Ethanol Metabolism by a Transplantable Hepatocellular Carcinoma

ROLE OF MICROSOMES AND MITOCHONDRIA*

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1. Ethanol metabolism in slices or homogenates of transplantable hepatocellular carcinoma HC-252 (HC-252) was 50 to 60% of the rate found in host liver slices or homogenates when they were expressed per gram of tissue wet weight and 70 to 80% of the liver when the rates were expressed per milligram of tissue protein. At 10 mM ethanol, the activities of alcohol dehydrogenase in tumor and liver supernatants were comparable.

2. Tumor microsomes did not oxidize ethanol in the presence of a NADPH-generating system, indicating the absence of the microsomal ethanol-oxidizing system and catalase-mediated peroxidation of ethanol. The HC-252 microsomes were contaminated with catalase, and acetaldehyde production occurred in the presence of a H2O2-generating system (xanthine oxidase). The virtual absence of ethanol oxidation and drug metabolism (aminopyrine demethylase and aniline hydroxylase) in HC-252 microsomes may be due to the low activities of NADPH-cytochrome c reductase, NADPH oxidase, and NADPH-dependent oxygen uptake.

3. Microsomal oxidation of ethanol was present in Morris hepatoma 5123C, a well-differentiated tumor of intermediate growth rate, while activity was negligible in microsomes from Morris hepatoma 7288CTC, a less differentiated tumor. Microsomal NADPH oxidase was present in the well differentiated tumor 5123C but was lacking in the less differentiated tumor 7288CTC. Several microsomal, mitochondrial, and cytosolic properties of HC-252 are similar to those of Morris hepatoma 7288CTC but differ from those of the more differentiated 5123C tumor and normal liver.

4. The content of mitochondrial protein in HC-252 was only 25% that of liver, and oxygen consumption per gram of tumor was only 28% that of the liver. When corrected for the mitochondrial protein content, oxygen uptake in tumor HC-252 and liver homogenates was comparable. Isolated tumor and liver mitochondria displayed comparable State 4 and 3 rates of oxygen consumption with succinate and glutamate as substrates. The activities of the reconstituted malate-aspartate and α-glycerophosphate shuttles were only slightly lower in isolated HC-252 mitochondria compared to liver mitochondria, when shuttles were reconstituted with purified enzymes.

5. Antimycin inhibited alcohol metabolism, and pyruvate stimulated alcohol metabolism, much less in tumor slices than in liver slices, suggesting the presence of an augmented mitochondria-independent, cytosolic mechanism for oxidizing reducing equivalents in the tumor. These factors suggest that oxidation of NADH is the limiting factor in ethanol metabolism. Whereas, in the liver mitochondrial reoxidation is predominant, in HC-252, cytosolic reoxidation of NADH also plays a major role.

The major pathway for the oxidation of ethanol in the liver involves the cytosolic enzyme, alcohol dehydrogenase. Most of the reducing equivalents produced in the cytosol are oxidized by the mitochondria. In view of the virtual impermeability of the mitochondria to NADH (1), the reducing equivalents are transported into the mitochondria by various substrate shut-}

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(MEOS) has been shown to share certain characteristics with the microsomal mixed function oxidase system (12, 13); the activities of MEOS and NADPH oxidase are both augmented by chronic ethanol consumption (14, 15). Another pathway using isolated microsomes in vitro involves catalase. The microsomal fraction is contaminated by catalase (16). Hydrogen peroxide, which participates in catalase-mediated peroxidation of ethanol, may be generated from NADPH oxidase activity (15, 17).

Of the major metabolic characteristics of rapidly growing neoplastic tissue is the accumulation of lactic acid (18, 19), possibly due to ineffective mechanisms for reoxidation of NADH produced during glycolysis (20), either owing to defects in the ability to transport reducing equivalents into the mitochondria via substrate shuttles or the inability of mitochondria to reoxidize the reducing equivalents at a sufficient rate. In addition, cytochrome P-450, postulated to be the terminal oxidase of MEOS (12), is virtually absent from the microsomes of some transplantable hepatocellular carcinomas (21, 22). The rate of ethanol metabolism may theoretically be affected by several factors, including the activity of alcohol dehydrogenase, the activity of the substrate shuttles, the ability of the mitochondria to oxidize reducing equivalents, and the activity of the microsomal ethanol-oxidizing system.

Since several of these activities may be altered in tumors, a study was made of ethanol metabolism in rat hepatocellular carcinoma 252 in order to shed light on the contribution of the microsomal mixed function oxidase system (12, 21). The rate of ethanol metabolism may theoretically be affected by several factors, including the activity of alcohol dehydrogenase, the activity of the substrate shuttles, the ability of the mitochondria to oxidize reducing equivalents, and the activity of the microsomal ethanol-oxidizing system.

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MATERIALS AND METHODS

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The disappearance of ethanol was assayed as described under "Materials and Methods." The data represent mean ±S.E.M. Experimental values are from six to eight preparations.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Specific activity</th>
<th>% of liver</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>HC-252</td>
<td></td>
</tr>
<tr>
<td>Ethanol metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slices</td>
<td>19.14 ± 1.32</td>
<td>11.05 ± 1.16</td>
<td>58</td>
</tr>
<tr>
<td>Homogenates</td>
<td>17.75 ± 1.82</td>
<td>8.36 ± 0.99</td>
<td>47</td>
</tr>
<tr>
<td>Protein content</td>
<td>100.8 ± 11.8</td>
<td>133.3 ± 12.9</td>
<td>70</td>
</tr>
<tr>
<td>Ethanol metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slices</td>
<td>0.101 ± 0.007</td>
<td>0.081 ± 0.008</td>
<td>80</td>
</tr>
<tr>
<td>Homogenates</td>
<td>0.093 ± 0.009</td>
<td>0.063 ± 0.007</td>
<td>68</td>
</tr>
</tbody>
</table>

* μmol/h/g wet weight.
+ μmol/h/mg of protein.

**Fig. 1.** Oxidation of ethanol by microsomes of liver and tumor HC-252. The production of acetaldehyde was assayed as described under "Materials and Methods." When present, the concentrations of ADP and FeCl₃ were 3.3 and 0.1 m, respectively. 3.1 units of bovine liver catalase were added to some experiments. Catalase activity was assayed with an oxygen electrode. Specific activity for all reactions except catalase refers to nanomoles of acetaldehyde produced per minute per milligram of protein. Specific activity for the catalase experiments is micromoles of perborate oxidized per minute per milligram of protein. Results are from two to six different preparations.

ADP plus ferric ions was reported to increase the activities of NADPH oxidase and MEOS (38). MEOS activity in the tumor was negligible even in the presence of ADP plus ferric chloride (Fig. 1). The inactivity of tumor microsomes to oxidize ethanol to acetaldehyde may reflect the absence of a component of MEOS or the catalase-mediated system. The lack of microsomal oxidation of ethanol by the tumor was not due to the absence of catalase, since the addition of bovine liver catalase to tumor microsomes did not lead to ethanol oxidation (Fig. 1). When the NADPH-generating system was replaced by an H₂O₂-generating system (xanthine plus xanthine oxidase), microsomal oxidation of ethanol by liver microsomes was increased 4 to 5 fold, whereas oxidation of ethanol by tumor microsomes was increased more than 100-fold (Fig. 1). The direct assay for contaminating catalase activity in tumor microsomes demonstrated that the activity of this enzyme is sufficiently active so as not to be a limiting factor in the peroxidation of ethanol (Fig 1).

Drug Metabolism—It has been suggested that MEOS shares certain characteristics with the microsomal mixed function oxidase system, and the addition of ethanol to microsomes gives rise to a modified type II binding spectrum (39). The activities of both aminopyrine demethylase (type I binding) and aniline hydroxylase (type II binding) were virtually absent in tumor microsomes (Table II). Substantial activity of NADH-linked partial electron transport reactions, e.g., NADH-ferricyanide reductase, was present in the tumor, terminal electron transport reactions are

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Activity</th>
<th>% Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Hepatoma</td>
</tr>
<tr>
<td>Aminopyrine demethylase</td>
<td>10.57 ± 2.17</td>
<td>0.45 ± 0.09</td>
</tr>
<tr>
<td>Aniline hydroxylase</td>
<td>0.45 ± 0.08</td>
<td>N.D.</td>
</tr>
<tr>
<td>NADPH-ferricyanide reductase</td>
<td>1.104 ± 0.51</td>
<td>5.119 ± 1.078</td>
</tr>
<tr>
<td>NADH-ferricyanide reductase</td>
<td>3,736 ± 900</td>
<td>10,314 ± 2,096</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase</td>
<td>43.4 ± 5.9</td>
<td>4.05 ± 0.5</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase</td>
<td>1,545 ± 120</td>
<td>1,806 ± 150</td>
</tr>
<tr>
<td>NADPH-dependent O₂ uptake</td>
<td>14.17</td>
<td>1.71</td>
</tr>
<tr>
<td>NADH-dependent O₂ uptake</td>
<td>8.90</td>
<td>1.44</td>
</tr>
<tr>
<td>NADPH oxidase</td>
<td>12.2 ± 2.95</td>
<td>1.4 ± 0.93</td>
</tr>
<tr>
<td>NADPH oxidase + ADP → Fe³⁺</td>
<td>17.08</td>
<td>1.68</td>
</tr>
</tbody>
</table>

* Not detectable.
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It appears likely that the catalase-mediated peroxidation of ethanol is limited by the rate of \( \text{H}_2\text{O}_2 \) generation, rather than by the activity of catalase itself (15, 40). It is possible that in tumor microsomes the catalase-mediated peroxidation of ethanol is inactive as a result of insignificant rates of \( \text{H}_2\text{O}_2 \) generation. The rate of total peroxide generation by tumor and liver microsomes was, therefore, studied. Peroxide generation associated with a NADPH-generating system by tumor microsomes was approximately 60% of the value found with liver microsomes (17.86 ± 1.95 nmol/min/mg of liver microsomal protein and 10.12 ± 1.66 nmol/min/mg for tumor microsomes, \( n = 10, p = 0.005 \)). Thus, although significant peroxide generation was detected in tumor microsomes, the peroxide is not \( \text{H}_2\text{O}_2 \), since NADPH-dependent catalase-mediated peroxidation of ethanol and NADPH oxidase activity are virtually absent. Measurements of total peroxide with the ferrithiocyanate method consequently do not reflect only \( \text{H}_2\text{O}_2 \) generation, and use of this method to determine whether the rates of \( \text{H}_2\text{O}_2 \) generation by liver microsomes are sufficient to account for the NADPH-dependent oxidation of ethanol by hepatic microsomes does not appear to be specific.

**MEOS Activity in Morris Hepatomas**—Rates of microsomal drug oxidation in hepatocellular carcinomas are less than those of liver microsomes (41–44). Therefore, the results obtained with HC-252, a tumor of intermediate to rapid growth rate, were compared with those obtained with Morris hepatoma 5123C, a well differentiated tumor (45–47), and Morris hepatoma 7288CTC, a tumor with a rapid growth rate (46). Microsomes from Morris hepatoma 7288CTC displayed insignificant rates of NADPH-dependent ethanol oxidation (Fig. 2).

By contrast microsomes prepared from the well differentiated Morris hepatoma 5123C displayed MEOS activity comparable to that of microsomes from host livers (Fig. 2). The addition of catalase caused a slight increase in MEOS activity, with both tumor and liver microsomes. The catalase-mediated peroxidation of ethanol was also present in tumor 5123C microsomes, as evidenced by acetaldehyde formation in the presence of xanthine-xanthine oxidase (Fig. 2). Assays of NADPH- and NADH-dependent oxygen uptake indicated that Morris 5123C microsomes displayed NADH and NADPH oxidase activities comparable to those of microsomes from host livers (Fig. 2). Microsomes from Morris 5123C were found by others to possess moderate activities of NADPH-cytochrome c reductase and NADPH oxidase (22).

**Integrity of Mitochondria**—With glutamate or succinate as substrates, the State 4 and State 3 rates of oxygen consumption were comparable with isolated liver and tumor HC-252 mitochondria (State 4-glutamate; liver 18.9 ± 3.1, tumor, 22.2...
The content of mitochondrial per gram of tissue was calculated from the rates of oxygen uptake by HC-252 and liver cell-free homogenates and mitochondria when 10 mM succinate (succinoxidase) or 5 mM ascorbate plus 0.2 mM N,N,N',N'-tetramethyl-p-phenylenediamine (cytochrome oxidase) served as substrates. Oxygen uptake was determined in the presence and absence of ADP. Results are means of two separate experiments. Oxygen uptake for homogenates is expressed as nanomoles per minute per gram of tissue while that for mitochondria is nanomoles per minute per milligram of protein.

**Table IV**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Tissue</th>
<th>Oxygen uptake</th>
<th>Content of mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Homogenate</td>
<td>Mitochondria</td>
<td>+ADP</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>HC-252</td>
<td>1064</td>
<td>1766</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>3512</td>
<td>6514</td>
</tr>
<tr>
<td>Succinate</td>
<td>HC-252</td>
<td>387</td>
<td>1119</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>1174</td>
<td>6567</td>
</tr>
</tbody>
</table>

**Integrity of mitochondria isolated from tumor HC-252 and liver**

Respiratory control (State 3/State 4) was assayed as described under "Materials and Methods." NADH oxidation was assayed polarographically in the presence of 1 mM NADH as substrate. Activity refers to nanomoles of oxygen consumed per minute per milligram of mitochondrial protein. Swelling was assayed by following the decrease in absorbance at a wavelength of 520 nm. Activity refers to change in absorbance/30 s/mg of mitochondrial protein. ATPase activity was assayed in the presence of either 2 mM Mg²⁺ or 50 μM dinitrophenol. Activity refers to nanomoles of P₇ released per minute per milligram of protein. The uptake of Ca²⁺ was assayed using 10 mM succinate plus 3 mM ADP, or 3 mM ATP (plus 1 μg of antimycin) as energy sources. Energy-dependent activity refers to counts per minute per milligram of protein and was obtained by subtracting the rate in the presence of 50 μM dinitrophenol from the total rate. Results are means ± S.E.M. and are from two to four experiments.

**Table III**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Plus bovine serum albumin</th>
<th>Minus bovine serum albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Tumor</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5.07 ± 0.51</td>
<td>3.91 ± 0.66</td>
</tr>
<tr>
<td>Succinate</td>
<td>4.38 ± 0.64</td>
<td>3.35 ± 0.55</td>
</tr>
<tr>
<td>NADH oxidation</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Swelling-ammonium phosphate</td>
<td>0.102</td>
<td>0.094</td>
</tr>
<tr>
<td>Swelling-potassium phosphate</td>
<td>0.012</td>
<td>0.016</td>
</tr>
<tr>
<td>Mg²⁺-ATPase activity</td>
<td>50 ± 10</td>
<td>60 ± 12</td>
</tr>
<tr>
<td>Dinitrophenol-ATPase activity</td>
<td>188 ± 14</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>Energy-dependent Ca²⁺ uptake</td>
<td>59,048</td>
<td>77,978</td>
</tr>
<tr>
<td>ATP</td>
<td>33,638</td>
<td>38,712</td>
</tr>
</tbody>
</table>
indicated that the content of mitochondrial protein in the tumor, per gram of tissue wet weight, was one-fourth that of the liver (Table IV). Even when expressed per milligram of tissue protein, the amount of mitochondrial protein per milligram of tumor protein (0.12 mg/mg of protein) was far less than the amount per milligram of liver protein (0.59 mg/mg of protein). The lower content of mitochondria in tumors has been well documented (45, 50, 56, 57). Oxygen uptake by cell-free homogenates was assayed to determine the respiratory state of the mitochondria as well as to confirm the data concerning the content of mitochondria. Oxygen uptake in homogenates of tumor HC-252 was only about one-fourth of the rate in liver homogenates when expressed per gram of tissue and 41% when expressed per milligram of tissue protein. However, when expressed per milligram of mitochondrial protein, the rates of oxygen uptake by tumor and liver homogenates were comparable (Table V). Thus, based on assays of oxygen uptake, the low content of mitochondria in tumor HC-252 was again apparent. In addition, the rates of oxygen consumption for both liver and tumor were somewhat higher than the State 4 rates but were considerably lower than the State 3 rates found for isolated mitochondria.

Reconstituted Substrate "Shuttles" for Transfer of Reducing Equivalents into Mitochondria—In the absence of any shuttle components, the endogenous rate of ethanol oxidation (a measure of NADH oxidation) was low for both liver and HC-252 mitochondria, confirming the maintenance of impermeability to NADH (Fig. 3). The addition of the components of the malate-aspartate shuttle stimulated ethanol oxidation with both mitochondrial preparations (Fig. 3). Similar results were obtained when the α-glycerophosphate shuttle was reconstituted (Fig. 3). Although slightly lower rates were obtained with the tumor mitochondria, isolated HC-252 mitochondria participated well in these two substrate shuttles.

Effect of Antimycin and Pyruvate on Ethanol Metabolism in Slices of Tumor and Liver—The fact that slices of tumor HC-252 showed rates of ethanol metabolism (per milligram of protein) that were 70 to 80% those of the liver, whereas the content of mitochondria (hence mitochondrial oxidation of reducing equivalents) was only 25% that of the liver, suggested that in the tumor the capacity of the mitochondria was not rate-limiting for ethanol metabolism. It is unlikely that pathways independent of alcohol dehydrogenase play a significant role in the tumor since microsomes from HC-252 had no MEOS or NADPH oxidase activity. Inasmuch as reoxidation of NADH is rate-limiting for ethanol metabolism via the alcohol dehydrogenase pathway, this suggested the possibility that significant rates of cytoplasmic NADH oxidation may occur in tumor HC-252 under these conditions. Therefore, the effects of antimycin, an inhibitor of mitochondrial oxygen consumption, and pyruvate, which promotes cytosolic NADH oxidation via the lactic dehydrogenase reaction, were tested. In the liver, antimycin caused approximately 80% inhibition of ethanol metabolism, while pyruvate stimulated ethanol metabolism by 125% (Fig. 4). In the tumor, antimycin inhibited ethanol metabolism by only 40%, while pyruvate stimulated ethanol metabolism by 70% (Fig. 4).

**DISCUSSION**

Factors which may influence the rate of ethanol metabolism include (a) the activity of alcohol dehydrogenase, (b) the activity of systems responsible for transporting reducing equivalents into mitochondria (shuttles), (c) the ability of mitochondria to oxidize these reducing equivalents, and (d) pathways of ethanol oxidation other than alcohol dehydrogenase. Although the rate of ethanol metabolism in the tumor is 20 to 30% lower per milligram of tissue protein than in liver, alcohol dehydrogenase activity in the tumor is 30% greater per milligram of soluble protein than in the liver, indicating that alcohol dehydrogenase activity is normally not rate limiting in ethanol metabolism.

Various estimates have been given for the proportion of ethanol metabolism accounted for by a nonalcohol dehydrogenase pathway (12, 13, 15, 58-63). When corrected for microsomal losses during the preparation, the activity of the microsomal pathway in vitro corresponds to 15 to 22% of the rate of ethanol metabolism in the rat (12, 13). At least 80% of

![Fig. 3. Transport of reducing equivalents into mitochondria of tumor HC-252 and liver by reconstituted substrate shuttles. The shuttles were reconstituted as described under "Materials and Methods" using purified enzymes as the source of either malate dehydrogenase and aspartate aminotransferase or α-glycerophosphate dehydrogenase. Results are from four separate experiments. There were no statistically significant differences in all experiments.](image-url)
The study investigated the ethanol metabolism in slices of tumor HC-252 and liver. Ethanol metabolism was analyzed as described in the legend to Table I. The concentration of antimycin was 3.6 μM, and that of pyruvate was 10 mM. Results from seven preparations showed that antimycin was dissolved in dimethylformamide; this amount of dimethylformamide (2 μL) had a negligible effect on ethanol metabolism.

The rate of H₂O₂ generation is probably not sufficient to support catalase activity in tumor microsomes, as suggested by the presence of catalase in the tumor microsomes and the high levels of catalase activity. This proves that catalase-mediated peroxidation of ethanol is limited by the rate of H₂O₂ generation rather than the activity of catalase itself.

There is controversy whether microsomes contain a unique MEOS system or whether MEOS activity can be accounted for by NADPH oxidase activity. The virtual absence of drug metabolism and NADPH oxidation in HC-252 microsomes suggests that MEOS activity would be quite low in these microsomes. This is consistent with the inability of tumor microsomes to metabolize drugs, perhaps owing to the low activities of NADPH-dependent partial electron transport reactions, oxygen consumption, and NADPH oxidase.

Since mitochondrial and microsomal properties of HC-252 tumors have not been described, it seems of interest to compare the results obtained with HC-252 with those obtained with the better characterized Morris hepatomas 5123C and 7288CTC, which resemble those in HC-252. By contrast, microsomes from Morris hepatoma 7288CTC resembled those in HC-252.

The high rate of aerobic lactic acid production in tumors may be due to ineffective mechanisms for reoxidation of NADH, either owing to defective shuttle mechanisms for the transport of reducing equivalents into the mitochondria or impaired capacity of the mitochondria to oxidize these reducing equivalents. In isolated HC-252 mitochondria, high activities of tumor mitochondrial α-glycerophosphate dehydrogenase (20, 73) have been suggested to be sufficiently active so as to not be rate-limiting for the α-glycerophosphate or phosphate shuttle. The activities of the reconstituted malate-aspartate and α-glycerophosphate shuttles are present when the shuttles are reconstituted with purified enzymes. This suggests that the activity of mitochondrial enzymes which participate in the shuttles is not impaired to any great extent in HC-252 mitochondria. The activities of tumor mitochondrial α-glycerophosphate dehydrogenase (68, 71, 72), aspartate aminotransferase, and malate dehydrogenase were found to be high.

The catalase-mediated pathway of peroxidation of ethanol is limited by the rate of H₂O₂ generation, rather than the activity of catalase itself (15, 17, 40). H₂O₂ generation from NADPH appears to be mediated principally via microsomal NADPH oxidase (15, 17), and NADPH oxidase activity in HC-252 microsomes is very low. Thus, the rate of H₂O₂ generation is probably not sufficient to allow significant rates of ethanol peroxidation via the catalase-mediated pathway. Consequently, it cannot be determined whether the absence of ethanol metabolism by HC-252 microsomes reflects a lack of the MEOS or catalase pathways since essential components of both pathways are missing or altered.
reducing equivalents into the mitochondria are much lower in HC-252 than host liver, transport and oxidation of reducing equivalents, which are believed to be the rate limiting steps of ethanol oxidation, should be considerably lower in the tumor than the liver. However, the rate of ethanol oxidation by HC-252 was 70 to 80% that of the liver. Thus, there is no correlation between transport and oxidation of reducing equivalents by mitochrondia and ethanol metabolism in the tumor. This becomes more apparent when the inability of HC-252 to display a microsomal ethanol-oxidizing system is taken into account. Thus, part of the explanation for the lower rate of ethanol metabolism in the tumor may be the lack of a microsomal ethanol-oxidizing system. Therefore, the activity of the alcohol dehydrogenase-dependent pathway may not be much lower in the tumor than the liver despite the lower content of mitochondria. It is possible that the capacity of mitochondrial oxidation in the tumor may still be sufficient to allow for the observed rates of ethanol metabolism. Assuming complete oxidation of ethanol to acetate by the tumor, 1 mol or 2 atoms of oxygen are required/mol of ethanol oxidized. Thus, an ethanol oxidation rate of 11 pmol/h g of tissue would require an oxygen consumption rate of 22 microatoms/h g of tissue.

From Table V, the oxygen consumption rate by the tumor was 11 pmol/h g of tissue, suggesting the possible presence of significant levels of both NAD+-dependent as well as flavin-linked substrate shuttles. Inhibited alcohol metabolism less in the tumor than in liver slices, emphasizing the lesser role that mitochondrial reoxidation plays in HC-252. By contrast, pyruvate stimulated alcohol metabolism to a smaller extent in tumor than in liver slices, suggesting the possible presence of significant levels of pyruvate or some other cytoplasmic oxidizing agent in the tumor preparations. Thus, the tumor may possess a NAD+-generating system that is independent of mitochondrial oxidation. This new model for studying ethanol metabolism has the advantage that all pathways independent of alcohol dehydrogenase are apparently absent. Since the activity of alcohol dehydrogenase is not rate limiting, these studies provide further evidence that mitochondrial and cytosolic reoxidation of NADH are the limiting factors in the rate of ethanol metabolism. Whereas, in the liver mitochondrial reoxidation is predominant, in the hepatocellular carcinomas cytosolic reoxidation of NADH also plays a major role.

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