ON THE LOSS OF URIDINE DIPHOSPHOGALACTOSE-4-EPIMERASE ACTIVITY IN L CELL CULTURES AND IN TUMOR CELLS*

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It has been reported (Maio and Rickenberg, 1961; Ebner et al., 1961) that L cell cultures and homogenates of these cells show no detectable epimerase** activity. Our own observations are somewhat at variance with this report. As will appear from Table 1, broken cell preparations of L cells or HeLa cells at the optimum pH (pH 8.7) and with excess DPN added show appreciable epimerase activity, whereas the transferase activity is very low and presumably rate-limiting.

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**The following abbreviations will be used:
gal-1-P: galactose-1-phosphate
UDP Hexose: Uridine diphosphohexose
UDPG: Uridine diphosphoglucose
UDP Gal: Uridine diphosphogalactose
Transferase: Galactose-1-phosphate uridyl transferase
Epimerase: Uridine diphosphogalactose-4-epimerase

Table 1

ACTIVITIES OF ENZYMES OF GALACTOSE METABOLISM IN HOMOGENATES OF L CELLS AND HeLa CELLS.

Galactokinase was assayed by paper chromatography (Paladini and Leloir, 1952) using 1-C^{14} galactose and a Vanguard coincident autoscanner (cf. Sundararajan et al., 1963). Transferase and epimerase were assayed according to Maxwell et al. (1962) except that glycylglycine (0.1 M, pH 8.7) was used as a buffer and that excess DPN (corresponding to 0.8 to 1.2 µmoles per ml) was used in the epimerase assays, which were done according to the two step procedure (cf. Maxwell et al. , 1962). UDP Gal (Sigma) was used in a concentration of 0.2 µmoles per ml.

	L Cells	HeLa Cells
	m µmoles per 10^6 cells per hour (37°).	
Galactokinase	160	120
Transferase	30	50
Epimerase	160	440

On the other hand, homogenates incubated with UDP Gal at pH 7 or 7.5 without fortification of DPN show scarcely any detectable epimerase activity. It is known that reduced DPN (DPNH) exerts a strong inhibition on mammalian epimerase (Maxwell, 1956; Kalckar, 1958). The DPNH/DPN ratio can be lowered either by hydrogen acceptors like pyruvate or by addition of DPN. As will appear from Table 2, homogenates of L cells and ascites tumor cells at pH 7.5 have very low epimerase activities but respond well to the addition of pyruvate. Addition of nicotinamide instead of pyruvate showed no ef:

It can be shown (cf. Table 3) that the DPNH inhibition of epimerase is much more pronounced at physiological hydrogen ion concentrations than at the pH optimum for epimerase (pH 8.7). It seems

Table 2

EFFECT OF PYRUVATE ON EPIMERASE ACTIVITY IN HOMOGENATES OF L CELLS AND EHRLICH ASCITES TUMOR.

Epimerase assay according to the two step procedure (Maxwell et al., 1962), except glycylglycine (0.1 M, pH 8.7) was used as a buffer and UDP Gal (Sigma) was used in a concentration of 0.2 μmoles per ml. No DPN was added to the preincubation samples unless specified in table.

	L Cells	Ascites Tumor
	m μmoles UDPG formed per mg protein per hour (37°).	
No additions	0.29	1.80
Addition of pyruvate, 10 μmoles	0.62	8.55
Addition of DPN, 0.1 μmoles		90.05

that the DPNH/DPN ratio as well as the pH are the crucial factors in this inhibition.

Since most tumors have aerobic glycolysis (Warburg, 1926) and therefore high hydrogen ion concentrations and also relatively high DPNH/DPN ratios, especially if supplied with glucose (Glock and McLean, 1957; Aeisenberg, 1961; Chance and Hess, 1959), it was thought that interference with epimerase activity might also occur in tumors. Table 4 shows that epimerase activity in intact L cells as well as in various tumors is indeed very low.

The studies of epimerase activity in intact cells were performed by means of administering radioactive galactose to the cells and scanning chromatograms of filtrates for C^{14} UDP Hexose and Gal-1-P (Paladini and Leloir, 1962) except that the C^{14} UDPG and C^{14} UDP Gal were separated by means of the enzyme UDPG dehydrogenase

Table 3

INHIBITION OF EPIMERASE FROM HOMOGENATES OF L CELLS OR HeLa CELLS BY DPNH AS A FUNCTION OF pH.

Assay procedures as in Table 2, except various buffers (phosphate, glycylglycine, glycine) were used according to pH. Moreover, DPN 0.2 μmoles per ml was replaced by a mixture of 0.05 μmoles of DPNH to 0.15 μmoles of DPN (per ml) the ratio DPNH to DPN being 1 to 4. The per cent inhibition is always referred to activity of the corresponding pH, with 0.2 μmoles of DPN. In no case did the activity with DPN go below 30% of the uninhibited activity at pH 8.7 (pH optimum).

pH	% inhibition
7.0	85-90
7.5	80
8.6	60
9.4	30
10.0	>10

*Activity with 0.05 μmoles DPNH + 0.15 μmoles DPN divided with activity at the same pH with 0.2 μmoles DPN (no DPNH added) times 100. The values are averages of figures obtained from L cells and HeLa cells.

(Strominger et al., 1957). It appears, from Table 4, that L cells, HeLa cells, Ehrlich ascites tumor cells and various mammary carcinomas show appreciable galactokinase activity but only minute amounts of transferase and epimerase activities. The latter is usually the rate-limiting factor. The low transferase activity in these cells gives rise to accumulation of galactose-1-phosphate if galactose is administered in appreciable amounts to the medium or to the circulation.

Table 4

ESTIMATES OF TRANSFERASE AND EPIMERASE ACTIVITIES IN INTACT NORMAL AND TUMOR CELLS FROM MICE. (1-C^{14} galactose used as markers)

Mice were injected intravenously with 1-C^{14} galactose in amounts corresponding to 5 μmoles (containing 1 μC/μmole). Tissue specimens were frozen after 30 to 60 minutes. Cell suspensions were incubated for 30 to 60 minutes with 1-C^{14} galactose (7.5 to 15 μmoles per ml incubation medium) and non-labelled glucose. Aliquots from protein-free filtrates were subjected to paper chromatography (ethyl alcohol-acetate mixture pH 3.5 (Paladini and Leloir, 1952)) and scanned. Other aliquots were subjected to the same treatment after a preincubation with UDPG dehydrogenase. Radioactive standard peaks were calibrated in advance in the liquid scintillation counter. The quantitative epimerase method was based on quantitative estimate of transferase. The latter was expressed on the amount of radioactive galactose incorporated in UDP Hexose (Robinson et al., 1963, unpublished).

	m μmoles converted per 10^6 cells per hr.	
	Transferase	Epimerase
L cells	0.6	0.3
HeLa cells	0.9	0.4
Ehrlich Ascites Tumor	0.4	0.1
Mammary Carcinoma (CH_3)	0.1	0.2[a]
Lactating Mammary Gland	3.0	3.0[a]
Normal Brain		1.5[a]

[a] UDP Gal/UDPG ratio had reached equilibrium, hence epimerase activity must be higher than transferase activity. Experiments with 1-C^{14} glucose indicated, however, that the epimerase activity in CH_3 mammary carcinoma could scarcely exceed o.5 μmoles per 10^6 cells per hour.

In L cells and HeLa cells 15 μmoles galactose per ml medium brought about an accumulation of 1 to 2 m μmoles of gal-1-P per 10^6 cells. Interestingly enough, this amount sufficed to stop any

de novo synthesis of UDPG from glucose which otherwise can be shown to be operative. This is borne out by the fact that simultaneous administration of 1-C^{14} labelled galactose and non-labelled glucose still permitted the cells to accumulate UDP Gal and UDPG with the same isotope concentration as that of the original radioactive galactose administered. Since UDPG synthetase (UDPG pyrophosphorylase) for HeLa cells was found to be insensitive to gal-1-P, whereas phosphoglucomutase is sensitive (Ginsburg, 1957; Sidbury, 1957), a block of the latter enzyme was probably responsible for the block in UDPG synthesis.

Details of these experiments will be submitted in a later publication.

It is important to emphasize, however, that the slow epimerase activity in tumor cells was also evident in experiments in which the glucose was labelled. The biological importance (especially with respect to malignancy) of an epimerase "choke" brought about by aerobic glycolysis is difficult to evaluate. However, as emphasized at other occasions (Kalckar, 1962; Kalckar, 1963), any block in a pathway leading to any one of the sugars which can endow antigenic specificity to the cell surface is of potential biological interest in connection with the problem of controlled and uncontrolled growth. In microorganisms it has been shown that epimerase defects greatly affect antigenic surface patterns as well as virus receptor sites (Fukasawa and Nikaido, 1960; Osborn et al., 1962).

The "traffic jam" (e.g. accumulation of gal-1-P in tumor cells brought about by galactose administration may also be important in a situation in which "galactose defective" metastases find them-

selves in a "galactose competent" cell population like liver. Administration of galactose might favor the normal cells at the expense of the metastases.

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