CHAPTER II

STUDIES ON GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN EHRlich
ASCITES CARCINOMA CELLS AND IN HOST'S LIVER
Glucose-6-phosphate dehydrogenase (G-6-PD) attracted the attention of several workers in the field of cancer research (Glock and Mclean, 1954; Van Vals et al., 1956; Williams-Ashman, 1953). Hershey et al. (1956) found an increase in G-6-PD activity in breast cancer. Wu and Homburger (1969) reported that the activity of G-6-PD increased with the growth of the Novikoff hepatoma. An increase in G-6-PD activity has also been observed in induced hepatocarcinoma (Rubenchik, 1969; Jacob, 1970 and Birk et al., 1976) and in transplanted hepatoma (Guenter, 1970). We have also observed an increase in G-6-PD activity in parallel with the growth of Ehrlich ascites carcinoma (EAC) (Chapter I).

Keeping in view the above mentioned findings as well as cancer being a metabolic disease, it was considered worthwhile to study the behaviour of this enzyme in liver during carcinogenesis in mice bearing fast-growing EAC and slow-growing methylcholanthrene (MC) induced carcinoma. It has also been decided to compare the behaviour of this enzyme in Ehrlich ascites carcinoma with that of the host's liver.

RESULTS

It can be seen from Fig. 3, that the G-6-PD activity in liver increases significantly with the development of the tumour. Within 4th day after transplantation, when the tumour
Fig. 3. Liver G-6-PD activity of Ehrlich ascites carcinoma bearing mice.
was not even perceptible, the enzyme activity increased by about 40% above the enzyme activity of the normal liver. The increase was very rapid between 2nd and 4th day and was about 80% on 10th day.

In case of slow growing induced carcinoma, liver G-6-PD showed 27% increase in activity over that of the control liver after one month (Table II). Incidentally, papilloma was not found at all during this period. Consequently, with the development of carcinoma, activity increased to about 60% on 3rd month. The results showed that during the course of epidermal carcinogenesis, the enzyme activity in liver increased and reached a higher level than that found in the liver of benzene-painted mice.

Hence, some antitumour agents were used to study their effect on tumour growth as well as on the enzyme activity. It can be observed from Table-III that the administration of mitomycin-C to the transplanted mice caused a regression of ascites tumour without any change in enzyme activity in liver. With rifampicin, however, the liver enzyme activity increased progressively along with the growth of the tumour.

Administration of mitomycin-C and rifampicin thus reveals a definite tumour-host relationship as regard to liver G-6-PD activity. Hence, the behaviour of host's liver G-6-PD was compared with that of the toluene-treated Ehrlich ascites cells.
Table II
Liver G-6-PD activity in 20-methylcholanthrene(MC) treated mice.

<table>
<thead>
<tr>
<th>Days of treatment (before treatment)</th>
<th>Weight of the liver (mg)</th>
<th>Specific activity (µmoles of NADP⁺ reduced/minute/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal mice</td>
<td>Benzene painted mice</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>700</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>950</td>
</tr>
</tbody>
</table>

Values are an average of three experiments.
### Table III
Effects of Mitomycin-C and Rifampicin on EAC-bearing mice.

<table>
<thead>
<tr>
<th>Days after transplantation</th>
<th>Packed cell volume (ml)</th>
<th>Specific activity (μmoles of NADP^+ reduced/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nontreated</td>
<td>Mitomycin-C treated</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------</td>
<td>---------------------</td>
</tr>
<tr>
<td>1</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>9</td>
<td>2.5</td>
<td>Nil</td>
</tr>
<tr>
<td>11</td>
<td>3.0</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Each value represents the average of three experiments.
pH Optima:

G-6-PD activities of ascites cells as well as host's liver at different pH are shown in Fig.4. Enzyme preparation from normal and host's liver showed peak at pH 8.0, whereas, the pH optima of ascites cell preparation was pH 7.5. It is also evident from the figure that the specific activity of the enzyme is much higher in tumour cells than that of the host's liver.

Thermal Stability:

Heat inactivation studies of enzyme preparations were carried out at 45°C and 55°C according to Messina et al. (1972). Enzymes in all preparations were inactivated at 55°C. It can be observed from Fig.5 that at 45°C, the host's liver enzyme retains about 60% of the initial activity at the end of 10 minutes. Whereas, it is evident from the figure that the tumour cell enzyme seems to be more heat labile.

Michaelis-Menten Constants:

In case of host's liver, Michaelis constant (Km) for G-6-P at 0.15 mM NADP⁺ concentration was 1.4 x 10⁻⁶ M which is similar in magnitude with that of the normal liver (2x10⁻⁶ M). Whereas, in ascites cells, the Km for G-6-P is significantly lower and is 5.7x10⁻⁸ M (Fig.6). G-6-P ranging from 0.1 mM to 1.5 mM and 0.01 to 0.5 mM was used for the liver enzymes and the EAC enzyme respectively.
Fig. 4. Activity of G-6-PD with respect to pH.
Fig. 5. Thermal stability studies of G-6-PD.
Fig. 6. Substrate concentration curves of G-6-PD.

Insets represent double reciprocal plot from where Kms are calculated.
The $K_m$ value of NADP$^+$ for normal and host's liver at 1.0 mM G-6-P is $1 \times 10^{-3}M$ and $7.7 \times 10^{-4}M$ respectively. While for ascites cells, the $K_m$ for NADP$^+$ at 0.1 mM concentration of G-6-P is $1.1 \times 10^{-4}M$ (Fig. 7). NADP$^+$ ranging from 0.015 to 0.21mM was used for the assays.

**Electrophoretic Mobility:**

Enzyme preparation from tumour cells and both normal and host's liver demonstrated one band on activity staining with same mobility on 5.5% polyacrylamide gel at pH 8.3. The band is more distinct in host's liver preparation in comparison to that of the toluene-treated ascites cells, as well as the normal liver.

**DISCUSSION**

It is well known that the tumour tissues have a high rate of aerobic glycolysis (Warburg, 1930). Hexose monophosphate shunt being an oxidative pathway is also higher in tumours (S.Kitt, 1956). The activity of G-6-PD, the enzyme of the shunt pathway, has been found to be increased in different types of induced and transplanted tumours (Birk et al., 1976; Livini et al., 1975; Hecker 1977).

Because of its location at a branch point in glucose metabolism and also because it is one of the few enzymes catalyzing the formation of the reduced form of NADP$^+$ from NADP$^+$, the regulation of this enzyme is of considerable
Fig. 7. NADP$^+$ concentration curves of G-6-PD. Insets represent double reciprocal plot from where Kms are calculated.
importance. Moreover, the activity of G-6-PD in some mammalian tissues show wide variations under conditions of altered metabolism which are correlated with the altered demand for NADPH or supply of NADP⁺.

On the basis of the above findings, it was thought to investigate the behaviour of this enzyme in host's liver during progression of the tumour. The fast growing Ehrlich ascites carcinoma and slow-growing methylcholanthrene-induced carcinoma were selected for the study, because any definite change in enzyme activity in both the cases will show how tumour growth affects the enzyme activity of the host's liver, in general.

The data obtained under these two conditions, show a definite increase in G-6-PD activity in host's liver when tumours were still very small. The increased level of activity was very significant when the tumour attained its maximum size. The packed cell volume can be taken as a measure of the growth of Ehrlich ascites tumour. The increase in enzyme activity in liver of benzene-painted animal may be due to some toxic effect. But this increase in activity is much less in comparison to that of the methylcholanthrene-painted mice.

Experiments with rifampicin and mitomycin-C show the effect of tumour growth on the enzyme activity in liver. The reason for rifampicin not having any inhibitory effect on the
growth of tumour could be due to the fact that a much higher dose of the antibiotic is needed for the inhibition of tumour growth (Sagiura, 1960). It could also be the reason that rifampicin is not very much effective in eukaryotic cells.

It can thus be concluded that the tumour development has definite correlation with liver G-6-PD activity, which is quite significant from the early stage of malignancy.

The comparative study of G-6-PD activity of Ehrlich ascites carcinoma cells with that of the host's liver, shows that the specific activity of G-6-PD is much higher in toluene-treated ascites cells in comparison to that of the host's liver. It may also be mentioned here that although the enzyme from EAC has a much higher affinity for G-6-P than the liver enzymes, it is much susceptible to G-6-P. This enzyme is significantly inhibited by G-6-P at a concentration higher than 0.6 mM whereas the liver enzymes (both normal and host's) are not inhibited even at a concentration of 1.5 mM (data not shown). This means that the EAC enzyme shows substrate inhibition at high concentration whereas the liver enzymes do not.

Although the proteins applied on the polyacrylamide gel for electrophoresis were almost equal, the low activity as shown by the intensity of colour in EAC cell enzyme may be due to partial inactivation of the enzyme. This inactivation may be due to the heat generated during electrophoresis as it has been already indicated in Fig.5 that EAC cell enzyme is more
labile to heat.

An attempt to compare the behaviour of this enzyme in liver and ascites cells, reveals a marked difference in thermal stability and pH optima. Moreover, the tumour G-6-PD shows higher affinity for the substrate than that of the liver enzyme.

It can thus be concluded that host's liver G-6-PD, the activity of which is proportional to the growth of both the slow-growing MC-induced carcinoma and fast-growing Ehrlich ascites carcinoma, behaves like the normal liver enzyme and thus differs significantly from the G-6-PD of toluene-treated ascites cells.