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MONOCLONAL ANTIBODY CHARACTERIZATION OF HEPATIC AND EXTRAHEPATIC CYTOCHROME P-450 ACTIVITIES IN RATS TREATED WITH PHENOBARBITAL OR METHYLCHOLANTHRENE AND FED VARIOUS CHOLESTEROL DIETS

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Abstract—Monoclonal antibodies (MAb) against 3-methylcholanthrene (MC)- and phenobarbital (PB)-inducible forms of cytochrome P-450 isozyme were used to characterize changes in aryl hydrocarbon hydroxylase (AHH) and ethoxycoumarin *O*-deethylase (ECDE) activities modulated by dietary cholesterol. Rats were induced by MC or PB, and immunochemical inhibition of AHH and ECDE activities was studied as an indication of changes in cytochrome P-450 isozyme patterns. Feeding of a cholesterol-free diet markedly decreased enzyme activities both in liver and in small intestinal mucosa, and the highest activities were observed after feeding rats a high (2%)-cholesterol diet for one month. As a control, a normal pelleted diet (0.1% cholesterol) was used; in rats fed this diet, intermediate levels of monooxygenase activities were present. Although no diet-dependent change in total AHH and ECDE activities was observed in kidneys and lungs, diet apparently modulated isozyme composition in the lungs, as indicated by a change in the immunochemical inhibition pattern with MAb; no such shift was observed in the kidneys. In liver and intestine, in addition to changes in total activity, isozyme composition was also altered, as indicated by inhibition of the catalytic activities of cytochrome P-450 by MAb.

Our data infer that dietary cholesterol can: (i) modulate total monooxygenase activities, especially in the intestine; (ii) change the cytochrome P-450 isozyme composition in liver and intestine; (iii) change isozyme composition without changing overall enzyme activity, e.g. in lungs; and (iv) have no effect in a tissue (e.g. kidney) that lacks constitutionally the P-450 isozyme responsive to cholesterol.

Both the liver and extrahepatic organs are important in the metabolism of drugs and other xenobiotics [1], and the rate of metabolism in extrahepatic organs may be largely determined by the degree of inducibility of different cytochromes [2, 3]. As carcinogens enter the body in relatively small quantities, even the low enzyme activity of extrahepatic tissues (in comparison with liver) might be sufficient to convert procarcinogens, e.g. *N*-nitroso compounds, into ultimate carcinogens [4], as suggested also by the fact that most known human carcinogens have target organs other than the liver [5].

Recently, evidence has been accumulating that different cytochrome P-450 isozymes preferentially metabolize certain carcinogens or produce certain types of metabolites [6–8]. Most studies have utilized the liver, from which various cytochrome P-450 isozymes have been purified and characterized [9–12]. Immunochemical inhibition of the reaction catalysed by a specific cytochrome P-450 isozyme is a useful and practical method for studying isozyme distribution in a tissue and the substrate specificity of various isozymes [13]. With MAb‖ against different cytochrome P-450 isozymes, each isozyme has been assigned a more specific role in metabolic reactions [14, 15]. An MAb, clone 1-7-1, has been produced against a rat liver MC-inducible cytochrome P-450 isozyme which completely inhibits the AHH and ECDE activities in a reconstituted enzyme system [16]. Another MAb, clone 2-66-3, inhibits enzyme activities catalysed by PB-inducible cytochrome P-450 isozyme [17]. These MAb can therefore be used to differentiate cytochrome P-450 isozymes of different antigenic phenotypes.

Dietary cholesterol has been shown to modify monooxygenase activities in both the liver [18] and extrahepatic organs such as the intestine [19]. Although diet is an important factor in the regulation of the intestinal monooxygenase activities [19–21], no study exists, to our knowledge, on how diet might modify cytochrome P-450 isozyme composition and thus modulate monooxygenase activities. Little attention has been paid, also, to extrahepatic monooxygenases, despite their potential importance in carcinogenesis and toxicology. We have studied whether modulation of dietary cholesterol content,

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‖ Abbreviations used: MAb, monoclonal antibody; MC, 3-methylcholanthrene; PB, phenobarbital; AHH, aryl hydrocarbon hydroxylase; ECDE, ethoxycoumarin *O*-deethylase; PMS, post-mitochondrial supernatant.

combined with treatment of rats with known enzyme inducers, alters the cytochrome P-450 isozyme patterns in liver and extrahepatic organs, using MAb for immunochemical inhibition of cytochrome P-450-catalysed reactions.

MATERIALS AND METHODS

Adult male Wistar rats were used. In the first part of the study, the inhibitory properties of MAb were graduated utilizing liver microsomes and intestinal postmitochondrial supernatant (PMS) and measuring ECDE and AHH activities at different MAb concentrations; concentrations that exceeded the threshold for maximal inhibition were used subsequently. Rats fed normal laboratory chow were treated either with PB (three doses of 100 mg/kg b.w. i.g. daily at 24-hr intervals in saline) or with MC (a single dose of 100 mg/kg b.w. i.g. in olive oil). Both groups of rats were killed 24 hr after the last dose; a third group served as untreated controls.

In dietary experiments rats were fed cholesterol-free or a high (2%)-cholesterol diet (ICN Nutritional Biochemicals, Cleveland, OH, U.S.A.) for one

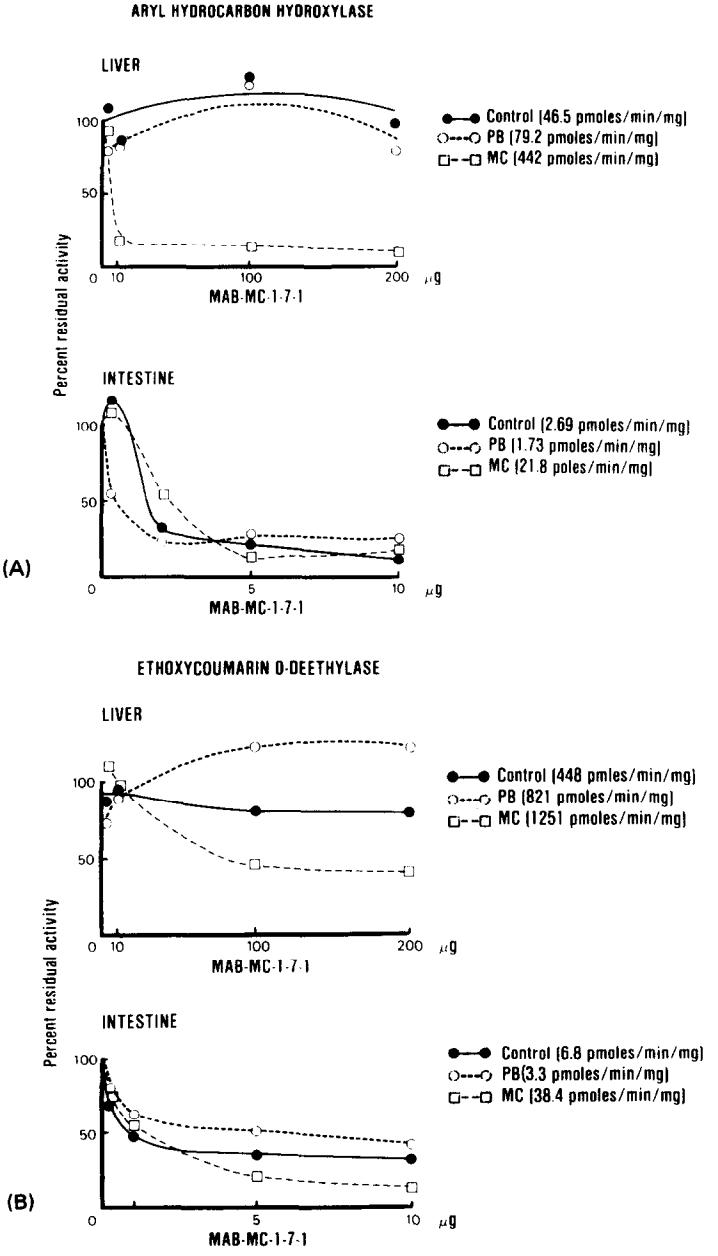

Fig. 1. The aryl hydrocarbon hydroxylase (A) and ethoxycoumarin *O*-deethylase (B) activities in the liver and in the intestine expressed as percentage of respective reference (NPS protein); activity is plotted as a function of MAb (MC-1-7-1) contents in the assay. The amount of liver microsomal protein was 10 µg per assay, and that of intestinal postmitochondrial supernatant protein 200 µg per assay. The symbols for variously treated rats and the initial enzyme activities (figures in parentheses) are shown.

month from the age of six weeks *ad libitum*. Animals were given either PB (in saline) at 100 mg/kg once a day for three days i.g., or MC (in olive oil) at 20 mg/kg once a day for three days i.g.; controls received respective amounts of saline or olive oil. The animals were killed 24 hr after the last dose. A further group of rats received a normal pelleted diet (cholesterol content 0.1%). Since the different control animals were similar in all of the parameters measured, they were combined.

After weighing the animals, the liver, kidneys, lungs and the mucosa of the excised 30-cm segment of the proximal small intestine were immersed in ice-cold saline and weighed. The tissues were homogenized in 0.15 M KCl–10 mM K_2EDTA buffer, pH 7.4 (1:4 wet w/v) and centrifuged at 12,000 *g* for 15 min (4°). This PMS was used for the enzyme assays of intestine and lungs; liver and kidney PMS was further centrifuged at 105,000 *g* for 60 min (4°), the supernatant was discarded and the microsomal pellet was resuspended in 0.2 M K-phosphate–1 mM K_2EDTA-1 mM dithiothreitol buffer, pH 7.4. After washing by ultracentrifugation, the microsomal pellet was finally resuspended in 0.2 M K_2HPO_4–20% glycerol–1 mM dithiothreitol buffer, pH 7.4, and used for enzyme assays.

MAb were produced by the hybridoma technique at the US National Cancer Institute, Laboratory of Molecular Carcinogenesis, using a modification of the method of Koehler and Milstein [22], tested and characterized as described previously [16, 17]. In the present study those clones of MAb against "MC-inducible" and "PB-inducible" cytochrome P-450 isozymes were used that were shown to cause maximal enzyme inhibition; clone 1-7-1 inhibits MC-inducible monooxygenases [16], and clone 2-66-3 inhibits PB-inducible monooxygenases. The latter MAb is less inhibitory than MAb 1-7-1 to MC-inducible monooxygenases [17]. As a reference protein, an NBS preimmune mouse peritoneal fluid was added to the assays, or a control MAb (Hy–Hel) against a chicken lysozyme was used, to determine any non-specific reaction. The MAb or reference protein was added to pooled microsomal or PMS suspensions 15 min prior to starting the assay at room temperature at the concentrations indicated in the results section. Thereafter, substrate–buffer solution of the respective (AHH, ECDE) enzyme assay was added to start the reaction. AHH was measured as described by Nebert and Gelboin [23] and ECDE as described by Greenlee and Poland [24]. The coefficients of variation (%) for the enzyme assays were: AHH ($\leq$ 16); ECDE ($\leq$ 18.5) (cf. 15). Protein content was measured according to Lowry *et al.* [25], and cytochrome P-450 concentration according to Omura and Sato [26].

Data obtained with different MAb concentrations are plotted for liver and intestine (Fig. 1); the concentrations of MAb were chosen to ensure maximal inhibition of AHH and ECDE activities and were used in further assays. Inhibition by MAb of AHH and ECDE was also tested for kidneys and lungs (not shown), prior to choosing the MAb concentration to be used in enzyme assays after dietary modulations. The MAb protein concentration (vs microsomal or PMS protein concentration) for use in enzyme assays

Table 1. Rat body and organ weights and protein and cytochrome P-450 concentrations after feeding various diets* and after treatment with phenobarbital (PB) or 3-methylcholanthrene (MC)

Treatment	Rat weight (g)†			Liver weight (g)‡			Liver MS protein (mg/g wet wt)†			Liver cyt. P-450 (nmol/mg protein)†		
	HC	CF	ND	HC	CF	ND	HC	CF	ND	HC	CF	ND§
MC	323 ± 4	338 ± 7	310 ± 5	16.0	17.3	13.7	28.8 ± 1.3	24.9 ± 0.7	27.6 ± 1.1	0.46 ± 0.01	0.34 ± 0.01**	0.41 ± 0.03
PB	305 ± 4	315 ± 7	309 ± 7	15.6	14.1	12.3	31.1 ± 1.5	29.7 ± 1.8	34.3 ± 0.6	0.94 ± 0.04***	0.46 ± 0.04***	0.76 ± 0.02
None	349 ± 1	327 ± 3	314 ± 7	15.1	13.9	11.6	24.1 ± 1.0	26.0 ± 1.0	26.9 ± 2.0	0.50 ± 0.01***	0.26 ± 0.01***	0.37 ± 0.01

* HC, 2% cholesterol; CF, 0% cholesterol; ND, normal diet; 20 rats per diet group, and 6–8 rats in each drug-treated subgroup; details of time and dose are described in Materials and Methods.
† Mean ± SEM.
‡ Mean.
§ Statistical comparisons were made between drug-treated (PB or MC) group and the normal diet group. **: $P < 0.05$; ***: $P < 0.001$.

was chosen such that for liver assays the MAb protein/microsomal protein ratio was 20; in extrahepatic tissues, the ratios of MAb protein/microsomal (kidney) and MAb protein/PMS (lungs and intestine) protein were 0.01.

RESULTS

In liver microsomes isolated from rats fed a commercial laboratory chow, both MC and PB treatment enhanced AHH and ECDE activities (Fig. 1A, B). In intestinal PMS, only MC was able to enhance monooxygenase activities (Fig. 1A, B). Using these data, complete inhibition curves for AHH and ECDE in the presence of MAb 1-7-1 were obtained (Fig. 1A, B). AHH activity in liver microsomes from MC-pretreated rats was inhibited maximally at MAb 1-7-1 protein concentration exceeding 10 μg MAb/10 μg microsomal protein, while ECDE activity was inhibited at MAb concentrations exceeding 100 μg/10 μg microsomal protein (Fig. 1). ECDE activity was inhibited to a lesser extent than AHH activity. AHH and ECDE activity was not inhibited by MAb 1-7-1 in liver microsomes of rats pretreated with PB or of control rats (Fig. 1). Intestinal AHH activity was inhibited by up to 80% by MAb 1-7-1 at a very low concentration exceeding 5 μg MAb protein/200 μg PMS protein (Fig. 1A); intestinal AHH activity from PB pretreated and control rats was inhibited to the same extent (Fig. 1A). Similarly, intestinal ECDE activity was inhibited by the MAb 1-7-1 protein to the same extent in control, PB-pretreated and MC-pretreated rats (Fig. 1B). Thus, the cytochrome P-450 isozyme catalysing AHH and ECDE activity in intestinal mucosa in control and PB-pretreated rats shares a common epitope with the MC-induced cytochrome P-450.

In the second part of the study, the organ-specific composition of cytochrome P-450 isozymes, as revealed by immunochemical inhibition of monooxygenase activities, was further characterized and modifications induced by different dietary cholesterol contents were studied. One-month feeding of any specific diet did not markedly alter final body weight, and changes in the liver paralleled the small changes in body weights (Table 1). The hepatic microsomal protein contents remained the same in dietary groups and in nontreated rats; PB treatment enhanced protein contents in all dietary groups. The hepatic cytochrome P-450 concentration was significantly lower in the cholesterol-free group and higher in the high-cholesterol group than in the normal diet group (Table 1). Although PB enhanced the P-450 concentration in all three groups, the relative differences remained; after MC treatment, the differences levelled off and P-450 concentration was enhanced only in the cholesterol-free group; only minor differences were present after MC treatment. In extrahepatic organs, there was no major difference in protein contents either between dietary groups or between treated groups (Table 2). The cytochrome P-450 concentration was below the level of detection except in renal microsomes, where it was elevated only in the high-cholesterol group after MC treatment.

Hepatic ECDE activity was lowest in the cholesterol-free group but was enhanced by MC to the same level as in rats fed normal or high-cholesterol diet; PB enhanced ECDE activity to a lesser degree (Table 3A). Hepatic AHH activity was lowest in the cholesterol-free group, but was enhanced to the same level as in other dietary groups by MC treatment; no increase was found after PB treatment. ECDE activity was very low in renal microsomes in all untreated dietary groups, and remained low after PB pretreatment; this activity was enhanced 12–30-fold after MC treatment. Renal AHH activity was enhanced 10 fold after MC treatment (Table 3B), but no diet-dependent difference was observed in ECDE or AHH activities. A 5–10-fold enhancement was seen in pulmonary ECDE and AHH activities of MC-treated rats as compared to untreated animals, independently of diet. The intestinal ECDE and AHH activities were lowest in the cholesterol-free group and 3–5-fold higher in both high-cholesterol and normal diet groups (Table 3). After MC treatment, the highest intestinal ECDE and AHH activities were found in the high-cholesterol group. In the cholesterol-free group, activity due to MC treatment was increased five fold, in the high-cholesterol and normal diet groups, induction of AHH and ECDE activities was much lower.

Hepatic ECDE activity was inhibited by 70% in MC-treated rats in all dietary groups by MAb 1-7-1, while in PB-treated rats MAb 2-66-3 caused a moderate inhibition in cholesterol-free and normal

Table 2. Mean microsomal protein (mg/g wet weight) and cytochrome P-450 (nmol/mg protein) contents of kidneys and postmitochondrial supernatant protein (mg/g wet weight) of lungs and small intestinal mucosa after feeding various diets* and after treatment with 3-methylcholanthrene (MC) or phenobarbital (PB)

	Kidney protein			Kidney cyt. P-450†			Lung protein			Intestinal protein		
Treatment	HC	CF	ND	HC	CF	ND	HC	CF	ND	HC	CF	ND
MC	16.6	22.6	20.9	0.08	0.04	0.04	158	144	166	78.4	73.6	72.8
PB	17.8	16.2	20.0	0.04	0.05	0.04	170	137	154	81.6	67.6	74.0
None	18.7	19.9	17.2	0.04	0.05	0.04	166	160	155	94.4	65.6	95.2

* HC, 2% cholesterol; CF, 0% cholesterol; ND, normal diet.

† Pulmonary and intestinal postmitochondrial supernatant cytochrome P-450 concentrations were below the detection limit.

Table 3. Ethoxycoumarin *O*-deethylase (ECDE) and aryl hydrocarbon hydroxylase (AHH) activities in liver and kidney microsomes and intestinal and pulmonary postmitochondrial supernatant (PMS) in rats fed various cholesterol diets* and treated with 3-methylcholanthrene (MC) or phenobarbital (PB)

Treatment	Liver			Kidney			Lung			Intestine		
	HC	CF	ND	HC	CF	ND	HC	CF	ND	HC	CF	ND
A. ECDE activity	nmol/min/mg (microsomal protein)						pmol/min/mg (PMS protein)					
MC	2.84	3.96	3.44	0.33	0.42	0.33	23.4	20.0	17.4	60.8	27.7	34.4
PB	1.04	0.56	0.89	0.01	0.02	0.02	4.3	5.7	5.7	29.2	15.1	15.1
None	0.77	0.25	0.69	0.01	0.03	0.01	6.0	6.0	7.5	26.8	4.5	18.9
B. AHH activity	pmol/min/mg (protein)											
MC	114.4	135.2	115.1	9.1	13.8	10.7	0.8	0.7	0.6	2.03	0.98	1.23
PB	74.2	27.7	43.7	0.8	1.1	1.3	0.06	0.04	0.09	1.53	0.63	0.75
None	68.6	28.4	44.4	0.8	1.6	0.9	0.09	0.08	0.16	1.04	0.24	0.74

* HC, 2% cholesterol; CF, 0% cholesterol; ND, normal diet.

diet groups; MAb 1-7-1 did not inhibit activity in any dietary group of PB-treated rats (Table 4). In liver microsomes from untreated rats, both clones inhibited the ECDE activity moderately when rats were fed normal diet, while in other dietary groups the inhibition was negligible. In renal microsomes, MAb 1-7-1 strongly inhibited ECDE activity after MC treatment in all dietary groups; 50–60% inhibition was seen after PB treatment and in untreated rats. MAb 2-66-3 did not inhibit renal ECDE activity after any treatment in any dietary group (Table 4). Interestingly, in lungs from both PB-treated and untreated rats, MAb 2-66-3 inhibited ECDE activity by 22–44%, but not in the MC-pretreated group, where MAb 1-7-1 inhibited 53–55% of the activity in all the dietary groups (Table 4). ECDE inhibition by MAb in intestinal mucosa was similar to that in kidneys, with strong inhibition by MAb 1-7-1 in all dietary groups after any treatment and in untreated rats, while MAb 2-66-3 caused no inhibition (Table 4).

Hepatic AHH activity in the high-cholesterol group after MC treatment was inhibited to a lesser extent by both MAb 1-7-1 and MAb 2-66-3 than in rats fed other diets (Table 5). Furthermore, inhibition of AHH activity was greater by MAb 2-66-3 in cholesterol-free group of untreated rats than in the high-cholesterol or normal diet groups (Table 5). In PB-treated rats of all dietary groups, MAb 2-66-3 inhibited hepatic AHH activity moderately (Table 5); in lungs and intestine MAb 2-66-3 strongly inhibited ECDE activity in the untreated cholesterol-free group. In kidneys of MC-treated rats in all dietary groups, MAb 1-7-1 strongly inhibited AHH activity, whereas MAb 2-66-3 caused no clear-cut inhibition. In lungs, MAb 1-7-1 inhibited AHH activity somewhat in the high-cholesterol and normal diet groups in all treatment groups, while in cholesterol-free groups, inhibition was present only after MC treatment. MAb 2-66-3 inhibited pulmonary AHH activity mainly in PB-treated rats fed high-cholesterol or normal diets. Intestinal AHH activity was inhibited by about 50% by MAb 1-7-1 in all dietary groups whether treated or not. MAb 2-66-3 inhibited the intestinal AHH activity only in untreated cholesterol-free rats where the total activity was the lowest.

DISCUSSION

We showed previously that dietary cholesterol may modify membrane cholesterol content in liver [27] and in small intestinal mucosa [19] and membrane fluidity [18]. In the present study, our aim was to study whether dietary cholesterol modifies the cytochrome P-450 isozyme composition in the liver and extrahepatic organs and whether there are organ-specific differences in isozyme composition, as seen by immunochemical inhibition of cytochrome P-450 catalyzed reactions.

Our results indicate that modification of dietary cholesterol contents may produce considerable changes in the activities of mixed function oxidases. A certain cholesterol content was found previously to be necessary to maintain mixed-function oxidase activity in the liver and in small-intestinal mucosa

Table 4. Inhibition of ethoxycoumarin *O*-deethylase activity by MAb 1-7-1 and MAb 2-66-3 in rats fed various cholesterol diets* and treated with 3-methylcholanthrene (MC) or phenobarbital (PB)†

		Liver			Kidney			Lung			Intestine		
Treatment	MAb	HC	CF	ND	HC	CF	ND	HC	CF	ND	HC	CF	ND
MC	1-7-1	30	28	28	23	28	26	47	47	45	39	34	37
	2-66-3	87	95	89	105	106	90	92	92	91	105	100	99
PB	1-7-1	102	94	92	47	54	35	92	91	76	45	48	47
	2-66-3	87	75	70	96	97	104	58	54	62	101	98	96
None	1-7-1	83	87	73	41	37	40	70	92	76	39	44	39
	2-66-3	87	92	72	102	97	96	59	56	78	97	89	101

* HC, 2% cholesterol; CF, 0% cholesterol; ND, normal diet.

† Results (% remaining activity) are expressed as: $\left(\frac{\text{activity with MAb}}{\text{activity with Hy–Hel}}\right)\times 100$.

Table 5. Inhibition of aryl hydrocarbon hydroxylase activity by MAb 1-7-1 and MAb 2-66-3 clones in rats fed various cholesterol diets* and treated with 3-methylcholanthrene (MC) or phenobarbital (PB)†.

		Liver			Kidney			Lung			Intestine		
Treatment	MAb	HC	CF	ND	HC	CF	ND	HC	CF	ND	HC	CF	ND
MC	1-7-1	68	53	59	48	46	50	47	53	54	49	53	57
	2-66-3	90	72	79	97	92	104	100	87	109	92	97	101
PB	1-7-1	90	92	108	78	85	70	33	91	51	57	59	58
	2-66-3	75	60	76	91	105	100	40	100	60	105	111	103
None	1-7-1	84	90	83	81	77	78	71	100	50	59	57	52
	2-66-3	78	65	93	114	94	89	78	60	142	122	36	105

* HC, 2% cholesterol; CF, 0% cholesterol; ND, normal diet.

† Results (% remaining activity) are expressed as: $\left(\frac{\text{activity with MAb}}{\text{activity with Hy–Hel}}\right)\times 100$.

[18, 27]. The inducibility of monooxygenase activities by MC in both the liver and small intestine was higher in the cholesterol-free group than in the other dietary groups, although the basic activity was much lower. Interestingly, even PB enhanced monooxygenase activities in small-intestinal mucosa in the cholesterol-free group, but not in the other groups. MC was also a potent inducer of AHH and ECDE activities in kidneys and lung, but independently of diet. Our data thus demonstrate that dietary cholesterol is of importance in regulating monooxygenase activities in liver and in small-intestinal mucosa but not in lung or kidney.

Previous studies in which intestinal cytochrome P-450 isozymes have been purified have suggested that they are mainly of the cytochrome P-448 type [28]. In the present study, however, the intestinal cytochrome P-450 isozyme in the cholesterol-free group with no drug treatment was detected mainly by MAb 2-66-3, based on inhibition of AHH activity (Table 5); AHH activity was only about 25% of that present in the untreated normal diet group (Table 3). Thus, even inhibition of a minor P-450 isozyme catalyzed enzyme activity became apparent due to loss of the major form of the isozyme, suggesting that under certain circumstances a minor P-450 isozyme may play a dominant role.

Table 6 gives a summary of inhibition of catalytic activities by MAb. In the livers of MC-treated rats, the MC-inducible form of cytochrome P-450 isozyme was always predominant, and no inhibition of catalytic activity was caused by MAb 2-66-3 in any dietary groups. In PB-treated or untreated rats, MAb 2-66-3 inhibited the catalytic activity, especially AHH activity in the cholesterol-free untreated group, probably owing to loss of P-448-type isozyme. In renal tissue, no inhibition of AHH or ECDE activity by MAb 2-66-3 was detected in any dietary group after any treatments, suggesting the absence of a "PB-type" isozyme in this tissue. No inhibition was seen even in the untreated cholesterol-free group, whereas some inhibition was present in the intestine when measured by AHH activity. Lungs showed a different response: in PB-treated or untreated rats of all dietary groups, a relatively strong inhibition of ECDE activity was produced by MAb 2-66-3; but in lungs of MC-treated rats in any dietary group, both MAb caused a strong inhibition of AHH activity after drug treatment, which was diet-dependent. This suggests that kidneys lack constitutionally the "PB-inducible" cytochrome P-450 isozyme or that gene regulates the induction of this isozyme, or lack the receptor that regulates this gene locus.

Our data reveal that cytochrome P-450 catalysed enzyme activities in liver and in small-intestinal mucosa respond to dietary modifications. Modu-

Table 6. Relative inhibition* of the ethoxycoumarin *O*-deethylase (ECDE) and aryl hydrocarbon hydroxylase (AHH) activities in various organs by MAb after feeding rats different cholesterol diets and treating them with 3-methylcholanthrene (MC) or phenobarbital (PB)

	High-cholesterol diet			Cholesterol-free diet			Normal diet		
Organ/MAb	MC	PB	None	MC	PB	None	MC	PB	None
A. ECDE									
Liver									
1-7-1	+++*	0	+	+++	0	0	+++	0	+
2-66-3	0	0	0	0	+	0	0	+	+
Kidney									
1-7-1	+++	+++	+++	+++	++	+++	+++	+++	+++
2-66-3	0	0	0	0	0	0	0	0	0
Lung									
1-7-1	+++	0	+	+++	0	0	+++	+	+
2-66-3	0	++	++	0	++	++	0	++	+
Intestine									
1-7-1	+++	+++	+++	+++	+++	+++	+++	+++	+++
2-66-3	0	0	0	0	0	0	0	0	0
B. AHH									
Liver									
1-7-1	++	0	+	++	0	0	++	0	+
2-66-3	0	+	+	+	++	++	+	+	0
Kidney									
1-7-1	+++	+	+	+++	+	+	+++	++	+
2-66-3	0	0	0	0	0	0	0	0	0
Lung									
1-7-1	+++	+++	+	++	0	0	++	++	+++
2-66-3	0	+++	+	0	0	++	0	++	0
Intestine									
1-7-1	+++	++	++	++	++	++	++	++	
2-66-3	0	0	0	0	0	0	0	0	0

* Inhibition grades as follows: 0, 0–14%; +, 15–24%; ++, 25–49%; +++, ≥ 50%.

lation due to dietary cholesterol content may arise from changes in cytochrome P-450 isozyme composition and/or membrane modifications, as shown previously. Those tissues, e.g. kidneys and lungs, that are unresponsive to dietary modulations, with an unchanged overall enzymic activity, such as the kidneys, lack either a regulatory mechanism or an isozyme; in the case of lungs, diet induces a change in the composition of P-450 isozymes (as in liver, but without altering total enzyme activity). The marked changes of cytochrome P-450 isozyme composition, as measured by immunochemical inhibition by MAb, suggest that diet may alter susceptibility to carcinogenesis by regulating metabolic activation/deactivation of chemicals and, as a consequence, may even alter the target organs for toxicity and carcinogenesis.

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