STUDIES ON VITAMIN B₆ METABOLISM OF CANCER CELLS AND TUMOR-BEARING RAT LIVER
I. ENZYME ACTIVITIES RELATED TO VITAMIN B₆ METABOLISM

Koji Ito, Isao Nakahara, and Yukiya Sakamoto
(Institute for Cancer Research, Osaka University Medical School)

Synopsis

The pyridoxal phosphate content of the liver of tumor-bearing rats decreased steadily after ascites hepatoma (AH-130) cells were inoculated into the animals intraperitoneally. However, pyridoxal phosphate content of total cancer cells in the rat increased as the tumor cells proliferated rapidly. The activities of both pyridoxal kinase and pyridoxine phosphate oxidase decreased in the liver of tumor-bearing rats. This decrease in pyridoxine phosphate oxidase activity was restored to the normal level by the addition of a coenzyme, flavin mononucleotide, either in vivo or in vitro. Livers of rats fed on a non-protein diet showed as low a level of pyridoxal kinase activity as those of tumor-bearing rats. These results showed that the decrease of pyridoxine phosphate oxidase activity in the liver of tumor-bearing rats was due to a decrease in the concentration of its coenzyme, flavin mononucleotide. The decrease in pyridoxal kinase activity in the early stage of tumor formation was a factor causing the depletion of pyridoxal phosphate in the liver of tumor-bearing rats.

Both pyridoxal kinase and pyridoxine oxidase activities of tumor cells were about one-half of the activities of normal rat liver as calculated per gram of wet tissue.

INTRODUCTION

In a previous paper, it was reported that pyridoxal phosphate synthesis via pyridoxine phosphate was more important physiologically than via pyridoxal, because the activity of pyridoxine oxidase was very weak and pyridoxine phosphate oxidase was the key enzyme in the synthesis of pyridoxal phosphate in the animals tested.

In the present work, the content of pyridoxal phosphate and activities of related enzymes in the liver of tumor-bearing rats and tumor cells (AH-130) was compared with that in normal rat liver.
EXPERIMENTAL MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 150 to 200 g were used in all the experiments. Rats were fed on normal chow (Oriental Yeast Co.) except for the animals on a non-protein diet which were given food containing 28.34% sucrose, 58% dextrin, 2% cellulose powder (Toyo Roshi), 4% salt mixture, 0.66% choline chloride, 5% vitamin mixture, and 2% oil.

The content of pyridoxal phosphate was determined with apo-tryptophanase obtained from E. coli strain K12. Pyridoxal kinase and pyridoxine phosphate oxidase were measured by the method of Hurwitz et al. and of Ichihara et al., respectively.

Pyridoxine phosphate was synthesized by the method of Heyl et al. and purified by the method of Peterson and Sober. Pyridoxal, pyridoxal phosphate, and flavin adenine dinucleotide were kindly provided by Wakamoto Pharmaceutical Co. and pyridoxine by Daiichi Pharmaceutical Co., Tokyo.

RESULTS

The pyridoxal phosphate content of the liver of tumor-bearing rats decreased steadily after the inoculation of AH-130 cells into the animals. The pyridoxal phosphate content of tumor cells per gram of wet tissue was about one-third of that of normal rat liver but the total pyridoxal phosphate of total tumor cells increased greatly and there was much cell proliferation, as shown in Fig. 1.

Fig. 1. Pyridoxal phosphate content of liver of tumor-bearing rats and tumor cells

Change in pyridoxal phosphate content of liver of tumor-bearing rats and tumor cells after AH-130 inoculation (0.5 ml of AH-130 ascites fluid).
- O - Pyridoxal phosphate content of liver of tumor-bearing rats.
- - - Pyridoxal phosphate content of tumor cells.
- - - Pyridoxal phosphate of whole tumor cells in rat.

Pyridoxal phosphate content was determined as µg/g wet tissue, except that of total tumor cells in rat. The values are expressed as the average of 10 estimations.

374
The pyridoxine phosphate oxidase activity of the liver of tumor-bearing rats showed a diphasic change, increasing in the early stage and decreasing subsequently, as shown in Fig. 2.

This decrease in pyridoxine phosphate oxidase activity was completely restored to the normal level \textit{in vivo} by injection of 400 \(\mu\)g of flavin mononucleotide, as shown in Fig. 2. Therefore, the decrease in pyridoxine phosphate oxidase activity in the liver of tumor-bearing rats is due to a decrease in the content of its coenzyme, flavin mononucleotide. The pyridoxal kinase activity of the liver of tumor-bearing rats

![Fig. 2. Pyridoxine phosphate oxidase activity of tumor-bearing rats and tumor cells](image)

![Fig. 3. Pyridoxal kinase activity of liver of tumor-bearing rats and tumor cells](image)
decreased rapidly from soon after tumor inoculation, as shown in Fig. 3.

This decrease of pyridoxal kinase activity might be due to a decrease in the content of apo-protein, because the activity was measured with co-factor, adenosine triphosphate and Mg\(^{2+}\), and the liver of rats fed on a non-protein diet and of fasting rats showed the same pattern of change of pyridoxal kinase as shown in Table I.

Table I. Pyridoxal Phosphate Content and Related Enzyme Activity in the Liver of a Rat fed on a Protein-free Diet and of a Fasting Rat

<table>
<thead>
<tr>
<th></th>
<th>Normal rat liver</th>
<th>Liver of rat fed on protein-free diet for 10 days</th>
<th>Liver of rat fasted for 3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of estimations</td>
<td>30</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Pyridoxine phosphate oxidase activity ((\mu)moles/30 min./20 mg tissue)</td>
<td>4.0 (3.5~5.0)</td>
<td>7.8 (6.8~8.4)</td>
<td>4.8 (4.3~5.2)</td>
</tr>
<tr>
<td>Pyridoxal kinase activity ((\mu)moles/30 min./20 mg wet tissue)</td>
<td>1.4 (0.9~2.0)</td>
<td>0.63 (0.6~0.7)</td>
<td>1.0 (0.8~1.1)</td>
</tr>
<tr>
<td>Pyridoxal phosphate content ((\mu)g/g wet tissue)</td>
<td>4.5 (3.3~6.7)</td>
<td>4.7 (4.3~5.1)</td>
<td>4.5 (4.1~4.8)</td>
</tr>
</tbody>
</table>

The range of experimental values are given in parentheses.

Therefore, it was concluded that one factor causing a decrease in the pyridoxal phosphate content of the liver of tumor-bearing rats was a decrease in the activities of enzymes related to vitamin B\(_6\) and especially of pyridoxal kinase which has a great influence on vitamin B\(_6\) metabolism from the early stage in the liver of tumor-bearing rats.

In tumor cells, the activities of both these enzymes were about one-half that of normal rat liver.

The pyridoxal phosphate content of the liver of fasting rats and of rats fed on a non-protein diet was normal when calculated as per gram of wet tissue but livers of these animals showed marked atrophy. Therefore, the total pyridoxal phosphate of the whole liver decrease in these animals.

**DISCUSSION**

As previously noted, one cause of the pyridoxal phosphate decrease in the liver of tumor-bearing rats was the decrease in the activities of enzymes related to vitamin B\(_6\). In addition to the decrease in these enzyme activities, pyridoxal phosphate in the liver of tumor-bearing rats might be taken up by tumor cells because the
pyridoxal phosphate content of whole tumor cells increased although tumor cells synthesized pyridoxal phosphate poorly because of their lower enzyme activities than that of rat liver.

As mentioned above, pyridoxine phosphate oxidase is the key enzyme in the metabolism of vitamin B₆ in the animal liver and there is a parallel between the pyridoxine phosphate oxidase activity and pyridoxal phosphate content. In contrast to normal liver, pyridoxal kinase is the limiting enzyme on the synthesis of vitamin B₆ in the liver of tumor-bearing rats because this enzyme quickly responds from early stages to the conditions causing internal protein deficiency such as fasting or a non-protein diet. Therefore, the decrease in the activity of pyridoxal kinase in the liver of tumor-bearing rats may be caused by internal protein deficiency.

**CONCLUSION**

1) The pyridoxal phosphate content of the liver of tumor-bearing rat decreased steadily after AH-130 cells had been inoculated into the animals intraperitoneally.
2) However, pyridoxal phosphate content of total cancer cells in the rat increased.
3) The activities of both pyridoxal kinase and pyridoxine phosphate oxidase decreased in the liver of tumor-bearing rats.
4) The decrease of pyridoxine phosphate oxidase activity in the liver of tumor-bearing rats was due to a decrease in concentration of its coenzyme, flavin mononucleotide.
5) The decrease of pyridoxal kinase activity in the liver of tumor-bearing rats may be caused by the internal protein deficiency from the early stage of tumor-bearing.
6) Both pyridoxal kinase and pyridoxine phosphate oxidase activities of tumor cells were about one-half of the activities of normal rat liver as calculated per gram of wet tissue.

This work was supported by a Grant-in-Aid of Scientific Research from the Ministry of Education.

*(Received June 17, 1964)*
REFERENCES