Activities of Some Enzymes of Pyrimidine and DNA Synthesis in a Rat Transplantable Hepatoma and Human Primary Hepatomas, in Cell Lines Derived from These Tissues, and in Human Fetal Liver

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ABSTRACT

Activities of the enzymes DNA polymerase, thymidine kinase, thymidylic acid kinase, thymidylate synthase, and deoxycytidylic deaminase have been measured in rat and human normal and neoplastic liver, in human fetal liver, and in cell lines derived from human hepatomas and rat transplantable hepatomas. The activities of these enzymes were increased in rat transplantable hepatomas, relative to rat normal or host liver, to a degree corresponding to the rapid growth rate of these tumors. With the exception of thymidine kinase, which did not change, the activities of these enzymes increased in human hepatomas relative to the corresponding host liver (apparently normal liver tissue from the same patient) and to human normal liver. The increases in enzyme activity observed in human hepatomas were small in comparison with those found in the rapidly growing rat hepatomas. The activities of deoxycytidylic deaminase in both human and rat liver tissues were 2 to 3 orders of magnitude higher than those of the other enzymes assayed. Activities of the enzymes of DNA synthesis in a slow-growing cell line derived from a human hepatoma were similar to those in human hepatoma tissues. In the case of rapidly growing cell lines derived from rat and human hepatomas, enzyme activities were higher than those in the corresponding tissues.

INTRODUCTION

Positive correlations between growth rate of mammalian tumors and the levels of activity of key enzymes of pyrimidine and DNA synthesis have been reported. The enzymes studied include dCMP deaminase (dCMP deaminase; EC 3.5.4.12), dTMP synthase (N',N'-methylene tetrahydrofolate 2'-deoxyuridylic acid Methyltransferase; EC 2.7.1.65), dTMP kinase (ATP:deoxythymidylate monophosphate phosphotransferase; EC 2.7.4.9) (27), DNA polymerase (deoxynucleoside triphosphate: DNA deoxynucleotidyltransferase; EC 2.7.7.7) (20), dThd kinase (ATP:thymidine 5'-phosphotransferase; EC 2.7.1.75) (5, 27). However, the activity of dThd kinase shows no increase in some tumors (10, 15). The findings for rat hepatomas generally support the correlation between tumor growth rate and incorporation of thymidine into DNA in the Morris spectrum of transplantable hepatomas. In human hepatomas, it has been shown that a key enzyme of glycolysis is increased, with a corresponding decrease in the activities of key enzymes of gluconeogenesis and glycogenesis (3, 11).

The present paper describes a comparative study of several of the key enzymes of DNA synthesis in a chemically induced (3'-Me-DAB) rat transplantable hepatoma and in human fetal, normal, and cancerous liver tissues. All 5 enzymes assayed had increased activities in the rat hepatomas and in human fetal livers compared to normal adult livers. Four of the enzymes were also higher in human hepatomas but to a lesser extent.

MATERIALS AND METHODS

Substrates. Crystalline deoxymethidine, disodium dTMP, sodium dTDP, sodium dUTP, sodium dUMP, sodium dCMP, sodium dCTP, sodium dGTP, disodium dATP, sodium DNA (type V), and dL-tetrahydrofolic acid, Grade III, were obtained from the Sigma Chemical Co., St. Louis, Mo. Disodium ATP was supplied by Boehhringer-Mannheim, Mannheim, West Germany. 5-Me-dCMP was purchased from Calbiochem AG, Lucerne, Switzerland. The ammonium salts of [methyl-3H]deoxymethidine, dTMP, dUTP, [5-3H]dCMP, and [5-3H]dUMP, were obtained from the Radiochemical Centre, Amersham, England. Radiochemicals were diluted with deionized water or unlabeled nucleotide solution.

Other Chemicals. 2-Mercaptoethanol and Folin-Ciocalteu’s phenol reagent were obtained from BDH Biochemicals, Ltd., Poole, England. Toluene and formaldehyde were purchased from E. Merck, Darmstadt, Germany. Norit A (activated charcoal) was obtained from Sigma. Crystalline bovine plasma albumin was obtained from Armour Pharmaceutical Co., Eastbourne, England. Reagents for scintillation counting (PPO, dimethyl-POPOP, p-bis(o-methylstyryl)benzene, and Instagel) were supplied by Packard Instrument Co., Downers Grove, Ill. Whatman DE81 DEAE-cellulose paper and Whatman GF/C glass fiber discs (2.1 cm diameter) were obtained from W & R Balston, Ltd., Maidstone, England. Other standard reagents, all of analytical grade, were obtained from Hopkin and Williams, Chadwell Heath, England, BDH, E. Merck, and Mallinckrodt Chemical Works, Inc., St. Louis, Mo.

Source of Tissues. Normal rat liver and transplantable hepatomas were obtained from albino Wistar rats. Transplantable hepatomas induced by the carcinogen 3'-Me-DAB (2) were provided by Dr. C. F. Albrecht and Dr. L. D. Nourse. The hepatomas were implanted into the hind legs of the rats. Rats were killed by cervical dislocation, and livers and hepatomas were removed. Necrotic tissue was discarded. Tissues were kept on ice and assayed immediately.

Caucasian normal human tissues, removed at autopsy, were provided by Dr. M. G. Maier of the Tissue Bank of The Southside Cancer Research Council and the Atomic Energy Board of South Africa.

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4 The abbreviations used are: dThd kinase, thymidine kinase; 5-Me-dCMP, 5-methyl-2'-deoxycytidine 5'-monophosphate; 3'-Me-DAB, 3'-methyl-4-dimethylaminoazobenzene.

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African Institute for Medical Research. Fetal tissues were obtained following spontaneous or therapeutic abortion from Professor L. G. R. van Dongen and staff. Livers and hepatomas obtained at autopsy from patients in whom primary hepatocellular carcinoma had been histologically confirmed were provided by Dr. E. W. Geddes and Professor I. Webster and staff. The patients were black males from Mozambique in East Africa. Gross liver weight ranged from 3 to 6 kg. Cancerous regions of liver tissue were separated from liver tissue which appeared to be grossly normal ('host liver'). Only hepatoma tissue which showed less than 15% necrosis on histological examination (by the Histopathology Department of The South African Institute for Medical Research) was assayed. On a single occasion, host liver and hepatoma were obtained during surgery on a black South African female. The tissues were provided by Dr. J. A. Hunt. Autopsy material was assayed within 6 hr after death, and fetal material was assayed within 1 to 6 hours after abortion. In studies on the stability of enzymes in these tissues to storage, samples of tissue were stored frozen at −20° or −70°.

Rat hepatoma cell lines, derived from the previously mentioned 3'-Me-DAB-induced hepatoma, were obtained from Dr. L. D. Nourse (1) and Professor O. W. Prozesky (22). These cell lines were designated NRH and PRH, respectively. Human hepatoma cell lines were developed and supplied by Professor O. W. Prozesky (PHH cells) and Dr. J. J. Alexander (AHH cells). The PHH cell line was grown as described by Prozesky et al. (22). The AHH cell line was grown at 37° in Eagle’s minimal essential medium containing essential amino acids, penicillin (0.1 mg/ml) and streptomycin (0.1 mg/ml) and supplemented with 10% fetal bovine serum [containing penicillin and streptomycin (0.2 mg/ml each), and mycostatin (0.1 mg/ml)]. In all cases, cells were harvested by trypsinization followed by washing with serum-free medium.

Preparation of Homogenates. Twenty % (w/v) tissue homogenates were prepared in 50 mM Tris-HCl-250 mM sucrose-5 mM β-mercaptoethanol-1 mM EDTA (pH 7.5), using a Potter-Elvehjem homogenizer. Preparations for the assay of dCMP deaminase also contained 0.5 mM dCTP, which stabilizes this enzyme (27). Tissue homogenates were centrifuged at 38,000 × g for 60 min at 4°, and the supernatant fractions were assayed for enzyme activities. The cell lines were prepared for assay by washing the harvested cells 3 times with 0.9% sodium chloride solution. The cell pellet was then suspended in 5 to 10 volumes (v/v) of the homogenizing buffer described above. The cells were disrupted by 3 cycles of rapid freezing and thawing. The lysed cell extracts were centrifuged at 38,000 × g for 60 min and the supernatants were retained for assay.

Assay Procedures. Reaction rates were linear with time and enzyme concentrations under the conditions used. A unit of enzyme activity is defined as the amount of enzyme catalyzing the production of 1 μmol of product per min at 37°, or, in the case of dTMP synthase, the utilization of 1 μmol of substrate per min at 30°.

DNA Polymerase. The method of Shepherd and Keir (24) was used, except that the concentration of unlabeled dTTP was decreased to 100 μM to increase the specific radioactivity of the [3H]dTTP. Rat DNA polymerases characteristic of non-dividing adult liver tissue and of rapidly dividing fetal, regenerating, or neoplastic liver can be distinguished by their preferences for, respectively, native and denatured template DNA (4). In the present study, levels of this enzyme were measured using both native and denatured calf thymus template DNA; the major form of DNA polymerase activity was determined from the ratio of the enzyme activities obtained. Heat-denatured DNA was prepared by heating a 2-mg/ml preparation of native DNA in a boiling water bath for 5 min and then cooling rapidly on ice.

dTth Kinase. The assay method described by Elford et al. (7) was used.

dTMP Kinase. The assay method was based on those of Gordon et al. (10) and Sneider et al. (27). Each assay mixture contained, in a final volume of 200 μl: 50 mM Tris-HCl buffer (pH 7.5); 7.5 mM MgCl₂; 5 mM ATP; 50 μM [3H]dTMP (20 μCi/μmol); and up to 2 mg of supernatant protein. The reaction was initiated by the addition of [3H]dTMP. After incubation for 20 min at 37°, the reaction was terminated by heating assay tubes in a boiling water bath for 4 min. [3H]dTMP and [3H]dTDP were separated with ascending chromatography on DE 81 paper, using 0.1 N formic acid:1 N ammonium formate as the solvent. The areas corresponding to the 2 nucleotides were cut out and counted in 10 ml of scintillation cocktail as described below.

dCMP Deaminase. The assay procedure was based on those described by Sneider et al. (27) and Maley and Maley (17). Tritiated substrate and product were separated and counted as for the dTMP kinase system. In addition, dCMP deaminase was occasionally measured using the spectrophotometric method described by Maley and Maley (17). This assay method was also modified to allow measurement of the rate of deamination of 5-Me-dCMP. In the latter case, assay mixtures contained, in a final volume of 0.5ml: 60 mM Tris-HCl buffer (pH 8.0); 40 μM dCTP; 6 mM MgCl₂; 2 mM 5-Me-dCMP, and up to 4 mg of supernatant protein. Assays were terminated by the addition of 1.5 ml of cold 0.6 M perchloric acid, and the decrease in absorbance at 287 nm over a 10-min assay period was determined. The isotopic and spectrophotometric assay methods were found to give comparable results for both human and rat dCMP deaminases.

dTMP Synthase. The assay procedure used was based on those described by Kamen (14) and Elford et al. (7). Assay mixtures contained, in a final volume of 200 μl: 20 mM Tris-HCl buffer (pH 7.5); 25 mM MgCl₂; 15.8 mM formaldehyde; 1.0 mM EDTA; 300 μM tetrahydrofolic acid; 196 μM β-mercaptoethanol; 10 μM [5-3H]dUMP (250 μCi/μmol); and up to 2 mg of supernatant protein. The reaction was terminated by adsorbing unconverted [3H]dUMP onto Norit A-activated charcoal and counting aliquots of the filtered supernatants in Instagel.

Estimation of Radioactivity. With the exception of dTMP synthase, scintillation counting was performed using a scintillation cocktail consisting of 5 g of PPO and 250 mg of β-bis(o-methylstyryl)benzene per liter of toluene. Radioactivity was determined using a Packard Tri-Carb Model 3380 liquid scintillation counter.

Protein Determinations. Protein determinations were performed using the method of Lowry et al. (16).

Statistical Evaluation of Results. The levels of activity for each enzyme assayed in the same types of tissue or cells are expressed as mean ± S.E. In comparing enzyme activities of corresponding enzymes in comparable tissues or cells, the significance of differences between the means and S.E. for the 2 sets of values was calculated using Student's t test. A value of p < 0.01 was considered to indicate a significant difference.
RESULTS AND DISCUSSION

Stability of the Enzymes Assayed in Human Liver Tissues. DNA polymerases and dTMP synthase from human fetal, normal, and cancerous liver tissues were unstable to storage at both -20° and -70°, as previously found for rat regenerating liver (18) and for Ehrlich ascites cells (12). Human liver dTTP kinase activity decreased on storage at these temperatures in contrast to the stability reported for the enzyme from rat liver (6). Thymidine kinase and dCMP deaminase activities were stable to storage at -20° and -70°. Because of the instability of dTMP synthase, of dTMP kinase, and of DNA polymerases, activities in all tissues were assayed immediately.

Enzyme Activities in Rat Tissues. The activities of the enzymes of pyrimidine and DNA synthesis in rat normal, host, and cancerous liver tissues and in cell lines derived from a rat transplantable hepatoma are summarized in Table 1.

Activities of the key enzymes of DNA synthesis were low in rat normal liver, as found previously (6, 27, 31). DNA polymerase activity in normal rat liver showed a preference for native DNA as a template, as also shown by others (4, 13, 20). dCMP deaminase activities were at least an order of magnitude greater than the levels of the other enzymes assayed in the present studies.

The activities of the enzymes assayed in the livers of rats bearing transplantable hepatomas (host livers) were also found to be low. However, with the exception of dTMP kinase activity, which was not detectable, the ranges of activity for each of the enzymes appeared to be slightly elevated in comparison with the levels of the corresponding enzymes in rat normal liver. This suggests that there may be a tumor-induced humoral factor which affects the enzymes of DNA synthesis in the host liver, as has been proposed by Shirasaka and Fuji (25).

Activities of the enzymes assayed in rat transplantable hepatoma tissue showed statistically significant increases over those in normal and host liver tissues. The predominant DNA polymerase form in the cancerous liver tissues was that preferentially using denatured template DNA, as also found by others (4, 13, 20). The activities of the enzymes of DNA synthesis in the 3'-Me-DAB-induced rat hepatoma correlate with those reported by Weber (29) for a rapidly growing Morris 3683F rat hepatoma, and with the observation that death of the host rats occurs within 3 to 4 weeks following tumor implantation. The elevated activities of these enzymes of DNA synthesis in the rat hepatoma tissue suggest that both de novo and salvage pathways leading to dTTP and DNA synthesis are markedly elevated in this rapidly growing tissue.

The activities of the enzymes of DNA synthesis in the rat hepatoma cell lines showed a wide scatter. It can, however, be seen that enzyme activities in the NRH cell line were generally higher than in the tumor tissue from which the cell line was derived. The cells had a doubling time of 22 hr and were dedifferentiated (2). The high enzyme activities assayed correlate with the rapid cell growth rate. The PRH cell line was cultured under conditions which were optimal for the growth of the PHH human hepatoma cells but suboptimal for growth of the PRH cell line. The growth rate of these cells was accordingly slower than that of the NRH cell line. The range of activities for each enzyme in the PRH cell line was similar to that of rat hepatoma tissue, with the exception of dThd kinase. The very low levels measured for this enzyme suggest that the salvage of thymidine may play an insignificant role in DNA metabolism of the PRH cells.

Enzyme Activities in Human Normal and Cancerous Liver Tissues. The activities of the enzymes of DNA synthesis in human normal, host, cancerous, fetal liver tissues and in cell lines derived from human hepatomas are summarized in Table 2.

Activities of DNA polymerase and dTMP synthase in human normal liver were low or, in some cases, undetectable, suggesting that little or no DNA synthesis occurs in differentiated human normal liver. The predominant form of DNA polymerase was that showing a preference for native template DNA, as was the case for rat liver. dThd kinase, dTMP kinase, and dCMP deaminase activities were notably higher in adult human liver tissue than in rat normal liver. High dCMC deaminase activities have been described in human liver (9). The metabolic significance of the elevated dThd kinase and dCMP deaminase activities in human normal liver is at present not clear.

Activities of DNA polymerase were significantly higher in human "host" liver, the predominant form still being that which preferentially used native template DNA. dThd kinase activity in the host livers was similar to that found in normal liver, while dTMP kinase activity was found to be about 3-fold higher than that in normal liver. The range of dThd kinase and dTMP kinase activities in human host liver was comparable with those measured in human kidney tissue adjacent to hypernephromas (10).

### Table 1

Comparison of the activities of key enzymes of DNA synthesis in rat normal and host livers, in transplantable 3'-Me-DAB-induced hepatomas, and in the NRH and PRH hepatoma cell lines

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity (milliunits/g protein)</th>
<th>Normal liver (N)</th>
<th>Host liver (H)</th>
<th>Hepatoma (Ca)</th>
<th>NRH cell line</th>
<th>PRH cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA polymerase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>With native DNA template</td>
<td>2.26 ± 0.38 (6)</td>
<td>3.17 ± 0.25 (3)</td>
<td>14.6 ± 1.7 (3)</td>
<td>63.2 ± 14.8 (5)</td>
<td>30.1 ± 2.6 (4)</td>
<td></td>
</tr>
<tr>
<td>With denatured DNA template</td>
<td>0.81 ± 0.18 (6)</td>
<td>1.31 ± 0.26 (15)</td>
<td>20.2 ± 3.2 (15)</td>
<td>95.9 ± 10.6 (5)</td>
<td>35.1 ± 10.9 (4)</td>
<td></td>
</tr>
<tr>
<td>Ratio: activities</td>
<td>0.38 ± 0.06 (6)</td>
<td>0.41 ± 0.02 (3)</td>
<td>1.52 ± 0.09 (3)</td>
<td>1.71 ± 0.22 (5)</td>
<td>1.12 ± 0.29 (4)</td>
<td></td>
</tr>
<tr>
<td>(denatured DNA/native DNA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dThd kinase</td>
<td>1.70 ± 0.11 (6)</td>
<td>2.18 ± 0.42 (15)</td>
<td>85.1 ± 8.4 (15)</td>
<td>241 ± 55 (5)</td>
<td>10.0 ± 8.9 (4)</td>
<td></td>
</tr>
<tr>
<td>dTMP kinase</td>
<td>1.42 ± 0.11 (6)</td>
<td>2.63 ± 0.26 (15)</td>
<td>151 ± 16 (3)</td>
<td>289 ± 55 (5)</td>
<td>274 ± 163 (4)</td>
<td></td>
</tr>
<tr>
<td>dTMP synthase</td>
<td>0.59 ± 0.12 (6)</td>
<td>0.93 ± 0.26 (12)</td>
<td>42.8 ± 3.2 (12)</td>
<td>34.9 ± 10.6 (5)</td>
<td>8.08 ± 4.28 (4)</td>
<td></td>
</tr>
<tr>
<td>dCMP deaminase</td>
<td>50 ± 10 (6)</td>
<td>112 ± 30 (5)</td>
<td>3680 ± 450 (5)</td>
<td>7470 ± 750 (5)</td>
<td>2890 ± 1220 (4)</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.E.
* Numbers in parentheses, number of samples assayed.
* N/D, No detectable activity.

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The activity of dCMP deaminase in host liver was slightly elevated in comparison with that in normal liver. dTMP synthase activity was not detectable in the host tissue. Raised DNA polymerase, dTMP kinase, and dCMP deaminase activities in human host liver could be due to a humoral factor produced by the tumor or may reflect regeneration or repair of damaged liver cells. In the latter respect, dCMP deaminase activity is elevated in most acute liver conditions in humans (19). Alternatively, since the hepatomas and host livers were from African blacks, whereas the "normal" livers were from Caucasians, the different enzyme activities could possibly be due to environmental (including dietary) and/or genetic differences.

DNA polymerase activities in human hepatomas were significantly higher than in normal or host liver. Moreover, the predominant form of the enzyme was that which showed a preference for denatured template DNA, as was found for rat hepatomas. This form of DNA polymerase also appears to increase in human carcinomas of the colon and breast and in melanomas (15) and shows increases which correlate with tumor growth rate in Morris hepatomas (4).

dThd kinase activities in the human hepatomas were not significantly different from those in human normal or host liver. These results are similar to those reported for human lung carcinomas and hypernephromas (10) and for human colonic cancer (15). The absence of increased dThd kinase activity in human neoplastic liver is the most striking difference noted between human and transplantable rat hepatoma tissues in the present study. Lack of increased dThd kinase activity is not, however, a property of all human cancerous tissues, since levels of the enzyme have been shown to increase in human carcinomas of the cecum, rectum, tonsil, tongue, larynx, and mammary gland (10). Activity of dTMP kinase was significantly increased in the human hepatoma in comparison with normal or host livers. Similar increases have been reported for a number of human carcinomas, bone sarcomas, and hypernephromas (10). Measurable dTMP synthase activity was present in human hepatoma tissue. This suggests that the de novo pathway of dTMP synthesis may be operative in human cancerous liver, although activities are very low in comparison with those in rat hepatoma.

Significantly higher dCMP deaminase was found in human hepatoma, in comparison with normal host liver. It has been tentatively suggested that dCMP deaminase may provide an alternative pathway of dTMP synthesis via the deamination of 5-Me-dCMP (26, 28). In the present study, a rat hepatoma preparation was found to deaminate 5-Me-dCMP 3 times faster than the normal substrate, dCMP. Using human liver and hepatoma preparations, similar rates of deamination were measured with either substrate. This alternative route of dTMP synthesis would be functional only if adequate 5-Me-dCMP is present in the cell. 5-Methylcytosine comprises 5 to 9% of the total cytosine bases in mammalian DNA (32) and 20% of the total deoxyribose compounds found in rat urine (23), but cellular concentrations of 5-Me-dCMP have not been determined.

Enzyme activities assayed in surgically removed or biopsied tissues were in the same range as those from tissues obtained at autopsy. These findings confirm that autopsy tissue can be reliably used for studies of this kind.

The relatively small or absent increases in the levels of these enzymes in comparison with those in the fast-growing rat

Table 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Human</th>
<th>Rat</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA polymerase</td>
<td>0.39 ± 0.10</td>
<td>0.12 ± 0.04</td>
<td>3.33</td>
</tr>
<tr>
<td>dThd kinase</td>
<td>0.18 ± 0.06</td>
<td>0.06 ± 0.02</td>
<td>3.00</td>
</tr>
<tr>
<td>dTMP synthase</td>
<td>0.34 ± 0.12</td>
<td>0.12 ± 0.04</td>
<td>2.80</td>
</tr>
<tr>
<td>dCMP deaminase</td>
<td>0.45 ± 0.15</td>
<td>0.15 ± 0.05</td>
<td>3.00</td>
</tr>
</tbody>
</table>

a Mean ± S.E. b Mean ± S.E. c N.D. No detectable activity.

Statistical analysis of DNA synthesis in human, normal, normal, and rat hepatomas using the Wilcoxon rank sum test revealed no significant differences in the enzyme activities.

Note: The comparison of the activities of key enzymes of DNA synthesis in human, normal, normal, and rat hepatomas using the Wilcoxon rank sum test showed no significant differences in the enzyme activities.
hepatoma suggest that human hepatomas may be characterized by a relatively slow growth rate. The present finding that dThd kinase activity is not increased in human hepatomas may explain the observation that thymidine incorporation into DNA in human hepatomas is comparable with that in human liver (30).

### Enzyme Activities in Human Hepatoma Cell Lines

Enzyme activities in the AHH cell line were in the same range as those in human hepatoma tissues. This cell line is characterized by a very slow growth rate. Of particular interest is the finding of dThd kinase and dCMP deaminase activities which are characteristic of human hepatoma tissue. In contrast to the slow growth rate of the AHH cell line, the PHH cells showed a rapid growth rate. This was reflected by the activities of the enzymes of DNA synthesis which were, with the exception of dCMP deaminase, higher than the highest activities in human hepatoma tissue. In general, the PHH cell line appears to represent a selection for a rapidly growing cell population which is not representative in terms of DNA metabolism of human cancerous liver tissue.

### Enzyme Levels in Human Fetal Liver

Potter (21) has suggested that the metabolic pattern of hepatomas may resemble that of fetal liver in a state of blocked maturation. Activities of some enzymes of DNA synthesis were accordingly assayed in a series of human fetal livers. With the exception of dCMP deaminase, enzyme activities were found to be significantly higher than those in human hepatomas. dCMP deaminase activities were significantly lower in the fetal liver. The finding of high dThd kinase activity in fetal liver suggests that the metabolism of human hepatoma cells is not identical with that of fetal liver. It is, however, important to note that the fetal liver is primarily a haemopoietic organ and that the relative proportions of haemopoietic to parenchymal cells, at different stages of gestation in the human, are not known. The present studies do not distinguish between the contribution due to each of these cell types.

Of the 5 enzymes of DNA synthesis studied, the activities of 4 were found to be elevated in both human and rat hepatomas relative to the corresponding normal and host livers; the fifth, dThd kinase, was elevated only in the rat hepatomas. The increased activities in the rapidly growing, poorly differentiated rat hepatomas were considerably greater than in the human hepatomas. This could indicate a slower growth rate for the human tumors, at least at the stage at which material used in the present studies was obtained. However, comparisons of enzyme activity and tumor growth rate in human hepatomas have not been carried out.

### REFERENCES


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