Comparative Biochemistry of Hepatomas

II. Isotope Studies of Carbohydrate Metabolism in Morris Hepatoma 5123*

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SUMMARY

Hepatoma 5123 as compared with liver showed a marked deficiency in incorporation of labeled carbon into glycogen, CO_2, and fatty acids; however, lactate production was normal. The lesion in glycogenesis agrees with the decreased phosphoglucomutase activity of this tumor. Incubation of hepatoma slices with pyruvate showed markedly depressed glucose production, revealing a decrease in gluconeogenesis. This is in line with the decreased glucose-6-phosphatase and fructose-1,6-diphosphatase activities of this tumor. The preferential oxidation of carbon-1 of glucose in Morris tumor, as in normal liver, is compatible with the normal glucose-6-phosphate dehydrogenase activities of these tissues. Thus, the increase in the direct oxidative pathway and in glucose-6-phosphate dehydrogenase activity as well as the high lactate production of the Novikoff tumor were not present in Hepatoma 5123. However, the decreased glycogen synthesis and the depressed phosphoglucomutase activity occurred in both tumors to a similar extent. The gluconeogenic pathway which failed to operate in the Novikoff tumor, partly as a result of the absent specific phosphatases, was functioning in the Morris tumor, where part of the activities of these enzymes was retained.

The extent of the examined carbohydrate metabolic lesions may be correlated roughly with the growth rates of Morris and Novikoff hepatomas.

The existence of liver tumors of different malignancy (rate of growth, metastasizing ability, length of host survival) has made it possible to compare the biological behavior of hepatomas with the metabolic makeup of these tumors. Recent investigations on the enzyme composition of a number of primary and transplantable liver tumors (10, 11) showed that the Morris hepatoma 5123 was nearest to normal liver, whereas Novikoff hepatoma was at the far end of the spectrum. Since the growth period of the Morris tumor is 3 months, in contrast to the Novikoff hepatoma, which kills the host in a week, a comparison of the intermediary metabolism of these tumors may throw light on the neoplastic significance of metabolic lesions in hepatomas.

The first paper in this series (18) showed that the marked increase in glucose-6-phosphate dehydrogenase activity in the Novikoff tumor (20) was not present in the Morris hepatoma. However, another enzymatic lesion of the Novikoff hepatoma, markedly decreased phosphoglucomutase activity (20), occurred in the Morris hepatoma to a similar extent. In contrast, some of the enzymes missing in the Novikoff hepatoma, glucose-6-phosphatase (19), and fructose-1,6-diphosphatase (16, 21), were operating, but in decreased activity, indicating a partial lesion in gluconeogenesis in Morris hepatoma.

The present investigation reports the results of studies of carbohydrate metabolism with differ-

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entially labeled glucose and pyruvate in the Morris hepatoma.

MATERIALS AND METHODS

Animals.—Adult, female, Buffalo-strain rats of 180–250 gm. were used in these studies. The control and tumor-bearing animals were shipped by air express from Dr. H. P. Morris of the National Cancer Institute, Bethesda, to Indiana University, Indianapolis. The isotope studies were carried out 3–10 days after arrival of the animals. The transplantable tumor used was the Morris hepatoma 5123, subline D, generation 19. The rats were given inoculations intramuscularly by trocar in both hind legs and killed when tumors were approximately 3 months old. Hepatoma 5123 was originally induced in a female Buffalo-strain rat in 1956 after ingestion of an adequate diet containing N-(2-fluorenyl)phthalamic acid (8). Since that time it has been maintained by serial passage into animals of the strain of origin. D represents one of four arbitrarily established sublines made from a tumor in the 16th transplant generation.

Upon arrival in the Indiana University laboratory, the tumor-bearing and control animals were kept in separate cages. Purina Laboratory Chow and water were available ad libitum until the time of sacrifice.

Experimental procedures.—The rats were stunned by a blow on the head, decapitated, and exsanguinated. Livers and tumors were rapidly removed and blotted on filter paper. Particular care was exercised in dissecting the tumor free of necrotic, hemorrhagic, or nontumor material to ensure the selection of viable tumor samples. Specimens were chilled in an ice-cold Petri dish on cracked ice before being sliced with a Stadie Riggs hand microtome. Half a gram of liver or tumor slices was weighed on a Roller-Smith torsion balance and incubated in 6.0 ml. of Hastings high-potassium medium (6) containing labeled substrate. Glucose-1-C^{14} (20 mmoles/liter), glucose-6-C^{14} (20 mmoles/liter), pyruvate-2-C^{14}, and pyruvate-1-C^{14} (40 mmoles/liter) were used. 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.

Chemical methods.—Initial and final concentrations of glucose in the medium were determined by the method of Nelson (9). Tissue slices were separated from the incubation medium and digested in KOH. Glycogen was precipitated by the addition of ethanol. Fatty acids were determined as previously described (12). Glucose and glycogen were isolated as the phenylglucosazone for the determination of specific activity. $C^{14}O_2$ was isolated from the incubation medium by acidification and diffusion into NaOH, precipitated, and counted as $BaCO_3$ (1).

Initial and final concentrations of pyruvate (5) and lactate (3) were determined colorimetrically on trichloroacetic acid filtrates of aliquots of the incubation medium. Lactic acid was isolated by ion exchange chromatography and oxidized with permanganate prior to radio assay (2). C^{14} assays were performed with the use of a Nuclear Chicago proportional flow counter, and C^{14} incorporation into the various metabolic products was computed in terms of μmoles of the original substrate/gm wet tissue.

Expression of biochemical results.—Since previous work (17, 18) showed that the cellularity of Morris hepatoma 5123 is in the same range as that of the normal liver, the isotope results in this paper are expressed per gram wet weight of tissue.

RESULTS

The Morris tumor proved adaptable to experimentation *in vitro* and can be sliced in a manner analogous to liver.

Metabolism of glucose in liver and hepatoma 5123.—Glucose uptake of the Morris hepatoma 5123 appeared to be markedly lower than that of the control liver. Both liver and tumor showed the same degree of lactic acid production in the presence of glucose as an added substrate.

The metabolism of glucose-1-C^{14} and of glucose-6-C^{14} by liver and hepatoma slices is summarized in Table 1. As compared with liver, the Morris hepatoma exhibited a pronounced deficiency in the incorporation of labeled carbon from glucose into glycogen, since the amount of radiocarbon found in tumor glycogen averaged only 2 per cent of that obtained in liver. Similarly, CO_2 production from labeled glucose in tumor slices was diminished to less than 15 per cent of that in the control liver. However, both liver and hepatoma slices showed a preferential oxidation of carbon-1 of glucose to CO_2.

The incorporation in the control liver of labeled carbon from glucose into fatty acids was 1.31 and 1.29 μmoles from glucose-1-C^{14} and glucose-6-C^{14}, respectively. In the Morris hepatoma under similar conditions incorporation of glucose carbons into fatty acids was less than 0.01 μmoles.

Metabolism of pyruvate in liver and Morris hepatoma 5123.—A comparison of the metabolism of pyruvate-2-C^{14} in liver and Hepatoma 5123 is given in Table 2. The pyruvate uptake of the Morris hepatoma was about 20 per cent of that in the control liver. The glycogen values show that there was little storage of this form of energy in the

TABLE 1

METABOLISM OF GLUCOSE (20 MMOLES) BY LIVER AND MORRIS HEPATOMA SLICES

Tissue	Uptake	Glucose-1-C^{14}					Glucose-6-C^{14}		
		Net lactate	To glycogen	To CO_2	To fatty acid	To lactate	To glycogen	To CO_2	To fatty acid
Liver:									
1	42	12.0	8.7	2.2	0.35		5.9	1.2	0.26
2	83	13.0	6.2	1.6	0.17		7.1	1.1	0.23
3	88	12.0	13.5	4.7	1.90		11.2	1.3	1.78
4	89	13.0	9.9	5.0	2.80		10.5	0.82	2.90
Mean*	76	12.5	9.6	3.4	1.31	3.0§	8.7	1.11	1.29
Per cent†	100	16	13	4	2	4	11	1	2
Hepatoma 5123:									
1	56	16.0	0.28	0.50	0.01		0.32	0.13	0.01
2	44	18.0	0.12	0.33	0.00		0.08	0.26	0.00
3	31	8.0	0.32	0.62	0.00		0.15	0.09	0.00
4		11.0	0.16	0.52	0.00		0.30	0.04	0.00
Mean*	44	13.3	0.22	0.49	0.00	2.5§	0.21	0.13	0.00
Per cent†	100	30	<1	1	0	6	<1	<1	0.00
Liver vs. hepatoma 5123:‡									
Liver	100	100	100	100	100	100	100	100	100
Hepatoma	58	106	2	14	<1	83	2	12	<1

* Data given in μmoles/gm wet tissue /90 min.

† The glucose uptake is used arbitrarily as 100 per cent.

‡ The values of the liver were taken arbitrarily as 100 per cent.

§ Pool of four samples.

TABLE 2

METABOLISM OF PYRUVATE (40 MMOLES) BY LIVER AND MORRIS HEPATOMA SLICES

Tissue	Uptake	Initial glycogen	Final glycogen	Net lactate	Net glucose	To glucose	To glycogen	To CO_2	To fatty acid
Liver:									
1	230	265	174	69	96	22.5	17.8	38	11.7
2	220	210	184	72	95	30.5	25.4	37	7.2
3	220	305	188	69	100	24.0	23.5	31	7.6
4	204	390	184	69	94	19.5	21.6	31	5.7
Mean*	219	293	183	70	96	24.1	22.1	34	8.1
Per cent†	100	100	100	100	100	100	100	100	100
Hepatoma 5123:									
1	36	6.4	2.4	34	14.8	3.6	2.4	5.4	0.28
2	51	7.0	1.6	32	12.8	3.2	1.7	8.7	0.16
3	63		2.0	24	10.5	2.3	2.8	5.2	0.08
4	49		1.9	26	10.5	2.8	2.0	6.3	0.08
Mean*	50	6.7	2.0	29	12.2	3.0	2.2	6.4	0.15
Per cent†	23	2	1	41	13	12	10	19	2

* Data given in μmoles/gm wet tissue/90 min.

† The liver values were taken arbitrarily as 100 per cent.

Morris tumor. A decrease in tissue glycogen occurs from both liver and hepatoma during the *in vitro* incubation procedure. Lactate production by tumor slices in the presence of pyruvate as added substrate was 40 per cent of that found in liver.

With the marked reduction of pyruvate uptake (utilization) by the Morris hepatoma there was a proportional reduction in CO_2 production which was 12–16 per cent of the pyruvate uptake in both normal and neoplastic liver. On the other hand, the percentage of substrate used that appeared in the various other end products of metabolism was decreased in the hepatoma. Thus, 11 per cent of the substrate went to glucose in the normal liver, but only 6 per cent in the hepatoma. In the liver 10 per cent of the pyruvate carbon taken up was recovered as glycogen, but only about 4 per cent was found in glycogen in the Morris hepatoma. The incorporation of pyruvate label into fatty acids in the hepatoma was negligible (0.3 per cent), since significant amounts of radioactivity were not recovered in this fraction; in the liver, however, 3.7 per cent of the pyruvate used could be accounted for in the fatty acid fraction.

A comparison of the metabolism of pyruvate-1-C^{14} and pyruvate-2-C^{14} in Morris hepatoma is presented in Table 3. When Morris hepatoma slices were incubated with pyruvate labeled in the 1- or 2-position there was no significant difference in the uptake of the substrates or in the extent of net glucose formation by the tissue. On the other hand, markedly less CO_2 was produced from pyruvate-2-C^{14} than from pyruvate-1-C^{14}. The metabolism of the two substrates by Morris hepatoma slices also differed in the conversion to glycogen, which was much higher from pyruvate-2-C^{14} than from pyruvate-1-C^{14}.

DISCUSSION

The present studies on glucose metabolism by Morris hepatoma 5123 revealed, as compared with liver, a marked deficiency in the incorporation of labeled carbon from glucose into glycogen, CO_2, and fatty acids. The lesion in glycogen synthesis can be correlated with the decreased phosphoglucomutase activity in this tumor.

Previous work on the Morris hepatoma showed that glucose-6-phosphatase activity was present in this tumor (10, 17, 18), and fructose-1,6-diphosphatase activity was decreased to 30 per cent in the average hepatoma cell (17, 18). The release of glucose from Morris hepatoma slices confirms the enzymatic indications for the presence of glucose-6-phosphatase activity. Incubation of hepatoma slices with pyruvate also showed that the gluconeogenic pathway is capable of operating in this tumor. However, the activity of this pathway appears to be markedly lower than that of the liver, which finding is in line with the decrease noted in activities of the specific phosphatases.

The preferential oxidation of carbon-1 of glucose in the Morris hepatoma and the normal liver is compatible with the presence of normal activities of glucose-6-phosphate dehydrogenase in these tissues. The fact that C-1 of glucose is recovered in CO_2 in greater yield than C-6 has been observed in many tumors, including several other hepatomas (22).

TABLE 3

COMPARISON OF C-1 AND C-2 LABELED PYRUVATE (40 MMOLES) METABOLISM BY MORRIS HEPATOMA SLICES

(All values μmole/gm wet tissue/90 min)

Pyruvate uptake	Net glucose produced	Pyruvate-1-C^{14}	
		Pyruvate to CO_2	Pyruvate to glycogen
11	8.3	6.4	0.35
26	7.9	6.6	0.61
11	9.6	9.2	0.44
7	10.7	9.8	0.43
Mean: 14	9.1	8.0	0.48
		Pyruvate-2-C^{14}	
9	9.6	3.3	2.9
19	11.5	3.0	2.5
16	9.3	3.5	2.6
36	8.9	2.6	1.8
Mean: 20	9.8	3.1	2.5

The study of pyruvate metabolism demonstrated that there was very poor utilization of this substrate in the Morris hepatoma. The decreased incorporation of pyruvate carbon into glycogen is in line with the findings that the gluconeogenic pathway operates to a decreased rate and that little glycogen was stored when glucose was used as a substrate. The decreased CO_2 production from pyruvate was proportional to the reduction of pyruvate uptake in the Morris hepatoma; however, the incorporation into fatty acids was much less than would be expected from a proportional reduction in pyruvate utilization.

The comparison of pyruvate-1-C^{14} and pyruvate-2-C^{14} metabolism by Morris hepatoma slices brought interesting information. Carbon-1 of pyruvate is expected to yield CO_2 in oxidative decarboxylation and production of acetyl CoA. Car-

bon-2 should give rise to CO_2 in the oxidation of acetyl CoA via the citric acid cycle. It has been established in liver that carbon-1 of lactate (4) and pyruvate (7) is preferentially oxidized 3/1 over carbon-2 oxidation. In the hepatoma a ratio of 2.0–3.8 was obtained. It has also been observed (13) that distribution of C^{14} in glucose formed from pyruvate-2-C^{14} suggests CO_2 fixation and randomization on the label in the dicarboxylic acid shuttle rather than a direct reversal of the pyruvic kinase reaction. Under these circumstances a loss of C^{14} from carbon-1-labeled pyruvate should occur, and in comparison with carbon-2-labeled pyruvate a marked reduction in labeled carbon incorporation into glucose or glycogen would be expected. This appears to be the situation in the hepatoma. In this case a C-2/C-1 ratio for liver slices of 2.6–3.6 (7) and a ratio for hepatoma of 4.2–8.2 were obtained.

A comparison of liver tumors of differing malignancy, such as the Morris and Novikoff tumors, is of interest. Such a study may make it possible to detect metabolic variations which may parallel the biological differences between these liver tumors. For the object of this comparison the carbohydrate isotope data of the Novikoff hepatoma (2) and the Morris hepatoma 5123 are brought together in Chart 1 and contrasted with values of normal liver which are taken as 100 per cent.

Similar lesions in Morris and Novikoff hepatomas.—A comparison of the carbohydrate metabolism (2) and enzyme pattern (14, 15, 21) of the Novikoff tumor and Morris hepatoma 5123 indicates that some of the metabolic lesions of the Novikoff hepatoma are present in the Morris tumor to a nearly identical extent. Examples of similar lesions are the diminished glucose uptake, decreased glycogen formation, decreased phosphoglucomutase, and depressed fatty acid formation from glucose or pyruvate.

Metabolism altered in Novikoff hepatoma, but normal in Morris tumor.—In the Novikoff hepatoma glucose-6-phosphate dehydrogenase was highly increased (20), and the isotope data were compatible with an increase in the pentose phosphate pathway (2). In the Morris tumor glucose-6-phosphate dehydrogenase was in the normal range (17, 18), and the isotope results indicate that this pathway in the Morris tumor contributes to the over-all metabolism of glucose to about the same extent as in liver. It is interesting that the increased lactic acid production found in the Novikoff hepatoma (2) and described in various other liver tumors (21) was not observed in Hepatoma 5123.

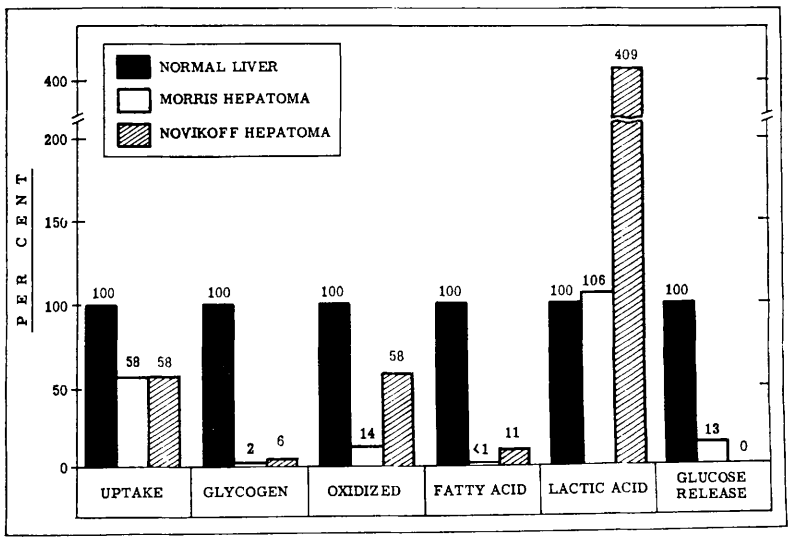

CHART 1.—Comparison of glucose metabolism in normal liver, Morris hepatoma 5123, and Novikoff hepatoma. Results were calculated as per cent; the values of the normal liver were taken as 100 per cent.

Metabolic alterations which occurred as complete lesions in Novikoff hepatoma, but appeared only as partial lesions in the Morris tumor.—In the Novikoff hepatoma no glucose was produced either from glycogen breakdown or through gluconeogenesis from pyruvate as demonstrated by isotope methods (2). The two enzymatic lesions which were responsible, partly at least, for the lack of glucose release and gluconeogenesis were the absence of glucose-6-phosphatase (10, 19) and fructose-1,6-diphosphatase (16, 21). On the other hand, in the Morris hepatoma the lesions which

occurred in glucose-6-phosphatase and fructose-1,6-diphosphatase activities were manifested only in sharp decreases in the normal activities to 50 and 30 per cent, respectively. Corresponding with these enzymatic activities the Morris hepatoma exhibited small but definite gluconeogenesis.

In the Novikoff hepatoma, as a result of decreased glycogenesis and absent gluconeogenesis, glucose-6-phosphate was channeled into glycolysis and pentose formation. However, in the Morris tumor a partial escape of glucose-6-phosphate is possible due to the presence of glucose-6-phosphatase and the operation of gluconeogenesis. As a result of metabolic lesions occurring to a smaller extent than those found in the Novikoff hepatoma, the Morris hepatoma may generate less energy for cell division and cell function. The relative lack of available energy is further emphasized by the fact that the oxidation of glucose is also markedly decreased in the Morris tumor.

It appears that the extent of the examined carbohydrate metabolic and enzymatic lesions may be correlated roughly with the low growth rate of the Morris hepatoma in contrast to the rapid growth of the Novikoff tumor.

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