Xenobiotic Metabolism: Nutritional Effects

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Xenobiotic Metabolism: Nutritional Effects

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that, in order to save time, the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable, because symposia may embrace both types of presentation.

PREFACE

OUR ENVIRONMENT exposes us daily to a wide variety of xenobiotics: in our food, in the air we breathe, or as a result of industrial exposure and toxic wastes. However, despite this exposure, most of us are living long, healthy lives. Certainly individual variation could account for some of the variability in resistance to disease, but other factors are undoubtedly involved. According to a growing body of evidence, diet may be extremely important in increasing resistance to chronic disease. One is tempted to speculate, or hope, that improved dietary habits could improve individual resistance to chemically induced chronic disease.

In this volume, we have focused on how xenobiotics are metabolized in higher animals and how this metabolism is mediated by the nutritional status of the target animal. Emphasis has been placed on the toxic, mutagenic, carcinogenic and potentially mutagenic or carcinogenic compounds. The topic is a natural follow-up and expansion of "Xenobiotics in Foods and Feeds" (ACS Symposium Series No. 234, Finley and Schwass, Eds.) in which sources of xenobiotics were identified and discussed at length. The authors in this volume discuss how and why these xenobiotics are toxic and how nutritional intervention can mediate some of the toxicities.

Current nutritional awareness in the western world is probably unparalleled in the history of man. One need only look in health food stores and supermarkets to see the results of this awareness. Many food companies now place major emphasis on the natural, pure, low-calorie, additive-free, health- and fitness-oriented ingredients in their products. Nutritionists have established requirements for normal individuals, and additional data are being acquired rapidly on diets designed especially for individuals who experience high stress due either to illness or life style.

This volume presents a state-of-the-art assessment of how diet can intervene and aid in the prevention of chronic disease. The editors hope this effort will stimulate further research in this important area of food biochemistry and nutrition.

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September 21, 1984

The editors would like to dedicate this volume to the memory of Morris N. Joselow whose untimely death in 1983 was a loss to all, particularly those in the area of preventative medicine. Dr. Joselow was Professor of Preventative Medicine and Community Health at the College of Medicine and Dentistry in Newark, New Jersey. One of his last published works, "Systematic Toxicity Testing for Xenobiotics in Foods," appeared in "Xenobiotics in Foods and Feeds" (ACS Symposium Series No. 234). In addition to his other duties, he was organizer and principal lecturer in the American Chemical Society Toxicology Short Course. Dr. Joselow's research interests included environmental sciences, industrial hygiene and safety, toxicology, trace metals, and biochemical monitoring. During his career he published more than 100 papers. Dr. Joselow will be missed as a friend, a coworker, and a scientist.

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Overview: The Influence of Nutrition on Xenobiotic Metabolism

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In the course of living in the modern world it is inevitable that man and animals will be exposed to compounds in the environment which are not essential for life or even "normal" from the standpoint of the evolution of the species. The term "xenobiotic" (from the Greek "xenos" and "bios", meaning stranger to life) was coined by Mason, et al (1) to describe the myriad of compounds including carbohydrates, lipids, proteins, alkaloids, natural and synthetic drugs, flavorings, pigments, preservatives, polycyclic hydrocarbons, flavonoids, terpenoids, etc., which may enter the organism as non-essential or non-functional materials. The assumption inherent in the use of the term is that one is speaking relative to an organism of reference. For example, the drug quinine is a xenobiotic relative to man but not to the South American tree, Chinchona officinalis, in which quinine is a major constituent of the bark. Xenobiotics which enter the biosphere of the organism are not necessarily toxic. In fact, based on the Mason definition, non-essential amino acids could be referred to as xenobiotics. For the purpose of this symposium, however, xenobiotic does not include non-essential nutrients which occur in the diet, but will be restricted to environmental compounds which are acutely toxic, potentially toxic requiring activation, or which exhibit long term effects, such as mutagens, carcinogens or teratogens. In general, the discussions in this symposium are relative to man and/or animals. It is important to remember that xenobiotics range from the inocuous (i.e. vanillin) through the chronically toxic (i.e. ethanol) to the acutely toxic (i.e. curare). Some xenobiotics, although not toxic in and of themselves, are metabolically converted to toxic substances. The metabolic conversion of xenobiotics to toxic substances can be dramatically influenced by the nutritional status of the organism.

Smoking, drugs, industrial chemicals and foods, represent the major sources of exposure to xenobiotics for modern man. Because diet furnishes the most variable and continuous array of xenobiotic exposure, the emphasis of this symposium is the

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influence of food-borne xenobiotics and how diet can mediate the metabolism of these compounds. Food borne xenobiotics can be endogenous to the foodstuff (flavinoids in tea), can result from processing and storage (lipid oxidation or non-enzymatic browning) or can be the result of deliberate addition (the antioxidant, butylated hydroxyanisole).

Exposure to xenobiotics is inevitable and although the degree of exposure can be controlled, it is impossible to prevent exposure altogether. Fortunately, the healthy, well-nourished individual or animal under normal circumstances can resist long-term effects of many of these compounds by metabolizing them and excreting them as metabolites or conjugates of metabolites.

A major source of exposure to xenobiotics is tobacco smoke. Cigarette smoke consists of a large variety of compounds including oxidants, free radicals, benzo-(a)-pyrene and carbon monoxide. Long term exposure to cigarette smoke entails exposure to both acutely toxic materials and chronically toxic materials. We are all exposed to smoke in various degrees both from tobacco and from the environment through the burning of fuels and from cooking. Exposure to industrial sources of xenobiotics can come via the air or through exposure and absorption by the skin. Although major industrial exposure receives much publicity when it occurs. This type of event usually accounts for exposure to relatively few compounds over a prolonged period of time. Fortunately, chemical companies have made great strides in reducing the incidence of such exposure, although it is likely that some exposure will continue to occur either through accidents or lack of knowledge. Industrial pollution in the form of toxic waste should be reduced significantly in the next several years as efforts continue to correct this problem. Exposure from toxic waste dumps is likely to continue but at lower levels. Pharmaceutical drugs can be considered xenobiotics and in the western world an individual might expect to be exposed significantly to two-to-three dozen compounds in a lifetime under normal circumstances. Frequently these exposures are over relatively short periods of time.

Food represents a large and continuous exposure to a vast array of xenobiotics. Xenobiotics from food can range from the inocuous to the extremely dangerous. Plants frequently produce a variety of xenobiotics which subsequently are consumed by man or animals. In addition, during storage and processing of foods, (including home cooking), xenobiotics can be produced. A recent symposium (Finley and Schwass) (2) reviewed many of these sources of xenobiotics in the diet.

Historically, the impact of diet on health has been a concern of man. In recent years we have seen greater emphasis on how nutrition relates to health and the prevention of chronic afflictions such as coronary heart disease, hypertension, obesity, and cancer. Consequently, several health organizations have proposed guidelines to promote better health and reduce risk

of chronic disease. The National Research Council (3) published an extensive study and guidelines regarding nutritional means to reduce the risk of cancer. In this volume, Palmer (4) discusses these guidelines in terms of current evidence. One of the problem areas discussed is dietary fat and its relationship to cancer as well as previously established relationships to coronary heart disease and obesity.

Throughout this symposium one observes a common thread in many of the papers: the many problems associated with lipids in the diet. It is important to note however, that the problems may not be due simply to fat but more likely to oxidized lipid products. Pryor (5) reviews the mechanisms of lipid oxidation and discusses the chemical basis for a relationship between lipid oxidation and chronic disease. It would seem from this and other evidence (6) (7) (8) that peroxidizing lipids could be a major dietary concern in the development of certain types of cancers. If one considers the early stages of tumor development to be initiation followed by promotion, one could speculate a number of roles for peroxidizing lipids. Initiation for the purpose of this discussion will be considered the initial chemical change in the DNA of a cell which has the potential to lead to the development of a tumor. In the promotion stage, the damaged cell begins to multiply as a result of chemical insult and tumor development proceeds. The initial damage or initiation can be caused by a variety of compounds, many of which are used as model compounds for the study of carcinogenesis. One might speculate that these compounds (i.e., DMBA, benzo-(a)-pyrene) could act as initiators and the oxidizing lipids as promoters. Indeed, much of the evidence in the present symposium will support this speculation. If one then considers the protective role against tumor development of retenoids, BHT, ascorbic acid and tocopherol, all of which are antioxidants, the argument is strengthened (Chow, (5) (8); King, (9); Newberne, (10) C. Reddy, (11); Anderson, (12); Seifter et al (13); Baird, (14). The issue is certainly more complicated than simple control of oxidation, but the evidence does suggest an important correlation. In addition, peroxidizing lipids represent a group of xenobiotics which can be controlled in the diet. Oxidized lipids are important in the development of certain flavors in foods such as frying and in the flavor profiles of certain meats. Through reduced consumption of meats and fried foods, as suggested in the NRC Guidelines (3), exposure to oxidized lipids can be reduced. Assuming that the oxidation products or intermediates act as promoters or modify the ability of the cell to metabolize the initiating carcinogen in a harmless way, reducing the level of these xenobiotics in the diet could significantly reduce the risk of tumor development.

The impact of xenobiotics on health can occur in a number of ways. As we have noted, the xenobiotic can be a carcinogen, be metabolized to a carcinogen, inhibit the detoxification of

another xenobiotic, act as a tumor promoter or act as an acute toxin. Clearly we are and will continue to be exposed to compounds which are both acutely toxic and those which exhibit a long term effect such as carcinogens. Acute toxins are usually detectable and can be controlled in diet. On the other hand, chronic xenobiotics can occur at extremely low levels in the diet over several years before any clinical manifestations are observed, and because of the extremely low levels and wide variety of candidate materials, chemical detection is difficult. Complete analysis for and subsequent control of these compounds is unlikely. Furthermore, these materials can result from processing, storage and home preparation, making control even more difficult. Dietary restraint and assuring that the detoxification system is functioning correctly would appear to be the best current means of preventing chronic disease.

In the process of attempting to detoxify materials, the animal of man frequently converts them to carcinogens in the process. One of the principle means of detoxification in higher animals is the microsomal mixed function oxidases (MFO). Xenobiotics including polycyclic hydrocarbons which appear in food cooked using hish temperatures, smoke, drugs, or chemical processes are frequently metabolized by the MFO system. These cytochrome P-450-linked systems, when coupled with other metabolically juxtaposed adjacent enzymes, provide cells with an important pathway whereby the various xenobiotics can be converted to metabolites that are water soluble, allowing them to be safely excreted. These systems are clearly beneficial and indeed essential to the organism. Unfortunately, these same systems can also convert xenobiotics to metabolites which are toxic, mutagenic, teratogenic and carcinogenic. A typical example of this type of enzyme is the aryl hydrocarbon hydroxylase (AHH) which oxygenates polycyclic aromatic hydrocarbons. This enzyme is nearly ubiquitous in animal tissues. It is a multicomponent system consisting of a cytochrome P-450, NADPH cytochrome P-450 reductase, and a phospholipid cofactor. The enzyme system is induced when the system is exposed to polycyclic aromatic hydrocarbons, or various drugs or hydrocarbons, with the activity increasing as much as one hundred fold. The ability of the organism to respond in this way is important for it to respond to normal exposures to xenobiotics. Typically, benzo-(a)-pyrene can be used to measure the activity of the enzyme so the phenolic procuts can be easily measured. The products also represent an excellent example of possible benefits and dangers of the enzyme system. Figure 1 shows that partial oxidation products can bind DNA, while a more favored route would be the formation of conjugates with glutathione. The activation of the enzyme and the levels of glutathione are factors that could be dramatically affected by the nutritional status of the organism. Also, one could speculate that other compounds in the cell could compete for

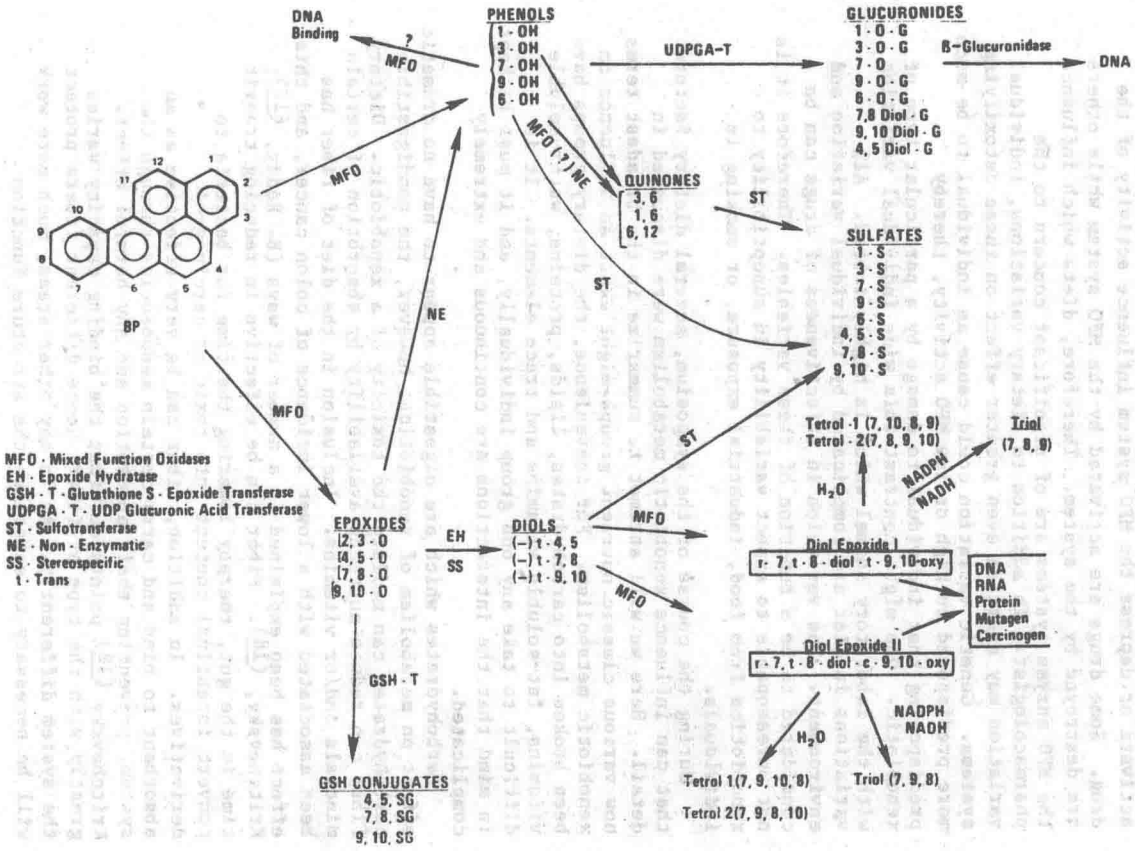

Figure 1. The metabolism and activation of benzo(a)pyrene. From Okano et al. (27).

glutathione conjugation and therefore increase the chance of DNA binding by the epoxide intermediates.

Examples of how variation in dietary status can influence drug metabolism in humans are discussed by Vesell (15) and Carr (16). Both report that diet can greatly influence the effectiveness of drugs. Dietary manipulations that would activate or depress the MFO system influence activity of the drug. Some drugs are activated by the MFO system while others are destroyed by the system. Therefore, diets which influence the MFO enzyme systems are of significant concern to the pharmacologist. In addition to dietary variations, individual variation may exert an even greater effect on these detoxifying systems. Genetic variation could cause an individual to be much more predisposed to high or low MFO activity, thereby predisposing that individual to damage by a particular type of xenobiotic. One might contrast this wide individual variation with the laboratory animal which is highly inbred. All variations in diet are complicated by individual variation and environment. The variation in effectiveness of drugs can be considered to be a function of these variables. Therefore it is not unreasonable to expect variability in suseptibility to xenobiotics from food, industrial exposure, or smoking in individuals.

During the course of the symposium, several dietary factors that can influence xenobiotic metabolism were discussed in detail. Here we will attempt to summarize in the broadest terms how various classic nutrient groups might exert an influence on xenobiotic metabolism. For convenience, the dietary groups have been broken into carbohydrates, lipids, proteins, water-soluble vitamins, fat-soluble vitamins and trace elements. It is difficult to take any one group individually, and it must be kept in mind that the interactions are continuous and extremely complicated.

Carbohydrates which are digestible appear to have no dramatic effect on metabolism of xenobiotics; however, the nondigestible carbohydrates can mediate the toxicity of a xenobiotic. Dietary fiber can reduce nutrient availability by absorbtion of certain minerals and/or vitamins. Inclusion in the diet of fiber has been associated with a lower incidence of colon cancer, and this effect has been explained in a number of ways (B. Reddy, (17); Kritchevsky, (18). Fiber can be effective in reducing transit time in the gut, thereby lowering the time for bacteria to convert intestinal content into toxic or carcinogenic derivatives. In addition, fiber can be very effective as an absorbant to bind and carry certain xenobiotics through the system, preventing their absorption and any harmful effect. Kritchevsky (18) points out that the binding capacity varies greatly with the type of fiber; hence different fibers protect the system differently. As in may other areas, much more work will be necessary to establish the structure function

relationship for these phenomena. Variations in dietary fiber also alter the nature of the gut flora. Inclusion of fermentable fiber such as pectin in the diet, results in a higher concentration of cecal bacteria, hence greater metabolism of 2, 6-dinitrotoluene or nitrobenzene. The result of the greater bacterial fermentation appears to be greater absorption and hepatic binding of the aromatics. The results suggest that non-fermentable fiber may offer the advantage that the bound xenobiotics would not be released and subsequently absorbed.

High levels of dietary fat have been associated with increased risk of colon, (B. Reddy, (17), prostrate, uterus, ovary and mammary cancers (Carroll, (19) in humans. It is important to recognize that diets higher in polyunsaturated fatty acids exhibit higher incidence of colon cancer than diets high in saturated or monounsaturated fatty acids (B. Reddy, (17). The polyunsaturated fatty acids appear to act at the promotion state of tumorogenesis rather than as initiators. Because diets containing polyunsaturated fatty acids are susceptible to oxidation, the question of the importance of lipid oxidation or lipid oxidation products in the initiation or promotion of tumor development becomes very important. Pryor (5) reports that the free radicals from the oxidation reactions can bind macromolecules and that the free radicals can themselves by involved in tumor promotion. Rahimtula et al (7) demonstrated that lipid peroxidation is involved in chemical carcinogenesis initiated by benzo-(a)-pyrene, aminofluorene, benzidine and methylaminobenzene. The products were shown to be bound irreversibly to protein and DNA. Wheeler, et al (20) demonstrated that varying the oxidation state of sulfur amino acids in the diet caused variation in the forms of MFO in the liver and therefore affected the nature of metabolites of benzo-(a)-pyrene formed. The results suggest again that oxidation state may be important. The various oxidation states of sulfur amino acids can be a secondary effect of lipid oxidation. As lipid oxidation proceeds, increased levels of oxidized sulfur amino acids would be expected in the diet.

The nature of protein in the diet also appears to alter xenobiotic metabolism. Clinton and Visek (6) and Clinton and Visek (21) observed that protein alters carcinogen metabolism and that fat level in the diet alters the absorption and distribution of DMBA in the tissue. The effects of protein nutriture also are observed when 1, 2-dimethylhydrazine is included in the diet. It appears that inadequate protein in the diet causes a significant decrease in hepatic MFO activity. Hayes (22) observed that in protein-deficient animals, aflatoxin B^1 was more acutely toxic and not as carcinogenic. This was explained by lower MFO activity in the liver. Animals with the decreased MFO activity were unable to detoxify the aflatoxin B^1, thus it was acutely toxic. Because the aflatoxin was not hydroxylated by the MFO, it was not converted to the

carcinogenic derivative. It appears that the state of the sulfur amino acids and the quantity of protein both influence the ability of the organism to metabolize xenobiotics.

Water-soluble vitamins and co-factors also appear to elicit an effect on xenobiotic metabolism. Ascorbic acid has been shown to inhibit chemically induced chemical carcinogenesis in test systems (Shamberger, (8). When diets are deficient in choline, the animals appear to become much more susceptible to chemically-induced carcinogenesis (Shinozuka, et al, (23). It was observed in this work that there was more lipid peroxidation in the livers of the choline-deficient animals. This may be important in relation to the tumor-promoting effects of lipid peroxides. Herbert (24) observed that chemically-induced tumors of the liver, colon and esophagus may be enhanced by diets deficient in folic acid, vitamin B^{12}, choline and methionine. Conversely, he observed that vitamin B^{12} may serve to enhance the carcinogenic effect of p-dimethylaminoazobenzene in rats fed a methionine-deficient diet. It is important to note, however, that this was only true in rats on a methionine-deficient diet.

Fat-soluble vitamins, in addition to their antioxidative effects on lipids, appear to exert a general protective effect in animals. Vitamin A and beta-carotenes protect lab animals from toxicity of citral, cyclophosphamide and some hydrocarbons (Seifter et al, (13). In related but independent studies, it was observed that high levels of vitamin A inhibit tumorogenesis and that low levels of vitamin A appear to enhance tumorogenesis (Baird, (14). Vitamin E inhibited chemically-induced carcinogenesis in test systems (Shamberger, (8) and also reduced the susceptibility of rats to cigarette smoke (Chow, (25). These observations support the NRC guidelines urging increased consumption of fruits and vegetables which are rich in these vitamins.

Selenium levels in the diet and blood have been shown to be inversely correlated with human cancer mortality (Shamnberger, (8). Also, selenium-dependent glutathione peroxidase activity has been shown to be reduced in selenium-deficient rats (C. Reddy, (11). Selenium appears to inhibit both the initiation and promotion phases of carcinogenesis (Milner, (26). These results suggest that selenium is critical for glutathione peroxidase activity, but apparently functions in other ways as well to inhibit carcinogenesis.

In summary, it is clear that a multitude of dietary factors can influence the metabolism of xenobiotics, particularly those that can be involved in carcinogenesis. We are and will continue to be exposed to xenobiotics of various types from our food, industrial sources, toxic wastes, drugs, and smoke. The well nourished animal or man appears, within the limits of individual variation, to be able to metabolize and excrete most of these xenobiotics harmlessly. It is important that we use the best diet possible to reduce fat in the diet, particularly where lipid