Glyoxalase Activity of Erythrocytes from Cancerous Rats and Human Subjects*

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The demonstration of a striking decrease in glyoxalase activity of liver from rats fed p-dimethylaminoazobenzene (1) suggested the possibility that other tissues rich in glyoxalase might be similarly affected. Since the glyoxalase activity of erythrocytes is very high (2, 3) it seemed of interest to determine the activity of this enzyme system in rats fed the compound just mentioned. Since a previous study (1) suggested that the greatest decrease in glyoxalase activity was associated with the extent of neoplasia, erythrocytes from human subjects with and without malignant neoplastic disease were also studied. While no significant variation from the normal was observed, some points of interest are suggested by the findings.

METHODS

Preparation of Erythrocytes

The glyoxalase activity of erythrocytes was estimated after hemolysis, using essentially the technic of Quastel (3). Blood was collected in 2 per cent sodium citrate solution (0.18 ml. per 1 ml. of blood). An aliquot was taken for hematocrit determination. After centrifugation and removal of the supernatant layer, the cells were suspended in saline and again centrifuged down. The supernatant layer was again decanted and 9 volumes of distilled water were added to lyse the cells. An aliquot of the 1:10 solution was then diluted with 4 volumes of 1.3 per cent sodium bicarbonate solution to give a final dilution of 1:50.

Estimation of Glyoxalase Activity

0.5 ml. of the 1:50 solution of lysed erythrocytes was placed in the side arm of Warburg flasks. The main compartment contained 1 ml. of methylglyoxal solution (1.2 mgm.) and 0.5 ml. of glutathione solution (2.0 mgm.) The final concentration of the former was 0.0083 M and of the latter 0.0035 M. This concentration of glutathione was established to be the optimum for the experimental conditions employed. Control flasks contained 0.5 ml. of 1.3 per cent sodium bicarbonate solution in place of the glutathione. All experiments were run anaerobically, the flasks being filled with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. The manometric procedures were otherwise the same as those employed for the estimation of liver glyoxalase activity (1). The rates of carbon dioxide production were found to be linear for 20 to 30 minutes, after which the rates started to fall off. The first 5 minute reading was disregarded. Glyoxalase activity is expressed as the microliters of carbon dioxide produced in 20 minutes. Approximately 50 per cent of the methylglyoxal was converted to lactic acid during this interval.

RESULTS

The glyoxalase activity of erythrocytes from normal and tumor-bearing rats is shown in Table I. As can be seen from the table, the glyoxalase activity of erythrocytes from the tumor-bearing animals falls within the same range as that found with normal animals. The animals with induced tumors had all been on a p-dimethylaminoazobenzene diet for 190 days, and the growths were all large. The transplanted neoplasms were large also, and had undergone some degeneration; the animals bearing them were in particularly bad shape, which probably accounts for the extremely low hematocrit values found in this group.

The glyoxalase activity of erythrocytes from normal human subjects and from patients with neoplastic and

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** CO2 formed in 20 min. per ml. of whole blood

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Table 1: Glyoxalase Activity of Erythrocytes from Normal and Tumor-Bearing Rats

<table>
<thead>
<tr>
<th>Animal</th>
<th>Hematocrit</th>
<th>R.B.C. whole blood</th>
<th>aCO2 formed in 20 min. per ml. of whole blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>45.0</td>
<td>204</td>
<td>92.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>42.5</td>
<td>198</td>
<td>84.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>43.0</td>
<td>210</td>
<td>90.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>39.1</td>
<td>202</td>
<td>79.0</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>34.2</td>
<td>208</td>
<td>71.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>39.3</td>
<td>185</td>
<td>72.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>30.2</td>
<td>201</td>
<td>60.8</td>
</tr>
<tr>
<td>Transplanted hepatoma</td>
<td>25.6</td>
<td>208</td>
<td>53.3</td>
</tr>
<tr>
<td>&quot;</td>
<td>28.9</td>
<td>190</td>
<td>55.0</td>
</tr>
</tbody>
</table>
other diseases is shown in Table II. Here, again, the glyoxalase activity of the erythrocytes shows no striking variation from the normal range.

In both rat and man the glyoxalase activity, expressed on the basis of whole blood, shows considerable variation due to the variation in hematocrit values.

**Table II: Glyoxalase Activity of Erythrocytes from Human Subjects**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Hematocrit</th>
<th>0.01 ml. R.B.C.</th>
<th>0.01 ml. whole blood</th>
<th>C02 formed in 20 min. per</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>45.8</td>
<td>146</td>
<td>67.0</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>41.6</td>
<td>144</td>
<td>60.0</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>48.5</td>
<td>138</td>
<td>67.0</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>39.5</td>
<td>150</td>
<td>59.3</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>41.3</td>
<td>140</td>
<td>57.7</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>49.2</td>
<td>132</td>
<td>65.0</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>51.0</td>
<td>138</td>
<td>70.5</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>46.5</td>
<td>135</td>
<td>63.0</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>44.8</td>
<td>133</td>
<td>59.6</td>
<td></td>
</tr>
</tbody>
</table>

Carcinoma (face) 45.2 138 62.5
Carcinoma (colon) 41.8 134 56.0
Carcinoma (bile duct) 39.6 134 53.2
Carcinoma (lung) 40.5 138 56.0
Carcinoma (pancreas) 37.3 126 47.0
Carcinoma (breast) 39.8 142 56.5
Carcinoma (stomach) 44.5 146 65.0
Carcinoma (rectum) 38.7 140 54.2
Carcinoma (chronic) 36.3 128 45.3
Leukemia, acute 26.9 134 36.0
Leukemia, chronic 35.3 140 48.4
Polycythemia vera 65.1 138 90.0
Pernicious anemia 40.5 126 51.0
Obstructive jaundice 36.1 126 45.5

**DISCUSSION**

The failure to find any significant difference in the glyoxalase activity or erythrocytes from cancer patients as compared with normal persons is perhaps not surprising. On the other hand, the finding of normal values for erythrocytes from animals with p-dimethylaminoazobenzene tumors was somewhat unexpected. These animals had been on the carcinogenic diet for 190 days and the glyoxalase activity of their livers was notably reduced as compared with normal. This finding suggests that either the metabolic intermediates of p-dimethylaminoazobenzene do not reach the blood stream in sufficient concentration to affect the intracellular contents of the erythrocyte, or that the decrease in glyoxalase activity observed in the livers of these animals is secondary to, or concurrent with, the neoplastic changes. Evidence against the former possibility is seen in the study of Miller and his associates (4), where it was demonstrated that certain of the p-dimethylaminoazobenzene intermediates, chiefly p-aminoazobenzene, were present in higher concentration within the erythrocyte than in any other tissue of the rat. It would thus appear that the decrease in glyoxalase activity in livers from rats fed this carcinogen is not due directly to the influence of this substance or its metabolic intermediates.

The physiological significance of a decreased glyoxalase activity on a whole blood basis as seen in cases with low hematocrit values is difficult to assess. In all probability this is without metabolic significance. Until the metabolic role of the glyoxalase system and the glyoxals are more clearly defined, the significance of such findings will continue to remain obscure.

**SUMMARY**

Erythrocytes from patients with neoplastic and other diseases, and erythrocytes from rats with p-dimethylaminoazobenzene hepatomas and transplanted hepatomas showed no significant change in glyoxalase activity as compared with erythrocytes from normal subjects.

**REFERENCES**

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