974).

SINNHUBER, R. O., J. H. WALES, J. L. AYRES, R. H. ENGEBRECHT, and D. L. AMEND: J. Nat. Cancer Inst. 41, 711 (1968).

TURNER, J. C., and R. S. GREEN: Bull. Environ. Contam. Toxicol. 12, 669 (1974).

WONG, J. J., and D. P. HSIEH: Proc. Nat. Acad. Sci. USA 73, 2241 (1976).

Fig. 1. Effect of length of metabolic incubation at 25°C upon mutagenic response of S. typhimurium TA 1538.

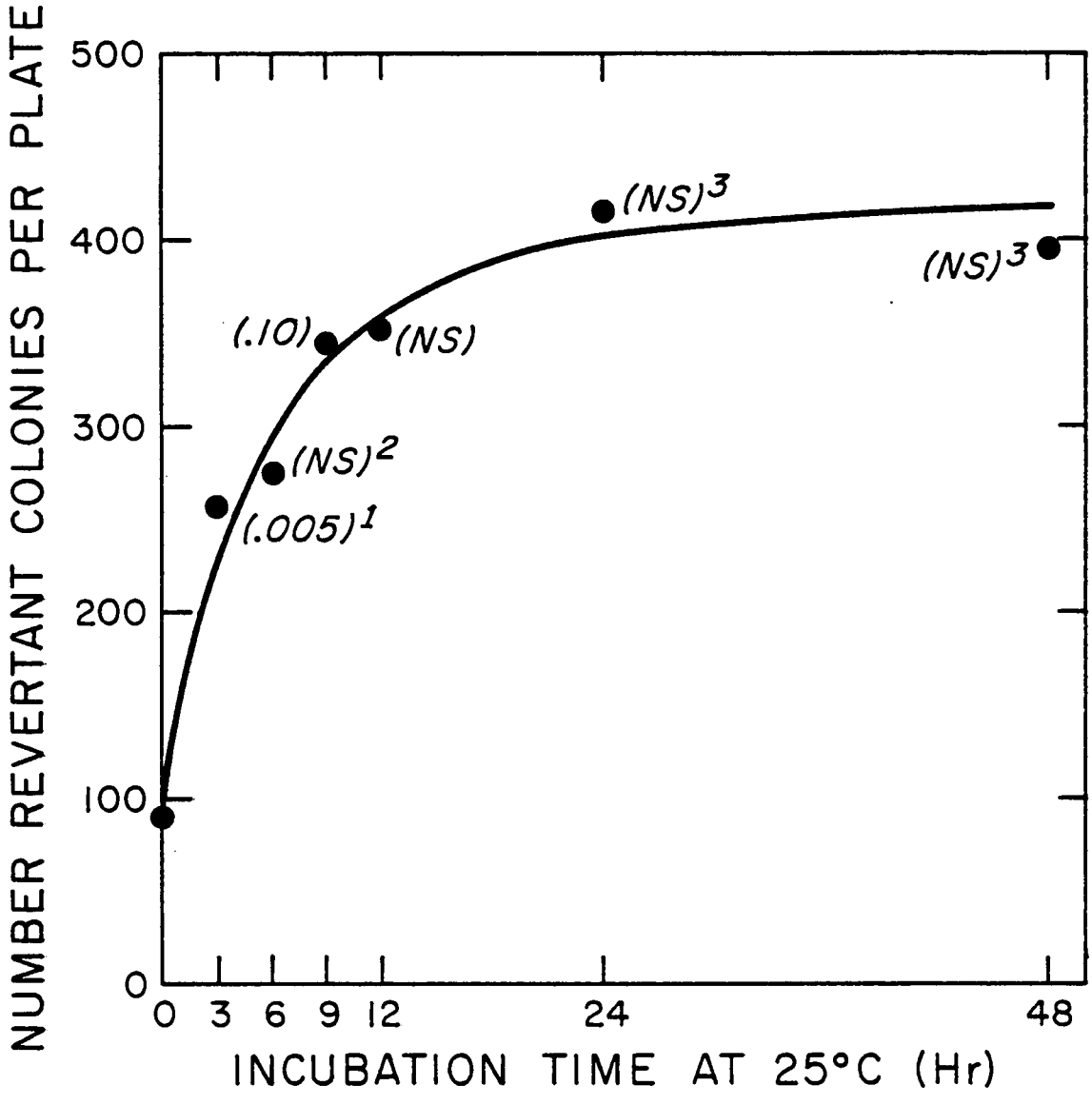

NUMBER REVERTANT COLONIES PER PLATE
500
400
300
200
100
0
0 3 6 9 12 24 48
INCUBATION TIME AT 25°C (Hr)
(.10)
(NS)
(NS)3
(NS)2
(.005)1
(NS)3

Figure 1.

Fig. 2. Effect of PCB pretreatments of fish 5 days prior to mutagen assay with S. typhimurium TA 1538.

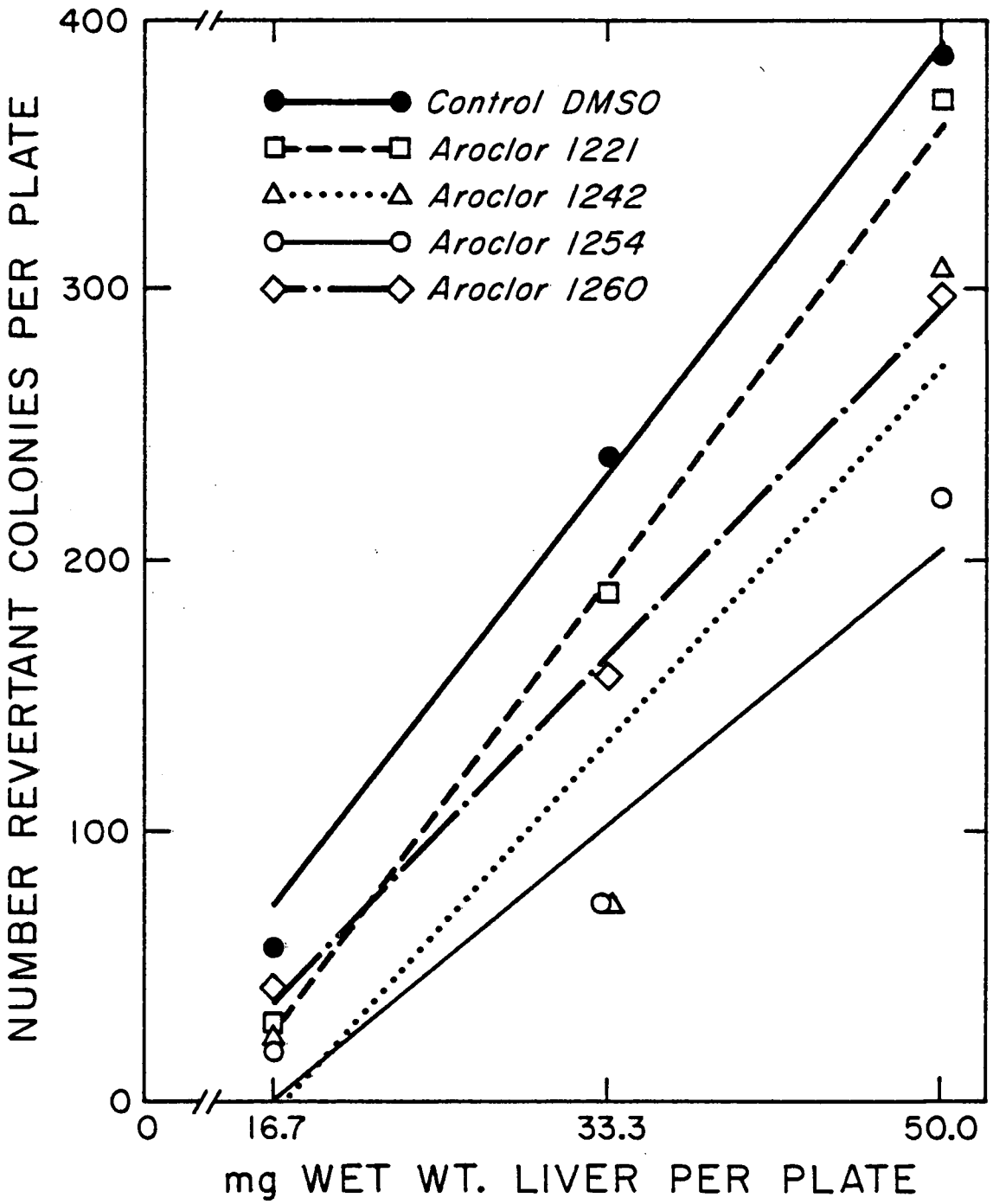

400
300
200
100
0
0
16.7
33.3
50.0
NUMBER REVERTANT COLONIES PER PLATE
mg WET WT. LIVER PER PLATE
Control DMSO
Aroclor 1221
Aroclor 1242
Aroclor 1254
Aroclor 1260

Figure 2.

Dietary Casein Levels and Aflatoxin B_1 Metabolism in Rainbow Trout (*Salmo gairdneri*)

W. T. Stott

R. O. Sinnhuber

Department of Food Science and Technology

Oregon State University

Corvallis, Oregon 97331

ABSTRACT

The effect of dietary protein (casein) levels (32%, 42%, 52%, 62%) upon the activities of several hepatic activating and detoxifying enzyme systems of aflatoxin B_1 (AFB_1) in rainbow trout (Salmo gairdneri) was investigated. It was observed that in fish fed increased casein, cytochrome P-450 content, in vitro epoxidase and glutathione epoxide transferase activity dropped, up to 21%, 32% and 25% respectively. Fish fed the higher levels of protein converted a greater amount of AFB_1 to aflatoxicol and showed a 62% increase in AFB_1 mutagen assay responses. In vitro cytochrome c reductase and epoxide hydrase activities were not observed to be affected by the diets. The enzyme data provided a possible explanation for the mutagen assay results.

The influence of nutrition on chemical carcinogenesis has been reviewed by Clayson (1975). Dietary protein reportedly affects the toxicity and carcinogenicity of a variety of chemical carcinogens in animals, presumably by altering the activities of enzymes involved in their activation and/or detoxification (McLean and Magee, 1970, Newberne and Rogers, 1970; Swann and McLean 1971).

Aflatoxin B_1 (AFB) is a mold metabolite which has been observed to be acutely toxic and carcinogenic to a wide variety of animals (Ciegler, 1975; Wogan, 1973) and has been implicated in human primary hepatic carcinoma (Peers and Linsell, 1973; Shank et al., 1972). Diets deficient in protein have been reported to increase the susceptibility of mammals to acute AFB toxicity and the induction of cancer (Madhavan et al., 1965; Madhavan and Gopalan, 1965; McLean and Magee, 1970; Rogers and Newberne, 1971; Sisk and Carlton, 1972; Todd et al., 1968). Increased dietary proteins have increased the carcinogenic activity of AFB fed to rats (Madhavan and Gopalan, 1968) and trout (Lee et al., 1978). Supportive of this later finding has been the reported direct relationship between dietary protein content and AFB-DNA adduct formation in vivo in rats (Allen-Hoffmann and Campbell, 1977; Preston et al., 1976).

AFB has been shown to require metabolic activation to its ultimate carcinogenic species (Garner et al., 1971; 1972; Garner and Wright, 1973; Gurtoo and Bejba, 1974; Gurtoo and Dave, 1973) which is believed to be a 2,3-epoxide form of AFB (OAFB) (Garner, 1973; Garner et al., 1972; Schoenhard et al., 1976; Schoental, 1970; Swenson et al., 1973; 1974; 1975; 1977). This epoxidation of AFB has been associated with aldrin epoxidase (AE) activity in trout (Schoenhard, 1974). As with other epoxide carcinogens, OAFB may be a substrate for epoxide metabolizing

enzyme systems such as epoxide hydrase (EH) (EC4.2.1.63) and glutathione-S-epoxide transferase (GTr) (EC4.4.1.7) found in mammals and fish (Boyland and Williams, 1965; James et al., 1976a; 1976b; Oesch et al., 1971). AFB also undergoes a variety of other reactions, generally to less toxic metabolites depending on the species of animal involved (Patterson, 1973; Roebuck and Wogan, 1977). The primary AFB metabolite in rainbow trout has been shown to be a reduced form of AFB, aflatoxicol (AFL) (Schoenhard et al., 1976).

The present study was undertaken to determine the influence of several levels of dietary casein upon the activities of trout hepatic enzyme systems which may be involved in the in vitro activation and detoxification of AFB. In addition, the effect of dietary casein upon the conversion of AFB to an active mutagen by trout hepatic enzymes is described.

EXPERIMENTAL PROCEDURES

Diets. Semipurified diets, as shown in Table I, were prepared as described by Sinnhuber, et al., (1977). Casein at levels of 32%, 42%, 52% and 62% (dry weight) provided the protein source along with an 8% gelatin binder. All diets were isocaloric and each test group received the same amount of feed each day.

Fish. One year old Mt. Shasta strain rainbow trout (Salmo gairdneri) weighing an average of 140g were used for the study and housed in circular tanks supplied with 15.2l of well water/min. at 11-12°C. Fish were fed twice daily for seven months prior to sacrifice at which time they weighed an average of 800g.

Hepatic Enzyme Preparations. Fish were killed by a cranial blow between 6 and 8 A.M. Livers were immediately removed, weighed and

perfused with ice cold saline (0.9%) and then homogenized with four volumes of 0.25M sucrose solution in a Potter-Elvejhem apparatus by four complete passes of the pestle. Homogenates were centrifuged for 15 minutes at 12,000xg, the supernatant recovered (PMF) and recentrifuged at 105,000xg for one hour. Fatty layers were discarded and the 105,000xg supernatant recovered. Microsomal pellets were resuspended in an original weight of either 0.25M sucrose or 0.073M potassium phosphate buffer (pH 7.6). All steps were carried out at 1°C and isolated fractions were frozen in dry ice and stored at -45°C until used (<2 days). Three livers were pooled from each diet and sampling was repeated in one week. For bacterial mutagen assays and AFL production assays six livers/diet were asceptically removed from fish and sterile solutions utilized. Protein content of each fraction was determined by the method of Lowry et al., (1951).

Cytochrome and Enzyme Assays. Liver fractions were thawed at R.T. and kept on ice until used. AE activity was determined using a slightly modified method of Chan et al., (1967). Basically, this involved the incubation at 25°C for 30 min. of 4mg microsomal protein in 0.25M sucrose, an NADPH generating system (Salhab and Hsieh, 1975) with 2U glucose-6-phosphate dehydrogenase, 260μmoles Tris buffer (pH8.2) and 25nm aldrin (gift of Shell Oil Company) in 10μl methyl cellusolve in a total volume of 6ml. Mixtures were extracted three times with 4ml n-hexane and extracts passed through a glass column containing 20g alumina (10% deactivated), concentrated and their dieldrin content determined by electron capture GC (Chan et al., 1967).

EH and GTr activities were assayed after the method of James et al., (1976a), which in the former case was a modification of Oesch

et al., (1971) method. Incubation mixtures contained 1μmole styrene-^{14}C-oxide (specific activity 47.7μCi/mmole) in 2μl tetrahydrofuran. Styrene-^{14}C-oxide was prepared from ^{14}C-styrene (purchased from California Bionuclear Corp.) after the method of Oesch et al. (1971). Incubations were carried out at 25°C for 15 minutes.

Cytochrome c reductase activity and cytochrome P-450 were determined by methods outlined by Mazel (1971) and the assay of AFB conversion to AFL was carried out by the method of Loveland et al. (1978).

Microbial Mutagen Assay. The conversion of AFB to an active mutagen for Salmonella typhimurium TA 98, a plasmid containing frameshift mutant, was accomplished using the Stott and Sinnhuber (1978) modification of the Ames mutagen assay (Ames et al., 1975). Several levels of AFB standard (0.05μg to 0.15μg AFB/assay) were utilized to ensure a linear relationship between mutagenic response and AFB concentration.

RESULTS AND DISCUSSION

As noted in Table II, the protein content of the hepatic microsomal fractions showed a significant increase with diets 32% through 52% casein. These observations are consistent with the findings of other workers with rats (Campbell and Hayes, 1976; Czygan et al., 1974; Kato et al., 1968; Pawar and Makhija, 1975; Sachan, 1975). The decrease noted in the protein content of the liver fractions of fish fed the 62% casein diet has also been observed by Sachan (1975) in rats fed a high protein diet.

Unlike hepatic proteins, cytochrome P-450 content in isolated trout microsomes was observed to decrease 21% with increasing casein in the diet (Table II). The large standard deviations noted were a result of

the averaging of two samplings data, but in each case the trend was identical. These findings are at variance with reported increases in cytochrome P-450 content of rats fed increasing levels of casein (Campbell and Hayes, 1976; Czygan et al., 1974; Kato et al., 1968; Marshall and McLean, 1969; Sachan, 1975). Only slight differences were observed in cytochrome c reductase activities between the diets, with the highest activity of 20nm cytochrome c reduced/min/mg protein occurring in fish fed the 32% casein diet (Table III).

The decrease in cytochrome P-450 content correlated with a significant lowering of trout AE activity observed in hepatic microsomes recovered from fish fed high levels of casein, versus those from fish fed low casein diets. As shown in Table III, up to a 32% decrease in the production of the epoxide dieldrin was noted. Similar results have been observed in 10 month old rainbow trout with a nearly identical maximum decrease (unpublished data). Since AFB activation has been shown to involve a cytochrome P-450 dependent enzyme system (Garner et al., 1972; Garner and Wright, 1973; Gurtoo and Dave, 1973) and trout aldrin epoxidase related directly to AFB activation (Schoenhard, 1974), these findings may reflect a decrease in the AFB activation potential of trout fed high casein diets. Similar effects of dietary protein on pesticide activation have been reviewed by Campbell and Hayes (1976). However, total cytochrome P-450 measurements may be deceiving as a poor correlation between total cytochrome P-450 contents and conversion of AFB to a mutagen has been noted (Ueno et al., 1978).

There was a significant $p<.05$) decrease in GTr activity in fish fed increasing levels of casein, while EH activity remained unchanged except

for a decrease in activity observed in fish fed a 42% casein diet (Figure 1). Trout hepatic GTr and EH activities were observed to be linear for up to 15 minutes incubation at 25°C with the use of up to 3mg cytosol and microsomal protein. The decrease in GTr activity from 171.8nm conjugate to 126.8nm conjugate/mg protein in fish fed 32% and 52% casein diets respectively may have represented a loss in the ability of fish fed the higher levels of protein to detoxify OAFB. Indeed, GTr has been implicated in the detoxification of activated AFB. Mgbodile et al., (1975) have reported that AFB induced hepatic necrosis was increased over the controls in rats depleted of glutathione (GSH) by treatment with diethyl maleate, while pretreatment with cysteine, a precursor of GSH, prevented necrosis. Similarly, Allen-Hoffmann and Campbell (1977) have noted that in rats fed a high casein diet and in those treated with diethyl maleate, hepatic GSH levels were depleted and in vivo DNA binding of administered AFB increased. Since GSH is required for GTr activity, decreased hepatic GSH may result in lowered GTr activity with the resultant drop in OAFB detoxification via this mechanism. The significance of free GSH interaction with activated AFB has been questioned as its addition to incubation mixtures failed to decrease toxicity of AFB to mutant bacteria (Garner and Wright, 1973; Schoenhard, 1974). The apparent lack of dietary casein effects upon in vitro hepatic EH activity may not be significant in terms of potential detoxification of OAFB. The addition of an EH inhibitor, cyclohexene oxide, to in vitro incubation mixtures reportedly failed to increase AFB binding to DNA (Gurtoo and Bejba, 1974) and the mortality of Salmonella typhimurium exposed to AFB (Garner and Wright, 1973).

Unlike EH and GTr activities, the production of AFL from AFB by trout hepatic enzymes was observed to increase in fish fed the higher casein diets (Table III). AFL has been shown to be carcinogenic (Schoenhard, 1974) and mutagenic (Wong and Hsieh, 1976) and may be oxidized back to AFB by trout hepatic enzymes (Loveland et al., 1977). It has been suggested that AFL represents a reserve pool of toxin *in vivo* (Patterson, 1973; Patterson and Roberts, 1972) and that its production is indicative of a sensitive animal species (Edwards et al., 1975; Salhab and Edwards, 1977). If AFL does indeed represent a reserve pool of AFB in trout, then its increased production by fish fed high levels of casein may predispose these particular animals to the induction of cancer.

Ames mutagen assay responses of *S. typhimurium* TA 98 to AFB followed a pattern similar to that of AFL production (Table IV). A 62% increase in the mutagenic response of these bacteria to AFB was noted in assays using PMF from trout fed high casein diets as compared to those using PMF from trout fed low casein diets. It has been suggested that the mutagenic character of compounds in this assay system is related to their carcinogenic activity (Ames et al., 1975). Evidence of this in rainbow trout treated with polychlorinated biphenyls and AFB has been observed (Hendricks et al., 1977; Stott and Sinnhuber, 1978). Thus, these findings indicate that trout fed higher casein diets produce a greater amount of an active form of AFB which may result in an enhanced carcinogenic activity of AFB.

The observed mutagenic responses to AFB reflect the overall effects of activation and detoxification systems on the *in vitro* metabolism

of AFB. It appeared that the effect of high casein levels fed to trout was that a greater amount of activated AFB was produced and/or that less could be detoxified by these fish than by those fed lower casein diets. If lowered cytochrome P-450 content and AE activities in fish fed the high casein diets represented a decrease in the activation of AFB, then these effects were overcome by the observed decreases in GTr activity and/or increases in AFB conversion to AFL relative to those of trout fed lower casein diets. Alternately, the results could be explained by dietary effects upon some unknown OAFB metabolizing enzyme system in trout, upon free GSH levels in hepatic tissue, or that the levels of the cytochrome P-450 involved in AFB activation were not reflected by the observed total cytochrome P-450 levels.

LITERATURE CITED

Allen-Hoffmann, B.L., and Campbell, T.C., Fed Proc. 36, 1116 (1977).

Ames, B.N., McCann, J., and Yamasaki, E., Mut. Res. 31, 347 (1975).

Boyland, E., and Williams, K., Biochem. J. 94, 190 (1965).

Campbell, T.C. and Hayes, J.R., Fed. Proc. 35, 2470 (1976).

Chan, T.M., Gillett, J.W. and Terriere, L.C., Comp. Biochem. Physiol. 20, 731 (1967).

Ciegler, A., Lloydia. 38, 21 (1975).

Clayson, D.B., Cancer Res. 35, 3292 (1975).

Czygan, P., Greim, H., Garro, A., Schaffner, F., and Popper, H., Cancer Res. 34, 119 (1974).

Edwards, G.S., Rintel, T.D., and Parker, C.M., Proc. Amer. Assoc. Cancer res. 133 (1975).

Garner, R.C., FEBS Lett. 36, 261 (1973).

Garner, R.C., Miller, E.C., and Miller, J.C., Cancer Res. 32, 2058 (1972).

Garner, R.C., Miller, E.C., Miller, J.A., Garner, J.V., and Hanson, R.S., Biochem. Biophy. Res. Com. 45, 774 (1971).

Garner, R.C., and Wright, C.M., Br. J. Cancer 28, 544 (1973).

Gurtoo, H.L., and Bejba, N., Biochem. Biophy. Res. Com. 61, 685 (1974).

Gurtoo, H.L., and Dave, C., Res. Com. Chem. Pathol. Pharmacol. 5, 635 (1973).

Hendricks, J.D., Putnam, T.P., Bills, D.D., and Sinnhuber, R.O., J. Natl. Cancer Inst. 59, 1545 (1977).

James, M.O., Fouts, J.R., and Bend, J.R., Biochem. Pharmacol. 25, 187 (1976a).

James, M.O., Van Stee, E.W., and Bend, J.R., Fed. Proc. 35, 244 (1976b).

Kato, R., Oshima, T., and Tomizawa, S., Jap. J. Pharmacol. 18, 356 (1968).

Lee, D.J., Sinnhuber, R.O., Wales, J.H., and Putnam, G.B., J. Nat. Cancer Inst. 60, 317 (1978).

Loveland, P.M., Sinnhuber, R.O., Berggren, K.E., Libbey, L.M., Nixon J.E., and Pawlowski, N.E., Res. Com. Chem. Pathol. Pharmacol. 16, 167 (1977).

Loveland, P.M., Nixon, J.E., Pawlowski, N.E., Eisele, T.A., Libbey, L.M., and Sinnhuber, R.O., Submitted, J. Nat. Cancer Inst. (1978).

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem. 193, 265 (1951).

Madhavan, T.V., and Gopalan, C., Arch. Pathol. 80, 123 (1965).

Madhavan, T.V., and Gopalan, C., Arch. Pathol. 85, 133 (1968).

Madhavan, T.V., Suryanarayana Rao, K., and Tulpule, P.G., Indian J. Med. Res. 53, 984 (1965).

Marshall, W.J., and McLean, A.E.M., Biochem. J. 115, 27p (1969).

Mazel, P., "General Principles and Procedures for Drug Metabolism In vitro." Williams and Wilkins Co., Baltimore, Md., 1971.

McLean, A.E.M., and Magee, P.N., Br. J. Exp. Pathol. 51, 587 (1970).

Mgbodile, M.U.K., Holscher, M., and Neal, R.A., Toxicol. Appl. Pharmacol. 34, 128 (1975).

Newberne, P.M., and Rogers, A.E., In Purchase, I.F.H., Ed., "Mycotoxins In Human Health", MacMillan Press Ltd., London and Basingstoke, 1970, pp 195-208.

Oesch, F., Jerina, D.M., and Daly, J., Biochem. Biophy. Acta. 227, 685 (1971).

Patterson, D.S.P., Food Cosmet. Toxicol. 11, 287 (1973).

Patterson, D.S.P., and Roberts, B.A., Food Cosmet. Toxicol. 10, 501 (1972).

Pawar, S.S., and Makhija, S.J., Bull. Environ. Contam. Toxicol. 14, 197 (1975).

Peers, F.G., and Linsell, C.A., Brit. J. Cancer 27, 473 (1973).

Preston, R.S., Hayes, J.R., and Campbell, T.C., Life Sci. 19, 1191 (1976).

Roebuck, B.D., and Wogan, G.N., Cancer Res. 37, 1649 (1977).

Rogers, A.E., and Newberne, P.M., Toxicol. Appl. Pharmacol. 20, 113 (1971).

Sachan, D.S., J. Nutrition 105, 1631 (1975).

Salhab, A.S., and Edwards, G.S., Cancer Res. 37, 1016 (1977).

Salhab, A.S., and Hsieh, D.P.H., Res. Com. Chem. Pathol. Pharmacol. 10, 419 (1975).

Schoenhard, G.L., Ph. D. Thesis, Oregon State University, 1974.

Schoenhard, G.L., Lee, D.J., Howell, S.E., Pawlowski, N.E., Libbey, L.M., and Sinnhuber, R.O., Cancer Res. 36, 2040 (1976).

Schoental, R., Nature 227, 401 (1970).

Shank, R.C., Bhamarapravati, N., Gordon, J.E., and Wogan, G.N., Food Cosmet. Toxicol. 10, 171 (1972).

Sinnhuber, R.O., Hendricks, J.D., and Wales, J.H., Ann. N.Y. Acad. Sci. 298, 389 (1977).

Sisk, D.B., and Carlton, W.W., Am. J. Vet. Res. 33, 107 (1972).

Stott, W.T., and Sinnhuber, R.O., Bull. Environ. Contam. Toxicol. 19, 35 (1978).

Swann, P.F., and McLean, A.E.M., Biochem. J. 124, 283 (1971).

Swenson, D.H., Lin, J.K., Miller, E.C., and Miller, J.A., Cancer Res. 37, 172 (1977).

Swenson, D.H., Miller, J.A., and Miller, E.C. Biochem. Biophy. Res. Com. 53, 1260 (1973).

Swenson, D.H., Miller, E.C., and Miller, J.A., Biochem. Biohy. Res. Com. 60, 1036 (1974).

Swenson, D.H., Miller, J.A., and Miller, E.C., Cancer Res. 35, 3811 (1975).

Todd, G.C., Shalkop, W.T., Dooley, K.L., and Wiseman, H.G., Am. J. Vet. Res. 29, 1855 (1968).

Ueno, Y., Kubota, K., Ito, T., and Nakamura, Y., Cancer Res. 38, 536 (1978).

Wogan, G.N., "Methods In Cancer Research," Vol. VII, 1973, p 309.

Wong, J.J., and Hsieh, D.P.H., Proc. Nat. Acad. Sci. USA 73, 2241 (1976).

Table I. Diets

Ingredient	Percent			
Casein	30.8[a]	42	52	62
Gelatin	7.7	8	8	8
Dextrin	20	15	10	5
α-Cellulose	17	12	7	2
Mineral mix[b]	4	4	4	4
Carboxymethyl cellulose[c]	1	1	1	1
Choline-chloride (70%)	1	1	1	1
Vitamin mix[d]	2	2	2	2
Fish oil (salmon or herring)	15	15	15	15

[a]Plus 0.6% arginine, 0.2% L-cysteine, 0.4% methionine and 0.3% DL-tryptophane.

[b]Calcium carbonate ($CaCO_3$, 2.100%), calcium phosphate ($CaHPO_4 \cdot 2H_2O$, 73.500%), potassium phosphate (K_2HPO_4, 8.100%), potassium sulfate (K_2SO_4, 6.800%), sodium chloride (NaCl, 3.060%), sodium phosphate ($Na_2HPO_4 \cdot 6H_2O$, 2.140%), magnesium oxide (MgO, 2.500%), ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$, 0.558%), manganese carbonate ($MnCO_3$, 0.418%), cupric carbonate [$2CuCO_3Cu(OH)_2$, 0.034%], zinc carbonate ($ZnCO_3$, 0.081%), potassium iodide (KI, 0.001%), sodium fluoride (NaF, 0.002%) cobalt chloride ($CoCl_2$, 0.020%), and citric acid ($C_6H_8O_7 \cdot H_2O$, 0.686%)

[c]Hercules Powder Company, San Francisco, Calif.

[d]Thiamine hydrochloride (0.3200%), riboflavin (0.7200%), niacinamide (2.5600%), biotin (0.0080%), D calcium pantothenate (1.4400%), pyridoxine hydrochloride (0.2400%), folic acid (0.0960%), menadione (0.0800%), vitamin B_{12} (cobalamine, 3000 μg/g, 0.2667%), i-inositol (meso, 12.5000%), ascorbic acid (6.0000%), p-aminobenzoic acid (2.0000%), vitamin D_2 (500,000 USP/g, 0.0400%), vitamin A (250,000 units/g, 0.5000%), dl-a-tocopherol (250 IU/g, 13.2%), and α-cellulose (60.0293%).

Table II. Liver weights, protein and cytochrome P-450 content of hepatic fractions in rainbow trout fed 32, 42, 52 and 62% casein diets[a]

Diet (% casein)	Liver Weight[b] (%Body weight)	Liver Fraction	Protein Content[c] (mg/g wet wt. liver)	Cytochrome P-450[d] (nmoles/ mg protein)
32	0.89	Microsomes	33.6(±4.1)[e]	0.160(±0.013)[1]
		Cytosol	55.6(±2.6)	
42	1.03	Microsomes	35.6(±4.2)	0.155(±0.035)[1,2]
		Cytosol	54.4(±6.0)	
52	1.00	Microsomes	39.6(±6.0)[1]	0.146(±0.020)[1,2]
		Cytosol	60.8(±3.5)	
62	1.07	Microsomes	31.6(±4.2)	0.127(±0.016)[2]
		Cytosol	56.8(±7.4)	

[a]Values with different superscript numbers are significantly different ($P<.05$, Students t Test).

[b]Average of 6 livers.

[c]$n=10$.

[d]$n=4$.

[e]standard deviation.

Table III. Effect dietary casein levels on the *in vitro* activities of trout hepatic cytochrome c reductase, aldrin epoxidase, and the conversion of aflatoxin B_1 to aflatoxicol.

Diet (% Casein)	Cytochrome c Reductase[b] (nmoles cytochrome c reduced/ min/mg protein)	Aldrin Epoxidase[c] (pmoles dieldrin/ mg protein)	Aflatoxin B_1 Conversion to Aflatoxicol[c] (nmoles aflatoxicol/nmoles total aflatoxin/mg protein)
32	20.0 (± 1.9)[d]	99.6^{1} (±13.2)	5.77^{1} (±1.63)
42	17.9 (± 2.7)	$72.4^{1,2}$ (±13.9)	$7.99^{1,2}$ (±2.05)
52	18.3 (± 5.7)	69.8^{2} (± 5.3)	9.66^{2} (±1.57)
62	18.9 (± 1.9)	67.2^{2} (± 9.2)	$9.02^{1,2}$ (±1.25)

[a]Values with different superscript numbers are significantly different ($P<.05$, Students t Test).

[b]n=4

[c]n=3

[d]± standard deviation

Table IV. Effect of casein intake upon conversion of aflatoxin B_1 by trout PMF to a mutagen for Salmonella typhimurium TA 98.[a]

Diet (% casein)	Response[b] (Number revertants/ µg aflatoxin B_1/mg protein)
32	502(±47)[c]
42	482(±80)
52	673(±96)[1]
62	814(±17)[d,2]

[a]Values with different superscript numbers are significantly different ($P<.05$, Students t Test).

[b]n=8

[c]± standard deviation

[d]n=4

Figure 1. Epoxide hydrase and glutathione-S-transferase of trout hepatic microsomes and cytosol respectively.

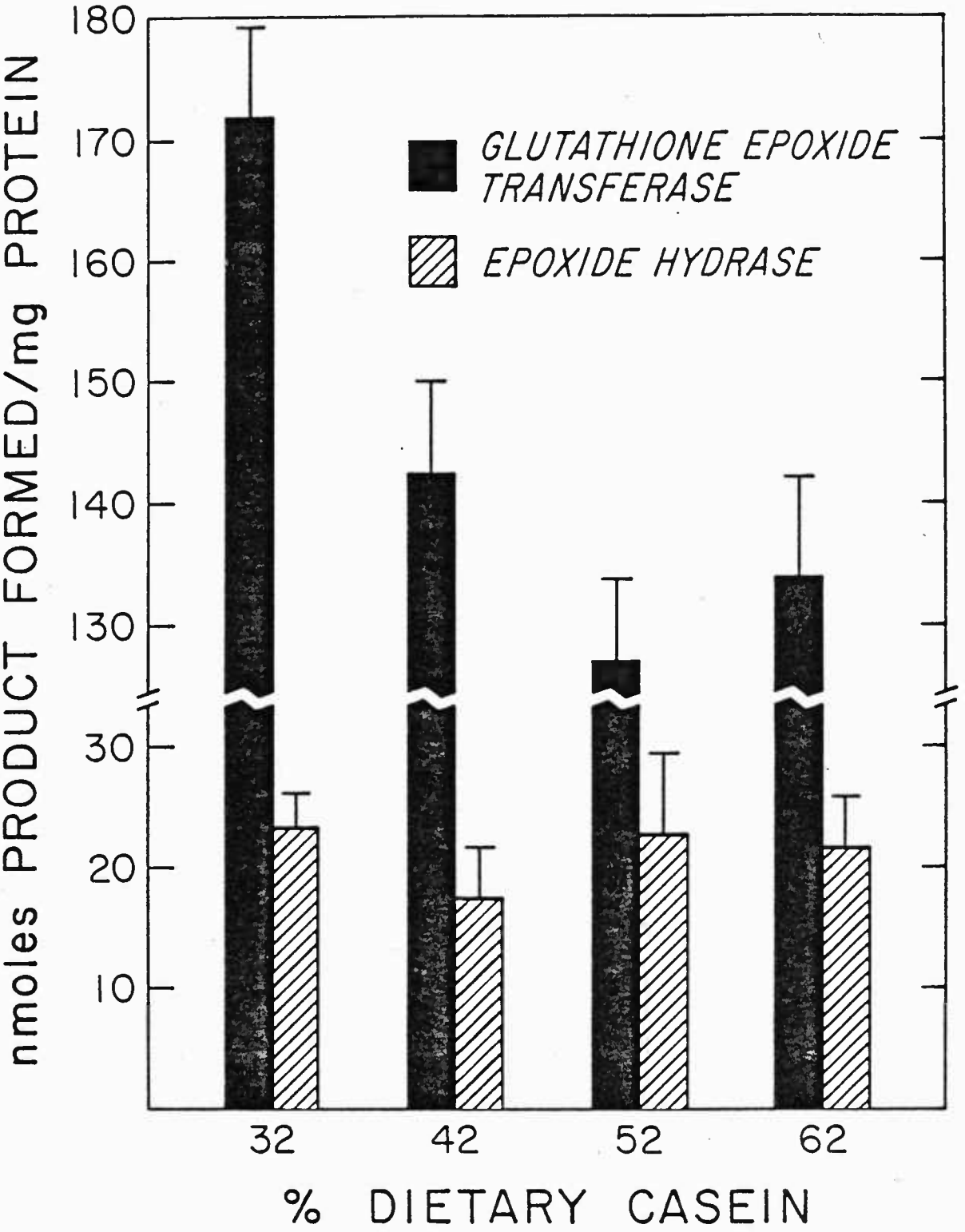

180
170
160
150
140
130
30
20
10
nmoles PRODUCT FORMED/mg PROTEIN
GLUTATHIONE EPOXIDE TRANSFERASE
EPOXIDE HYDRASE
32
42
52
62
% DIETARY CASEIN

Figure 1.

Dietary Protein Levels and Aflatoxin B_1 Metabolism in Rainbow Trout (*Salmo gairdneri*)

W. T. Stott

R. O. Sinnhuber

Department of Food Science and Technology

Oregon State University

Corvallis, Oregon 97321

In an attempt to understand dietary protein effects upon aflatoxin B_1 induced liver cancer in rainbow trout, the activities of several suspected aflatoxin B_1 metabolizing enzyme systems were studied relative to protein intake. Fish fed diets containing 32%, 52% and 62% fish protein concentrate (FPC) were examined for hepatic cytochrome P-450 content and *in vitro* cytochrome c reductase, glutathione-S-epoxide transferase (GTr), epoxide hydrase (EH) and aldrin epoxidase (AE) activity. In addition, aflatoxin B_1 conversion to aflatoxicol (AFL) was examined. A direct correlation was observed between increased FPC intake and cytochrome P-450 content and AFL production, with increases of 14% and 41% respectively. With increased FPC intake, decreases in EH (15%), GTr (20%), cytochrome c reductase (13%) and AE (50%) activities were noted. These findings are discussed in relation to AFB feeding trials reported earlier.

Dietary proteins, both type and level, have been reported to influence the acute toxicity and carcinogenicity of a variety of chemical carcinogens as reviewed by Clayson (1975). It has been suggested that these effects are related to the activities of enzymes involved in carcinogen activation and/or detoxification (McLean and Magee, 1970; Newberne and Rogers, 1970; Swann and McLean, 1971). One compound which has been extensively studied is aflatoxin B_1 (AFB), a mold metabolite which has been shown to be carcinogenic to a wide variety of animals (Wogan, 1973) and has been implicated in the formation of liver cancer in humans (Shank et al., 1972; Peers and Linsell, 1973). Numerous authors have reported an increased sensitivity of animals fed low protein or lipotrope deficient diets to the acute toxicity of AFB relative to adequate diets. (Madhavan and Gopalan, 1965; Madhavan et al., 1965; Rogers and Newberne, 1970; 1971; Sisk and Carlton, 1972; Todd et al., 1968). A low casein diet has also been found to protect against AFB carcinogenesis in rats (Madhavan and Gopalan, 1968).

Our laboratory has reported that rainbow trout fed increasing levels of protein as fish protein concentrate (FPC), showed a higher incidence of AFB induced hepatocellular carcinoma (Lee et al., 1978). In this study, fish fed 32% FPC were found to have a significantly lower incidence of hepatocellular carcinoma as compared to those fed 50% FPC diets. Supportive of Lee's et al. (1978) findings has been the reportedly direct relationship between dietary protein content and AFB induced mortality of Bacillus subtilis GSY 1057 (Schoenhard, 1974), and the formation of AFB-DNA adducts in vivo in rats (Allen-Hoffman and Campbell, 1977; Preston et al., 1976).

AFB has been shown to require metabolic activation to the ultimate carcinogen (Garner et al., 1971; 1972; Garner and Wright, 1973; Gurtoo and Bejba, 1974; Gurtoo and Dave, 1973) which is believed to be 2,3-epoxide AFB (OAFB) (Garner 1973; Garner et al., 1972; Schoental, 1970; Swenson et al., 1973; 1974; 1975; 1977). Similar to other epoxides, OAFB may be a substrate for low specificity epoxide metabolizing enzymes such as epoxide hydrase (EH) (EC4.2.1.63) and glutathione-S-epoxide transferase (GTr) (EC4.4.1.7) found in mammals and fish (Boyland and Williams, 1965; James et al., 1976a; 1976b; Oesch et al., 1971). The conversion of AFB to a variety of metabolites has also been reported, depending upon the species of animal involved (Patterson, 1973; Roebuck and Wogan, 1977). The primary AFB metabolite in rainbow trout has been shown to be a reduced form of AFB , aflatoxicol (AFL) (Loveland et al., 1978; Schoenhard et al., 1976).

The present study was undertaken to determine the influence of several levels of FPC upon the in vitro activities of rainbow trout hepatic enzyme systems which may be involved in the metabolism of AFB and provide a possible explanation for the variation in cancer incidence noted by Lee et al., (1978).

METHODS

Ten month old Mount Shasta strain rainbow trout weighing an average of 100g were utilized in the study. Fish were housed in circular tanks supplied with 15.2 l of 12°C well water/min. and fed semipurified, isocaloric diets (Sinnhuber et al., 1977) containing 32%, 52% or 62% FPC (Table 1) twice daily for 2.5 months prior to sacrifice. Each dietary group received the same amount of feed per day and were fasted 48 hours prior to sampling.

Fish were killed by a cranial blow between 6 and 8 AM and their livers immediately removed, weighed and perfused with ice cold saline (0.9%). Perfused livers were then homogenized with four volumes of 0.25 M sucrose solution in a Potter-Elvejhem apparatus by four complete passes of the pestle. Homogenates were centrifuged for 15 minutes at 12,000xg and the supernatant recentrifuged at 105,000xg for one hour. Upon removal of fatty layers, the supernatant was recovered and microsomal pellets resuspended in an original weight of either 0.25M sucrose or 0.073M potassium phosphate buffer (pH 7.6). Protein content was determined after the method of Lowry et al., (1951). All steps were carried out at 1°C and isolated fractions were frozen in dry ice and held at -45°C until used (<2 days). Six livers from immature males and females were pooled from each diet.

In all assays liver fractions were thawed at room temperature and held on ice until used. Trout hepatic AE activity was determined by a slightly modified method of Burns (1976). This involved an incubation mixture of 0.8-1.0mg of microsomal protein in 0.25M sucrose, an NADPH generating system (0.5mM NADPH, 1.0mM $NADP^+$, 10mM glucose-6-phosphate, 2U G-6-P dehydrogenase and 5mM $MgCl_2 \cdot 6H_2O$), 0.1mmoles Tris-HCL buffer (pH 7.4) and 30nmoles aldrin (gift of Shell Oil Co.) in 10µl ethanol, in a final volume of 2ml. Mixtures were incubated for 15 minutes at 25°C with shaking. Extraction and analysis of dieldrin were as described by Burns (1976).

GTr and EH activities were assayed according to James et al. (1976a) which in the latter case was a modification of the Oesch et al. (1971) method. Incubation mixtures were 1mM in styrene-^{14}C-oxide (Specific activity 47.7µCi/mmole) in 2 µl tetrahydrofuran. Styrene-^{14}C-

oxide was prepared from ^{14}C-styrene (purchased from California Bionuclear Corp.) after the method of Oesch et al. (1971) and incubations were carried out at 25°C for 15 minutes. Cytochrome c reductase activity and cytochrome P-450 analysis were determined after methods outlined by Mazel (1971).

Conversion of AFB to AFL was assayed utilizing an incubation mixture consisting of 105,000xg supernatant equivalent to 0.25g wet weight liver, the NADPH generating system as described above with 5U G-6-P dehydrogenase, 324 µmoles potassium phosphate buffer (pH 7.6) and 5µg AFB (purchased from Cal Biochemical Corp.) in 0.1ml DMSO, in a final volume of 4.5ml. Mixtures were incubated at 25°C for 60 minutes prior to extraction three times with 5ml of acetone: chloroform (6:4). Extracts were passed through a Na_2SO_4 filled funnel, concentrated under N_2 stream and quantitated as described by Loveland et al., (1978).

RESULTS

As indicated in Table 2, rainbow trout fed 52% and 62% FPC diets were observed to have larger livers, when calculated on a % body weight basis, than those fed a 32% FPC diet. Likewise, hepatic microsomal and cytosol protein contents were noted to be directly related to protein intake.

Cytochrome P-450 has been shown to be involved in the conversion of AFB to its active form (Garner et al., 1972; Garner and Wright, 1973; Gurtoo and Dave, 1975) and significantly higher levels of cytochrome P-450 occurred in trout fed the 52% and 62% FPC diets than in those fed the 32% protein diet (Table 2). A maximum increase of 30 pmoles cytochrome P-450/mg protein was noted in fish fed a 52% FPC diet while

a slight decrease occurred in fish consuming the highest FPC diet. A 13% decrease in the activity of another component of the mixed function oxidase system, cytochrome c reductase, was observed in fish fed increasing levels of FPC.

The production of the epoxide dieldrin from aldrin in trout has been related to AFB activation (Schoenhard, 1974), therefore the assay of hepatic microsomal AE in fish was undertaken as a potential measure of AFB activation. As shown in Table 3, a two fold decrease in *in vitro* AE activity was noted in fish fed 52% versus those fed 32% FPC. A smaller decrease in activity (40%) was recorded in trout fed a 62% FPC diet.

To better understand the potential of the trout to detoxify OAFB, the activities of two potential OAFB detoxifying enzyme systems, hepatic GTr and EH, were also assayed. The activities of both enzymes were observed to decrease with increasing FPC content of the fishes diet (Table 3). A 20% drop in *in vitro* GTr activity and a 15% decrease in *in vitro* EH activity was observed in fish fed the highest versus the lowest FPC diets. EH and GTr activities were observed to be linear with incubation time for up to 15 min. at 25°C with the use of up to 3mg microsomal or cytosol protein respectively.

It has been suggested that the production of AFL from AFB represents a "reserve pool" of activatable toxin (Patterson, 1973; Patterson and Roberts, 1972), thus *in vitro* conversion of AFB to AFL by trout hepatic enzymes was assayed. As shown in Table 3, the production of AFL from AFB was significantly increased in fish fed the higher FPC diets relative to those fed the low FPC diet. A maximum increase of 8.1pm AFL/total nmoles aflatoxin/mg protein was recorded.

DISCUSSION

The data presented here provides evidence that the level of dietary protein can influence the in vitro activities of rainbow trout hepatic xenobiotic metabolizing enzyme systems. Dietary FPC effects trout cytochrome P-450 content, in vitro cytochrome c reductase and AE activities, the in vitro activities of several epoxide metabolizing enzyme systems and the in vitro conversion of AFB to AFL.

The recorded increases in fish hepatic cytochrome P-450 content with increasing FPC intake parallel findings by other workers with rats using casein diets. (Campbell and Hayes, 1976a;Czygan et al., 1974; Kato et al., 1968; Pawar and Makhija, 1975; Sachan, 1975). These observations indicate that an increased activation of AFB can occur in fish fed the higher FPC diets versus those fed a 32% FPC diet. Similar findings with MFO activated pesticides have been reviewed by Campbell and Hayes (1976a). Contrary to these findings, the decrease in cytochrome c reductase and AE activity in trout fed increasing levels of FPC seem to indicate a decrease in the potential of these fish to form OAFB. It appears that total cytochrome P-450 measurement may not correlate well with AFB activation, as Ueno et al. (1978) have reported in studies involving the activation of AFB to a bacterial mutagen. Burns et al. (1976) have noted that total cytochrome P-450 levels of induced estuarine fish did not correlate well with aldrin epoxidase activity. If trout aldrin epoxidase activity is linked to AFB activation as observed by Schoenhard (1974), then the above findings indicated a decrease in the ability of fish fed increasing levels of FPC to activate AFB regardless of the total cytochrome P-450 increases observed.

Once AFB has been metabolized to its 2,3-epoxide, it may serve as a substrate for trout microsomal EH and/or cytosol GTr. The decreases observed in the *in vitro* activities of these two enzyme systems in fish fed higher FPC diets may reflect a general decrease in enzymatic OAFB detoxification *in vivo*. Indirect evidence for GTr involvement in the detoxification of activated AFB exists. Mgbodile et al. (1975) have reported that AFB induced hepatic necrosis increased, relative to controls, in rats depleted of glutathione (GSH) by pretreatment with diethyl maleate (DEM) while pretreatment with cysteine, a precursor of GSH, prevented necrosis. Similarly, Allen-Hoffman and Campbell (1977) have noted that in rats fed a high casein diet and in those treated with DEM, hepatic GSH levels were depleted and *in vivo* binding of DNA by AFB increased. Contrary to these reports, Garner and Wright (1973) could observe no protective effect of GSH in the *in vitro* conversion of AFB to a mutagen. As GSH is a substrate of GTr, decreases in *in vivo* GSH content may result in lowered GTr activity and a decrease in OAFB conjugation. The role of EH in the detoxification of OAFB has been questioned. The addition of an EH inhibitor, cyclohexene oxide, to incubation mixtures failed to increase AFB binding to DNA (Gurtoo and Bejba, 1974) and the mutagenic response of *Salmonella typhimurium* to AFB (Garner and Wright, 1973). In the present study lowered hepatic *in vitro* EH activity in high FPC diets correlated with the higher AFB induced tumor incidence observed in trout (Lee et al., 1978). The observed decreasing EH and GTr activities in fish fed increasing levels of FPC have also been observed to occur in two yr. old spawning rainbow trout (unpublished data).

The noted increase in the *in vitro* conversion of AFB to AFL by fish fed increasing levels of FPC may have resulted in the increased production of a reserve pool of activated toxin *in vivo* (Patterson and Roberts, 1972; Patterson, 1973). AFL has been observed to be carcinogenic (Schoenhard, 1974) and mutagenic (Wong and Hsieh, 1976), conceivably by its observed reconversion back to AFB (Patterson and Roberts, 1972; Loveland et al., 1977; Salhab and Edwards, 1977) and subsequent activation. It has been suggested that AFL production is indicative of AFB sensitive animal species, such as rainbow trout (Edwards et al., 1975; Salhab and Edwards, 1977). This possibility however, has been questioned by Campbell and Hayes (1976b).

In summary, although some question remained about the potential for the activation of AFB in rainbow trout relative to dietary protein content, a correlation between decreasing activities of the epoxide detoxifying enzymes GTr and EH, the production of an activatable pool of toxin (AFL), and increasing FPC intake was noted. These results correspond to observed increases in the conversion of AFB to a mutagen (Schoenhard, 1974; unpublished data) and the increased cancer incidence in trout fed high FPC diets (Lee et al., 1978). If the influence exerted by dietary FPC intake upon the induction of cancer by AFB in the trout is mediated through the activities of AFB metabolizing enzymes, then the data may provide some insight regarding Lee's et al. (1978) feeding trial results.

REFERENCES

Allen-Hoffmann, B.L., and Campbell, T.C.: The relationship between hepatic glutathione levels and the formation of aflatoxin B_1-DNA adducts as influenced by dietary protein intake. Fed. Proc. 36:1116, 1977.

Boyland, E., and Williams, K.: An enzyme catalyzing the conjugation of epoxides with glutathione. Biochem. J. 94:190-197, 1965.

Burns, K.A.: Microsomal mixed function oxidases in an estuarine fish, Fundulus heteroclitus, and their induction as a result of environmental contamination. Comp. Biochem. Physiol. 53:443-446, 1976.

Campbell, T.C., and Hayes, J.R.: The effect of quantity and quality of dietary protein and drug metabolism. Fed. Proc. 35:2470-2474, 1976a.

Campbell, T.C., and Hayes, J.R.: The role of aflatoxin metabolism in its toxic lesion. Toxicol. Appl. Pharmacol. 35:199-222, 1976b.

Clayson, D.B.: Nutrition and experimental carcinogenesis: A review. Cancer Res. 35:3292-3300, 1975.

Czygan, P., Greim, H., Garro, A., Schaffner, F., and Popper, H.: The effect of dietary protein deficiency on the ability of isolated microsomes to alter the mutagenicity of a primary and a secondary carcinogen. Cancer Res. 34:119-123, 1974.

Edwards, G.S., Rintel, T.D., and Parker, C.M.: Aflatoxicol as a possible predictor for species sensitivity to aflatoxin B_1 (AFB_1). Proc. Amer. Assoc. Cancer Res. 1975, p. 133.

Garner, R.C.: Chemical evidence for the formation of a reactive aflatoxin B_1 metabolite, by hamster liver microsomes. FEBS Lett. 36:261-264, 1973.

Garner, R.C., Miller, E.C., Miller, J.A., Garner, J.V., and Hanson, R.S.: Formation of a factor lethal for S. typhimurium TA 1530 and TA 1531 on incubation of aflatoxin B_1 with rat liver microsomes. Biochem. Biophy. Res. Com. 45:774-780, 1971.

Garner, R.C., Miller, E.C., and Miller, J.A.: Liver microsomal metabolism of aflatoxin B_1 to a reactive derivative toxic to Salmonella typhimurium TA 1530. Cancer Res. 32:2058-2066, 1972.

Garner, R.C., and Wright, C.M.: Induction of mutations in DNA-repair deficient bacteria by a liver microsomal metabolite of aflatoxin B_1. Br. J. Cancer. 28:544-554, 1973.

Gurtoo, H.L., and Bejba, N.: Hepatic microsomal mixed function oxygenase: Enzyme multiplicity for the metabolism of carcinogens to DNA-binding metabolites. Biochem. Biophy. Res. Com. 61:685-692, 1974.

Gurtoo, H.L., and Dave, C.: Interaction of aflatoxin B_1 metabolite with rat liver RNA. Res. Com. Chem. Pathol. Pharmacol. 5:635-645, 1973.

James, M.O., Fouts, J.R., and Bend, J.R.: Hepatic and extrahepatic metabolism, in vitro, of an epoxide (8-^{14}C-styrene oxide) in the rabbit. Biochem. Pharmacol. 25:187-193, 1976a.

James, M.O., Van Stee, E.W., and Bend, J.R.: In Vitro metabolism of epoxides in marine species. Fed. Proc. 35:244, 1976b.

Kato, R., Oshima, T., and Tomizawa, S.: Toxicity and metabolism of drugs in relation to dietary protein. Jap. J. Pharmacol. 18: 356-366, 1968.

Lee, D.J., Sinnhuber, R.O., Wales, J.H., and Putnam, G.B.: Effect of dietary protein on the response of rainbow trout (Salmo gairdneri) to aflatoxin B_1. J. Nat. Cancer Inst. 60:317-320, 1978.

Loveland, P.M., Sinnhuber, R.O., Berggren, K.E., Libbey, L.M., Nixon, J.E., and Pawlowski, N.E.: Formation of aflatoxin B_1 from aflatoxicol by rainbow trout (Salmo gairdneri) liver in vitro. Res. Com. Chem. Pathol. Pharmacol. 16:167-170, 1977.

Loveland, P.M., Nixon, J.E., Pawlowski, N.E., Eisele, T.A., Libbey, L.M. and Sinnhuber, R.O.: Aflatoxin B_1 and aflatoxicol metabolism in rainbow trout (Salmo gairdneri) and the effects of dietary cyclopropene. Submitted, J. Nat. Cancer Inst., 1978.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275, 1951.

Madhavan, T.V., and Gopalan, C.: Effect of dietary protein on aflatoxin liver injury in weanling rats. Arch. Pathol. 80:123-126, 1965.

Madhavan, T.V., and Gopalan, C.: The effect of dietary protein on carcinogenesis of aflatoxin. Arch. Pathol. 85:133, 1968.

Madhavan, T.V., Suryanarayana Rao, K., and Tulpule, P.G.: Effect of dietary protein level on susceptibility of monkeys to aflatoxin liver injury. Indian J. Med. Res. 53:984-989, 1965.

Mazel, P.: General principles and procedures for drug metabolism in vitro. In LaDu, B.N., Mandel, H.G., and Way, E.L. (Editors): Fundamentals of Drug Metabolism and Drug Disposition. Baltimore, Williams and Wilkins Co., 1971, pp. 573-577.

McLean, A.E.M., and Magee, P.M.: Increased renal carcinogenesis by dimethylnitrosamine in protein deficient rats. Br. J. Exp. Pathol. 51:587-590, 1970.

Mgbodile, M.U.K., Holscher, M., and Neal, R.A.: A possible protective role for reduced glutathione in aflatoxin B_1 toxicity: Effect of pretreatment of rats with phenobarbital and 3-methylcholanthrene on aflatoxin toxicity. Toxicol. Appl. Pharmacol. 34:128-142, 1975.

Newberne, P.M., and Rogers, A.E.: Aflatoxin carcinogenesis in rats: Dietary effects. In Purchase, I.F.H. (Editor): Mycotoxins in Human Health. London and Basingstoke, MacMillan Press, Ltd., 1970, pp. 195-208.

Oesch, F., Jerina, D.M., and Daly, J.: A radiometric assay for hepatic epoxide hydrase activity with [7-H^3]styrene oxide. Biochem. Biophy. Acta. 227:685-691, 1971.

Patterson, D.S.P.: Metabolism as a factor in determining the toxic action of the aflatoxins in different animal species. Fd. Cosmet. Toxicol. 11:287-294, 1973.

Patterson, D.S.P., and Roberts, B.A.: Aflatoxin metabolism in duck-liver homogenates: The relative importance of reversible cyclo-pentenone reduction and hemiacetal formation. Fd. Cosmet. Toxicol. 10:501-512, 1972.

Pawar, S.S., and Makhija, S.J.: Effect of insecticide intoxication on the hepatic microsomal electron transport reactions during dietary protein variations in young rats. Bull. Environ. Contam. Toxicol. 14:197-204, 1975.

Peers, F.G., and Linsell, C.A.: Dietary aflatoxins and liver cancer--a population based study in Kenya. Brit. J. Cancer. 27:473-484, 1973.

Preston, R.S., Hayes, J.R., and Campbell, T.C.: The effect of protein deficiency on the *in vivo* binding of aflatoxin B_1 to rat liver macromolecules. Life Sci. 19:1191-1198, 1976.

Roebuck, B.D., and Wogan, G.N.: Species comparison of *in vitro* metabolism of aflatoxin B_1. Cancer Res. 37:1649-1656, 1977.

Rogers, A.E., and Newberne, P.M.: Nutrition and aflatoxin carcino-genesis. Nature. 229:62-63, 1970.

Rogers, A.E., and Newberne, P.M.: Diet and aflatoxin B_1 toxicity in rats. Toxicol. Appl. Pharmacol. 20:113-121, 1971.

Sachan, D.S.: Effects of low and high protein diets on the induction of microsomal drug-metabolizing enzymes in rat liver. J. Nutrition. 105:1631-1639, 1975.

Salhab, A.S., and Edwards, G.S.: Comparative *in vitro* metabolism of aflatoxicol by liver preparations from animals and humans. Cancer Res. 37:1016-1021, 1977.

Schoenhard, G.L., Ph.D. Thesis, Oregon State University, 1974.

Schoenhard, G.L., Lee, D.J., Howell, S.E., Pawlowski, N.E., Libbey, L.M., and Sinnhuber, R.O.: Aflatoxin B_1 metabolism to aflatoxicol and derivatives lethal to *Bacillus subtilis* GSY 1057 by rainbow trout (*Salmo gairdneri*) liver. Cancer Res. 36:2040-2045, 1976.

Schoental, R.: Hepatotoxic activity of retrorsine, senkirkine and hydroxysenkirkine in newborn rats, and the role of epoxides in carcinogenesis by pyrrolizidine alkaloids and aflatoxins. Nature. 227:401-402, 1970.

Shank, R.C., Bhamarapravati, N., Gordon, J.E., and Wogan, G.N.: Dietary aflatoxins and human liver cancer. IV. Incidence of primary liver cancer in two municipal populations of Thailand. Fd. Cosmet. Toxicol. 10:171-179, 1972.

Sinnhuber, R.O., Hendricks, J.D., Wales, J.H., and Putnam, G.B.: Neoplasms in rainbow trout, a sensitive animal model for environmental carcinogens. Ann. N.Y. Acad. Sci. 298:389-408, 1977.

Sisk, D.B., and Carlton, W.W.: Effect of dietary protein concentration on response of miniature swine to aflatoxins. Am. J. Vet. Res. 33:107-114, 1972.

Swann, P.F. and McLean, A.E.M.: Cellular injury and carcinogenesis. The effect of a protein-free high-carbohydrate diet on the metabolism of dimethylnitrosamine in the rat. Biochem. J. 124: 283-288, 1971.

Swenson, D.H., Lin, J.K., Miller, E.C., and Miller, J.A.: Aflatoxin B_1-2,3-oxide as a probable intermediate in the covalent binding of aflatoxin B_1 and B_2 to rat liver DNA and ribosomal RNA *in vivo*. Cancer Res. 37:172-181, 1977.

Swenson, D.H., Miller, J.A., and Miller, E.C.: 2,3-Dihydro-2,3-dihydroxy-aflatoxin B_1: An acid hydrolysis product of an RNA-aflatoxin B_1 adduct formed by hamster and rat liver microsomes *in vitro*. Biochem. Biophy. Res. Com. 53:1260-1267, 1973.

Swenson, D.H., Miller, E.C., and Miller, J.A.,: Aflatoxin B_1-2-3-oxide: Evidence for its formation in rat liver *in vivo* and by human liver microsomes *in vitro*. Biochem. Biophy. Res. Com. 60:1036-1043, 1974.

Swenson, D.H., Miller, J.A., and Miller, E.C.: The reactivity and carcinogenicity of aflatoxin B_1-2,3-dichloride, a model for the putative 2,3-oxide metabolite of aflatoxin B_1. Cancer Res. 35: 3811-3823, 1975.

Todd, G.C., Shalkop, W.T., Dooley, K.L., and Wiseman, H.G.: Effect of ration modifications of aflatoxicosis in the rat. Am. J. Vet. Res. 29:1855-1861, 1968.

Ueno, Y., Kubota, K., Ito, T., and Nakamura, Y.: Mutagenicity of carcinogenic mycotoxins in Salmonella typhimurium. Cancer Res. 38:536-542, 1978.

Wogan, G.N.: Aflatoxin carcinogenesis. In Methods In Cancer Research Vol. VII:309-345, 1973.

Wong, J.J., and Hsieh, D.P.H.: Mutagenicity of aflatoxins related to their metabolism and carcinogenic potential. Proc. Natl. Acad. Sci. USA. 73:2241-2244, 1976.

Table 1. Diets

Ingredient	Percent		
FPC	32	52	62
Gelatin	8	8	8
Dextrin	20	10	5
α-Cellulose	18.5	8.1	3.4
Mineral Mix[a]	2.5	2.9	2.6
Carboxymethyl cellulose[b]	1	1	1
Choline-chloride (70%)	1	1	1
Vitamin mix[c]	2	2	2
Fish oil (salmon or herring	15	15	15

[a]Calcium carbonate ($CaCO_3$, 2.100%), calcium phosphate ($CaHPO_4 \cdot 2H_2O$, 73.500%), potassium phosphate (K_2HPO_4, 8.100%), potassium sulfate (K_2SO_4, 6.800%), sodium chloride (NaCl, 3.060%), sodium phosphate ($Na_2HPO_4 \cdot 6H_2O$, 2.140%), magnesium oxide (MgO, 2.500%), ferric citrate ($FeC_6H_5O_7 \cdot 3H_2O$, 0.558%), manganese carbonate ($MnCO_3$, 0.418%), cupric carbonate [$2CuCO_3Cu(OH)_2$, 0.034%], zinc carbonate ($ZnCO_3$, 0.081%), potassium iodide (KI, 0.001%), sodium fluoride (NaF, 0.002%), cobalt chloride ($CoCl_2$, 0.020%), and citric acid ($C_6H_8O_7 \cdot H_2O$, 0.686%).

[b]Hercules Powder Company, San Francisco, California.

[c]Thiamine hydrochloride (0.3200%), riboflavin (0.7200%), niacinamide (2.5600%), biotin (0.0080%), D calcium pantothenate (1.4400%), pyridoxine hydrochloride (0.2400%), folic acid (0.0960%), menadione (0.0800%), vitamin B_{12} (cobalamine, 3000 μg/g, 0.2667%), i-inositol (meso, 12.5000%), ascorbic acid (6.0000%), p-aminobenzoic acid (2.0000%), vitamin D_2 (5000,000 USP/g, 0.0400%), vitamin A (250,000 units/g, 0.5000%), dl-a-tocopherol (250 IU/g, 13.2%)k- and α-cellulose (60.0293%).

Table 2. Effects of FPC consumption in trout upon liver weights, hepatic microsomal and cytosol protein and microsomal cytochrome P-450 content[a].

Diet (% FPC)	Avg. Liver Weight[b] (% Body Wt.)	Microsomes[c] (mg protein/g liver)	Cytosol[d] (mg protein/g liver)	Cytochrome[e] P-450 (nmoles P-450/mg protein)
32	0.96	32.8 (± 1.1)[f]	49.6 (± 0.0)	0.183 (±0.000)
52	1.33[1]	39.5 (± 1.2)[1]	59.0 (± 1.2)[1]	0.213 (±0.000)[1]
62	1.39[1]	37.9 (± 2.3)[1]	64.8 (± 2.1)[2]	0.204 (±0.002)[2]

[a] Values with different superscript numbers are significantly different ($P<.05$, Student t Test).

[b] n=12

[c] n=7

[d] n=3

[e] n=2

[f] ± Standard deviation.

Table 3. Effect of FPC consumption in trout upon the *in vitro* activities of hepatic cytochrome c reductase, glutathione-S-epoxide transferase, epoxide hydrase and AFB conversion to AFL[a].

Diet (% FPC)	Cytochrome c Reductase[b] (nmoles cytochrome c reduced/min/mg protein)	Aldrin Epoxidase[b] (nmoles dieldrin/ mg protein)	Glutathione-S-Epoxide Transferase[c] (nmoles styrene-^{14}C-GSH conjugate/ mg protein)	Epoxide Hydrase[d] (nmoles styrene-^{14}C-diol/mg protein)	Aflatoxin B_1 Conversion to Aflatoxicol[b] (pmoles AFL/nmole total aflatoxin/mg protein)
32	34.8 (± 1.1)[e]	0.32 (± 0.05)	92.6 (± 3.9)	24.4 (± 1.9)	19.7 (± 1.3)
52	32.8 (± 2.8)	0.16 (± 0.04)[1]	76.9 (± 2.2)[1]	23.5 (± 1.0)	26.5 (± 0.9)[1]
62	30.2 (± 1.9)[1]	0.19 (± 0.04)[1]	73.9 (± 2.9)[2]	20.5 (± 1.8)[1]	27.8 (± 1.0)[1]

[a] Values with different superscript numbers are significantly different ($P<.05$; Student t Test).

[b] n=4

[c] n=10

[d] n=5

[e] ± Standard deviation.