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EFFECTS OF VITAMIN A DEFICIENCY ON HEPATIC AND EXTRAHEPATIC MIXED-FUNCTION OXIDASE AND EPOXIDE-METABOLIZING ENZYMES IN GUINEA PIG AND RABBIT

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Abstract—Male guinea pigs and male rabbits were fed a vitamin A deficient diet for 9 weeks and for 12 weeks respectively. Hepatic levels of vitamin A were significantly reduced in the vitamin A deficient animals. The activities of some xenobiotic-metabolizing enzymes were measured in the liver, lung and small intestine. Aryl hydrocarbon hydroxylase, aniline hydroxylase, and 7-ethoxycoumarin deethylase activities were decreased in the vitamin A deficient guinea pig liver. However, in the guinea pig small intestine, aniline hydroxylase, 7-ethoxycoumarin deethylase, aminopyrine demethylase, and aryl hydrocarbon hydroxylase specific activities were increased. In rabbits, vitamin A deficiency decreased hepatic aniline hydroxylase and 7-ethoxycoumarin deethylase activities but increased intestinal aminopyrine demethylase activity. Enzyme activities in lung were not altered by vitamin A deficiency in guinea pig or rabbit. Microsomal epoxide hydrase and microsomal supernatant glutathione *S*-transferase activities in the three tissues of both species were not altered by vitamin A deficiency.

The cytochrome P-450-dependent MFO system localized in the microsomal fraction of liver and several extrahepatic tissues metabolizes a wide variety of xenobiotics and endogenous substrates. This enzyme system is responsible for the activation of various olefinic and aromatic hydrocarbons to intermediary alkene and arene oxides [1, 2]. Microsomal epoxide hydrase converts alkene and arene oxides to diols and *trans*-dihydrodiols respectively [3]. Epoxides are also conjugated with glutathione by a family of soluble enzymes, the glutathione *S*-transferases [4, 5]. This reaction appears to be a major detoxication pathway.

Certain alkene and arene oxides are hepatotoxic [6], carcinogneic [6, 7], and mutagenic [8, 9]. The dihydrodiols resulting from the hydration of epoxides are normally weak or inactive as cell-transforming agents [10] and mutagens [2, 7, 11]. However, in the case of BP, the formation of benzo(a)pyrene 7,8-dihydrodiol by epoxide hydrase is an activation step, since the diol formed is the precursor for the isomeric benzo(a)pyrene 7,8-dihydrodiol-9,10-epoxides, which are potent mutagens and carcinogens [12, 13]. Similar activation reactions have been described for various other polycyclic aromatic hydrocarbons. The carcinogenicity of polycyclic aromatic hydrocarbons apparently depends, to some extent, on the balance between activation and inactivation of parent compounds by MFO and secondary metabolizing enzymes respectively.

Nutritional status is one of several factors which can alter the activities of microsomal MFO enzyme activities, a subject that has been reviewed extensively [14–16]. However, the influence of nutritional factors on epoxide hydrase and glutathione *S*-transferase activities has not been thoroughly investigated. The role of vitamin A in carcinogenesis has been studied by several investigators [17–24]. In these studies, the enhanced susceptibility of vitamin A deficient animals to chemical carcinogens and the protective effect of vitamin A on cancer development in experimental animals have been demonstrated.

The present investigation was designed to study the effects of vitamin A deficiency on the activities of a number of MFO enzyme activities, including AHH (involved in metabolic activation of BP), and of epoxide hydrase (involved in both activation and inactivation of BP metabolites) and glutathione *S*-transferase (involved in activation of BP metabolites) in liver, lungs, and small intestine of the guinea pig and rabbit.

MATERIALS AND METHODS

Animals. Male guinea pigs (Hartley strain, weighing 250 ± 20 g) and male New Zealand rabbits (weighing 1 ± 0.2 kg) were obtained from Charles River Breeding Laboratories, Wilmington, MA, and from Dutchland Rabbitry, Denver, PA, respectively. Animals were divided into three groups and fed a complete semi-purified diet (Takland Test Diets, Madison, WI.) with or without retinyl palmitate. In vitamin A supplemented diets, the concentration of retinyl palmitate was 35 I.U./g of guinea pig diet and 15 I.U./g of rabbit diet. The supplemented diets were given in restricted amounts (pair-fed controls) to equal the food intake of

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‡ Abbreviations used are: MFO, mixed-function oxidase; BP, benzo(a)pyrene AHH, aryl hydrocarbon hydroxylase; and BPO, benzo(a)pyrene 4,5-oxide.

animals fed the vitamin A deficient diet. The guinea pigs were fed for 9 weeks and the rabbits were fed for 12 weeks. All animals were killed by gassing them with CO_2 in an enclosed chamber. The livers, lungs, and first 30 (for guinea pig) or 60 cm (for rabbit) of the small intestine starting from the pylorus were quickly removed. The intestines were flushed with physiological saline and then the mucosa was scraped for the preparation of microsomes. Microsomes were prepared from liver, lung and intestinal mucosa according to procedures reported previously [25].

Enzyme assays. Microsomal protein was assayed by the method of Lowry *et al.* [26]. Microsomal cytochrome P-450 content was measured as described by Omura and Sato [27].

The activities of the following enzymes were determined in microsomal fractions: aminopyrine demethylase, by a method based on measuring the formaldehyde formed [28]; aniline hydroxylase, as described by Chhabra *et al.* [29]; AHH, by the method of Hansen and Fouts [30]; 7-ethoxycoumarin deethylase, by the method of Ullrich and Weber [31], as modified by Pohl *et al.* [32]; and epoxide hydrase (using BPO as substrate), by the thin-layer chromatographic procedure of Jerina *et al.* [33]. Glutathione *S*-transferase (using BPO as substrate) activity was determined in the microsomal supernatant fraction by the method of James *et al.* [34].

Concentrations of vitamin A in the liver were determined by the method of Bayfield [35, 36].

RESULTS

Body weight and hepatic vitamin A content of guinea pigs and rabbits. Guinea pigs or rabbits fed a vitamin A deficient diet for 12 weeks had body weight similar to those of the corresponding pair-fed controls. The levels of hepatic vitamin A in both guinea pigs and rabbits fed the deficient diets were significantly lower than the pair-fed controls (Table 1).

Hepatic, pulmonary and intestinal drug metabolism in guinea pigs. Specific xenobiotic-metabolizing enzyme activities and cytochrome P-450 content in liver remained unchanged after feeding the vitamin A deficient diet for 3 weeks (data not shown). However, by 9 weeks, aminopyrine demethylase, aniline hydroxylase, and 7-ethoxycoumarin deethylase, and AHH activities, and cytochrome P-450 content were significantly reduced in the vitamin A deficient animals as compared to controls. Epoxide hydrase and glutathione *S*-transferase activities were not altered by vitamin A deficiency. Since no significant differences in the xenobiotic-metabolizing enzyme activities studied were observed between pair-fed and *ad lib.* control animals, the data on the latter are not presented.

None of the xenobiotic-metabolizing enzyme activities in the lung was significantly altered by vitamin A deficiency (Table 2). In the small intestine, aminopyrine demethylase, aniline hydroxylase, 7-ethoxycoumarin deethylase, and AHH activities were significantly higher in guinea pigs fed the vitamin A deficient diet (Table 2).

Drug metabolism in rabbit liver, lung, and small intestine. Rabbits fed the vitamin A deficient diet for 12 weeks had significantly lower hepatic aniline hydroxylase, AHH and 7-ethoxycoumarin deethylase activities than the pair-fed controls (Table 3). On the other hand, intestinal aminopyrine demethylase activity was significantly increased in the vitamin A deficient rabbits. Enzyme activities in lung were not altered by vitamin A deficiency. The vitamin A deficient diet also did not produce an alteration in the epoxide hydrase or glutathione *S*-transferase activity in any tissue examined. Partial food restriction had no effect on hepatic, pulmonary or intestinal xenobiotic-metabolizing enzyme activities (data not shown). The food intake of vitamin A deficient and pair-fed rabbits was approximately 87 percent of *ad lib.* controls. This was also true for guinea pigs.

DISCUSSION

Although the role of vitamin A on chemical carcinogenesis has been demonstrated by several investigators [17–24], the underlying mechanisms are not well understood. The protective effect of vitamin A on carcinogenesis seems to be related to the function of the vitamin in the control of normal differentiation of epithelial tissues. However, other mechanisms may also be of some significance. For example, some carcinogens stimulate guanylate cyclase and increase cellular cyclic guanosine 3′:5′-monophosphate [37], and retinol has been found to suppress this activation process [38].

This study shows that vitamin A deficiency has different effects on AHH activity depending upon the animal species and the tissue used. The AHH activities

Table 1. Effects of vitamin A deficiency on growth and liver vitamin A content of guinea pig and rabbit *

Dietary group	Weeks of test	Final body weight (g)	Liver vitamin A (μg/g)
Guinea Pig			
Deficient	9	531 ± 18 (14)	0.5 ± 0.1† (4)
Pair-fed control	9	522 ± 13 (13)	145 ± 31 (4)
Rabbit			
Deficient	12	2853 ± 93 (8)	1.3 ± 0.2† (4)
Pair-fed control	12	2694 ± 101 (7)	71 ± 4 (4)

* Values are means ± S.E. (number of animals).
† Significantly different from pair-fed control, $P < 0.05$.

Table 2. Effects of vitamin A deficiency on drug metabolism in guinea pig liver, lung and small intestine*

Organ and dietary group	P-450 content	Enzyme activities					
		AMD	ANH	AHH	7-ECD	EH	GST
Liver							
A-deficient	0.55 ± 0.11†	3.20 ± 0.70†	0.40 ± 0.10†	830 ± 99†	0.33 ± 0.13†	13.10 ± 0.70	82 ± 7
Pair-fed control	0.90 ± 0.17	5.80 ± 0.30	0.80 ± 0.03	1722 ± 303	0.69 ± 0.06	13.80 ± 0.80	86 ± 5
Lung							
A-deficient	0.17 ± 0.04	0.70 ± 0.10	0.20 ± 0.01	75 ± 13	0.15 ± 0.02	0.53 ± 0.04	5.0 ± 0.6
Pair-fed control	0.10 ± 0.02	0.90 ± 0.20	0.25 ± 0.04	97 ± 19	0.28 ± 0.05	0.49 ± 0.03	5.1 ± 0.9
Small intestine							
A-deficient	0.23 ± 0.03	0.98 ± 0.11†	0.07 ± 0.01†	185 ± 24†	0.07 ± 0.01†	5.4 ± 0.30	24.0 ± 2.8
Pair fed control	0.16 ± 0.02	0.59 ± 0.04	0.04 ± 0.01	118 ± 19	0.05 ± 0.01	5.3 ± 0.40	24.1 ± 3.9

* Values are expressed as means ± S.E. Tissues were pooled from four animals (N = 4 individual pooled samples). The following abbreviations were used: P-450, cytochrome P-450; AMD, aminopyrine demethylase; ANH, aniline hydroxylase; AHH, aryl hydrocarbon hydroxylase; 7-ECD, 7-ethoxycoumarin deethylase; EH, epoxide hydrase; and GST, glutathione *S*-transferase, P-450 content is expressed as nmoles/mg of microsomal protein. Enzyme activities are expressed as nmoles product/mg of microsomal protein/min except for AHH and GST. AHH is expressed as relative fluorescence units/mg of microsomal protein/min. GST is expressed as nmoles conjugate formed with benzo(a)pyrene 4,5-oxide/min/mg of supernatant protein.

† $P < 0.05$, from pair-fed controls.

Table 3. Effects of vitamin A deficiency on drug metabolism in rabbit liver, lung and small intestine*

Organ and dietary group	P-450 content	Enzyme activities					
		AMD	ANH	AHH	7-ECD	EH	GST
Liver							
A-deficient	0.52 ± 0.12	4.20 ± 0.87	0.20 ± 0.02†	319 ± 70†	0.27 ± 0.02†	7.82 ± 0.54	6.61 ± 1.70
Pair-fed control	0.64 ± 0.03	5.60 ± 0.60	0.39 ± 0.05	691 ± 102	0.46 ± 0.06	9.16 ± 0.69	5.45 ± 0.45
Lung							
A-deficient	0.28 ± 0.03	1.90 ± 0.13	0.26 ± 0.04	77 ± 2	0.46 ± 0.10	0.45 ± 0.03	0.67 ± 0.05
Pair-fed control	0.29 ± 0.03	2.40 ± 0.19	0.32 ± 0.06	85 ± 3	0.66 ± 0.06	0.45 ± 0.02	0.67 ± 0.05
Small intestine							
A-deficient	0.33 ± 0.02	0.44 ± 0.03†	0.14 ± 0.01	65 ± 2	0.06 ± 0.01	6.79 ± 0.30	2.32 ± 0.16
Pair-fed control	0.35 ± 0.02	0.31 ± 0.04	0.16 ± 0.04	53 ± 6	0.06 ± 0.01	7.01 ± 0.39	1.97 ± 0.27

* Abbreviations and enzyme activities are the same as in Table 2 except that the issue were not pooled and the data are derived from four individual animals.

† $P < 0.05$, from pair-fed control.

in rabbit intestine were not affected, but were increased in guinea pig small intestine. The guinea pig liver AHH activities were decreased, showing tissue specific differences within the same species. Vitamin A deficiency had no effect on epoxide hydrase or glutathione *S*-transferase activity in all tissues studied in both the rabbit and the guinea pig. These observations indicate that vitamin A deficiency may influence the oxidative metabolism of polycyclic hydrocarbons. Further work is being undertaken currently to determine whether hypervitaminosis A could alter the metabolism of BP.

The activities of MFO enzymes other than AHH were also affected by vitamin A deficiency and were species- and tissue-dependent. Intestinal aniline hydroxylase and intestinal 7-ethoxycoumarin deethylase activities were increased by vitamin A deficiency in guinea pig but not in rabbit. It is possible that these species differences in MFO activities (including AHH activity) in response to vitamin A deficiency could influence the susceptibility of various species to chemical toxicity.

There is a lack of information on the effect of nutritional deficiencies on lung xenobiotic-metabolizing enzymes. In our study, the pulmonary enzymes were insensitive to vitamin A deficiency. Similarly, vitamin C deficiency has been shown to affect glutathione *S*-transferase activity in liver and kidney of guinea pig but not in lung [39]. Further study is indicated in this area since vitamin A deficiency exists in certain populations [40] and some members of malnourished groups may also be concomitantly exposed to airborne pollutants.

Investigations on the effects of dietary factors on intestinal xenobiotic-metabolizing enzymes have focused mainly on inducers found or included in the diet [30, 40–42]. Less attention has been focused on the role of nutritional deficiencies in intestinal metabolism of foreign compounds although toxic chemicals may enter the body with ingested food and interact with the small intestine. In this study, we found that the ability of the small intestine to metabolize xenobiotics could be affected by vitamin A deficiency. In the vitamin A deficient guinea pig, intestinal AHH, aminopyrine demethylase, aniline hydroxylase and 7-ethoxycoumarin deethylase activities were increased. Enhanced metabolism of xenobiotics by small intestine in a vitamin A deficient environment could possibly modify the relative amounts of parent compound and metabolites to be transported to other tissues from the small intestine. For instance, it has been reported that changes in intestinal metabolism of phenacetin can influence the biavailability of phenacetin when administered orally [42, 43].

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