Enzyme Patterns in a Group of Transplantable Mouse Hepatomas of Different Growth Rates

E. Bresnick, E. D. Mayfield, Jr., A. G. Liebelt, and R. A. Liebelt

SUMMARY

A series of transplantable hepatomas which arose spontaneously in mice or in mice treated with goldthioglucose, urethan, or 3-methylcholanthrene has been tested for the extent of deviation of enzyme activity from that of normal liver. The growth rates of these hepatomas varied from 21 to 211 days. Deoxythymidine kinase, uridine kinase, carbamylphosphate synthetase, aspartate transcarbamylase, ornithine transcarbamylase, orotidylic acid pyrophosphorylase and decarboxylase, uracil reductase, histidase, tyrosine α-ketoglutarate transaminase, threonine-serine dehydrase, and tryptophan pyrrolase activities were examined in control liver and in the hepatomas. Deoxythymidine kinase and aspartate transcarbamylase activities correlated to some extent with the growth rate of these hepatomas. Threonine-serine dehydrase, histidase, and carbamylphosphate synthetase activities were almost undetectable in all the hepatomas, while uracil reductase was present in detectable amounts in only two of the hepatomas.

INTRODUCTION

In a previous study (6), the activities of a group of enzymes that function in the synthesis of pyrimidines and of TdR kinase were determined in rat kidney adenocarcinomas of varying growth rates, i.e., 80 to 400 days. In this series, the activity of TdR kinase appeared to correlate with the growth rate, while no such relationship was observed between the rapidity in growth and the activities of ATC, dihydroorotase, and OMP transcarbamylase dehydrogenase, and OMP pyrophosphorylase and decarboxylase. A series of transplantable mouse hepatomas with varying growth rates has been developed by Dr. A. G. Liebelt. It was of interest to ascertain whether the growth of these mouse hepatomas might be directly related to the extent of deviation of enzyme activity from normal liver and to compare the results in this system with those obtained in the Morris hepatomas (32).

Accordingly, the activities of host liver, normal liver, and hepatoma enzymes in the metabolism of pyrimidines and amino acids were determined. The data are somewhat suggestive of a correlation between growth rate and the activity of ATC and TdR kinase in the mouse hepatoma system. The results also clearly depict the individuality of each of the enzymic patterns of the hepatomas.

MATERIALS AND METHODS

Radiochemicals. Thymidine-2-14C (30 μCi/μmole), uridine-2-14C (28 μCi/μmole), sodium bicarbonate-14C (5 μCi/μmole), uracil-2-14C (5 μCi/μmole), DL-aspartic acid-3-14C (23 μCi/μmole), uracil-2-14C (10 μCi/μmole), and potassium cyanate-14C (5 μCi/μmole) were purchased from New England Nuclear, Boston, Mass. Carbamylphosphate-14C was prepared from cyanate-14C by the method of Spector et al. (40). The final product had a specific activity of 0.125 μCi/μmole; the product was dissolved in water immediately before use.

Animals and Tumors. The mice used in these experiments were from inbred strains (or F1 hybrids) maintained by brother X sister mating in the Kirschbaum Memorial Laboratory at Baylor College of Medicine. The animals were housed 5 to a cage and were given Purina laboratory chow and water ad libitum.

The GTG-induced hepatomas were found in C3Hf and CBA mice that had received at 2 months of age 1 i.p. injection of GTG in 0.9% NaCl solution, at 0.8 mg/g and 0.4 mg/g body weight, respectively.

Hepatomas were also induced in the inbred strains (a) by feeding 3-methylcholanthrene at a level of 1 mg in 0.2 ml olive oil once a week for 9 weeks beginning at 6 to 10 weeks of age, and (b) by i.p. injection of urethan, 0.8 mg/g body weight, into newborn CBA mice.

Transplantation was done by s.c. implantation into the right side of isogenic mice with a trocar containing the 1- to 2-mm fragments of viable hepatoma tissue. Specific information about the mouse strains and tumor lines is given in Table 1.

The mice were observed, the tumor growth was recorded, and when transplants were 1 to 1.5 cm in size the animals were killed by cervical dislocation. A representative piece, free of necrotic tissue, was placed in 10% neutral buffered formalin.
and processed for histological examination. The latter included hematoxylin and eosin stain, periodic acid-Schiff-Alcian Blue with and without diastase treatment for the presence of glycogen, and occasionally an additional piece was fixed with Oil Red O to stain neutral fat. A second piece was used for the next transplantation passage while enzyme activity was determined in the rest of the tumor tissue (generally, the tumor mass from about 4 to 6 mice was combined).

**Enzyme Assays.** All assays were conducted under conditions in which the activity was directly proportional to the amount of enzyme and the time of incubation. TdR kinase was assayed as described previously (7). The assay system consisted of ATP, 5 mM; MgCl₂, 5 mM; TdR-2-'⁴C, 0.1 μCi (3.3 pmol/mole); supernatant fraction, 0.01 to 0.05 ml; 0.05 M Tris buffer, pH 8, to a total volume of 0.25 ml. The enzyme consisted of a supernatant fraction obtained after the centrifugation of a 20% homogenate of the tumor or liver in 0.25 M sucrose at 100,000 X g for 60 min. The incubations were conducted in test tubes at 37° for 15 min after which time the enzymatic reaction was stopped by immersing the tubes in a boiling water bath for 3 min.

The specific activity of ATC was determined by a modification of existing methods (5, 14). The assay system included DL-aspartic acid-3-'⁴C, 0.1 μCi (2 μmol/mole); carbamylphosphate, 5 μmol/mole; supernatant preparation, 0.2 ml; and 0.2 M Tris buffer, pH 9.2, to 1.5 ml. The incubation was conducted at 37° for 10 min, after which time the 0.2 ml 4 N perchloric acid was added. The deproteinized extract was passed through a column of Dowex 50-H⁺, 200 to 400 mesh (0.6 x 5 cm). Carbamyl aspartic acid-1-'⁴C passed through the ion exchange column whereas aspartic acid was retained. The column was washed twice with 1 ml water. The radioactivity of the combined eluates was estimated in a Packard Tri-Carb liquid scintillation spectrometer, with the use of Bray's solvent system (18). The efficiency of counting was 75%.

Uridine kinase activity was assayed in a system similar to the one reported by Skold (39). The assay mixture included uridine-2-'⁴C, 0.5 μmol (0.1 μCi); ATP, 2.5 μmol/mole; MgCl₂, 10 μmol/mole; 3-phosphoglycerate, 7 μmol/mole; enzyme; and 0.1 M Tris buffer, pH 7.5, to 0.25 ml. The incubation was conducted at 37° for 15 min. The radioactive product was assayed in the same manner as in the TdR kinase assay.

OMP pyrophosphorylase and decarboxylase activities were assayed by the method of Rubin et al. (37). The assay mixture included phosphoribosylpyrophosphate, 0.5 μmol/mole; orotic acid-7-'⁴C, 0.1 μmol (0.1 μCi); ATP, 1 μmol/mole; 3-phosphoglycerate, 2 μmol/mole; MgCl₂, 1.5 μmol/mole; potassium phosphate, 1 μmol/mole; ribose 5-phosphate, 0.5 μmol/mole; supernatant preparation, 0.1 ml; and 0.05 M Tris buffer, pH 7.4, to 1.5 ml. The incubation was conducted for 15 min in a 50-ml Erlenmeyer flask containing a center well in which was placed 0.1 ml 5 N KOH and a folded square of paper, 1 x 1 cm. The reaction medium was placed around the center well, and the flasks were capped with rubber serum caps. At the end of the incubation, 0.2 ml 4 N perchloric acid was added through the caps to the reaction medium by means of a syringe and needle. The flasks were then shaken for 30 min to ensure complete release of 14CO₂. At the end of this time, the fluted filter paper was removed and placed in a scintillation vial containing 10 ml Bray's scintillation mixture (3), and the radioactivity was ascertained in a Packard spectrometer.

Uracil reductase was assayed by a modification of the method of Potter et al. (36). The assay mixture included ATP, 14 μmol/mole; NADP, 1 μmol/mole; glucose 6-phosphate, 6 μmol/mole; MgCl₂, 30 μmol/mole; uracil-2-'⁴C, 0.5 μmol (0.1 μCi); supernatant preparation as described above, 0.1 ml; and buffered sucrose, pH 8.0 (0.20 M sucrose-0.04 M nicotinamide-0.012 M Tris-6 mM KCl) to 2.0 ml. The incubation was conducted for 15 min at 37° in 50-ml flasks containing 0.1 ml 5 N KOH in the center well, and radioactive 14CO₂ was assayed as described above.

Carbamylphosphate synthetase was determined by modifications of the methods of Brown and Cohen (9) and Marshall and Cohen (28). The assay system consisted of NH₄Cl, 5 μmol/mole; sodium bicarbonate-14C, 10 μmol/mole (1 μCi); ATP, 5 μmol/mole; enzyme; L-ornithine, 5 μmol/mole; MgCl₂, 10 μmol/mole; N-acetylglutamate, 5 μmol/mole; and 0.05 M glycylglycine buffer, pH 8.0, to a total volume of 1.0 ml. The enzyme consisted of a supernatant fraction obtained after the centrifugation at 4000 x g for 15 min of a 10% homogenate of liver or tumor in 0.1% cetyltrimethylammonium bromide. After a 15-min incubation at 37°, the mixture was deproteinized with 0.2 ml 4 N hydrochloric acid, an aliquot of the deproteinized material was placed on a planchet, and the planchet was heated at 105° for 30 min in a ventilated hood. The radioactivity on the planchet that represented the acid-stable 14C, i.e., citrulline, was then determined.

The specific activity of OTC was determined by a modification of the method of Brown and Cohen (9). The assay system consisted of L-ornithine, 20 μmol/mole; carbamylphosphate-14C, 2.5 μmol/mole (0.07 μCi); supernatant preparation; and 0.045 M glycylglycine buffer, pH 8.0, to 2.0 ml. The enzyme source was the same as described in the carbamylphosphate synthetase assay. The incubation was conducted for 15 min at 37°, and the reaction was terminated with 0.2 ml 4 N hydrochloric acid. Citrulline-14C was assayed as described above.

The protein in the homogenates and extracts was estimated by the colorimetric procedure of Lowry et al. (27), with bovine serum albumin as the reference standard.

The specific activity of threonine-serine dehydrase was assayed by the method of Sayre et al. (38) as modified by Bottomley et al. (2). The assay system consisted of L-threonine or L-serine, 50 μmol/mole; pyridoxal phosphate, 10 μmol/mole; phosphate buffer, pH 8.0, 100 μmol/mole; enzyme preparation; and distilled water to 2.0 ml. The enzyme consisted of a 20% homogenate in 0.25 M sucrose. The reaction was carried out in test tubes for 30 min at 37° and was stopped with 2.0 ml 10% trichloracetic acid. Aliquots were assayed for keto acid production by the method of Friedman and Haugen (16).

The procedure used for the assay of tryptophan pyrrolase was a modification (35) of the method of de Castro et al. (15), with the use of the coupling procedure of Brown and Price (10). The assay system consisted of L-tryptophan, 9 μmol/mole; Tris-HCl buffer, pH 7.5, 100 μmol; 0.025% hematin, 0.02 ml; enzyme preparation (see above); and water to 2.0 ml. The reaction was run in test tubes at 37° for 30 min and stopped.
with 1.0 ml 10% trichloracetic acid. The kynurenine was assayed colorimetrically by the method described by Pitot et al. (35).

Histidase activity was assayed by the method of Tabor and Mehler (41) as modified by Pitot et al. (35). The assay was run in quartz cuvets containing 2-mercaptoethanol, 5 μmoles; reduced glutathione, 10 μmoles; L-histidine, 30 μmoles; supernatant fraction; and 15 mM sodium pyrophosphate to 3.0 ml. The reaction was followed in a Beckman DU spectrophotometer at 277 nm at 2- to 3-min intervals. A blank consisted of a cuvet containing all constituents except enzyme. The enzyme consisted of a supernatant fraction obtained after the centrifugation at 100,000 X g for 60 min of a 20% homogenate in 0.25 M sucrose.

Tyrosine α-ketoglutarate transaminase was assayed by the method described by Pitot and Morris (34). The assay medium of 3 ml consisted of pyridoxal phosphate, 10 μmoles; sodium diethyldithiocarbamate, 10 μmoles; α-ketoglutarate, pH 7.0, 50 μmoles; 0.2 M K2HPO4, pH 8.0; L-tyrosine, 6 μmoles; supernatant preparation (see above). The incubation was carried out in test tubes at 37°C for 15 min and stopped with 1.0 ml 6 N perchloric acid. The enol-borate complex was determined by the method of Lin et al. (26).

RESULTS

Description of Tumors. The origin, the growth characteristics, and the morphological features of the transplanted hepatomas used in this study are listed in Table 1. The original hepatomas arose in mice of the CBA/S, CBA/Ki, C3Hf, and DBA/2 inbred strains; all transplantation lines were maintained by serial transplantation in mice of the same strain of origin except for hepatoma C3Hf 1719A which was passed in C3Hf X C57 hybrids from the 12th transplant generation (T-12) to the 18th transplant generation. A separate transplant line designated C3Hf 1719B was derived from the same hepatoma (C3Hf 1719) after T-4 and maintained exclusively in C3Hf hosts up to and beyond T-16.

The original hepatomas arose either spontaneously in untreated mice or in mice treated with GTG (25), urethan (24), or 3-methylcholanthrene (20) and all but 1 tumor developed in male mice. Two of the hepatomas studied, CBA 2192 and DBA/2 5199, have been discussed previously with respect to “morphological transformation” as well as physiological changes during the course of serial transplantation (21, 23).

No relationship was found between the type of experimental treatment or age of host at time of tumor diagnosis and morphological appearance of the original hepatoma. The age of the hosts in which the primary hepatomas were found ranged from 421 to 840 days. The growth period, that is, the time from trocar inoculation until the tumor reached approximately 1 cm in diameter, for the first transplant generation ranged from 162 to 403 days. However, with subsequent transplantation generations, the growth period for each tumor became progressively less. CBA 8379 had an initial growth period in T-1 of 307 days, but after 5 transplant generations the growth period was reduced to 21 days. On the other hand, hepatoma CBA 29 had an initial growth period of 162 days and stabilized at 110 days after 9 transplant generations. The transplantation generation for the various tumors used in this particular study ranged from T-2 to T-20 and the growth period for these same tumors at this time was 211 to 21 days, respectively.

The architecture of the primary tumors was similar to normal liver with parenchymal-type cells arranged in cords around a central blood vessel but lacking a true lobular pattern (Fig. 1). This morphological pattern in general remained stable in the majority of the transplanted tumors used (Fig. 2), with the exception of 2 hepatomas, C3Hf 1719A and CBA 8379. These 2 hepatomas changed from a liver-like architecture to a tumor composed of sheets of squamous-type cells (Fig. 5).

The distribution of glycogen was similar to that of normal liver (Fig. 3) in approximately one-half of the tumor lines in the first transplant generation (Fig. 4). However, 5 of these tumors were free of histochemically demonstrable glycogen following subsequent transplantation as well as at the time when used for enzyme analyses. Several of the transplanted tumor lines showed evidence of an accumulation of neutral lipid droplets as determined by histochemical stains. In routine histological techniques, the accumulation of lipid droplets was manifested by highly vacuolated cells. Several of the tumor lines showed an increase in nuclear size as compared with normal liver, but this observation was not followed up with precise measurements. One tumor (CBA/S 16) had, upon examination by electron microscopy, numerous cytoplasmic infoldings into the nucleus proper which appeared to be nucleoli when observed with light microscopy. In general, the number and size of the nucleoli in all tumors were similar to those of normal liver.

Cytoplasmic inclusion bodies were present in all of the C3Hf primary tumors (Fig. 6) but were not present in tumors following subsequent transplantation. These inclusion bodies are protein-rich structures found in hepatomas arising in only certain strains of mice (22, 24).

Detailed mitotic counts were not carried out, but if 4 to 6 mitotic figures were seen in an area covered by approximately 100 parenchymal cells, the tumor was classified as a mitotically active tumor. This classification correlated well with the rapidly growing tumors with growth periods between 21 and 47 days.

Several of these transplanted tumors were followed beyond the transplantation generation used for enzyme analysis. Hepatoma CBA/S 16 has remained morphologically similar from T-2 to T-7 and hepatoma CBA 2192, except for an increase in lipid droplets, has remained essentially the same in appearance between T-15 and T-24. Two tumors, C3Hf 2275 and CBA 29, have undergone a morphological transformation since they were used in the present study similar to that described earlier (21).

Enzymes of Pyrimidine Metabolism. The activities of the enzymes involved in pyrimidine metabolism are compared in Table 2. ATC and OTC represent competitive pathways for the utilization of carbamylphosphate in pyrimidine and urea synthases, respectively. ATC is elevated in all hepatomas except one, Tumor 16, and is maximum in 1719B hepatoma. On the other hand, the activity of the catabolic enzyme, OTC,
is decreased in all hepatomas. The reduction in enzymatic activity was least in hepatoma 29 (CBA host) and maximal in hepatoma 1719A (C3Hf host). The initial enzyme for carbamylphosphate synthesis, carbamylphosphate synthetase, was also assayed, but no activity was detected in any hepatoma except hepatoma 7727, which contained 10% of that present in normal liver.

The ability of the tumors to degrade pyrimidines was assessed by a measurement of the activity of the rate-limiting enzyme in the catabolic pathway, uracil reductase. The

Table 1
Characteristics of hepatomas used for enzyme studies

<table>
<thead>
<tr>
<th>Strain and sex of tumor-bearing mouse</th>
<th>Treatment</th>
<th>Age of mouse with primary hepatoma (days)</th>
<th>Growth period of T-1 (days)</th>
<th>Morphological characteristics of tumor of first transplantation generation</th>
<th>Transplant generation</th>
<th>Growth period (days)</th>
<th>Morphological changes as compared with T-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA/S 16d</td>
<td>GTG</td>
<td>840</td>
<td>266</td>
<td>Hepatic-like cells arranged in cords. Increased vascularity. Increased glycogen. Increased vacuolization (lipid).</td>
<td>T-2</td>
<td>211</td>
<td>Decreased glycogen.</td>
</tr>
<tr>
<td>CBA/S 150d</td>
<td>Spontaneous</td>
<td>675</td>
<td>246</td>
<td>Hepatic-like cells arranged in cords. Increased vascularity. Increased glycogen. Increased nuclear size.</td>
<td>T-3</td>
<td>140</td>
<td>No change.</td>
</tr>
<tr>
<td>CBA 2192d</td>
<td>MC</td>
<td>723</td>
<td>224</td>
<td>Hepatic-like cells arranged in cords. Vascularity, glycogen, vacuolization similar to normal liver, normal nuclear size. Squamous type cells arranged in sheets. Increased vascularity. Increased vacuolization (lipid).</td>
<td>T-15</td>
<td>113</td>
<td>Increased vascularity, glycogen, and vacuolization (lipid).</td>
</tr>
<tr>
<td>CBA 29d</td>
<td>UR</td>
<td>535</td>
<td>162</td>
<td>Squamous type cells arranged in sheets. Increased vascularity. Increased vacuolization (lipid). Normal nuclear size.</td>
<td>T-9</td>
<td>110</td>
<td>No change.</td>
</tr>
<tr>
<td>CBA 7727d</td>
<td>Spontaneous</td>
<td>612</td>
<td>377</td>
<td>Hepatic-like cells arranged in cords. Increased vascularity. Increased glycogen. Increased vacuolization. Normal nuclear size.</td>
<td>T-3</td>
<td>64</td>
<td>Increased nuclear size.</td>
</tr>
</tbody>
</table>

\[a\] MC, 3-methylcholanthrene; UR, urethan.
\[b\] As compared with normal liver morphology.
\[c\] Adenocarcinoma-like cell type.
Hepatoma Growth Rate and Enzymatic Activity

Table 2

Enzyme activity in mouse liver and hepatoma

<table>
<thead>
<tr>
<th>Tissue</th>
<th>ATC</th>
<th>Carbamyl-phosphate synthetase</th>
<th>OTC</th>
<th>Uridine kinase</th>
<th>TdR kinase</th>
<th>Conversion of orotic acid to UMP</th>
<th>Uracil reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control liver</td>
<td>41.0 ± 3.9 (17)b</td>
<td>36.0 ± 3.7 (18)</td>
<td>139 ± 21 (17)</td>
<td>6.7 ± 1.6 (17)</td>
<td>11.1 ± 0.1 (17)</td>
<td>85.1 ± 10.6 (17)</td>
<td>716 ± 100 (22)</td>
</tr>
<tr>
<td>CBA/S tumor 16</td>
<td>41.0; 42.0</td>
<td>0; 0</td>
<td>25.6; 43.6</td>
<td>3.6; 4.4</td>
<td>15.2; 16.0</td>
<td>n.d.</td>
<td>11.0 ± 2 (4)</td>
</tr>
<tr>
<td>CBA tumor 3375</td>
<td>n.d.</td>
<td>n.d.</td>
<td>17.6; 28.8</td>
<td>15.1; 10.6</td>
<td>45.6; 46.4</td>
<td>76.2; 76.8</td>
<td>734; 846</td>
</tr>
<tr>
<td>CBA tumor 150</td>
<td>76.8 ± 2.3 (4)</td>
<td>0 (3)</td>
<td>21.2 ± 3.2 (3)</td>
<td>4.4 ± 0.3 (3)</td>
<td>116.4 ± 2.2 (4)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>CBA tumor 2192</td>
<td>115.8 ± 11.6 (4)</td>
<td>0 (3)</td>
<td>61.2 ± 9.2 (3)</td>
<td>10.8 ± 1.6 (3)</td>
<td>142.8 ± 37.1 (3)</td>
<td>77.4 ± 1.8 (8)</td>
<td>184 ± 62 (3)</td>
</tr>
<tr>
<td>CBA tumor 29</td>
<td>72.6 ± 18.0 (4)</td>
<td>0 (3)</td>
<td>72.0 ± 10.6 (3)</td>
<td>5.2 ± 0.6 (3)</td>
<td>27.1 ± 7.3 (3)</td>
<td>140.2 ± 13.2 (4)</td>
<td>43 ± 10 (4)</td>
</tr>
<tr>
<td>CBA tumor 7727</td>
<td>64.8 ± 7.5 (10)</td>
<td>3.6 ± 0.4 (10)</td>
<td>42.0 ± 5.1 (10)</td>
<td>12.4 ± 2.0 (10)</td>
<td>47.8 ± 5.2 (10)</td>
<td>n.d.</td>
<td>19 ± 5 (6)</td>
</tr>
<tr>
<td>C3Hf tumor 1719B 315.6 ± 34.7 (4)</td>
<td>0 (4)</td>
<td>34.4 ± 8.5 (4)</td>
<td>30.4 ± 2.6 (4)</td>
<td>744 ± 110 (4)</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>C3Hf tumor 1719A 207.6 ± 54.0 (4)</td>
<td>0 (4)</td>
<td>8.4 ± 1.9 (4)</td>
<td>15.2 ± 4.3 (4)</td>
<td>425 ± 114 (4)</td>
<td>142 ± 10 (13)</td>
<td>14 ± 4 (13)</td>
<td></td>
</tr>
<tr>
<td>DBA/2 tumor 5199</td>
<td>131.4 ± 41 (3)</td>
<td>0 (3)</td>
<td>29.2 ± 1.9 (3)</td>
<td>6.8 ± 1.5 (4)</td>
<td>104.4 ± 73.3</td>
<td>n.d.</td>
<td>11 ± 2 (4)</td>
</tr>
<tr>
<td>CBA tumor 8379</td>
<td>209 ± 50 (3)</td>
<td>0 (3)</td>
<td>10.8; 11.2</td>
<td>39.6 ± 8.3 (3)</td>
<td>788 ± 140 (3)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

The enzyme activities in livers of DBA, CBA/S, CBA, and C3Hf mice did not differ significantly and have been grouped together in this category. Unless the values for host liver are given, it can be assumed that these did not significantly vary from control liver presented in the table. The table is arranged according to increasing growth rate of the hepatomas.

activity of this enzyme was reduced in almost all hepatomas (Table 2). The activity was less than 10% of the corresponding control liver value except in hepatomas 2192 and 3375, which contained approximately 25 and 100% of the normal liver value, respectively.

The relative conversion of orotic acid to UMP by normal liver and by the hepatomas is also shown in Table 2. The combined activities of OMP pyrophosphorylase and decarboxylase showed no reduction from control liver in any of the hepatomas which were assayed; an increase was apparent in hepatomas 29 and 1719A.

Uridine kinase, a "salvage" enzyme involved in UMP formation, showed a wide variation in activity (Table 2). Three hepatomas showed decreased activity while 6 others were 2- to 6-fold higher than the corresponding host liver value. The most active in this regard was hepatoma 8379, a fast-growing tumor.

The specific activity of TdR kinase is markedly elevated in all of the hepatomas (Table 2). The enzymatic activities ranged from 1.5- to 75-fold higher than normal liver activity. The slowest growing tumors, hepatomas 16 and 3375, were elevated only slightly, while the fast-growing hepatomas, 1719A, 1719B, and 8379, exhibited the highest activities.

Enzymes of Amino Acid Metabolism. The activities of tryptophan pyrrolase and tyrosine α-ketoglutarate transaminase are shown in Table 3. It is evident that a wide range of tryptophan pyrrolase activity was present in the hepatomas. The enzyme level varied from 15 to 200% of the corresponding normal liver value. The slowest growing tumors, hepatomas 16 and 3375, were elevated only slightly, while the fast-growing hepatomas, 1719A, 1719B, and 8379, exhibited the highest activities.

Table 3

Amino acid metabolism in mouse liver and hepatoma

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Histidase</th>
<th>Tyrosine α-ketoglutarate transaminase</th>
<th>Tryptophan pyrrolase</th>
<th>Serine-threonine dehydrase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control liver</td>
<td>1.0 ± 0.1 (19)b</td>
<td>220 ± 61 (6)</td>
<td>512 ± 78 (21)</td>
<td>7120 ± 560 (25)</td>
</tr>
<tr>
<td>CBA/S tumor 16</td>
<td>0 (4)</td>
<td>n.d.c</td>
<td>280 ± 35 (4)</td>
<td>n.d.</td>
</tr>
<tr>
<td>CBA tumor 3375</td>
<td>0 (2)</td>
<td>568; 968</td>
<td>n.d.</td>
<td>0 (4)</td>
</tr>
<tr>
<td>CBA tumor 2192</td>
<td>0 (3)</td>
<td>133 ± 28 (6)</td>
<td>412 ± 73 (4)</td>
<td>98 ± 30 (6)</td>
</tr>
<tr>
<td>CBA tumor 29</td>
<td>0 (4)</td>
<td>112 ± 33 (4)</td>
<td>400 ± 90 (3)</td>
<td>10 ± 3 (8)</td>
</tr>
<tr>
<td>CBA tumor 7727</td>
<td>0.1 ± 0.1 (6)</td>
<td>n.d.</td>
<td>80 ± 30 (6)</td>
<td>700 ± 140 (6)</td>
</tr>
<tr>
<td>DBA/2 tumor 5199</td>
<td>n.d.</td>
<td>n.d.</td>
<td>200 ± 15 (4)</td>
<td>0 (4)</td>
</tr>
<tr>
<td>C3Hf tumor 1719A</td>
<td>n.d.</td>
<td>136; 156</td>
<td>1140 ± 160 (3)</td>
<td>590 ± 50 (6)</td>
</tr>
<tr>
<td>CBA tumor 8397</td>
<td>0 (9)</td>
<td>n.d.n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Histidase activity is expressed as the change in absorbance at 270 nm/mg protein/hr. All others are expressed as mmol product produced/mg protein/hr.

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DISCUSSION

Up to 1960, the Novikoff tumor was the most commonly used and most extensively studied hepatoma. The latter, however, differs markedly from normal liver both in terms of morphology and in biochemistry (reviewed in Ref. 19). It appeared desirable to develop a series of hepatomas which would deviate only minimally from normal tissue. Accordingly, a number of tumors of varying growth rates were obtained in inbred strains of rats, largely through the administration of N-(2-fluorenyl)phthalamic acid and N-(2-fluorenyl)diacetamide by Morris et al. (31, 32).

The tumors were divided according to growth rate into 3 series, the slow-growing, the medium-growing, and the rapidly growing hepatomas. The slow-growing tumors were those that required transplantation every 1.5 to 6 months, were histologically well-differentiated trabecular hepatocarcinomas, and had few mitotic figures. The medium-growing tumors required transplantation every 4 to 6 weeks, were well-differentiated, and showed more mitotic figures. The rapidly growing hepatomas had an anaplastic histology, required less than 3 weeks for transplantation, and were mitotically quite active. Some of the hepatomas showed increasing growth rates with increasing numbers of transfers; others showed little change. Each tumor line, apparently, possessed its own growth characteristics. The histology of these Morris hepatomas has been extensively studied (30).

Although the well-differentiated hepatomas are characterized by many biological and biochemical similarities to normal liver, the less well-differentiated and more rapid-growing tumors possess more extensive differences in this regard. For example, bile secretion was observed in a number of the former. Glycogen was also seen, although, in general, the content decreased from transplant generation to generation.

In a recent review by Weber and Lea (42), the enzyme content of Morris hepatomas has been classified according to the correlation of activity to the growth rate of the tumor. The activities of certain key enzymes involved in carbohydrate, lipid, protein, and nucleic acid metabolism exhibited a remarkable relationship in this regard.

A decrease in gluconeogenesis and a concomitant rise in glycolysis were noted in the rapidly growing hepatomas when compared with either normal liver or the slow-growing tumors (42). The activities of the key gluconeogenic enzymes, i.e., pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-diphosphatase, and glucose 6-phosphatase, were depressed in parallel with the increase in growth rate. On the other hand, the key glycolytic enzymes, hexokinase, phosphofructokinase, and pyruvate kinase, increased in activity in direct relation to increasing growth rate.

A close inverse relationship was also observed between the increasing growth rate of the Morris hepatomas and the activities of certain enzymes that functioned in the catabolism of amino acids (1, 29, 42). Thus, tryptophan pyrrolase, serine-threonine dehydrase, glutamate dehydrogenase, and OTC were reduced in activity in proportion to the increasing growth capacity of the tumors.

Certain of the enzymes involved in nucleic acid metabolism exhibited a similar close correlation in the Morris hepatomas (reviewed in Ref. 42). Thus, it had previously been shown (18, 44) that incorporation of thymidine into tumor DNA closely correlated with the growth rate of the hepatomas. A comparable finding was reported for a group of kidney adenocarcinomas (17). We had previously reported on the activities of TdR kinase in the Morris hepatomas (8) and in the kidney adenocarcinomas (7). The quantitative aspects bore a direct relationship to growth rate in these cases. In other rapidly proliferating tissues TdR kinase activity was markedly elevated (7, 11, 29).

The activities of two of the enzymes involved in the de novo synthesis of pyrimidines, carbamylphosphate synthetase and ATC, were also influenced by the growth rate of the hepatomas, but in different directions. The former was progressively reduced with increasing growth (33); the latter was increased (4). ATC activity was also markedly elevated in other rapidly proliferating tissues (5, 7, 12, 13).

We have reported here the characteristics of a group of mouse hepatomas that arose either spontaneously or as a result of administration of urethan, GTG, or 3-methylcholanthrene. The hepatomas were observed in mice that were between 421 and 840 days old. The first transplantation time (T-1) varied between 162 and 403 days. The second transplantation period (T-2) of Tumors 16 and 3375 was little changed. A substantial decrease in subsequent transplantation time was noted in many of the other hepatomas. The morphology of the hepatomas resembled that of normal liver in T-1, although several demonstrated an increased nuclear size and increased glycogen deposition; cytoplasmic inclusion bodies were also noted in several. Upon subsequent transplantations, glycogen was decreased in hepatomas 16, 3375, 1719B, and 1719A. Increased mitosis was observed in 1719A and 1719B, in 8379, and in 5199.

In this mouse hepatoma series, uracil reductase activity proved a sensitive marker. With the exception of hepatomas 3375 and 2192, enzyme activity was markedly reduced, almost to the point of nonexistence in the other tumors. In the latter 2 tumors, uracil reductase was present at approximately 100 and 25%, respectively, of control liver activity.

With a singular exception of hepatoma 7727, carbamylphosphate synthetase activity was not present in any of the tumors. In this tumor, enzyme activity was only 10% of control liver level. Thus, in contrast to the Morris hepatoma series (see above), no direct correlation to growth rate was seen with the mouse tumors.

ATC and TdR kinase were markedly elevated in most of the mouse hepatomas, with a suggestion of correlation to growth rate. Hepatoma 7727 seemed somewhat unique in that the magnitude of the increase was only 1.5- and 4-fold, respectively. The other transcarbamylase, OTC, was markedly depressed in activity in the mouse hepatomas, with no apparent correlation with growth rate.

Amino acid metabolism was profoundly altered in the mouse hepatomas. Thus, histidase activity was almost nonexistent in 5 hepatomas and serine-threonine dehydrogenase activity was depressed. No correlation of the magnitude of the reduction of the latter enzyme was observed with growth rate.
Tryptophan pyrrolase activity, on the other hand, was elevated in the 1719A hepatoma. In the other tumors, this activity was reduced but again in no direct relationship to growth.

In summary, a series of transplantable mouse hepatomas have been developed, which exhibit many of the morphological characteristics of normal liver. However, these tumors vary greatly in their enzymic complement and are quite deviant from the normal liver pattern. Each tumor exhibited its own individuality in this respect, which could be an expression of genetic origin or of the manner of induction of the neoplasia.

REFERENCES

Bresnick, Mayfield, Liebelt, and Liebelt


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Fig. 1. A primary hepatoma from CBA mouse 2192. H & E, x 512.
Fig. 2. Transplanted CBA 2192 hepatoma after the 15th transplant generation. H & E, x 500.
Fig. 3. Distribution of glycogen as determined by the periodic acid-Schiff staining reaction in normal liver of CBA mouse. x 610.
Fig. 4. Distribution of glycogen as determined by the periodic acid-Schiff staining reaction in transplanted hepatoma CBA 9214. x 628.
Fig. 5. Transplanted C3Hf 1719A hepatoma which has undergone a morphological transformation from a typical hepatic parenchymal type of cell population as in Fig. 1 to a squamous-type cellular arrangement. H & E, x 518.
Fig. 6. A primary hepatoma of a C3Hf mouse demonstrating the presence of cytoplasmic inclusion bodies. H & E, x 1320.
Enzyme Patterns in a Group of Transplantable Mouse Hepatomas of Different Growth Rates


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