Two Forms of Thymidine Kinase in Normal and Tumor Tissues of Animals

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SUMMARY

The thymidine kinases extracted from the spleen of mice infected with Friend virus and from Yoshida sarcoma in rats were separated into two active peaks by diethylaminoethy cellulose column chromatography, while those of normal tissues have been found to consist of only the first peak (P-I). The second peak (P-II) was also found in the enzyme from the extract of the spleen when the animals were treated by i.p. injections of 1-acetyl-2-phenylhydrazine. The two P-II peaks from tumor tissue and from spleen enlarged by anemia-inducing agents were indistinguishable on the chromatographic profile. On the other hand, the thymidine kinase extracted with Triton X-100 from a mitochondrial fraction of normal liver was found to consist of only one peak in the same position as the above P-II's on the chromatogram, but its faculty for deoxythymidine triphosphate inhibition was not identical to that of tumor tissue. This treatment with the detergent might cause dissociation of a certain component from the enzyme complex to make the extra peak (P-II), but it eventually shifts to P-II on the chromatogram.

INTRODUCTION

A number of reports have shown the importance of thymidine kinase as one of the enzymes that catalyze the serial reactions from thymidine to TTP needed for DNA synthesis (2-4, 12, 13, 19, 31, 39). The activity of thymidine kinase is correlated to the proliferation rate of cells. Thymidine kinase activity was high in regenerating liver (2-4, 12, 28, 31), fetus (9, 29, 35, 40), thymus (3), spleen (36), bone marrow (15), and virus-infected cells (27, 32) but low in nongrowing tissues such as normal liver (2-4, 12, 31).

The relationship between the activity of thymidine kinase and the growth rate has been investigated in tumor cells. A good correlation was found between the growth rate of tumors and [3H]thymidine incorporation into DNA (30). A correlation was also found between the growth rate and thymidine kinase activity in a series of transplantable hepatomas having different growth rates (7, 9, 11).

The presence of 2 forms of thymidine kinase in KB cells and Yoshida sarcoma cells was found by electrophoresis on Pevikon blocks and by DEAE-cellulose column chromatography, as reported by Hashimoto et al. (14, 15). These 2 peaks having thymidine kinase activity were also observed in tumors such as Yoshida ascites hepatoma (AH-130), Morris hepatomas (7793 and 7794A), mouse Sarcoma 180, and Ehrlich ascites carcinoma in the patterns of DEAE-cellulose chromatography (33). However, only 1 peak of thymidine kinase could be found in the extracts of normal tissues and regenerating liver, embryonic liver, and bone marrow, which had a high thymidine kinase activity (33). In these reports, 1 of the 2 peaks of thymidine kinase was claimed to be a tumor-specific enzyme. Otherwise, a tumor-specific enzyme has not been reported.

Friend virus is an oncovirus (oncogenic RNA virus), and it causes splenomegaly in a mouse. Splenomegaly can also be induced in animals by the injection of 1-acetyl-2-phenylhydrazine (36) or other hemolytic agents. Histological examination showed an increase of immature blast cells in the spleen of mice infected with Friend virus, just as in the spleen of animals given injections of the above drugs. We have previously reported that the activities of thymidine kinase, uridine kinase, and DNA-directed DNA polymerase in the spleen increase with infection by Friend virus (36).

This paper shows that a kind of thymidine kinase, which was assumed to be tumor specific (33), can be induced in mouse or rat spleen by the administration of 1-acetyl-2-phenylhydrazine, and the characteristics of thymidine kinases in cytoplasmic and mitochondrial fractions are also discussed.

MATERIALS AND METHODS

Tissues and Virus. The partial hepatectomy of mouse and rat was carried out by removing about two-thirds of the liver by the method of Higgins and Anderson (16). The amounts of liver removed in the operation are summarized in Table 1. Enlargement of the spleens of C3H/He mice and Wistar rats was induced by the injection of 1-acetyl-2-phenylhydrazine, as reported by Ito and Tatibana (17), who treated animals with 4 injections in doses of 50 mg per kg body weight per day and sacrificed the animals 2 days after the last injection. Identical injection schedules for hydroxylamine and aniline were also performed, using the same procedure as for 1-acetyl-2-phenylhydrazine administration. Spleen weights of treated and untreated animals used in this experiment are shown in Table 1.

The maintenance and inoculation of Friend virus in our laboratory were carried out as follows. Several C3H mice (20 to 30 g body weight) were inoculated i.p. with 0.1 ml of 20% (w/v) homogenate in 0.95% NaCl solution of spleens from...
Table 1  
Summaries of the weights of body, liver, and spleen measured in mice and rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Body (g)</th>
<th>Liver (g)</th>
<th>Removed liver (g)</th>
<th>Spleen (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (control)</td>
<td>10</td>
<td>19.8 ± 2.1</td>
<td>1.03 ± 0.08</td>
<td>0.12 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Friend virus-infected</td>
<td>5</td>
<td>23.4 ± 2.1</td>
<td></td>
<td>1.26 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>1-Acetyl-2-phenylhydrazine-injected</td>
<td>5</td>
<td>30.7 ± 2.6</td>
<td></td>
<td>0.61 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Hydroxylamine-injected</td>
<td>5</td>
<td>24.3 ± 2.0</td>
<td></td>
<td>0.58 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Aniline-injected</td>
<td>5</td>
<td>35.6 ± 4.6</td>
<td></td>
<td>0.24 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Partially hepatectomized</td>
<td>5</td>
<td>26.3 ± 2.2</td>
<td>(0.63 ± 0.05)</td>
<td>0.94 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (control of spleen)</td>
<td>5</td>
<td>470.8 ± 40.8</td>
<td></td>
<td>0.71 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>1-Acetyl-2-phenylhydrazine-injected</td>
<td>5</td>
<td>329.3 ± 29.1</td>
<td></td>
<td>3.72 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Normal (control of liver)</td>
<td>5</td>
<td>200.5 ± 12.3</td>
<td>10.43 ± 0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partially hepatectomized</td>
<td>3</td>
<td>198.7 ± 7.1</td>
<td>(3.89 ± 0.34)</td>
<td>7.88 ± 0.54</td>
<td></td>
</tr>
</tbody>
</table>

* Average ± S.D.  
* Numbers in parentheses, weights of regenerating liver.

mice with Friend disease, which had been maintained by serial inoculation since original Friend disease mice were supplied by Dr. C. Friend (Sloan-Kettering Institute for Cancer Research, New York, N. Y.).

Yoshida sarcoma in Donryu strain rats was a gift from Dr. T. Tashiro (Cancer Institute, Tokyo, Japan).

Chemical Compounds. Chemicals used and their sources were as follows: [3H]thymidine from Daiichi Pure Chemicals Co., Tokyo, Japan; thymidine (cold) from Sigma Chemical Co., St. Louis, Mo.; ATP and RNase from Boehringer Mannheim-Yamanouchi Co., Tokyo, Japan; 3-phosphoglyceric acid, extra pure grade, from Wako Pure Chemicals Co., Osaka, Japan; pancreatic DNase I (RNase-free) from Worthington Biochemical Corp., Freehold, N. J.; DEAE-cellulose powder from Bio-Rad Laboratories, Richmond, Calif.; and Whatman DEAE-cellulose DE-81 paper from W. & R. Balston, Ltd., Maidstone, Kent, United Kingdom.

Extraction of Thymidine Kinases. The enzyme preparations of cytoplasmic fractions from fetal liver, regenerating liver, the spleens of mice and rats, and Yoshida sarcoma were obtained as follows. The tissue was homogenized in a Teflon homogenizer with 2 volumes of 0.05 M Tris-HCl buffer (pH 8.0) containing 0.34 M sucrose, 25 mM KCl, and 5 mM MgCl₂ (Buffer A). The homogenate was centrifuged at 100,000 × g for 120 min and the supernatant was used as the thymidine kinase fraction of cytoplasm. Thymidine kinase fraction from mitochondria was prepared from mouse liver. The freshly removed liver was rinsed with 0.85% NaCl solution and was homogenized with 2 volumes of Buffer A using a Teflon homogenizer for 3 min. The homogenate was then centrifuged at 1,000 × g for 10 min and the slightly turbid supernatant was spun down at 10,000 × g for 10 min. The crude mitochondrial fraction was then suspended in an equal volume of 0.1 M Tris-HCl buffer, pH 8.0, containing 1 mM 2-mercaptoethanol and 1% Triton X-100. The suspension was homogenized again by a Polytron (Kinematica GMBH, Luzern, Switzerland) for 1 min and then centrifuged at 100,000 × g for 60 min. The supernatant was fractionated by the addition of saturated ammonium sulfate solution to make a final 40% saturation. The precipitates that were formed were spun down at 20,000 × g for 30 min and then dissolved in as small a volume as possible of equilibrium solution for DEAE-cellulose chromatography. The above dissolved material was transferred to a Visking tube and dialyzed against 50 volumes of the same equilibrium solution for 3 hr in a cold room at 4°C. After dialysis the inner solution was spun down again at 1,000 × g for 10 min, and the clear supernatant was used as the enzyme extract from the mitochondrial fraction.

Assay of Enzyme Activity. Thymidine kinase activity was measured by the procedure of Bresnick and Karjala (6) with slight modifications. The incubation medium was composed of 0.4 μCi (4.0 nmoles) of [3H]thymidine, 1.2 μmoles of ATP, 1.2 μmoles of MgCl₂, 1.4 μmoles of 3-phosphoglyceric acid, 20 nmoles of 2-mercaptoethanol, 4.0 μmoles of NaF, and 0.1 ml of enzyme extract in a total volume of
 Separation of Thymidine Kinases by DEAE-cellulose Chromatography. In the cytoplasmic fraction from the spleens of mice infected with Friend virus, at 2 weeks after infection, thymidine kinase activity was separated into 2 peaks by DEAE-cellulose column chromatography. The 1st peak, P-I, was eluted with 0.05 M NaCl in Tris-HCl buffer (pH 8.0) containing 1.0 mM 2-mercaptoethanol, and the 2nd peak, P-II, was eluted with 0.4 M NaCl concentration in the same buffer after 2 steps of different elution conditions. On the other hand, the P-II peak of thymidine kinase could not be detected in the cytoplasmic fraction from normal mouse spleen under the same conditions (Chart 1A).

The injection of 1-acetyl-2-phenylhydrazine can lead to severe hemolytic anemia and a splenomegaly in mice and rats. Hematopoiesis in the spleen of animals given the drug was a compensatory regeneration to supply the loss of blood corpuscles. At this time, the synthesis of thymidine kinase was also induced by the same treatment. The time course of this induction of the enzyme was quite similar to the case of Friend virus disease (36). Consequently, comparison of the molecular species of thymidine kinase in both cases was carried out on the DEAE-cellulose column chromatography. As shown in Chart 1C, there were several
peaks of thymidine kinase activities on the chromatogram when the mouse was treated with 1-acetyl-2-phenylhydrazine. Two of them corresponded to the P-I and P-II peaks, which were observed in the extract from the spleens of mice infected with Friend virus (Chart 1B). In addition, an extra peak, P-IIb, was found widely distributed between 0.075 M and 0.1 M NaCl in the eluate.

Hydroxylamine and aniline have been known to cause hemolytic anemia in animals and these chemicals were also tested to see whether they were able to induce the P-II peak of thymidine kinase as does 1-acetyl-2-phenylhydrazine. The spleen of mice given injections of either of these chemicals was enlarged, and their thymidine kinase activity was also induced to remarkably higher levels than those of normal animals (Tables 1 and 2). Although the enzyme activity on DEAE-cellulose column chromatography was separated into 2 peaks, the P-II fraction detected was low in activity and the thymidine kinase corresponding to P-IIb, which was induced by 1-acetyl-2-phenylhydrazine, was not present in a large amount when the animals were treated with hydroxylamine or aniline.

The frequent appearance of the P-IIb fraction seemed to be influenced by the procedure of preparation and extraction of the crude enzyme that could be associated with other cellular components. To confirm this assumption, the crude extract that had been shown to contain the P-IIb fraction was treated with RNase, DNase I, or Triton X-100 and then chromatographed on the DEAE-cellulose column. The P-IIb fraction disappeared by the pretreatment with DNase I or Triton X-100, while other fractions, P-I and P-II, were found to change only slightly, increasing their activities. This result suggested that P-IIb might be a loose complex of thymidine kinase, probably that of P-II with DNA. We have not yet analyzed the changes of P-IIb at the molecular level, although the active peak (P-IIb) was shifted into the position of P-II by the addition of either DNase I or Triton X-100 in vitro.

The activity of thymidine kinase in the cytoplasm of mouse liver was in general low, and only 1 peak of P-I was found, even when the activity was increased by the regenerating or fetal state. As an attempt to induce P-II or P-IIb of thymidine kinase, probably that of P-II with DNA, we have not yet analyzed the changes of P-IIb at the molecular level, although the active peak (P-IIb) was shifted into the position of P-II by the addition of either DNase I or Triton X-100 in vitro.

The thymidine kinase in the cytoplasm of mouse liver was eluted with 0.4 M NaCl in Tris-HCl buffer (Chart 2). On DEAE-cellulose column chromatography, mitochondrial thymidine kinase was identical with P-II in the cytoplasm from tumor tissue. In spite of the high activity of mitochondrial thymidine kinase, P-I peak was not observed in 0.05 M NaCl eluate from DEAE-cellulose column. This result seems to eliminate the possibility of contamination of any thymidine kinase from cytoplasm in the mitochondrial fraction.

The experiment with the enzyme preparation extracted with the buffer without Triton X-100 indicated that mitochondrial thymidine kinase was not fully extracted. When the extract was fractionated with a DEAE-cellulose column, a low and broad peak of thymidine kinase was eluted with 0.075 M NaCl (P-IIb). This fact suggested that mitochondrial thymidine kinase might be bound to certain mitochondrial components.

End Product Inhibition. Shiosaka et al. (34) reported that thymidine was phosphorylated by nucleoside phosphotransferase using ADP or AMP as a phosphate donor. Furthermore, in the presence of ATPase and nucleoside phosphotransferase in the enzyme preparation, TMP was produced in the reaction mixture containing ATP and thymidine, even if the thymidine kinase was absent. Such a reaction by ATPase and nucleoside phosphotransferase is apparently similar to the phosphorylation of thymidine by thymidine kinase. However, thymidine kinase was inhibited by dTTP, which is an end product in the process of thymidine phosphorylation (5, 6, 8, 9, 18, 22, 29, 40), but the reactions by ATPase and nucleoside phosphotransferase were not inhibited by the end product. Examinations were made on the inhibitory effect of dTTP on the active peak of P-I and P-II from cytoplasm of the spleens from mice treated with 1-acetyl-2-phenylhydrazine, and also on the P-II peak of mitochondrial thymidine kinase from normal liver. As shown in Chart 3, all the fractions were inhibited by the end product, dTTP, but P-II of mitochondria was inhibited much less than the others. In this experiment, all the active fractions were found to contain no detectable nucleoside phosphotransferase activity, using AMP, UMP, TMP, and dCMP as phosphate donors. These results indicated that thymidine-phosphorylating activities in the P-I and P-II fractions from the cytoplasm and the P-II fraction from mitochondria were due to catalysis by the true thymidine kinase, but P-II from the former was different from the latter with respect of the inhibitory behavior.

Thymidine Kinases of Rat Tissues. The thymidine kinases in different species of animals or in some viruses (21, 25) are not identical and can be separated by electrophoresis. However, the enzymes originating from rats and mice were not distinguishable by the elution patterns in DEAE-cellulose column chromatography. In the cytoplasmic extract from Yoshida sarcoma 5 days after inoculation, there were 2 peaks of thymidine kinase activities on the chromatogram, and only the peak of thymidine kinase at the position of P-I could be detected in the preparation from regenerating liver tissue. This observation agrees with the results by Hashimoto et al. (15). Appearance of the P-II peak by the administration of 1-acetyl-2-phenylhydrazine to rats was quite similar to the mouse experiments. The presence of
### Table 2

Summary of total activities of the thymidine kinase and total protein contents obtained from various enzyme sources

Values were calculated from a number of experiments as shown in Column 3. Thymidine kinase was assayed as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Enzyme source, treatment and tissue</th>
<th>Animal</th>
<th>No of experiments</th>
<th>Crude extract</th>
<th>Peak I</th>
<th>Peak II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total activity (nmol)</td>
<td>Protein (mg)</td>
<td>Total activity (nmol)</td>
</tr>
<tr>
<td>Normal, liver</td>
<td>Mouse</td>
<td>3</td>
<td>9.09 ± 1.36</td>
<td>245.0 ± 32.2</td>
<td>6.19 ± 1.92</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>3</td>
<td>10.61 ± 0.24</td>
<td>288.7 ± 46.2</td>
<td>ND</td>
</tr>
<tr>
<td>Fetal liver</td>
<td>Mouse</td>
<td>3</td>
<td>171.19 ± 16.22</td>
<td>296.8 ± 35.5</td>
<td>188.68 ± 47.17</td>
</tr>
<tr>
<td>Regenerating liver</td>
<td>Mouse</td>
<td>5</td>
<td>243.76 ± 72.2</td>
<td>213.5 ± 3.9</td>
<td>179.32 ± 17.21</td>
</tr>
<tr>
<td>Yoshida sarcoma</td>
<td>Rat</td>
<td>6</td>
<td>473.67 ± 1.73</td>
<td>304.5 ± 15.3</td>
<td>274.31 ± 20.30</td>
</tr>
<tr>
<td>Normal, spleen</td>
<td>Mouse</td>
<td>2</td>
<td>321.15 ± 7.07</td>
<td>68.6 ± 9.3</td>
<td>261.24 ± 24.89</td>
</tr>
<tr>
<td>Friend virus-infected, spleen</td>
<td>Mouse</td>
<td>3</td>
<td>98.54 ± 10.82</td>
<td>481.3 ± 43.3</td>
<td>93.10 ± 23.28</td>
</tr>
<tr>
<td>1-Acetyl-2-phenyldrazine-injected, spleen</td>
<td>Mouse</td>
<td>3</td>
<td>221.90 ± 27.21</td>
<td>285.1 ± 43.7</td>
<td>358.42 ± 36.10</td>
</tr>
<tr>
<td>Hydroxylamine-injected, spleen</td>
<td>Mouse</td>
<td>4</td>
<td>251.17 ± 4.98</td>
<td>514.0 ± 20.6</td>
<td>409.18 ± 81.02</td>
</tr>
<tr>
<td>Aniline-injected, spleen</td>
<td>Mouse</td>
<td>2</td>
<td>180.31 ± 6.21</td>
<td>525.4 ± 15.8</td>
<td>297.82 ± 32.76</td>
</tr>
<tr>
<td>Normal, liver mitochondria</td>
<td>Rat</td>
<td>3</td>
<td>127.31 ± 26.75</td>
<td>788.0 ± 142.9</td>
<td>0.00</td>
</tr>
</tbody>
</table>

a In all experiments the total weight of collected wet tissue was 3.5 ± 0.2 g.
b Crude extracts were prepared as described in "Materials and Methods."
c Peaks I and II were shown in the values calculated from the DEAE-cellulose column chromatographic determination.
d Total activities were calculated by conversion to the amount of product, nmol of TMP, from a given cpm (1 nmol = 1250 cpm) in total amounts of a fraction.
\[ \text{Total proteins in extract or eluted fraction of peak} \]
\[ \times \text{average S.D.} \]
\[ \text{ND} \] not done.
a Fetal liver was obtained from 7 different female mice.
h Yoshida sarcoma was grown in an ascites fluid. Cells were collected and washed with 0.85% NaCl solution by low-speed centrifugation. Three ml of cell pellet were applied to the enzyme preparation.
i Eighty g wet weight of the liver was used to prepare the mitochondrial fraction for an experiment.
ties are expressed by cpm $\times 10^{-3}$ of products, phosphorylated [~H]TMP (1 nmole of product was equivalent to 1250 cpm).

The final preparation was 25 ml of enzyme extract containing 37.5 mg protein per ml. The amount of the enzyme extract loaded to the column was 836 mg protein per ml. The chromatographic conditions and the assay of enzyme activities in each fraction were as described for Chart 1. The activities are expressed by cpm $\times 10^{-3}$ of products, phosphorylated [H]TMP (1 nmole of product was equivalent to 1250 cpm).

DISCUSSION

Hashimoto et al. (14, 15) and Okuda et al. (33) reported that there were 2 forms of thymidine kinases in tumor tissues when the crude extract was analyzed by electrophoresis or DEAE-cellulose column chromatography. Their results seemed to suggest strongly the presence of a tumor-specific enzyme. Our experiments confirmed these phenomena, and thymidine kinases in cytoplasmic extracts from Yoshida sarcoma and spleens of mice infected with Friend virus were separated into at least 2 active fractions according to Hashimoto’s chromatographic procedures.

However, the 2nd peak, P-II, which was eluted with 0.4 M NaCl in buffer on the DEAE-cellulose column was found not only in tumor tissue itself and in the spleens of mice infected with oncornavirus but also in normal hepatic mitochondria and in the spleens of mice and rats administered 1-acetyl-2-phenylhydrazine, hydroxylamine, or aniline. These chemicals produced severe hemolytic anemia in the animals and induced a net synthesis of thymidine kinase at a high rate, but they have never been shown to induce malignant transformation. From these observations, appearance of the 2nd peak of thymidine kinases seems not specific for tumors, and a question arises why P-II was not found generally in normal tissues.

One possible reason is the presence of a “spare enzyme” that is masked normally by a certain regulation of the gene expression and is not expressed by the genome throughout the normal life-span of the cells. Nevertheless, this spare enzyme is expressed in emergencies. Since the P-II fraction was not detected in regenerating or fetal liver, the condition of release from regulation may be extremely specific, such as mobilization of young RBC into the peripheral blood stream induced after the administration of 1-acetyl-2-phenylhydrazine. The appearance of P-II in tumor tissue may be due to the gene expression of spare enzyme corresponding to the above extremely specific state.

There is also the possibility of the presence of a tumor-specific enzyme in the P-II fraction, if different molecular species of thymidine kinases are eluted at the same 2nd peak on DEAE-cellulose column chromatography. To test this possibility we need to use other separation methods or characterizations of the enzyme by immunological methods or enzyme kinetics.

Bresnick and Thompson (8), and Bresnick et al. (9), have reported that there were 2 kinds of thymidine kinases in the Walker sarcoma and that they were composed of associated and dissociated forms, depending on the ionic strength of the solution. Toide et al. (38) suggested that thymidine kinase in crude extract was associated with some RNA as a modifier, which could be digested by RNase. We also carried out experiments to confirm the presence of the modifier by treating P-I and P-II fractions with RNase and the result did not support their suggestion at all. On the other hand, in the parallel experiments, treatment of the cytoplasmic extract with DNase I or Triton X-100 did not result in any changes in the profiles of P-I and P-II elution patterns in chromatography, but the P-IIb fraction disappeared after the treatment with either of them, and the P-II fraction increased slightly at the same time. We think that these treatments do not effect the activity of P-IIb itself and just effect the dissociation of P-IIb and the chromatographic behavior. These results supported the assumption that P-IIb might be a complex form of thymidine kinase and DNA.

Taylor et al. (37) also separated 2 kinds of thymidine kinase from cultivated human cells, KB or HeLa cells, by polyacrylamide gel electrophoresis. They showed that there...
were 2 types, adult and fetal thymidine kinase molecules. The result of our experiment along lines similar to those of Taylor et al. showed the presence of only 1 peak of the P-I fraction in fetal liver of mice. This discrepancy in results must be clarified by further experiments, but it might be suggested that the fetal type of enzyme, according to Taylor's observation, if present, should be eluted at the same position as the P-I fraction in DEAE-cellulose column chromatography. If that is the case, P-II of thymidine kinase should be completely independent of the fetal enzyme.

Recently, Kit et al. (21–26) have demonstrated that thymidine kinase in mitochondria can be separated into 2 or 3 bands by polyacrylamide gel electrophoresis in a condition for the enzyme assay. They concluded that 2 or 3 thymidine kinases were involved in mitochondrial fraction, that 1 of them might be peculiar to mitochondrion itself, and that the others might be derived from the cytoplasmic fraction. In our experiment, a large amount of mitochondria was collected and subjected to assay in order to detect the enzyme activity, because the normal liver of mice or rats has very low activity of the enzyme in the cytoplasm, similar to TK cells that had only 1 band on the gel, according to Kit et al. (25). The mitochondrial thymidine kinase was eventually found to contain only 1 peak of P-II fraction in DEAE-cellulose chromatography. Electrophoretic studies on P-II fractions from our preparations are under way in our laboratory to confirm the results of Kit et al. (24).

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Two Forms of Thymidine Kinase in Normal and Tumor Tissues of Animals

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