

Glucose-6-phosphatase Activity in Normal, Precancerous, and Neoplastic Tissues*

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Studies on carbohydrate metabolism have revealed important differences between hepatoma and normal liver.

It has been pointed out by Olson (14) that the carbohydrate metabolism of the hepatoma is altered in such a way that glucose is diverted almost entirely from its storage as glycogen to its degradation to lactic acid. Olson demonstrated increased phosphohexo-isomerase activity in the hepatoma, which might have an important role in channeling the G-6-P¹ ester to the glycolytic pathway. On the other hand, Goranson, McBride, and Weber (10) suggested that the diversion of glucose from glycogen synthesis may be explained in part, at least, by the low activity of the phosphorylase system, which is the rate-limiting step in the G-6-P \rightleftharpoons G-1-P \rightleftharpoons glycogen reaction.

The decreased storage of energy-rich material, such as glycogen, in the hepatoma has also been explained by Zamecnik *et al.* (18) on the basis that the metabolic activities of the hepatoma cell are adjusted to protein synthesis and growth. The findings of Dickens and Glock (6) that the direct oxidation of G-6-P and 6-phosphogluconate is increased in liver tumor extract might partially account for this latter metabolic change from the normal liver.

It seems that the deviation of the carbohydrate metabolism of the hepatoma from that of the normal liver starts at the utilization of G-6-P ester. Therefore, the elucidation of the metabolic pathways of G-6-P would bring us nearer to an understanding of this metabolic change in the hepatoma. One of the most important pathways of G-6-P in normal liver is its hydrolysis into glucose and phosphate by the enzyme G-6-Pase.

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¹ The following abbreviations are employed: G-6-Pase = glucose-6-phosphatase; G-6-P = glucose-6-phosphate; G-1-P = glucose-1-phosphate; F-6-P = fructose-6-phosphate.

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Fantl and Rome (8, 9), Swanson (16), and De Duve (5) demonstrated the specificity of G-6-Pase and distinguished it from other nonspecific phosphatases by its pH optimum, thermal instability, and substrate specificity for G-6-P with little or no activity toward glycerophosphate, G-1-P, or F-6-P. The enzyme has been demonstrated in different organs, but it is most active in the liver and kidney. By the histochemical methods used by Chiquoine (3) it was demonstrated that the enzyme is more abundant in the peripheral third of the hepatic lobule than in the inner two-thirds. Distribution studies showed that the liver enzyme is associated mainly with the microsome fraction (12, 13). Cori and Cori reported the almost complete absence of the enzyme from liver in human cases of glycogen storage disease (4). Weber and Cantero (17) demonstrated that the G-6-Pase activity of the liver in mice and rats is greatly increased in fasting. This paper reports the investigation of the G-6-Pase activity of various normal, precancerous and neoplastic tissues. The liver G-6-Pase activity was also examined in mice bearing transplanted E 0771 adenocarcinomas and in rats bearing intraperitoneally transplanted Novikoff hepatomas.

MATERIALS AND METHODS

Normal tissues were taken from adult Wistar rats (200–250 gm.). Transplantable tumors were obtained from albino mice bearing Sarcoma 37; C57 BL/6 mice bearing E 0771 adenocarcinoma; C3H mice carrying Gardner lymphosarcoma 6C3HED; and Carworth Farm Wistar rats carrying intraperitoneally transplanted Novikoff hepatoma.² The mouse tumors were transplanted subcutaneously on the lower back region and assayed when 8–10 days old. Rat Novikoff hepatomas were 7 or 8 days old when used. All animals were male and were kept on Purina Fox Chow and water ad libitum. Precancerous livers were taken from male

² We are indebted to Professor Arthur W. Ham, Dept. of Anatomy, University of Toronto, for the original Novikoff hepatoma transplant.

Wistar rats of 200 gm., which were fed for up to 150 days a semisynthetic diet (1) in which 4-dimethylaminoazobenzene (DAB) was incorporated at a concentration of 0.06 per cent. Control animals received the semisynthetic diet alone. Rats were sacrificed when they had been on the diets for 50-60 days. Primary induced liver tumors were obtained from rats fed the DAB diet for 150 days, followed by a period of 30 days on Purina Fox Chow.

The animals were killed by a blow on the head and were decapitated and bled. The tissue was quickly excised, placed into a small beaker standing on ice, chilled for 5 minutes, then blotted on filter paper and minced with scissors. From the

TABLE 1
GLUCOSE-6-PHOSPHATASE ACTIVITY IN
NORMAL RAT ORGANS*

Organ	Activity†	Per cent‡	Specific activity§	Per cent‡
Liver	213	100	7.10	100
Kidney	203	95	7.30	103
Spleen	16	8	0.54	8
Brain	11	5	0.49	7
Intestine	8	4	0.42	6
Lung	<1	<1	<0.10	<1
Muscle	<1	<1	<0.10	<1

* The values are the mean of six or more determinations.

† Micrograms of phosphorus liberated in 15 minutes at 31° C/100 mg wet weight.

‡ Liver arbitrarily assigned a value of 100 per cent.

§ Micrograms of phosphorus liberated in 15 minutes at 31° C/mg nitrogen.

minced tissue a 5 or 10 per cent homogenate was prepared in 0.25 M ice-cold sucrose, according to the procedure outlined by Allard, Mathieu, Lamirande, and Cantero (2). The enzyme was assayed according to Cori and Cori (4). Incubation time was 15 or 30 minutes at 31° C. as specified in the text. The phosphorus was determined by the method of Fiske and Subbarow. The colorimeter readings were done at 450 m μ with Beckman Quartz Spectrophotometer, Model DU, in glass cells No. 2097. The micro-Kjeldahl procedure was employed for total nitrogen determinations.

RESULTS

The G-6-Pase activities of various normal rat organs are compared in Table 1. The G-6-Pase activity was the highest in the liver and kidney, low in the brain, spleen, small intestine, hardly appreciable in lung and different muscles.

There was no significant difference between the G-6-Pase activity of normal rat liver and that in the liver of rats bearing intraperitoneally transplanted Novikoff hepatoma. On the other hand, the G-6-Pase activity was significantly decreased on both wet weight and nitrogen bases in the livers

of mice bearing adenocarcinoma E 0771 (Table 2).

There was no demonstrable G-6-Pase activity in any of the transplantable mouse tumors or in the Novikoff hepatoma. The histological picture of the Novikoff tumor used in this laboratory is shown in Figures 1 and 2.

To investigate the possible presence of a G-6-Pase inhibitor in some of the tumors, homogenates were prepared from E 0771 adenocarcinoma and Novikoff hepatoma, and their effect on the G-6-Pase activity of normal liver homogenates was tested. The tumor homogenates showed no inhibitory effect. A 1-hour incubation of these tumor homogenates with normal liver homogenate, prior to the enzyme assay, was also without any effect on the G-6-Pase activity of the normal liver.

Investigation was carried out to determine whether G-6-Pase activity could be demonstrated in the Novikoff hepatoma under experimental conditions different from those described above. Since fasting greatly increases the normal liver G-6-Pase activity (17), male Wistar rats bearing 6-day-old transplanted Novikoff hepatoma were fasted for 24 hours and then sacrificed. The livers from these rats showed a 60 per cent increase in

TABLE 2

COMPARISON OF THE G-6-PASE ACTIVITY IN NORMAL MOUSE LIVER AND IN LIVERS OF MICE BEARING SUBCUTANEOUSLY TRANSPLANTED E 0771 MAMMARY ADENOCARCINOMA

ACTIVITY*		SPECIFIC ACTIVITY†	
Normal (8)‡	Tumor-bearing (8)‡	Normal (8)‡	Tumor-bearing (8)‡
649.3 ± 79.5§	479.3 ± 96.8	17.0 ± 0.6	13.9 ± 2.9
P < 0.01		P < 0.05	

* Micrograms of phosphorus liberated in 30 minutes at 31° C/100 mg wet weight.

† Micrograms of phosphorus liberated in 30 minutes at 31° C/mg nitrogen.

‡ Number of rats.

§ Means with standard deviations. "P" is the probability for chance occurrence of the difference between the means.

G-6-Pase activity, but again no activity could be demonstrated in the intraperitoneally transplanted hepatoma.

The liver G-6-Pase activity of control and DAB-fed rats in presented in Chart 1. The enzyme activity is expressed in per cent change as compared with the normal, fox chow-fed animals. The feeding of the control semi-synthetic diet increased the G-6-Pase activity. On the other hand, the G-6-Pase activity of the liver progressively decreased during DAB feeding, and at 180 days the activity was almost completely absent from the induced hepatomas. The difference between the values for control and DAB-fed rats is significant on both wet weight and nitrogen bases.

DISCUSSION

The data on G-6-Pase activity for homogenates of normal rat organs generally confirm the results of De Duve *et al.* obtained with extracts. It is interesting that the G-6-Pase activity of the kidney is as high as that of the liver. Possibly this high G-6-Pase activity is connected with the recently reported ability of the kidney to release sugar into the blood stream (7, 15), since we find high G-6-Pase activity only in those organs (Table 1) which are able to supply glucose for the whole organism.

It has been reported that tumors may affect the metabolism of distant tissues (11). A reduction in the liver phosphorylase activity and in liver glycogen was recently reported in animals bearing tumor transplants (10). The decreased G-6-Pase activity of the liver of mice bearing transplanted adenocarcinoma (Table 2) is in line with the above data. However, the mechanism of this decrease is not clearly understood, especially since there was no similar decrease in the liver of Novikoff hepatoma-bearing animals.

The present study demonstrated that there was no G-6-Pase activity in transplanted adenocarcinoma E 0771, lymphosarcoma 6C3HED, and Sarcoma 37. The absence of G-6-Pase activity in the Novikoff tumor is of special interest. The histological picture of the Novikoff tumor (Figs. 1 and 2) shows that one is justified in calling this tumor a hepatoma. If this tumor were a cholangioma, the increased number of bile duct cells, which do not contain G-6-Pase, might explain its lack of G-6-Pase activity. However, since this tumor is composed of parenchymal cells, which normally contain much G-6-Pase, the absence of activity is real and not caused by a change in the cell population of the tissue. Furthermore, it was shown that the absence of G-6-Pase activity in neoplastic tissues is not due to the presence of an inhibitor, since homogenates of adenocarcinoma or hepatoma did not inhibit reaction mixtures containing normal liver.

The studies with DAB present several interesting points. Chart 1 shows that the feeding of the control semisynthetic diet increases the liver G-6-Pase activity. Since this semisynthetic diet has a very high carbohydrate content, the increased G-6-Pase activity might be considered an adaptive response of the organism. On the other hand, when DAB is incorporated into the semisynthetic diet, very little increase is observed, and after 40 days the enzyme activity progressively decreased. The almost complete absence of G-6-Pase activity in the induced hepatomas agrees well with the finding that this enzyme is absent in the transplanted Novikoff hepatoma.

According to Cori the role of G-6-Pase is the transformation of the cellular stores of carbohydrate into blood glucose. The absence of G-6-Pase in Sarcoma 37 is difficult to evaluate, since this enzyme is present only in very small quantities in the *gastrocnemius* and *rectus abdominis* of rats or mice. A similar consideration applies to the absence of G-6-Pase activity in transplanted adenocarcinoma, since the G-6-Pase activity of normal breast tissue is not known. If the function of G-6-

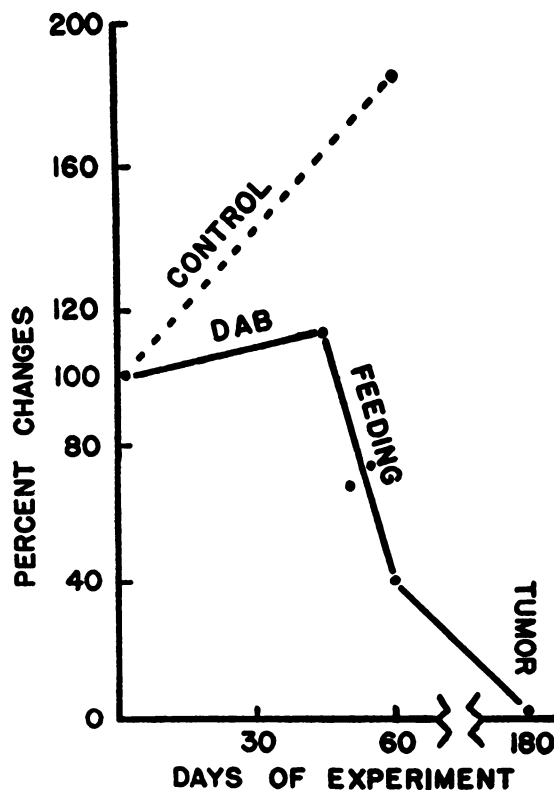


CHART 1.—Glucose-6-Pase activity during carcinogenesis. Rats were on DAB diet for 150 days, then on fox chow for 30 days. Activity is expressed as per cent change from control fox chow-fed rats, killed at the same time. Each point represents the average of at least three animals.

Pase is the splitting of the G-6-P ester to release glucose for the use of the other cells in the body, we can understand its presence in the liver cell, since the liver is the main carbohydrate store of the organism. On the other hand, muscle, which also contains glycogen, uses it through glycolytic channels and does not supply the organism with glucose. This consideration is of interest in relation to the biochemical changes occurring in liver tumor. When the neoplastic liver loses its G-6-Pase activity and utilizes glucose for glycolysis and for protein synthesis, its G-6-P metabolism becomes similar to that of the muscle.

It is apparent from the above discussion that the fate of the G-6-P ester is of great interest. In considering the decrease in phosphorylase activity and decrease and absence of G-6-Pase activity, as well as the known increased lactic acid production of neoplastic tissues, our attention is drawn to the glycolytic pathway and the oxidative utilization of G-6-P.

Whether the above-outlined carbohydrate metabolism changes are specific to neoplasia or simply characterize the changes in fast-growing tissue is not known. The study of G-6-Pase in regenerating liver is in progress.

SUMMARY

The glucose-6-phosphatase (G-6-Pase) activity was investigated in normal and precancerous tissues, in transplanted and induced tumors, and in the liver of tumor-bearing animals.

1. Highly active G-6-Pase was found only in those normal organs which are able to release glucose into the blood stream (liver, kidney). Other organs (small intestine, brain, lung, spleen, muscle) contained only low or negligible activities.

2. The liver G-6-Pase activity of mice bearing E 0771 adenocarcinoma was significantly lower than the activity of normal mouse liver. No difference was found between normal rat liver and the liver of rats bearing transplanted Novikoff hepatoma.

3. Homogenates of Sarcoma 37, E 0771 transplantable adenocarcinoma, 6C3HED lymphosarcoma, and Novikoff hepatoma possessed no G-6-Pase activity. It has not been possible to demonstrate a G-6-Pase inhibitor in any of these neoplastic tissues. The G-6-Pase activity of liver decreased progressively during carcinogenesis produced by 4-dimethylaminazobenzene and was almost completely absent in the hepatomas.

4. The pathways of glucose-6-phosphate utilization in hepatoma were discussed.

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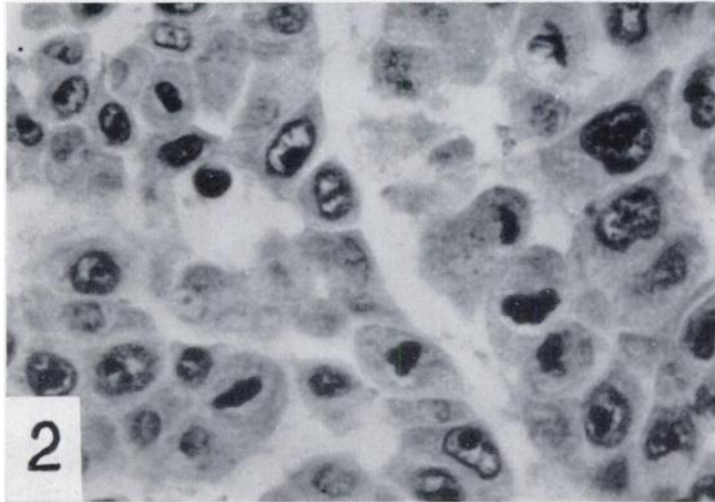
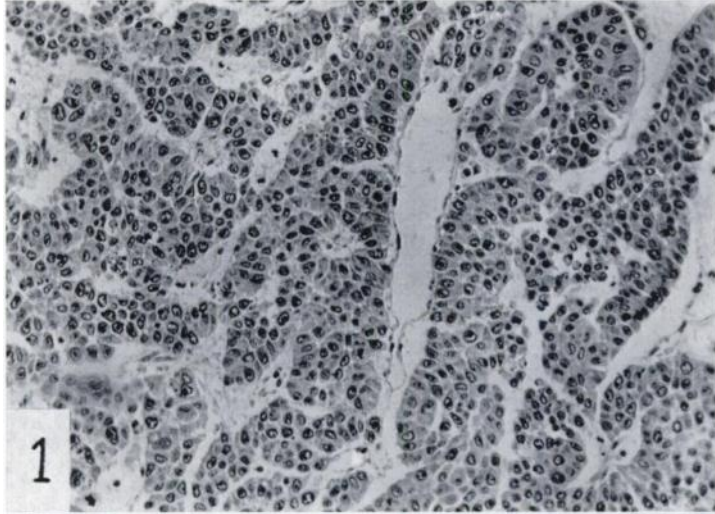
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FIG. 1.—Novikoff hepatoma, 7-day-old transplant. Large irregular cells in disorganized trabeculae, stratifications and capillary outgrowths. H & E stain. Mag. $\times 150$.

FIG. 2.—Novikoff hepatoma, higher magnification from the same section. Large irregular vacuolized nuclei, with prominent nucleoli. Numerous mitotic figures. H & E stain. Mag. $\times 760$.



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