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Hexokinase, Differentiation and Growth Rates of Transplanted Rat Tumors[1]

W. Eugene Knox,[2] S. C. Jamdar,[3] and Patricia A. Davis

Department of Biological Chemistry, Harvard Medical School, and The Cancer Research Institute, New England Deaconess Hospital, Boston, Massachusetts 02215

SUMMARY

Concentrations of hexokinase in 18 types of transplanted rat tumors were significantly correlated with the measured growth rates and also paralleled the degrees of loss of histological differentiation of these tumors. The correlations confirm the similar relationship already found among hepatomas and extends it to some nonhepatic tumors of the rat. The normal tissues, fetal and regenerating liver, grew equally as fast as the fastest tumors but contained significantly less hexokinase.

INTRODUCTION

The limitations on glycolytic rates in tumors are becoming clearer from studies on hepatomas of various growth rates. Glycolysis, long held to be typically high in neoplasms, was actually low or absent in slow-growing hepatomas (2, 3), and rose only in parallel with the growth rates of 5 of these (23) and of 10 other hepatomas (7). Since the rate of glycolysis in homogenates was limited by the rate of glucose phosphorylation by ATP (8, 16), it might therefore be predicted that growth rate, too, was related to glucose phosphorylation in hepatomas. Weinhouse (27) described 20 different hepatomas with hexokinase activities covering a 10-fold range in which enzyme concentrations approximately paralleled the relative growth rates of the hepatomas, insofar as they are known from the "transplantation times" published by Morris (17).

It was desirable that this possible biochemical correlative of cellular growth rate be tested more widely, on nonhepatic tumors as well as hepatomas, and on normal tissues. Actual growth rates of the hepatomas had not been measured in the earlier work. For this purpose, we measured hexokinase activities in fetal, adult, and regenerating livers and in 18 different types of transplanted hepatic and nonhepatic tumors of the rat, and compared them with the measured rates of growth in volume of the same tissues and tumors. Comparisons were also made with degrees of loss of histological differentiation in the tumors and with their concentrations of glutaminase (K),[4] which were earlier found to be proportional to growth rate in many of the same tumors (12, 13, 15).

MATERIALS AND METHODS

NEDH inbred rats were the sources of regenerating livers, taken 36 hours after partial ($^2/_3$) hepatectomy of adult males and of fetal livers taken at 20 days of gestation. Each generation of tumors was grown, 1 to a rat, in at least 6 males of the Buffalo, Fischer, or NEDH strains of rats in which each tumor arose. All rats were fed Purina chow without limitation. Tumor volumes between 1 and 30 ml were determined at intervals by measurements through the skin of 3 orthogonal diameters. The simple exponential rate constant of growth in volume, b_{vol}, was calculated by least squares from the early, linear part of the relation, $\log_{10}$ vol $= a + b_{vol}$ (days). This was done for each tumor and averaged for all the tumors of that type and generation as described in detail (12, 13). During the period of study, the growth rates did not change appreciably in successive generations of any of the tumors. They are similar to the previously published rates for single generations of many of these tumors. The same procedure was used to measure the growth of liver weight in fetal rats from Day 14 of gestation and in control adult rats. The weight of liver was measured in control animals killed at intervals at the same ages as the host animals. Adult liver weight increased at a rate equivalent to a b_{vol} less than 0.005 and was assigned a value of $b_{vol} = 0.0$. Rate of regeneration of liver in partially hepatectomized rats during the first 2 postoperative days, as given in 4 published series, was also calculated in this way (1, 5, 9, 10).

Tissues and the viable cortex of the tumors were homogenized in 4 volumes of cold 0.14 M KCl. Counts of cell nuclei were made in a hemocytometer in duplicate on suitable dilutions in KCl of the homogenates. Hexokinase

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[4]The abbreviations used are: glutaminase (L), liver-type glutaminase; glutaminase (K), kidney-type glutaminase.

and glucokinase were immediately determined on the soluble fraction after centrifugation at 100,000 X *g* for 60 min at 0° as described by Sharma *et al.* (19). The activities were linearly proportional to tissue concentration and to time. The hexokinase and glucokinase activities in adult male rat (NEDH) liver as the reference standard were measured regularly under identical conditions of assay. Activities are expressed in units of μmoles/min of glucose-6-P formed at 25° either per g tissue, wet weight, or per 10^6 cell nuclei, as the means ± S.D. of a given number of individual assays.

RESULTS

The varieties of tumors studied are listed in Table 1. Regenerating and fetal livers with their measured growth rates are included for comparison. They grow as fast as or faster than most of the tumors.

Relative histological differentiation of the tumors was determined by an averaged ranking procedure for comparison with the measured growth rates. Four overlapping groups of the tumors were ranked from the most to the least differentiated by a number of experienced pathologists, as already described for some of these tumors (12, 13).

The coefficient of concordance between the judges was high and significant (about 0.7 to 0.8) for each of these groups. By use of the ranks of tumors common to 2 groups, the tumors were fitted into an overall series in the order of increasing undifferentiation with the rank of each tumor as that given in Table 1. The tumors are actually arranged in Table 1 in the not very different order of increasing measured growth rates. The rank by loss of histological differentiation cited in Table 1 and the actual order in Table 1 by measured growth rates for the tumors were very significantly correlated (r_s = 0.920, d.f. = 18). It is highly unlikely that the 2 orders are independent.

Hexokinase and cell nuclei in the tissues studied are given in Table 1 as the means, standard deviations, and number of determinations in 1 or more generations. These or a number of other measurements were made in each of the generations of these tumors. Like the growth rates, the compositions did not differ appreciably for a tumor type between generations. Hexokinase per g tissue in adult liver and the tumors was significantly correlated with the measured growth rate (r = 0.533, d.f. = 17, $p < 0.02$). The number of cell nuclei per g tissue was independent of growth rate (r = −0.002). With cell nuclei as the tissue base for expressing hexokinase concentration, the correlation with growth rate was very much increased. The correlation coefficient of hexokinase (given in milliunits) per 10^6 cell nuclei with growth rate was r = 0.751 (d.f. = 17, $p < 0.001$) (Chart 1).

Hexokinase concentrations were low in regenerating and fetal livers, either per g tissue or per cell nuclei, in relation

Table 1

Growth, differentiation, and hexokinase content of tissues and tumors

Measurements on tissues and tumors are recorded as the means, S.D., and (in parentheses) the number of determinations. For b_{vol}, the numbers in parentheses are the generations or separate groups in which growth rate was determined. The value for liver was <0.005 b_{vol} and is set at 0.0. Histological rank is the order of increasing undifferentiation as defined in the text.

Tissue or tumor	Histological rank	b_{vol} (day^{-1})	Hexokinase (μmoles/min/g)	Cell nuclei (10^6/g)	Hexokinase / 10^6 cell nuclei (milliunits)
Liver[a]	1	0.000 (2)	0.45 ± 0.15 (5)	245 ± 10 (3)	1.84
Morris hepatoma 7793[b]	3	0.010 (1)	0.28 ± 0.04 (5)	346 ± 60 (12)	0.81
Morris hepatoma 9618A[b]	2	0.019 (1)	0.22 ± 0.06 (6)	203 ± 22 (12)	1.08
Mammary carcinoma 211[a]	5	0.027 ± 0.007 (6)	0.88 ± 0.22 (13)	220 ± 36 (4)	4.29
Morris hepatoma 7777[b]	4	0.030 (2)	1.49 ± 0.27 (4)	512 ± 50 (11)	2.91
Morris hepatoma 5123tc[b]	6	0.031 (1)	1.16 (2)	510 ± 50 (12)	2.27
Mammary carcinoma 8B[c]	9	0.033 ± 0.011 (6)	3.86 ± 0.36 (7)	714 ± 184 (3)	5.41
Mammary carcinoma 7A[c]	10	0.038 ± 0.002 (3)	1.31 ± 0.15 (6)	366 ± 18 (3)	3.58
Renal cell carcinoma 9789K[b]	7	0.038 (1)	3.67 ± 0.35 (5)	761 ± 118 (5)	4.82
Mammary carcinoma 1C[c]	8	0.052 ± 0.011 (6)	3.25 ± 0.40 (5)	592 ± 43 (4)	5.49
Fibrosarcoma 208[a]	16	0.085 ± 0.014 (4)	2.40 ± 0.61 (18)	601 ± 120 (33)	3.99
Dunning hepatoma LC-18[c]	11	0.086 ± 0.015 (3)	2.50 ± 0.54 (12)	385 ± 88 (15)	6.49
Mammary carcinoma 1A[c]	15	0.099 ± 0.019 (6)	3.13 ± 0.25 (5)	402 ± 54 (5)	7.79
Mammary carcinoma 3230AC[c]	12	0.102 ± 0.005 (3)	3.29 ± 0.59 (18)	550 ± 50 (4)	5.98
Mammary carcinoma 3230C[c]	17	0.102 ± 0.019 (5)	2.52 ± 0.35 (5)	372 ± 19 (4)	6.77
Mammary carcinoma 205A[a]	14	0.106 ± 0.007 (3)	2.27 ± 0.54 (26)	505 ± 69 (32)	4.50
Mammary carcinoma 3230A[c]	13	0.127 ± 0.019 (8)	1.76 ± 0.25 (16)	369 ± 38 (6)	4.77
Mammary carcinoma 5A[c]	18	0.153 ± 0.028 (8)	3.41 ± 0.62 (16)	272 ± 20 (5)	12.54
Undifferentiated carcinoma Walker[a]	19	0.162 ± 0.012 (7)	2.44 ± 0.66 (15)	420 ± 66 (22)	5.80
Regenerating liver[a]		0.129 ± 0.013 (4)	0.56 ± 0.08 (4)	226 ± 30 (4)	2.48
Fetal liver[a]		0.218 (2)	0.48 ± 0.05 (4)	760 ± 70 (6)	0.63

[a, b, c] Rat strains used were [a], NEDH; [b], Buffalo; and [c], Fischer.

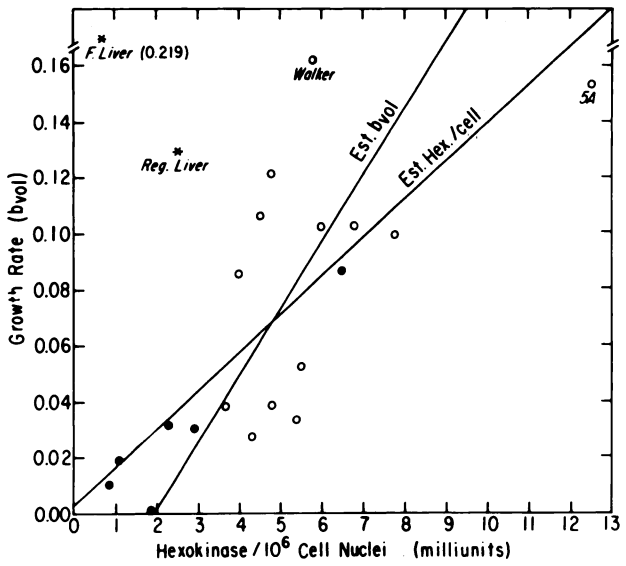

Chart 1. Relation of hexokinase to growth rate of 18 transplanted rat tumors and adult liver. Hepatomas (•) and nonhepatic tumors (○) are distinguished. Hexokinase (nmoles/min at 25°) is expressed per 10^6 cell nuclei, and growth rate is expressed as average b_{vol} of each tumor type. The correlation coefficient is $r = 0.751$ (d.f. = 17; $p < 0.001$). The estimating equations of the 2 regression lines are:

$(b_{vol}) = 0.003 + 13.6$ (hexokinase/10^6 cell nuclei)

(Hexokinase/10^6 cell nuclei) $= 0.002 + 0.042\ (b_{vol})$

Fetal and regenerating livers (*) were not included in the correlation. They grow faster than tumors with the same hexokinase concentrations.

to the concentrations in tumors that grew at similar rates. The values for these normal tissues are plotted in Chart 1 for comparison with those of tumors.

Glutaminase (K) concentrations in many of these tumors were generally proportional to their growth rates (12, 13, 15). Remeasured in the present series, its concentration per 10^6 nuclei was again correlated with growth rate but less strongly than was hexokinase ($r = 0.406$), primarily because of the very high concentration of glutaminase (K) (42 μmoles/min/g) found in the relatively slow-growing renal cell carcinoma, 9789K (18). Glutaminase (K) and hexokinase concentrations were, however, significantly correlated with each other ($r = 0.619$, d.f. = 17, $p < 0.01$).

Glucokinase was measured at the same time as hexokinase in all of the tissues by the extra activity obtained with a higher glucose concentration. The difference was not significant in most of the tissues, especially in view of the high base line of hexokinase activity in many of the tumors. The results for various hepatic tissues as well as the average for all the nonhepatic tumors of Table 1 are given in Table 2. Of the tumors, only hepatoma 9618A showed significant activity. It was higher than in liver. The next highest activities (0.16 μmole/min/g in hepatoma 7777 and mammary carcinoma 8B) and all the lower values were judged not to be significant. Glucokinase was decreased significantly in livers of rats bearing at least the faster-growing tumors (the lowest values, with Tumor 5A, are given

Table 2

Glucokinase activities in liver, hepatomas, and nonhepatic tumors

Tissue or tumor	Generation[a]	Glucokinase (μmoles/min/g)
Liver, normal adult		1.78 ± 0.16 (5)
Liver, regenerating		1.59 ± 0.47 (4)
Liver, tumor-bearing (5A)		0.74 ± 0.08 (4)
Liver, fetal		0.02 ± 0.02 (6)
Morris hepatoma 7793	14	0.00 ± 0.02 (5)
Morris hepatoma 9618A	3	2.01 ± 0.38 (6)
Morris hepatoma 7777	30	0.16 ± 0.09 (3)
Morris hepatoma 5123tc	59	0.02 (2)
Dunning hepatoma LC-18	20	0.05 ± 0.02 (7)
Nonhepatic tumor means[b]		0.06 ± 0.05 (13)

[a]Generation of transplantations as recorded by Dr. H. P. Morris, except for LC-18. Its number is the generation maintained by us.

[b]Average ± S.D. of the *means* for the 13 types of nonhepatic tumors in Table 1 (range, 0.0 to 0.16).

in Table 2). Levels of this enzyme in liver are known to vary with the physiological state (19). Persistence of glucokinase in rapidly growing regenerating liver contrasted with its absence from fetal liver and its loss from all but the 1 hepatoma among the tumors studied.

DISCUSSION

Absence of significant amounts of glucokinase from all but one of the variety of tumors studied appears here in a different perspective than in studies limited to hepatomas. This enzyme is uniquely characteristic of adult liver. Its level varies with diet and physiological state, but even so at least half the normal level persisted in the livers of rats bearing the most rapidly growing tumors (Table 2). In contrast, it was not measurable in most of the hepatomas or any of the other tumors. It persisted in only 1 hepatoma (9618A). This one was highly differentiated and was also the one most recently induced. Weinhouse (27) found comparable levels up to the 5th generation in one other hepatoma (7787) not examined here, and lower but detectable activity in earlier generations of hepatoma 7793, which now lacked activity. Loss of glucokinase is clearly not obligatory in hepatomas, but it does disappear in the later generations or from the more undifferentiated hepatomas, and this loss is generally correlated with growth (26). In this respect, hepatomas thus approach the nonhepatic tumors which do not contain glucokinase and arise from tissues that do not contain it. Glucokinase therefore appears to be a "dispensable" (11) enzyme for rat tumors.

Previous observations on hepatomas established that they could be divided into at least 2 classes: well-differentiated, slow-growing hepatomas with low hexokinase concentrations, and poorly differentiated, fast-growing hepatomas with high hexokinase concentrations (20–22, 26). Weinhouse (27) presented data showing that the hepatomas actually formed a spectrum of increasing hexokinase concentrations with increasing rates of growth. Hexokinase was also higher in 1 fast-growing hepatoma than in 2 slow-growing ones (26),

higher in 5 fast-growing hepatomas (and in Walker tumor) than in 8 slow-growing ones (4, 21), and higher in 14 fast-growing hepatomas than in 3 slow-growing ones (22). A significant quantitative relationship between the enzyme concentrations in 5 hepatomas and their measured growth rates is confirmed here. Enzyme concentrations and growth rates also parallel the degrees of histological undifferentiation in these tumors (Table 1). The same relationships hold for all the tumors studied as well as for the hepatomas. Since the tumors in the series studied included 13 nonhepatic tumors, the relationship is not limited to hepatomas but holds more generally among different kinds of transplanted rat tumors. Apparently, the concentration of biochemical work on hepatomas can, as hoped, lead to findings of general applicability to tumors.

Because hexokinase concentrations parallel the neoplastic characteristics (growth and histological undifferentiation) of these tumors from different tissues, this enzyme can be recognized as a significant component of these neoplasms. The same conclusion about glutaminase (K) in neoplasms was drawn with the same types of evidence from many of the same tumors (12, 13, 15). Hexokinase and glutaminase (K) concentrations were also parallel to one another in these tumors. It is important to recognize that these 2 enzymes, although judged to play significant roles in neoplastic type metabolism, occur in low to high concentrations in the different tumors. Their significance, and possibly the significance of other components measured in neoplasms, is not obvious from the concentrations in a particular tumor. Significance can be recognized only in relation to the nature of the tumor. Growth rate and degree of histological differentiation are particularly relevant characteristics of the nature of tumors. Of these characteristics, the direct measurements of growth in volume are quantitative and therefore more like the quantitative enzyme measurements than are the morphological classifications. Even with averaging of the very real discrepancies between judges, the latter has the inherent weakness of ranked data. However, the 2 enzymes and 2 biological characteristics together comprise a beginning description of the graded nature of neoplasia among the examples studied. Since hexokinase concentrations limit glycolysis (8, 16), these findings support the view that glycolysis is also a graded function of these neoplasms, as has been indicated by the actual measures of others (2, 3, 7, 11, 23). In this case, glycolysis would be dependent in the first instance on the concentration and not the activity of the limiting enzyme.

Glucokinase and glutaminase (L) are both uniquely characteristic of adult liver and are both replaced in hepatomas to greater or lesser extents by related isozymes [hexokinase and glutaminase (K)]. The latter are characteristic of fetal liver (6, 14, 24). The appearance of these 2 fetal-type isozymes provides concrete evidence that the hepatomas containing them are in some degree dedifferentiated from the composition of adult liver toward an earlier type of enzyme pattern.

Fetal and regenerating livers grow as fast or faster than most of the tumors studied here, yet they have concentrations of hexokinase, whether per g or per cell, that are considerably less than in most of the tumors. It seems likely that hexokinase in the tumors, and perhaps in normal tissues, may be directly concerned with growth processes as an initial reaction leading to energy production. The rapid growth of these 2 normal tissues, however, while containing less hexokinase than the tumors, suggests a much greater efficiency of the growth processes in the normal tissues than in the tumors.

The relationships between concentrations of hexokinase in Chart 1 and of glutaminase (K) (12, 13, 15) to the growth of tumors are the closest that have been found between chemical composition and growth rate of living tissues. A linear relationship between growth and enzyme concentrations has been assumed in Chart 1 because it is the simplest, and the data do not yet compel more elaborate curve fitting that could produce an even higher correlation and deeper insight into the mechanisms controlling growth. It is also not known whether to expect growth rates to be more closely proportional to the concentrations of a significant enzyme per g tissue (concentration) or per cell (amount). Now that some data are available, hexokinase as well as glutaminase (K) both appear to correlate more closely with growth when expressed per cell (or DNA), as Weber (25) and some others have done in the past. The marginal superiority of the per cell over the per g base is not yet decisive, however.

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