Glucose-6-phosphate Dehydrogenase in Normal and Malignant Mouse Tissues and Cells Propagated *in Vitro* (34177)

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(Introduced by J. Kieler)

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Recent studies in this laboratory (1) have shown a pronounced decrease in the rates at which ^{14}C-acetate is incorporated into fatty acids of mouse cells which have undergone "spontaneous" malignant transformation during cultivation *in vitro*. The incorporation was about five times higher in the nonmalignant cells than in the malignant cells. In both types of cells the extramitochondrial pathway first described by Wakil (2) and by Lynen (3) was active.

The observation of this difference raised the question whether the NADPH formation in the malignant cells was adequate. NADPH is required for the reductive steps which follow the malonyl reaction in the nonmitochondrial fatty acid synthesis. Since glucose was found to stimulate ^{14}C-acetate incorporation (1), while various metabolites of the Embden-Meyerhof metabolic pathway did not, it is conceivable that the nonmitochondrial fatty acid synthesis is mainly supplied with NADPH by the reactions of the pentose shunt, *i.e.*, the glucose-6-*P* dehydrogenase (G-6-PDH) reaction and the 6-phosphogluconate dehydrogenase (6-PGDH) reaction. A difference in these reactions between nonmaligant and malignant cells might account for the differences in the ^{14}C-acetate incorporation mentioned above.

Previous studies of G-6-PDH activity in normal and malignant tissues (4–7) have not revealed any clear differences between the G-6-PDH activity of the groups, but a direct comparison of nonmalignant and malignant material has only been made by a few authors (5–8). Very little is also known of the G-6-PDH activity in cultured cells.

TABLE I. Material Studied: Murine Tissues.

Malignant
DLB mammary carcinoma
ST/a chondroma
cholangioma
Ehrlich's near tetraploid ascites tumor (ELTa) from ST/a mice
Ehrlich's near diploid ascites tumor (ELDa) from DBA/2 mice
Nonmalignant
C3H embryos
spleen
lung
ST/a lung
liver
Primary explants
ST/a lung

The purpose of the present work was to study the G-6-PDH activity in various nonmalignant and malignant mouse tissues and to elucidate the changes which may occur during explantation and propagation *in vitro*.

Material and Methods. The material studied is presented in Tables I–II. Part of it was obtained either directly from inbred mice of the C3H/Fib, ST/a-Fib or DLB/Fib strains or from primary explants maintained *in vitro* for a few days (Table I), while another part was obtained from six *in vitro* propagated permanent cell lines shown in Table II.

Two of these (ELDc and ELTc) originated from a near diploid (ELDa) and a near tetraploid (ELTa) subline of Ehrlich's ascites tumor (9, 10). The C3H-E, C3H-M, and C3H-L cells originated from normal tissues which during cultivation *in vitro* underwent apparently spontaneous malignant trans-

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TABLE II. Cell Lines Propagated *in Vitro* and Tumors Resulting from Reinoculation into Susceptible Mice.

Cell line	Origin	Culture age at time of investigation (years)	Tumors resulting from reinoculation	
			Host mice	Site of reinoculation[a]
C3H-E	19-day-old C3H-mouse embryos	4	Newborn C3H	sc
C3H-M	Spleen of adult C3H mouse	4	Newborn C3H	sc
C3H-L	Lung of same adult mouse	4	Newborn C3H	sc
L-929	Subcutis of adult C3H mouse	28	Newborn C3H	sc
ELDc	ELDa tumor	6	Adult DBA/2	sc
			Adult DBA/2	ip
ELTc	ELTa tumor	4	Newborn C3H	sc

[a] sc = subcutaneous; ip = intraperitoneal.

formation in the course of 6 months to 1 year (11). Earle's strain L fibroblasts became malignant after treatment *in vitro* with methylcholanthrene (12).

The transplanted tumors were propagated by subcutaneous transplantation or intraperitoneal inoculation. Only nonnecrotic subcutaneous material was used. The ascites tumors investigated were harvested during their exponential growth phase. The primary explants as well as the permanent cell lines were grown in a semisynthetic medium (Fib 14B) containing 20% fetal bovine serum and 80% Eagle's minimum essential medium (13) modified to contain twice the recommended concentration of amino acids and four times the concentration of vitamins and glutamine.

Primary explants of ST/a mouse lung were prepared by cutting the lung tissue with scissors into small pieces with a diameter not more than 0.5 mm. The cut tissue was placed in a tidal flow culture chamber constructed by Langvad (14) and gassed continuously with a gas mixture of 5% CO_2, 20% O_2, and 75% N_2. This permits the preservation of tissue up to 7–8 days with only descrete histological changes.

The cultured material was harvested by gentle scraping without the use of trypsin. Like the rest of the material it was washed twice in a solution containing 0.154 *M* NaCl and 6.6×10^{-4} *M* EDTA.

The same solution was used as suspension medium during homogenization, which was carried out in a Potter-Elvehjem homogenizer with a Teflon pestle. The material was suspended at a concentration of 1:9 (w/v) and homogenized for 4–8 min. The supernatant was obtained by centrifugation in a MSE high speed centrifuge at 26.000*g* at 4° for 20 min.

The G-6-PDH was determined in the supernatants according to the principle of Glock and McLean (15). A NADP/G-6-P ratio of not less than 5:1 was found to be optimal. Tris 0.4 *M* was used as assay buffer. All procedures of harvesting, washing, and homogenization were carried out at 2–4°. Protein determinations were performed according to the method of Lowry *et al.* (16). Statistical evaluations were performed with Wilcoxon's rank test (17, 18), which allows the treatment of small populations of random distribution.

Results. The G-6-PDH activity in various normal mouse tissues and in transplantable tumor is shown in Table III. In normal mouse tissues the highest values were found in C3H spleen. Somewhat lower values were found in 19-day-old mixed C3H embryonic tissues. By the Wilcoxon rank test this difference was found to be significant ($p < 0.01$). Significantly lower than in embryonic tissues was the activity in C3H lung ($p < 0.01$). No differences were found between lung tissue from C3H and ST/a mice but ST/a liver showed a significantly lower G-6-PDH activity than ST/a lung ($p < 0.01$).

No significant differences were demonstrated between the activities of the five

TABLE III. The Glucose-6-*P* Dehydrogenase (G-6-PDH) Activity in Malignant and Nonmalignant Mouse Tissues.

Tissue	Strain of mice	G-6-PDH (Wroblewski units/mg of protein) $\bar{x}$	s	No. of expts. n
Nonmalignant				
Spleen	C3H	62.0	5.1	9
Embryo	C3H	44.6	2.4	7
Lung	C3H	25.2	10.3	9
	ST/a	22.6	5.4	4
Liver	ST/a	10.8	4.4	13
Malignant				
Mammary carcinoma	DLB	49.2	26.8	15
Chondroma	ST/a	41.2	6.3	9
Cholangioma	ST/a	52.0	4.2	2
ELTa	ST/a	61.0	17.8	17
ELDa	DBA/2	49.7	22.3	11

transplantable tumors, except for the ELT-a cells, which showed significantly higher activity than the ELDa cells ($p < 0.05$). The activities of all tumors were within the ranges of the values found in spleen and embryonic tissues, but were significantly higher than the values of lung tissue ($p < 0.01$) and liver ($p < 0.01$).

Table IV shows the results of four experiments in which the changes in G-6-PDH activity in primary explants of the ST/a lung tissue was followed for up to 8 days. It appears from Table IV that the values gradually increased approaching the level of the transplantable tumors in 4–7 days.

In permanent malignant cell lines propagated for years *in vitro* the G-6-PDH activity was 2–5 times higher than in tumors propagated *in vivo* (Table V). As compared to the tissues of origin these differences were found to be significant ($p < 0.01$). The three cell lines (C3H-E, C3H-M, and C3H-L) which had undergone "spontaneous" malignant transformation *in vitro* and the L-929 fibroblasts did not differ much, only the C3H-L cells showed significantly smaller activity than C3H-E cells ($p < 0.01$) and C3H-M cells ($p < 0.02$). The ELDc cells showed a little higher values, but the difference was only significant when compared to the C3H-L cells ($p < 0.01$). The ELTc cells on the other hand showed a significantly higher activity than the other five cell lines ($p < 0.01$).

TABLE IV. Changes in the Activity of G-6-PDH in Primary Explants of Lung Tissue from ST/a Mice.[a]

Expt.	Duration of cultivation (days) 0	1	2	3	4	5	6	7	8
1	28.2		29.6		37.0			49.6	
2	18.1		27.0		34.5		47.5		
3	17.8				63.8		60.8		
4	26.4		34.0		49.3		50.6		58.0

[a] Units of enzyme activity: Wroblewski units/mg of protein.

TABLE V. The Glucose-6-*P* Dehydrogenase (G-6-PDH) Activity in Permanent Cell Lines Propagated *in Vitro*.

Cell line	G-6-PDH (Wroblewski units/mg of protein) $\bar{x}$	s	No. of expts. n
C3H-E	123.1	15.4	10
C3H-M	132.7	22.8	10
C3H-L	99.7	10.5	9
L-929	113.0	5.5	10
ELDc	150.5	46.3	10
ELTc	330.2	71.7	10

TABLE VI. The Glucose-6-*P* Dehydrogenase (G-6-PDH) Activity in Tumors Produced by the Reinoculation of Cultured Cell Lines.

Cell line	Host	Site of inoculation[a]	G-6-PDH (Wroblewski units/mg of protein) $\bar{x}$	s	No. of expts. n
C3H-E	Newborn C3H	sc	71.3	11.9	8
C3H-M	Newborn C3H	sc	144.4	33.2	5
C3H-L	Newborn C3H	sc	62.9	10.0	11
L-929	Newborn C3H	sc	107.5	11.8	6
ELDc	Adult DBA/2	ip	109.0	22.6	7
ELDc	Adult DBA/2	sc	114.3	64.5	8
ELTc	Adult C3H	sc	152.8	18.1	5

[a] sc = subcutaneous; ip = intraperitoneal.

On reinoculation into susceptible mice the cells propagated *in vitro* produced tumors still showing very high activities, although not always quite as high as the cells of origin (Table VI). The decrease was statistically significant in the case of subcutaneous tumors produced by C3H-E ($p < 0.01$), C3H-L ($p < 0.05$), and ELTc cells ($p < 0.01$). On intraperitoneal, but not on subcutaneous reinoculation of ELDc cells a significant decrease was also seen ($p < 0.02$ and > 0.1, respectively). However, all the tumors produced by reinoculated cultured cells showed significantly higher G-6-PDH activities than the tissues listed in Table III from which the cultured cell lines were originally established ($p > 0.01$).

Discussion. Previous studies of G-6-PDH activity in a variety of normal and malignant mammalian tissues (4) have shown widely divergent values with very high activities in normal tissues such as lactating rat mamma and rat spleen, while other normal organs had low activities.

Various tumors, especially those of lymphatic origin, showed higher values. Recent investigations also showed significantly higher activities in mammary tumors than in normal mamma when measured per milligram of protein (5, 6). In hepatomas the G-6-PDH activity was 3–5 times higher than in normal liver (7).

In agreement with these reports we found high G-6-PDH activity to be characteristic of various malignant cell lines propagated *in vivo* and *in vitro*. However, the high values found in some nonmalignant tissues, the rapid increase in the activity of this enzyme in primary nonmaligannt explants, and the increase which is also seen in autonomous tumor cells during cultivation *in vitro* do not suggest a direct correlation between the G-6-PDH concentration and the process of malignant transformation.

The increased activity in the cultured material was partially retained when the transformed cells produced tumors on reinoculation into susceptible hosts. The presence of stroma cells might account for the about 30% decrease which was seen in subcutaneous tumors, but this hardly explains why the ELDc cells lost some of their activity on ip reinoculation into susceptible hosts.

The primary purpose of this investigation was to clarify whether the decreased fatty acid synthesis which has been observed in cultured tumor cells (1) could be explained by a decreased G-6-PDH activity. Obviously this is not the case. The highest G-6-PDH activity was found in those cells (ELDc and ELTc) which showed the lowest rate of ^{14}C-acetate incorporation into fatty acids.

G-6-PDH is not the only glycolytic enzyme which shows increasing activity in primary explants. Similar observations have been made with hexokinase (19), pyruvate kinase, and lactate dehydrogenase (20). However, the G-6-PDH activity does not seem to reflect the glycolytic capacity directly. Thus, the ELD and ELT cells propagated *in vitro*

showed much higher G-6-PDH activities than the same cells propagated *in vivo* as ascites tumors, but according to Biczowa *et al.* (9) only small differences between the glycolytic activities of these cell lines can be demonstrated. The absence of a positive correlation between glycolysis and G-6-PDH activity in cultured cells was previously reported by Paul (8), who found that infection with polyoma virus may stimulate the glycolytic activity of hamster kidney cells (BHK 21) in culture and at the same time depress the G-6-PDH activity as measured per cell. Thus, even if a high rate of glycolysis often seems to be accompanied by a high G-6-PDH activity, no strict correlation has been demonstrated.

Summary. Previous studies showed overlapping values of G-6-PDH activity in normal and malignant tissues. This is confirmed by the present work. Malignancy may be accompanied by high G-6-PDH activity; similar values, however, may be found in embryonic tissues, spleen, and in primary lung explants after a few days of cultivation *in vitro*. In long-term cultures a further increase in G-6-PDH activity was seen. Although the G-6-PDH activity was usually high in cells and tissues with a high glycolytic capacity, the observations do not indicate a direct correlation between glycolysis and G-6-PDH activity or between acetate incorporation and G-6-PDH activity in tissue cultured cells.

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