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COMPARATIVE ALTERATIONS IN EXTRAHEPATIC DRUG METABOLISM BY FACTORS KNOWN TO AFFECT HEPATIC ACTIVITY

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Abstract—Various factors known to alter hepatic drug metabolism were examined for their effects on drug metabolism in certain extrahepatic organs, viz. lung and kidney. The prominent sex-related differences in drug metabolism in rat liver were not seen in either lung or kidney. Pretreatment of rats with phenobarbital produced the expected large increases in hepatic NADPH cytochrome *c* reductase, cytochrome P-450, aminopyrine demethylase and biphenyl hydroxylase activities without concomitant changes in any of these parameters in lung, and only scattered and smaller changes in kidney. 3-Methylcholanthrene (3-MC) pretreatment significantly increased cytochrome P-450 levels in all three organs. Pretreatment of rats with carbon tetrachloride (CCl_4) produced consistent inhibition of mixed-function oxidation in hepatic microsomes, but the extrahepatic effects were less predictable and were both organ- and enzyme-specific. An increase in renal UDP-glucuronyltransferase activity was observed after CCl_4 treatment that paralleled a similar but larger increase observed in liver. Extrahepatic NADPH cytochrome *c* reductase and *N*-methyl-*p*-chloroaniline demethylase values were unaffected by CCl_4. Lung and kidney responded in a like manner to liver to the additions *in vitro* of β-diethylaminoethyl diphenylpropylacetate (SKF-525A). Losses in enzyme activities in lung and kidney microsomes roughly paralleled those of liver when stored as pellets for up to 14 days at −70°. Two or 4 days of starvation produced substrate-specific changes in enzyme-specific activity in liver and kidney, with lung appearing resistant to the effect. When enzyme activity was expressed on a whole organ basis, however, lung cytochrome P-450 values decreased significantly and parameters from liver and kidney increased or decreased in a substrate-specific manner. It is concluded that some physiological and pharmacological factors that influence hepatic drug metabolism produce similar effects in lung and kidney, while other factors produce organ-specific effects.

Much interest has been recently demonstrated in extrahepatic drug metabolism. Our group has compared mixed-function oxidation and conjugation of xenobiotics in lung and liver [1, 2] and has described the microsomal drug-metabolizing capabilities of lung, liver and kidney from a number of common laboratory species [3, 4]. Similar work has been carried out by other groups using lung or kidney, or other organs, such as intestine, adrenal or placenta. In addition, numerous investigators have studied the effects of various xenobiotics on drug metabolism in extrahepatic systems. Thus, Reid *et al.* [5] have examined the effect of bromobenzene on lung microsomal drug metabolism, and Zampaglione and Mannering [6] have examined the effect of a number of potential inducers or inhibitors of microsomal drug metabolism in adrenals and intestinal mucosa. The data that have accumulated on extrahepatic drug metabolism from these studies are fragmentary and present no concise and comprehensive picture of the comparability of extrahepatic and hepatic drug metabolism to exogenous influences. In a continuing attempt to arrive at a more thorough understanding of the biological control mechanisms active in the extrahepatic tissues, we have therefore studied the response of lung and kidney microsomal enzymes to some factors that have a well-documented effect on liver microsomal drug metabolism [7].

METHODS

General design

Adult male and female Sprague–Dawley-derived rats (170–230 g) or New Zealand rabbits (2 to 2.5 kg) were used throughout. Rabbits were housed two/cage on wire mesh floors and rats were housed four/cage on hardwood bedding chips in clear plastic cages. All animals were allowed food and water *ad lib.* except where specifically noted. Experiments were conducted on tissue pools from two rabbits or four rats and repeated a minimum of four times; data were expressed as the mean ± S.D. A two-tailed Student's *t*-test was used to distinguish significant differences between treated and control groups, except where it was appropriate to use a Dunnett's test for statistical analysis [8].

Experimental design

Sex differences. Possible sex differences in extrahepatic drug metabolism were investigated using adult male and female Sprague–Dawley rats.

Starvation. In experiments designed to determine the effect of starvation on drug metabolism, control (non-starved) animals were sacrificed for the preparation of microsomes on the morning of day 0 (control group 1). Remaining animals were either fed *ad lib.* (control groups 2 and 3) or starved for 48 or 96 hr,

and microsomal enzyme activities were determined simultaneously on fed and starved animals. Since no differences were found between control groups 1, 2 and 3, only the day 0 control data are presented for brevity (see Tables 2 and 3). Similar dual controls were run in the carbon tetrachloride experiments described below with similar results (see Table 4).

Storage. The effect of storage on microsomal drug metabolism was determined using microsomes from rats and rabbits. Microsomes were prepared as described below and either analyzed immediately upon resuspension (day 0) or were overlaid with 1.15% KCl containing 0.05 M Tris–HCl (pH 7.4) and frozen at −70° until analysis. On the day of the assay, pellets were thawed on ice prior to being resuspended as described below.

Carbon tetrachloride. Carbon tetrachloride (CCl_4) was examined for its effect on drug metabolism by injecting rats i.p. with 1.5 ml/kg of CCl_4 as a 25% (v:v) solution in corn oil. Control animals were injected with corn oil only. At various times after injection with CCl_4, animals were killed and analyzed as described below.

Inducers and inhibitors. Rats were injected once daily for 4 days with 80 mg/kg of Na phenobarbital in physiological saline or once daily for 3 days with 25 mg/kg of 3-methylcholanthrene (3-MC) dissolved in corn oil. Control animals received equivalent volumes of the solvents only. All injections were made i.p. Twenty-four hr after the final dose, animals were killed and enzyme activities determined. The response of microsomal enzymes to the addition *in vitro* of β-diethylaminoethyl diphenylpropylacetate (SKF-525A) was studied utilizing microsomes from untreated rabbits.

Preparation of microsomes, enzyme assays and analytical procedures

Animals were killed by cervical dislocation, and liver, lungs and kidneys were promptly removed and chilled in ice. The tissue was carefully trimmed and minced into small pieces with scissors and then homogenized in 3 vol. of cold 0.25 M sucrose containing 50 mM Tris–HCl (pH 7.4) in a Potter-type glass homogenizer with a motor-driven Teflon pestle. The homogenate was centrifuged for 20 min at 9,000 *g*, after which the supernatant was centrifuged for an additional 60 min at 105,000 *g*. The supernatant was aspirated and discarded and the microsomal pellet suspended in 1.15% KCl containing 0.05 M Tris HCl (pH 7.4). Protein content was determined by the method of Lowry *et al.* [9] and the microsomes were diluted with KCl Tris to contain 3 mg protein/ml.

All enzyme assays have been described in detail elsewhere [3]. Briefly, mixed-function oxidase activity was estimated by aerobically incubating substrates [biphenyl, 15 mM; aminopyrine, 25 mM; *N*-methyl-*p*-chloroaniline (NMPCA), 5 mM] with microsomes (1 mg protein/ml, final), Tris HCl buffer (100 mM, pH 7.4) and an NADPH-generating system at saturating concentrations for 15 min. UDP-glucuronyltransferase activity utilizing *p*-nitrophenol (PNP) as substrate (1 mM) was determined in the presence of saturating concentrations of UDPGA. Standard methods were then used to detect the products formed [3]. Cytochrome P-450 levels were determined by the method of Omura and Sato [10] by its dithionite difference spectrum, and NADPH cytochrome *c* reductase was determined as described by Williams and Kamin [11], and modified by Gigon *et al.* [12].

RESULTS

Sex differences

In rat liver, the expected significant sex-related differences were noted in aminopyrine *N*-demethylase and in cytochrome P-450 levels (Table 1). No corresponding differences were noted in lung or kidney for these parameters (Table 1). Similar observations (data not presented) were made with rabbit lung and kidney.

Starvation

Table 2 shows the response of hepatic and extrahepatic drug-metabolizing enzymes to 2 or 4 days of starvation. The lung appears to be resistant to changes induced by starvation, while kidney and liver show only isolated and bidirectional changes in drug metabolism. The liver shows the expected minor changes in specific activity that have previously been shown by others [13, 14]. Table 3 shows that, when the data are expressed on a whole organ basis, the changes in the activity of different enzyme pathways are more marked than was the case when data were expressed relative to microsomal protein (Table 2). In addition, significant changes are observed in lung, where cytochrome P-450 decreases significantly after only 2 days of starvation. It is interesting to note that when data are normalized in this way, the content of cytochrome P-450 and the activity of biphenyl

Table 1. Lack of sex difference in extrahepatic microsomal drug metabolism in adult Sprague–Dawley rats*

	Liver	Lung	Kidney
Aminopyrine *N*-demethylase			
Male	9.29 ± 1.16	0.48 ± 0.17	0.71 ± 0.22
Female	4.74 ± 1.37†	0.47 ± 0.18	0.72 ± 0.06
Cytochrome P-450 (ΔO.D./mg protein)			
Male	0.084 ± 0.005	0.007 ± 0.003	0.012 ± 0.005
Female	0.066 ± 0.007†	0.006 ± 0.003	0.014 ± 0.007
NADPH cytochrome *c* reductase			
Male	148 ± 11	42 ± 4	35 ± 6
Female	153 ± 32	58 ± 15	32 ± 3

* Enzyme activities are expressed as nmoles product formed/mg of protein/min. Values represent mean ± S.D.; N = 4.
† Male and female values significantly different at $P < 0.05$.

Table 2. Effect of starvation on hepatic and extrahepatic microsomal drug metabolism in rats*

	Days starved		
	0	2	4
Cytochrome P-450 (ΔO.D./mg protein)			
Liver	0.077 ± 0.012	0.086 ± 0.009	0.076 ± 0.019
Lung	0.009 ± 0.001	0.008 ± 0.003	0.009 ± 0.004
Kidney	0.013 ± 0.002	0.014 ± 0.015	0.020 ± 0.005†
NADPH cytochrome *c* reductase			
Liver	101 ± 29	112 ± 11	100 ± 20
Lung	36 ± 6	42 ± 4	42 ± 7
Kidney	26 ± 6	27 ± 2	24 ± 1
Biphenyl-4-hydroxylase			
Liver	0.83 ± 0.12	1.43 ± 0.15†	1.06 ± 0.08†
Lung	0.07 ± 0.01	0.10 ± 0.04	0.06 ± 0.02
Kidney	0.03 ± 0.01	0.06 ± 0.01	0.07 ± 0.01†
Aminopyrine *N*-demethylase			
Liver	5.32 ± 1.11	7.22 ± 0.80†	7.05 ± 1.12
Lung	0.21 ± 0.18	0.27 ± 0.25	0.33 ± 0.16
Kidney	0.35 ± 0.15	0.36 ± 0.10	0.64 ± 0.29
UDP-glucuronyl transferase (PNP)			
Liver	3.56 ± 0.86	2.81 ± 0.44	2.37 ± 0.88
Lung	1.65 ± 0.38	1.28 ± 0.39	1.29 ± 0.43
Kidney	2.72 ± 0.81	1.32 ± 0.36†	1.51 ± 0.40
Microsomal protein (mg/g)			
Liver	36.2 ± 5.3	28.9 ± 3.3	32.9 ± 5.9
Lung	11.1 ± 2.1	9.1 ± 1.8	9.7 ± 2.9
Kidney	22.4 ± 2.0	22.7 ± 1.5	24.9 ± 3.5

* Enzyme activities are expressed as nmoles product formed/mg of microsomal protein/min. Values are the mean ± S.D.; N = 4.
† Values at day 2 or 4 significantly different from day 0 at $P < 0.05$.

Table 3. Effect of starvation on microsomal drug metabolism in lung, liver and kidney of rats*

	Days starved		
	0	2	4
Organ weights			
Body	207 ± 55	181 ± 58	155 ± 51
Liver	9.20 ± 2.41	6.39 ± 1.85	5.09 ± 1.43†
Lung	1.16 ± 0.32	1.15 ± 0.20	1.02 ± 0.23
Kidney	1.59 ± 0.26	1.45 ± 0.63	1.46 ± 0.26
Cytochrome P-450 (ΔO.D./organ)			
Liver	25,241 ± 3,934.0	15,884.0 ± 1,662.0†	12,730.0 ± 3,182.0†
Lung	116 ± 13	84 ± 13†	89 ± 19
Kidney	463 ± 71	461 ± 164	727 ± 181†
NADPH cytochrome *c* reductase			
Liver	33,108.0 ± 9,506	20,686.0 ± 2,032†	16,750.0 ± 3,350†
Lung	464 ± 77	439 ± 42	415 ± 69
Kidney	926 ± 214	888 ± 66	872 ± 36
Biphenyl-4-hydroxylase			
Liver	272 ± 38	264 ± 28	177 ± 13†
Lung	0.90 ± 0.13	1.05 ± 0.41	0.59 ± 0.19
Kidney	1.07 ± 0.35	1.97 ± 0.32†	2.54 ± 0.36†
Aminopyrine *N*-demethylase			
Liver	1,744 ± 364	1,333 ± 129	1,181 ± 187
Lung	2.71 ± 2.32	‡	3.26 ± 1.58
Kidney	12.5 ± 5.3	11.8 ± 3.3	23.3 ± 8.7
UDP-glucuronyltransferase (PNP)			
Liver	1,167 ± 282	519 ± 81†	397 ± 147†
Lung	21.3 ± 4.9	13.4 ± 4.1	12.8 ± 4.2
Kidney	96.8 ± 25.3	43.4 ± 11.8†	54.9 ± 14.5†

* All enzyme activities are expressed as nmoles product/whole organ/min. Values are the mean ± S.D.; N = 4.
† Values at day 2 or 4 significantly different from day 0 at $P < 0.05$.
‡ Not measured.

Table 4. Effect of CCl_4 treatment on microsomal drug metabolism in lung, liver and kidney of male rats*

	Time post treatment (hr)					
	0	2	12	24	48	144
Cytochrome P-450 (ΔO.D./mg protein)						
Liver	0.076 ± 0.007	0.052 ± 0.007†	0.025 ± 0.002†	0.024 ± 0.007†	0.033 ± 0.011†	0.055 ± 0.006†
Lung	0.006 ± 0.001	0.003 ± 0.002†	0.003 ± 0.001†	0.002 ± 0.002†	0.002 ± 0.003	0.005 ± 0.003
Kidney	0.012 ± 0.003	0.014 ± 0.002	0.013 ± 0.000	0.014 ± 0.003	0.012 ± 0.002	0.012 ± 0.004
NADPH cytochrome *c* reductase						
Liver	171 ± 3	192 ± 34	138 ± 22†	117 ± 12†	112 ± 21†	99 ± 27†
Lung	51 ± 2	47 ± 5	51 ± 4	50 ± 2	49 ± 12	54 ± 10
Kidney	39 ± 2	34 ± 2	40 ± 3	51 ± 7	41 ± 5	34 ± 10
Biphenyl-4-hydroxylase						
Liver	0.77 ± 0.06	0.29 ± 0.05†	0.38 ± 0.25†	0.11 ± 0.06†	0.19 ± 0.12†	0.26 ± 0.24†
Lung	0.05 ± 0.01	0.01 ± 0.01†	0.01 ± 0.01†	ND‡	0.03 ± 0.02†	0.03 ± 0.04
Kidney	0.03 ± 0.02	0.04 ± 0.00	ND‡	0.01 ± 0.01	0.02 ± 0.02	ND‡
NMPCA *N*-demethylase						
Liver	5.18 ± 0.95	4.97 ± 0.75	2.04 ± 0.34†	1.89 ± 0.47†	2.09 ± 0.53†	3.56 ± 0.77†
Lung	1.34 ± 0.45	1.53 ± 0.16	0.57 ± 0.11	1.32 ± 0.22	1.27 ± 0.55	1.86 ± 0.47
Kidney	2.66 ± 1.01	2.65 ± 0.14	1.44 ± 0.50	2.10 ± 0.30	2.29 ± 1.42	4.41 ± 0.80
UDP-glucuronyl transferase						
Liver	2.76 ± 0.53	5.28 ± 1.02†	9.63 ± 1.34†	6.14 ± 0.75†	4.04 ± 0.67†	2.63 ± 0.22
Lung	1.20 ± 0.07	1.05 ± 0.33	1.18 ± 0.12	1.38 ± 0.48	1.37 ± 0.60	1.27 ± 0.35
Kidney	1.55 ± 0.33	2.28 ± 0.23†	2.06 ± 0.26†	2.08 ± 0.53	2.25 ± 0.47†	1.76 ± 0.61
Microsomal protein (mg/g)						
Liver	34.9 ± 1.6	30.8 ± 4.1	26.1 ± 4.1	22.0 ± 2.9†	25.1 ± 2.3†	32.4 ± 5.3
Lung	8.9 ± 1.3	11.8 ± 4.0	8.9 ± 0.7	11.3 ± 1.3	8.3 ± 2.6	8.2 ± 1.1
Kidney	24.4 ± 1.1	21.8 ± 2.6	22.4 ± 2.0	26.7 ± 2.6	23.7 ± 1.5	22.3 ± 1.4

* Enzyme activities are expressed as nmoles product formed mg of protein min. Values represent mean ± S.D.; N = 4.
† Values significantly different from 0 time at $P \geq 0.05$.
‡ Non-detectable.

Table 5. Effect of pretreatment of rabbits with phenobarbital or 3-methylcholanthrene (3-MC) on hepatic and extrahepatic microsomal drug metabolism

	Liver	Lung	Kidney
Biphenyl-4-hydroxylase			
Control	1.06 ± 0.68	1.07 ± 0.50	0.11 ± 0.05
Phenobarbital	2.97 ± 0.72†	1.38 ± 1.11	0.22 ± 0.03†
3-MC	0.90 ± 0.08	1.04 ± 0.35	0.17 ± 0.10
NMPCA *N*-demethylase			
Control	4.05 ± 0.28	2.62 ± 0.55	0.69 ± 0.47
Phenobarbital	9.44 ± 0.43†	2.89 ± 0.20	1.43 ± 0.66
3-MC	‡	‡	‡
UDP-glucuronyltransferase (PNP)			
Control	7.11 ± 3.00	0.12 ± 0.28	2.40 ± 0.95
Phenobarbital	5.20 ± 0.84	0.00 ± 0.00	2.84 ± 1.09
3-MC	7.39 ± 2.95	0.33 ± 0.27	3.05 ± 0.20
NADPH cytochrome *c* reductase			
Control	133 ± 25.8	85.8 ± 14.4	29.3 ± 3.0
Phenobarbital	194 ± 37.4†	96.9 ± 4.9	41.6 ± 10.8
3-MC	131 ± 17.3	110 ± 9.4†	30.8 ± 4.0
Cytochrome P-450 (ΔO.D./mg protein)			
Control	0.128 ± 0.022	0.021 ± 0.001	0.014 ± 0.003
Phenobarbital	0.232 ± 0.028†	0.022 ± 0.006	0.030 ± 0.008†
3-MC	0.224 ± 0.034†	0.027 ± 0.004†	0.025 ± 0.004†
Microsomal protein (mg/g)			
Control	27.7 ± 6.2	6.61 ± 0.86	15.1 ± 3.3
Phenobarbital	43.0 ± 7.2†	6.13 ± 2.70	18.4 ± 3.4
3-MC	28.1 ± 5.5	5.08 ± 0.52†	15.0 ± 1.4

* Enzyme activities are expressed as nmoles product formed/mg of protein/min. Values represent mean ± S.D.; N = 4.
† Values significantly different from control at $P < 0.05$. Student's *t*-test was used to compute the significance of differences between 3-MC-treated vs corn oil-treated and between saline-treated vs phenobarbital-treated rats. ($P \leq 0.05$). "Control" refers to the combined corn oil and saline data, which were found not to differ statistically.
‡ Not measured.

hydroxylase in kidney actually increase significantly after fasting, while in the liver these parameters either decrease or show no change.

CCl_4

Alterations produced by CCl_4 administration on mixed-function oxidases were complex and varied from organ to organ. For example, on the one hand, CCl_4 administration significantly reduced biphenyl hydroxylase activities in liver, lung and kidney (Table 4). On the other hand, CCl_4 significantly reduced NADPH cytochrome *c* reductase activity in liver without influencing this activity in lung or kidney. Levels of cytochrome P-450 in liver and lung were reduced by CCl_4 administration but kidney levels were unaffected. Demethylation of NMPCA was reduced in liver but unaltered in lung and kidney. Thus, the enzyme activities were consistently reduced in hepatic microsomes from CCl_4-treated animals, but the extrahepatic response was far less predictable and was both organ- and enzyme-specific (Table 4). Finally, it is of interest that hepatic UDP-glucuronyltransferase activity was markedly stimulated (> 3-fold) by CCl_4 pretreatment (Table 4). A similar but less significant trend was observed in kidney, although the pulmonary system was unresponsive.

Table 6. Effect of SKF-525A on drug oxidation by microsomes from rabbit lung, liver and kidney*

Substrate	SKF-525A concn (mM)	Liver	Lung	Kidney
Biphenyl	0	0.62 ± 0.07	0.74 ± 0.04	0.11 ± 0.02
	0.01	0.47 ± 0.06	0.70 ± 0.09	0.11 ± 0.02
	0.1	0.31 ± 0.05†	0.39 ± 0.04†	0.07 ± 0.02
	1	0.14 ± 0.0†	0.03 ± 0.01†	0.02 ± 0.01†
Aminopyrine	0	7.26 ± 1.08	1.64 ± 0.39	0.51 ± 0.03
	0.01	6.20 ± 0.89	1.45 ± 0.30	0.31 ± 0.14†
	0.1	5.88 ± 0.93	0.70 ± 0.18†	0.27 ± 0.14†
	1	2.91 ± 0.70†	0.12 ± 0.09†	0.11 ± 0.12†
Ethylmorphine	0	2.56 ± 0.43	0.44 ± 0.09	0.08 ± 0.09
	0.01	2.64 ± 0.68	0.32 ± 0.09	0.19 ± 0.14
	0.1	1.49 ± 0.33†	0.19 ± 0.11†	0.07 ± 0.10
	1	1.26 ± 0.27†	0.03 ± 0.04†	0.09 ± 0.11

* Enzyme activities are expressed as nmoles product formed/mg of protein/min. Values represent mean ± S.D.; N = 3.
† Values significantly different from control at $P < 0.05$.

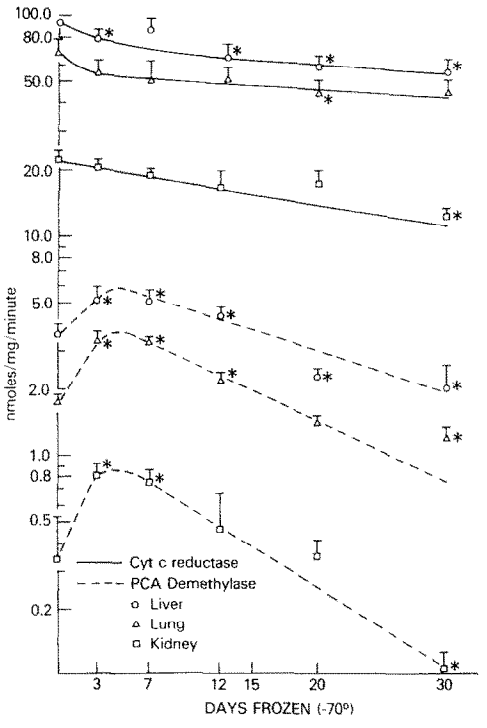

Fig. 1. Effect of storage at −70° on specific activity of NADPH cytochrome *c* reductase (——) and *N*-methyl-*p*-chloroaniline (– – –) *N*-demethylase in rabbit liver (○), lung (△) and kidney (□). Each point is the mean ± S.D. of six replicates from pools of tissue that were prepared on 2 separate days. Values which are significantly different from the 0 day control are indicated by an asterisk ($P \leq 0.05$).

Induction and inhibition

Phenobarbital pretreatment produced the expected significant increases in hepatic biphenyl hydroxylase, NMPCA *N*-demethylase, cytochrome P-450, NADPH cytochrome *c* reductase activities, and in microsomal protein (Table 5), but produced only scattered and generally smaller increases in extrahepatic parameters. 3-MC treatment significantly increased microsomal cytochrome P-450 content in liver, lung and kidney (Table 5). Table 6 shows the response of selected substrates of the mixed-function oxidase system in rabbit organs to SKF-525A *in vitro*. It can be seen that enzymes from all three organs responded similarly to this inhibitor of drug metabolism.

Storage

Figure 1 shows the influence of storage at −70° on NADPH cytochrome *c* reductase and NMPCA demethylase activities in microsomes from rabbit liver, lung and kidney. It can be seen that in all cases the lung and kidney activities responded qualitatively similarly to those from liver in their rate of loss in activity. In addition, the transient increase in NMPCA activity during week 1 of storage was seen in all three organs, although the extent and duration of the increase were organ-specific. Storage experiments conducted with rat microsomes (not shown) yielded similar results, except that the rat enzyme activities decayed more rapidly than those of rabbit, usually reaching a 50 per cent activity level within 10–14 days.

DISCUSSION

Kato [15] suggested that the well-documented sex difference in hepatic microsomal drug oxidation in rats may be related to higher plasma testosterone levels in males as compared with females. Our data demonstrating a lack of sex difference in rat lung and kidney drug metabolism (see Table 1) suggest a more complex system which must involve differences in end-organ responsiveness. In another study [16], comparable to the present investigation, no significant sex differences in drug metabolism were observed in rat lung or intestine with a number of drug substrates different from those used here.

The effects of starvation on hepatic drug metabolism show some apparently conflicting results, and various authors have shown no change, increases or decreases in enzyme activities depending on the specific parameter studied and the duration of starvation [14, 17, 18]. Furthermore, changes in activity become more significant or even reverse direction when data are normalized per whole organ rather than per unit of microsomal protein [17–20]. The importance of conducting inter-organ comparisons in the same laboratory and under the same conditions is, therefore, of great importance. The data shown in Table 2 suggest that lung microsomal drug-metabolizing enzymes appear relatively unresponsive to starvation-induced changes, while liver and kidney enzymes appear more susceptible. When the data are recalculated on the basis of organ weight, however (see Table 3), one sees, for extrahepatic organs, changes that are similar to those reported for the liver.

The storage of liver microsomes from various species has been thoroughly investigated, and the stability of various enzymes shown to be non-uniform. Little comparable work, however, has been done utilizing microsomes from extrahepatic sources [21]. The data in Fig. 1 demonstrate that the enzyme activity from extrahepatic organs responded qualitatively similarly to hepatic activities. Although only two pathways are shown in the figure, the response of other parameters showed the same inter-organ relationships. Furthermore, parallel experiments with rat microsomes duplicated these results with rabbit microsomes except that the time course was much shorter (data not presented).

The relative lack of effect of CCl_4 on drug metabolism in extrahepatic organs may seem surprising, particularly in view of the nephrotoxicity and to a lesser extent pneumotoxicity of CCl_4 [22, 23], and the fact that the lung is a major excretory route for this compound [24, 25]. However, it is now generally accepted that CCl_4 itself is inactive and must be activated to the proximate toxicant by mixed-function oxidases [26]. Although the presence of these enzymes has been demonstrated in both lung and kidney [3, 4], it is possible that the levels of this activating system in these organs may be too low to produce sufficient amounts of the toxic metabolite after a single dose of CCl_4. Alternatively, the proximate toxicant may be rapidly inactivated or cleared by lung and kidney. It is of interest to note that CCl_4 produces an increase

in the specific activity of UDP-glucuronyltransferase particularly in liver but to a lesser extent in kidney (see Table 4). One might suspect that this represents an activation of UDP-glucuronyltransferase rather than induction of synthesis of enzyme protein. Support for this suggestion can be found in the ease with which UDP-glucuronyltransferase is known to be activated [27] by a variety of solvents, detergents and other compounds and the recent findings of Aitio [28] and Otani *et al.* [29], who demonstrated that CCl_4 produced an activation of hepatic UDP-glucuronyltransferase activity.

The induction of mixed-function oxidase activity in extrahepatic organs has previously been demonstrated [30–33], but most work has dealt with either the induction of aryl hydrocarbon hydroxylase or with induction by polycyclic aromatic hydrocarbon administration [28, 30, 32]. Some work, however, has been done with phenobarbital and other drugs, and most of these studies suggest that the activity of extrahepatic enzymes may be relatively refractory to induction by drug treatment [31, 33, 34]. Data presented in Table 5 show mixed-function oxidases in the lung and kidney to be relatively resistant to induction, and to differ in substrate specificity and responsiveness to inducing agents. It can be seen that the lung cytochrome P-450 is responsive to 3-MC treatment but not to phenobarbital, although phenobarbital did provide significant increases in several hepatic parameters. The kidney, on the other hand, responds to both 3-MC and phenobarbital, as does the liver, but to different degrees. Mixed-function oxidases from all three tissues responded similarly to the addition *in vitro* of SKF-525A, a known inhibitor of microsomal drug metabolism (see Table 6).

The present study has shown that drug metabolism in the lung and kidney responds in a parameter-specific manner to factors that produce consistent and significant effects on hepatic drug metabolism. Because the liver also often responds in a substrate-specific manner to exogenous stimuli, this suggests that the non-uniform responsiveness of the mixed-function oxidase system in the three organs may be related to separate control mechanisms. It should be recalled that both the lung and kidney, in addition to the parameter specificity of their responses, responded in an inconsistent manner relative to the liver response to the various factors examined in this work. This lack of general similarity in responsiveness suggests that drug metabolism in each organ may be under individual control and not interrelated.

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