Fructose-1,6-diphosphatase and Lactic Dehydrogenase Activity in Hepatoma and in Control Human and Animal Tissues*

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Previous studies on the carbohydrate metabolism of neoplastic liver have established definite qualitative and quantitative alterations in the glucose-6-phosphate (G-6-P) utilization of this tumor. The G-6-P metabolism was examined in homogenates and supernatant fluid of Novikoff hepatoma and control tissues by studying the behavior of enzymes directly involved in the utilization of this hexose monophosphate ester (26). It was found that glucose-6-phosphatase activity was absent (23), phosphoglucomutase activity markedly decreased (24), and G-6-P dehydrogenase activity increased (22), whereas phosphohexoseisomerase activity was maintained essentially at the level of normal liver values (25). The enzymatic alterations in G-6-P metabolism seem to be specific to the neoplastic liver, because no similar changes were found in regenerating, embryonic, or newborn rat liver (24–26).

These enzymatic studies on the four metabolic pathways of G-6-P were followed up recently by studying the fate of glucose-1-C14 and glucose-6-C14 in tissue slices of liver and hepatoma (1, 20). The isotope work confirmed the absence of glucose release and showed a marked decrease in glycogen formation in the neoplastic liver. The results were also in line with a possible increased shunt. An especially striking finding was the accumulation of large quantities of lactic acid in hepatoma slices.

However, it was noted that the markedly increased lactate production in the presence of normal phosphohexoseisomerase activity in the hepatoma presents an apparent contradiction. Several factors could be responsible for the accumulation of lactic acid. One possibility is the lack of reversibility of the reactions between pyruvate and G-6-P. In the pyruvate → G-6-P step series there is an important one-way reaction which is catalyzed by the enzyme fructose-1,6-diphosphatase. Preliminary studies demonstrated that fructose-1,6-diphosphatase activity was indeed absent in the hepatoma (19). However, the pyruvate =⇒ lactate conversion itself is mediated by another enzyme, lactic dehydrogenase.

The present study confirms the absence of fructose-1,6-diphosphatase activity in the Novikoff hepatoma and establishes the specificity of this lesion in the neoplastic liver, as compared with various resting and fast-growing normal livers. The behavior of lactic dehydrogenase in liver tumor and control livers is also described. A comparison of the cytochemistry of carbohydrate enzymes in normal human and rat liver is presented.

MATERIALS AND METHODS

Animals and experimental procedures.—Male, adult Wistar rats weighing 180–220 gm. were used in these experiments. Animals were given free access to food and water, except for the fasted ones which were placed in individual cages with only water available ad libitum. The Novikoff hepatomas were transplanted intraperitoneally (23, 26); the tumors were 7 or 8 days old when used for biochemical assays. For regenerating liver studies, the animals were partially hepatectomized, and sham operations were performed at the same time on rats of the same weight. The experimental results of the regenerating liver were always compared with the data from the sham-operated animals (21, 96). For embryo studies, pregnant rats were sacrificed at 20 days of gestation. The livers of 1-day-old rats were also examined.

Human liver specimens were obtained during abdominal operations from patients with clinical diagnosis of cholecystitis and cholelithiasis. Hematoxylin and cosin studies were made of each specimen. Only those biopsy results which showed normal liver structure on histological examination were included in the present series.

Biochemical procedures.—Preparation of homogenates (20) and determination of cellularity (96) have been described previously. Both carbohydrate enzymes were assayed in the supernatant fluid, which was obtained by centrifuging the homogenate for 30 minutes at 0°C. at 100,090 g in a refrigerated Spinco model L centrifuge. Fructose-1,6-diphosphatase activity was followed in preliminary studies (19) by the method of Fogell and McGilvery (15). However, for all later studies described in the preliminary report (19) and throughout the
present paper the following standard assay procedure was employed. Fructose-1,6-diphosphatase activity was measured at 37°C with preincubation in the presence of glycylglycine buffer (0.25 M), pH = 7.0-7.4; sodium borate (0.1 M); MgSO4 (0.05 M); fructose-1,6-diphosphate (0.05 M); and an appropriate amount of enzyme (supernatant fluid) to give linear kinetics in a final reaction mixture volume of 1.0 ml. One ml of 5 per cent trichloroacetic acid was used to stop the reaction at 0, 5, and 10 minutes. The liberated inorganic phosphate was measured by the method of Fiske and Subbarow. All assays were run in duplicate, thus giving quadruplicate determinations of 5-minute runs. For the lactic dehydrogenase determination, the reaction cuvette contained a mixture of the following reagents in final molarity: sodium pyruvate (2.5 × 10⁻⁴ M); DPNH (1.25 × 10⁻⁴ M); phosphate buffer (0.1 M), pH = 7.4; enzyme and distilled water to give a final reaction mixture of 3.0 ml. The blank contained all the reagents except DPNH. The assay was started by addition of an appropriate amount of enzyme (supernatant fluid) to give linear kinetics over a period of 10 minutes at 37°C. The course of the reaction was followed by observing the decrease in optical density at 340 m~ using a spectrophotometer, model DU with glass cells no. 2097. Enzymatic activities were calculated as #moles of substrate metabolized per hour at 37°C. Enzymatic activities were expressed per nitrogen and per average cell. The micro-Kjeldahl procedure was employed for nitrogen determinations.

With both enzymes preliminary kinetic studies were conducted to check pH and substrate optima. Proportionality of enzymatic activity with amount added and with reaction time was established. After these criteria were fulfilled, the effect of fasting was examined (31). Studies were also begun to investigate hormonal effects on these enzymes (31). After sufficient information had been obtained on the physiological behavior of these enzymes, the experiments were started which are described in the present communication.

RESULTS AND DISCUSSION

Distribution of fructose-1,6-diphosphatase activity in normal organs.—When the liver specific activity is taken arbitrarily as 100, the following is a comparison of fructose-1,6-diphosphatase activity in various rat organs in order of magnitude: kidney, 100; intestine, 17; brain, <0.1; lung, <0.1; muscle, <0.1. It is of interest that there is a striking agreement with the organ distribution of glucose-6-phosphatase activity (23). It appears that both specific phosphatases occur in high activity in liver and kidney where gluconeogenesis has been demonstrated. The biological significance of these enzymes in the intestine in minimal activity needs further evaluation.

Comparison of cellularity and nitrogen content in neoplastic and in resting and rapidly growing normal rat liver.—Table 1 draws attention to the fact that the hepatoma contained more than twice as many cells as did the liver of normal, fed animals (26). On the other hand, 1-day fasting also caused a significant increase in the cell count in normal liver (29). The cellularity of embryonic and newborn liver was not indicated because of the presence of the different developmental forms of red and white blood cells series (31). At present we have no data on the proportion of hepatic versus hemopoietic cell elements in fetal and newborn rat liver.

When the nitrogen content was expressed per milligram of dry weight, the following data were obtained:

<table>
<thead>
<tr>
<th>Liver tissues</th>
<th>No. ANIMALS</th>
<th>Cytological data</th>
<th>Enzymatic data†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Per wet weight</td>
<td>Per cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mg/cm)</td>
<td>(mg × 10⁻⁴)</td>
</tr>
<tr>
<td>Normal fed</td>
<td>11</td>
<td>214 ± 10</td>
<td>5.4 ± 1.3</td>
</tr>
<tr>
<td>Normal fasted†</td>
<td>4</td>
<td>298 ± 12</td>
<td>16.7 ± 1.0</td>
</tr>
<tr>
<td>Regenerating§</td>
<td>3</td>
<td>188 ± 30</td>
<td>15.5 ± 0.4</td>
</tr>
<tr>
<td>Sham-operated§</td>
<td>3</td>
<td>223 ± 16</td>
<td>14.9 ± 0.5</td>
</tr>
<tr>
<td>Newborn (1-day-old)</td>
<td>4</td>
<td>10.0 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Embryonic (30-day-old)</td>
<td>4</td>
<td>8.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Neoplastic</td>
<td>5</td>
<td>460 ± 9</td>
<td>9.1 ± 1.0</td>
</tr>
</tbody>
</table>

* Means and standard deviations are given. Means represent groups of animals consisting of three to eleven rats, as indicated.
† Enzymatic activities are expressed as μmoles of substrate metabolized per hour at 37°C.
‡ Fasted for 1 day, with water available ad libitum.
§ Animals were killed and assayed 1 day after operation.

Table 1

Comparison of cellularity, nitrogen content, fructose-1,6-diphosphatase and lactic dehydrogenase activity in normal, neoplastic, and rapidly growing normal liver*
unit wet weight an apparent increase was found in fasting, and a decrease in regenerating liver. The neoplastic and embryonic liver contained only half and the newborn liver about 65 per cent of normal liver nitrogen. When the nitrogen values were expressed per average cell the regenerating liver showed no change. However, there was a significant decrease after 1-day fasting (29), and the nitrogen content was only 39 per cent in the average hepatoma cell. These results clearly suggest that the markedly decreased nitrogen content and highly increased cellularity of the hepatoma have to be taken into consideration in the expression of the biochemical data (26).

Fructose-1,6-diphosphatase studies in hepatoma and in control livers.—During these investigations it was noted that storage of liver supernatant fluid for 2-3 months at -20°C. did not appreciably affect fructose-1,6-diphosphatase activity. In Table 1 fructose-1,6-diphosphatase activity was expressed on nitrogen and per average cell bases. The previously reported absence of fructose-1,6-diphosphatase activity in the Novikoff hepatoma has been confirmed. On the other hand, this enzymatic activity was not affected in fasting or in regenerating liver. Table 1 also shows that fructose-1,6-diphosphatase activity in the embryonic liver was 63 per cent and in the newborn rat liver was 79 per cent of the normal rat liver values. It appears that the absence of fructose-1,6-diphosphatase activity is specific to the Novikoff hepatoma, since no similar lack of activity was found in resting or rapidly growing normal rat liver.

The absence of glucose-6-phosphatase and fructose-1,6-diphosphatase seems to be of importance in explaining the lack of gluconeogenic response in tumors (5, 12, 13, 33). The lack of gluconeogenic response, along with the decreased glycogenesis and absent glucose release in the presence of normal phosphohexoseisomerase, favors the irreversible channeling of G-6-P into pyruvate formation.

Lactic dehydrogenase studies in hepatoma and in control livers.—Storage of liver supernatant fluid for several months at -20°C. failed to affect lactic dehydrogenase activity. Table 1 shows that, on a nitrogen basis, lactic dehydrogenase activity was decreased to 68 per cent in the neoplastic liver, whereas the enzyme was in the normal range in fetal, newborn, and regenerating liver and in the liver of 1-day fasted rats. However, when lactic dehydrogenase activity was expressed per average cell, it was only 17 per cent of the normal liver activity. Even 6-day fasting was able to decrease the lactic dehydrogenase activity of the average normal liver cell to only 50 per cent.1

It appears that the marked depletion of the lactic dehydrogenase activity is specific to the neoplastic liver, since no similar alterations have been noted in the control fetal, resting, or rapidly growing livers. The decreased lactic dehydrogenase activity in the Novikoff hepatoma is in line with lowered activity of this enzyme in various rodent tumors (4, 8, 10).

Attention may be drawn to the fact that, whereas the hepatoma lactic dehydrogenase activity (as determined in the supernatant fluid) was markedly depleted, the lactate production (as determined in hepatoma slices) was highly increased (1, 20). This apparent discrepancy between the enzymatic assay and the actual determination of the metabolite end-product brings up the following points of importance.

1. The magnitude of enzymatic activity.—Lactic dehydrogenase is a highly active enzyme in the liver. Under optimal in vitro assay conditions it produced 71.7 X 10^-6 μmoles per hour in the average normal liver cell. This activity decreased to 12.7 X 10^-6 μmoles per hour (17 per cent) in the average hepatoma cell. However, determinations in hepatoma slices showed that 0.0414 X 10^-6 μmoles of lactic acid per hour were generated (1). It is evident that the markedly decreased lactic dehydrogenase activity present in this tumor was still about 300-fold the activity necessary to produce the actual amount of lactate obtained. Clearly, it is not sufficient to determine the activity of an enzyme and register a statistically significant increase or decrease, but it is necessary also to take into consideration the performance (activity) of the enzyme to gain an indication regarding the possible biological significance of the alteration. The difference between statistical significance and biological significance has been discussed elsewhere (28).

2. Role of cofactors, e.g. coenzymes.—When lactic dehydrogenase activity was determined in vitro, DPNH was added in optimal concentration. However, under physiological or pathological conditions the availability of this coenzyme might be a significant limiting condition in tissue slice studies when this factor is not added.

3. Presence of alternate metabolic pathways.—It is not possible to predict from the activity of lactic dehydrogenase the amount of lactic acid "to be produced," because this is but one of the various pathways of pyruvate metabolism. It is probable that only a complete knowledge of the behavior of all the enzymes involved in pyruvate utilization could give us information on the expected fate of the pyruvate molecule, which includes its dehydrogenation into lactate.

1 G. Weber and A. Cantero, unpublished observation.
Comparison of carbohydrate cytochemical values in normal human and rat liver.—The previously reported similarity in the cellularity and nitrogen content of human and rat liver (27) is confirmed in Table 2. However, there is not such a complete agreement in the absolute values of the enzymatic activities. The human liver fructose-1,6-diphosphatase activity was 50 per cent, and the lactic dehydrogenase activity 30 per cent, of the normal rat liver values on both specific activity and per average cell bases. The presence of such a species difference is in line with previous findings on the comparison of G-6-P-utilizing enzymes in human and rat liver (27). It was reported that the specific activity of human hepatic glucose-6-phosphatase was 60 per cent of rat liver activity. However, not all human carbohydrate enzyme activities were lower, because the specific activity of human hepatic phosphohexoseisomerase, phosphogluco-

TABLE 2
COMPARISON OF CYTOCHEMISTRY OF HUMAN AND RAT LIVER CARBOHYDRATE ENZYMES

Means and standard deviations represent seven or more human liver samples. Enzymatic activities are expressed in mmoles of substrate metabolized/hour at 37°C. For definition of data, see Table 1.

<table>
<thead>
<tr>
<th>Enzymatic activities</th>
<th>Human liver data</th>
<th>Per cent of rat liver values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose-1,6-diphosphatase:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific activity</td>
<td>10.8±6.4</td>
<td>48</td>
</tr>
<tr>
<td>Activity/cell</td>
<td>0.8±0.5</td>
<td>50</td>
</tr>
<tr>
<td>Lactic dehydrogenase:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific activity</td>
<td>208±111</td>
<td>30</td>
</tr>
<tr>
<td>Activity/cell</td>
<td>21±9.6</td>
<td>29</td>
</tr>
<tr>
<td>Enzymes of G-6-P utilization:*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Specific activities)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphohexoseisomerase</td>
<td>1.122±320</td>
<td>156</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>711±312</td>
<td>154</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>8.4±5.8</td>
<td>58</td>
</tr>
<tr>
<td>G-6-P dehydrogenase</td>
<td>5.2±1.4</td>
<td>226</td>
</tr>
</tbody>
</table>

* Previously published data (27).

metase, and G-6-P dehydrogenase was 163–200 per cent of the rat liver values (27). It is noteworthy that a comparison of the relative activities of these six enzymes shows a similarity in the pattern of carbohydrate enzymes in both rat and human liver.

ENERGY

MITOSIS

CHO

NUCLEIC ACIDS

SYN-
THESIS

BREAK-
DOWN

SYN-
THESIS

BREAK-
DOWN

1. G-6-PASE; 2. FDPASE; 3. XANTHINE OXIDASE; 4. DNAASE;
5. URICASE.

CHART 1.—A schematic and tentative representation of some of the lesions discovered in the Novikoff hepatoma. Arrows in the Chart mean synthetic ↑ or catabolic ↓ pathways. However, arrows in boxes indicate the final results of the metabolic imbalance; e.g., urea synthesis ↓ = decreased; amino acid synthesis ↑ = increased. In the carbohydrate metabolism the anabolic pathway is interrupted by absence of glucose-6-phosphatase (G-6-Pase) and fructose-1,6-diphosphatase (FDPase). The energy obtained by glucose catabolism is coupled with nucleic acid metabolism. The nucleic acid metabolites are preserved and recycled by the interruption of the catabolic processes (absent xanthine oxidase, uricase, and deoxyribonuclease [DNAase]). Amino acids are preserved, but various cell functions are decreased; e.g., synthesis of fat and phospholipide. Data on absent urea synthesis were obtained in another liver tumor (7).

Metabolic features of the Novikoff hepatoma.—On the basis of a combination of biochemical results a meaningful metabolic pattern of this tumor appears to take shape. Chart 1 demonstrates the following points. In the carbohydrate metabolism of the Novikoff hepatoma, the anabolic pathway is interrupted at two steps at least (absent glucose-6-phosphatase and fructose-1,6-diphosphatase [23,
The storage and synthesis of carbohydrate in form of glycogen are also markedly decreased (1). In the nucleic acid metabolism, on the other hand, the catabolic processes are interrupted at two enzymatic steps (absent xanthine oxidase and uricase [6, 11]). Recent histochemical studies also demonstrated a great decrease in deoxyribonuclelease activity in liver tumors. The catabolism of amino acids is decreased (2), and the amount (11, 30) and turnover rate (32) of phospholipides and fatty acids (1) are markedly diminished.

These biochemical alterations in the Novikoff hepatoma cell result in a metabolic pattern in which glucose is conserved for the cell and utilized for the production of energy and the synthesis of nucleic acids. The nucleic acid metabolites are also trapped, recycled (3), and thus retained for the synthesis of nucleoprotein for the mitotic processes. The energy made available by glucose breakdown is coupled with mitosis to drive the mitotic mechanism. The amino acids are conserved for the preferential production of the biosynthesis of enzymes which are involved in the maintenance of cell life and multiplication.

It is possible that the loss of strategic enzyme systems in carbohydrate anabolism results in the stimulation of the catabolic pathway; whereas the absence of enzymes along the catabolic pathway of nucleic acid metabolism may stimulate the synthesis of these building blocks. Such a rearrangement of the alternate pathways may result from the enzyme deletions by providing less competition for the metabolites or by increasing the amount of enzyme produced by feedback mechanisms acting on enzyme-forming systems (16, 17). It is a possibility that by these and other as yet unknown enzyme alterations cell division processes might be enhanced and sustained.

Work is in progress to find means of uncoupling energy production from the mitotic mechanism and to elucidate factors governing the biosynthetic processes of the enzymes involved in G-6-P utilization.

Comments on the relation of the Novikoff hepatoma to other liver tumors.—Whether the biochemical alterations described in the Novikoff hepatoma are specific to this tumor or may characterize other liver tumors also requires further studies. A number of lesions similar to those found in the Novikoff hepatoma have been noted in other liver tumors. For instance, glucose-6-phosphatase activity was also absent in primary liver tumors induced by carcinogenic diet (23, 18). A decreased fatty acid synthesis, comparable to that found in the Novikoff hepatoma, has previously been reported in primary liver tumors (9). On the other hand, some of the carbohydrate anabolic enzymes which could not be demonstrated in the Novikoff tumor were reported present in the Dunning hepatoma in decreased but measurable activity (14). It is of interest that the Dunning hepatoma, in which the lesion in gluconeogenesis was only partially developed, had a growth period of about 21 days, which is 3 times longer than that of the Novikoff hepatoma, in which no gluconeogenesis was demonstrable either by enzyme assay (23, 26) or by isotope procedures (1).

An attempt at a complete comparison of various liver tumors appears precocious at this time owing to lack of information on the scale of that already obtained in the Novikoff hepatoma. However, it may not be superfluous to say at this point that, since various liver tumors differ in their histological structure, cellular population, biological behavior, and growth rate, it is not unexpected that the biochemical lesions or alterations which underlie morphological and biological differences will be present in varying qualitative or quantitative extent. 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.

Therefore, it appears that a careful comparison of the metabolism, histology, and biological behavior may bring a new understanding of the role of biochemical alterations in the pathological behavior of various liver tumors.

SUMMARY

The behavior of fructose-1,6-diphosphatase and lactic dehydrogenase activities was studied in the Novikoff hepatoma and in human and rat control livers.

1. The distribution of fructose-1,6-diphosphatase activity was examined in normal rat organs. The following was the order of magnitude of the specific activity in per cent: liver, 100; kidney, 100; intestine, 17; brain, <0.1; lung, <0.1; muscle, <0.1.

2. The previously reported absence of fructose-1,6-diphosphatase activity in the neoplastic liver was confirmed. This lesion seems to be specific to the neoplastic liver, since no similar alteration was found in regenerating, embryonic, or newborn rat liver or in the liver of fed or fasted rats.

3. The lactic dehydrogenase activity was markedly decreased in the hepatoma. This enzymatic activity was decreased (3), and the amount (11, 30) and turnover rate (32) of phospholipides and fatty acids (1) are markedly diminished.

R. Daoust, personal communication.
activity was in the normal range in all examined control livers.

4. A comparison of human and rat liver carbohydrate cytochemical data was presented.

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