Chemical, Enzymatic, and Cytochrome Assays of Microsomal Fraction of Hepatomas with Different Growth Rates

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Summary

The microsomal fractions from hepatomas of different growth rates have been analyzed for cytochrome components, enzymatic activities, and some chemical constituents. Regenerating liver and embryonal liver were assayed in the same way for comparison. The hepatomas examined included minimal deviation type Morris hepatomas 7793, 7794A, 7795, and 7316A, and fast-growing Yoshida hepatomas AH 130 and AH 371.

Microsomal cytochromes, cytochromes b5 and P-450, and the microsomal enzymes glucose-6-phosphatase, sulfatase C, NADH-cytochrome b5 oxidoreductase, NADH-cytochrome c oxidoreductase, NADH-cytochrome c oxidoreductase, and aromatic hydroxylating activity have been found in minimal deviation hepatomas at equivalent or somewhat lower levels than those of normal or regenerating liver. In Yoshida hepatomas AH 130 and AH 371, and in embryonal liver, these cytochromes and enzymes were extremely depressed or deleted. The decrease in activity in these components correlated roughly with increasing rates of growth. Magnesium-activated adenosine triphosphatase in the microsomal fraction was elevated in all hepatomas examined.

The RNA content in the microsomal fractions increased while phospholipid content decreased with increasing growth rate.

Introduction

The microsomal fraction of the liver consists mainly of 2 morphologic entities, namely, ribosomes and endoplasmic reticulum. Some ribosomes exist in the free state, and others in association with reticulum structures comprise the rough surfaced reticulum. Reticulum structures which are not associated with ribosomes are called the smooth surfaced reticulum (17). On the reticulum of hepatic cells, various microsomal enzymes, including hydrolytic, hydroxylating, and electron-transporting enzymes and specific hemoproteins such as cytochrome b5 (18) and carbon monoxide-binding pigment, P-450 (12-14) are found to be firmly bound. The existence of these enzymes and hemoproteins is understood to be 1 of the characteristics of the differentiated hepatic cells. It is also reported that the tissue specific antigen is contained in the microsomal fraction (21).

The idea has been generally accepted that components which were related to the differentiated function and the tissue specific antigens are deleted in hepatoma. However, the biochemical and enzymatic studies of minimal deviation type Morris hepatomas showed that these hepatomas resemble the normal liver in many morphologic and biochemical aspects (10). To learn more about minimally deviated hepatomas and to study the differentiation of microsomes of the liver, the microsomal fractions of 4 lines of minimally deviated hepatomas and 2 Yoshida hepatomas were subjected to enzyme and chemical analyses. The results are presented in this paper in comparison with that of the regenerating liver and the embryonal liver.

The investigation shows a rough correlation between the growth rates of the liver tumors and changes in certain microsomal enzyme activities and chemical composition.

Materials and Methods

TUMORS. The minimally deviated Morris hepatomas, Nos. 7794A, 7316A, 7793, and 7795, were used. Of these hepatomas 7793, 7794A, and 7795 were induced by ingesting a diet containing N-(2-fluorenyl)phthalamic acid in an experiment similar to that used for the induction of hepatoma 5123 (11). Numbers 7794A and 7795 were males, and 7793 was a female as was 5123. Hepatoma 7316A was induced in a female rat that ingested a diet containing 2,4,6-trimethyl-aniline continuously for 18 months. All of these Morris hepatomas were slowly growing tumors as measured by the time between transplantation. They were described histologically as well-differentiated trabecular carcinomas except 7316A, which was described as a hepatocellular adenocarcinoma (9). The tumors were transplanted by trocar i.m. in both hind legs of Buffalo-strain rats. The tumor-bearing and control animals were shipped air express from Dr. H. P. Morris of the National Cancer Institute, Bethesda, Maryland, to the National Cancer Center, Tokyo, Japan. They were maintained in separate cages at least for 1 month after arrival. Pellet diet, Oriental No. CA-1, and water were given ad libitum.

The Yoshida hepatomas AH 130 and AH 371 were transplanted i.p. or s.c. in the back of Donryu strain rats by injection of ascites fluid. The animals bearing hepatoma in the ascites form were kept for 10-14 days on pellet diet No. CE-2 and water ad libitum, and those bearing the subcutaneous tumors were kept for 14-21 days.

NORMAL ADULT, REGENERATING, AND EMBRYONAL LIVERS. The regenerating liver was obtained 24 hr after the removal of about two-thirds of the liver of male Donryu strain rats weighing about 200 gm. The embryonal liver was collected from embryos from pregnant rats of the same strain, 1-3 days prior to the ex-
Table 1

Comparison of Yields of Microsome from Normal Liver, Hepatoma, Regenerating Liver, and Embryonal Liver

<table>
<thead>
<tr>
<th></th>
<th>Microsomal protein from 1 gm tissue wet wt. (mg)</th>
<th>% of normal values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver (12)*</td>
<td>10.0 ± 1.6</td>
<td>100</td>
</tr>
<tr>
<td>Morris hepatoma 7794A (6)</td>
<td>9.6 ± 1.0</td>
<td>96</td>
</tr>
<tr>
<td>Morris hepatoma 7910A (6)</td>
<td>9.0 ± 0.7</td>
<td>90</td>
</tr>
<tr>
<td>Morris hepatoma 7993 (6)</td>
<td>9.5 ± 0.9</td>
<td>95</td>
</tr>
<tr>
<td>Morris hepatoma 7979 (7)</td>
<td>9.9 ± 1.0</td>
<td>99</td>
</tr>
<tr>
<td>AH 371 (6)</td>
<td>13.9 ± 5.3</td>
<td>139</td>
</tr>
<tr>
<td>AH 130 (6)</td>
<td>11.9 ± 2.7</td>
<td>119</td>
</tr>
<tr>
<td>Embryonal liver (3)</td>
<td>7.5 ± 0.8</td>
<td>75</td>
</tr>
<tr>
<td>Regenerating liver (4)</td>
<td>10.0 ± 0.9</td>
<td>100</td>
</tr>
</tbody>
</table>

* Values of normal liver are taken as 100%.

Numbers of determinations are given in parentheses in this column.

The normal liver and the regenerating liver were thoroughly perfused with cold 1.15% KC1. In the case of the embryonal liver, it was carefully rinsed with cold isotonic KC1 and blotted on filter paper. The perfusion procedure was carried out to avoid contamination of the microsomal fraction with blood pigment.

Preparation of Microsomal Fractions. When the i.m. transplanted Morris hepatomas or the s.c. transplanted Yoshida hepatomas grew to the appropriate size, the animals were killed by decapitation. The tumors were carefully dissected free of necrotic, hemorrhagic, and nontumorous material, rinsed with cold isotonic KC1 and minced with scissors. Twenty % homogenate was prepared by disrupting cells in cold isotonic KC1 with a Waring Blender for 3 min.

The ascites cells of the Yoshida hepatomas were obtained as follows: The ascites fluid was pipetted out through the incision of the abdominal wall of the rats killed by decapitation. The ascites were diluted about 5 times with cold physiologic saline, and centrifuged at 100 × g for 3 min. Most of the hepatoma cells were loosely packed, while most of the red cells remained as a suspension in the supernatant and were pipetted off. The repetition of this procedure twice more yielded hepatoma cells free of red cells and the hepatoma cells were finally well packed by centrifuging for 5 min at 1400 × g. The homogenate of packed ascites hepatoma cells was prepared by disrupting the cells by means of a Vir-Tis 45 homogenizer operating at top speed with 4 volumes of 0.24 M sucrose solution containing 5 × 10⁻⁴ M magnesium chloride.

The homogenate prepared as described above, was centrifuged to remove cell debris and nuclei at 600 × g for 8 min. The resulting supernatant fluid was centrifuged at 9000 × g for 10 min to remove mitochondria. The final supernatant fluid was centrifuged at 105,000 × g for 30 min to obtain a pellet of “microsomes.” The microsomal pellet was washed by dispersing in isotonic KC1 and recentrifuging at 105,000 × g. Finally, the washed particulate preparation was dispersed in 0.1 M Tris (tris(hydroxymethyl)aminomethane) buffer, pH 7.5, at the rate of 1 gm of original tissue equivalent per ml and assayed for several chemical constituents and enzyme activities.

The livers were removed from the normal control rats after perfusing in situ with cold isotonic KCl, as above described, and subjected to a similar centrifuge fraction.

Chemical Assays. The protein content of the microsomal fraction was determined by the method of Lowry et al. (7). The hot trichloroacetic acid extract was assayed for DNA and RNA by the method of Burton (2) and Mejbaum (8), respectively. Phospholipid was determined on the ethanol-ethyl ether extract by the method of Fiske and SubbaRow (6).

Enzyme Assays. Glucose-6-phosphatase was measured according to Swanson (20). Sulfatase C was assayed by using potassium p-nitrophenyl sulfate as substrate according to Roy (16). Adenosine triphosphatase was measured according to Ernst and Jones in the presence of magnesium ion (4). NADH₂-cytochrome c oxidoreductase and NADPH₂-cytochrome c oxidoreductase were determined by following the increase in absorbancy at 550 mμ with 1 × 10⁻⁴ M NADH₂ or NADPH₂ as electron donor and 5 × 10⁻³ M oxidized cytochrome c as electron acceptor, adding antimycin A in each case at the level of 1 μg/ml (19). NADH₂-cytochrome b₅ oxidoreductase was determined by following the increase in absorbancy at 424 mμ with 1 × 10⁻⁴ M NADH₂ and 4.4 × 10⁻³ M cytochrome b₅ in the presence of KCN at 10⁻³ M, according to Ernst et al. (5). Hydroxylating activity was measured by the formation of p-aminophenol due to hydroxylation of aniline in the presence of the NADH₂ generating system (1). All enzymes were measured under linear conditions.

Difference Spectra. Microsomal cytochrome b₅ was determined by the differential spectrum between NADH₂ reduced and oxidized forms (12). NADH₂ reduced form was obtained by adding 0.5 mg of NADH₂ into 3 ml of microsomal suspension. Another kind of hemoprotein in microsomes, carbon monoxide-binding pigment (P-450), was determined by differential spectrum CO-bound reduced form against reduced form (13, 14). Reduced form was obtained by adding a few mg of sodium dithionite. CO-bound reduced form was made by further bubbling CO gas for 20 sec. Spectra were read in Hitachi model EPS-2 recording spectrophotometer.

Reagents. NADH₂, NADPH₂, and cytochrome c were obtained from Sigma Chemical Company. Cytochrome b₅ was purified from rabbit liver according to Strittmatter and Velick (19).

Evaluation of Experimental Results. Data are expressed as the mean value of 3–12 determinations with S.E.’s. Since the values determined in normal rats are not significantly different between strains or sexes, they were pooled and the mean values presented in the table.

Results

The results of this investigation are presented in Tables 1–5. Comparison of the Yield of Microsomal Fractions from Normal Liver, Hepatoma, Regenerating Liver, and Embryonal Liver. The yields of the microsomal fractions from various tissues are not greatly different. To obtain the microsomal fraction as free as possible from contamination from other fractions, no attention was paid to obtaining the exact yield of
### TABLE 2
**Comparison of Chemical Composition of Microsomal Fraction from Normal Liver, Hepatoma, Regenerating Liver, and Embryonal Liver**

<table>
<thead>
<tr>
<th></th>
<th>RNA a</th>
<th>Lipid P b</th>
<th>DNA c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver</td>
<td>122 ± 31 (100) a</td>
<td>12.4 ± 4.0 (100) a</td>
<td>4.1 ± 1.6 (100) a</td>
</tr>
<tr>
<td>Morris hepatoma 7794A</td>
<td>191 ± 12 (157)</td>
<td>9.3 ± 2.5 (75)</td>
<td>6.7 ± 2.4 (163)</td>
</tr>
<tr>
<td>Morris hepatoma 7316A</td>
<td>173 ± 5 (142)</td>
<td>8.8 ± 0.4 (71)</td>
<td>8.7 ± 0.7 (212)</td>
</tr>
<tr>
<td>Morris hepatoma 7793</td>
<td>130 ± 16 (107)</td>
<td>11.2 ± 3.2 (90)</td>
<td>4.9 ± 1.3 (120)</td>
</tr>
<tr>
<td>Morris hepatoma 7795</td>
<td>186 ± 33 (152)</td>
<td>8.4 ± 4.6 (68)</td>
<td>3.7 ± 1.1 (90)</td>
</tr>
<tr>
<td>AH 371</td>
<td>203 ± 54 (166)</td>
<td>6.1 ± 1.4 (49)</td>
<td>3.9 ± 3.2 (55)</td>
</tr>
<tr>
<td>AH 130</td>
<td>251 ± 105 (206)</td>
<td>7.5 ± 1.1 (60)</td>
<td>3.9 ± 1.6 (55)</td>
</tr>
<tr>
<td>Embryonal liver</td>
<td>262 ± 32 (215)</td>
<td>5.4 ± 3.4 (44)</td>
<td>9.2 ± 4.3 (224)</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>186 ± 34 (152)</td>
<td>14.3 ± 1.5 (115)</td>
<td>3.1 ± 0.5 (76)</td>
</tr>
</tbody>
</table>

a These are μg/mg of microsomal protein.
b Numbers of determinations are given in parentheses in this column.
c Value of normal liver is 100%; comparative % is listed in parentheses, after mean ± S.E.

### TABLE 3
**Comparison of Microsomal Hydrolytic and Hydroxylating Enzyme Activities of Normal Liver, Hepatoma, Embryonal Liver, and Regenerating Liver**

<table>
<thead>
<tr>
<th></th>
<th>G-6-Pase a</th>
<th>ATPase b</th>
<th>Sulfatase c</th>
<th>Hydroxylation d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver</td>
<td>98.3 ± 20.6 (100) a</td>
<td>72 ± 2.5 (100) a</td>
<td>3.8 ± 1.4 (100) a</td>
<td>0.448 ± 0.164 (100) a</td>
</tr>
<tr>
<td>Morris hepatoma 7794A</td>
<td>28.3 ± 20.6 (20)</td>
<td>171 ± 38.2 (238)</td>
<td>6.1 ± 0.33 (01)</td>
<td>0.110 ± 0.050 (25)</td>
</tr>
<tr>
<td>Morris hepatoma 7316A</td>
<td>78.4 ± 17.0 (80)</td>
<td>90 ± 16.3 (125)</td>
<td>4.2 ± 1.0 (111)</td>
<td>0.060 ± 0.020 (13)</td>
</tr>
<tr>
<td>Morris hepatoma 7793</td>
<td>97.8 ± 13.0 (99)</td>
<td>190 ± 30.0 (264)</td>
<td>1.4 ± 0.64 (37)</td>
<td>0.212 ± 0.045 (47)</td>
</tr>
<tr>
<td>Morris hepatoma 7795</td>
<td>33.1 ± 6.8 (34)</td>
<td>130 ± 39.4 (181)</td>
<td>0.31 ± 0.11 (8)</td>
<td>0.084 ± 0.035 (19)</td>
</tr>
<tr>
<td>AH 371</td>
<td>4.2 ± 1.0 (4)</td>
<td>130 ± 30.8 (181)</td>
<td>0.50 ± 0.06 (13)</td>
<td>Not detected</td>
</tr>
<tr>
<td>AH 130</td>
<td>4.4 ± 1.0 (5)</td>
<td>115 ± 40.2 (160)</td>
<td>0.22 ± 0.07 (6)</td>
<td>Not detected</td>
</tr>
<tr>
<td>Embryonal liver</td>
<td>48.3 ± 22.6 (49)</td>
<td>42 ± 3.9 (58)</td>
<td>0.50 ± 0.06 (13)</td>
<td>0.022 ± 0.012 (5)</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>108.0 ± 18.0 (110)</td>
<td>49 ± 2.2 (68)</td>
<td>3.4 ± 0.15 (89)</td>
<td>0.200 ± 0.021 (58)</td>
</tr>
</tbody>
</table>

a μg of inorganic phosphate released/15 min/mg of protein.
b μg of inorganic phosphate released/20 min/mg of protein.
c μg of p-nitrophenol released/30 min/mg of protein.
d mmoles of formed p-aminophenol/min/mg of protein.
Numbers of determinations are given in parentheses in this column.
Value of normal liver is 100%; comparative % is listed in parentheses, after mean ± S.E.
TABLE 4
COMPARISON OF MICROSONAL OXIDOREDUCTASE ACTIVITIES OF NORMAL LIVER, HEPATOMA, EMBRYONAL LIVER, AND REGENERATING LIVER

<table>
<thead>
<tr>
<th></th>
<th>NADH-cytochrome b oxidoreductase</th>
<th>NADH-cytochrome c oxidoreductase</th>
<th>NADPH-cytochrome c oxidoreductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver (7)</td>
<td>125 ± 29 (100)</td>
<td>1120 ± 334 (100)</td>
<td>202 ± 135 (100)</td>
</tr>
<tr>
<td>Morris hepatoma 7794A (5)</td>
<td>18 ± 4 (14)</td>
<td>371 ± 164 (33)</td>
<td>102 ± 14 (50)</td>
</tr>
<tr>
<td>Morris hepatoma 7316A (5)</td>
<td>35 ± 6 (28)</td>
<td>767 ± 95 (68)</td>
<td>43 ± 9 (21)</td>
</tr>
<tr>
<td>Morris hepatoma 7793 (5)</td>
<td>76 ± 8 (61)</td>
<td>951 ± 75 (85)</td>
<td>115 ± 28 (57)</td>
</tr>
<tr>
<td>Morris hepatoma 7795 (5)</td>
<td>54 ± 15 (43)</td>
<td>393 ± 93 (35)</td>
<td>71 ± 24 (35)</td>
</tr>
<tr>
<td>AH 371 (4)</td>
<td>16 ± 5 (13)</td>
<td>32 ± 10 (3)</td>
<td></td>
</tr>
<tr>
<td>AH 130 (4)</td>
<td>9 ± 2 (7)</td>
<td>53 ± 24 (5)</td>
<td>19 ± 8 (9)</td>
</tr>
<tr>
<td>Embryonal liver (3)</td>
<td>36 ± 5 (29)</td>
<td>316 ± 84 (28)</td>
<td>27 ± 8 (13)</td>
</tr>
<tr>
<td>Regenerating liver (4)</td>
<td>138 ± 4 (110)</td>
<td>1415 ± 95 (126)</td>
<td>148 ± 4 (73)</td>
</tr>
</tbody>
</table>

* Numbers of determinations are given in parentheses in this column.

* Value of normal liver is 100%; comparative % is listed in parentheses, after mean ± S.E.

Table 5
COMPARISON OF MICROSONAL HEMOPROTEINS OF NORMAL LIVER, HEPATOMA, EMBRYONAL LIVER, AND REGENERATING LIVER

<table>
<thead>
<tr>
<th></th>
<th>Cytochrome b₄ (μmoles/mg protein)</th>
<th>P-450 (μmoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver (7)</td>
<td>0.377 ± 0.038 (100)</td>
<td>0.484 ± 0.179 (100)</td>
</tr>
<tr>
<td>Morris hepatoma 7794A (5)</td>
<td>0.211 ± 0.022 (56)</td>
<td>0.127 ± 0.014 (26)</td>
</tr>
<tr>
<td>Morris hepatoma 7316A (5)</td>
<td>0.387 ± 0.001 (103)</td>
<td>0.225 ± 0.065 (46)</td>
</tr>
<tr>
<td>Morris hepatoma 7793 (5)</td>
<td>0.299 ± 0.025 (79)</td>
<td>0.163 ± 0.064 (34)</td>
</tr>
<tr>
<td>Morris hepatoma 7795 (5)</td>
<td>0.192 ± 0.058 (51)</td>
<td>0.084 ± 0.059 (17)</td>
</tr>
<tr>
<td>AH 371 (4)</td>
<td>0.020 (5)</td>
<td>0.040 (8)</td>
</tr>
<tr>
<td>AH 130 (4)</td>
<td>0.020 (5)</td>
<td>0.040 (8)</td>
</tr>
<tr>
<td>Embryonal liver (3)</td>
<td>0.125 ± 0.027 (33)</td>
<td>0.044 ± 0.024 (9)</td>
</tr>
<tr>
<td>Regenerating liver (4)</td>
<td>0.511 ± 0.017 (135)</td>
<td>0.482 ± 0.011 (100)</td>
</tr>
</tbody>
</table>

* Numbers of determinations are given in parentheses in this column.

* Value of normal liver is 100%; comparative % is listed in parentheses, after mean ± S.E.

The same activity as found in the normal liver. Morris hepatoma 7316A had about three-fourths, and Morris hepatoma 7794A and 7795 each had about one-third of the activity of the normal liver. The fast growing hepatomas are practically devoid of this enzyme.

The activity of sulfatase C in the microsome was in the same range for the normal liver, Morris 7316A and 7793 hepatomas. The activity of Morris hepatoma 7795 is about one-third of that of the normal liver, and that of the fast growing hepatoma, is less than one-tenth or one-twentieth.

The microsomal adenosine triphosphatase activity showed a quite different behavior from the above described 2 enzymes. All hepatomas examined in this investigation possessed the higher activity than the normal liver. This observation might be pointed out as 1 common deviation accompanied by malignan change which occurred in slowly growing and fast growing tumors.

COMPARISON OF HYDROXYLATING ACTIVITIES IN MICROSONES.
Hydroxylation of aromatic compounds was assayed by conversion of aniline to p-aminophenol in the presence of the NADPH₂-generating system. The results are given in Table 3. All Morris hepatomas maintain this activity although less than the normal liver. In the fast growing hepatoma this activity was completely deleted.

COMPARISON OF OXIDOREDUCTASE ACTIVITIES IN MICROSONAL FRACTIONS. Table 4 compares the values of oxidoreductase activities in microsomes. NADH₂-cytochrome b₄ oxidoreductase activities decrease in the order of 7793 > 7795 > 7316A > 7794A. NADH₂-cytochrome c oxidoreductase decreases in the order of 7793 > 7795 > 7316A > 7794A. NADPH₂-cytochrome c oxidoreductase decreases in the order of 7793 > 7794A > 7795 > 7316A. AH 371 and AH 130 showed far less activity than Morris hepatomas, as was observed for other microsomal enzymes.
Comparison of Microsomal Hemoproteins. The data on the hemoproteins in microsomal fractions are given in Table 5. Number 7316A has cytochrome b₅ at almost the same level as the normal liver. Hepatomas 7793, 7795, and 7794A have one-half to two-thirds of the value of the normal liver. Yoshida hepatomas lack almost completely this cytochrome. P-450 is also contained in all 4 kinds of Morris hepatomas at the more diminished rate than cytochrome b₅. However, P-450 was not found in the microsomes from Yoshida hepatomas.

Regenerating Liver and Embryonal Liver. The data on the regenerating liver and the embryonal liver for the various analyses are given in Tables 1–5. The conclusion can be drawn that the patterns of enzymatic activities and chemical composition of the regenerating liver resemble those of the normal liver. On the other hand, several patterns of the embryonal liver are common to the normal liver. Hepatomas 7793, 7795, and 7794A have one common change which was found in the microsomal fraction of this was observed in the regenerating liver and the embryonal liver, both of which are rapidly growing tissues. At the present, this elevation of adenosine triphosphatase is the only common change which was found in the microsomal fraction of all hepatomas studied herein. The function or the biological significance of microsomal adenosine triphosphatase in liver and hepatoma is not fully understood. The presence of K-Na-activated and ouabain-sensitive adenosine triphosphatase in the membranous fraction of the liver has been reported (3). The characterization of this elevated enzyme activity in hepatomas is now under investigation. The possibility exists that this elevation is somehow related to the changed character of the membrane showing an increased tendency for cellular dissociation in hepatoma cells, and might lead to the development of metastases.

Webb et al. (22, 23) observed an increase in free polyribosomes in hepatomas 7793 and 7794A as well as in other hepatomas. It was suggested (22, 23) that there may be structural defects in the enzyme-forming system possibly involving endoplasmic reticulum in addition to messenger RNA (mRNA) ribosomes, and amino acid transfer RNA. Further studies of these microsomal components need to be carried out. The present study on hepatoma 7793 indicates that hydroxylating activity, P-450 and NADPH₂-cytochrome c oxidoreductase are depressed to about one-third of the value of the normal liver, although glucose-6-phosphatase and sulfatase C activities are retained at the normal level. It was suggested that the hydroxylation, P-450 and NADPH₂-cytochrome c oxidoreductase, in the microsomal fraction are functionally connected with each other since those 3 are enhanced by phenobarbital administration in rats (15).

Discussion

The analyses of microsomes show that the microsomal enzymes, glucose-6-phosphatase, sulfatase C, antymycin A-insensitive NADH₂-cytochrome c oxidoreductase, antymycin A-insensitive NADPH₂-cytochrome c oxidoreductase, and hydroxylation of aromatic compounds and microsomal hemoproteins, cytochrome b₅ and P-450, are not deleted in these slowly growing Morris hepatomas, although they are completely lacking or extremely diminished in the rapidly growing ascites hepatomas. The existence of glucose-6-phosphatase at a somewhat decreased level of activity on the microsomal fraction found for hepatomas 7794A and 7316A was reported by Weber and Morris (24) in Morris hepatoma 5123 TC, and is in agreement with the present results. However, hepatomas 7316A and 7795 had glucose-6-phosphatase activity in the microsomal fraction quite similar to normal liver. This indicates that the deletion of those enzymes and cytochromes is not necessarily concomitant with the malignant conversion of hepatic cells. Among these hepatomas it was observed that the microsomal enzymes and cytochromes decrease in the rapidly growing hepatomas. This general decrease in these components might be due to the decrease of a certain fraction in microsomes which carry them. The decrease in phospholipid phosphorus in the microsomal fraction indicating the probable membranous nature of the phospholipid-protein complex supports the above explanation.

It is also possible that enzymes that are related to functions of differentiation, as microsomal enzymes, are synthesized in the resting phase of the cellular cycle and the fast growing tumor cells have too short a resting phase to develop the full synthesis of these enzymes before going into the next cell division. A similar idea was once proposed by Weinhouse (25).

It is interesting to note that microsomal adenosine triphosphatase activity measured in the presence of magnesium ion was elevated in all hepatomas without exception, although no elevation of this was observed in the regenerating liver and the embryonal liver, both of which are rapidly growing tissues. At the present, this elevation of adenosine triphosphatase is the only common change which was found in the microsomal fraction of all hepatomas studied herein. The function or the biological significance of microsomal adenosine triphosphatase in liver and hepatoma is not fully understood. The presence of K-Na-activated and ouabain-sensitive adenosine triphosphatase in the membranous fraction of the liver has been reported (3). The characterization of this elevated enzyme activity in hepatomas is now under investigation. The possibility exists that this elevation is somehow related to the changed character of the membrane showing an increased tendency for cellular dissociation in hepatoma cells, and might lead to the development of metastases.
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