

## Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat

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The effect of 16 d intake of 300 mg carotenoids/kg diet ( $\beta$ -carotene ( $\beta$ C), bixin (BX), lycopene (LY), lutein (LU), canthaxanthin (CX) or astaxanthin (AX)) on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of male Wistar rats was assessed. A control group received the basal diet (AIN-76) without carotenoids and a positive control group for enzyme induction received 3-methylcholanthrene (3-MC) at 666 mg/kg diet. Cytochrome P450 activity was assessed using the substrates ethoxyresorufin for P450 1A1, methoxyresorufin for P450 1A2, pentoxyresorufin for P450 2B1/2 and benzyloxyresorufin for P450 types 1A1/2, 2B1/2 and 3A. Glutathione-S-transferase (*EC* 2.5.1.18) and reduced glutathione status were assessed. Carotenoid uptake by the tissues was also determined. 3-MC and the carotenoids BX, CX and AX led to significant increases compared with control in liver, lung and kidney ethoxyresorufin-O-deethylation. Methoxyresorufin-O-demethylation activity was significantly increased in liver and lung by BX, CX and AX but only CX and AX significantly increased activity in kidney. Pentoxyresorufin-O-depentylation and benzyloxyresorufin-O-dearylation increased in liver of 3-MC-, BX-, CX- and AX-treated rats, but to a much lesser degree than for the other two substrates. Benzyloxyresorufin-O-dearylation in lung was significantly decreased by all carotenoids. Activities of any of the measured enzymes in the small intestine were undetectable in all treatment groups except the 3-MC group. Glutathione status was unaffected by any of the treatments. This is the first study identifying the carotenoids BX, CX and AX as inducers of rat lung and kidney xenobiotic metabolizing enzymes.

### Cytochrome P450: Enzyme induction: Carotenoids

Data from numerous epidemiological studies have suggested that fruit and vegetable-enriched diets are associated with reduced risk of many cancers (Steinmetz & Potter, 1996). Much interest has focused on the identification of protective nutrients and phytochemicals in fruit and vegetables. Carotenoids are a family of fat-soluble pigments found in fruit and vegetables and a large body of work involving epidemiological and animal models has investigated their potential role in the carcinogenic process. Most work has focused on  $\beta$ -carotene ( $\beta$ C) as a potential anti-carcinogen.  $\beta$ C is a nutritionally significant carotenoid as it can be converted to vitamin A and represents the principal dietary source of this vitamin for the majority of the world's population. Other provitamin A carotenoids include  $\alpha$ -carotene and  $\beta$ -cryptoxanthin.  $\beta$ C has been shown to function as an antioxidant in many *in vitro* systems (Krinsky, 1991). A plausible mechanism for potential anti-carcinogenic effects of  $\beta$ C is its ability to scavenge reactive

oxygen species that cause oxidative DNA damage. However, two recent major intervention trials, one in Finland (Heinonen & Albanes, 1994) and one in the USA (Omenn *et al.* 1996) unexpectedly demonstrated an increased risk of lung cancer in smokers who were given high-dose  $\beta$ C supplements. Van Poppel *et al.* (1995) reported no effect of  $\beta$ C supplementation on oxidative DNA damage as assessed by urinary excretion of 8-oxo-7,8-dihydro-2-deoxyguanosine in male cigarette smokers. However, other studies have shown beneficial effects of  $\beta$ C in reducing cancer risk (Toma *et al.* 1995).  $\beta$ C has been shown to have a number of other functions that are completely independent of its antioxidant role. It has been reported to influence immune function (Santos *et al.* 1996; Hughes *et al.* 1997) and enhance gap junction intercellular communication (Zhang *et al.* 1992) and, indeed, other cancer preventive mechanisms for this molecule cannot be excluded.

In addition, fruits and vegetables are a rich source of other

**Abbreviations:** AX, astaxanthin; BHT, butylated hydroxytoluene; BROD, benzyloxyresorufin-O-dearylation; BX, bixin;  $\beta$ C,  $\beta$ -carotene; CX, canthaxanthin; EROD, ethoxyresorufin-O-deethylation; GST, glutathione-S-transferase; LU, lutein; LY, lycopene; 3-MC, 3-methylcholanthrene; MROD, methoxyresorufin-O-demethylation; PROD, pentoxyresorufin-O-depentylation.

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carotenoids and there is increasing evidence that these compounds may have anticarcinogenic properties. Carotenoids are effective quenchers of singlet oxygen. Lycopene (LY) exhibits the highest singlet oxygen quenching activity (Stahl & Sies, 1996) and has also been shown to increase communication of growth regulatory signals between cells (Zhang *et al.* 1992). Carotenoids function as chain-breaking antioxidants protecting cells and other body components from free-radical attack (Rock *et al.* 1996). The intakes or plasma levels of carotenoids are associated with a diminished risk of various cancers. The intake of an LY-rich diet has been associated with a diminished risk for prostate cancer (Giovannucci *et al.* 1995). High dietary intake of lutein (LU)/zeaxanthin has been associated with decreased risk of lung cancer (Ziegler *et al.* 1996) and high dietary intake of  $\beta$ -cryptoxanthin has been associated with decreased incidence of cervical cancer (Batieha *et al.* 1993). The mechanism by which these carotenoids may be protective is not known.

Very little work has been conducted investigating the effect of carotenoids on xenobiotic metabolizing enzyme systems. Xenobiotic metabolizing enzymes can be divided into phase I and phase II enzymes. The majority of phase I reactions are catalysed by one enzyme system, cytochrome P450 monooxygenase. The cytochrome P450 system is actually a collection of isoenzymes which catalyse different types of oxidation reactions. Phase II reactions, also known as conjugation reactions, involve the addition of a polar group to the foreign molecule. There are a number of phase II enzymes but glutathione conjugation catalysed by glutathione-S-transferase (*EC* 2.5.1.18; GST) is a particularly important route of phase II metabolism as it is often involved in the removal of reactive intermediates. One group (Astorg *et al.* 1994; Gradelet *et al.* 1996a) has reported that canthaxanthin (CX) and astaxanthin (AX) are excellent inducers of cytochrome P450 1A1 and 1A2 activity in liver of male Wistar rats. However, LY, LU and  $\beta$ C had negligible effect. Rats fed with unrefined red palm oil which is rich in  $\beta$ C and  $\alpha$ -carotene exhibited a 23% increase in liver GST activity compared with rats fed with the refined oil which contained negligible carotenoids (Manorama *et al.* 1993). No differences in liver P450, aminopyrine *N*-demethylase or ethoxyresorufin-O-deethylation (EROD) were reported between the groups treated with unrefined and refined oil. No work has been reported, however, on the effects of carotenoids on xenobiotic metabolizing enzyme systems in organs other than liver.

The objective of our work, therefore, was to investigate the effects of a range of carotenoids on xenobiotic metabolizing enzyme systems (cytochrome P450 and GST) and glutathione status in liver, lung, kidney and small intestine in Wistar rats.

## Materials and methods

### Materials

Commercial carotenoid preparations were obtained from Quest International Ireland Ltd (Carrigaline, Co. Cork, Republic of Ireland). These included Annatto R35 bixin (BX) powder containing 42 g BX/kg; Lutein OS30, a refined

xanthophyll oleoresin extracted from marigold (*Calendula officinalis*) flowers containing 34 g LU/kg and natural mixed tocopherols (mainly  $\gamma$  and  $\delta$ ); Lycopene OS4, a tomato oleoresin containing 36 g LY/kg, lecithin, natural mixed tocopherols (mainly  $\gamma$  and  $\delta$ ), and polysorbate 80 (emulsifier); and Astaxanthin OS5, an oleoresin extracted from adonis (*Adonis vernalis*) flowers containing 49 g AX/kg. All oleoresins were dissolved in maize oil. CX in a water-dispersible powder formulation (CX (10 g/kg), sucrose, gelatin, starch) and pure carotenoids used as HPLC standards were obtained from Hoffmann-La Roche, Basel, Switzerland. Carotenoid preparations were evaluated for their purity before use in diet formulation. Carotenoids were extracted from the preparations using hexane, dried down under  $N_2$  and redissolved in HPLC mobile phase as described later. The purity of the extracts was then determined by HPLC analysis. In all cases it was >99%.  $\beta$ C (>95% pure), 3-methylcholanthrene (3-MC) and all other chemicals were obtained from Sigma Chemical Co. (Poole, Dorset, UK). Solvents were of HPLC grade and used without further purification.

### Animals and diets

Male Wistar rats (obtained from the Biological Service Unit, University College, Cork, Republic of Ireland) 26 d old with a mean body weight of 46.0 g ( $n$  64) were housed individually in stainless steel cages under a 12 h light-cycle (light on 08.00 hours). Animals were fed on a basic AIN-76 rat diet (American Institute of Nutrition, 1977) supplemented with 50 g maize oil/kg, and tap water *ad libitum* for 7 d to allow acclimation. The animals were subsequently divided into eight experimental groups with eight animals per group. The groups were: control, 3-MC, BX,  $\beta$ C, LY, LU, CX and AX. The control group received the basal diet, the 3-MC group received the basal diet supplemented with 666 mg 3-MC/kg diet and the remaining groups received the basal diet supplemented to provide 300 mg of the appropriate carotenoid/kg diet. The purity of each carotenoid preparation was established and the appropriate amount used in formulation of the diet to achieve the desired level of carotenoid. 3-MC is a classical inducer of cytochrome P450 enzymes and was used in this study as a positive control. Carotenoids or 3-MC were mixed thoroughly with maize oil, stored frozen and added to the diets, which were prepared daily, to give a final concentration of 50 g oil/kg. All animals received 15 g diet each day. Given the level of carotenoid in the diet, this represents an approximate daily intake of 45 mg/kg body weight for a rat weighing 100 g. The human intake of  $\beta$ C has been reported to be in the range of 1.5 mg/d (LaChance, 1988). This represents an intake of 0.02 mg/kg body weight for a 70 kg male. Drinking water was supplied *ad libitum* and the animals were weighed every second day. After 16 d of feeding the rats were killed by cervical dislocation. The proximal 150 mm of the small intestine, liver, lung and kidney were excised, washed in phosphate buffer and weighed. In addition, the small intestine was syringed and washed thoroughly with phosphate buffer before homogenization to remove any loose material within the gut. A sample of each tissue was retained for immediate analysis of GSH as described later. The remaining tissue

was rapidly frozen in liquid N<sub>2</sub> and stored at -70°. On the day of analysis samples were homogenized on ice in a glycerol buffer (100 ml glycerol/l, 150 mM-sucrose, 50 mM-Trisma, 0.1 mM-KCl and 0.1 mM-EDTA, pH 7.4) using an Ultra-Turrax T25 homogenizer (IKA Labortechnik, Janke & Kunkel GmbH & Co KG, Staufen, Germany) and used to prepare sub-cellular fractions (microsomes and cytosol).

#### *Glutathione and enzyme assays*

GSH levels were measured in freshly excised tissues by a modification of the method of Cotgreave & Moldéus (1986). Tissue (50 mg) was homogenized in 900 µl bromobimane (0.5 mg/ml) in *n*-ethylmorpholine buffer (0.05 M, pH 8). After 30 min in the dark, 100 µl methane sulfonic acid (100 ml/l) was added to precipitate protein and the mixture was centrifuged for 10 min at 2000g. The supernatant fraction was assayed for GSH content by HPLC using a 150 mm C18 column followed by fluorimetric detection. The system consisted of a Shimadzu SIL-6B auto sampler, SCL-6B system controller, two LC-6A pumps and RF-551 spectrofluorimetric detector (Shimadzu, Kyoto, Japan). The bromobimane-thiol complexes were detected at an excitation wavelength of 350 nm and an emission wavelength of 477 nm. The mobile phase was freshly prepared acetonitrile (100 ml/l) in acetic acid (0.25 ml/l) (pH 3.7) for 10 min, followed by 100% acetonitrile for 5 min to remove excess bromobimane, at a flow rate of 1.5 ml/min. Data were collected by a personal computer with Millennium Chromatography Manager data software (Waters<sup>TM</sup> Corporation, Milford, MA, USA).

Cytochrome P450 monooxygenase (EC 1.14.14.1) activity in the microsomal fraction of the tissues was determined using the substrates ethoxyresorufin (EROD, specific for P450 1A1), methoxyresorufin (methoxyresorufin-O-demethylation, MROD, P450 1A2), pentoxyresorufin (pentoxyresorufin-O-depentylation, PROD, P450 2B1/2), and benzyloxyresorufin (benzyloxyresorufin-O-dearylation, BROD, non-specific for P450 types 1A1/2, 2B1/2, and 3A) by the method of Burke *et al.* (1985). The rate of the reaction was monitored using a fluorimeter (Shimadzu RF-551) containing a water-jacketed cell holder at 37° and connected to a chart recorder. The fluorimeter was set at an excitation wavelength of 530 nm and emission wavelength of 585 nm. GST activity was determined in the cytosolic fractions by the method of Habig *et al.* (1974) using dinitrochlorobenzene as substrate. Protein concentration of the microsomal and cytosolic fractions was determined by the method of Smith *et al.* (1985).

#### *Determination of carotenoid levels in tissues*

Tissue samples (300 mg) were homogenized in 2 ml ethanol (500 ml/l) containing 0.25 mg diethyl triamine pentaacetic acid/ml and 0.25 mg butylated hydroxytoluene (BHT)/ml. A 1 ml sample was removed and 1 ml ethanol (500 ml/l), containing 0.25 mg BHT/ml, was added. This solution was extracted three times with 1 ml methylethylketone-cyclohexane (1:1, v/v) containing 0.1 mg BHT/ml. The organic layers were transferred to amber vials and dried down under

N<sub>2</sub>. The extract was redissolved in dichloromethane (100 µl) followed by acetonitrile (700 µl) and methanol (200 µl). Extracts were analysed using HPLC by the method of Craft & Wise (1992). The HPLC system consisted of Shimadzu SCL-10A system controller, LC10-AD pump, SIL-10A autoinjector, SPD-10AV u.v.-visible detector, 2×150 mm×4.6 mm C18 columns, secured with Teflon frits, water-jacketed and maintained at 25°. Instrumentation was connected to a personal computer with Millennium Chromatography Manager data collection software as described earlier. Mobile phase consisted of acetonitrile-methanol-dichloromethane (15:4:1, by vol.) containing 10 mM-ammonium acetate, 4.5 mM-BHT and 3.6 mM-triethylamine, and was used at a flow rate of 1.5 ml/min.

#### *Statistical analysis*

All data were analysed by ANOVA followed by Dunnett's test using the InStat statistical package (GraphPad Software Inc., San Diego, CA, USA).

### **Results**

For the duration of the study the food intakes and body-weight gains of the rats in all test groups were similar. At the end of the study body-weight changes and organ weights were found to be similar in all groups (results not shown).

The effects of feeding diets containing 3-MC or carotenoids on cytochrome P450 monooxygenase activity in rat liver, lung and kidney tissues are presented in Tables 1, 2 and 3. As expected, the compound 3-MC significantly induced, above control levels, all the liver P450 enzymes examined (EROD, MROD, BROD and PROD activities, Table 1). Of the carotenoids examined BX, CX and AX significantly induced liver EROD, MROD, BROD and PROD activities with CX and AX demonstrating the largest effect. CX induced EROD, MROD, BROD and PROD activities by factors of 44, 34, 21 and 15 respectively, compared with the control group. AX induced the same pattern of enzyme activities (factors of 56, 35, 21 and 19 respectively). BX induced activities of these liver enzymes to a lesser extent (factors of 6.2, 4.0, 2.6 and 5.5 respectively). In contrast, βC, LY and LU did not induce any of the liver microsomal enzymes activities measured (Table 1).

In the lung 3-MC significantly increased EROD and MROD activities (Table 2). The three carotenoids BX, CX and AX significantly induced activity of these lung microsomal enzymes; however, in the case of CX and AX the induction of both enzymes was less than that seen in the liver. EROD activity increased by factors of 4.3, 3.1 and 3.1, and MROD activity increased by factors of 2.1, 1.2 and 1.4 for BX, CX and AX respectively. βC, LY and LU had no effect on EROD and MROD activities in the lung. Lung BROD activity was significantly decreased below control levels in animals fed on each of the carotenoid diets. In contrast, 3-MC had no effect on BROD activity (Table 2). The activity of PROD in lung tissue was not affected by any of the treatments.

In the case of the kidney, the positive control 3-MC significantly induced EROD, MROD and BROD activities (Table 3). The carotenoids CX and AX significantly

**Table 1.** Activities (pmol/min per mg microsomal protein) of microsomal P450 enzymes in the livers of rats fed on a control diet, or the same diet supplemented with different carotenoids or 3-methylcholanthrene (3-MC)†  
(Mean values with their standard errors for eight rats per group)

Group	EROD		MROD		BROD		PROD	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	51.9	2.4	45.1	0.9	35.3	2.2	14.6	0.7
3-MC	222.1*	27.1	140.2*	13.8	123.3*	3.5	26.7*	1.8
Bixin	323.7*	20.8	182.3*	8.1	90.6*	6.9	80.9*	5.0
β-Carotene	54.0	3.8	45.3	6.4	30.5	2.5	12.5	1.2
Lycopene	54.8	2.5	55.5	3.2	43.5	3.4	12.6	0.9
Lutein	68.2	4.3	50.1	3.4	44.9	4.6	14.8	1.1
Canthaxanthin	2268.4*	117.9	1533.7*	25.9	734.5*	23.7	217.3*	27.4
Astaxanthin	2913.4*	243.9	1598.4*	141.6	735.5*	41.6	279.1*	10.9

EROD, ethoxyresorufin-O-deethylation, a marker of P450 1A1; MROD, methoxyresorufin-O-demethylation, a marker of P450 1A2; BROD, benzyloxyresorufin-O-dearylation, an unspecific activity catalysed by P450 1A1/2, 2B1/2, and 3A; PROD, pentoxyresorufin-O-depentylation, a marker of P450 2B1/2. Mean values were significantly different from those for the control group: \* $P < 0.05$  (ANOVA and Dunnett's test).

† For details of diets and procedures, see pp. 236–237.

**Table 2.** Activities (pmol/min per mg microsomal protein) of microsomal P450 enzymes in the lungs of rats fed on a control diet, or on the same diet supplemented with different carotenoids or 3-methylcholanthrene (3-MC)†  
(Mean values with their standard errors for eight rats per group)

Group	EROD		MROD		BROD		PROD	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	1.9	0.2	4.7	0.3	161.3	13.9	13.1	0.6
3-MC	120.9*	10.7	21.9*	1.8	168.0	16.1	13.7	0.9
Bixin	8.2*	0.7	10.1*	0.3	112.7*	17.9	11.9	0.6
β-Carotene	2.2	0.2	4.4	0.3	97.3*	6.8	12.1	1.3
Lycopene	2.6	0.4	4.4	0.5	66.2*	4.9	13.1	1.3
Lutein	1.9	0.2	4.0	0.7	39.6*	5.2	11.8	0.9
Canthaxanthin	5.9*	0.5	5.7*	0.6	56.8*	5.9	12.9	1.5
Astaxanthin	5.9*	0.6	6.8*	0.5	52.4*	2.5	15.5	0.9

EROD, ethoxyresorufin-O-deethylation, a marker of P450 1A1; MROD, methoxyresorufin-O-demethylation, a marker of P450 1A2; BROD, benzyloxyresorufin-O-dearylation, an unspecific activity catalysed by P450 1A1/2, 2B1/2, and 3A; PROD, pentoxyresorufin-O-depentylation, a marker of P450 2B1/2.

Mean values were significantly different from those for the control group: \* $P < 0.05$  (ANOVA and Dunnett's test).

† For details of diets and procedures, see pp. 236–237.

**Table 3.** Activities (pmol/min per mg microsomal protein) of microsomal P450 enzymes in the kidneys of rats fed on a control diet, or on the same diet supplemented with different carotenoids or 3-methylcholanthrene (3-MC)†  
(Mean values with their standard errors for eight rats per group)

Group	EROD		MROD		BROD		PROD	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	4.8	0.7	0.5	0.0	2.9	0.5	0.1	0.0
3-MC	183.2*	20.4	2.3*	0.2	14.3*	0.3	0.1	0.0
Bixin	50.6*	6.3	0.6	0.1	7.8*	0.1	0.1	0.0
β-Carotene	7.0	2.9	0.5	0.0	3.2	0.5	0.1	0.0
Lycopene	1.9	0.6	0.5	0.1	2.8	0.1	0.1	0.0
Lutein	1.9	0.7	0.5	0.0	2.8	0.1	0.1	0.0
Canthaxanthin	155.9*	19.1	3.4*	0.2	10.1*	0.6	0.1	0.0
Astaxanthin	54.3*	3.9	2.2*	0.1	5.6*	0.3	0.1	0.0

EROD, ethoxyresorufin-O-deethylation, a marker of P450 1A1; MROD, methoxyresorufin-O-demethylation, a marker of P450 1A2; BROD, benzyloxyresorufin-O-dearylation, an unspecific activity catalysed by P450 1A1/2, 2B1/2, and 3A; PROD, pentoxyresorufin-O-depentylation, a marker of P450 2B1/2.

Mean values were significantly different from those for the control group: \* $P < 0.05$  (ANOVA and Dunnett's test).

† For details of diets and procedures, see pp. 236–237.

induced kidney microsomal EROD, MROD and BROD activities (for CX fold-inductions of 32, 6.8 and 3.5 above control for the three enzymes respectively, and AX fold-inductions of 11, 4.4 and 2.0 respectively). On the other hand, BX induced EROD ( $\times 10.5$ ) and BROD ( $\times 2.7$ ) but not

MROD in kidney microsomes. βC, LY and LU had no effect on any of the measured kidney microsomal enzymes (Table 3). Activity of kidney PROD was very low in all treatment groups.

In the small intestine 3-MC induced EROD (43.8 (SE 5.4)),

MROD (131.6 (SE 17.2)) and BROD (29.2 (SE 2.9) pmol/min per mg microsomal protein) activities. However, activities of all of the measured enzymes were undetectable in all other treatment groups including the control.

GST activity was significantly induced above control levels by 3-MC in liver and small intestine (Table 4). Carotenoid treatment had no effect on this phase II enzyme in any of the tissues examined. GSH status was

not affected by any of the treatments (Table 5). The carotenoid content of the liver, lung, kidney and small intestine increased in animals fed on the enriched diets (Table 6). All organs tested accumulated various amounts of the carotenoids except for undetectable levels of LY in kidney.  $\beta$ C, LY, LU and CX accumulated primarily in the liver. In this study BX and AX accumulated primarily in the small intestine (Table 6).

**Table 4.** Activity (nmol/min per mg cytosolic protein) of glutathione-S-transferase in the liver, lung, kidney and small intestine of rats fed on a control diet or the same diet supplemented with different carotenoids or 3-methylcholanthrene (3-MC)†

(Mean values with their standard errors for eight rats per group)

Group	Liver		Lung		Kidney		Small Intestine	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	440	18	82	9	200	6	499	30
3-MC	612*	48	79	11	198	3	595*	91
Bixin	388	59	70	8	206	5	495	23
$\beta$ -Carotene	447	22	68	9	192	10	500	28
Lycopene	430	27	75	9	210	13	478	25
Lutein	495	29	77	8	196	8	500	41
Canthaxanthin	470	50	64	12	201	9	479	87
Astaxanthin	490	45	78	7	212	10	499	63

Mean values were significantly different from those for the control group: \* $P < 0.05$  (ANOVA and Dunnett's test).

† For details of diets and procedures, see pp. 236–237.

**Table 5.** Concentration of reduced glutathione (nmol/g tissue wet weight) in the liver, lung, kidney and small intestine of rats fed on a control diet or the same diet supplemented with different carotenoids or 3-methylcholanthrene (3-MC)†

(Mean values with their standard errors for four rats per group)

Group	Liver		Lung		Kidney		Small Intestine	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	3.71	0.06	0.74	0.08	2.08	0.16	1.37	0.12
3-MC	3.73	0.15	0.73	0.06	1.96	0.15	1.46	0.16
Bixin	3.50	0.08	0.82	0.06	2.06	0.19	1.39	0.16
$\beta$ -Carotene	3.77	0.06	0.73	0.04	2.12	0.18	1.33	0.16
Lycopene	3.46	0.21	0.74	0.07	1.92	0.13	1.41	0.19
Lutein	3.53	0.19	0.73	0.08	2.12	0.17	1.53	0.17
Canthaxanthin	3.41	0.19	0.75	0.04	2.00	0.23	1.56	0.09
Astaxanthin	3.42	0.10	0.73	0.02	1.95	0.20	1.45	0.08

Mean values were significantly different from those for the control group: \* $P < 0.05$  (ANOVA and Dunnett's test).

† For details of diets and procedures, see pp. 236–237.

**Table 6.** Carotenoid content (nmol/g tissue wet weight) of the liver, lung, kidney and small intestine of rats fed on a control diet, or the same diet supplemented with different carotenoids\*

(Mean values with their standard errors for four rats per group)

Group	Liver		Lung		Kidney		Small Intestine	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bixin	225.3	10.4	84.6	3.4	40.3	0.1	1856.3	289.1
$\beta$ -Carotene	74.6	4.7	3.6	0.2	0.9	0.1	25.9	0.1
Lycopene	145.0	6.0	15.9	0.1	0.0	0.0	92.8	5.6
Lutein	547.7	13.6	57.7	0.6	7.3	0.6	15.7	3.7
Canthaxanthin	1200.8	14.9	582.0	36.6	75.9	0.1	224.6	22.8
Astaxanthin	57.1	6.6	12.8	0.2	11.2	0.3	209.5	26.2

\* For details of diets and procedures, see pp. 236–237.



## Discussion

Our study confirms previous findings (Gradelet *et al.* 1996a) that CX and AX, when administered in the diet, are potent inducers of rat liver phase I enzymes. However, in contrast with Gradelet *et al.* (1996a), we found AX to be equally as effective as CX in its ability to induce liver EROD, MROD, BROD and PROD activities (Table 1). The same strain of rat was used in both studies, however, the sources of the carotenoids differed as did the basal diets. We used the standard AIN-76 rat diet (g/kg: sucrose 487, casein 200, maize starch 150, fibre 50, maize oil 50, DL-methionine 3, choline chloride 2.98, and vitamins and minerals 57) while Gradelet *et al.* (1996a) used a semi-liquid purified diet consisting of (g/kg dry weight) casein 230, starch 330, sucrose 250, maize oil 100, cellulose 20, agar 20, mineral mix 50, and a vitamin mix as described by Astorg (1988). Previous studies have demonstrated that dietary composition, especially the type of lipid, influences plasma and tissue levels of  $\beta$ C in rats fed with this carotenoid (Alam *et al.* 1989, 1990). It is unknown whether diet administered in a dry powdered form *v.* a semi-liquid consistency influences carotenoid absorption. Interestingly, when we measured uptake of carotenoids into rat tissues (Table 6) liver CX concentration was significantly higher

than liver AX concentration. However, in contrast to the findings of Gradelet *et al.* (1996a) the liver content of CX and AX in our study was 21-fold, whilst it was 105-fold in theirs.

Additionally, this is the first study to demonstrate the induction of rat lung and kidney cytochrome P450 enzymes by CX and AX. The few *in vivo* studies that have been published to date on the effects of carotenoids on phase I and II enzymes have focused exclusively on liver (Edes *et al.* 1989; Blakely *et al.* 1990; Manorama *et al.* 1993; Astorg *et al.* 1994, 1997; Gradelet *et al.* 1996a,b). Induction of drug metabolizing enzymes in extrahepatic tissue is also of significance since lung is a route of both entry and excretion of toxicants and kidney is the primary route of excretion of xenobiotics from the body. The activity of the P450 enzymes is much lower in extrahepatic tissue, for example control EROD activities in liver, lung and kidney were 51.9 (SE 2.4), 1.9 (SE 0.2) and 4.8 (SE 0.7) pmol/min per mg microsomal protein respectively; nevertheless a 3-fold induction of activity occurred in response to both CX and AX in lung and 32 and 11-fold inductions in response to CX and AX respectively, in kidney. A similar pattern of response to CX and AX was seen in lung and kidney MROD activities; however, the level of induction was not as great as that for EROD.

BX, a carotenoid extracted from the seeds of the tropical annatto tree (*Bixa orellana*) (Ash & Ash, 1995) is one of the few naturally occurring *cis* carotenoids and has a structure similar to the skeleton of carotene with a free and an esterified carboxyl as end groups (Fig. 1). No work has previously been published on the effects of BX on phase I and II enzymes. This compound induced liver EROD, MROD, BROD and PROD activities. However, the induction in the liver was not as large as that seen with CX and AX for any of the enzymes. Interestingly, in the lung BX had a more pronounced effect on inducing EROD and MROD activities compared with CX and AX, while in the kidney only EROD and BROD activities were induced by BX. Another unexplained finding in our study was the significant decrease below control in lung BROD activity of all carotenoid-fed rats. No previous work has been published on the effects of carotenoids on lung phase I enzymes and, therefore, the reason for these differing responses is not known at this time. The additional carotenoids examined in this study,  $\beta$ C, LY and LU, had no effect on P450 enzyme activities in any of the other tissues. Gradelet *et al.* (1996a) also reported no effect of these three carotenoids on liver drug metabolizing enzymes in male Wistar rats.

When we examined the activities of EROD, MROD, BROD and PROD in the small intestine of rats from all treatment groups, we found induction of EROD and MROD activities in the 3-MC group but enzyme activities in all other groups were below the limit of detection. Very high levels of BX were measured in the small intestine and significant levels of CX and AX were also detected, indicating uptake by this tissue (Table 6).

GST and GSH levels were not affected by any of the carotenoids in any of the tissues examined (Tables 4 and 5 respectively). Blakely *et al.* (1990) and Astorg *et al.* (1994) also reported no effect of  $\beta$ C or CX on rat liver GST

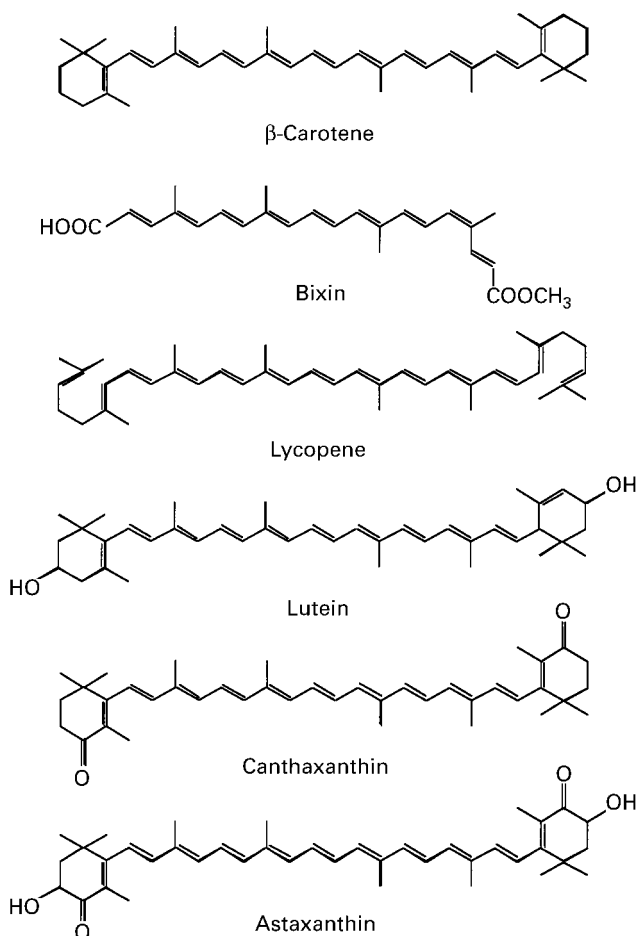


Fig. 1. Chemical structures of the carotenoids used in the present study.

activity. In contrast, a 23% increase in liver GST activity was reported by Manorama *et al.* (1993) in rats fed on unrefined red palm oil compared with rats fed on refined palm oil containing no carotenoids. The unrefined palm oil contained a mixture of carotenoids (37 mg  $\beta$ C and 18 mg other carotenoids) and therefore it is hard to define the active component in this study.

Table 6 shows the carotenoid content of rat tissues measured in our study. The liver was the major depot organ for  $\beta$ C, LY, LU and CX. Mathews-Roth *et al.* (1990) also reported that liver was the major depot organ for CX and LY in rats. In general, no obvious pattern was noted between carotenoid content of the tissues and induction of xenobiotic metabolizing enzymes. For example,  $\beta$ C was poorly taken up by all tissues and had little inducing effect on P450 enzymes. However, AX was also poorly taken up by tissues but had a significant effect on P450 induction. LU was incorporated well into tissues but led to no induction.

In summary, our findings confirm that AX and CX are potent inducers of selected liver P450 enzymes. In addition we have demonstrated that BX is capable of inducing these liver enzymes. Interestingly, the three carotenoids also induced some lung and kidney P450 enzymes but had no effect on small-intestine enzymes. Modulation of P450 enzyme activity in liver and other tissues can significantly affect the toxic potential of endogenous and exogenous chemicals and hence can influence toxicological endpoints such as carcinogenicity, mutagenicity and teratogenicity. Gradelet *et al.* (1997) demonstrated that dietary intake of carotenoids (CX, AX and  $\beta$ -apo-8'-carotenal) which are inducers of EROD and MROD reduced the carcinogenicity of aflatoxin B1, diverted aflatoxin B1 metabolism into the less genotoxic aflatoxin M1 form and reduced aflatoxin B1-induced single-strand breaks in rat liver. However, it should also be noted that induction of P450 enzymes can be potentially deleterious in certain situations, for example if induction enhances the conversion of procarcinogens to active metabolites. Further work on the effects of carotenoids and other phytochemicals on P450 enzymes in rats and other species is warranted. The relatively poor correlation between findings in rats and mice in relation to factors influencing xenobiotic metabolizing enzyme activities emphasizes the necessity for a conservative attitude in extrapolating findings from rodents to man. Ultimately, studies using human tissue biopsies from subjects fed on controlled levels of carotenoids will be required to investigate these effects in man.

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