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FURTHER STUDIES ON THE INCREASE IN DRUG-METABOLIZING CAPACITY ADJACENT TO INTRAHEPATIC MORRIS HEPATOMAS

LESTER G. SULTATOS, DAI K. LIU AND ELLIOT S. VESELL*
Department of Pharmacology, The Pennsylvania State University, College of Medicine, Hershey, PA 17033, U.S.A.

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Abstract—Microsomal cytochrome P-450 content was higher in histologically non-tumorous liver adjacent to intrahepatically implanted Morris hepatomas 5123D or 7795 than in histologically normal liver far removed from each tumor. V_{max} values for microsomal benzo[*a*]pyrene monooxygenase activity and cyclophosphamide activation were also significantly higher in tumor-adjacent liver than in normal liver far removed from tumor. K_m values of these reactions were unchanged. After intrahepatic implantation, inert spheres of several different materials produced no regional differences in hepatic microsomal cytochrome P-450 content. Both intrahepatic Morris hepatomas exhibited markedly reduced cytochrome P-450 content and benzo[*a*]pyrene monooxygenase activity. Cyclophosphamide biotransformation could not be detected in microsomes from either Morris hepatoma. Similar recoveries from microsomes of far-removed and tumor-adjacent liver indicated that differences between these regions in drug-metabolizing activity could not be attributed to different stabilities or sedimenting properties of their microsomes. Although microsomal recovery was significantly less from hepatomas than from far-removed or tumor-adjacent liver, this loss of tumor microsomes accounted for only a small part of the reductions in cytochrome P-450-mediated monooxygenases observed within tumors. Compared to control rats, tumor-bearing rats exhibited no change in hepatic drug-metabolizing capacity measured *in vivo* by hexobarbital sleeping times and antipyrine elimination rates. Phenobarbital (PB) pretreatment of tumor-bearing rats induced cytochrome P-450 to different extents within far-removed liver, tumor-adjacent liver, and both hepatomas. The same differential inducibility occurred with PB pretreatment for cyclophosphamide activation. After PB induction, differences in drug-metabolizing activity between far-removed and tumor-adjacent liver disappeared; though induced, these activities remained lower in the hepatomas than in other regions. These changes in drug-metabolizing activity in both basal and PB-induced states of various hepatic regions were related to changes in cellularity of tumor-adjacent tissue. Hepatocellular nuclei prepared from tumor-containing liver were separated into diploid and tetraploid classes by sucrose density gradient centrifugation. Compared to far-removed liver, tumor-adjacent liver contained significantly more diploid nuclei and less tetraploid nuclei.

Recently, we described regional differences in microsomal cytochrome P-450 content of rat liver containing intrahepatically implanted Morris hepatomas 5123D or 7795 [1]. Histologically non-tumorous liver directly adjacent to either hepatoma had more cytochrome P-450 than normal liver far removed from tumor, whereas each hepatoma exhibited markedly reduced cytochrome P-450 content [1]. Furthermore, tumor-adjacent liver had less DNA (expressed per g liver) than far-removed liver. The present study extends these observations by describing similar regional differences in benzo[*a*]pyrene monooxygenase activity and cyclophosphamide activation, both cytochrome P-450-mediated reactions. Specificity of these regional differences in liver implanted with tumors (allographs) was investigated by asking whether regional differences occurred in drug metabolizing enzyme activity after inert spheres of several different sizes and materials were implanted *in vivo*. Nuclear ploidy was measured in the three distinct regions of tumor-bearing liver. The results showed a change in cellularity of tumor-adjacent liver compared to liver far removed from tumor. Regional studies in liver that contained implanted Morris hepatomas were also performed after phenobarbital (PB) induction. In addition (using hexobarbital and antipyrine as test compounds), we investigated the *in vivo* hepatic drug-metabolizing capacities of rats bearing transplanted (allographic) intrahepatic Morris hepatomas 5123D or 7795.

* Address all correspondence to: Elliot S. Vesell, Department of Pharmacology, Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033, U.S.A.

MATERIALS AND METHODS

Male Buffalo rats supplied by Microbiological Associates (Walkersville, MD), weighing 200–250 g at the start of the studies, were maintained as described previously [2]. Seventeen milligrams of Morris hepatoma 5123D or 7795 (both originally induced in Buffalo rats) was surgically implanted under anesthesia intrahepatically in our male Buffalo rats within the left lateral lobe; a 13-gauge trochar

was used. Small portions of Gel Foam (The Upjohn Co., Kalamazoo, MI) were packed into the needle tract to minimize bleeding and to prevent extrusion of the tumor. Tumors developed intrahepatically until they weighed approximately 1.3 g (30 days for 5123D and 54 days for 7795), at which time the rats were killed. Seventeen milligrams of normal liver from a Buffalo rat was implanted in livers of control Buffalo rats. Approximately 1 week later, however, no evidence of this implanted allograph was apparent, thereby making further analyses impossible.

To determine if the previously reported increase in cytochrome P-450 content of liver adjacent to intrahepatic hepatoma [1] was tumor-specific, or if it might have arisen as a non-specific response that could be elicited by many kinds of foreign bodies, we implanted assorted sizes of nylon, stainless steel, glass, teflon, polypropylene, or lucite spheres (Small Parts Inc., Miami, FL) within the left lateral hepatic lobes of Buffalo rats. Some of these rats were killed 30 days later; the remainder were killed 54 days after implantation.

Rats were decapitated, and livers were immediately removed and immersed in ice-cold 0.02 M Tris–HCl buffer (pH 7.4) containing 1.15% KCl. Each tumor that appeared discrete was dissected free; all liver surrounding tumor within 0.5 cm was removed and considered "adjacent tissue". In the case of non-tumorous implants, all liver surrounding the implant within 0.5 cm was considered "adjacent tissue". Portions of the remaining unaffected lobes were considered "far-removed" normal liver. Previous studies using normal male Buffalo rats revealed no detectable inter- or intra-lobular differences in hepatic microsomal cytochrome P-450 [1]. Each experiment required pooled tissues from at least six animals.

Tissues were homogenized in 4 vol. of ice-cold 1.15% KCl solution buffered with 0.02 M Tris–HCl (pH 7.4) using a Potter–Elvehjem homogenizer having a motor-driven teflon pestle. The homogenates, transferred to polypropylene centrifuge tubes, were spun at 9,000 *g* for 20 min in a refrigerated Sorvall Superspeed RC2-B centrifuge. Fatty layers were removed and supernatant fractions were collected and centrifuged at 78,000 *g* in a Spinco model L ultracentrifuge for 60 min. The 78,000 *g* pellets, resuspended in 4 vol. of 1.15% KCl solution with 0.02 M Tris–HCl using a glass homogenizer, were centrifuged again at 78,000 *g* for 60 min. Pellets were again resuspended in 1.15% KCl buffered with 0.02 M Tris–HCl, pH 7.4, using a glass homogenizer, to give a final protein concentration of approximately 10 mg/ml. Protein was estimated by the method of Lowry *et al.* [3].

Microsomal cytochrome P-450 content was determined using an Aminco DW2 UV-Vis recording spectrophotometer by the method of Omura and Sato [4]. Cytochrome P-450 content of whole homogenate was determined after correcting for contamination by hemoglobin, methemoglobin, and cytochrome oxidase [5–7].

Benzo[*a*]pyrene monooxygenase activity was measured according to the method of DePierre *et al.* [8], using [G-^{3}H]benzo[*a*]pyrene (Amersham/Searle, Arlington Heights, IL). Radioactivity was counted on a Searle Analytic 92 liquid scintillation counter. Recorded activity was adjusted by reference to quench curves of ^{3}H-labeled standards. Incubation times were 10 min. Each reaction vessel contained 0.4 mg of microsomal protein. Under these conditions, benzo[*a*]pyrene monooxygenase activity proceeded in a linear fashion using microsomes from tumor, tumor-adjacent liver, or liver far removed from tumor.

Microsomal activation of cyclophosphamide was determined by the method of Sladek [9], as modified by Allen and Creaven [10]. Incubation times were 15 min; 3 mg of microsomal protein was present in each incubation vial. Under these conditions, cyclophosphamide activation proceeded linearly. A standard curve of alkylating activity was prepared using mechlorethamine (Sigma Chemical Co., St. Louis, MO). Forty nanomoles of mechlorethamine gave an absorbance of 0.65, using a Gilford 2400-S spectrophotometer. A small percentage of activated cyclophosphamide is capable of binding to microsomal protein [11], thereby becoming undetectable with the aforementioned procedure. Preliminary experiments using [^{3}H-chloroethyl]cyclophosphamide and [^{14}C-4]cyclophosphamide (New England Nuclear Corp., Boston, MA) did not detect differences in extent of metabolite binding between microsomes of far-removed and tumor-adjacent liver. No metabolite was detectable in microsomes from either hepatoma.

Pretreated rats received i.p. injections of phenobarbital (PB) (Merck & Co., Rahway, NJ) (75 mg/kg) dissolved in physiological saline at 8:00–9:00 a.m. daily for 3 days. The PB solution (pH 8.3) was made in a concentration such that each animal received 1 ml volume/100 g body weight. Control animals received physiological saline i.p. (adjusted to pH 8.3) (also 1 ml/100 g body weight). Rats were killed 24 hr after the last PB dose.

Hexobarbital sleeping times were determined after administration i.p. of sodium hexobarbital in physiological saline at a dose of 125 mg/kg. Each group consisted of twelve rats. The animals were considered awake when they righted themselves on all four paws. Controls consisted of sham-operated rats. Experimental groups contained intrahepatic Morris hepatomas 5123D or 7795.

Antipyrine half-lives were determined in sham-operated controls and experimental rats after implantation of teflon catheters as described previously [2]. After purification by thin-layer chromatography using the solvent system of Stahl [12] as described by Sultatos *et al.* [2], [*N*-methyl-^{14}C]antipyrine (11.1 mCi/mmoles, ICN, Irvine, CA) was added to a solution of unlabelled antipyrine (1.8 mg/ml) and administered i.p. to rats in which catheters had been implanted. The dose was 18 mg/kg of unlabeled antipyrine and 18 μCi/kg of [^{14}C]antipyrine. The antipyrine solution was made up so that 1 ml was administered per 100 g body weight. Blood samples of 0.1 ml were withdrawn from each rat at 15 min, 30 min, 45 min, 1 hr, 1.5 hr, 2 hr and 3 hr after antipyrine administration. These samples were then processed and counted by liquid scintillation techniques previously described for aminopyrine [2].

Nuclei were isolated and separated into different size classes according to the procedure of Albrecht [13] as modified by Kulick and Liu [14, 15]. Immediately after resuspension of nuclei, 0.33 ml aliquots were applied to either a 37 ml 25–60% linear sucrose gradient containing 1.0 mM MgOAc (for adjacent and far-removed liver) or a 37 ml 25–60% linear sucrose gradient containing 3.3 mM MgOAc (for hepatoma). Samples were centrifuged in a refrigerated PR-6 International centrifuge at $700g$ for 15 min. Immediately after centrifugation, the gradients were fractionated using a Beckman fraction recovery system. Samples were collected in a volume of 0.4 ml per fraction using a ISCO fraction collector, and the nuclei in each fraction were counted under a microscope using an improved Neubauer hemocytometer. Rounded nuclei were classified as hepatocytic, whereas all others were considered non-hepatocytic [16].

Several rats from every experimental group were used routinely for light microscopic analyses of liver. Total body perfusions were performed under light ether anesthesia using 10% buffered formalin injected through the left ventricle, and exiting via the right atrium. Sections cut from paraffin-embedded blocks were stained with hematoxylin and eosin.

Statistical analyses were performed using analysis of variance followed by the Newman Keul's test [17].

RESULTS

Light microscopy revealed that non-tumorous liver directly adjacent to Morris hepatoma 5123D or 7795 was histologically indistinguishable from liver far removed from tumor (Fig. 1). In addition, light microscopy disclosed either minimal or no necrosis in the hepatomas.

Table 1 shows that microsomes from liver directly

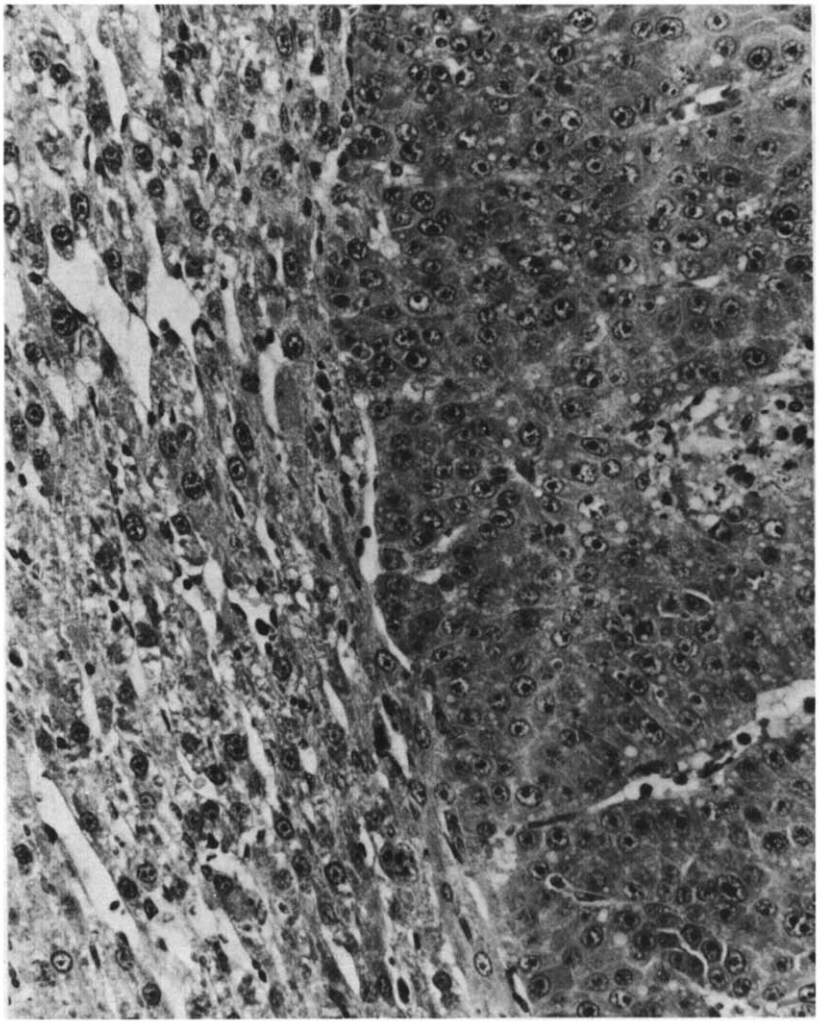

Fig. 1. Photomicrograph of a hematoxylin and eosin stained section of tumor-bearing rat liver showing the sharply defined border between histologically normal liver (on the left) and adjacent Morris hepatoma 5123D (on the right) (magnification × 470).

Table 1. Cytochrome P-450 content of three regions of tumor-bearing rat liver*

Morris hepatoma or foreign mass implanted	Tissue region	Cytochrome P-450 content (nmoles/g tissue)	Recovery ratio†
5123D Tumor	Far-removed liver	8.21 ± 0.39	0.64 ± 0.15
	Tumor-adjacent liver	11.27 ± 0.89‡	0.65 ± 0.10
	Tumor	1.73 ± 0.12§	0.44 ± 0.05§
7795 Tumor	Far-removed liver	8.34 ± 0.45	0.64 ± 0.15
	Tumor-adjacent liver	10.95 ± 0.44‡	0.63 ± 0.14
	Tumor	2.74 ± 0.16§	0.45 ± 0.10§
Stainless steel‖	Far-removed liver	8.39 ± 0.35	
	Stainless steel-adjacent liver	8.26 ± 0.41	
Nylon‖	Far-removed liver	8.25 ± 0.40	
	Nylon-adjacent liver	7.86 ± 0.36	
Teflon‖	Far-removed liver	8.29 ± 0.39	
	Teflon-adjacent liver	8.31 ± 0.45	
Polypropylene‖	Far-removed liver	8.26 ± 0.39	
	Polypropylene-adjacent liver	8.35 ± 0.30	
Lucite‖	Far-removed liver	8.41 ± 0.29	
	Lucite-adjacent liver	7.50 ± 0.44	
Glass‖	Far-removed liver	8.29 ± 0.36	
	Glass-adjacent liver	7.86 ± 0.39	

* Each value is the mean ± S.E.M. of four experiments; each experiment used pooled tissue from at least six rats. Values for cytochrome P-450 content were not corrected for the yield of microsomes.

† Ratio of total microsomal cytochrome P-450 to total whole homogenate cytochrome P-450.

‡ Significantly different ($P < 0.05$) from far-removed liver and tumor by two-way analysis of variance followed by Newman Keul's test.

§ Significantly different ($P < 0.05$) from far-removed and tumor-adjacent liver by two-way analysis of variance followed by Newman Keul's test.

‖ The inert sphere measured 3/32 to 3/16 inch diameter and was implanted in the liver for 30 days.

adjacent to either intrahepatically implanted Morris hepatoma 5123D or 7795 had significantly more cytochrome P-450 than microsomes from liver far-removed from tumor. To test the possibility that this difference in microsomal cytochrome P-450 content might have developed during isolation, as a result of differences in sedimentation characteristics between tumor-adjacent and far-removed liver, we determined for each hepatic region the ratio of total microsomal cytochrome P-450 to total whole homogenate cytochrome P-450 (recovery ratio). Identical recovery ratios for tumor-adjacent and far-removed liver (Table 1) indicates that regional differences of cytochrome P-450 content in tumorous liver were not artifacts of the isolation procedure.

The microsomal cytochrome P-450 content of both of the intrahepatic tumors was markedly reduced from that of far-removed or tumor-adjacent liver (Table 1). Microsomal P-450 recovery, however, was significantly less from hepatomas than from tumor-adjacent or far-removed liver (Table 1), suggesting that dissimilar sedimentation characteristics between

Table 2. V_{max} and K_m of benzo[*a*]pyrene monooxygenase activity in microsomes from three regions of tumor-bearing rat liver*

Morris hepatoma implanted	Region	V_{max} [nmoles · (g liver)$^{-1}$ · min^{-1}]	K_m (mM)
5123D	Far-removed liver	16.05 ± 0.40	19.03 ± 0.43
	Tumor-adjacent liver	19.08 ± 0.32†	19.34 ± 0.39
	Tumor	3.92 ± 0.41‡	9.02 ± 0.57‡
7795	Far-removed liver	16.14 ± 0.25	18.86 ± 0.33
	Tumor-adjacent liver	19.04 ± 0.62†	19.09 ± 0.29
	Tumor	4.83 ± 0.31‡	10.69 ± 0.33‡

* Each value is the mean ± S.E.M. of four experiments; each experiment used tissues pooled from at least six rats.

† Significantly different ($P < 0.05$) from far-removed liver and tumor by a two-way analysis of variance followed by Newman Keul's test.

‡ Significantly different ($P < 0.05$) from far-removed and tumor-adjacent liver by a two-way analysis of variance followed by Newman Keul's test.

Table 3. Effect of phenobarbital pretreatment on microsomal cytochrome P-450 content and microsomal cyclophosphamide activation within hepatoma, tumor-adjacent tissue, and far-removed liver*

Intrahepatic tumor	Pretreatment	Tissue region	Cytochrome P-450 content (nmoles/g tissue)	Cyclophosphamide activation V_{max} [μmoles mechlorethamine equivalents · (g tissue)$^{-1}$ · hr^{-1}]	K_m (mM)
5123D	Saline	Far-removed liver	8.41 ± 0.42	0.94 ± 0.05	5.59 ± 0.24
		Tumor-adjacent liver	10.93 ± 0.76†	1.31 ± 0.09†	5.78 ± 0.20
		Tumor	1.64 ± 0.19	NDA‡	NDA
5123D	Phenobarbital	Far-removed liver	19.48 ± 1.01§	1.97 ± 0.11§	6.78 ± 0.21§
		Tumor-adjacent liver	18.97 ± 1.32§	1.83 ± 0.15§	6.82 ± 0.28§
		Tumor	2.84 ± 0.53§	NDA	NDA
7795	Saline	Far-removed liver	7.93 ± 0.51	0.89 ± 0.04	5.81 ± 0.19
		Tumor-adjacent liver	11.09 ± 0.64†	1.22 ± 0.06†	5.68 ± 0.16
		Tumor	2.62 ± 0.21	NDA	NDA
7795	Phenobarbital	Far-removed liver	20.14 ± 1.21§	1.85 ± 0.04§	6.93 ± 0.20§
		Tumor-adjacent liver	19.71 ± 1.07§	1.99 ± 0.18§	7.00 ± 0.27§
		Tumor	3.88 ± 0.86§	NDA	NDA

* Rats were pretreated for 3 days with i.p. injections of phenobarbital (75 mg/kg) or saline. Each value is the mean ± S.E.M. of four determinations; each determination used pooled tissues from at least six rats.

† Significantly different ($P < 0.05$) from far-removed liver by three-way analysis of variance followed by Newman Keul's test.

‡ NDA = no detectable activity.

§ Significantly different ($P < 0.05$) from its corresponding control.

tumorous and non-tumorous liver might have contributed, at least partially, to their different microsomal cytochrome P-450 contents.

To determine if regional variations in hepatic cytochrome P-450 occurred only in response to tumor, or if any intrahepatically implanted foreign mass might elicit a similar response, spheres of glass, stainless steel, nylon, teflon, polypropylene or lucite were implanted in rat livers for 30 or 54 days. None of these implants resulted in more microsomal cytochrome P-450 in liver adjacent to the implants compared to liver far removed from the implants (Table 1).

V_{max} values of microsomal cytochrome P-450-mediated benzo[*a*]pyrene monooxygenase activities of liver adjacent to the intrahepatically implanted hepatomas were higher than those of microsomes from liver far removed from tumor, but the K_m values in these regions did not differ significantly (Table 2). Both the V_{max} and K_m for benzo[*a*]pyrene monooxygenase activity in tumor microsomes were reduced compared to tumor-adjacent and far-removed liver (Table 2).

More activation of cyclophosphamide occurred in microsomes from tumor-adjacent liver than in microsomes from far-removed liver (Table 3). Although the V_{max} values for cyclophosphamide activation from these regions differed, their K_m values did not. Microsomal activation of cyclophosphamide in both intrahepatic hepatomas was so low that it could not be detected (Table 3).

Pretreatment of tumor-bearing rats with PB resulted in induction of cytochrome P-450 content within far-removed liver, tumor-adjacent liver, and in both Morris hepatomas, whereas cyclophosphamide activation was enhanced only in far-removed and tumor-adjacent liver (Table 3). Moreover, after PB pretreatment cytochrome P-450 content and cyclophosphamide activation within tumor-adjacent liver and far-removed liver were similar. PB induction of cyclophosphamide activation could not be detected using microsomes from either Morris hepatoma (Table 3).

Drug metabolizing capacity *in vivo* of rats bearing intrahepatic hepatoma 5123D for 30 days or hepatoma 7795 for 54 days was investigated, using hexobarbital and antipyrine as test compounds. Table 4 shows that no significant differences between tumor-bearing rats and sham-operated controls were observed in hexobarbital sleeping times or antipyrine half-lives.

Table 4. Hexobarbital sleeping times and antipyrine half-lives in tumor-bearing and control Buffalo rats

Experimental group	Hexobarbital sleep times* (min)	Antipyrine half-lives† (min)
Controls	28.6 ± 2.1	73 ± 4.6
Rats bearing Morris hepatoma 5123D	71 ± 5.4	35.8 ± 2.7
Controls	33.5 ± 2.6	75 ± 4.3
Rats bearing Morris hepatoma 7795	28.0 ± 2.8	77 ± 4.6

* Each value is the mean ± S.E.M. of twelve rats.
† Each value is the mean ± S.E.M. of eight rats.

Hepatocellular nuclei from far-removed and tumor-adjacent liver were separated by sucrose density centrifugation into two distinct classes representing diploid nuclei (band 1 in Fig. 2) and predominantly tetraploid nuclei (band 2 in Fig. 2). Nuclei from either hepatoma could not be separated into discrete bands, but instead formed a single continuous band several centimeters in width. Figure 2 represents examples of the fractionation of nuclei from far-removed liver, tumor-adjacent liver, and hepatoma 5123D. Tumor-adjacent liver had significantly more nuclei within band 1 (diploid) and fewer neclei within band 2 (tetraploid) compared to far removed liver (Fig. 2 and Table 5). These regional differences resulted from alterations in the number of hepatocyte nuclei, since the number of non-hepatocyte nuclei (differentiated from hepatocytes by morphological examination under light microscopy) was the same in tumor-adjacent and far-removed liver (Table 5).

DISCUSSION

Rat livers containing intrahepatically implanted Morris hepatomas 5123D or 7795 exhibit regional differences in microsomal cytochrome P-450 content [1]. In addition to confirming these observations (Table 1), the present study reveals that these metabolic differences do not result from differences in subcellular sedimentation characterisitics of adjacent and far-removed liver, since microsomal P-450 recoveries from both areas were identical (Table 1).

To determine whether these regional metabolic differences are tumor-specific, arising only after hepatoma implants, or if they might represent a non-specific response of normal liver to a foreign mass, we implanted inert spheres of several different materials and sizes in rat livers. None of these implants caused elevations of cytochrome P-450 in liver adjacent to implants compared to liver far removed from implants (Table 1). Therefore, regional differences in hepatic cytochrome P-450 content observed in the present and previous [1] studies might be tumor specific, suggesting that some interaction between tumor and adjacent hepatocytes enhances cytochrome P-450 within tumor-adjacent tissue. However, experiments using a foreign mass (summarized in Table 1) cannot exclude entirely the possibility that the regional variations in cytochrome P-450 content within tumor-bearing liver were non-specific, since implanted inert spheres do not adequately simulate an expanding foreign mass.

V_{max} values for microsomal cytochrome P-450-mediated benzo[*a*]pyrene monooxygenase activity and cyclophosphamide activation in tumor-adjacent liver were significantly higher than those in liver far removed from tumor. K_m values for these reactions were unchanged (Table 2). These data suggest that, although more cytochrome P-450 exists in tumor-adjacent liver than in far-removed liver, substrate affinities of the enzyme with respect to benzo[*a*]pyrene and cyclophosphamide are similar in both regions. Within both Morris hepatomas,

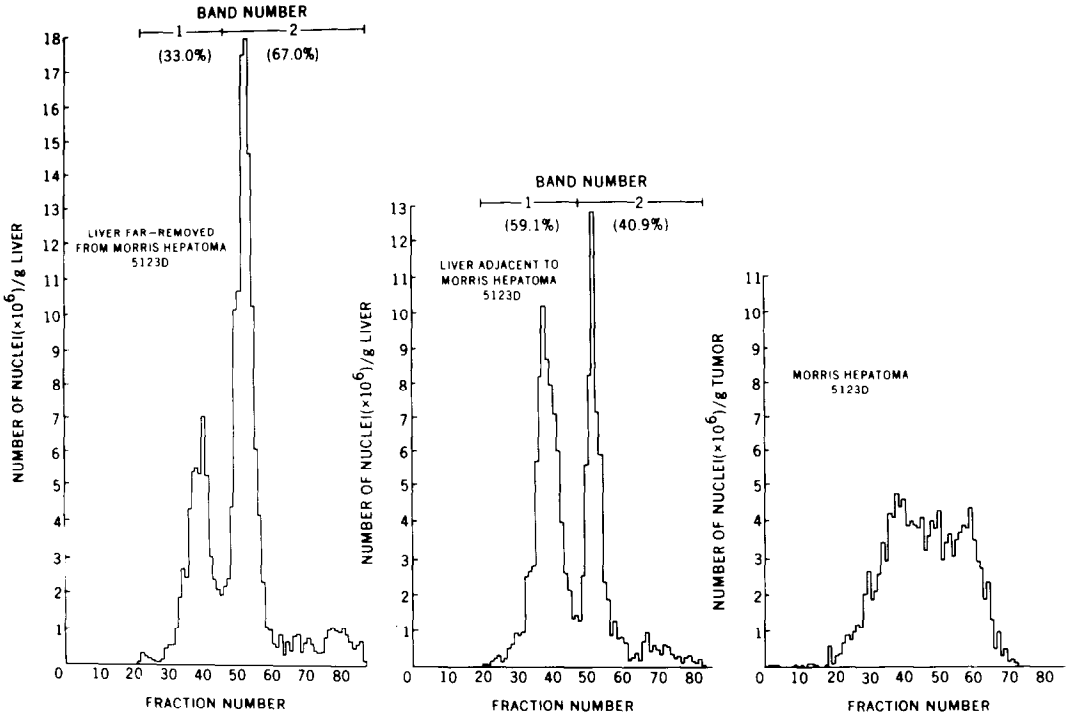

Fig. 2. Distribution according to size of nuclei from liver containing Morris hepatoma 5123D. Nuclei were isolated as described in Materials and Methods. Gradients were fractionated and the number of nuclei in each fraction was determined.

Table. 5. Distribution of hepatocyte and non-hepatocyte nuclei within tumor-adjacent and far-removed liver after sucrose density gradient centrifugation

Morris hepatoma implanted	Tissue region	Band number	Type of nuclei within band*	% of Total number of nuclei classified as non-hepatocyte or hepatocyte nuclei†	% of Total number of nuclei within band†
5123D	Far-removed liver	1	Non-hepatocytes	16.0 ± 1.1	37.2 ± 3.3
			Hepatocytes	21.3 ± 0.9	
5123D	Tumor-adjacent liver	1	Non-hepatocytes	14.7 ± 1.0	56.4 ± 4.7§
			Hepatocytes	41.7 ± 2.6‡	
5123D	Far-removed liver	2	Non-hepatocytes	0	63.1 ± 1.7
			Hepatocytes	63.1 ± 1.7	
5123D	Tumor-adjacent liver	2	Non-hepatocytes	0	43.7 ± 2.0§
			Hepatocytes	43.7 ± 2.0‖	
7795	Far-removed liver	1	Non-hepatocytes	15.9 ± 2.1	37.3 ± 4.8
			Hepatocytes	21.4 ± 1.6	
7795	Tumor-adjacent liver	1	Non-hepatocytes	15.5 ± 1.1	62.2 ± 4.5§
			Hepatocytes	46.7 ± 2.1‡	
7795	Far-removed liver	2	Non-hepatocytes	0	62.8 ± 2.1
			Hepatocytes	62.8 ± 2.1	
7795	Tumor-adjacent liver	2	Non-hepatocytes	0	37.6 ± 1.80§
			Hepatocytes	37.6 ± 1.80‖	

* Differentiation of nuclei was based on examination by light microscopy as described in Materials and Methods.

† Each value is the mean ± S.E.M. for four determinations and each determination used pooled tissue from six animals.

‡ Significantly different ($P < 0.05$) from the percent of hepatocyte nuclei in band 1 from far-removed liver by one-way analysis of variance followed by Newman Keul's test.

§ Significantly different ($P < 0.05$) from the percent of nuclei within the corresponding band from far-removed liver by one-way analysis of variance followed by Newman Keul's test.

‖ Significantly different ($P < 0.05$) from the percent of hepatocyte nuclei in band 2 from far-removed liver by one-way analysis of variance followed by Newman Keul's test.

markedly decreased microsomal cytochrome P-450 content was accompanied by decreased V_{max} and K_m values for tumor benzo[*a*]pyrene monooxygenase activity, confirming previously published reports on extrahepatic Morris hepatomas [18, 19]. No detectable activation of cyclophosphamide by tumor was observed (Table 2). Although the recovery ratio for microsomal cytochrome P-450 was lower in each hepatoma than in tumor-adjacent or far-removed liver (Table 1), diminished microsomal recoveries from each hepatoma accounted for only a small portion of the reduced cytochrome P-450 content and drug-metabolizing enzyme activities observed within hepatomas.

Several consequences of regional variations in cytochrome P-450-mediated production of cytotoxic metabolites from cyclophosphamide within tumor-containing liver merit discussion. Failure of tumor to activate cyclophosphamide suggests that, in the case of Morris hepatomas 5123D and 7795, non-tumorous liver is the only source of cytotoxic cyclophosphamide metabolites. For these reasons the antitumor activity of cyclophosphamide may vary in different stages of liver tumors, depending on the size and location of the tumors, as well as on the amount and location of histologically non-tumorous, tumor-adjacent liver.

In rats bearing extrahepatic hepatomas, previous investigations showed diminished metabolism of host livers, but only after tumors became extremely large (10 per cent of total body weight) with extensive necrosis [18, 19]. Lack of effect of either minimum deviation intrahepatic hepatoma on the *in vivo* hepatic drug-metabolizing capacity of tumor-bearing rats (Table 4) reflects the small mass of both tumor and tumor-adjacent tissue compared to the much larger mass of normal surrounding liver. A 30–40 per cent increase in cytochrome P-450 content of tumor-adjacent liver could make a substantial contribution to *in vivo* metabolism of some drugs. The actual change in drug metabolism in such a tumor-bearing animal would depend on the proportion of tumor-adjacent liver to normal liver.

Liver adjacent to intrahepatic Morris hepatoma 5123D or 7795 contained less DNA (per g liver) than liver far removed from tumor [1]. Differences between tumor-adjacent and far-removed liver in the ploidy of nuclei observed in the present study probably account for the lower DNA content of adjacent tissue. Since liver directly adjacent to tumor had more diploid and less tetraploid nuclei than liver far removed from tumor (Fig. 2 and Table 5), a lower DNA content would be anticipated in tumor-adjacent than in far-removed liver. Within tumor-adjacent tissue, change in hepatocyte nuclei from predominantly tetraploid to diploid could indicate a response to non-specific injury produced by the expanding adjacent hepatoma. A similar response occurs after physically or chemically induced injury to liver [20].

After PB pretreatment of tumor-bearing rats, hepatic microsomal cytochrome P-450 increased differentially among hepatoma, tumor-adjacent, and far-removed liver (Table 3). After PB induction, no differences between tumor-adjacent and far-removed liver occurred in cytochrome P-450 content or cyclophosphamide activation. This differential response of tumor-adjacent and far-removed hepatocytes to PB, together with the differences in nuclear ploidy between these two regions (Fig. 2 and Table 4), suggests that tumor-adjacent hepatocytes have a change in cellularity.

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