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# ALTERATIONS IN HEPATIC HEME AND CYTOCHROME P-450 METABOLISM IN MURPHY–STURM LYMPHOSARCOMA-BEARING RATS

## IMPLICATIONS FOR DRUG METABOLISM

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**Abstract**—Previous studies have shown that tumor-bearing rats have significantly decreased hepatic microsomal cytochrome P-450 content and NADPH–cytochrome *c* reductase activity with, consequently, significantly decreased capacity for microsomal oxidative drug metabolism. Subsequent investigations have revealed that the rates of hepatic cytochrome P-450 apo-protein synthesis and degradation are decreased significantly and hepatic microsomal heme oxygenase activity is increased significantly in rats bearing an extra-hepatic tumor. Further studies have been done to attempt to clarify the pathogenesis and significance of these observations. Hepatic delta-aminolevulinic acid (ALA) synthetase activity in male Wistar rats declined to a nadir of 162 ± 34 (S.E.) pmoles ALA per mg protein per 30 min 6 days following i.m. transplantation of Murphy–Sturm lymphosarcoma (vs control = 218 ± 36 pmoles per mg per 30 min). Turnover of $^3$H-labeled heme in microsomal CO-binding particles (i.e. cytochrome P-450 heme) was increased significantly 8 days following i.m. transplantation of Murphy–Sturm lymphosarcoma with a $T_{\frac{1}{2}}$ of 5.5 hr for the fast phase of hepatic cytochrome P-450 heme disappearance in tumor-bearing rats as compared with a $T_{\frac{1}{2}}$ of 7 hr in control rats. Hepatic cytochrome P-450 apo-protein concentration was slightly, but not significantly, increased in Murphy–Sturm lymphosarcoma-bearing rats as compared with control rats up to 10 days following tumor transplantation. These results suggest that, in Murphy–Sturm lymphosarcoma-bearing rats, decreased microsomal cytochrome P-450 concentration is the result of both decreased cytochrome P-450 apo-protein synthesis and increased cytochrome P-450 heme turnover. Apo-cytochrome P-450 concentration was not appreciably altered because increased cytochrome P-450 heme turnover and decreased cytochrome P-450 apo-protein degradation were balanced by decreased cytochrome P-450 apo-protein synthesis. Because of their effects on cytochrome P-450 concentration and action, these alterations in heme and hemoprotein metabolism may be of importance in regulating oxidative drug metabolism in the tumor-bearing state.

Several lines of evidence now suggest that hepatic microsomal oxidative drug metabolism is impaired in tumor-bearing animals [1–4]. Diminished hepatic microsomal drug metabolism in this situation is associated with decreased levels of hepatic cytochrome P-450, cytochrome $b_5$ and NADPH–cytochrome *c* reductase [1, 3, 5–7] and, consequently, prolonged duration of action of many drugs normally metabolized by the hepatic microsomal electron transport system in tumor-bearing animals [7–12]. These effects are produced by tumor which is present at an extra-hepatic site and which does not involve the liver by direct metastases. It has been suggested that these changes in microsomal function may be mediated by a humoral factor(s) produced by tumor cells [13–15], and studies in parabiotic rats have demonstrated that such substances may be released into the circulation and cause depression of hepatic microsomal electron transport system function [16].

Recent studies have suggested that decreased hepatic microsomal cytochrome P-450 content in tumor-bearing animals may be related to decreased synthesis of this hemoprotein [1, 7]. The enzyme responsible for hepatic heme catabolism, microsomal heme oxygenase, is increased in activity [1, 7] and delta-aminolevulinic acid (ALA) synthetase, the rate-controlling enzyme in heme biosynthesis, is reduced in activity in tumor-bearing rats [7, 17]. It has been suggested that alterations in the activity of key enzymes in heme biosynthesis and degradation may be related to an increase in size of the free heme pool in tumor-bearing rats as the result of decreased apo-cytochrome P-450 synthesis [1, 7], causing substrate-mediated induction of heme oxygenase and inhibition of ALA synthetase by heme. It therefore appears that alterations in microsomal oxidative drug metabolism in tumor-bearing animals are associated with profound alterations in the regulation of hepatic heme and hemoprotein synthesis and degradation.

Further investigations regarding the mechanisms producing these changes are presented here. Specifically, hepatic cytochrome P-450 heme turnover and apo-cytochrome P-450 content in Murphy–Sturm lymphosarcoma bearing rats were examined. The findings suggest that decreased hepatic microsomal cytochrome P-450 content in rats bearing Murphy–Sturm lymphosarcoma results from both decreased

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apo-cytochrome P-450 synthesis and increased cytochrome P-450 heme turnover. The implications of these results with respect to regulation of heme and hemoprotein metabolism and microsomal oxidative drug metabolism in tumor-bearing rats are discussed.

## MATERIALS AND METHODS

*Animals and tumor passage.* Male Wistar rats weighing 150–250 g were used in these experiments. The Murphy–Sturm lymphosarcoma cell line was provided by Dr. I. Kline, Microbiological Associates, Bethesda, MD. The tumor cells were prepared for inoculation following removal from a Wistar rat under sterile conditions by mincing them in Fischer's medium on ice, followed by homogenization with a glass homogenizer. The homogenate was filtered through a sieve, and the filtrate was centrifuged at 1000 rpm for 10 min. The supernatant fraction was discarded, and the cells were resuspended in sterile Fischer's medium containing 10% horse serum. The resuspended cells were then diluted 1:20 (v:v) in sterile 0.9% NaCl. Following cell counting, male Wistar rats for experiments were each injected with $3–6 \times 10^6$ cells i.m. in the thigh. Tumors were also passaged every 10 days in this matter. Control rats were animals of the same age and weight kept in the same animal room but in separate cages.

*Tissue preparation.* Groups of three to five control or experimental animals that had been fasted were killed by decapitation at the time intervals indicated in each experiment. The livers were perfused *in situ* via the portal vein with ice-cold 0.9% NaCl until bleached of hemoglobin, and then removed. The methods of tissue preparation from this point varied, depending on the experiment, and are described below.

*Determination of ALA synthetase activity.* ALA synthetase was assayed by the incorporation of [1,4-$^{14}$C]succinic acid into ALA [18] as measured by sequential ion exchange column chromatographic isolation of ALA [19]. Pooled samples from three livers in each experimental group and the control group were prepared as a 1:10 (v/v) homogenate in 0.9% NaCl containing 0.5 mM EDTA and 10 mM Tris buffer, pH 7.4. Each assay was performed in duplicate, as previously described, with less than 10% error noted between duplicate assays. Radioactivity was counted in a Beckman LS9000 counter.

*Determination of cytochrome P-450 heme turnover.* The method of Levin and Kuntzman [20] was used to radiolabel hepatic microsomal hemoproteins and then observe the rate of decrease of radioactive hemoprotein in CO-binding particles in control and tumor-bearing rats. Control rats and rats bearing Murphy–Sturm lymphosarcoma (8 days following i.m. transplantation of tumor) were fasted for 18 hr and then given [3,5-$^3$H]ALA (sp. act. = 1.3 Ci/mmole) intravenously by tail vein injection at a dose of 0.37 mCi/kg. Animals were then killed at various time intervals from 3 to 72 hr following injection. Liver microsomes were prepared using 1.15% KCl in 0.05 M Tris buffer, pH 7.5, as previously described [20]. The microsomal pellets were washed once with 1.15% KCl in 0.05 M Tris buffer, pH 7.5, and the final microsomal pellets were layered with 3 ml of 0.1 M $KH_2PO_4$–$K_2HPO_4$ buffer, pH 7.4, and frozen at −15°. Within 7 days, the microsomal pellets were thawed at room temperature and then resuspended in 0.1 M $KH_2PO_4$–$K_2HPO_4$ buffer, pH 7.4, to a concentration of 250 mg of wet weight liver/ml. The microsomal suspensions were purged with nitrogen for 10 min and incubated with 0.2% steapsin for 1 hr at 37° under nitrogen in order to selectively solubilize cytochrome $b_5$. After incubation, the samples were centrifuged at 105,000 *g* for 2 hr. The pellet obtained in this way contains 80–90% of the CO-binding hemoprotein, mostly as cytochrome P-450 [21]. The CO-binding particles were resuspended in 0.1 M $KH_2PO_4$–$K_2HPO_4$ buffer, pH 7.4, and 0.2 to 0.4-ml aliquots were counted in a Beckman LS9000 liquid scintillation counter after addition of 10 ml Aquasol.

*Determination of apo-cytochrome P-450 content.* The method of Correia and Meyer [21] was used to quantitate hepatic apo-cytochrome P-450 content in control rats and in Murphy–Sturm lymphosarcoma-bearing rats at various time intervals after tumor transplantation. In each experiment, livers from three rats were pooled and homogenized in 0.1 M potassium phosphate buffer, pH 7.4, to make a 50% suspension. Aliquots (10 ml) of this suspension were supplemented with 1 mM phosphatidylcholine and 0.25 mM phosphatidylethanolamine and incubated in either the presence or absence of 40 μM hemin for 20 min at 37° in a shaking water bath. Hemin was prepared by dissolving appropriate amounts in 0.1 M NaOH and then adjusting the pH to 7.4 with 0.1 M potassium phosphate buffer, pH 7.4. Following incubation, the suspensions were centrifuged at 10,000 *g* for 15 min at 4°, and the resulting supernatant fraction was centrifuged at 105,000 *g* for 1 hr at 4°. The microsomal pellet was washed with 1.15% KCl and resedimented. The microsomal fraction was then resuspended in 0.1 M potassium phosphate buffer, pH 7.4, for cytochrome P-450 assay. Apo-cytochrome P-450 content was determined as the difference in cytochrome P-450 concentration between heme-supplemented and non-supplemented incubations of each homogenate.

*Other analytical methods.* Hepatic microsomal cytochrome P-450 was measured by the method of Omura and Sato [22] using a millimolar extinction coefficient of 91 $mM^{-1}$ $cm^{-1}$ [22]. To minimize spectral distortion by exogenous heme adsorbed to microsomes in the apo-cytochrome P-450 experiments, cytochrome P-450 in these studies was measured as the difference in absorbance between the reduced cytochrome P-450–CO complex and oxidized cytochrome P-450 [23], with a millimolar extinction coefficient of 100 $mM^{-1}$ $cm^{-1}$ for the wavelength pair 450 and 490 nm [23]. Proteins were measured by the method of Lowry *et al.* [24] with bovine serum albumin used as standard. All spectrophotometric assays were done using an Aminco DW-2a UV-Vis spectrophotometer (American Instrument Co., Inc., Silver Springs, MD). Statistical analysis of significance was done with Student's two-tailed *t*-test.

*Materials.* Phosphatidylethanolamine, phosphatidylcholine, ATP, coenzyme A, and recrystallized hemin were obtained from the Sigma Chemical Co., St. Louis, MO. Glutathione was purchased from

Calbiochem, San Diego, CA, and steapsin from Nutritional Biochemicals. [3,5-$^3$H]delta-Aminolevulinic acid (sp. act. 1.3 Ci/mmole) was purchased from the New England Nuclear Corp., Boston, MA. [1,4-$^{14}$C]Succinic acid (sp. act. 8 mCi/mmole) was purchased from ICN Pharmaceuticals, Irvine, CA. Dowex 1-acetate and Dowex 50-H$^+$ columns were prepared by and purchased from Biorad Laboratories, Richmond, CA. All other chemicals used were reagent grade.

## RESULTS

*Effect of Murphy–Sturm lymphosarcoma on hepatic delta-aminolevulinic acid (ALA) synthetase activity.* Previous studies have provided evidence for decreased synthesis of hepatic cytochrome P-450 in tumor-bearing rats [1, 7], suggesting that heme biosynthesis, which is required for cytochrome P-450 synthesis, might be deficient in the tumor-bearing state. Therefore, ALA synthetase, which is the rate-limiting enzyme in hepatic heme biosynthesis, was studied in rats bearing Murphy–Sturm lymphosarcoma at an extra-hepatic site. The results (Fig. 1) illustrate that hepatic ALA synthetase activity was moderately depressed compared to control rats 4–6 days following i.m. transplantation of the tumor at an extra-hepatic site. Hepatic ALA-synthetase activity was not decreased 2 days following tumor transplantation, and activity of this enzyme returned toward normal 8 and 10 days following tumor transplantation. The decrease observed in hepatic ALA-synthetase activity in Murphy–Sturm lymphosarcoma-bearing rats confirms previous observations [7, 17] with other transplantable tumors, although the effect of extra-hepatic tumor on ALA synthetase activity was generally more marked in the previous studies.

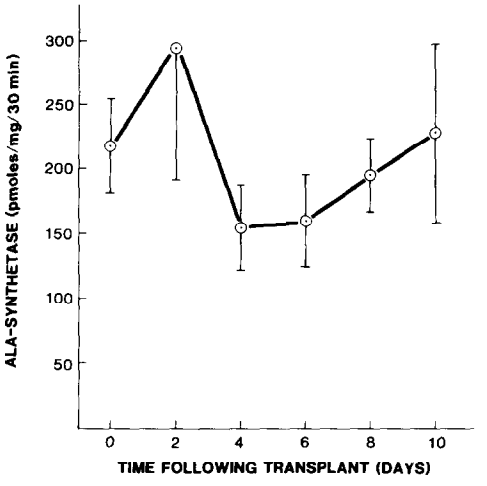


Fig. 1. Hepatic ALA–synthetase activity in Murphy-Sturm lymphosarcoma-bearing rats at various intervals following tumor transplantation. Groups of three rats were inoculated i.m. with $3 \times 10^6$ Murphy–Sturm lymphosarcoma cells on day 0. On days 2, 4, 6, 8, and 10 following tumor inoculation, the groups of animals were killed, and their livers removed, pooled and assayed for ALA synthetase activity as described in Materials and Methods. Each point represents the mean ± S.E. of four experiments.

*Effect of Murphy–Sturm lymphosarcoma on hepatic microsomal cytochrome P-450 heme turnover.* While the observed decrease in cytochrome P-450 apo-protein synthesis in tumor-bearing rats might alone account for decreased cytochrome P-450 content, an alternative mechanism might be related to increased turnover of cytochrome P-450 heme. To explore this possibility, the turnover of hepatic cytochrome P-450 heme was compared in control rats and in rats bearing Murphy–Sturm lymphosarcoma 8 days following transplantation. This time point was chosen because it corresponds with the greatest decrease in cytochrome P-450 concentration [1] and with previous observations of significant decreases in cytochrome P-450 apo-protein synthesis and degradation [1]. The method of Levin and Kuntzman [20] was used to label microsomal hemoproteins with [$^3$H]ALA *in vivo* and then monitor the rate of degradation of CO-binding particles in hepatic microsomes. CO-binding particles are prepared by selective solubilization and subsequent removal of microsomal cytochrome $b_5$ by steapsin digestion of the microsomal fraction, resulting in retention of most of the radioactive label in the particles as radiolabeled heme in association with the CO-binding pigment [20]. The CO-binding particles obtained are essentially devoid of cytochrome $b_5$, and the only spectrally distinguishable hemoprotein present within them is cytochrome P-450 [22].

Figure 2 shows the disappearance of radioactivity with time from the CO-binding particles in control and Murphy–Sturm lymphosarcoma-bearing rats 8

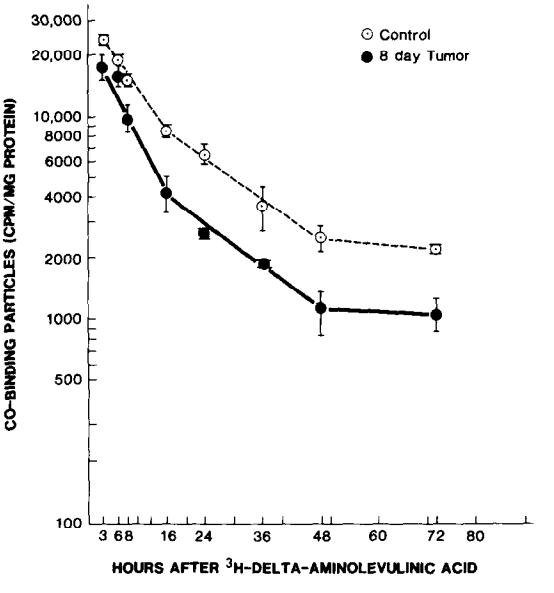


Fig. 2. Disappearance of $^3$H-labeled hepatic hemoprotein from CO-binding particles obtained from control and Murphy–Sturm lymphosarcoma-bearing rats (8 days following i.m. transplantation of tumor). Samples were prepared from hepatic microsomal fractions as described in Materials and Methods at various time intervals as indicated following i.v. injection of [3,5-$^3$H]ALA (0.37 mCi/kg body wt). Each point represents the mean ± S.E. from three experiments. Key: (○) control rats; and (●) rats 8 days following transplantation of Murphy–Sturm lymphosarcoma.

days following i.m. transplantatioin. The disappearance of radioactivity with time in both control and tumor-bearing rats appears to be at least biphasic, as demonstrated in previous studies in similar systems [20, 25]. In control rats, the corrected half-life of radioactivity in the initial fast phase was calculated to be 7 hr, while in the Murphy–Sturm lymphosarcoma-bearing rats the corrected half-life in the initial fast phase was determined as 5.5 hr. The slopes of the lines representing the initial fast phase of disappearance of radioactivity in control and tumor-bearing rats were significantly different from each other ($P < 0.02$). The corrected half-life of the fast phase was calculated as described previously [20] by subtracting the counts per minute per milligram of protein contributed by the slow phase after extrapolating the slow phase line back to zero time. The ratio of cytochrome P-450 content in the fast phase as compared with the slow phase in tumor-bearing rats was calculated as 18.2:1 [20]. The lower total cpm/mg protein found in the CO-binding particles from Murphy–Sturm lymphosarcoma-bearing rats reflects the significantly decreased content of hepatic cytochrome P-450 8 days following tumor transplantation [1, 7]. These data suggest that the turnover of microsomal cytochrome P-450 heme was significantly faster in the Murphy–Sturm lymphosarcoma-bearing rats, and this could have contributed to the decreased levels of microsomal cytochrome P-450 observed.

*Effect of Murphy–Sturm lymphosarcoma on hepatic apo-cytochrome P-450 content.* In view of the fact that the rate of hepatic cytochrome P-450 degradation is decreased in Murphy–Sturm lymphosarcoma-bearing rats [1], and since it appears that cytochrome P-450 heme turnover may be slightly accelerated in this situation, it was thought possible that increased levels of hepatic cytochrome P-450 apo-protein might accumulate in tumor-bearing animals. To test this hypothesis, the method of Correia and Meyer [21] was used to determine hepatic microsomal apo-cytochrome P-450 content in both control and tumor-bearing rats at various times after transplantation of Murphy–Sturm lymphosarcoma. Table 1 illustrates cytochrome P-450 content of hepatic microsomal fractions from control and tumor-bearing rats before and after supplementation of whole liver homogenates with hemin. Cytochrome P-450 concentrations were similar in the control groups but were much lower just 2 days following tumor transplantation, and continued to decline 8 and 10 days following transplantation to levels approximately 50% of control values, consistent with previous data [1, 7]. Addition of hemin resulted in an increase in hepatic cytochrome P-450 concentration in both control and Murphy–Sturm lymphosarcoma-bearing rats, presumably representing apo-cytochrome P-450 reconstituted by the addition of hemin. The percent increase in cytochrome P-450 following incubation with hemin, representing apo-cytochrome P-450, ranged from 6 to 11% in controls and from 5 to 17% in tumor-bearing rats. While in most of the groups, the percent increment representing hepatic apo-cytochrome P-450 content in tumor-bearing rats was larger than in controls, none of these differences was statistically significant. Moreover, the absolute increase in hepatic cytochrome P-450 concentration following hemin treatment was in most instances only slightly larger in tumor-bearing rats and, in fact, was smaller in the 2-day and 10-day treatment groups (Table 1). These data suggest that the perturbations in heme and hemoprotein metabolism brought about by transplantation of Murphy–Sturm lymphosarcoma at an extra-hepatic site did not result in a significant accumulation of hepatic apo-cytochrome P-450 protein.

## DISCUSSION

A systematic examination of the processes that might alter hepatic microsomal cytochrome P-450 content in tumor-bearing animals was undertaken in view of the recent observation by ourselves and others that the tumor-bearing state is associated with decreased hepatic cytochrome P-450 apo-protein synthesis [1, 7]. While the relative rate of degradation of hepatic cytochrome P-450 was found to be decreased following tumor transplantation [1], the fall in hepatic cytochrome P-450 concentration following tumor transplantation [1, 3, 5–7] at times when the rate of cytochrome P-450 synthesis was diminished significantly [1, 7] suggested that the decreased rate of synthesis of cytochrome P-450 was a more important factor in regulating cytochrome P-450 levels. Another mechanism that could contribute to the decrease in cytochrome P-450 concentration would be increased turnover of cytochrome P-450 heme. Increased turnover of hepatic cytochrome P-450 heme associated with a decrease in cytochrome P-450 concentration has been observed following treatment of rats with allylisopropylacetamide [25, 26] or with carbon tetrachloride [26] by monitoring the rate of disappearance of [$^3$H]ALA incorporated into heme in submicrosomal CO-binding particles constituting cytochrome P-450. Using this methodology, a small but significant increase in the rate of turnover of hepatic cytochrome P-450 heme in Murphy–Sturm lymphosarcoma-bearing rats was seen, with a shortening of the half-life of fast phase radioactivity in CO-binding particles from 7 hr in control rats to 5.5 hr in tumor-bearing animals. The half-life of fast phase radioactivity in control rats was identical with previous results using this methodology [20, 27]. This 22% increase in the rate of turnover of cytochrome P-450 heme probably contributed to the decrease in cytochrome P-450 content in tumor-bearing rats, since a substantial proportion of cytochrome P-450 turnover (about 95%) was associated with the fast phase. However, the decrease in the relative rate of synthesis of cytochrome P-450 [1, 7], in which incorporation of radioactivity into cytochrome P-450 protein may be reduced by approximately 40% in tumor-bearing rats 7 days after transplantation [7], may have been quantitatively more important in causing this reduction. Unfortunately, our present data do not allow a determination as to whether only some or all of the multiple forms of cytochrome P-450 are affected in this way.

The combination of a diminished rate of cytochrome P-450 apo-protein degradation and increased cytochrome P-450 heme turnover could re-

Table 1. Hepatic microsomal cytochrome P-450 and apo-cytochrome P-450 content in control and tumor-bearing rats*

| Days following tumor transplant | Cytochrome P-450 content† (nmol/mg microsomal protein) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control rats‡ | | | | Tumor-bearing rats‡ | | | |
| | −Hemin | +Hemin | Increment§ | % Increment | −Hemin | +Hemin | Increment§ | % Increment |
| 2 | 1.53 ± 0.17 | 1.70 ± 0.19 | 0.17 | 11.1 | 0.96 ± 0.17 | 1.01 ± 0.19 | 0.05 | 5.2 |
| 4 | 1.45 ± 0.11 | 1.59 ± 0.12 | 0.14 | 9.6 | 0.91 ± 0.10 | 1.07 ± 0.10 | 0.16 | 17.5 |
| 6 | 1.51 ± 0.13 | 1.62 ± 0.17 | 0.11 | 7.3 | 0.99 ± 0.15 | 1.16 ± 0.12 | 0.17 | 17.2 |
| 8 | 1.48 ± 0.15 | 1.57 ± 0.14 | 0.09 | 6.1 | 0.86 ± 0.06 | 0.97 ± 0.08 | 0.11 | 12.8 |
| 10 | 1.38 ± 0.04 | 1.47 ± 0.04 | 0.09 | 6.5 | 0.66 ± 0.03 | 0.73 ± 0.03 | 0.07 | 10.6 |

* Transplantation (i.m.) of Murphy–Sturm lymphosarcoma was done as described in Materials and Methods.
† Results are expressed as mean ± S.E. of six experiments, in each of which three livers were pooled.
‡ Liver homogenates were incubated in the presence or absence of 40 μM hemin as described in Materials and Methods. Subsequently, microsomes were prepared, and cytochrome P-450 was assayed as described in Materials and Methods.
§ The increment in cytochrome P-450 content following incubation of liver homogenate with hemin represents apo-cytochrome P-450 content.

sult in the accumulation of apo-cytochrome P-450 in tumor-bearing rats. When apo-cytochrome P-450 content was examined and compared in control and Murphy–Sturm lymphosarcoma-bearing rats, a small increase was found in tumor-bearing rats as compared with controls. In most cases, however, apo-cytochrome P-450 did not accumulate in large amounts, and neither the absolute nor the percent increases in apo-cytochrome P-450 content in tumor-bearing rats were statistically significant. This suggests that the mechanisms described which tend to increase apo-cytochrome P-450 content must be nearly balanced by the decreased relative rate of synthesis of cytochrome P-450 apo-protein in the tumor-bearing state.

The data do not allow a precise determination as to which of the mechanisms regulating hepatic hemoprotein metabolism was the primary and rate-limiting event in the regulation of cytochrome P-450 synthesis and degradation under these circumstances. However, as apo-cytochrome P-450 levels were relatively unaffected and because the rate of cytochrome P-450 protein synthesis may be significantly affected earlier following tumor transplantation than the rate of cytochrome P-450 protein degradation [1], it is possible that the primary effect of tumor transplantation may be to decrease the rate of hepatic cytochrome P-450 protein synthesis. Consistent with this is the finding that, under conditions of maximal induction of cytochrome P-450 by phenobarbital or 3-methylcholanthrene, apo-cytochrome P-450 accumulates, suggesting that heme synthesis lags behind apo-cytochrome P-450 synthesis [21], and that apo-protein synthesis is the primary and rate-limiting event in the regulation of cytochrome P-450 formation [21]. Decreased activities of microsomal NADPH–cytochrome *c* reductase, the peroxisomal hemoprotein catalase, and other hepatic enzymes including glucokinase, ornithine aminotransferase, malate-NADP dehydrogenase, and glutamine synthetase have been reported in tumor-bearing animals [1, 28–33], suggesting that alterations in hepatic protein metabolism are not confined to cytochrome P-450 under these conditions.

Hepatic ALA-synthetase activity was decreased slightly following transplantation of Murphy–Sturm lymphosarcoma, confirming previous studies [7, 17]. The decrease in hepatic ALA synthetase activity and concomitant increase in hepatic heme oxygenase activity previously shown [1, 7] may be explainable by the perturbations in cytochrome P-450 metabolism observed in tumor-bearing rats. The combination of decreased synthesis of cytochrome P-450 apoprotein and increased turnover of cytochrome P-450 heme in tumor-bearing rats might increase intracellular hepatic heme concentration. As the size of the hepatic heme pool appears to be regulated by changes in the rates of synthesis and catabolism of heme [34–36], hepatic heme present in excess of requirements for cytochrome P-450 synthesis could then cause repression of the synthesis of ALA synthetase, thus decreasing net heme synthesis [37, 38], and simultaneously increase heme oxygenase by substrate-mediated induction [39–41].

While the exact mechanisms responsible for these alterations in heme and hemoprotein metabolism in

tumor-bearing rats have not yet been established, it is clear that these changes may have important implications for hepatic drug metabolism in the tumor-bearing state. It is now clearly established that hepatic microsomal oxidative drug metabolism is impaired in tumor-bearing animals [1–4] due to decreased levels of hepatic microsomal electron transport components including cytochrome P-450, cytochrome $b_5$, and NADPH–cytochrome *c* reductase [5–7, 13], resulting in prolonged duration of drugs normally metabolized by this system [7–12]. As this hepatic biotransformation mechanism is required for the metabolism of many drugs used in clinical cancer chemotherapy, including cytophosphamide [42], procarbazine [43], 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) [44], and mitomycin C [45], the pharmacokinetics and potential toxicity of these agents may be modified by the noted alterations in hepatic oxidative drug metabolism in tumor-bearing animals. While studies of cyclophosphamide pharmacokinetics in an animal tumor model system demonstrated no clear effect on cyclophosphamide action [46, 47], and other drug metabolism studies in human subjects have also been inconclusive [48, 49], the actual importance of modification of the rates of activation or detoxification of chemotherapeutic agents and other drugs due to decreased hepatic microsomal oxidative activity in tumor-bearing animals or in man has not yet been fully clarified. Further study is required to determine the pathogenesis of these alterations in hepatic heme and hemoprotein metabolism and their ultimate significance for hepatic oxidative drug metabolism, pharmacokinetics, and drug toxicity in tumor-bearing animals and man.

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