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SODIUM CHOLATE EXTRACTION OF RAT LIVER NUCLEAR XENOBIOTIC-METABOLIZING ENZYMES*

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Abstract—DNA is the purported target of several carcinogenic and mutagenic agents. Nuclear enzymes which could generate or detoxify reactive metabolites are of major concern. Several such enzymes have been identified within nuclei, but obtaining samples with enriched content or activity is difficult, time-consuming, and uses harsh isolation techniques. Extraction of rat liver nuclear suspensions with cholate-containing buffer results in solubilization of 25–30% of the protein. Linear extraction was obtained for total protein and cytochromes P-450 and b_5, NADPH–cytochrome P-450 reductase, NADH–cytochrome b_5 reductase, DT-diaphorase, and microsomal-like epoxide hydrolase with specific activities comparable to values reported for isolated nuclear membrane, while the yield was five to ten times greater. Detergent extracts of rat liver nuclei were employed to study the comparative response of microsomal and nuclear enzymes to chemical treatment. While the responses to acute inductive (phenobarbital and 3-methylcholanthrene) and toxic (carbon tetrachloride and dibromochloropropane) treatments were qualitatively similar, an initiation–promotion protocol (diethylnitrosamine with phenobarbital promotion) resulted in divergent responses between the enzymes in the two subcellular fractions. Detergent extracts of nuclei offer an efficient means of recovering xenobiotic-metabolizing enzymes from rat liver nuclei, and have been utilized to demonstrate a differential response of nuclear enzymes during preneoplastic development.

The metabolic activation site of xenobiotics plays an important role in the ensuing toxic cellular responses. Much attention has focused on the content of nuclear xenobiotic-metabolizing enzymes due to their proximity to cellular DNA, the proposed target of many carcinogens and mutagens. Cytochrome P-450, NADPH–cytochrome P-450 reductase (EC 1.6.2.4), cytochrome b_5, NADH-cytochrome b_5 reductase (EC 1.6.2.2), UDP glucuronyl transferase (EC 2.4.1.17), epoxide hydrolase (EC 3.3.2.3), and flavin-containing monooxygenase (EC 1.14.13.8) have been localized in liver nuclei or nuclear envelope preparations [1–7], and nuclear enzymes may be important in the metabolism of a number of genotoxins [4, 8–12]. The qualitative similarity in enzymatic content of the endoplasmic reticulum (microsomes) and nuclear envelope is not surprising as the two membranes are continuous and share certain additional biochemical similarities. Whether these similarities extend to their responses to chemical stimulation is still a matter of controversy.

The responses of liver nuclear enzymes to the inductive effects of polycyclic hydrocarbons and phenobarbital have been well studied; however, investigations on their responses to toxic compounds or chronic treatments have been more limited. Whole nuclei, nuclear envelopes, and microsomal enzymes have repeatedly been found to respond in a similar manner to treatment with 3-methylcholanthrene or β-naphthoflavone [2–4, 7–10, 13–21]. Conflicting reports are numerous, however, on the response of nuclear membrane cytochrome P-450, NADPH-cytochrome P-450 reductase, and expoxide hydrolase to phenobarbital treatment (compare findings of Refs. 2 and 21–23 with those of 4, 14 and 24; see Ref. 25 for a thorough review). This discrepancy may arise from the techniques utilized to isolate nuclear membranes [19]; while liver nuclei can be rapidly isolated in high yield [26], the preparation of nuclear subfractions involves lengthy and potentially harsh treatments.

Most xenobiotics are lipophilic in nature and enzymes involved in their metabolism commonly reside in a lipophilic environment. As an alternative means of obtaining nuclear xenobiotic-metabolizing enzymes, we tested the utility of detergent extraction of nuclei. This procedure rapidly provides enriched preparations of cytochromes P-450 and b_5 [19, 27]. Here, we have tested the utility of this procedure to extract other xenobiotic-metabolizing enzymes from liver nuclei. This procedure was used to compare the response of nuclear and microsomal enzymes to the inductive effects of phenobarbital and 3-methylcholanthrene, the heme protein-lowering effects of the hepatotoxins carbon tetrachloride (CCl_4) and

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1,2-dibromo-3-chloropropane (DBCP),* and an initiation–promotion protocol using a single dose of diethylnitrosamine in hepatectomized, phenobarbital-challenged rats. Divergent responses between the two organelles to the latter treatment suggested a differential response of nuclear xenobiotic-metabolizing enzymes.

MATERIALS AND METHODS

Animals and treatment. Male Sprague–Dawley rats (CD strain, Charles Rivers, Wilmington, MA) weighing 200–300 g were housed in steel-screened cages in environmentally controlled rooms with food (Purina Lab Chow) and water provided *ad lib.* Specified animals were treated with three daily i.p. injections of phenobarbital (50 mg/kg), 3-methylcholanthrene (25 mg/kg) or the respective solvents 0.9% saline and corn oil (10 ml/kg). CCl_4 (1.0 ml/kg) and DBCP (0.1 ml/kg) were dissolved in mineral oil and administered as a single oral dose by gastric intubation. Controls received a similar dose of mineral oil (5 ml/kg). The initiation–promotion protocol was similar to that described by Pitot *et al.* [28]. Male rats were given a two-thirds partial hepatectomy or sham-operation while under ether anesthesia. At 24 hr, animals received a single oral dose of diethylnitrosamine (10 mg/kg) or the vehicle (5 ml/kg water). After 8 weeks, specified animals received 0.05% phenobarbital in the drinking water (made fresh twice weekly) until termination of the experiment at 32 weeks. All rats were fasted 16 hr prior to being killed at the times indicated. Under light ether anesthesia, livers were perfused *in situ* with cold 0.9% saline and then were removed and weighed.

Preparation of cell fractions. Livers were homogenized in 0.1 M potassium phosphate (pH 7.4), 5 mM $MgCl_2$ and centrifuged for 10 min at 18,000 *g*. Total, or rough and smooth microsomes and cytosol were prepared from the supernatant fluid [27]. Nuclei were prepared from resuspensions of the 18,000 *g* pellet essentially as described by Blobel and Potter [26] except that the cushion was 2.1 M STKM2 [2.1 M sucrose, 50 mM Tris–HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol] [19, 27]. Nuclei were rinsed in 0.25 M STKM2 and then in 0.88 M STKM2. Next, nuclear pellets were resuspended in extraction buffer [0.1 M potassium phosphate (pH 7.4), 1 mM 2-mercaptoethanol, 20% glycerol, 5 mM $MgCl_2$, 1 mM EDTA, and 0.6% sodium cholate], incubated for 15 min at 4°, and then centrifuged for 10 min at 2000 *g* as previously described [19]. The resulting supernatant fraction was the detergent extract of nuclei used for assays. Nuclear envelopes were prepared using a discontinuous high-salt sucrose gradient as previously described [19].

Morphology of isolated nuclei. For fluorescent microscopy, nuclei were resuspended in 0.25 M STKM2 and mixed (1:1) with 0.8% Sea Plaque low gelling temperature agar and then placed on ice until gelled. Agar blocks were then coated with Tissue Tek and frozen in liquid N_2; sections were cut on a cryostat. Sections were then processed for non-specific indirect immunofluorescence using normal rabbit IgG, examined, and photographed as previously described [29]. For electron microscopy, nuclei were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), post-fixed in 1% osmium tetroxide, and embedded in Epon epoxy resin. Thin sections were cut on a Sorvall MT2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a JEOL 100S electron microscope.

Biochemical assays. Protein was determined by the method of Lowry *et al.* [30]. Cytochromes P-450 and b_5 were determined from difference spectra as described by Omura and Sato [31]. NADPH–cytochrome P-450 reductase was measured as the NADPH-mediated reduction of cytochrome *c* [32], NADH–cytochrome b_5 reductase as the ferricyanide-mediated oxidation of NADH [33], microsomal epoxide hydrolase as diol production from [^{3}H]*cis*-stilbene oxide at pH 9.0, cytosolic epoxide hydrolase as diol production from [^{3}H]*trans*-stilbene oxide at pH 7.4, and glutathione-*S*-transferase as conjunction of [^{3}H]*cis*-stilbene oxide to glutathione at pH 7.4, as described by Gill *et al.* [34]. Diethylmaleate was added to cytosolic and nuclear epoxide hydrolase assays to prevent conjugate formation from endogenous glutathione as previously described [35]. DT-Diaphorase activity was determined using a modification of the procedure of Lind and Ernster [36] with NADPH and NADH (0.5 mM) as hydrogen donors, menadione (6.5 μM) as hydrogen acceptor, and continuous reoxidation by cytochrome *c* (61.4 μg/ml) in 0.1 M potassium phosphate (pH 7.4). DT-Diaphorase activity was the difference between rates of cytochrome *c* reduction in the presence and absence of dicoumarol (10 μM). All spectrophotometric assays were performed with an Aminco DW 2a spectrophotometer. Scintillation counting was performed with an LKB 1217 Rackbeta liquid scintillation counter. Polypeptides in whole microsomes and detergent extracts of nuclei were separated by SDS–PAGE, and the gels were scanned using an LKB laser densitometer as previously described [29].

RESULTS AND DISCUSSION

Principles of extraction. Liver nuclei were isolated using a method [19, 37, 38] derived from Blobel and Potter [26], which utilizes a 2.1 M, rather than a 2.3 M, sucrose buffer cushion. Nuclear preparations sedimented throught the lighter cushion were similar to those obtained with the original method by light and electron microscopy (Fig. 1) and by enzymatic assay ([37], see below).

Comparison of the protein recovered in detergent extracts with those from nuclear envelope preparations readily demonstrates the efficiency of detergent extraction. In control, phenobarbital-, and 3-methylcholanthrene-treated rats, 32.0, 34.4, and 32.2% of nuclear protein was recovered in sodium cholate extracts of nuclei versus 4.1, 2.8, and 4.3% of

* Abbreviations: DBCP, 1,2-dibromo-3-chloropropane; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; and STKM2, sucrose (at molarity specified), 50 mM Tris (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol.

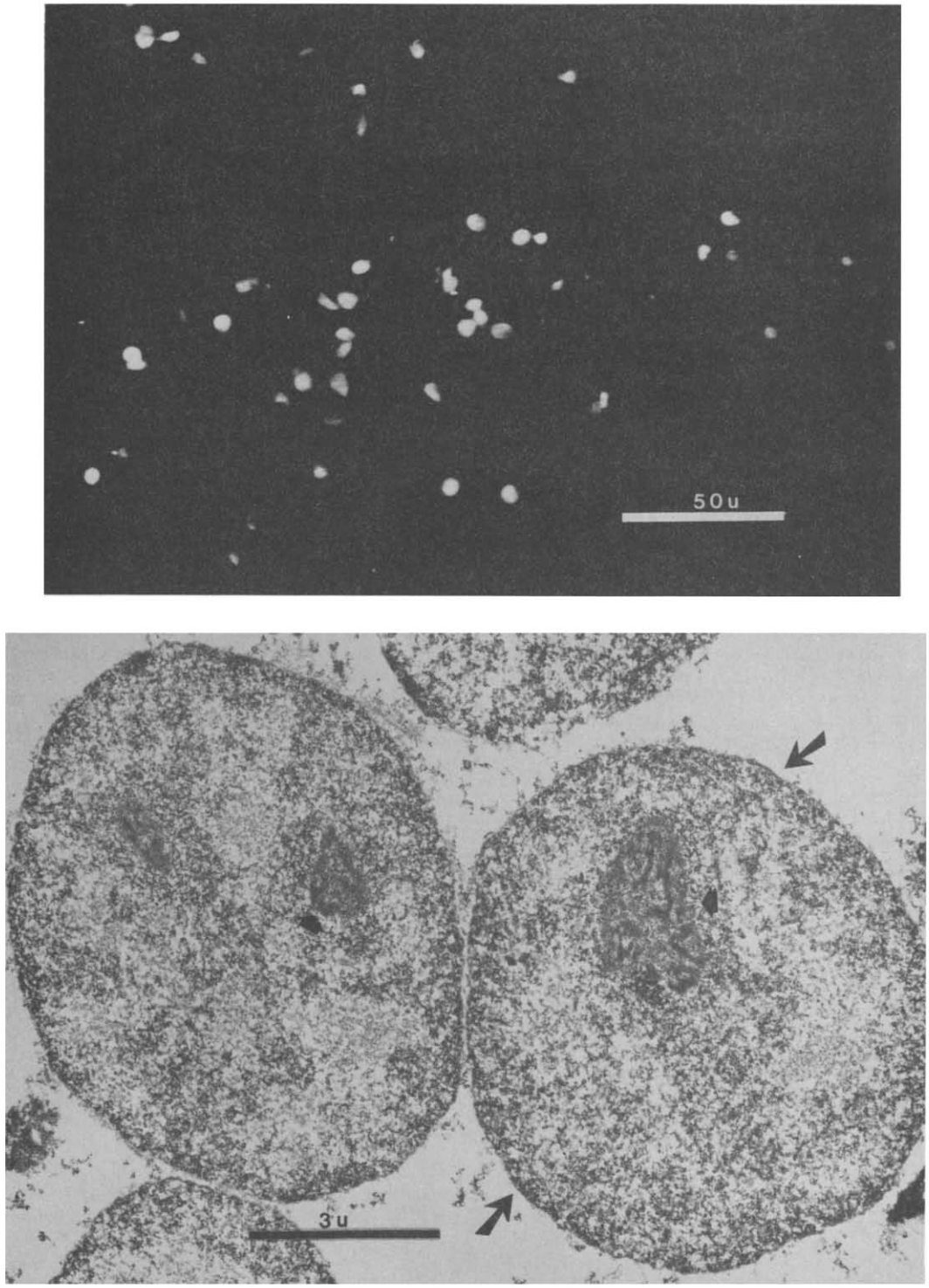

Fig. 1. Morphology of isolated liver nuclei used for detergent extraction. Nuclei were prepared from control male rat livers by pelleting through 2.1 M STKM2 with subsequent resedimentation through 0.25 and 0.88 M STKM2. (Upper panel): Resuspended nuclei were processed for indirect immunofluorescence. Nonspecific fluorescence demonstrates intact nuclei with minimal contamination from other cell fractions (600). (Lower panel): Electron microscopic examination of nuclear pellets. The majority of the nuclei remained intact with clearly-defined hetero- and euchromatin, prominent nucleoli (arrowheads), and intact nuclear envelope (arrows). Occasional nuclei were disrupted, which accounts for debris. Contamination from endoplasmic reticulum, mitochondria, and other cytoplasmic organelles was minimal (12,000).

Table 1. Protein recovered in liver nuclear subfractions

Treatment	Protein recovered (mg/g liver)				
	2.3 M Cushion		2.1 M Cushion		
	Whole nuclei	Detergent extract	Whole nuclei	Detergent extract	Nuclear envelope
A. Control – 3 day	1.90 ± 0.29	0.56 ± 0.89	2.25 ± 0.18	0.72 ± 0.01	0.092 ± 0.018
PB	1.46 ± 0.17*	0.33 ± 0.09*	1.83 ± 0.21*	0.63 ± 0.01*	0.051 ± 0.016*
3-MC	1.51 ± 0.26	0.46 ± 0.06	2.05 ± 0.18	0.66 ± 0.03*	0.088 ± 0.018
B. Control – 48 hr	2.12 ± 0.33	0.54 ± 0.02	2.86 ± 0.35	1.04 ± 0.13	0.074 ± 0.009
CCl_4	0.68 ± 0.29*	0.32 ± 0.09*	1.52 ± 0.32*	0.60 ± 0.04*	0.064 ± 0.006*
DBCP	1.94 ± 0.70	0.45 ± 0.04*	2.68 ± 0.31	0.78 ± 0.05*	0.071 ± 0.014
C. Control – 32 week		0.73 ± 0.19		0.81 ± 0.18	
PH/DEN/PB		0.58 ± 0.04*		0.65 ± 0.06*	

Male rats received (A) three daily injections of the inducers phenobarbital (PB) and 3-methylcholanthrene (3-MC); (B) a single oral dose of CCl_4 or DBCP; and (C) initiation of partially hepatectomized rats with a single dose of diethylnitrosamine with phenobarbital promotion from 8 to 32 weeks (PH/DEN/PB). Nuclear subfractions were prepared from rat livers as described in Materials and Methods. Values are the means ± SD for at least three rats.

* Significantly different from appropriate controls, $P < 0.05$.

the nuclear protein in nuclear envelope preparations respectively (Table 1). A similar increase in protein recovery in detergent extracts as compared to nuclear envelopes was found in rats treated with CCl_4 and DBCP (Table 1). Nuclear envelope yields reported here were comparable with those for several other techniques [1, 2, 17], which are approximately one-tenth that found in detergent extracts.

Resuspension of control rat liver nuclei in the extraction buffer resulted in linear recovery of protein (24%), cytochrome P-450 (0.13 nmol/mg protein), and cytochrome b_5 (0.12 nmol/mg protein)

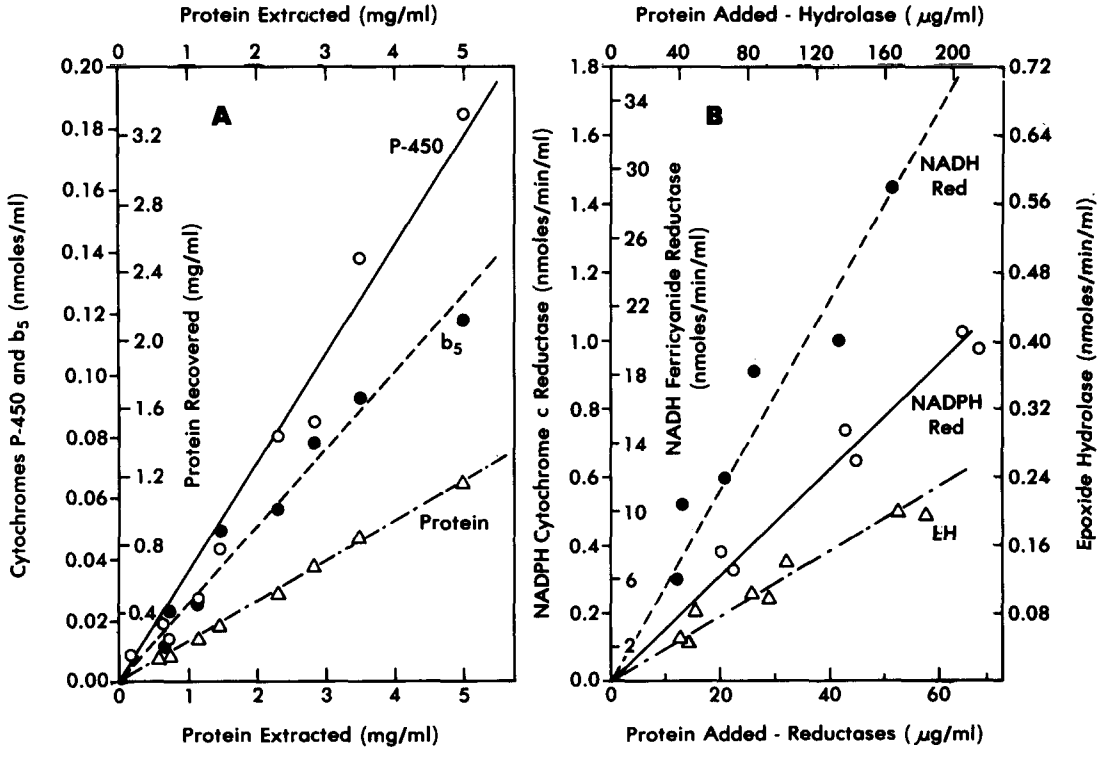

Fig. 2. Enzyme recovery in detergent extracts of nuclei. Nuclei were isolated from male rat liver, washed in 0.25 and 0.88 M STKM2, resuspended at specified protein concentrations in 0.1 M potassium phosphate (pH 7.4), 1 mM 2-mercaptoethanol, 20% glycerol, 5 mM $MgCl_2$, 1 mM EDTA, and 0.6% sodium cholate (extracting buffer), and then incubated for 15 min at 4°. (A) The recovery of protein (—·—) and cytochromes P-450 (———) and b_5 (– – –) in the detergent extracts when a range of nuclear protein was extracted. (B) Product formation for epoxide hydrolase (—·—), NADPH–cytochrome P-450 reductase (———), and NADH–cytochrome b_5 reductase (– – –) versus detergent extract protein added to assay. Values are the mean of three separate experiments.

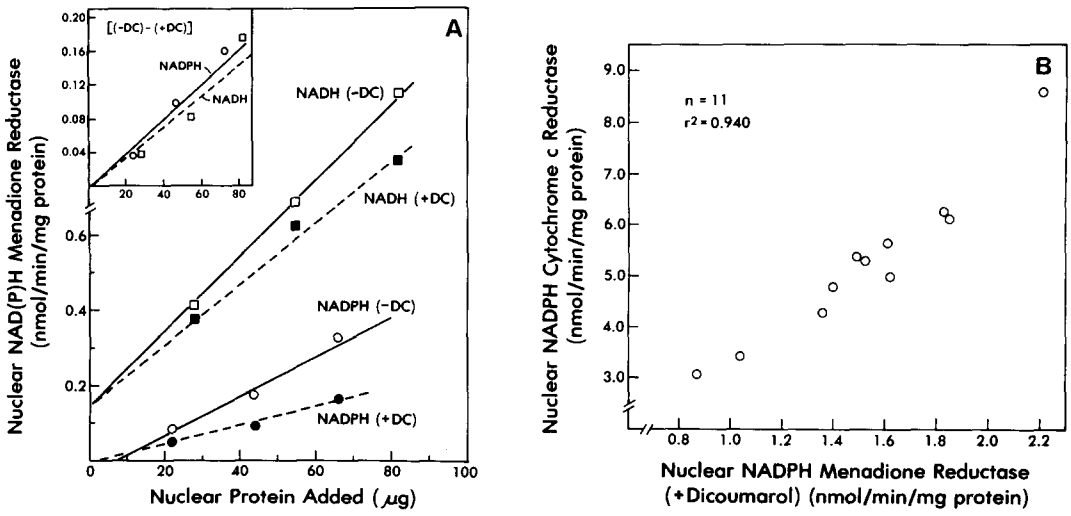

Fig. 3. DT-diaphorase activity in detergent extracts of nuclei. Cytochrome *c* reduction in the presence of menadione (menadione reductase) was used as an assay for DT-diaphorase activity [36] in detergent extracts of rat liver nuclei. (A) Menadione reductase activity versus addition of nuclear extract protein with NADPH (○, ●) or NADH (□, ■) as reducing equivalents in the absence (solid lines) or presence (dashed lines) of dicoumarol, an inhibitor of DT-diaphorase. The difference between inhibited and non-inhibited activity (DT-diaphorase) is shown in the inset; note the similarity when either NADH or NADPH was used as reducing equivalent. (B) Samples of detergent extracts from eleven rat liver nuclei preparations were assayed for NADPH–cytochrome *c* reductase and NADPH menadione reductase (and dicoumarol). The intersample variation in activity is plotted as a scatter diagram and demonstrates the strong correlation between these two activities.

with a range of 0.7 to 5.0 mg nuclear protein as starting material (Fig. 2A). NADPH-cytochrome P-450 reductase (15 nmol/min/mg protein) and the microsomal-like epoxide hydrolase (1.1 nmol/min/mg protein) activities were linear with protein added to the assays (Fig. 2B). No enzymatic activity or cytochromes were recovered in the pellet after extraction (data not shown). The detergent extraction of isolated nuclei took less than 1 hr to perform, it did not require adjustment of nuclear protein levels prior to extraction, and the specific enzymatic activities and cytochrome contents recovered were similar to reported values for nuclear membranes.

Cytosolic enzymes in detergent extracts. DT-Diaphorase (EC 1.6.99.2), glutathione *S*-transferase (EC 2.5.1.18), and the cytosolic epoxide hydrolase are predominantly localized in the cytosol. All three have also been localized in microsomes of certain species [36, 39, 40], and may, therefore, be associated with nuclei. Sodium cholate extracts were employed to examine this possibility.

Detection of DT-diaphorase was measured as the difference in menadione reduction with or without dicoumarol, a specific inhibitor of DT-diaphorase [36], since the NADPH–cytochrome P-450 reductase and NADH–cytochrome b_5 reductase present in the nuclear extracts could compete with DT-diaphorase for substrates and reducing equivalents [41–43]. Reduction of cytochrome *c*, with menadione as an intermediate hydrogen acceptor, was diminished in the presence of dicoumarol with either NADPH or NADH as reducing equivalents (Fig. 3A). The loss

Table 2. DT-diaphorase and other cytosolic enzyme activities in rat liver subfractions

Activity	Cell fraction			
	Cytosol	Microsomes (nmol/min/mg protein)	Nuclei	Nuclei/ Cytosol
Menadione reductase				
(−Dicoumarol)	19.8 ± 3.1	20.7 ± 2.2	4.3 ± 0.4	0.22
(+Dicoumarol)	0.7 ± 0.2	15.2 ± 1.8	1.9 ± 0.2	2.71
DT-Diaphorase	19.0 ± 2.9	5.6 ± 1.5	2.4 ± 0.3	0.13
Glutathione *S*-transferase	41.7 ± 24.8	0.78 ± 0.33	1.16 ± 0.71	0.03
Cytosolic epoxide hydrolase	0.087 ± 0.012	0.072 ± 0.041	ND*	

Cell fractions were prepared from livers of control rats, and assays were performed as described under Materials and Methods. DT-Diaphorase activity is the difference between NADPH menadione reductase assayed in the presence (+) or absence (−) of 10 μM dicoumarol. Values are the mean ± SD of at least four different tissue preparations.

* Not detectable.

Table 3. Effects of phenobarbital and 3-methylcholanthrene on xenobiotic-metabolizing enzymes in nuclear extracts and microsomes. Comparison with reported values in whole nuclei and nuclear membranes

		Current values		Reported values					
		Microsomes	Nuclear extracts	Whole nuclei			Nuclear membranes		
				Lab 1 [15]	Lab 2 [20, 48]	Lab 3 [8]	Lab 4 [19]	Lab 5 [2, 13, 23]	Lab 6 [16, 17]
Component	Treatment	Specific content or activity (ratio of nuclear to microsomal as %)							
Cytochrome P-450 (nmol/mg protein)	Cont	1.23 ± 0.11	0.21 ± 0.03 (16.7)	0.018 (3.3)	0.025	0.019 (2.7)	0.089 (7.4)	0.204 (21.3)	0.302 (2.6)
	PB	1.85 ± 0.14*	0.30 ± 0.01* (16.4)	0.032 (3.1)	0.045		0.086 (3.9)	0.225 (8.1)	0.079 (2.8)
	3-MC	1.71 ± 0.22*	0.34 ± 0.03* (19.9)	0.047 (5.5)		0.043 (3.1)	0.174 (8.8)	0.340 (21.0)	0.081 (2.4)
Cytochrome b_5 (nmol/mg protein)	Cont	0.66 ± 0.13	0.24 ± 0.04 (36.9)	0.005 (1.0)		0.033 (6.2)	0.313 (50.4)	0.145 (26.2)	
	PB	0.82 ± 0.01*	0.35 ± 0.08* (42.9)	0.008 (1.1)			0.377 (55.4)	0.167 (23.6)	
	3-MC	0.72 ± 0.01	0.23 ± 0.08 (32.3)	0.005 (0.8)		0.039 (6.8)	0.354 (47.8)	0.138 (22.4)	
NADPH–cytochrome P-450 reductase (nmol/min/mg protein)	Cont	83 ± 12	10.1 ± 3.4 (12.2)	4.33 (3.6)	7.5	8.8 (6.1)	5.5 (14.1)	0.109 (31.8)	81 (18.0)
	PB	156 ± 12*	20.0 ± 2.0* (12.8)	5.51 (2.1)	13.0		6.1 (9.7)	0.124 (20.6)	
	3-MC	87 ± 11	10.6 ± 1.4 (12.2)	3.90 (3.6)		8.2 (5.3)	4.6 (12.4)	0.125 (33.9)	
NADH–cytochrome b_5 reductase† (μmol/min/mg protein)	Cont	2.69 ± 0.11	0.76 ± 0.14 (28.3)	0.21 (67.8)			1.77 (78.7)	0.55 (56.3)	
	PB	2.03 ± 0.28*	0.79 ± 0.01 (38.9)	0.21 (52.9)			1.08 (72.5)	0.40 (61.4)	
	3-MC	2.03 ± 0.25*	0.86 ± 0.12 (42.4)	0.21 (67.0)			1.60 (50.3)	0.44 (62.6)	
Epoxide hydrolase‡ (nmol/min/mg protein)	Cont	8.2 ± 1.7	1.41 ± 0.38 (17.2)		0.38 (5.1)	0.17 (2.1)		5.32 (51.6)	0.98 (10.1)
	PB	19.5 ± 1.8*	3.94 ± 0.86 (20.2)		0.90 (3.8)			3.64 (12.2)	2.40 (9.3)
	3-MC	8.0 ± 1.1	1.16 ± 0.25 (14.4)		0.46 (4.7)	(2.2)		3.07 (26.4)	1.01 (9.8)

Current values were from control (Cont) rats and rats receiving three daily i.p. injections of phenobarbital (PB, 50 mg/kg) or 3-methylcholanthrene (3-MC, 25 mg/kg). Microsomes and detergent extracts were prepared and assayed as described under Materials and Methods. Values are the mean ± SD for three rats. Reported values were selected from laboratories that have performed assays on at least two of the components measured in this report and used one of the treatments. For Lab 1, data from β-naphthoflavin-treated rats was substituted for 3-MC (both are AHH-receptor inducers). References for the manuscripts reviewed are listed in brackets along with the lab designation.

* Significantly different from controls, $P < 0.05$, used for current values only.

† NADH-cytochrome b_5 reductase was assayed as ferricyanide reduction (current values and Lab 4) and cytochrome *c* reduction (Lab 5).

‡ The epoxides used as substrates were *cis*-stilbene oxide (current values), styrene oxide (Labs 2 and 3), and benzo[a]pyrene 4,5-oxide (Labs 5 and 6).

of activity with dicoumarol was linear with nuclear protein added and was equivalent with NADPH and NADH as hydrogen donors (Fig. 3A, insert), as is characteristic of DT-diaphorase [41]. NADPH-dependent menadione reduction in the presence of dicoumarol correlated with NADPH–cytochrome P-450 reductase activity ($r^2 = 0.94$). The data in Fig. 3B also demonstrate that the addition of menadione to the assays decreased the rate of cytochrome *c* reduction to one-fourth of the activity. This was probably due to the ability of menadione to transfer reducing equivalents to oxygen and back to the reductase [42]. The presence of DT-diaphorase in rat liver nuclei may ameliorate the production of oxygen radicals from some quinones [43, 44], but it has also been reported to activate some compounds to toxic metabolites [45].

The ratio of nuclear to cytosolic activity of DT-diaphorase was at least four times that of the other two cytosolic enzymes (Table 2). The glutathione-dependent transferase of *cis*-stilbene oxide was present in detergent extracts at only 3% of its cytosolic activity, while the *trans*-stilbene oxide hydrolase, cytosolic epoxide hydrolase, was not detectable in nuclear extracts (Table 2). These data suggest that the latter two enzymes are unlikely to have a nuclear localization in rat liver. The microsomal-like and cytosolic-like epoxide hydrolases both act on a number of xenobiotic epoxides, but with distinct substrate specificities [46]. The hydrolysis of epoxides in rat liver nuclei will therefore be defined by the specificities of the microsomal-like hydrolase. The absence of the cytosolic epoxide hydrolase from nuclear fractions in rat liver is in contrast to a recent finding in mouse liver [47]. The activity of this enzyme is 10- to 20-fold higher in the mouse, and while its presence in microsomes has been demonstrated in mouse liver, it does not appear to share this characteristic with the rat [35]. The difference in the nuclear:cytosolic ratios of the three enzymes also suggests that contamination of the nuclei by cytosolic components was minimal.

Phenobarbital and 3-methylcholanthrene inductive response in microsomes and nuclear extracts. Previous studies with whole nuclei or nuclear envelopes from rats treated with phenobarbital and 3-methylcholanthrene have compared the inductive responses with these agents (reviewed in Table 3). As with whole nuclei or nuclear envelopes, the ratio of nuclear extract to microsomal activities ranged from 10 to 40%. Had a significant amount of the activities arisen from microsomal contamination, a more homogeneous ratio of nuclear to microsomal activity would be expected. The heterogeneity of these ratios, therefore, is consistent with minimal levels of microsomal contamination occurring in these nuclei prepared through the 2.1 M STKM2 cushion. Detergent extraction of nuclei resulted in specific contents/activities that were comparable to nuclear envelope preparations and 5- to 10-fold greater than whole nuclei (Table 3). A similar enhancement of cytochrome P-450 has been reported in nucleolar preparations [49], whereas nucleolar epoxide hydrolase and NADPH–cytochrome P-450 reductase were at lower levels than those found in whole nuclei [50]. Although a similar enhancement of enzymes was achieved by detergent extraction of nuclei and envelope (but not nucleoli) isolation, enzymes in the three preparations appeared to respond differently to inductive treatment.

Phenobarbital treatment resulted in significant increases in cytochrome P-450, cytochrome b_5, NADPH–cytochrome P-450 reductase, and epoxide hydrolase. Similar responses were found in microsomes and have been reported in whole nuclei. In studies using nuclear envelope, one laboratory reported increases in cytochrome P-450 and epoxide hydrolase, whereas two other laboratories found no increases in content or activity of nuclear membrane enzymes after phenobarbital treatment (Table 3). Phenobarbital also has no effect on the enzymes measured in nucleolar preparations [50]. 3-Methylcholanthrene treatment increased only cytochrome P-450 (448) content in detergent extracts of nuclei and microsomes, with similar findings reported with whole nuclei and nuclear membranes (Table 3).

The polypeptide contents of microsomes and detergent extracts of nuclei from livers of control and treated rats were examined after separation by SDS–PAGE (Fig. 4). A similar enhancement of one (*ca.* 52 kD) and two bands (*ca.* 55 and 57 kD) were found in microsomes and detergent extracts of nuclei from phenobarbital- and 3-methylcholanthrene-treated rats respectively (Fig. 4). These bands correspond to the specific isozymes of cytochrome P-450 induced by phenobarbital and 3-methylcholanthrene respectively [5, 23, 27]. While the population of protein extracted from nuclei with sodium cholate may include nonmembranous polypeptides, this experiment suggests that changes in the polypeptide population with drug treatment, for the most part, arise from specific induction as opposed to differential extraction. As with total cytochrome P-450 determined from spectral content, phenobarbital treatment did not induce any nuclear polypeptides when nuclear envelope preparations were employed [22].

Discrepancies appeared in the reported responses of nuclear xenobiotic-metabolizing enzymes to inductive treatment. For the most part, these were limited to the effects of phenobarbital on whole or in detergent extracts of nuclei where increases in enzyme content or activity were found, as opposed to a limited response reported when nuclear envelopes were studied. Previously, we suggested that the discrepancies found between whole nuclei and nuclear membranes from phenobarbital-treated rats may arise from selective extraction of enzymes during the isolation of nuclear membranes [19]. This possibility has now been extended to additional enzymes, and suggests that the topology of the phenobarbital-induced enzymes may be different, as they are more susceptible to extraction. The converse control of comparing detergent extracts of microsomes with those of nuclei has not been directly approached in this or previous studies. This may, indeed, offer an alternative approach for testing induction-associated topological differences in enzymes. They were not chosen for controls in these experiments, however, in order to allow comparison between conventionally prepared microsomes and a new method of nuclear enzyme preparation. These data are consistent with studies on whole nuclei which show that nuclear

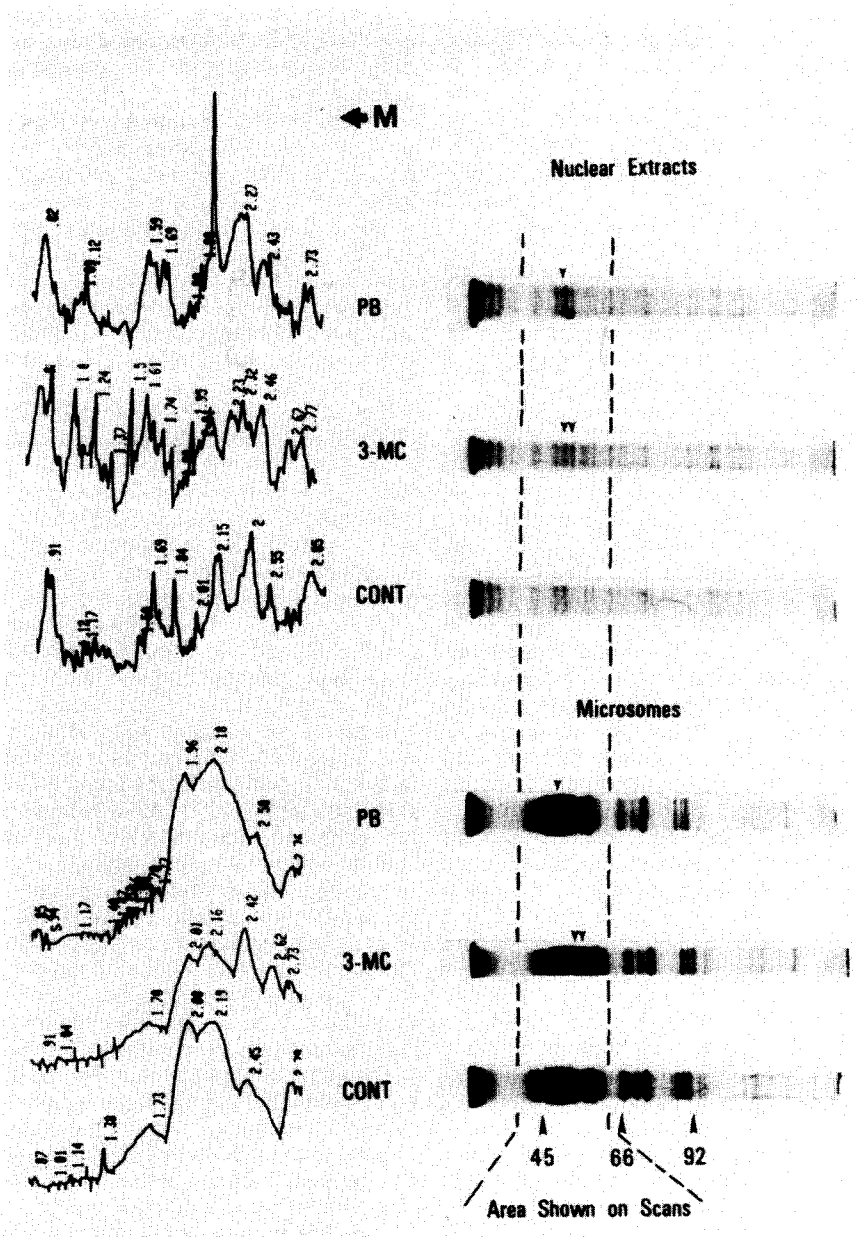

Fig. 4. SDS–PAGE separation of polypeptides from detergent extracts of nuclei and whole microsomes from livers of control (CONT), phenobarbital (PB), and 3-methylcholanthrene (3-MC) treated rats. Slab gels were loaded with 5 μg of nuclear extract protein and 10 μg of microsomal protein and run as previously described [29]. Gels are shown on the right. Gels were scanned by laser densitometer with the tracings of the 40–60 kD region shown on the left. Small arrowheads designate bands enhanced by treatment; large arrowheads designate the position of molecular weight standards. The direction of migration (M) is noted.

enzymes respond to phenobarbital and 3-methylcholanthrene in a fashion similar to microsomes.

Detergent extracts were used to compare the time course of induction of cytochrome P-450 to that in smooth and rough microsomal fractions (Fig. 5). Cytochrome P-450 levels increased in nuclear and smooth microsomal fractions at a similar rate, after phenobarbital. Treatment with 3-methylcholanthrene resulted in a rapid rise in nuclear cytochrome P-450, with maximal induction seen at 24 hr, while a progressive increase occurred in smooth microsomes (Fig. 5). Relatively little induction of the cytochrome occurred in the rough microsomes.

Degenerative effects of CCl_4 *and DBCP.* While induction of nuclear enzymes has been studied extensively, little is known concerning the response of

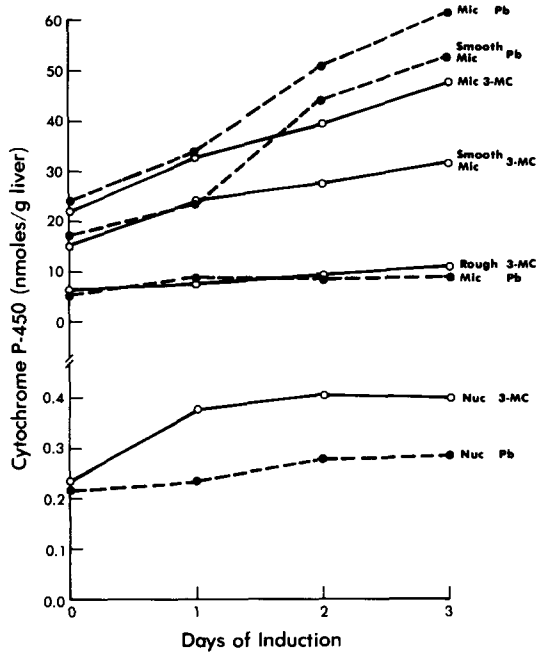

Fig. 5. Time–course for the induction of cytochrome P-450 in total, smooth, and rough microsomes (MIC), and nuclear extracts (NUC) from livers of rats treated with phenobarbital (PB) and 3-methylcholanthrene (3-MC).

these enzymes to compounds which decrease xenobiotic-metabolizing enzymes in nuclei. The hepatotoxins CCl_4 and DBCP disrupt hepatic heme synthesis and cause a decrease in hepatic microsomal cytochrome P-450 [51, 52]. Total microsomal cytochrome P-450 was decreased significantly within 4 hr of a single dose of CCl_4, with a more gradual decreased in content noted through 48 hr. DBCP caused a more gradual loss of cytochrome P-450, which was significant only at the latter time periods. A comparative loss was seen in rough and smooth microsomes (Fig. 6A). Cytochrome b_5, in contrast, was refractory to either hepatotoxin in total microsomes, decreased in smooth microsomes, and at selected times actually increased in rough microsomes (Fig. 6B).

In detergent extracts of liver nuclei, cytochrome P-450 loss was similar to that seen in the microsomal fractions after CCl_4 treatment. Treatment with DBCP resulted in an increase in cytochrome P-450 content at 4 hr, with significant decreases found at the later time periods (Fig. 7A). Cytochrome b_5 content in nuclear extracts was increased at 4 hr by both compounds (Fig. 7B). This was most evident in nuclei recovered in the 2.3 M cushion. These studies, as well as the preceding time–course studies on induction, demonstrate that nuclear enzymes respond to chemical stimuli at a different rate, and occasionally in a different manner, from microsomal enzymes. Gonzalez and Kasper [24] have also observed temporal differences in nuclear and microsomal response to acute treatments with regard to specific xenobiotic-metabolizing enzyme mRNAs.

Table 4. Response of hepatic microsomal and nuclear extract enzymes to initiation with partial hepatectomy and diethylnitrosamine (PH, DEN) with or without phenobarbital (PB) promotion

Cell faction	Treatment PH, DEN	Treatment PB	Cytochrome P-450 (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)	NADPH-Cytochrome P-450 reductase (nmol/min/mg protein)	NADH-Cytochrome b_5 reductase (μmol/min/mg protein)	Epoxide hydrolase (nmol/min/mg protein)
Microsomes	–	–	0.73 ± 0.08	0.63 ± 0.02	110 ± 10	2.04 ± 0.11	13.2 ± 1.5
	+	–	0.53 ± 0.04	0.54 ± 0.04*	123 ± 26	2.31 ± 0.56	12.9 ± 2.2
	–	+	1.22 ± 0.10†	0.90 ± 0.06†	215 ± 31†	2.37 ± 0.24	30.6 ± 4.9
	+	+	0.88 ± 0.32*	0.80 ± 0.07*	167 ± 17*	1.80 ± 0.18*	42.3 ± 2.3*
Nuclear extracts	–	–	0.076 ± 0.010	0.049 ± 0.002	5.19 ± 0.89	0.22 ± 0.01	1.06 ± 0.21
	+	–	0.068 ± 0.006	0.036 ± 0.006*	4.11 ± 0.70*	0.21 ± 0.02	1.04 ± 0.17
	–	+	0.099 ± 0.017†	0.055 ± 0.006†	6.67 ± 1.33†	0.19 ± 0.06	1.76 ± 0.17†
	+	+	0.106 ± 0.019	0.064 ± 0.015	7.15 ± 0.95	0.25 ± 0.06	2.07 ± 0.43

Male rats received an initiation of two-thirds partial hepatectomy followed by a single oral dose of DEN (10 mg/kg) 24 hr later. At 8 weeks, promoted rats were given 0.5% phenobarbital in their drinking water until termination of the experiment at 32 weeks. Values are the mean ± SD for four to six rats.
* Significant difference between initiated and non-initiated control, $P < 0.05$.
† Significant difference between phenobarbital non-initiation and non-initiated control, $P < 0.05$.

Initiation–promotion effects on nuclear extract enzymes. Several microsomal xenobiotic-metabolizing enzymes change their activities during hepatocarcinogenesis in a predictable manner. This includes reported decreases in cytochromes P-450 and b_5 and their reductases, and an increase in epoxide hydrolase activity [53–56]. Whether comparable changes occur in nuclear xenobiotic-metabolizing

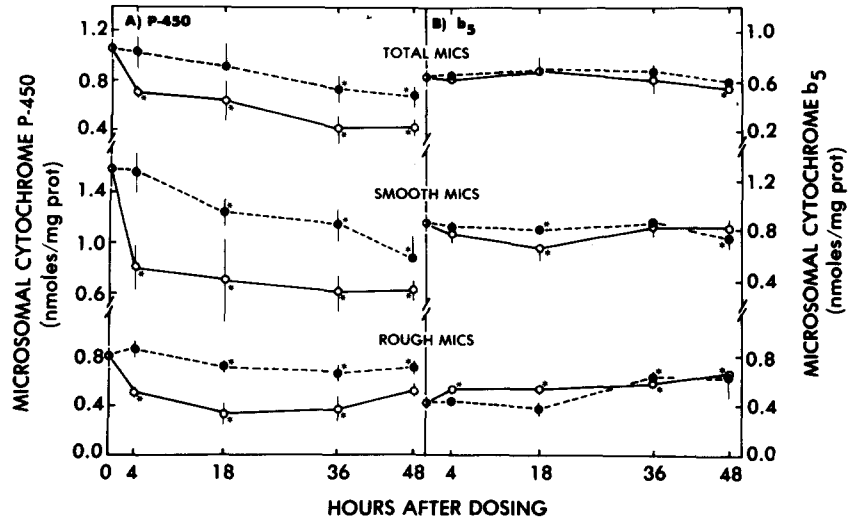

Fig. 6. Time–course of the response of cytochrome P-450 (A) and cytochrome b_5 (B) in total, smooth, and rough microsomes (MICS) to a single oral dose of CCl_4 (———) and DBCP (— — —). Key: (*) significantly different from control ($P < 0.05$).

enzymes was studied in rats receiving an initiation–promotion protocol of partial hepatectomy followed at 24 hr with a single dose of diethylnitrosamine and promotion with phenobarbital from 8 to 32 weeks. The initiation–promotion protocol used in this study resulted in no gross changes to the liver, but hyperplastic foci were evident (data not shown). Treatments included initiation alone, promotion alone, and initiation–promotion (Table 4).

The nuclear and microsomal xenobiotic-metabolizing enzymes responded to chronic phenobarbital treatment in a similar fashion (Table 4). However, the response to initiation with a chemical carcinogen, whether followed by promotor or not, was not similar. In microsomes from initiated–promoted rats, changes in these enzymes were similar to those reported in other studies using a number of different protocols [53–56]. In detergent extracts of liver nuclei from initiated–promoted rats, however, only the induction of epoxide hydrolase was noted, with no change in activity or content of the cytochromes and their reductases (Table 4).

The significance of these differences to the neoplastic event is not known. These results point to

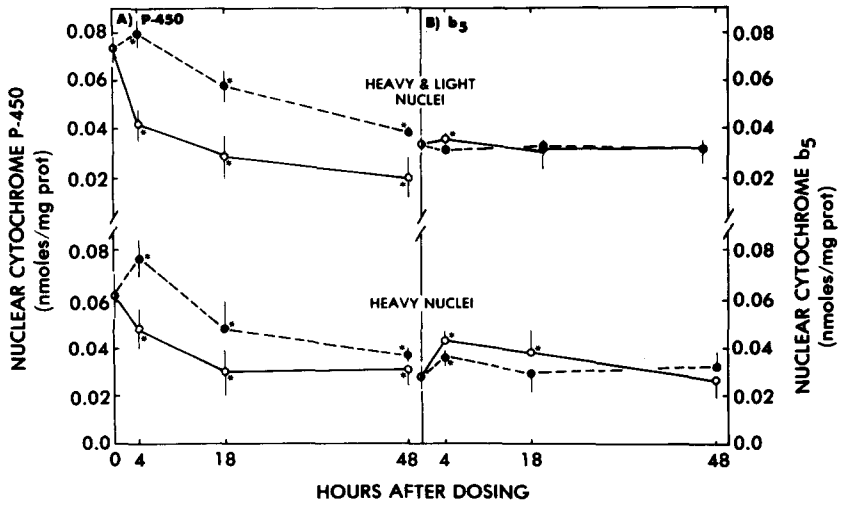

Fig. 7. Time–course of the response of cytochrome P-450 (A) and cytochrome b_5 (B) in detergent extracts of heavy and light nuclei (sedimented through 2.1 M STKM2 cushion) and heavy nuclei (sedimented through 2.3 M STKM2 cushion) to a single dose of CCl_4 (———) and DBCP (— — —). Key: (*) significantly different from control ($P < 0.05$).

a differential response of the nuclear xenobiotic-metabolizing enzymes to initiation with chemical carcinogens, which extends beyond the temporal difference in response seen to acute induction and cytochrome loss.

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