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Role of Nutrition in the Drug-Metabolizing Enzyme System

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I. Introduction

THE LITERATURE is replete with evidence that dietary composition represents an important environmental determinant of the pharmacotoxicological activities of chemicals which are foreign to the living system. However, most of this information has been derived from experimental laboratory investigations rather than empirical epidemiological or clinical data.

Reasons for the paucity of clinical data on this type of interaction could be 1) that the separation of cause and effect for the toxicity in question may be far removed in time, 2) that the information on pathological symptoms characteristic of environmental toxins is not yet complete, and 3) the expected symptoms are rather subclinical, nondescript, and chronic in nature.

Although the significance of nutritional-pharamcological interactions is not fully realized, valuable clinical contributions will be made by better understanding the fundamental mechanisms of these potential interactions. For example, a survey of the limited literature in this area readily demonstrates the proposed clinical significance: 1) there is a great variety of nutrients affecting drug metabolism and disposition; 2) experiments with both adequate and deficient diets have

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shown impressive differences in the activities of drug-metabolizing enzymes.

The interaction of nutritional status with drug or foreign compound toxicity may take many forms. Nutritional imbalances may affect toxicity *via* physiological, immunological, and/or biochemical mechanisms. Most of the experience on these interactions, however, involves the effects of nutritional imbalances on the drug-metabolizing enzymes located in the endoplasmic reticulum of the liver hepatocyte. This enzyme complex, experimentally fractionated and prepared as "microsomes," is conventionally classified as a mixed function oxidase according to the nomenclature of Mason (138). This enzyme complex is comprised of cytochrome P-450. phosphatidylcholine, and a flavoprotein reductase, is closely invested into the structural membrane, requires oxygen and NADPH,[1] and possesses a broad substrate "specificity," since a wide variety of drugs and foreign compounds appear to be metabolized. Even endogenous steroid hormones may serve as substrates. In addition to the broad range of potential substrates, versatility is also enhanced by its capacity to be readily "induced" by a wide variety of so-called environmental factors. The term "induction" has been used broadly, however, and includes not only classical stimulation of protein synthesis, but apparently also involves activation or modification of existing enzyme. These facts are amply documented in several excellent reviews on the microsomal enzyme system (33, 72, 73, 133). Since this enzyme system appears to be the predominant catalyst of the metabolism of foreign compounds to products of greater or lesser toxicity, this review will accordingly be limited to a description of the effects of nutritional imbalance on the activity of this enzyme system, hereinafter referred to as the mixed function oxidase (MFO). There is no intention to disregard the significance of other intervening systems; only an arbitrary division of labor needs to be imposed. The interposition of the cell-mediated immunity system appears to be particularly relevant (189) and should be mentioned as a potentially impressive mediator of nutrient-toxin interaction.

II. Macronutrient Deficiencies

The influence of individual macronutrients is generally difficult to assess independently by experiment. Interpretations of independent effects are difficult since the caloric intakes and densities of the diet, stress effects, and "sparing" actions that result from the supplementation of one macronutrient upon the deficiency of another are often not discernible. Also, inadequate attention has been given to the necessity of establishing equivalent feed intakes between experimental groups. In spite of these difficulties, independent nutritional properties for specific macronutrients have been reported and may be clinically valid; secondary interactions may be difficult to assess experimentally but are nevertheless representative of clinical situations and are worthy of presentation. This review will present these macronutrient imbalances independently, albeit with an awareness that secondary interactions may be equally important.

A. Carbohydrates

A specific biochemical role for carbohydrates in the MFO system has not been demonstrated. Dietary manipulation of carbohydrates would therefore appear to relate through the interventions of more generalized effects on intermediary metabolism, caloric intake, and/or hormonal-stress relationships. Specific alterations of MFO activities have, however, been reported.

Strother *et al.* (202) have shown that a high intake of various sugars (glucose, sucrose, or fructose), especially glucose, will increase the duration of sleep induced by

[1] The abbreviations used are: MFO, mixed function oxidase; PB, phenobarbital; and MC, 3-methylcholanthrene; NADP, NADPH, nicotinamide adenine dinucleotide phosphate and its reduced form; NADH, reduced nicotinamide adenine dinucleotide.

barbiturates in mice. The longer sleeping time seems to be correlated with a decrease in the metabolism of these compounds, although the animals tended to compensate for this effect after a few days. These authors suggested that the intravenous administration of 5% glucose over extended periods to ill patients could have marked effects on barbiturate-induced sleep time. Boyd *et al.* (18) reported that high sucrose diets, compared with starch, fed to rats potentiated the lethal reaction to benzylpenicillin presumably due to lower rates of conversion to its less toxic product. Dickerson *et al.* (57) also found that rats fed sucrose or glucose plus fructose had lower levels of biphenyl 4-hydroxylase, which were correlated with lower levels of cytochrome P-450.

B. Lipids

From 30 to 55% of the dry weight of the hepatic endoplasmic reticulum is lipid (191), comprised of 0.07 mg of cholesterol esters, 0.07 mg of free fatty acids, 0.5 mg of triglycerides, 0.6 mg of cholesterol, and 4 to 6 mg of phospholipids per g of liver (75). A specific lipid role in the MFO system is that shown for phosphatidylcholine, whose enzymatic degradation with phospholipase C (39) decreased the metabolism of hexobarbital and ethylmorphine. Morover, iso-octane extraction (118) and phospholipase C treatment (39, 65) of microsomes depressed type I binding[2] while tending to increase type II binding.[2] Coon and co-workers (9, 121, 201) established the requirement for phosphatidylcholine, together with cytochrome P-450 and NADPH-cytochrome P-450 reductase, as one of the three required fractions in their reconstituted microsomal enzyme system. In addition to this specific but as yet unidentified role for phosphatidylcholine, lipid substances may also function in other ways. Maintenance of membrane integrity is suggested by the fact that phenobarbital induction of MFO activity coincides with similar increases in microsomal phospholipid, cholesterol, and triglyceride concentrations to approximately the same extent as that for protein (75). Another role for lipid substances in MFO activity is illustrated by the finding that endogenous lipids such as steroids (114, 115, 186) and fatty acids (50, 56) may occupy cytochrome P-450 binding sites, thereby displacing exogenous substrates and perhaps interfering with their metabolism. It was reported by Diehl *et al.* (58) that endogenous substrates may occupy as much as 20% of the total type I binding sites in freshly prepared microsomes.

The effects of dietary lipid on MFO activity should, therefore, depend on the specific functional role as well as the type of dietary fat considered. From a nutritional point of view, lipids are apparently required in the diet only in the case of the essential fatty acids. Caloric density and palatability are also important considerations, but cannot be considered as absolute requirements. In the case of the essential fatty acids Caster *et al.* (31) found that feeding a diet deficient in linoleic fatty acid depressed the activities of certain drug-metabolizing enzymes in rats. Norred and Wade (157) examined more fundamental kinetic parameters associated with the hepatic MFO system in relation to essential fatty acid deficiency. Feeding a fat-free diet to rats for three weeks, compared with a 3% corn oil diet, depressed cytochrome P-450 levels and decreased the V_{max} and K_m estimations for ethylmorphine demethylase and hexobarbital oxidase. Moreover, the V_{max} for aniline hydroxylase was significantly depressed although the K_m was not affected. Microsomal contents of linoleic (18:2) and arachidonic (20:4) acids increased as dietary corn oil was increased. Century (37) has also reported increases in the microsomal contents of these acids when

[2] Type I ligands produce a difference spectrum characterized by an absorption peak at 385–390 nm and a trough at 419–425 nm, whereas type II substrates produce a difference spectrum characterized by an absorption peak at 426–435 nm and a trough at 390–405 nm.

dietary levels of polyunsaturated fatty acids are increased. Since NADPH-cytochrome *c* reductase activities were not altered by the essential fatty acid deficiency, Norred and Wade (157) suggested that the defect in the enzyme system may be at some point beyond the initial oxidation of NADPH. They indicated that changes in the lipid composition of microsomal membranes may alter the abilities of the intimately associated microsomal enzymes to bind substrate, although supplementation of their fat-free diets with either 3% or 10% dietary levels of corn oil did not consistently increase the binding of either type I or type II substrate. Sex was a contributing variable even though its relationship to substrate binding could not be ascertained. From this same laboratory a later report (32) showed that the absolute amount of saturated fat in the diet linearly increased aniline metabolism and resulted in a 2-fold increase when that fat contributed 35% of the calories rather than 15%. Manipulation of the dietary content of saturated fat produced no measurable effect on the rate of hexobarbital metabolism, however.

An interesting observation by Marshall and McLean (137) showed that, in order to permit maximum induction of cytochrome P-450 synthesis after administration of phenobarbitone, the dietary addition of either herring oil, linoleic acid, or 0.1% oxidized sitosterol was required. Since neither coconut oil, 5% olive oil, nor saturated fatty acids were effective, they suggested that some aspect of the polyunsaturation was responsible for the "permissive" effect. They proposed that either the degree of unsaturation or the lability to lipid peroxidation was a possibility. Semipurified diets, adequate for life and reproduction in the rat, did not allow maximal induction of cytochrome P-450 by phenobarbitone until these types of lipids were included (137). Gillette *et al.* (72) pointed out, however, that these effects could have been due to the presence of antioxidants (70, 71) added to oils by the manufacturer or to peroxidized lipids and steroids (21), which are well known MFO inducers (33).

More recently, Century (37) obtained results similar to those of Marshall and McLean (137) in which the ability of phenobarbital pretreatment to stimulate the metabolism of hexobarbital and aminopyrine was significantly increased with the dietary inclusion of menhaden oil and/or linseed oil, particularly when compared with the dietary addition of beef fat. They observed a correlation between the "*omega* status" of the microsomal fatty acid composition of microsomes and the inductive ability of phenobarbital but did not suggest any apparent mechanism for the relationship. Whether there were differences in the contents of the above cited additives (21, 70, 71) between beef fat and the menhaden and/or linseed oils should be evaluated.

In addition to these dietary lipid effects on MFO activities, a deficiency of essential fatty acids may also affect target site sensitivity. Kaschnitz (99), for example, reported that, whereas a deficiency of essential fatty acids resulted in markedly lower aryl 4-hydroxylase and cytochrome P-450 levels, hexobarbital narcosis was not affected.

C. Lipotropes

Lipotropes are compounds which either function as methyl donors or assist in methyl group transfer during the synthesis of phospholipids which are required for triglyceride transport.

Lipotrope deficiency is characterized by the accumulation of triglycerides in the liver because of the ineffective transport mechanism. The resulting fatty liver syndrome, sometimes extended with time to liver cirrhosis, has been suggested as a predisposing condition to the development of primary liver carcinogenesis (111, 129, 199). Research workers in the laboratory of Newberne and Rogers were pioneers in the studies on possible interactions between dietary lipotropes and toxic response (155, 156, 173, 174, 176–178). For example, they examined the effect of dietary lipotrope deficiency in rats on the

hepatocarcinogenic effect of aflatoxin B_1 and found that a diet marginally deficient in lipotropes, which induced fatty liver but not cirrhosis, enhanced the development of tumors (173). On the other hand, a severely deficient diet, which induced cirrhosis, did not affect aflatoxin B_1 carcinogenesis. In later studies (174), they observed that rats marginally deficient in dietary lipotropes were resistant to the toxicity of a single dose (7–9 mg/kg) of aflatoxin B_1, which killed 60 to 100% of the animals fed the complete diet. In contrast, the marginally deficient rats, which had been shown to be more susceptible to aflatoxin B_1 carcinogenesis (173), were also more sensitive to the acute toxicity of repeated small doses of aflatoxin B_1. They proposed that this dietary influence may be explained by low basal levels of the microsomal enzymes in the livers of the marginally deficient rats and the failure of the enzyme levels to respond to repeated doses of aflatoxin B_1. The effects of aflatoxin B_1 on MFO induction are rather complex, however, since they are not only dosage dependent, but are also influenced by the various capabilities of aflatoxin B_1 to inhibit protein synthesis, activate existing enzyme activities, and specifically induce MFO synthesis (81). It would appear that the effects of aflatoxin B_1 on MFO activities will need to be independently elucidated before diet-toxin interactions can be adequately explained in terms of enzyme induction.

An observation which merits further comment is that aflatoxin B_1 was specific for the induction of demethylation as opposed to hydroxylation. This assumes that the suggested specificity of induction, however, would contrast with the expected toxicity. That is, the O-demethylated metabolite P_1 is much less toxic (200) than is a hydroxylated product such as M_1 (165, 166, 193). The marginally deficient diets substantially decreased aminopyrine demethylase, *p*-nitroanisole demethylase, and benzpyrene hydroxylase activities and the administration of aflatoxin B_1 induced demethylase but markedly depressed benzpyrene hydroxylase. Of course, other factors, such as lack of cellular transport, may also account for the reported absence of aflatoxin P_1 toxicity. This attempt to extrapolate metabolism data to toxicity patterns also clearly illustrates the necessity of first obtaining a clearer understanding of complete metabolism schemata for foreign chemicals, both in terms of reaction rate constants *in vivo* and in terms of qualitative alterations in patterns.

Butler and Neal (27), who recently confirmed the findings of Rogers and Newberne (174), showed that marginal lipotrope deficiency depressed the toxicity of a single dose of aflatoxin B_1. The lipotrope deficiency prevented the usual increase in smooth endoplasmic reticulum often found in aflatoxin poisoning. The inability of aflatoxin B_1 to stimulate proliferation of smooth endoplasmic reticulum, which is generally associated with higher MFO activities, agrees with the observations of Rogers and Newberne (174) wherein repeated daily doses of aflatoxin B_1 in lipotrope-deficient rats also did not increase MFO activities. Whereas Rogers and Newberne (174) found demethylation and hydroxylation reactions to be reduced in lipotrope-deficient rats, Butler and Neal (27) on the other hand, observed no difference between hexobarbitone sleeping times. Hexobarbitone narcosis, however, may not be an uncomplicated indicator of MFO activities (99). Both groups (27, 174) believe that marginal lipotrope deficiency may offer a good model system to distinguish between those changes in the liver associated with acute aflatoxicosis from those associated with aflatoxin carcinogenicity. There is also evidence here, as well as in reports to be cited later, that increased MFO activities are generally associated with lower tumor yield but higher acutely toxic response from chemical carcinogens capable of being metabolized by the MFO system.

The hepatotoxic effect of monocrotaline one of the pyrrolizidine alkaloids, is due to its conversion to its pyrrolic metabolite (139). A low-lipotrope diet fed to rats did

not permit conversion of the parent compound to its active metabolite at a sufficient rate to allow the characteristic toxicity to be expressed (156).

The intervention of lipotrope deficiency with tumorigenesis caused by the nitrosamines has also been examined (177, 178). Increased tumor yields were seen with N-nitrosodiethylamine and N-nitrosodibutylamine, but not with the simpler N-nitrosodimethylamine. As expected, the metabolism of the N-nitrosodimethylamine was not altered by the lipotrope deficiency; similar measurements of metabolism of N-nitrosodiethylamine and N-nitrosodibutylamine were not undertaken.

Whereas hepatic MFO activity is considered the principal intervenor of toxicity modification due to metabolism, other sites of MFO activity must not be overlooked. Rogers and Newberne (176), for example, showed that the carcinogenicity of symmetrical dimethylhydrazine was enhanced during lipotrope deficiency in the colon but not in the small intestine, thereby suggesting the intervention of bacterial MFO activity found in the gastrointestinal flora.

The biochemical role of lipotropes in MFO activity would seem to be most apparent with the activity of the phosphatidylcholine function (39, 65, 118, 121) discussed earlier. For example, Cooper and Feuer (46) and Sosa-Lucero *et al.* (197) have shown that, although a lack of choline caused no significant overall changes in the total phospholipid level in the liver, the phospholipid content of the microsomes was reduced significantly. This alteration paralleled decreases in microsomal hydroxylase activities. Both reports concluded that the MFO inducibility was also impaired, but since the fold-increase was approximately the same in the control and deficient diets (46), the limitation may have been associated more with secondary effects of choline deficiency on total microsomal protein. Lower basal levels of microsomal protein would be expected to accommodate less total inducibility; that is, the same fold-difference does not indicate direct impairment by choline deficiency.

D. Protein

The dietary constituent most studied is protein. A dietary reduction in either quantity or quality causes a depression of hepatic microsomal mixed function oxidase activities. As pointed out by Boyd (16), these results have a direct application to the toxicity of drugs and chemical agents in persons whose dietary protein is low. Such a deficiency is often widespread in many developing countries and occurs in the abnormal diets of chronic alcoholics, narcotic and other drug addicts, depressed psychopaths, and food faddists. Patients with advanced cancer who tend to develop protein deficiency symptoms could also be included.

The original reports of increased toxicity of drugs during protein deficiency were submitted by Drill in 1952 (64) and Rouiller in 1964 (183). Protein deficiency has been shown to decrease the microsomal reaction rates *in vitro* for a variety of oxidative pathways with substrates such as phenobarbital (107), strychnine (107), aminopyrine (107, 140), benzopyrene (140), zoxazolamine (107), pyramidon (136, 140), ethylmorphine (143), and aniline (107, 143) and to increase the disappearance *in vivo* of pentobarbital (107) and heptachlor (217).

The toxicities *in vivo* of foreign compounds administered to protein-deficient animals apparently depend on whether the principal reaction step represents intoxication or detoxication. The best known illustrations of this phenomenon are those shown for certain pesticides. For example, the toxicity of a compound such as heptachlor (217) is decreased in protein deficient rats, since its principal metabolite, heptachlor epoxide, is more toxic. Also, Kato *et al.* (107) found that the toxicity of octamethylpyrophosphoramide is also decreased in protein deficient rats and McLean and McLean (141) observed that the acute toxicity of carbon tetrachloride in rats likewise is decreased during protein deficiency. In each case, the respective authors noted that these compounds are activated to more toxic metabolites whose production is apparently decreased during protein deficiency. In spite of

these few examples of decreased toxicities, the toxicities of most pesticides are increased during protein deficiency. Examples of such compounds include a series of 16 pesticides reported by Boyd and Krupa (19). Chlordane, diazinon, endrin, lindane, and malathion were up to twice as toxic in animals previously fed a diet containing 3.5% casein as in animals fed 26% of dietary casein; chlorpropham, DDT, diuron, endosulfan, monuron, toxaphene, and demeton were 3 to 5 times more toxic; carbaryl is a naphthylcarbamate and was 6 times more toxic; parathion is a *p*-nitrophenyl organic phosphorothioate and was 8 times more toxic; and captan is a mercaptophthalidimide related structurally to thalidimide and was 26 times more toxic. They concluded that organic chloro pesticides of the DDT-lindane-chlordane type and organic phosphorothioates except parathion, which possesses a *p*-nitrophenyl group, would appear the least likely to produce augmented acute oral toxicity in countries where the diet is low in protein. Carbaryl, parathion, and particularly captan would seem most likely to produce augmented toxicity reactions. In another report from this laboratory, Krijnen and Boyd (113) showed that with rats maintained for 28 days on non-protein diets, the toxicities of these pesticides were increased still further and captan was 2100 times more toxic than with control rats. With male weanling rats pair-fed for 10 days semipurified diets containing either 5% or 20% casein, Webb *et al.* (218) showed that both of the organophosphates, malathion and parathion, as well as their more toxic oxygen analogue metabolites, malaoxon and paraoxon, are more toxic after protein deficiency is established. The important pharmaceutical, phenacetin, is another compound whose toxicity is increased by protein deficiency (17).

The mutagenicity of certain chemical carcinogens is different when these compounds are metabolized by microsomes prepared from protein deficient animals. Hepatic microsomes isolated from protein-deficient mice possessed lower activities for the inactivation of the primary carcinogen, N-methyl-N′-nitro-N-nitrosoguanidine and the activation of the secondary carcinogen, dimethylnitrosamine, when their mutagenicities for bacteria were compared (48).

A lower quantity of dietary protein reduces microsomal enzyme oxidations; reduced protein quality, as defined by conventional protein utilization ratios, has the same effect. Webb and Miranda (219) showed that weanling rats fed different types of dietary protein for 10 days exhibit different susceptibilities to the lethal effects of heptachlor. At either 10% or 18% dietary protein levels, unsupplemented gluten diets reduced toxicity when compared with casein diets. These authors postulated that the impairment in the MFO conversion of the heptachlor to its more toxic epoxide was responsible for this effect. Chadwick *et al.* (38) also attempted to differentiate the abilities of proteins of dissimilar quality to support pesticide (lindane plus DDT)-induced stimulation of microsomal O-demethylation of *p*-nitroanisole and the oxidative hydrolysis of O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate (EPN). They concluded that "poor quality protein supports and even potentiates some metabolic responses in DDT + lindane-treated guinea pigs." However, all of their diets contained an excessive level of 30% protein which would not be expected to permit differentiation of protein quality. In fact, lower quality protein generally results in a lower level of body depot fat (219) which reduces tissue storage of pesticides and increases circulating levels, thereby permitting greater induction of the MFO activity. Presumably, poorer quality protein tends to mimic quantitative protein deficiency. For example, feeding a gluten diet to rats for 10 days, compared with a casein diet, reduced hepatic microsomal protein, cytochrome P-450 contents, and drug metabolism *in vivo* and *in vitro* (147).

Dietary protein sufficiency is also an important factor to be considered in the toxicities of chemical carcinogens, although acute toxicities may respond quite differently from

carcinogenicities. Reports from India have shown (131, 132) that protein deficiency decreased tumor induction but increased the toxicity of repeated aflatoxin B_1 doses. Increased acute toxicity to aflatoxin during protein deficiency has also been observed with chicks (196) and swine (194). The fact that toxin response modified by a dietary variable differs for acute and chronic exposure to aflatoxin B_1 was also found by Rogers and Newberne (174) in their studies of lipotrope deficiency. However, if we consider the MFO system to be the principal mediator of this response, then these dietary deficiencies of protein and lipotropes (*vide supra*) appear to produce contrasting effects when toxic and carcinogenic responses are considered.

Those who have worked with protein deficiency have noted other mediating mechanisms which should be considered in toxin-nutrient interactions. For example, moderate protein deficiency was found to enhance cellular immune response due to a lack of blocking serum antibody; such a phenomenon resulted in marked inhibition of tumor growth (98). Perhaps of even greater significance are the findings of Ross and Bras (181) who clearly established that the protein/calorie ratio of the diet influenced tumor yield unpredictably and the degree of inhibition or enhancement was related to the tissue origin, tissue type, and degree of malignancy. Moreover, feeding *ad libitum* yielded results different from restricted feeding. In addition to the intervention of the MFO metabolic disposition mechanism considered in this review, Ross and Bras (181) present an impressive argument for the consideration of the endocrine and cell-mediated immune systems as intervenors of dietary modification of chemical tumorigenesis. Hopefully, research in this area will parallel that for the microsomal metabolic system so that interpretation of research will not become too narrow in scope.

In order to extrapolate data generated from normal well nourished experimental animals to man lacking adequate dietary protein, more information is needed on the fundamental biochemical mechanism(s) by which dietary protein deficiency modifies MFO activities. Among the approaches used toward this type of understanding include measurements of the apparent kinetic constants of V_{max} and K_m for several substrates with whole microsomes, examinations of fractionated individual components of the enzyme system, determinations of rates of reduction of cytochrome P-450 (or cytochrome *c*), apparent substrate binding constants, and degree of induction.

Mgbodile and Campbell (143) observed that with weanling rats pair-fed for 14 days semipurified diets containing either 5% or 20% casein, part of the depression of microsomal enzyme activity caused by protein deficiency was due to retardation in the normal rate of liver cell proliferation and part was due to modification of the specific enzyme activity.

In additional studies from this same laboratory, Hayes *et al.* (J. R. Hayes, A. H. Merrill, L. M. Nerurkar and T. C. Campbell, unpublished observations) have found that the decrease in the rate of liver cell proliferation caused by protein deficiency can essentially be restored by replacing the deficient diet with a diet containing adequate protein; this indicates that the regenerative capacity of the liver permits recovery—unlike the effect of protein deficiency on cell proliferation in the cerebrum of the young animal. Generally speaking, the microsomal protein content in hepatic tissue is limited by the quantity of cells, as measured by deoxyribonucleic acid content (142, 143).

The effect of protein deficiency on the specific enzyme activity has been studied principally by determining the apparent kinetic binding and catalytic constants for substrates such as ethylmorphine (EM, type I) and aniline (AN, type II)[2] (87, 88, 144). Important information can also be obtained when these same kinetic parameters are measured after pretreatment of each group

of animals with inducers such as PB and MC.[3]

Protein deficiency causes an equivalent 50 to 75% depression of cytochrome P-450 content and flavoprotein reductase activity (measured either with cytochrome P-450 or with added cytochrome *c*) after 14 days of feeding, both of which correlate with similar decreases in ethylmorphine demethylase and aniline hydroxylase activities (87, 88, 144). Binding of ethylmorphine to cytochrome P-450 was shown to be directly correlated with the ratio of phosphatidylcholine to hemoprotein, whereas the binding of aniline was correlated with the quantity of cytochrome P-450, *per se* (88). However, binding of these substrates to cytochrome P-450 is not necessarily correlated with catalysis (87, 144). On the other hand, there was an inverse relationship between the total quantity of type I binding sites and the K_m for metabolism which suggested that the binding site, as measured spectrophotometrically, may be better visualized as a "substrate effector" site (87). With that interpretation, therefore, the influence of a dietary variable, such as protein, on metabolism may also be mediated by an effect on phosphatidylcholine and its association with cytochrome P-450. The influence on metabolism caused by a functional phosphatidylcholine association would, in turn, depend on the concentrations *in vivo* of substrate presented to the catalytic site. The K_m, whose interpretation remains uncertain for MFO activities, may nevertheless reflect "availability" of a substrate to the active site; determination of this parameter certainly permits estimation of enzyme velocities at low substrate concentrations. According to this argument, an influence of dietary protein on the "function" of phosphatidylcholine, as it relates to an alteration of the apparent K_m, could have very pronounced effects at the presumably lower concentrations *in vivo* of substrate.

Moreover, this enzyme system has been shown to possess the very unusual characteristic of having the K_m and V_{max} vary with substrate concentration, particularly at the lower concentrations (89). In that case, the phosphatidylcholine influence may be magnified even further at the lower physiological concentrations of substrate presumably encountered. This observation, coupled with the above interpretation, indicates that one role for phosphatidylcholine may be as a component which can be affected by exogenous substances and which modulates short-term control of MFO activities.

The role proposed for phosphatidylcholine in mediating the effect of protein deficiency on the microsomal drug-metabolizing enzyme system must be examined in greater depth. This is particularly true when considered with reference to the earlier finding that phosphatidylcholine (together with cytochrome P-450 and cytochrome P-450 reductase) is required for fatty acid, hydrocarbon, and drug hydroxylation in a reconstituted liver microsomal enzyme system (201). Moreover, removal of phosphatidylcholine has been shown to depress microsomal binding of type I substrates (39, 65, 118). It has also been shown that protein deprivation enhances incorporation of ^{32}P into phosphatidylcholine (84, 179), which supports the proposal of Harada and Suzuki (84) that this type of dietary deficiency has a generalized effect on phospholipid metabolism through a modification of phosphatides, as measured in mitochondria and whole liver. Of course, a clear understanding of the

[3] PB is a classical broad-spectrum inducer, stimulating varied pathways of metabolism of liver microsomes, including oxidation and reduction reactions and glucuronide formation and characterized by increased quantities of cytochrome P-450 (33). MC is a classical narrow-spectrum inducer causing the appearance of a cytochrome exhibiting a reduced-CO complex peak at 448 nm for the spectrophotometric difference spectrum (4), and possessing a preferential catalysis for type II substrates (33, 190) such as aniline, as well as polycyclic hydrocarbons such as benzpyrene (3). This latter cytochrome is called cytochrome P-448 or P_1-450.

mechanism by which phosphatidylcholine may mediate the influence of protein deficiency on microsomal enzyme metabolism must await information on the specific role for phosphatidylcholine in the enzymatic mechanism itself. Several laboratories have submitted reports on the manner in which this component functions in the enzyme system, particularly in the resolved system originally reported by Strobel *et al.* (201) and more lately studied in the laboratories of Coon and co-workers (7, 44, 126) and Lu and co-workers (122–125, 127, 160, 220). Coon *et al.* (45) recently published a summary of their findings on the proposed role for phosphatidylcholine and have favored the interpretation that this component is in some way involved in the electron transfer step from NADPH to cytochrome P-450 rather than in the step(s) involving oxygen activation or insertion into the substrate.

In considering the various modes in which phosphatidylcholine may participate in modulating MFO activity during protein deficiency, one might also consider the change in the fatty acid substitution of phosphatidylcholine caused by protein deficiency (180). For example, different fatty acids esterified to phosphatidylcholine provide different degrees of activity in a reconstituted system (201).

Although phosphatidylcholine may function as a modulator of MFO activity *in vivo*, fractionation of the MFO system into its three major components has shown that dietary protein deficiency does not affect the independent characteristics of the phospholipid fraction. In other words, reconstitution of reductase and cytochrome P-450 fractions from rats fed 20% casein with the lipid fraction isolated from rats fed 5% casein did not impair activity (L. S. Nerurkar and T. C. Campbell, unpublished observations). Restoration of MFO activity with the lipid fraction therefore does not appear to depend on its compositional characteristics. The activities for the cytochrome P-450 and flavoprotein reductase fractions are each independently depressed when weanling rats are fed 5% casein diets for 14 days compared to rats fed 20% casein.

The effects of protein deficiency on MFO activity also depends on the species and/or severity of protein deprivation. Rumack *et al.* (184) used *Macaca nemestrina* monkeys, which were severely deprived of protein and which showed classic features of marasmic kwashiorkor, and found lower activities of cytochrome P-450 reductase, but no differences in cytochrome *c* reductase, ethylmorphine demethylase, and aniline hydroxylase, even when these latter two activities were either expressed per mg of microsomal protein or per kg of body weight. This absence of differences contrasts sharply with the results of Mgbodile and Campbell (143) and Mgbodile *et al.* (144) who demonstrated greatly reduced activities for both substrates when expressed on the same weight bases for rats fed 5% protein for 14 days. The animals of Rumack *et al.* (184) may have been much more stressed, thereby causing a rise in MFO activities and confounding the expected differences.

In addition to diet-toxin interactions which are derived from altered MFO toxin metabolism, we must remain aware that dietary manipulation may also affect reactions other than the often measured MFO hydroxylases and demethylases. Other reactions may include the enzymatic formation of the epoxides generally considered to occur prior to the nonenzymatic formation of phenols or the enzymatic formation of the diols (158). Moreover, follow-up conjugation reactions occur with a wide variety of oxidized metabolites.

To understand the effect of dietary manipulation on the metabolic disposition of xenobiotics, it becomes important to evaluate all such reactions before there can be an appreciation of either how the dietary-generated difference in toxicity is manifested or which chemical intermediate predominates in the toxic lesion. A good example is that presented by Wood and Woodcock (228) and Woodcock and Wood (229), who found that whereas dietary protein deficiency

decreases MFO activities, it increases uridine diphosphate glucuronic acid transferase activities. These increases were real and were not related either to microsomal uridine diphosphate glucuronic acid pyrophosphatase or 6-glucuronidase activities. Moreover, the microsomal epoxide hydrase, which may be coupled with the mono-oxygenase system (158), is also subject to activity alterations when inducers such as MC and PB are administered (97, 159), and Oesch (158) has theorized that modulation of epoxide hydrase activity represents an important determinant of toxin disposition. An experimental evaluation of the effect of protein deficiency as an interactant with chemical carcinogenic potential on epoxide hydrase activities would be highly desirable.

In addition to the basal MFO activities being reduced because of protein deficiency, the inducibility of those activities are also known to be impaired (87, 102, 135, 136, 144). One interesting feature of the induction is that the percentage increases seen in the protein-deficient animals are generally of the same magnitude as their counterpart protein-adequate controls. Perhaps the existing basal levels in some way control the maximum activities which are permitted to be induced.

III. Micronutrient Deficiencies

Many known vitamins and nutritionally important trace minerals have been examined for their effects on the metabolism of foreign chemicals by the microsomal MFO system. The rationale for all of these studies, however, is not entirely clear. A judicious choice of which vitamin or mineral to study should depend in large measure on whether the micronutrient has been shown to participate in the synthesis or function of some component of the enzyme system; such has not always been the case. Most investigators who have studied these deficiencies report effects on MFO activities not as striking as those observed for protein deficiency although activities are affected at least to some degree if the deficiency is severe enough.

A. Vitamins

1. Vitamin A. Since vitamin A influences the state of differentiation in many epithelial tissues (54, 85, 227) as well as decreasing the incidence of tumor yields in certain tissues (41, 185), recent reports have focused on its mechanism of interaction. Much attention has been given to the metabolism of the carcinogen under study and, of course, the effect of dietary vitamin A status upon microsomal MFO activities has been a particularly relevant consideration, although only very recently has this specific interaction been examined (11).

Reports from the laboratory of Newberne and Rogers (154) and Rogers *et al.* (175) have shown that, although vitamin A deficiency did not seem to affect the usual hepatoma incidence induced by aflatoxin B_1 feeding in rats, there was a significant induction of colon carcinomas in the deficient animals. Since the liver carcinomas were induced with the expected frequency, they suggested that alternative mechanisms, *i.e.*, nonmicrosomal metabolism might be considered relative to the induction of the colonic carcinomas. The diets contained refined sources of carbohydrates which may have affected the metabolism and status of the intestinal microflora or slowed intestinal transit time, both of which are related to the epidemiological characterization of colon carcinomas (24, 211, 212). These investigators pointed out that since vitamin A is required in the normal differentiation of intestinal mucosa and ciliated cells from the basal cells, a deficiency of this nutrient and the consequent change in the pattern of differentiation might predispose the epithelium to neoplastic changes (154, 175). It is assumed that the authors meant that the neoplastic potential of their refined carbohydrate diets was enhanced both by vitamin A deficiency and aflatoxin exposure. One might also consider the possibility that the gastrointestinal microflora were capable of metabolizing aflatoxin and the vitamin A deficiency shifted the metabolism to a rate or product with greater carcinogenic potential.

Other vitamin A-aflatoxin interactions are also known to occur. For example, acute aflatoxin poisoning may reduce hepatic and serum levels of vitamin A in animals (2, 112) and birds (29), which could have further enhanced the interaction observed earlier (154, 175). Also, Reddy *et al.* (168) administered a single intraperitoneal dose (3.5 mg/kg) of aflatoxin B_1 to rats maintained for 9 weeks on a vitamin A-free diet and observed increased susceptibility shown by rapid mortality and severe liver damage. The female animals remained relatively unaffected.

Rogers *et al.* (175) succeeded in inducing colon carcinomas in rats fed dimethylhydrazine and found that, although a high level of vitamin A in the diet did not change the incidence of tumors, the number of tumors per rat for the highest dose was decreased. Moreover, a chronic vitamin A deficiency slightly increased the incidence and may have reduced the retention time. Hepatic microsomal demethylase activities were not reduced after seven weeks of feeding.

The effects of vitamin A deficiency on MFO activities were examined more extensively by Becking (11), who found that although hepatic N-hydroxylase and N-demethylase activities were reduced in rats fed diets without vitamin A for 20 to 25 days, nitroreductase activity remained unchanged. Microsomal protein levels and cytochrome *c* reductase activities were unaffected but cytochrome P-450 levels were depressed. Becking (11) concluded that the vitamin A deficiency did not affect microsomal protein metabolism; rather he theorized that the deficiency may have affected the structure of the endoplasmic reticulum leading to impairment in the function of the closely integrated MFO system. Roels *et al.* (172) had proposed earlier that vitamin A deficiency could impair the normal structure of this membrane.

Although the effect of vitamin A status on MFO enzyme activities may vary, Genta *et al.* (69) observed a rather specific consequence of such an interaction. They showed that tracheas, which were taken from vitamin A-deficient hamsters and which were incubated *in vitro* with tritium-labeled benzopyrene, incorporated greater quantities of radioactivity into deoxyribonucleic acid. Since 7,8-benzoflavone, an inhibitor of the aryl hydrocarbon hydroxylase system (55, 223), dramatically reduced this incorporation, an impairment in the steady-state levels (formation/degradation?) of the reactive carcinogenic intermediate is clearly indicated. Indeed, as implied earlier, vitamin A status on both the MFO activities and the epoxide hydrase should be examined.

According to Becking (11), MFO activities were significantly depressed in animals where no obvious clinical signs of vitamin A deficiency were apparent, although liver stores were markedly depressed. Since great numbers of people show clinical signs of vitamin A deficiency in many parts of the world (6, 93, 167, 205), particularly in developing countries where more than 90% of the rural children in certain developing countries are known to suffer night blindness (F. Solon, personal communication), the implications of vitamin A status on the MFO system may be especially significant.

2. Vitamins B. Niacin and riboflavin nutrition have been studied because of their requirements for the pyridine nucleotides and the flavoprotein cytochrome P-450 reductase, respectively. Thiamine has been studied because of its ". . . importance . . . in several pathways involving carbohydrate metabolism . . . " (207).

Thiamine deficiency enhances the metabolism of heptachlor and aniline, according to a study by Wade *et al.* (207), but depresses somewhat the metabolism of hexobarbital. They pointed out that currently accepted experimental diets (47), which provide 20 μg of thiamine per day to the rat (far in excess of the amounts shown to reduce heptachlor and aniline metabolism), may result in marked depression in the metabolism of some drugs and that further additions of thiamine may increase this depression. In a later study (79) they found that high thial mine diets, which supplied 2 mg per anima per day, also reduced zoxazolamine me-

tabolism which was correlated, in turn, with an increase in zoxazolamine paralysis. Aminopyrine metabolism was decreased in male but not in female rats. Lower cytochrome P-450 and cytochrome b_5 contents and NADPH-cytochrome P-450 reductase activities were also found. These workers ruled out an effect of the deficiency on alteration of sex hormone levels, starvation, glucose 6-phosphate dehydrogenase activity and/or cofactor availability. They thought that an indirect mechanism may be involved and that possibly the thiamine deficiency exerted its effect through a decrease in *d*-glucose intestinal transport, as shown by the data of Hara *et al.* (83) and consistent with the findings of others (57, 202) that lower dietary glucose levels can increase certain microsomal enzyme activities. More recently Wade *et al.* (209) have suggested that low dietary thiamine appears to enhance the ratio of a new species of microsomal cytochrome analogous to the P_1-450 of Sladek and Mannering (195). The higher binding (ΔA_{max}/mg of protein) and metabolism of aniline, the unaffected hexobarbital oxidase activities, and the higher proportion of P_1-450, suggested to Wade *et al.* (209) a phenomenon analogous to enzyme induction promoted by MC (195). Such a finding merits further study and illustrates the manner in which a study of diet-toxin interactions often leads to information on fundamental MFO enzyme mechanisms.

Riboflavin availability would appear to be an important vitamin requirement for microsomal enzyme activity because of the flavoprotein reductase component. Such an interpretation is supported by the long-standing observation that riboflavin is able to modify the carcinogenicity of certain azo dyes such as 4-dimethylaminoazobenzene (146). Williams *et al.* (224) showed that the azoreductase catalyzing the 4-dimethylaminoazobenzene reaction was present not only in liver microsomes but was also present at even higher levels in the bacteria found in the cecum. Rats maintained on low riboflavin (2 ppm) diets for six weeks exhibited highly significant depressions of activity in both the liver (85–90%) and the cecal contents (15–35%). Unlike most other nutrient deficiencies, the direct addition *in vitro* of riboflavin to the assay mixtures caused restoration of normal activities. These authors called attention to other reports (40, 74, 77) which have shown that the toxicity and biological effects of several drugs and foreign compounds are related to their metabolism by gut microflora and, thus, the intervention of nutrient deficiency with this system could be of considerable importance.

Catz *et al.* (34), with mice of two ages, studied the effect of riboflavin deficiency on the microsomal oxidations of aminopyrine, hexobarbital, aniline, and 3,4-benzpyrene; the reduction of *p*-nitrobenzoic acid; and the conjugations of *o*-aminophenol and *p*-aminophenol. Although hepatic flavin contents were consistently reduced, the effect of the deficiency on these enzyme activities was quite variable. "Oxidative" reactions (aminopyrine, hexobarbital, and aniline) tended to increase (with the exception of hexobarbital in young animals); benzpyrene metabolism was decreased; *p*-nitrobenzoic acid reduction was decreased as in the study by Williams *et al.* (224); and the conjugations of the *o*- and *p*-aminophenols were variable. They did not find agreement between hexobarbital sleep time and hexobarbital metabolism and suggested, as one alternative, that an altered neuronal receptor sensitivity may have been the responsible mechanism. They also cited preliminary work showing that since flavin compounds inhibit aniline, aminopyrine, and benzpyrene metabolism, a deficiency condition could indicate the removal of a flavin-like inhibitor. However, that interpretation was not consistent with their data on the metabolism of benzpyrene, unless, as they suggested, benzpyrene is hydroxylated by a system different from that which oxidizes type I and type II substrates.

Some of the MFO responses to riboflavin deficiency appear to be contradictory. Shargel and Mazel (188) have shown that cyto-

chrome P-450 content and aminopyrine demethylase activity were independent of liver flavin levels but cytochrome *c* reductase activity was depressed during riboflavin deficiency. In this study, the hydroxylation of 3,4-benzpyrene was also depressed during riboflavin deficiency and became greater when the microsomal flavin content was higher. That induction of benzpyrene metabolism is dependent upon a flavoprotein enzyme was also indicated by the observation that the inducibility of benzpyrene hydroxylase by MC-pretreatment increased with an increased microsomal flavin content. These authors have postulated that azo-dyes may be reduced by hepatic microsomal enzymes in three separate pathways. The first is represented by azoreductase contributed by NADPH-cytochrome *c* reductase and is decreased by flavin deficiency; the second is CO-insensitive, is MC-inducible, and is decreased by flavin deficiency; and the third is CO-sensitive, is PB-inducible, and is not affected by flavin deficiency. They concluded that the second pathway depends upon a flavoprotein enzyme since it is decreased by riboflavin deficiency, is induced by MC and can be stimulated by flavins added *in vitro* (187). According to Shargel and Mazel (187), administration of MC caused induction of azoreductase, which was flavin-dependent. However, they also suggested that part of the mechanism of azoreductase induction by MC was due to an alteration in the structure or composition of the microsomal flavoprotein since induction in riboflavin-deficient rats required a minimal flavin level, was not compensated by an increase in microsomal flavin, and augmented flavin mononucleotide stimulation of microsomal azoreductase *in vitro*. Considerably more research is required before the various and interesting postulates can be accepted.

To the authors' knowledge, an examination of niacin deficiency has been limited to the single report by Levy and DiPalma (119). They reported that the deficency produced an increased response to thiopental and suggested that the partial depletion of reduced NADPH may have been responsible.

3. Vitamin C. A consideration of vitamin C deficiency has been limited, of course, to those species (man, monkey, guinea pig) whose requirements must be met *via* their diets. Many years ago, Richards *et al.* (170) and Richards (169) showed that vitamin C-deficient guinea pigs were more sensitive than normal guinea pigs to pentobarbital and procaine. Later, Axelrod *et al.* (8) found that the hydroxylations *in vivo* of acetanilide and aniline were depressed in vitamin C-deficient guinea pigs. Conney *et al.* (42) reported a close parallel between the duration of zoxazolamine paralysis in guinea pigs and reduced zoxazolamine metabolism by liver microsomes caused by vitamin C deficiency. Decreased hydroxylation of acetanilide and coumarin by microsomal preparations from scorbutic guinea pigs has also been observed by Degkwitz and Staudinger (53) and Degkwitz *et al.* (52), respectively. Kato *et al.* (109) examined the effect of vitamin C deficiency on the metabolism of several drugs which are metabolized *via* demethylation reactions and concluded that demethylation activity was much less sensitive than hydroxylation activity to vitamin C deficiency.

The effect of vitamin C deficiency on the O-demethylation of *p*-nitroanisole and the hydroxylation of aniline was studied by Wagstaff and Street (210), who found that the major effect of this dietary deficiency was its impairment of enzyme induction by organochlorine pesticides. The inductive process, which responds to pesticide pretreatment, did not appear to be caused by protein synthesis; presumably some type of activation was involved (210). In any case, these investigators discovered a very interesting phenomenon showing that, in order to permit maximum induction, not only were dietary levels of vitamin C required in excess of that needed to prevent scurvy, but these levels were even in excess of the minimum dietary levels recommended by the National Research Council (153).

Zannoni *et al.* (232) essentially confirmed

the effects of vitamin C deficiency on the depression of drug enzyme activities and found that restoration of normal enzyme activities required six days of vitamin C treatment, even though normal levels of liver ascorbic acid were established within three days. Moreover, significant decreases in enzyme activities were achieved only after liver ascorbic acid levels were reduced to 30% of control. These lag times noted for modification of enzyme activities, either after complete restoration of liver ascorbic acid levels in deficient animals or after highly significant depression of liver ascorbic acid levels in normal animals, require more research. They appear to indicate a rather complex effect *in vivo* of vitamin C on microsomal enzyme activity. For example, several reports (8, 109, 232) have shown that the direct addition of vitamin C to the incubation medium *in vitro* did not restore enzyme activities.

Kato *et al.* (109), Zannoni *et al.* (232), and Wade *et al.* (208) have attempted to determine the mechanism of the vitamin C effect by determination of enzyme kinetic parameters, together with activities of individual enzyme components after the onset of deficiency. Kato *et al.* (109) found no change after 12 days of feeding a deficient diet to 400-g guinea pigs in either the activities of NADPH-cytochrome *c* reductase, NADPH-neotetrazolium reductase, NADH-cytochrome *c* reductase, NADH-ferricyanide reductase, NADPH oxidase, and NADH oxidase, or in the contents of cytochromes P-450 and b_5. Zannoni *et al.* (232) observed decreases in the activity of NADPH-cytochrome P-450 reductase and the content of cytochrome P-450 in 250-g animals fed the deficient diet for 21 days. Leber *et al.* (117) and Wade *et al.* (208) also found depressions of cytochrome P-450 in deficient animals. Because Zannoni *et al.* (232) found atypical aniline-cytochrome P-450 binding spectra in deficient animals, they proposed that the integrity of the microsomal phospholipid membrane associated with cytochrome P-450 may be altered and may require vitamin C for its maintenance, although they did not report any effects of dietary manipulation of vitamin C on phospholipid function. They found that although direct addition *in vitro* of ascorbic acid did not restore the normal spectrum, the more lipophilic ascorbyl palmitate analogue was effective. Zannoni *et al.* (232) also called attention to the demonstration (198, 208) that there is present in microsomes an ascorbic acid-dependent NADH oxidase system, which may be an important auxiliary pathway for drug metabolism in the absence of a functioning microsomal NADPH-cytochrome P-450 reductase system. Such a system may have confounded interpretation of the above cited reports.

Luft *et al.* (128) demonstrated that the amounts of cytochromes P-450 and b_5 could be raised to normal values within 48 hr after resupplementation with vitamin C by the administration of δ-aminolevulinic acid, thus providing evidence for the participation of vitamin C in the biosynthesis of heme. This same research group had earlier ruled out the possibility that cytochrome levels were raised by vitamin C administration through an inhibition of cytochrome decomposition (92, 117, 128). Equally active to ascorbate is 5-oxo-D-gluconate in supporting cytochrome levels and D-arabinoascorbate is partially active (51). That the 5-carbon D-arabinoascorbate was at least partially active indicates a certain heretofore unknown specificity for ascorbate in the biosynthesis of heme (51). Further studies are indicated to establish what correlations may exist between the synthesis of δ-aminolevulinic acid, vitamin C deficiency, and the levels of cytochromes P-450 and b_5.

Burns *et al.* (26) many years ago suggested that an increase in urinary ascorbic acid excretion in rats was indicative of MFO induction since drugs possessing completely unrelated chemical and pharmacological properties could stimulate ascorbate excretion (25, 26, 43, 120). Because man, monkeys, and guinea pigs are unable to synthesize ascorbic acid due to the absence of gulonolactone oxidase (E.C. 1.1.1.20), the

urinary excretion of glucaric acid, an intermediate on the pathway prior to the missing enzyme, was suggested by Hall *et al.* (82) to be a potentially useful MFO induction index in these species. Evidence in support of this suggestion was provided by Chadwick *et al.* (38) who showed that pesticide (DDT + lindane) administration stimulated glucaric acid excretion in rats after three days on the pesticide-containing diets.

4. Vitamin E. Dietary deficiency of vitamin E reduces rat liver MFO activities (14, 30, 94, 95, 197) but, like most other nutritional deficiencies, the specific biochemical basis for this effect remains obscure.

One explanation which has been considered is that, during the deficiency condition, the synthesis of heme and hemoproteins is impaired (134, 164, 222). The enzymatic defect in the rat has been purported to be δ-aminolevulinic acid dehydratase in liver and δ-aminolevulinic acid synthase in bone marrow (151). Although decreased levels of cytochromes b_5 and P-450 have been observed in one laboratory (28, 86, 151, 152), other investigators could not confirm these results (35, 94).

Another possible role for vitamin E is that represented by the work of Diplock and colleagues (36, 60, 61), which led to the proposal (62) that a primary function *in vivo* of this vitamin may be its inhibition of the oxidation of selenide-containing proteins. It was further suggested by these authors that the selenide may form a part of the active center of an uncharacterized class of non-heme proteins which are located in the endoplasmic reticulum and which may function in microsomal electron transport (62), although no requirement for a non-heme iron has been shown. Evidence in support of this role is suggested by the fact that in rats deprived of vitamin E, or of vitamin E and selenium, PB had no effect on the incorporation of radiolabeled selenium but when vitamin E was provided, there was a large increase in the incorporation of selenium. That selenium is essential for MFO induction is shown by Burk *et al.* (23) who observed that PB induction of microsomal cytochrome levels was markedly impaired in selenium-deficient rats whereas basal levels of cytochromes P-450 and *b*, as well as the inducibility of the NADPH-cytochrome *c* reductase, were not affected.

B. Minerals

Deficiencies in dietary calcium, magnesium, iron, iodine, zinc, selenium, and copper affect in varying degrees the microsomal MFO system.

1. Calcium. Dingell *et al.* (59) found that rats maintained on calcium-deficient diets for 40 days, but not for 33 days, exhibited microsomal MFO activities for the oxidations of hexobarbital and aminopyrine and the reduction of *p*-nitrobenzoic acid which were only 30 to 50% of those of the calcium-supplemented animals. Although the depression of metabolism for hexobarbital did not occur until 40 days of feeding, sleeping times were found to be prolonged at 33 days, thus suggesting an earlier involvement of the deficiency at sites other than the microsomal system.

2. Magnesium. That hypomagnesemia often coexists with hypocalcemia (130) prompted Becking and Morrison (13) to study the effects of magnesium deficiency on the MFO system. They found that magnesium deficiency caused a much earlier depression of enzyme activity (11–14 days) than did calcium deficiency, although the magnesium deficiency lowered the rates of metabolism only for aniline hydroxylation (*in vitro*) and (*in vivo*) and aminopyrine N-demethylation (*in vitro*) and not for the reduction of *p*-nitrobenzoic acid (*in vitro*) or the oxidation of pentobarbital (*in vitro*). Cytochrome P-450 levels were also reduced. The effect of magnesium deprivation was specific and was neither related to decreased food utilization nor to starvation effects; moreover, magnesium repletion restored the depressed activities. Peters and Fouts (162, 163) concluded from studies *in vitro* that magnesium activation of microsomal metabolism may be the result of an effect of

the magnesium in altering the microsomal membrane structure which, in turn, allows a more rapid interaction of the enzyme and substrate, thereby enhancing the flow of electrons from the electron donor through several of the various chain components to the substrate. Magnesium activation of NADPH oxidase and NADPH-cytochrome *c* reductase activities differed qualitatively and quantitatively; the NADPH oxidase activation kinetics correlated with the microsomal metabolism of aminopyrine, aniline, benzphetamine, *p*-chloromethylaniline, hexobarbital, and zoxazolamine whereas the reductase activation kinetics correlated well with nitro- and azoreductases. Such differing effects may explain the contrasting effects of dietary magnesium deficiency on aminopyrine and hexobarbital metabolism as opposed to the metabolism of *p*-nitrobenzoic acid reported by Becking and Morrison (13), but would not appear to account for the absence of an effect of dietary magnesium deficiency on pentobarbital metabolism (13).

3. Iron. Catz *et al.* (34) reported that chronic iron deficiency in adult mice resulted in statistically significant increases in hexobarbital side chain oxidation, aminopyrine N-demethylation, and microsomal cytochrome b_5 concentration, but no significant change either in sleeping times or in cytochrome P-450 content. Also, no significant changes were noted either in the MFO metabolism of aniline, 3,4-benzopyrene, and *p*-nitrobenzoic acid or in the conjugations of the *o*- and *p*-aminophenols. Although the hematocrit values and hemoglobin concentrations in whole blood were reduced to levels only 35 to 50% of that of controls, they believed that the microsomal iron was still not sufficiently reduced to cause an effect, although the latter determinations were not undertaken. In support of this postulation, Dallman (49) had earlier pointed out the difficulty in producing intracellular iron deficiency in liver and other organs.

In contrast to these results are those of Becking (10) who found that aniline metabolism in rats is increased both *in vitro* and *in vivo* as soon as 18 days after feeding the deficient diet or when hemoglobin levels were reduced to approximately 65% of the controls. Aminopyrine N-demethylation was also increased, but at a time when iron deficiency was somewhat more advanced. Pentobarbital metabolism *in vivo* and *in vitro* was not changed, although deficient animals did exhibit longer sleeping times than did the control animals. *p*-Nitrobenzoic acid reduction likewise was not altered. Similar to Catz *et al.* (34), they showed that cytochrome P-450 levels remained unchanged. Cytochrome *c* reductase activities were increased after advanced deficiency developed. In general, MFO activities are either increased or left unaltered with iron deficiency.

Wills (226) attempted to explain the fundamental mechanism associated with iron status by following certain microsomal enzyme properties after injections into mice of large doses of iron as an "iron-dextran" complex. The iron injections elicited an increase in the rate of NADPH-dependent lipid peroxidation; this was closely correlated with reduced rates of oxidative demethylation of aminopyrine or of *p*-chloro-N-methyl aniline. Lipid peroxidation, enhanced by iron overload, was thought to lead to a degradation of endoplasmic reticulum membranes which was associated, in turn, with diminished MFO hydroxylation (225) and demethylation (226) activities. Presumably, a dietary deficiency may have the unusual property of stabilizing the membranes against peroxidative attack and thereby allow increased MFO enzyme activities. Wills (226) has warned that human patients, heavily loaded with iron, are much more vulnerable to the formation of lipid peroxide than normal subjects. The precise participation of iron in microsomal enzyme metabolism remains an open question, however, since Wills (226) interprets his findings in terms of the presence in microsomes of non-heme iron although Montgomery *et al.* (150) provided data showing that, if a non-heme iron-sulfur protein is present in hepatic

microsomes, it contributes less than 10% of the total iron.

4. Trace minerals. That thyroid hormone appears to regulate hepatic levels of flavins (171) suggested to Catz *et al.* (34) that a dietary deficiency of iodide may affect microsomal drug metabolism. Thyroid hormone deficiency, for example, has been shown to depress the metabolism of both types I and II substrates (22, 108, 203). However, apparent iodide deficiency in mice given propylthiouracil to block thyroid-stimulating hormone caused large increases in microsomal enzyme metabolism of aminopyrine, hexobarbital, aniline, and 3,4-benzopyrene. *p*-Nitrobenzoic acid reduction and *p*-nitrophenol conjugation were not affected. No explanation was offered for these effects.

Zinc deficiency may cause alterations of certain microsomal MFO activities in rats but only after prolonged feeding (12). The rate of oxidation *in vitro* of pentobarbital was significantly reduced after 37 days on the deficient diet; the decreased metabolism of aminopyrine and *p*-nitrobenzoic acid were seen only after 44 and 58 days, respectively. On the other hand, no alteration of either aniline or zoxazolamine metabolism was seen even after 58 days on the deficient diet. Cytochrome P-450 levels were reduced after 42 days. These authors considered that their animals exhibited modest deficiency symptoms after 14 days and severe symptoms after 50 days. They suggested that the lower enzyme activities were most likely due to an unidentified mechanism associated with lower cytochrome P-450 levels.

Selenium deficiency is generally associated with vitamin E status; together, these were considered earlier (23, 28, 35, 36, 60–62, 86, 151, 152, 197).

Moffitt *et al.* (148) showed that the PB induction of MFO activities resulted in marked increases in rat liver microsomal copper contents which were associated with altered microsomal enzyme activities. Moreover, since other reports had also shown microsomal copper contents to be influenced by the administration of microsomal inducers and inhibitors (67, 90, 116), Moffitt and Murphy (149) undertook a systematic study on the optimal levels of dietary copper eliciting maximal MFO activities. Both excesses and deficiencies of copper resulted in diminished enzyme activities. Only benzopyrene hydroxylase remained unaffected. On the other hand, copper deficiency did not prevent the induction of liver MFO activity by PB. Factors which influence the involvement of copper in MFO activities most likely do so by a redistribution of tissue and whole body copper.

Ho and Elliott (91) examined a very practical problem to determine the role of dietary copper supplementation on the softening of depot fat (204) and the consequent decrease in oxidative stability of pork (5). Significant increases in stearoyl-CoA desaturase activities were observed in hepatic and adipose microsomes of copper-supplemented pigs. They suggested that this would cause the observed replacement of the higher-melting sterate to the lower-melting oleate.

IV. General Nutritional Status

General inanition resulting from starvation, restricted feeding, or protein-calorie malnutrition has also been considered to be an important nutrient-toxin interaction. As pointed out earlier, independent effects and precise mechanisms associated with this type of imbalance are particularly difficult to evaluate. These are therefore discussed in a more general manner.

Numerous reports have appeared on the effects of starvation in hepatic drug-metabolizing enzyme activities but, to date, the mechanism involved remains obscure. The interaction remains important, however, since fasting is frequently a prerequisite to certain schedules of chemotherapy. Dixon *et al.* (63) in 1960 were the first to show that starvation of male mice for 36 hr depressed hepatic microsomal drug metabolism measured both *in vitro* and *in vivo*. Oxidative pathways were affected more than reductive pathways. Roth and Bukovsky (182) observed that rats starved overnight (16 hr)

showed complete loss of meperidine N-demethylation *in vitro*, which could be restored by the addition of glucose 6-phosphate or isocitrate; such restoration was attempted but was not possible in the studies of Dixon *et al.* (63). Roth and Bukovsky (182) also found that microsomes isolated from starved animals possessed an inhibitor which could be reversed by high levels of NADP and nicotinamide. This latter observation suggested the presence of a nucleotidase cleaving the NADP. In 1962, Kato *et al.* (102) showed that the *in vivo* metabolism of meprobamate was increased in female rats fasted for 36 hr. However, Kato and Gillette (103) later reported that these apparently contrasting results cited above might be explained in part by sex differences (63, 102, 182). Enzymes having the greatest sex difference in activities are impaired in fasted male rats but enhanced in fasted female rats. Since they found that starvation impairs the microsomal enzymes that are markedly sex-dependent and does not significantly alter the enzymes which are less sex-dependent, Kato and Gillette (103) tentatively suggested that starvation may cause impairment by interfering with the stimulatory effects of androgenic steroids.

In an effort to determine the mechanism by which starvation exerts its effects, Kato (100) found that the content of cytochrome P-450, as well as the activity of NADPH-cytochrome *c* reductase, were reasonably well correlated with enzyme activities. Starvation of female rats for 72 hr increased the metabolism of hexobarbital and aminopyrine, whereas refeeding a standard chow diet or sucrose rather quickly depressed activities, even below the initial control levels. Later, Kato and co-workers (101, 103, 104, 106, 110) presented evidence to support the hypothesis that starvation decreases the sex-dependent drug-metabolizing enzymes most likely through impairment of an androgen-dependent activation mechanism. In a further test of this hypothesis, Kato and Onoda (105) showed that the decrease in the capacity of cytochrome P-450 to interact with hexobarbital and aminopyrine in liver microsomes of fasted male rats was correlated with decreases in the activities of hexobarbital hydroxylation and aminopyrine N-demethylation. Also, the spectral dissociation constant, K_s, and the K_m for metabolism of these substrates increased after starvation. Since the administration of androgen decreases both K_s and K_m values for hexobarbital and aminopyrine (105), they concluded that these data supported the theory that starvation impaired the action of androgen on the K_s and K_m values as well as on the ΔOD_{max} and V_{max} values.

Gram *et al.* (76) conducted further studies on the effect of 72-hr starvation of male and female rats upon the kinetic properties associated with the N-demethylation of ethylmorphine and the hydroxylation of aniline. Like Kato and Gillette (104), their findings showed a pronounced sex difference in the response to starvation in rats; males tend to be highly responsive to the effects of food deprivation, whereas females are relatively unresponsive. However, they observed a rather complex array of kinetic changes after starvation, including increases, decreases, and no change in the K_m and V_{max} kinetic parameters, depending on both the sex and the substrate type. Other than the sex dependence, they were unable to advance a specific suggestion on the mechanism of the effect of starvation. The rather pronounced changes in the K_m values after starvation indicated to these authors that the effects of a variable such as starvation on microsomal enzyme activities must be interpreted with caution since different physiological effects would be observed at different substrate concentrations.

Further complicating the starvation effect is the fact that it is species dependent. Furner and Feller (68) attempted to determine the effect of species on starvation for 1 to 3 days in a study including the rat, the guinea pig, and the mouse. Metabolism of aniline was stimulated in mice, rats, and guinea pigs; *p*-nitroanisole metabolism was increased only in mice and rats; and the metabolism of

ethylmorphine was stimulated by starvation only in mice.

More recently, Greim (78) found that in male rats, starvation permitted an increased induction of cytochrome P-450 in response to PB administration; this increase was due to a reduced breakdown of the cytochrome. They concluded that during starvation this increase is accelerated by stabilization of the endoplasmic reticulum components. Brodeur and Lambert (20) confirmed these data by showing that a five-day period of starvation in rats markedly potentiates the inducing effect of a simultaneous pretreatment with PB on the amount of P-450, as well as on the activity of aniline hydroxylase and aminopyrine N-demethylase.

Bock *et al.* (15) warned that the method of expression of cytochrome P-450 contents must be carefully considered before making interpretations. For example, although three days of fasting of male rats caused an increase in cytochrome P-450 when expressed per g of liver or per mg of microsomal protein, there really was a decrease when expressed on a whole liver basis. They also clearly showed that the induction of cytochrome P-450 by PB is markedly reduced in fasted animals, when the P-450 is expressed on a whole liver basis. Their claim that the decreased cytochrome P-450 contents (15) correlated well with the report of Kato and Gillette (103) is in error, however. Kato and Gillette (103) showed more increases than decreases; moreover, their data were expressed per g of liver.

Another factor which may be responsible for the increases in microsomal enzyme activities generally seen with starvation could be the stress associated with this condition. For example, Mgbodile (142) showed that when consumption of a 20% casein semipurified diet by growing rats is restricted to approximately 60% of control animals for 14 days, the serum levels of deoxycorticosterone were significantly increased; deoxycorticosterone levels were indicative of stress.

More careful analysis of the effects of starvation on MFO activity is required before an assessment of clinical, epidemiological, and/or therapeutic value can be made, particularly with respect to the methods of expressing MFO activities. The activities of *in vivo* significance must eventually be based on 1) substrate concentrations *in vivo* and 2) enzyme activities expressed on the same weight basis (enzyme units/kg of body weight) as that used for the administration of foreign compounds. For example, findings of reduced tumor yield during restriction of food intake (80) could be accounted for by the increases in the activities of MFO enzymes which metabolize carcinogens (1, 66, 96, 145, 161, 192, 206, 215, 216, 221, 230), and which are presumably increased in response to the restricted food intake. However, the relationship must be more complex than this since an increase in MFO activities could also lead to higher levels of the proposed ultimate carcinogens such as the aryl hydrocarbon epoxides. Information on *in vivo* enzyme activities for the *in vivo* substrate concentrations in question would be important; also activities of other enzyme activities which affect the disposition of the ultimate carcinogens should be considered.

V. Natural *vs.* Synthetic Diets

When natural dietary ingredients are used rather than synthetic ones, there is often an increase in MFO activities (214) and a decrease in tumor yield. This type of dietary influence should not be considered as a nutrient-toxin interaction since the major effect of the diet is owed to certain foreign residues and vegetable components which are not known to be nutrients. It is, however, a very interesting property of natural diets from the point of view expressed by Wattenberg who has speculated (213, 214) that the MFO system may provide a broad range survival mechanism since its inducibility by the consumption of natural ingredients tends to decrease tumor yield (1, 66, 96, 145, 161, 192, 206, 215, 216, 221, 230). Not only is the hepatic MFO system a possibility for this property, but also included are the MFO activities found in the tissues of other major

portals of body entry, *i.e.*, the lung and intestine (214). In addition to unnatural dietary residues such as polycyclic hydrocarbons, phenothiazines and phenylthiazoles, the principal group of naturally occurring substances appears to be the flavones found in a wide variety of plant materials. These relationships have been succinctly reviewed by Wattenberg (214).

Further studies by Zampaglione and Mannering (231) have shown that the cytochrome P-450 system is the normal enzyme system in liver and is replaced in large part by the cytochrome P_1-450 system upon the administration of MC. The P_1-450 system is present much more extensively in the portals of entry (intestine, skin, lung) where its induction functions in the biotransformation of noxious exogenous compounds, particularly those causing its induction.

VI. Concluding Remarks

A clearer understanding of toxin metabolism which is altered by nutrient intakes is of considerable importance. It is not so much the possibilities represented by the severe nutrient deficiencies presented in many of the laboratory investigations reviewed here as it is the fact that marginal nutritional deficiencies are extensive throughout the world and these less severe deficiencies could easily represent major effects when considered in terms of total cancer incidence or in terms of more subtle and as yet undefined illnesses associated with toxicological insult. In addition, more effective chemotherapy may result from a careful analysis of the nutritional status of the patient.

From an experimental point of view, more knowledge in this area would allow greater reproducibility in toxicological evaluation procedures. Also, experimental animals subjected to specified nutritional conditioning provide good models for fundamental studies on toxicological mechanisms.

The chief recommendation which we wish to propose is that a more comprehensive evaluation of nutrient intervention in *all* reactions involved in toxin metabolism schemata should be pursued. The control mechanisms, together with the interrelationships of such metabolic reactions, have barely been considered up to the present, and a clearer understanding of all such reactions relative to nutrient intake would be of considerable practical value.

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