

Isotope Studies on the Pathways of Glucose-6-phosphate Metabolism in the Novikoff Hepatoma*

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The importance of carbohydrate metabolism in neoplasia has long been recognized. During the past few years a special effort has been expended to study the metabolism of G-6-P in normal and neoplastic liver by investigating the four enzymes directly involved in the utilization of this hexose monophosphate ester. These enzymatic studies have revealed definite qualitative and quantitative metabolic alterations in the Novikoff hepatoma. It was found that G-6-Pase activity was absent (16), phosphoglucomutase activity markedly decreased (19), and glucose-6-phosphate dehydrogenase activity (18) increased—while phosphohexoseisomerase activity (20) was essentially maintained at the level of normal liver values.

The enzymatic pattern associated with G-6-P-related enzymes in the hepatoma appears to be unique in that no similar pattern has been found in the livers of normal or fasted animals or in embryonic, newborn, or regenerating livers (17, 19, 20, 22).

In a recent review of the enzymatic studies of G-6-P utilization (22), it was emphasized that the data obtained do not necessarily mean that these reactions actually take place *in vivo* and that the quantitative relationships of the pathways to one another correspond to the maximum capacities of the enzymes determined under optimal *in vitro* conditions. Therefore, it seemed necessary

* Supported in part by the United States Atomic Energy Commission and Swift & Co. This work was performed at Harvard Medical School. Preliminary reports on this study were given at the meeting of the Canad. Physiol. Soc., Oct. 10-12, 1957 (*Rev. Can. Biol.*, 16:524, 1957) and at the meeting of the Am. Assoc. for Cancer Research, April 11-13, 1958 (*Proc. Am. Assoc. Cancer Research*, 2:277, 1958).

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The following abbreviations are employed in this paper: G-6-P = glucose-6-phosphate; G-6-Pase = glucose-6-phosphatase; FDPase = fructose-1,6-diphosphatase; TCA = trichloroacetic acid.

Received for publication May 18, 1958.

to reinvestigate G-6-P utilization of the same hepatoma by using isotope methods. By the use of differentially labeled glucose in the present studies it was possible to calculate the relative rates of G-6-P metabolism via the alternate pathways available and to compare these results with those obtained in enzymatic studies.

MATERIALS AND METHODS

Experimental procedures.—Male, adult Wistar rats weighing 180-220 gm. were used. Rats were maintained on Purina Laboratory Chow ad libitum. The Novikoff hepatomas were transplanted intraperitoneally by the large-inoculum method with the injection of 50 ± 10 million cells (22). The animals were sacrificed on the 7th or 8th day after transplantation. Livers were quickly removed, blotted on filter paper, and chilled on cracked ice. Hepatomas were similarly removed, chilled, and dissected free of connective tissue and peritoneal fat.

Tissues were sliced with a Stadie Riggs slicer and weighed wet on a Roller-Smith torsion balance. Two incubation media were used: (a) a high-potassium medium containing K = 110, Ca = 10, Mg = 20, Cl = 135, and $\text{HCO}_3 = 40$ $\mu\text{moles/l}$ and (b) a high-sodium medium differing from medium (a) only in that Na was used instead of K. Five per cent CO_2 : 95 per cent O_2 and bicarbonate were used to provide buffering at pH 7.4-7.5 in the presence of the tissue. Glucose-1- C^{14} and glucose-6- C^{14} were added as substrates at a concentration of 20 $\mu\text{moles/l}$.

Cellularity determinations.—One gram of liver slices was weighed, and 10 per cent homogenates were prepared for the enumeration of the nuclei. The procedure followed the one employed previously for minced tissue samples (21). The cellularity was expressed as the number of nuclei/gm wet tissue (14).

Chemical methods.—Initial and final concentrations of glucose in the medium were determined by the method of Nelson (8). The tissue slices were separated from the medium and digested in KOH. Glycogen was isolated from the KOH digest by alcoholic precipitation (5) and was determined by the Nelson glucose method after acid hydrolysis. Long-chain fatty acids were isolated from the alcoholic KOH digest of tissue by acidification and extraction with petroleum ether (12). Glucose and glycogen were isolated as the phenylglucosazone for the determination of specific activity. The specific activity of the CO_2 was determined on a 1-ml. sample of the medium at the end of incubation by acidification with 1 N H_2SO_4 , and the evolved CO_2 was collected in CO_2 -free alkali (3) and counted as BaCO_3 .

An aliquot of the medium was deproteinized with trichloroacetic acid (TCA), and lactic acid was determined colori-

metrically by the method of Barker and Summerson (4). Carrier lactic acid was added to another portion of the medium, deproteinized with TCA, and the lactate was separated on a Dowex-1 column (3). Lactic acid was plated and counted as the Zn salt. Long-chain fatty acids were plated on lens paper as the free acids. C¹⁴ was assayed on a Robinson proportional flow windowless counter (13).

RESULTS

The Novikoff tumor proved particularly adaptable to slice experimentation, because it is easily separated free from fat, connective tissue, and blood vessels and can be sliced in a manner analogous to liver.

Metabolism of glucose by normal and neoplastic liver.—In Table 1, net changes in tissue glycogen,

fed liver and 25–30 times that observed with fasted liver slices.

Effect of ionic composition of the incubation medium.—A comparison of the data in Table 1 shows several metabolic changes associated with changes in the ionic composition of the incubation medium. Both liver and hepatoma slices exhibited less glycogen breakdown in the high-potassium medium than in the high-sodium medium. However, with regard to net change in glucose and lactic acid production, liver and hepatoma did not react similarly with respect to the influence of the cationic environment. Hepatoma slices exhibited greater glucose uptake in a high-sodium

TABLE 1
METABOLISM OF GLUCOSE (20 MILLIMOL/LITER) BY LIVER SLICES IN NORMAL FED AND FASTED ANIMALS AND NOVIKOFF HEPATOMA
(All values $\mu\text{moles/gm wet tissue/90 min}$)

Tissue	Initial glycogen	HIGH-SODIUM MEDIUM			HIGH-POTASSIUM MEDIUM		
		Net change glycogen	Net change glucose	Net change lactic acid	Net change glycogen	Net change glucose	Net change lactic acid
Normal fed rat liver	284	-158	+ 87	+ 25.6	-156	+20	+14.7
		-161	+ 70	+ 22.3	-159	+10	+11.0
	347	-193	+ 58	+ 21.6	-112	+40	+11.4
		-186	+ 61	+ 23.2	-101	+32	+ 9.5
	Means 316	-175	+ 69	+ 23.2	-132	+23	+11.7
Normal fasted rat liver	1.8	+ 0.3	- 8	+ 1.6	+ 6.4	- 5	+ 5.7
		- 0.2	- 15	+ 4.2	+ 5.9	-10	
	1.4	+ 0.1	+ 11	+ 2.6	+ 0.8	+ 5	+ 5.3
		+ 0.1	+ 11	+ 5.8	+ 3.8	+ 3	+ 6.2
	Means 1.6	+ 0.1	- 0.3	+ 3.6	+ 4.2	- 1.8	+ 5.7
Hepatoma	1.3	+ 1.2	- 76	+ 86	+ 2.1	-44	+61
		+ 1.0	- 87	+ 81	+ 1.9	-58	+62
	0.6	+ 0.7	- 80	+ 66	+ 1.4	-48	+44
		+ 0.1	- 65	+ 72	+ 0.9	-63	+53
	2.3	- 0.4	- 74	+ 86	- 0.7	-54	+59
		- 0.9	- 96	+ 96	- 0.9	-54	+59
	1.3	- 0.1	- 68	+111	+ 0.5	-59	+84
		+ 0.2	- 98	+127	+ 0.8	-73	+80
1.1	+ 0.5	- 43	+129	+ 1.0	-64	+85	
	+ 0.6	-111	+136	+ 1.1	-54	+94	
	Means 1.3	+ 0.3	- 80	+ 99	+ 0.8	-58	+68

medium glucose, and lactic acid in liver and hepatoma slices incubated *in vitro* are compared. The glycogen content of the hepatoma was similar to that of liver in a 24-hour fasted rat. Glycogen change during incubation was very small and again compared with changes observed in liver slices from fasted rats. In livers from fed rats the concentration of glucose in the medium increased during the course of the incubation, presumably from the breakdown of the tissue glycogen. In tissue slices from fasted rats there was no significant change in the medium glucose concentration, while hepatoma showed a significant net glucose utilization. Lactic acid production by the hepatoma was 4 times that observed with

medium than in a high-potassium medium, which observation is exactly the opposite of what is seen in liver slices (6). Both liver and hepatoma slices had a greater lactic acid production in the sodium medium. The lactic acid production of liver is probably associated with increased glycogenolysis, while in the hepatoma lactate production parallels glucose utilization.

Metabolism of differentially labeled glucose in hepatoma slices.—The metabolism of glucose-1-C¹⁴ and glucose-6-C¹⁴ by hepatoma slices incubated in a high-potassium medium and a high-sodium medium is shown in Table 2. Again, it seems that glucose utilization is greater in the sodium medium than in the potassium medium. Incorporation

of label from glucose into glycogen was greater in the potassium medium than in the sodium medium. Although lactic acid was equally labeled in both media, glucose oxidation appeared to be slightly greater in the sodium medium. With respect to the oxidation of Carbon 1 of glucose in relation to Carbon 6, a preferential oxidation of Carbon 1 occurred in both media.

Metabolism of differentially labeled glucose in liver slices.—In Table 3 the metabolism of glucose-1-C¹⁴ and glucose-6-C¹⁴ by liver is presented for comparison. These data also show greater incorporation of C¹⁴ from labeled glucose into glycogen in the high-potassium medium. A better comparison of liver and hepatoma can be obtained, however, from the summaries of these data presented

in Table 4. In this table the metabolism of glucose by liver slices has been compared with glucose utilization in hepatoma slices under similar conditions, i.e., in the high-potassium medium. Glucose utilization/gm wet tissue was essentially the same in the liver and hepatoma. The disposition of the glucose utilized by the two tissues was markedly different. In the liver, glycogen accounted for 36 per cent of the glucose utilized; in hepatoma only 3 per cent of the glucose utilized could be accounted for as glycogen. Carbon-1 of glucose was oxidized to C¹⁴O₂ to the same extent in both tissues and accounted for 12 per cent of the glucose used. The most remarkable difference in metabolism between the two tissues occurred with respect to the incorporation of C¹⁴

TABLE 2
METABOLISM OF GLUCOSE (20 MILLIMOL/LITER) BY NOVIKOFF HEPATOMA SLICES (ALL VALUES μ MOLE/GM WET TISSUE/90 MIN)

	Up- take	GLUCOSE-1-C ¹⁴			Up- take	GLUCOSE-6-C ¹⁴		
		To gly- cogen	To CO ₂	To lactic acid		To gly- cogen	To CO ₂	To lactic acid
HIGH-POTASSIUM MEDIUM								
1	44	3.24	6.50	21	58	1.79	1.32	17
2	48	1.96	6.20	—	68	0.97	1.16	15
3	54	1.56	6.22	29	54	1.09	1.00	—
4	59	1.13	7.62	18	78	0.66	1.34	35
5	64	1.24	5.92	43	53	0.78	0.87	60
Mean	54	1.83	6.49	27.8	62	1.06	1.14	31.8
HIGH-SODIUM MEDIUM								
1	76	1.53	9.25	30	87	0.95	1.62	19
2	80	1.14	7.98	36	65	0.77	1.32	29
3	74	1.18	8.43	30	96	0.74	1.15	35
4	68	0.70	9.09	15	98	0.53	1.48	34
5	43	0.56	9.81	25	111	0.47	1.53	41
Mean	68	1.02	8.91	27.2	91	0.69	1.42	31.6

TABLE 3
METABOLISM OF GLUCOSE BY RAT LIVER
All values are expressed in μ moles/gm wet tissue/90 min.

	Up- take	GLUCOSE-1-C ¹⁴			Up- take	GLUCOSE-6-C ¹⁴		
		To gly- cogen	To CO ₂	To lactic acid		To gly- cogen	To CO ₂	To lactic acid
HIGH-POTASSIUM MEDIUM								
Fed	32	19	5.5		55	16	4.0	
"	48	20	2.7		55	14	2.1	
"	50	15	8.9		35	17	4.2	
"	40	16	7.2		22	14	4.2	
"		9.3	3.3	3.1		8.1	2.6	6.0
"		11	2.8			11	2.4	
Mean	42 ± 4.1*	15 ± 1.7	5.1 ± 1.1		41 ± 8.1	13 ± 1.3	3.3 ± 0.4	
Fasted		2.5	1.8			2.1	2.9	
"		2.8	3.6			3.2	2.3	
HIGH-SODIUM MEDIUM								
Fed		4.6	3.0			3.9	2.2	
"		3.7	2.6			3.5	2.2	
Fasted		0.82	3.3			0.99	2.2	
"		0.63	3.0			0.56	1.8	

* Standard error.

from glucose into lactic acid. In liver slices lactic acid accounted for only 7 per cent of the glucose utilized, whereas in the hepatoma slices lactic acid accounted for 51 per cent of the glucose uptake.

Consideration of data on per average cell basis.—Actual cell counts showed that a 1-gm. liver

that 12 per cent of the glucose used could be accounted for as CO₂ and 51 per cent as lactic acid. A comparison of the Novikoff tumor with liver is similar in other respects to the comparison of the primary DAB-induced hepatoma with hepatic tissue. In the tumor incubated *in vitro* a net glucose uptake is found, whereas with liver slices

TABLE 4

PER CENT OF GLUCOSE-1-C¹⁴ UTILIZED ACCOUNTED FOR IN LIVER AND HEPATOMA

TISSUE	Up- take	Gly- cogen	CO ₂	Lactic acid	Fatty acids	Total
Liver						
μmoles/gm.*	42	15	5.1	3.1	0.9	24.1
μmoles/cell†	208	73.5	25.0	15.2	4.4	118
Per cent	100	36	12	7	2	57
Hepatoma						
μmoles/gm	54	1.83	6.49	27.8	0.2	36.3
μmoles/cell	121	4.1	14.5	62.1	0.5	81.2
Per cent	100	3	12	51	0.3	67
Liver vs. hepatoma						
Liver	100	100	100	100	100	100
Hepatoma (wet weight basis)	129	12	127	897	22	118
Hepatoma (per cell basis)	58	6	58	409	11	118

* μmoles/gm wet weight.

† Values are expressed as μmoles × 10⁸.

slice of normal fed rats contained 204 ± 29 million nuclei, while a 1-gm. hepatoma slice contained 447 ± 64 million nuclei. These data agree with previously reported values obtained from homogenates (22). Glucose metabolism in hepatoma slices, expressed on a per cell basis, has been summarized in Chart 1. Values have been expressed as the per cent of conversion found in normal liver. For example, per cell, the hepatoma utilized only 58 per cent of the glucose of liver and incorporated only 6 per cent as much C¹⁴ from glucose into glycogen. Glucose oxidized per cell in the hepatoma was unchanged relative to glucose uptake, but represented only 58 per cent of the amount oxidized by liver. Conversion of glucose to lactic acid was increased fourfold, while the incorporation of C¹⁴ from glucose into fatty acids was reduced to 11 per cent of that of liver.

DISCUSSION

The present studies on glucose metabolism by the Novikoff tumor are in good agreement with previous studies on the metabolism of labeled glucose by hepatoma *in vitro* (1, 10, 25). For example, Olson has reported that 10 per cent of the glucose utilized by *m'*-methyl-*p*-dimethylaminoazobenzene (DAB) hepatoma slices could be accounted for as C¹⁴O₂, while 30 per cent of the glucose used appeared as lactic acid. In the present study with the Novikoff hepatoma, it was found

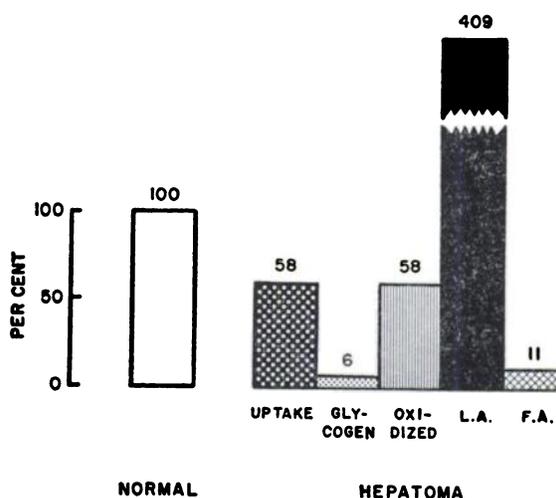


CHART 1.—Glucose metabolism in hepatoma slices. The results were calculated on average cell basis and expressed as per cent. The values of the liver of normal fed rats were taken as 100 per cent.

one usually observed a net glucose production (10, 25). The absence of glucose-6-phosphatase activity (16) in the hepatoma undoubtedly accounts for the lack of glucose release and probably accounts for the net glucose uptake by this tissue, since utilization of added radioactive glucose is essentially the same in liver and hepatoma.

Although the preferential oxidation of carbon 1 of glucose to CO₂ by hepatoma slices might indicate an increase in the direct oxidation of hexose phosphate via the phosphogluconate oxidation pathway over that found in the liver, the maximum contribution of this pathway to the over-all metabolism (3) of glucose is essentially the same in liver and hepatoma (12 per cent). The contribution of the phosphogluconate pathway relative to the glycolytic pathway can be calculated from the contribution of carbons 1 and 6 of glucose to fatty acids and to lactic acid (2, 3, 24) (see Table 5). These calculations are based on the assumption that any C-1 of glucose appearing in fat or lactic acid must have arrived via the classical Embden-Meyerhof scheme, while carbon 6 of glucose could have been derived by either glycolysis or phosphogluconate oxidation.

that lactate oxidation proceeded as well in hepatoma slices as in liver slices.

To account for the increased lactic acid production in hepatoma slices Olson (10) suggested that the increased activity of phosphofructokinase in this tissue might contribute to the over-all increased rate of glycolysis. However, the interconversion of hexose phosphate and hexose diphosphate is mediated by two enzymes, a kinase and a phosphatase. An increased flow of substrate down the glycolytic pathway might be achieved by a decrease in phosphatase activity as readily as by an increase in the kinase activity. We have, therefore, assayed supernatant fluid of liver and hepatoma for fructose-1,6-diphosphatase activity by the method of Pogell and McGilvery (11). Having failed to detect FDPase in hepatoma, we feel that a lack of this enzyme may con-

TABLE 5
LIPOGENESIS IN LIVER AND HEPATOMA

Tissue	Medium	Fatty acid (mg/gm tissue)	μmoles glucose to fatty acids			Per cent fatty acid carbons derived via glycolysis
			C-1	C-6	C-1/C-6	
Fed liver	High potassium	22	0.85	1.04	0.82	82
		22	0.93	1.19	0.78	78
Fed liver	High sodium	22	0.67	0.59	1.1	100
		22	0.54	0.80	0.67	67
Hepatoma	High potassium	33	0.08	0.09	0.9	90
		34	0.15	0.19	0.79	79
Hepatoma	High sodium	34	0.20	0.17	1.2	100
		27	0.45	0.35	1.3	100

The rationale for these calculations has been discussed in relation to the relative contribution of these two pathways in other tissues (2, 7, 24). The data presented in Table 5 would indicate that most of the carbons incorporated into fatty acids in liver and hepatoma slices are derived via the glycolytic system. The contribution of an extra-glycolytic pathway does not appear to be of great import in either of these tissues. A similar conclusion was reached by Wenner and Weinhouse in studies on a number of animal tumors (24). The decreased fatty acid synthesis of the Novikoff hepatoma is compatible with the observed diminished phospholipid content of this tumor (9, 23).

Apart from glycogen synthesis and glucose production the major metabolic difference between liver and hepatoma appears to be the extent to which glucose is converted to lactic acid. That this represents an increased glycolysis of hexose phosphate rather than an accumulation of lactic acid owing to decreased oxidation of pyruvate is suggested by the work of Olson (10) and of Zamecnik *et al.* (25). Both of these workers found

tribute to the over-all increased rate of glycolysis observed in this tissue (15).

SUMMARY

The metabolism of glucose-6-phosphate in normal rat liver and Novikoff hepatoma slices was studied by utilization of glucose-1-C¹⁴ and glucose-6-C¹⁴. The tissue slices were incubated in two different media: a high-sodium and a high-potassium medium. Glucose uptake was determined, and balance sheets were drawn up by determining glycogen, lactic acid, fatty acid, and CO₂. Results were expressed on wet weight basis and per average cell.

1. Liver and hepatoma were similar in ionic response in two respects: (a) Liver and hepatoma slices exhibited less glycogen breakdown in the high-potassium medium than in the high-sodium medium. (b) Both liver and hepatoma slices showed a greater lactic acid production in the sodium medium. On the other hand, hepatoma slices exhibited greater glucose uptake in the high-sodium medium than in the high-potassium

medium, which was the opposite of what was seen in the liver.

2. When glucose metabolism of the hepatoma slices was expressed on a per cell basis, it was noted that the glucose uptake was 58 per cent and the incorporation of glucose-C¹⁴ into glycogen was only 6 per cent of that of the liver. Glucose oxidation in the hepatoma was unchanged relative to glucose uptake but represented only 58 per cent of the amount oxidized by the liver. The incorporation of C¹⁴ from glucose into fatty acids was reduced to 11 per cent, while conversion of glucose to lactic acid was increased to fourfold of the liver values.

3. The review of the four pathways of glucose-6-phosphate utilization in the hepatoma showed complete absence (glucose release) or nearly complete abolition (glycogen synthesis) of two pathways. On the other hand, the hexose monophosphate shunt (as calculated on the basis of CO₂, lactate, and fatty acid data) appeared to be present, and the lactic acid production was markedly increased. These data are essentially in line with the previously obtained enzymatic indications of the alterations of glucose-6-phosphate metabolism in the neoplastic liver.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Prof. A. Baird Hastings for making this cooperative work possible and for his criticism of the manuscript. Grateful acknowledgment is made to Dr. Alex B. Novikoff and Sidney Goldfisher for supplying the hepatoma, and to Sylvia Zottu for valuable technical assistance.

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