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BIOCHEMICAL BASIS FOR IMPAIRED DRUG METABOLISM IN TUMOR-BEARING RATS

EVIDENCE FOR ALTERED REGULATION OF HEPATIC MICROSOMAL HEMEPROTEIN SYNTHESIS

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Abstract—The pharmacologic effects of many drugs are enhanced in animals bearing tumors. This apparently stems from a decrease in the microsomal metabolism of these compounds in liver cells, owing to a decrease in either the activities of certain enzymes or in the content of cytochrome P-450 in microsomes, or both. Since impaired drug metabolism may have a direct bearing on the outcome of chemotherapy, a study of the biochemical basis for this alteration was begun. In female Sprague–Dawley rats bearing Walker 256 solid tumors i.m., pentobarbital metabolism was impaired as judged from prolonged sleeping-times. This effect was accompanied by a decrease in the microsomal content of cytochrome P-450. Analysis of hepatic microsomes by sodium dodecylsulfate-polyacrylamide gel electrophoresis revealed that a microsomal protein of ~53,000 daltons was diminished in livers of tumor-bearing animals, when compared to normal controls, as determined by staining with Coomassie Blue. When rats were injected with [^{3}H]leucine, although the dpm/100 μg of microsomal protein were the same for both normal and tumor-bearing rats, ~40% less ^{3}H was associated with the microsomal proteins in the cytochrome P-450 region (~43,000 to ~58,000 daltons) of tumor-bearing animals than with those of normal controls. This was attributed to a decrease in the rate of synthesis of these, but not of other, microsomal proteins. Either staining of microsomal proteins with 3,3′,5,5′-tetramethylbenzidine, or labelling of them with δ-[^{14}C]aminolevulinic acid, revealed that the overall content of microsomal hemeproteins of tumor-bearing rats was reduced considerably, when compared to normal controls (~70% as judged by the incorporation of ^{14}C). These observations prompted investigations of the apparent rate-limiting synthetic and degradative enzymes of heme. We found that in the livers of 7-day tumor-bearing rats, the activity of δ-aminolevulinic acid synthetase was only 16% of control activity; conversely, the activity of hepatic microsomal heme oxygenase in the tumor-bearing rats was nearly eight-times greater than that of the normal animals. Together, these data indicate that perturbations in heme- and hemeprotein-synthesis cause the reduced content of cytochrome P-450 seen in tumor-bearing rats, and they provide a partial explanation for diminished drug metabolism by hepatic microsomes in the presence of a growing, transplantable, non-hepatic tumor.

It has become evident that microsomal cytochrome P-450 from liver has multiple forms, as demonstrated by spectral [1, 2], antigenic [3, 4], and electrophoretic [5–9] evidence. Also clear is that these P-450 cytochromes are affected differently by drug and chemical treatments [6, 9–12] and by certain pathologic conditions [11, 13].

We as well as others have reported [14–17] that the pharmacologic effects of many drugs are exaggerated in animals bearing non-hepatic neoplasms; for example, the pentobarbital-induced sleeping-times of tumor-bearing mice [14] or rats [15–17] were prolonged by comparison with normal control values. Such effects have been ascribed to a decrease in the microsomal metabolism of the drug by liver cells. This alteration in metabolism has been attributed, in turn, to a decrease in the activities of the microsomal mixed-function oxidase enzymes [18], as well as to a decrease in the amount of cytochrome P-450 in microsomes [18, 19]. The basis for these changes has not been resolved. It was of interest, therefore, to learn more of the nature of this decrease in microsomal cytochrome P-450 content. The experiments presented herein indicate that the presence of an experimental non-hepatic neoplasm in rats is associated with decreased synthesis of hepatic microsomal hemeproteins, a phenomenon most likely due to an altered regulation of hepatic heme synthesis. A preliminary account of this work has been presented [20].

MATERIALS AND METHODS

Animals and tumor. Female Sprague–Dawley rats, weighing 140–160 g, were purchased from ARS/Sprague–Dawley (Madison, WI). The Walker 256 carcinosarcoma, which was provided by Dr. Arthur Bogden of Mason Research Laboratories (Worcester, MA), was maintained in rats in the ascites form. For experiments, 1–2 × 10^6 cells in 0.1 or 0.2 ml of 0.9% NaCl were injected i.m. in the right thigh; under these conditions the tumor grew as a solid mass. Tumor weights were calculated as described by Simpson-Herron *et al.* [21]: tumors were meas-

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ured in two perpendicular directions with calipers and, assuming that the tumors were prolate spheroids with a density of 1.0, the weight (mg) =(length [mm]) × (width [mm]^2/2). The rats were fed standard laboratory chow (Ralston Purina, St. Louis, MO) and were housed in cages with hardwood-chip bedding (Ab-Sorb-Dri, Knoxville, TN).

Preparation of hepatic microsomes. Microsomal proteins were prepared for spectral determination of cytochrome P-450 by the method of Omura and Sato [22] with slight modification, and for gel electrophoresis by a modified version of the methods of Haugen *et al.* [6] and Mackinnon *et al.* [11]. The rats were stunned and decapitated, and their livers were placed in ice-cold 0.9% NaCl solution, blotted dry, weighed, and homogenized in 4 vol. of 1.15% KCl. For analysis of microsomal proteins and heme-staining, livers were first perfused *in situ* through the superior vena cava with ~200 ml of ice-cold 0.25 M sucrose/0.01 M Tris–HCl [11] and homogenized in 4 vols. of the same. Homogenates were centrifuged at 12,000 *g* for 1 hr, after which the supernatant fractions were removed and centrifuged at 100,000 *g* for 1 hr. The microsomal pellets were collected, washed with cold 0.9% NaCl solution, quick-frozen in liquid nitrogen, and stored at −20°.

Determination of cytochrome P-450 content. Microsomal suspensions were analyzed for cytochrome P-450 content by the method of Omura and Sato [22], using an Aminco DW-2 dual-beam spectrophotometer in the split-beam mode. The amount of cytochrome P-450 was calculated from the change in absorption between 450 and 490 nm in a difference spectrum for CO-treated, $Na_2S_2O_4$-reduced sample, with a value of 91 cm^{-1} mM^{-1} for the molar extinction coefficient. Protein was determined by the method of Lowry *et al.* [23].

Analysis of hepatic microsomal proteins. Microsomal proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemmli system [24] and a modification of the method of Haugen *et al.* [6]. Microsomal pellets were dissolved in Laemmli disruption buffer [24], and protein was determined by the method of Ross and Schatz [25]. The stacking gel contained 3.5% acrylamide and the running gel 7.5% acrylamide, with 3.0% polyacrylamide (mol. wt >5,000,000) for stability [26]. Usually, 50 μg of microsomal protein was added to each well. The slabs were 1.5-mm thick and, unless otherwise indicated, the gels, cooled by tap-water, were run at 5 mA/gel for 60–80 min for stacking and, then, at 40 mA/gel until the tracking dye reached the bottom of the gel, usually an additional 120–150 min (using a PE 500 apparatus and PE 101 power supply from Hoefer Scientific, San Francisco, CA). The gels were stained for protein with Coomassie Brilliant Blue R (CB), as described earlier [27]. Molecular weights of proteins in the gels were calculated by the method of Weber and Osborn [28].

In some experiments, animals were injected intraperitoneally with [^{3}H]leucine (~1 mCi/200 g) or δ-[^{14}C]aminolevulinic acid (10–20 μCi/200 g); after a 6-hr or a 3- or 5-hr labeling period, respectively, the rats were killed. The hepatic microsomes were prepared for electrophoresis as described above. In these instances, the stained gels were sliced into 2-mm sections and incubated at 60° in 0.5 or 1.0 ml of 30% H_2O_2 [29] until the slices were dissolved; after cooling, each sample was suspended in RIA-Solve II (Research Products International Corp., Elk Grove Village, IL) and counted for radioactivity for 10 min.

Heme-staining of microsomes. Hepatic microsomes were prepared as described above. Polyacrylamide slab gels were prepared using the method of Thomas *et al.* [30] and, after electrophoresis at 4° in the dark, gels were stained for heme with 3,3′5,5′-tetramethylbenzidine/hydrogen peroxide (TMBZ/H_2O_2) [30]. With this method, the gels can be destained completely and subsequently re-stained for protein with CB. For best results, we found that it was necessary, before loading the samples on the gels, to extract "exogenous" heme from microsomes by a minor modification of the method of White *et al.* [31], using bovine serum albumin rather than human serum albumin. Preliminary experiments also demonstrated that, to obtain sufficient staining of heme, it was necessary to electrophorese 100–200 μg of "heme-extracted" microsomal protein and to use two times the concentrations of both TMBZ and H_2O_2 recommended by Thomas *et al.* [30] (12.6 and 60 mM respectively). Moreover, because of the relative lability of microsomal heme during electrophoresis [30], the best results were obtained when the gel was run quickly, as described.

Determination of δ-aminolevulinic acid synthetase (δ-ALAS) activity. Whole homogenates of rat liver were analyzed for δ-ALAS activity according to the sensitive method of Condie and Tephly [32], which measures the formation of δ-[^{14}C]aminolevulinic acid (δ-[^{14}C]ALA) from the condensation of [^{14}C]succinyl CoA and glycine. Each liver homogenate was assayed in duplicate, and activity for each sample was corrected for its own blank.

Determination of heme oxygenase (HO) activity. Hepatic microsomes were analyzed for HO activity according to the coupled assay described by Maines and Kappas [33], which measures the amount of bilirubin formed from biliverdin, using the 100,000 *g* supernatant solution from normal animals as the source of biliverdin reductase. (We very much appreciate the helpful comments of Dr. Maines in our performance of this assay.) Freshly prepared microsomal pellets used in these assays were snap-frozen and stored in liquid nitrogen until a few hours before the assay was run; samples were stored in this manner for no longer than 7–10 days.

Chemicals. L-[4,5-^{3}H]Leucine (sp. act. 55–65 Ci/mmole) was purchased from either the ICN Corp. (Irvine, CA) or Schwarz/Mann (Orangeburg, NY); δ-[4-^{14}C]aminolevulinic acid hydrochloride (sp. act. 40–60 mCi/mmole) and [2,3-^{14}C]succinic acid (sp. act. 17 and 50 mCi/mmole) were purchased from the New England Nuclear Corp. (Boston, MA). Pentobarbital, TMBZ, sodium succinate, δ-aminolevulinic acid hydrochloride, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, hematin, pyridoxal-5′-phosphate, bovine serum albumin, dithioerythritol, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) and NADP were purchased from the Sigma Chemical Co. (St. Louis,

MO). Dowex 50W-8X was obtained from the Bio-Rad Laboratories (Richmond, CA), and high molecular weight (>5,000,000 daltons) polyacrylamide (BDH Chemicals [Poole, England]) was purchased from the Gallard–Schlesinger Chemical Mfg. Corp. (Carle Place, NY). Allylisopropylacetamide was a gift of Hoffmann–LaRoche Inc., Nutley, NJ, through the courtesy of Dr. Allan H. Conney. Chemicals for gel electrophoresis were purchased from vendors as described previously [26], and all other chemicals were reagent grade and were obtained from standard sources.

RESULTS

Effect of the Walker 256 tumor on pentobarbital-induced sleeping-times and microsomal cytochrome P-450 content. Since our previous experiments, using mice carrying the Ehrlich ascites carcinoma, demonstrated that an increase in pentobarbital-induced sleeping-time was related to a decrease in the metabolism of this compound [14], it was desirable to confirm and extend these results in rats bearing the Walker 256 tumor. When injected with pentobarbital (25 mg/kg, i.p.), normal rats slept an average of 85–90 min, whereas similarly treated rats bearing the Walker tumor 7 days after implantation i.m. slept for an average of 163–195 min, or about twice as long as controls (Table 1). Such results are in agreement with those of others [15, 16] and can be attributed to a decrease in the metabolism of this compound [14]. Also consistent with findings by others [17, 18], the livers of tumor-bearing animals contained significantly less cytochrome P-450 than did the livers of normal animals (0.21 to 0.29 vs 0.54 to 0.55 nmole/mg protein, or decreases of 47–61%). The observed differences in the contents of cytochrome P-450 between the groups were probably not due to gross differences in the relative contents of total microsomal proteins, since it was estimated that, while there was about 41% more total microsomal protein in the livers of tumor-bearing rats, when compared to normal animals, their livers also weighed about 49% more, so that the values for mg microsomal protein per g liver were the same for both groups.

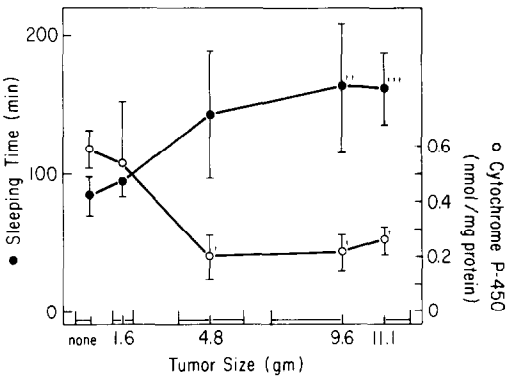

Fig. 1. Demonstration of a reciprocal relationship between the pentobarbital-induced sleeping-time and hepatic microsomal cytochrome P-450 content in the presence of a non-hepatic tumor. Groups of five rats each were inoculated i.m. with 1×10^6 Walker 256 tumor cells on day 0. On days 3, 6, 7 and 9 after inoculation, the pentobarbital-induced sleeping-time was determined for each of five tumor-bearing and five normal control rats, after which the animals were killed, and their livers were prepared for determination of cytochrome P-450 content. The sleeping-time was the time between the loss and the return of the righting reflex after an i.p. injection of pentobarbital (2.5 mg per 100 g per 0.125 ml). Key: (+) $P < 0.02$; (++) $P < 0.05$; (+++) $P < 0.001$.

That the alterations in sleeping-time and cytochrome P-450 are in general reciprocally related to each other is demonstrated clearly in Fig. 1. Shown on the abscissa is the mean tumor size (±1 S.D.) 3, 6, 7 and 9 days after i.m. inoculation of 1×10^6 Walker 256 cells. At those times, pentobarbital-induced sleeping-times were assessed, after which the animals were killed and their hepatic microsomes were prepared for analysis of cytochrome P-450 content. (It was established that a single injection of pentobarbital had no acute effect on the content of cytochrome P-450.) It is evident that a reciprocal relationship exists between the content of cytochrome P-450 and the metabolism of pentobarbital (as measured by the sleeping-time [14]), and this relationship is affected by the presence of a non-hepatic tumor.

Table 1. Pentobarbital-induced sleeping-times and hepatic microsomal cytochrome P-450 content in normal versus tumor-bearing rats

Experiment no.	Animal groups	No. of rats	Average tumor size (g)	Sleeping-time*		Cytochrome P-450 content	
				Minutes	% of Control	nmoles/mg protein	% of Control
I	Normal controls	5		85 ± 56†		0.54 ± 0.05	
	Tumor-bearing‡	5	9.59 ± 2.61	163 ± 46§	192	0.21 ± 0.07‖	38.9
II	Normal controls	10		90 ± 50		0.55¶	
	Tumor-bearing‡	7	10.45 ± 2.09	195 ± 50‖	217	0.29	52.7

* Sleeping-time is defined as the time between the loss and the return of the righting reflex after an intraperitoneal injection of pentobarbital (2.5 mg/100 g).

† Mean ± S.D. All animals were fasted overnight before being killed.

‡ Seven days after i.m. injection of 1×10^6 cells.

§ $P < 0.05$.

‖ $P < 0.001$.

¶ Liver homogenates from each group were pooled before preparing microsomes in this experiment.

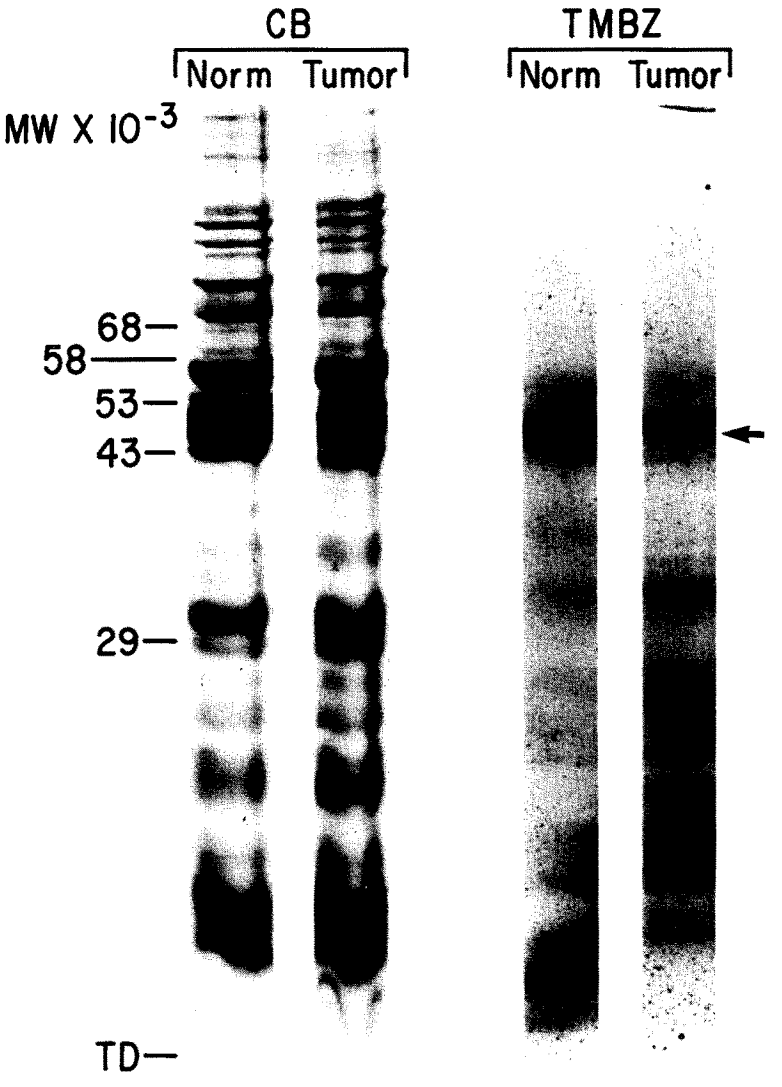

Fig. 2. Polyacrylamide gel electrophoresis of rat-liver microsomal proteins. The details of preparation and electrophoresis of microsomes are given in Materials and Methods. Representative profiles of microsomes from single rats are shown; however, microsomes from more than ten rats each have yielded similar results. Microsomes were from either normal (Norm) or 7-day tumor-bearing (Tumor) rats. Abbreviations: CB, Coomassie Blue-stained; and TMBZ, 3,3′,5,5′-tetramethylbenzidine-stained.

Analysis of microsomal proteins and hemeproteins by gel electrophoresis. The hepatic microsomal proteins from these animals were subjected to SDS-PAGE, after removal of "exogenous" heme [31]. Under these conditions, electrophoresis of 50 μg of microsomes (Fig. 2) revealed that there was less of a protein of ~53,000 daltons in hepatic microsomes of tumor-bearing animals, when compared to normal controls, as determined by staining for protein with Coomassie Blue (CB). Moreover, electrophoresis of 150 μg of microsomes, and subsequent staining of the gels for heme with TMBZ/H_2O_2, revealed that there was apparently less heme in the cytochrome P-450 region (*arrow*; see below) of the microsomes from tumor-bearing rats, when compared to the controls.

Microsomal proteins in the molecular weight range of ~43,000 to ~58,000 daltons have been identified as cytochromes P-450 [5–9], although non-P-450 proteins co-migrate in this region [34–36]. While the P-450 cytochromes are the major hemeproteins of liver microsomes, other hemeproteins have been shown to be associated with this microsomal fraction [37]. The major TMBZ-staining region on our gels was in the range of 43,000 to 58,000 daltons, indicating that these were probably the cytochrome(s) P-450; that their amount was apparently diminished in microsomes from tumor-bearing animals is in agreement with the results of staining with CB. The TMBZ-stained material below 40,000 daltons most likely represented heme that had dissociated from the cytochrome(s) P-450 during electrophoresis [30], and was notable only in that it was a variable artifact of the method.

Incorporation of [^{3}H]*leucine or* [^{14}C]*aminolevulinic acid by microsomal proteins.* To examine

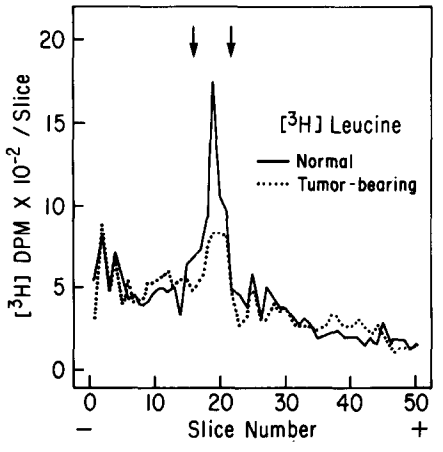

Fig. 3. Distribution of radioactivity in rat liver microsomal proteins after an injection of [^{3}H]leucine. Rats were injected i.p. with [^{3}H]leucine (~1 mCi/200 g) and killed 6 hr later. Hepatic microsomal proteins were prepared, subjected to electrophoresis, and assayed for radioactivity as described in Materials and Methods. Representative profiles of microsomes from single rats are shown; microsomes from ~10 rats each have yielded similar results. The arrows mark the molecular weight range of the cytochromes P-450, ~58,000 daltons (left) and ~43,000 daltons (right). Solid line, normal rat liver; dotted line, liver from rat bearing the 7-day Walker 256 tumor.

the synthesis of the apoproteins of the hepatic microsomal cytochromes P-450, normal or 7-day tumor-bearing rats were injected i.p. with ~1 mCi of [^{3}H]leucine per 200 g body weight and killed 6 hr later. The livers were removed, microsomes were prepared, and proteins were separated by gel electrophoresis as before. The gels were then sliced into 2-mm sections, which were solubilized in H_2O_2, and assayed for radioactivity.

Shown in Fig. 3 is a profile of the distribution of ^{3}H radioactivity in microsomal proteins. It is evident that in normal rats an appreciable amount of radioactivity was incorporated into the cytochrome P-450-region proteins (area between the arrows). In contrast to this labeling pattern, the profile of ^{3}H radioactivity incorporated into microsomes from 7-day tumor-bearing rats is quantitatively different, notably in the cytochrome P-450 region. Indeed, it can be seen in Table 2 that, while there was no difference in the incorporation of ^{3}H into the microsomes of normal versus tumor-bearing rats (7700 ± 1594 vs 8661 ± 5028 dpm/100 μg protein respectively), the amount of radioactivity incorporated into proteins in the cytochrome P-450 region was reduced to 59 ± 29% of control values.

The synthesis of microsomal heme was examined by following the incorporation of δ-[^{14}C]aminolevulinic acid into hemeproteins [38, 39]. In these experiments, animals were injected i.p. with 10–20 μCi of δ-[^{14}C]aminolevulinic acid per 200 g and killed 3 or 5 hr later. The liver microsomes were prepared and run on gels as detailed in Materials and Methods for TMBZ/H_2O_2 staining, followed by CB-staining, and were then sliced and solubilized for the determination of ^{14}C as described. As there were no apparent differences in either the total or specific labeling of microsomes and microsomal hemeproteins 3 or 5 hr after injection of δ-[^{14}C]aminolevulinic acid, the results of three seperate experiments were pooled.

In contrast to the results with [^{3}H]leucine, the incorporation of ^{14}C into the liver microsomes of tumor-bearing rats was inhibited by 69%, when compared to the incorporation of label into microsomes of normal animals (7770 ± 825 vs 2443 ± 1303 dpm/mg protein respectively; Table 2). Since the microsomal hemeproteins are primarily the cytochromes P-450, it was expected that the incorporation of ^{14}C into this region would also be inhibited to a similar extent in the microsomes of the tumor-bearing rats versus control animals, and this is what was observed.

Activities of δ-aminolevulinic acid synthetase (δ-ALAS) and heme oxygenase (HO) in normal and tumor-bearing rats. The results shown above provided strong support for the consideration that the regulation of heme metabolism is perturbed in tumor-bearing rats. To explore this possibility, we examined the hepatic activities of both the rate-limiting enzyme in heme synthesis, δ-ALAS [40], as well as the primary heme-degrading enzyme, HO [41]. Table 3 indicates that the activity of δ-ALAS in livers from 7-day tumor-bearing rats was only 16%

Table 2. Incorporation of [^{3}H]leucine or δ-[^{14}C]aminolevulinic acid into hepatic microsomal proteins of normal and tumor-bearing rats*

	[^{3}H]Leucine		δ-[^{14}C]Aminolevulinic acid	
Animal groups	Total† (dpm/100 μg protein)	"P-450"‡ (relative weight)	Total† (dpm/mg protein)	"P-450"‡ (relative weight)
Normal	7700 ± 1594		7770 ± 825	
Tumor-bearing§	8661 ± 5028 (1.12)¶	0.59‖	2443 ± 1303 (0.31)¶	0.39‖

* Animals (six to eight per group) were injected with isotopes as described in Materials and Methods.
† ^{3}H or ^{14}C incorporated into total microsomes.
‡ ^{3}H or ^{14}C incorporated into the cytochrome P-450 region (43,000 to 58,000 daltons).
§ Seven days after inoculation of 1 × 10^6 Walker 256 cells.
‖ Hepatic microsomal proteins were run on gels, which were then sliced, dissolved, and counted for radioactivity, as described in Materials and Methods. The data were graphed, and the P-450-region peaks were cut out and weighed; these weights are presented as values relative to normalized control weights.
¶ Tumor-bearing/normal.

Table 3. δ-Aminolevulinic acid synthetase activities in rat liver

Treatments	δ-ALAS activity* ALA formed [pmoles · (mg protein)$^{-1}$ · (30 min)$^{-1}$]	% of Control
Control	3.66 ± 2.31†	
AIA-treated	27.3 ± 10.2	746
Tumor-bearing‡	0.59 ± 0.46	16

* Hepatic enzyme activity was measured in whole homogenates of liver according to the sensitive method of Condie and Tephly [32], which measures the formation of δ-[^{14}C]aminolevulinic acid from the condensation of [^{14}C]succinyl CoA and glycine; see Ref. 32 for details of the method.

† Mean ± S.D., six rats/group.

‡ Seven days after i.m. injection of 1×10^6 Walker 256 cells.

of the normal control values (0.59 ± 0.46 vs 3.66 ± 2.31 pmoles · (mg protein)$^{-1}$ · (30 min)$^{-1}$ respectively). Allylisopropylacetamide (AIA) induces the activity of δ-ALAS [40], and animals pretreated with this agent were used as positive controls in these experiments to ensure that the assay system was functioning properly. It is clear from the results shown in Table 3 that the assay system worked well, the AIA-pretreated animals having an enzyme activity nearly 7.5 times that of the controls. The depressed activity of δ-ALAS seen in these tumor-bearing rats is in basic agreement with the results of Bonkowsky *et al.* [42], and indicates that the synthesis of heme is impaired significantly in animals with tumors, when compared to healthy controls.

When we examined the activity of HO, it was found to be *increased* nearly 8-fold in liver microsomes from 7-day tumor-bearing rats, when compared to normal controls (2.32 ± 0.26 vs 0.30 ± 0.21 nmoles bilirubin formed · (mg protein)$^{-1}$ · (15 min)$^{-1}$ respectively; Table 4). Cobaltous chloride has been shown to induce the activity of HO [33], and animals pretreated with this chemical were used as positive controls in these experiments to ensure that the HO assay system was functioning properly. It is clear from the results in Table 4 that pretreatment with $CoCl_2$ overnight induced the activity of HO nearly 20-fold, as compared to controls, a finding in general agreement with the results of others (e.g. Ref. 33). That the activity of HO was markedly elevated in tumor-bearing rats, when compared to controls, is taken as further evidence that the presence of a tumor causes a derangement in the metabolism of heme. Moreover, preliminary findings have indicated that HO activity is demonstrably elevated by 3 days after i.m. inoculation of 10^6 Walker 256 cells.*

* W. T. Beck, M. A. Ouellette and M. L. Dedmon, unpublished results.

DISCUSSION

Changes in the pharmacologic effects of many drugs in tumor-bearing animals are often due to an impairment in the hepatic biotransformation of such agents [14, 15, 17]. Little progress has been made, however, in clarifying the mechanism of these alterations. Because such effects on drug metabolism may have important pharmacokinetic and toxicologic implications for the design of antineoplastic chemotherapy, it was deemed important to learn more about this altered pharmacologic response to drugs in animals with tumors, with a view to extension of this knowledge to patients.

The results presented here demonstrate that the amount of cytochrome(s) P-450 in hepatic microsomes is decreased in tumor-bearing rats, when compared to healthy controls, and this may account in part for the impaired rate of microsomal drug metabolism in these animals. The results of the [^{3}H]leucine incorporation experiments probably represent *de novo* protein synthesis, as described by Haugen *et al.* [6]. The amounts of radioactivity per μg of micro-

Table 4. Heme oxygenase activity in rat liver microsomes

Treatments	Heme oxygenase activity* Bilirubin formed [nmoles · (mg protein)$^{-1}$ · (15 min)$^{-1}$]	% of Control
Control	0.30 ± 0.21†	
$CoCl_2$-treated	5.86 ± 1.43	1953
Tumor-bearing‡	2.32 ± 0.26	773

* Enzyme activity was measured in hepatic microsomes according to the method of Maines and Kappas [33], which is a coupled assay that measures the amount of bilirubin formed from biliverdin; see Ref. 33 for details of the method.

† Mean ± S.E. (three experiments, three to six rats per experiment).

‡ Seven days after i.m. injection of 1×10^6 cells.

somal protein in normal and tumor-bearing rats were roughly the same, indicating that the overall synthesis of microsomal proteins proceeds at a normal rate in tumor-bearing animals.

When analyzed by SDS-PAGE, however, those microsomal proteins in the molecular weight range of ~43,000 to ~58,000 daltons appeared to be affected by the presence of the tumor. Microsomal proteins in this range of molecular weight have been identified as cytochrome(s) P-450 [5–9], although some non-P-450 proteins have also been shown to migrate in this region [34–36]. Accordingly, the designation of the proteins of interest as cytochrome(s) P-450 rests, in the absence of purified material, on either the staining of heme with TMBZ/H_2O_2 [30] or the labeling of heme with δ-[^{14}C]aminolevulinic acid [38, 39]. The results in this paper demonstrate that there is stained and labeled material in the cytochrome P-450 region, indicating that these are indeed hemeproteins and, most likely, represent cytochrome(s) P-450.

The presence of other heme-stained material in the hepatic microsomes migrating below ~40,000 daltons was a variable finding and most likely represents an artifact of the method. In this regard, Thomas *et al.* [30] observed that heme, which is noncovalently bound to the cytochromes P-450, may dissociate and subsequently bind to other proteins during electrophoresis. Indeed, the lower molecular weight TMBZ-staining material, representing an artifact of dissociated heme, was observed by us in our initial attempts at heme-staining of total microsomal proteins on gels. If the livers were not extensively perfused, we found heavy staining throughout the gel; this was most likely due to trapped hemoglobin. Exhaustive perfusion of the liver altered the heme-staining pattern dramatically, leaving prominent bands migrating at (1) 43,000 to 58,000 daltons, (2) ~35,000 daltons, and (3) near the tracking-dye. Although the molecular weight of cytochrome b_5 is 25,000 daltons [43], it was unlikely to be seen in these microsomal preparations [44]. Accordingly, suspecting that the TMBZ-stained material migrating with an apparent molecular weight of ~35,000 daltons was artifactual, we resorted to the exogenous heme "stripping" technic of White *et al.* [31], the results of which are shown in Fig. 2. This procedure not only removes the prominent heme-band at 35,000 daltons, but it also removes CB-stained material of the same molecular weight.* The nature of this material, which is less prominent in the microsomes from tumor-bearing animals, is at present unknown.

The perturbations in the major synthetic and degradative enzymes of heme metabolism, seen in the tumor-bearing rats, were of much interest. Our results showing a significant decrease in the activity of δ-ALAS confirmed earlier observations of Bonkowsky *et al.* [42] and indicated that heme biosynthesis is considerably reduced in the tumor-bearing host. Our demonstration, however, that the major heme-catabolizing enzyme, HO, is elevated in such animals has not been reported before and, in light of the δ-ALAS results, was an unexpected finding.

It has been shown that the activity of HO can be elevated by fasting [45]: moreover, it has been documented clearly that HO activity can be elevated by heavy metal cations [33, 46]. The mechanism(s) by which these effects on HO are mediated is unclear at this time. We do not believe, however, that fasting is a significant factor in the elevation of hepatic microsomal HO activity in tumor-bearing rats, because this increase was observed in animals only 3 days after the inoculation of the tumor; at that time, gross food consumption was no different from that of controls.† Alternatively, it is of considerable interest that polyamines, organic bases that are polycationic at physiological pH, are elevated above normal levels in the blood, urine and tissues of tumor-bearing animals [47]. Whether these cations play a role in the elevation of HO activity is unknown at this time.

The increase in the activity of HO under conditions of apparent decreased synthesis of heme, however, is enigmatic. It is possible that the tumor, through nutritional perturbations, polyamines or hormonal effects, impairs the coordinated syntheses of heme and those apoproteins that have a relatively fast turnover. As a consequence, more free heme might be available both to "induce" the activity of HO, as well as to inhibit the activity of δ-ALAS. (A precedent for this can be found in the study of Yoshida *et al.* [48], who observed that administration of "host-mediating" antitumor drugs to rats not only caused a decrease in hepatic microsomal cytochrome P-450 content, but also produced an increase in the activity of HO and a decrease in the activity of δ-ALAS.) Alternatively, it is conceivable that HO may have several "induction" pathways, one responsive to excess heme, another responsive to heavy metals (or polyamines); some of these possibilities are now under investigation. We can only conclude from our results at this time that hepatic microsomal hemeprotein synthesis is perturbed by the presence of a non-hepatic tumor.

Finally, it is well-known that the presence of a tumor impairs the synthesis of the peroxisomal hemeprotein, catalase [49], and this phenomenon has been attributed to a ubiquitous tumor-produced factor, "toxohormone" [50]. Additionally, toxohormone has been implicated as a factor that inhibits drug metabolism in animals with tumors [51]. In this regard, serum from tumor-bearing rats, when injected into normal rats, has been reported to inhibit the microsomal metabolism of drugs *in vitro* [17]. Although we have also treated normal rats i.v. with serum obtained from tumor-bearing rats, according to the schedule described by Rosso *et al.* [17], we have been unable to demonstrate any serum effects on drug metablism *in vivo*, nor were we able to show any effect of such serum on the [^{3}H]leucine-labeling of either P-450-region proteins or of total microsomal proteins.‡ These findings are consistent with our earlier observation [14] that a tumor has no *acute* effect on drug metabolism.

Drugs [6, 11] and such pathologic conditions as cholestasis [11] and diabetes [13] have been shown

* W. T. Beck and M. L. Dedmon, unpublished results.

† W. T. Beck, M. A. Ouellette and M. L. Dedmon, unpublished results.

‡ W. T. Beck and M. A. Ouellette, unpublished observations.

to have effects on the syntheses of various hepatic microsomal cytochromes P-450 in rodents, but the mechanism(s) by which this occurs is not known. Data presented in this paper indicate that another pathologic condition, the presence of a neoplasm, not only impairs the metablism of drugs by hepatic microsomes, but also can perturb the synthesis of the microsomal P-450 cytochromes, apparently through effects on apoproteins and on heme metabolism. The clinical significance of such observations is unknown at the present time. Although attempts have been made to study such phenomena in cancer patients [52–54], the data were generally inconclusive: no significant changes in drug metabolism were seen in these patients when compared to controls, although some impaired metabolism was observed. In general, however, the data could not be properly evaluated because of difficulties in obtaining appropriate control groups. Nevertheless, impaired drug metabolism in patients with cancer would have implications for drug therapy in terms of drug toxicity and altered pharmacokinetics.

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