Comparative Biochemistry of Hepatomas

I. Carbohydrate Enzymes in Morris Hepatoma 5123*

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SUMMARY

A systematic study of the carbohydrate-metabolizing enzyme systems of the Morris hepatoma 5123 was carried out, and results were interpreted in the light of normal liver data and in comparison with previously established values of Novikoff hepatoma.

The cellularity of the Morris tumor was in the same range, whereas the nitrogen content of the average cell was significantly decreased, as compared with that of the control liver.

In the average Morris hepatoma cell glucose-6-phosphatase (56 per cent), phosphohexoseisomerase (66 per cent), lactic dehydrogenase (65 per cent), and 6-phosphogluconate dehydrogenase (50 per cent) decreased to the same extent as the nitrogen level (58 per cent). On the other hand, in relation to the decreased nitrogen content glucose-6-phosphate dehydrogenase activity was preferentially maintained in normal range, whereas phosphoglucomutase (15 per cent) and fructose-1,6-diphosphatase (30 per cent) were preferentially depleted.

A comparison of these enzymes in the two examined liver tumors of differing growth rates showed that the marked increase in glucose-6-phosphate dehydrogenase activity in the Novikoff tumor was not present in the Morris hepatoma. However, another enzymatic lesion of the Novikoff hepatoma (markedly decreased phosphoglucomutase) occurred in Morris hepatoma to a similar extent. On the other hand, some of the enzymes missing in the Novikoff tumor (glucose-6-phosphatase, fructose-1,6-diphosphatase) were present but in diminished activity, manifesting only a partial lesion in gluconeogenesis in the Morris hepatoma.

It appears that the extent of the examined enzymatic lesions may be correlated roughly with the growth rates of the Morris and Novikoff hepatomas.

During hepatic carcinogenesis the liver undergoes extensive morphological and biochemical alterations which culminate in the development of cancerous tissue. Since the various liver tumors differ in histological structure, cellular population, biological behavior, and growth rate, it is expected that the metabolic lesions or alterations which underlie morphological and biological properties will emerge in varying qualitative and quantitative extent. It has been suggested that "such a concept agrees well with common medical experience of finding many variations of the same disease from subclinical through mild or severe manifestations to the rarely encountered, full-blown case in which all symptoms and signs are present to their maximum development" (22).

The most detailed studies on transplantable liver tumors have been carried out on the Novikoff hepatoma, in which a number of enzyme lesions and metabolic alterations have been described (2, 3, 6, 11, 14, 17–20, 22) and correlated (15, 16). Recently enzymatic investigations were conducted on a spectrum of eleven primary and transplantable liver tumors (12, 13). These studies showed...
that the Morris hepatoma 5123 was nearest to normal liver, whereas the Novikoff was least like normal liver. Since the growth period of the Morris tumor is 3 months, in contrast to the Novikoff hepatoma, which kills the host in a week, it appears that a comparison of the enzymatic activities of these two tumors may throw light on the neoplastic significance of the enzymatic lesions in the hepatomas.

The present investigation demonstrated that, in correlation with the slower growth rate of the Morris hepatoma, alterations in the examined glucose-6-phosphate-metabolizing enzymes also occurred to a smaller degree than in the Novikoff hepatoma.

MATERIALS AND METHODS

Animals.—Female, Buffalo-strain rats, weighing 180–250 gm., were used in these experiments. The tumor-bearing and control animals were shipped by air express from Dr. H. P. Morris of the National Cancer Institute, Bethesda, to Indiana University, Indianapolis. The biochemical studies were carried out 3–10 days after arrival of the rats. The transplantable tumor used was the Morris hepatoma No. 5123, subline D, generation 19. The animals were given inoculations on 7/13/60 by trocar intramuscularly in both hind legs. Tumor 5123 was originally induced in female Buffalo-strain rats in 1956 after ingestion of an adequate diet containing N-(2-fluorenyle)phthalamic acid (9). It has been maintained since that time by serial passage into animals of the strain of origin. D represents one of four arbitrarily established sublines made from a tumor in the sixteenth transplant generation. The tumors used in this study were approximately 3 months old when harvested.

The tumor-bearing and control animals were kept in separate cages upon arrival in the Indiana University laboratory. Purina Laboratory Chow and water were available ad libitum.

Preparation of homogenates and supernatant fluid.—The animals were stunned, decapitated, and exsanguinated. Livers and tumors were rapidly removed and blotted on filter paper. Tumors were carefully dissected free of necrotic, hemorrhagic, and nontumorous material. Tissues were chilled in a beaker on cracked ice for 5 minutes, then minced with scissors. Ten percent homogenates were prepared in isotonic KCl. Supernatant fluid was obtained by centrifuging the tissue homogenates at 100,000 × g for 30 minutes at 0° C. A refrigerated Spinco Model L centrifuge was used.

**Determination of cellularity.**—The number of nuclei/gm fresh tissue was counted by the technic described previously (21). Cellularity was expressed in millions of nuclei/gm wet weight of tissue.

**Biochemical procedures.**—Nitrogen content was determined by the micro-Kjeldahl procedure. Glucose-6-phosphatase (G-6-Pase) was measured in the homogenate; all other enzymes were assayed in the supernatant fluid. G-6-Pase was measured according to Cori and Cori (5), as modified by Weber and Cantero (18). Phosphoglucomutase was assayed according to Najjar (10). Phosphohexoseisomerase was measured according to Bruns and Hinsberg (4), as modified by Glock et al. (8). Fructose-1,6-diphosphatase and lactic dehydrogenase were measured according to Weber and Cantero (22). G-6-P dehydrogenase and 6-phosphogluconate dehydrogenase were determined by the method of Glock and McLean (7). All enzymes were assayed under linear kinetic conditions.

Enzymatic activities were calculated as moles of substrate metabolized in 1 hour at 37° C. Enzymatic activities were expressed per nitrogen and per average cell.

RESULTS

Tables 1 and 2 compare the cellularity, nitrogen content, and activities of carbohydrate-metabolizing enzymes in Morris hepatoma and in the liver of control animals.

**Nitrogen content and cellularity.**—When the data are expressed per unit wet weight, the homogenate nitrogen content in the Morris hepatoma was decreased to 54 per cent and supernatant nitrogen content to 69 per cent of control liver (Table 1). The cellularity of the Morris tumor and control liver are in about the same range (Table 2). The average Morris hepatoma cell contained 58 per cent of the nitrogen content of the average control liver cell. The decrease is also reflected in the supernatant nitrogen, which was 73 per cent of the normal liver value (Table 2).

**Enzyme results.**—Enzyme activities are given as specific activities in Table 1. It appears that, when the enzyme data are expressed on a nitrogen basis, there were normal G-6-Pase, phosphohexoseisomerase, lactic dehydrogenase, and G-6-P dehydrogenase activities in hepatoma 5123. On the other hand, 6-phosphogluconate dehydrogenase was 67 per cent, fructose-1,6-diphosphatase was 40 per cent, and phosphoglucomutase was 20 per cent in this tumor, as compared with activities found in control liver. The specific activities of...
G-6-Pase and G-6-P dehydrogenase in the Morris hepatoma were previously reported (12, 13).

Enzyme activities are expressed on an average cell basis in Table 2. This table demonstrates that, when tumor cellularity is taken into consideration, a number of enzymes show statistically significant alterations from the normal values. Attention is also drawn to the fact that certain enzymes are depleted to about the same extent as the total nitrogen content of the average tumor cell. Such a parallelism with the behavior of the nitrogen content which decreased to 58 per cent is exhibited by G-6-Pase (56 per cent), phosphohexoseisomerase (66 per cent), lactic dehydrogenase (65 per cent), and 6-phosphogluconate dehydrogenase (50 per cent). On the other hand, G-6-P dehydrogenase, which shows no statistically significant alteration, was preferentially maintained in the normal range. In contrast, phosphoglucomutase (15 per cent) and fructose-1,6-diphosphatase

### TABLE 1

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Normal liver</th>
<th>Morris hepatoma</th>
<th>Per cent of normal values</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate nitrogen§</td>
<td>34.6 ± 1.2</td>
<td>18.7 ± 0.5</td>
<td>54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Supernatant nitrogen§</td>
<td>17.0 ± 0.4</td>
<td>11.7 ± 0.4</td>
<td>69</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>20.3 ± 0.6</td>
<td>19.5 ± 0.6</td>
<td>96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fructose-1,6-diphosphatase</td>
<td>24.6 ± 0.4</td>
<td>19.9 ± 0.2</td>
<td>40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>173.0 ± 7.6</td>
<td>35.4 ± 5.6</td>
<td>20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phosphohexoseisomerase</td>
<td>812.0 ± 35.8</td>
<td>730.0 ± 19.4</td>
<td>90</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>883.0 ± 23.1</td>
<td>775.0 ± 17.5</td>
<td>88</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydro-</td>
<td>10.3 ± 0.95</td>
<td>11.6 ± 0.9</td>
<td>113</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>genase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Phosphogluconate dehydro-</td>
<td>38.1 ± 2.9</td>
<td>25.6 ± 2.3</td>
<td>67</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>genase</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Activities are expressed in μmoles of substrate metabolized/hour/mg nitrogen at 37°C. Means and standard errors represent 4 or more samples studied.
† Values of normal liver are taken as 100 per cent.
‡ Calculated P value as compared with normal fed animals. When no values are given, the difference is >0.05, which is not accepted as significant.
§ Milligrams of nitrogen/gm wet weight of tissue.

### TABLE 2

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Normal liver</th>
<th>Morris hepatoma</th>
<th>Per cent of normal values</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellularity‡</td>
<td>267.0 ± 4.3</td>
<td>250.0 ± 6.6</td>
<td>94</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Homogenate nitrogen§</td>
<td>1.30 ± 0.05</td>
<td>0.75 ± 0.02</td>
<td>58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Supernatant nitrogen§</td>
<td>0.64 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>73</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>26.3 ± 0.6</td>
<td>14.6 ± 0.4</td>
<td>56</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fructose-1,6-diphosphatase</td>
<td>15.7 ± 0.4</td>
<td>4.7 ± 0.2</td>
<td>30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>110.0 ± 4.3</td>
<td>16.6 ± 1.4</td>
<td>15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Phosphohexoseisomerase</td>
<td>516.0 ± 24.0</td>
<td>342.0 ± 7.2</td>
<td>66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>563.0 ± 17.9</td>
<td>365.0 ± 19.6</td>
<td>65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydro-</td>
<td>6.6 ± 0.6</td>
<td>5.4 ± 0.4</td>
<td>82</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>genase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Phosphogluconate dehydro-</td>
<td>24.2 ± 2.3</td>
<td>12.0 ± 1.1</td>
<td>50</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td></td>
<td>genase</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Activities are expressed in μmoles of substrate metabolized/hour/average cell x 10^-7 at 37°C. Means and standard errors represent 4 or more samples studied.
† Calculated P value as compared with normal fed animals. When no values are given, the difference is >0.05, which is not accepted as significant.
‡ Millions of cells/gm wet weight of tissue.
§ Milligrams of nitrogen/cell x 10^-7.
(30 per cent) were preferentially depleted, as compared with the decrease in nitrogen content.

**DISCUSSION**

A comparison of two liver tumors of differing malignancy (as indicated by different rates of growth of the tumor and length of survival of the host), such as the Morris and Novikoff tumors, is of interest. Thus, there is the possibility of detecting biochemical variations which can be correlated with biological differences of these hepatocellular tumors. For the purpose of such a comparison the carbohydrate enzyme data of the average cell of the Novikoff hepatoma (20) and the Morris tumor are brought together in Chart 1 and contrasted with values of normal liver, which are taken as 100 per cent.

**Enzymes which have similar lesions in the Morris and Novikoff hepatomas.**—In both types of neoplastic livers there was a very marked decrease in phosphoglucomutase activity. It was shown that the Novikoff hepatoma can store very little glycogen (2); recently it was demonstrated that the Morris hepatoma is also unable to deposit glycogen.\(^2\) The lactic dehydrogenase activity (not shown in Chart 1) was statistically significantly decreased in both liver tumors. However, this enzyme is present in such an excess that no necessary correlation with lactate production has been possible (22).

\(^2\) Dr. Sidney Weinhouse, personal communication.


As a result of these enzymatic lesions no glucose was produced in this tumor either from glycogen breakdown or through gluconeogenesis, as demonstrated by isotope methods (2). On the other hand, in the Morris hepatoma the lesions which occur in G-6-Pase and fructose-1,6-diphosphatase activities were manifested only in sharp decreases. In interpreting these enzymatic lesions in the Morris tumor it may be pointed out that these are decreases which in normal liver can be obtained only under extreme conditions as in fasted, hypophysectomized animals which are approaching hypoglycemia and death (1, 21, 28). The biological significance of the absence of G-6-Pase and fructose-1,6-diphosphatase in the Novikoff hepatoma has been discussed, and it was emphasized that, as a result of these lesions, the Novikoff hepatoma cell can conserve 75 per cent more G-6-P than the normal liver cell (15, 16). The extent to which the enzymatic lesions in the specific phosphatases

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**Chart 1.**—Comparison of carbohydrate enzyme activities in normal and neoplastic livers. Activity per average cell is given, with normal liver taken as 100 per cent.
in the Morris hepatoma would allow release of glucose from the tumor cell is under investigation. However, it may be suggested that the functioning of these gluconeogenic key enzymes may play a role in the decreased growth rate of the Morris hepatoma by allowing the G-6-P draining pathway to function.

From Chart 1 it appears that the extent of the metabolic lesions revealed in the examined carbohydrate-metabolizing enzyme activities may be correlated roughly with the comparatively low growth rate of the Morris hepatoma. Most of the carbohydrate enzymatic lesions which occurred in the Novikoff hepatoma in an extensive form of metabolic derangement also appeared in the Morris hepatoma in nearly identical or greatly attenuated extent. In the Novikoff hepatoma, in the presence of insignificant glycogen synthesis and lack of glucose release, all G-6-P formed was channeled into glycolysis or through the highly active G-6-P dehydrogenase into pentose formation and nucleic acid synthesis. However, in the Morris hepatoma due to the presence of decreased but still functioning gluconeogenic enzyme systems glucose may escape from the tumor cells, and thus the presence of gluconeogenesis limits the available G-6-P for glycolysis and direct oxidation.

The presented results further illustrate the view that the various tumors of the same tissue may differ markedly in histological structure, cellular population, biological behavior and growth rate, partly at least on the basis of the quality and extent of enzymatic lesions which underlie morphology and pathological behavior.

The study of the metabolic pathways of the Morris tumor with isotope methods is in progress.

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REFERENCES

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