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THE EFFECT OF DIETARY LIPIDS AND ANTIOXIDANTS ON THE ACTIVITY OF EPOXIDE HYDRATASE IN THE RAT LIVER AND INTESTINE

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Abstract—The effect of varying the fatty acid composition of the lipid components of the diet on the activity of epoxide hydratase in the rat liver and intestinal mucosa has been studied. Feeding a 10% cod liver oil diet (containing 18% $C_{20:5}$ and 11% $C_{22:6}$) resulted in a 3-fold increase in epoxide hydratase activity in the liver and a 1.6-fold increase in the intestine compared to rats fed a fat-free diet. The activity of epoxide hydratase in rats fed a cod liver oil diet was significantly greater than that for the group fed a lard diet (containing mainly saturated and mono-unsaturated fatty acids) containing the same quantity of vitamin E. Thus, the enhancing effect of the cod liver oil diet was due to the polyunsaturated fatty acids in this oil. Dietary corn oil (58% $C_{18:2}$) also stimulated epoxide hydratase activity in the liver but not in the intestine. Vitamin E levels of up to 500 mg/kg diet were ineffective at inducing epoxide hydratase activity in both the liver and intestine. Significant changes in the fatty acid composition of hepatic and intestinal microsomes took place when rats were fed diets of different fatty acid composition. These changes were such that the proportions of polyunsaturated fatty acids in the microsomal fractions reflected the amounts of these fatty acids in the dietary fat. Hepatic epoxide hydratase activity was found to be positively correlated to the proportion of polyunsaturated fatty acids in the microsomal fractions of the liver.

Epoxide hydratase catalyses the hydration of a wide range of arene epoxides, formed by the cytochrome P-450 monooxygenase system, to less chemically reactive *trans*-dihydrodiols [1]. While this can be considered to be a detoxification pathway, certain *trans*-dihydrodiols (e.g. benzo(*a*)pyrene 7,8-diol) can be further oxidised by the monooxygenase system to highly mutagenic and carcinogenic diol-epoxides [2]. Thus epoxide hydratase plays a complex role in the process of chemical mutagenesis and carcinogenesis induced by polycyclic aromatic hydrocarbons.

Epoxide hydratase is predominantly a microsomal enzyme which is closely associated with the cytochrome P-450 monooxygenase system [3]. Hepatic epoxide hydratase activity is increased by pretreatment of animals with phenobarbitone but is only slightly affected by polycyclic aromatic hydrocarbons [4]. Antioxidants are inducers of epoxide hydratase. Feeding a diet containing 0.75% 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) to rats has been found to result in a 3-fold enhancement of hepatic epoxide hydratase activity [5] and 0.1% 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) or ethoxyquin (EQ) in the diet significantly increased epoxide hydratase activity in the rat liver [6].

We have previously shown that the activity of the aryl hydrocarbon monooxygenase system in the rat liver and intestinal mucosa is enhanced when diets containing polyunsaturated fats are fed [7–9]. The rate of benzo(*a*)pyrene metabolism was correlated with the proportion of polyunsaturated fatty acids in the microsomal fractions as a result of feeding different diets. It is possible that an increase in the polyunsaturated fatty acid content of the membrane phospholipids may alter the configuration of the cytochrome P-450 or P-448 complex which enables it to metabolise carcinogens more rapidly [10].

In view of the location of epoxide hydratase in the endoplasmic reticulum and its close association with the cytochrome P-450 monooxygenase system, this study was carried out to determine whether dietary polyunsaturated fats also affect epoxide hydratase activity in the rat liver and intestinal mucosa. In order to account for the possible effect of the vitamin E content of the oils used in this study, a series of experiments were also carried out to determine the effect of dietary vitamin E on epoxide hydratase activity.

MATERIALS AND METHODS

Materials. Benzo(*a*)pyrene-4,5-oxide used as the substrate for determining epoxide hydratase activity and benzo(*a*)pyrene-4,5-dihydrodiol used to calibrate the assay were kindly provided as gifts by the National Cancer Institute Chemical Repository (ITT Research Institute, Chicago, IL).

Rats and diets. Groups of male albino rats, 5–6 weeks old, weight 100–130 g were fed *ad libitum* on synthetic powdered diets for 15–30 days. The purified

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diets contained, by weight, 25% casein (supplied as a gift from the Scottish Milk Marketing Board), 30% sucrose, 20% wheat starch (or 30% wheat starch in the case of fat-free diets) (Rank-Hovis-MacDougall, Ashford, Kent), 10% dried yeast (Bovril, Burton-on-Trent, Staffs), 5% Cox's salt mix [11] and 10% fat. All diets were supplemented with Rovimix A-D_3® (supplied as a gift from Roche, Welwyn Garden City, Herts) supplying 5000 IU Vit. A and 1000 IU Vit. D_3/kg diet. α-Tocopherol (Sigma) (100 or 500 mg/kg diet) was added to some diets after mixing into the lard.

The lipid components of the diets were lard (antioxidant-free; Unigate), corn oil ("Mazola" which contains 100 mg vitamin E/100 g [12]; Brown & Polson, Division of CPC Ltd) and cod liver oil ("Maxepa" which contains 100 mg α-tocopherol acetate/100 g; supplied as a gift from British Cod Liver Oils Ltd, Marfleet, Hull).

Preparation of microsomal fractions. Rats were killed by cervical dislocation and the liver and the distal half of the intestine (50 cm nearest to the caecum) were removed, washed and weighed.

Samples of liver were homogenised in ice-cold 125 mM KCl (10% w/v) using a Potter-Elvehjem homogeniser and centrifuged at 9000 g_{av} for 25 min. The supernatant was removed and the microsomal pellet prepared by centrifugation at 100,000 g_{av} for 60 min and was resuspended in 125 mM KCl (0.5 g liver/ml).

Intestinal microsomes were prepared according to the method of Stohs *et al.* [13]. The mucosa was removed by scraping with the edge of a glass slide and was suspended in 50 mM Tris–HCl buffer pH 7.8 containing 125 mM KCl, trypsin inhibitor (5 mg/g wet weight intestine), glycerol (20% v/v) and heparin (3 U/ml). The suspension was homogenised and microsomes were prepared and resuspended in the same manner as liver microsomes.

The protein concentration of the microsomal suspensions were determined by the method of Lowry *et al.* [14] using bovine serum albumin as the standard.

Determination of epoxide hydratase activity. Epoxide hydratase activity was determined by the fluorimetric method of Hukkelhoven *et al.* [15] using benzo(*a*)pyrene-4,5-oxide as substrate. The incubation mixture (1 ml) contained 0.1 M Tris–HCl buffer (pH 8.7) and approximately 60 μg of hepatic microsomal protein or 1 mg of intestinal microsomal protein. The reaction was initiated by the addition of benzo(*a*)pyrene-4,5-oxide in DMSO (10 μl) (final concentration 20 μM) and continued for 15 min at 37°. The reaction was stopped by the addition of petroleum ether (3.5 ml), shaking and cooling on ice. DMSO (0.5 ml) was then added and the tubes shaken for 60 sec. After centrifugation to separate the phases, the ether fraction containing the unreacted substrate was removed and the extraction repeated. The product, benzo(*a*)pyrene-4,5-dihydrodiol was then extracted into ethylacetate (3 ml) by mixing for 90 sec and separation of the phases by centrifugation. The amount of product was determined using a Perkin Elmer LS3 spectrofluorimeter at an excitation wavelength of 312 nm and an emission wavelength of 388 nm. A standard curve was constructed using a series of benzo(*a*)pyrene-4,5-dihydrodiol standards of known concentration. In routine experiments, quinine sulphate in 0.1 N H_2SO_4 was used for the calibration of the fluorimeter.

Determination of the fatty acid composition of the dietary lipids and the microsomal fractions. The fatty acid compositions of the dietary lipids and the lipids of the hepatic and intestinal microsomes were determined by gas liquid chromatography (150 cm × 4 mm i.d. column packed with 10% diethylene glycol stearate on chromosorb W 80–100 mesh) [16]. Lipids were extracted with chloroform/methanol [16] and transmethylated with BF_3–methanol complex [17].

RESULTS

Diet analysis

The fatty acid composition of the lard, corn oil and cod liver oil used in the preparation of the diets are shown in Table 1. Lard contained mainly saturated and mono-unsaturated fatty acids, particularly $C_{18:1}$ (44.6%) and $C_{18:2}$ comprised 10.4% of the total fatty acids. Corn oil contained a high proportion of $C_{18:2}$ (58.2%). Cod liver oil contained only a small amount of $C_{18:2}$ (7.7%) but a relatively large proportion of the highly unsaturated ω3 fatty acids $C_{20:5}$ (17.7%) and $C_{22:6}$ (10.7%).

The effect of dietary lipids on epoxide hydratase activity

The rate of epoxide hydratase in the rat liver changed significantly when different diets were fed (Fig. 1). The addition of lipid (10%) to the fat-free diet resulted in increases in epoxide hydratase activity which were significant ($P < 0.01$) when the 10% corn oil and cod liver oil diets were fed. The activity of epoxide hydratase in hepatic microsomes of rats fed the highly unsaturated cod liver oil diet was significantly greater ($P < 0.02$) than when lard was fed which contains little polyunsaturated fat (Fig. 1).

Changes in epoxide hydratase activity in the intestine on feeding different diets were less marked than those in the liver (Fig. 2). There are no differences in epoxide hydratase activity between rats fed a fat-free diet and diets containing 10% corn oil or lard. However, epoxide hydratase activity was significantly greater ($P < 0.05$) in the intestinal mucosa of

Table 1. Fatty acid analysis of dietary lipids

Fatty acid	% Total fatty acids		
	Lard	Corn oil	Cod liver oil
$C_{16:0}$	24.1	10.9	17.4
$C_{16:1}$	3.2	0.3	8.4
$C_{18:0}$	13.4	1.7	4.4
$C_{18:1}$	44.6	24.4	17.4
$C_{18:2}$	10.4	58.2	7.7
$C_{20:1}$	1.1	0.2	2.8
$C_{20:5}$	0	0	17.7
$C_{22:1}$	0.3	0.1	2.7
$C_{22:5}$	0	0	2.8
$C_{22:6}$	0	0	10.7
Minor fatty acids	2.9	4.2	8.0

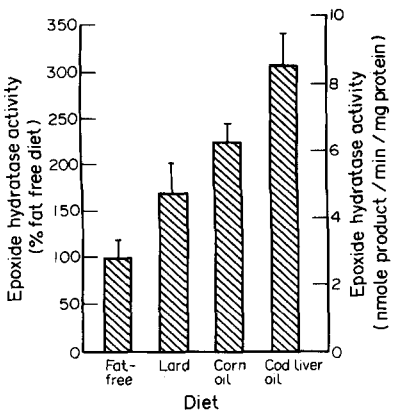

Fig. 1. The effect of dietary lipid (10% of diet) on the activity of epoxide hydratase in hepatic microsomes. Values represent the mean ± S.E.M. of duplicate determinations on 6 rats.

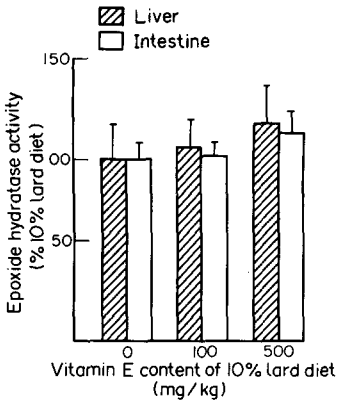

Fig. 3. The effect of dietary vitamin E on the activity of epoxide hydratase in microsomes prepared from the liver, and intestinal mucosa of rats fed a 10% lard diet. Values represent the mean ± S.E.M. of duplicate determinations on 4 rats.

rats fed the cod liver oil diet than in the groups of rats fed fat free, corn oil or lard diets.

The effect of dietary vitamin E on epoxide hydratase activity

In order to determine the effect of the vitamin E present in the corn oil (100 mg/100 g) and cod liver oil (100 mg/100 g) diets on epoxide hydratase activity, groups of rats were fed on 10% lard diets containing 0, 100 and 500 mg vitamin E/kg diet. The results (Fig. 3) show that supplementation of the diet with vitamin E of up to 500 mg/kg diet produced only very small and insignificant increases in epoxide hydratase activity in both the liver and intestinal mucosa.

The effect of the composition of dietary fat on the fatty acid composition of the liver and intestinal mucosa

The total amount of lipid extracted from the microsomal fractions of the liver and intestinal mucosa (expressed both in terms of the microsomal protein content and on a per g whole tissue basis) did not vary when rats were fed different diets. Feeding different diets did cause significant changes in the relative proportions (and thus the amounts) of polyunsaturated fatty acids in these fractions. These changes were such that the proportions of the major polyunsaturated fatty acids in the microsomal fractions of the liver and intestinal mucosa reflected the proportion of these fatty acids in the diet (Fig. 4). Thus, feeding a corn oil diet resulted in substantially increased proportions of $C_{18:2}$ in hepatic microsomes (25.7% of the total fatty acids) and intestinal microsomes (22.8% of the total fatty acids) (Fig. 4a). Microsomes prepared from the livers of rats fed the highly unsaturated cod liver oil diet contained significant proportions of $C_{20:5}$ (Fig. 4b) and $C_{22:6}$ (Fig. 4c) which were present only in low or undetectable levels when rats were fed fat-free, lard or corn oil diets. The microsomal content of $C_{20:5}$ in the intestinal mucosa after feeding a cod liver oil diet was similar to that of the liver but less $C_{22:6}$ was found in the intestinal fractions (2.8% of the total fatty acids in intestinal microsomes compared to 15.9% of the total fatty acids in hepatic microsomes). The proportion of $C_{18:2}$ in hepatic and intestinal microsomes decreased to a low level when rats were fed the cod liver oil diet (Fig. 4a).

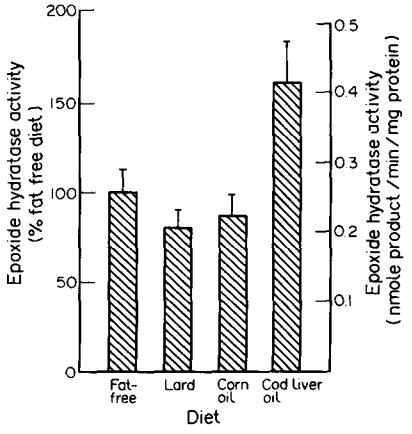

Fig. 2. The effect of dietary lipid (10% of diet) on the activity of epoxide hydratase in microsomes prepared from the intestinal mucosa. Values represent the mean ± S.E.M. of duplicate determinations on 6 rats.

DISCUSSION

Addition to the fat-free diet of cod liver oil (10%), which is rich in $C_{20:5}$ and $C_{22:6}$ and contains 100 mg vitamin E/100 g, resulted in a 3-fold increase in epoxide hydratase activity in the liver but was less effective in the intestine (1.6-fold increase). Supplementation of a 10% lard diet with vitamin E (0.01%, the same content as in the cod liver oil diet) did not increase epoxide hydratase activity and raising the vitamin E content to 0.05% only resulted in a 17% increase in activity in the liver and a 12% increase in the intestinal mucosa. The failure of these doses of vitamin E to enhance epoxide hydratase activity is not surprising as a minimal enhancing

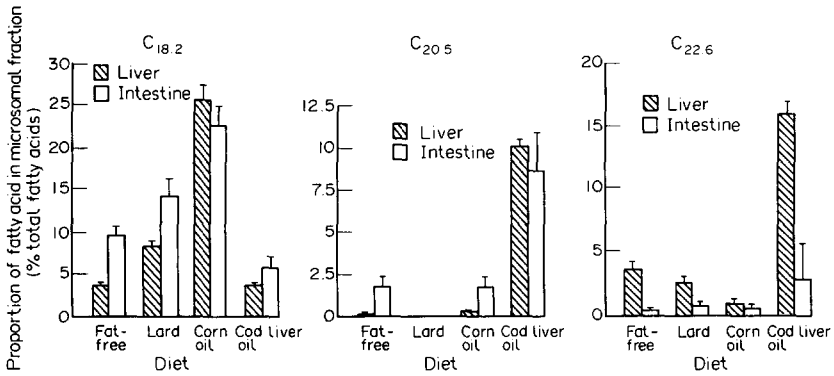

Fig. 4. The proportion of linoleic acid ($C_{18:2}$), eicosapentaenoic acid ($C_{20:5}$), and docosahexaenoic acid ($C_{22:6}$) in the microsomal fractions of the liver and intestinal mucosa of rats fed diets containing different lipids (10% of diet). Values represent the mean ± S.E.M. of 6 determinations.

concentration of 0.1% of other antioxidants (ethoxyquin and butyl-hydroxytoluene) in food has been established for hepatic epoxide hydratase [6]. Thus, the activity of epoxide hydratase in the liver and intestine of rats fed a 10% cod liver oil diet was significantly ($P < 0.05$) greater than when a 10% lard diet containing 0.01% vitamin E was fed. This demonstrates that the highly polyunsaturated $\omega 3$ fatty acids ($C_{20:5}$ and $C_{22:6}$) in the cod liver oil were responsible for elevating the activity of epoxide hydratase in the rat liver and intestine. The lower stimulation of epoxide hydratase activity in the intestine compared to the liver by a cod liver oil diet may therefore have been due to the fact that intestinal microsomes contained significantly less $C_{22:6}$ than hepatic microsomes after feeding this diet.

Feeding a 10% corn oil diet (58.2% $C_{18:2}$) increased hepatic epoxide hydratase activity to 227% of that found for rats fed the fat-free diet. This significant increase cannot have been due to the vitamin E present in this oil (100 mg/100 g; 0.01% of diet) and thus dietary $C_{18:2}$ was also able to elevate epoxide hydratase activity in the liver. However, dietary $C_{18:2}$ was not as effective at stimulating epoxide hydratase as were the highly polyunsaturated fatty acids present in the cod liver oil diet.

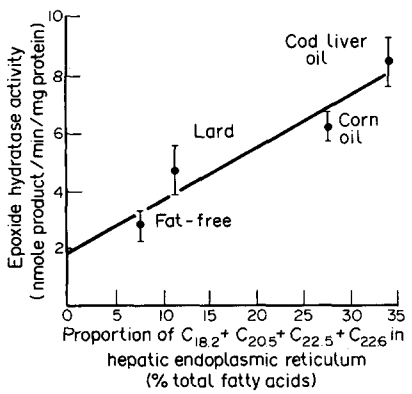

Fig. 5. The relationship between the activity of epoxide hydratase and the proportion of polyunsaturated fatty acids in the hepatic endoplasmic reticulum prepared from rats fed different diets containing 10% lipid. Values represent the mean ± S.E.M. of duplicate determinations on 6 rats.

A good correlation ($R = 0.96$) was found to exist between the proportion of polyunsaturated fatty acids ($C_{18:2} + C_{20:5} + C_{22:5} + C_{22:6}$) in hepatic microsomes and the activity of epoxide hydratase in these fractions prepared from rats fed diets containing a wide range of fatty acid compositions (Fig. 5). The endoplasmic reticulum is the predominant component of the microsomal fraction [18] and thus the changes in the fatty acid composition of microsomes, observed when feeding different diets, are likely to represent changes in the fatty acid composition of the membrane phospholipids of the endoplasmic reticulum. The components of the endoplasmic reticulum are known to turnover rapidly [18] and the dependence of the fatty acid composition of this membrane system on the composition of the dietary fat has been extensively reported [7–9, 16]. An increase in the polyunsaturated fatty acid content of the phospholipids of the endoplasmic reticulum will result in an increase in the fluidity of this intracellular membrane and there have been indications that this may in turn affect a number of different membrane functions (e.g. [19]). Epoxide hydratase is thought to be located deep in the hydrophobic phase of the membrane [20], and it is therefore possible that an increase in its activity when diets rich in polyunsaturated fats are fed is the result of changes in the lipid environment of this enzyme. This may cause an alteration of the configuration of epoxide hydratase and an increase in its activity towards benzo(*a*)pyrene-4,5-oxide. Indeed, increases in the unsaturated fatty acid content and hence the fluidity of the hepatic endoplasmic reticulum have been suggested to play a part in the induction of epoxide hydratase by *trans*-stilbene oxide [21]. The relationship shown in Fig. 5 is very similar to that previously found between the activity of aryl hydrocarbon hydroxylase and the polyunsaturated fatty acid content of hepatic microsomes [7]. This therefore supports the idea of a close association between these two carcinogen metabolising activities in the hepatic endoplasmic reticulum.

Although, like the liver, epoxide hydratase activity in the intestinal mucosa was stimulated by an increase

in the microsomal content of the polyunsaturated fatty acids present in the cod liver oil diet, it was not enhanced when a corn oil diet was fed. Feeding a corn oil diet resulted in a similar increase in the proportion of $C_{18:2}$ in the microsomal fraction of the intestinal mucosa as that which took place in the liver. It is therefore possible that the enzyme present in the intestinal mucosa has slightly different properties to that of the liver and consequently responds differently to changes in its lipid environment.

The activity of epoxide hydratase in the distal half of the intestine was 10–30-fold less than the activity of hepatic epoxide hydratase. However, the intestine is a major route by which carcinogens enter the body and polycyclic aromatic hydrocarbons, which are present in significant amounts in many foodstuffs [22], are readily absorbed into the mucosa [23]. The enzymes of the small intestine therefore play a major role in the metabolism of ingested carcinogens. Thus, alterations in the activities of these enzymes are likely to affect the initiation of intestinal cancer and the distribution of ingested carcinogens and their metabolites to other sites in the body.

Epoxide hydratase has multiple effects on the metabolism of polycyclic aromatic hydrocarbons (PAHs) [1] and it is difficult to assess the effect which the elevation of epoxide hydratase activity by dietary polyunsaturated fats has on the overall carcinogenicity of PAHs. Both increases and decreases in the mutagenicity of benzo(*a*)pyrene in the *Salmonella typhimurium* mutagenicity assay containing liver microsomes have been observed, depending on the source of microsomes and the concentration of benzo(*a*)pyrene [24, 25]. The lack of specific inducers and inhibitors of epoxide hydratase have limited *in vivo* studies on the effect of epoxide hydratase activity on PAH-induced carcinogenesis, although there is a suggestion that epoxide hydratase may play a protective role against 3-methylcholanthrene-induced skin tumours [26].

The elevation of both epoxide hydratase activity and P-448 catalysed benzo(*a*)pyrene oxidation [7–10] by dietary polyunsaturated fats, will lead to an increase in benzo(*a*)pyrene-7,8-epoxide formation by the monooxygenase system and an increase in its subsequent hydration to benzo(*a*)pyrene-7,8-diol by epoxide hydratase (this has been confirmed by h.p.l.c. [9]). The formation of benzo(*a*)pyrene-7,8-diol, 9,10-epoxide, which is the ultimate carcinogenic metabolite of benzo(*a*)pyrene [2] is also likely to be increased due to the raised level of benzo(*a*)pyrene-7,8-diol and the elevation of the monooxygenase system which catalyses this reaction. Thus, dietary polyunsaturated fats are likely to play an important role in the metabolism of pro-carcinogens to active carcinogens and thus the likelihood of PAH-induced carcinogenesis. This may have important implications in view of the epidemiological evidence which has associated a high level of dietary fat intake with an increased cancer incidence in man of several organs including the intestine and breast [27], and the increasing consumption of polyunsaturated fats in the western world.

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