HEME REGULATION IN MOUSE MAMMARY CARCINOMA AND LIVER OF TUMOR BEARING MICE—I. EFFECT OF ALLYL-ISOPROPYLACETAMIDE AND VERONAL ON δ-AMINOLEVULINATE SYNTHETASE, CYTOCHROME P-450 AND CYTOCHROME OXIDASE*

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Abstract—1. Basal levels and allyl-isopropylacetamide (AIA) or veronal induced levels of δ-aminolevulinate synthetase (ALA-S), cytochrome P-450 (cyt P-450) and cytochrome oxidase were determined in tumor (T) and liver of both normal mice (NM) and T bearing mice (TBM).
2. Basal levels of ALA-S were nearly the same in either source. The amount of cyt P-450 was lower in TBM liver than in NM liver, and no detectable in T. While the basal activity of cytochrome oxidase in TBM liver and T were higher than those of NM liver.
3. In AIA intoxicated animals there was a lower induction of ALA-S in liver of TBM than in NM liver. There was no induction in T ALA-S. The loss of cyt P-450 was less in TBM liver when compared with NM liver.
4. The induction level of cyt P-450 after veronal administration was nearly the same in liver of both TBM and NM.
5. We conclude that lower induction of liver ALA-S activity in TBM liver is due to correspondingly lower drug metabolism ability of TBM liver. Otherwise our results suggest that the control mechanism operating in T and probably in its original tissue are different from those described for normal liver.

INTRODUCTION
δ-Aminolevulinate synthetase (ALA-S) (EC 2.3.1.37) is the rate limiting and regulatory enzyme in heme pathway. Administration of porphyrinogenic drugs to animals greatly increased liver ALA-S activity (Granick, 1966).
A number of unsaturated compounds including allyl-isopropylacetamide (AIA) destroy cytochrome P-450 (cyt P-450), leading to a concomitant acceleration of heme biosynthesis, to restore the levels of heme protein (De Matteis, 1970).
On the other hand compounds such as veronal produce a significant decrease in the pool of regulatory free heme by increasing apocytochrome P-450 synthesis.

MATERIALS AND METHODS
Animals
Male mice strain BALB/c (20-25 g) were used. Animals were fed Purina 3 diet and given water ad libitum. Spontaneous mouse mammary carcinoma from strain BALB/c were used, mice received a 1 mm3 inocula of T injected under skin overlaying the flanks up to the axilla. The animals were killed one month after the implementation. The mice previously heparinized were killed under ether anesthesia by cardiac puncture and bled. In all cases animals were fasted 24 hr before death.
**AIA intoxication**

Animals were intoxicated with two s.c. injection of AIA (350 mg/kg body wt) dissolved in 50% ethanol saline, given with an interval of 12 hr. Control mice were injected with the vehicle (50% ethanol saline). Animals were killed 3 hr after the last injection.

**Veronal intoxication**

Animals were intoxicated with 3 s.c. injection of veronal (167 mg/kg body wt) dissolved in saline solution given every 24 hr. Control mice were injected with saline. Animals were killed 24 hr after the last injection.

**Tissue preparation and enzyme assays**

**ALA-S:** Tissues were homogenized (1:3, w/v) in a solution containing: 0.9% NaCl, 0.1 mM Tris-HCl pH 7.4; 0.5 mM EDTA. An aliquot of homogenate was used as enzyme source without previous centrifugation and activity measured as described by Marver et al. (1966). An extinction coefficient of 58 mM$^{-1}$ cm$^{-1}$ was used to calculate δ-aminolevulinic acid concentration.

**Cyt P-450:** The microsomal fraction of the homogenates were used and cyt P 450 determined by the dithionite-CO absorbance difference, as described by Omura and Sato (1964).

**Cytochrome oxidase:** The mitochondrial fraction of the homogenates was used and measured according to Yonetani and Ray (1965).

Enzyme units (U) were defined as the amount of enzyme that catalyses the formation of 1 nmol of product under the standard incubation conditions. Specific activity (SA) was expressed as U/mg protein. Protein concentration was determined by the method of Lowry et al. (1951).

**RESULTS**

**ALA-S**

As shown in Fig. 1 the basal level of ALA-S was nearly the same in either source. In AIA intoxicated animals, liver ALA-S was two-fold increased ($P < 0.05$) in TBM and three-fold increased in NM ($P < 0.001$) when compared to controls. The activity of tumoral ALA-S was not induced by AIA. Veronal produced no changes in ALA-S.

**Cyt P-450**

Basal levels of liver cyt P-450 from TRM were decreased when compared to controls ($P < 0.05$) (Fig. 2). When animals were intoxicated with AIA, liver cyt P-450 from NM was 32% ($P < 0.01$) decreased and from TBM 43% ($P < 0.05$) decreased. Veronal instead provoked a significant enhancement of cyt P-450 content in liver from both NM and TBM. Tumoral cyt P-450 levels were not detectable in any case.

**Cytochrome oxidase**

Levels of cytochrome oxidase in TBM liver and T were significantly higher ($P < 0.01$) than those of NM liver (Fig. 3). Neither AIA nor veronal produced any variation, in any tissue.

**DISCUSSION**

Bonkowsky et al. (1973) had already reported 16 yr ago that AIA induction of liver ALA-S was lower in T bearing rats than in normal rats, our
results are in good agreement with the Americans' findings. But we could now provide some more evidence as to explain the reasons why the levels of ALA-S activity in liver of TBM treated with AIA are lower than in NM. If we take into account that the basal levels of cyt P-450 were also lower in TBM liver than in NM liver, that the amount of cyt P-450 was nearly equal in liver of both TBM and NM after AIA administration, in other words that the loss of cyt P-450 was less in TBM liver and that AIA exerts its porphyrinogenic action only after having been metabolized by cyt P-450, it is clear that lower induction of liver ALA-S activity in TBM liver is due to the corresponding lower drug metabolizing ability of TBM liver.

We have also found that veronal did not induce ALA-S, but slightly reduced in either liver from TBM or NM and it did increase cyt P-450 content up to nearly the same levels in both TBM and NM liver. Beck et al. (1982) have proposed that because of nutritional disorders, and polyamines or hormonal effects occurring in the T, there might be an impairment in the coordinated synthesis of heme and some of those apoproteins having a fast turnover, as a consequence, excess of free heme would be available to both induced heme oxygenase and decreased ALA-S activities, thus explaining the results of these authors but only part of ours. We could justify the observed enhancement of cyt P-450, assuming that veronal acts also inducing the synthesis of the cytoprotein. It is worth taking notice of that when doses >350 mg/kg body wt of veronal were used all TBM died within the second and third day after the first injection, while no NM did, suggesting that the lower initial cyt P-450 in TBM liver would diminish the threshold of the veronal lethal dose.

On the other hand basal levels of cytochrome oxidase were higher in TBM liver than in NM liver, this is expected if we consider that there might be an increase of the regulatory free heme pool, according to Beck et al. (1982) hypothesis and that free heme mitochondrial fraction which is in equilibrium with that of the regulatory pool, would control cytochrome oxidase levels (Kappas et al., 1983), therefore, enhancement of mitochondrial heme would result in enhancement of cytochrome oxidase.

Regarding now results obtained in T, while ALA-S activity basal levels were the same as those found in liver from either TBM or NM, neither AIA nor veronal produced any changes; more or less the same picture arises for cytochrome oxidase if we compare basal levels in TBM liver and T and we could not detect cyt P-450, even after veronal administration.

These findings are clearly indicating that heme regulation in T should be different from that operating in liver. It has been shown however that T has the same capacity for heme synthesis as it has liver at least up to protoporphyrin formation but it does not seem to produce cyt P-450; which is then the fate of the heme produced. It is very likely, therefore, that T makes use of its heme to synthesize heme proteins other than cyt P-450, this possibility is supported by the fact that cytochrome oxidase is greatly increased in T compared with NM liver which emphasizes the hypothesis that the control mechanisms operating in T and probably in its original normal tissue are different from those described for normal liver.

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