Melting point 249°–250° (uncorr.). The yield was 2·4 g (74 per cent). Calc. for $C_{24}H_{21}N_3$, C, 82·05; H, 5·98, N, 11·96 per cent; found, C, 82·04; H, 6·01; N, 12·10 per cent. It was oxidized in almost quantitative yield to IId by ferric chloride in acetic acid, exactly as described by Fischer for the analogous phenyl compounds[2].

<div align="center">Thomas A. Scott</div>

Department of Biochemistry,
University of Leeds.

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Glycolysis and Respiration of Transformed BHK21 Cells

Warburg et al.[1] observed that tumour tissues almost always had a higher rate of aerobic glycolysis than normal cells. Warburg attributed this to irreversible damage to the respiratory pathways. The theory was a source of contention from the beginning. It was abandoned by most workers when it was found that glycolysis very rapidly increased (in a few days) when tissues were explanted and maintained as tissue cultures[2-4]. Since then many factors, such as pH and oxygen tension, have been shown to influence both glycolysis and respiration in cultured cells[5-7]; the increase of glycolysis on explantation can easily be explained by these findings. Reduced respiration and increased glycolysis in tumours in vivo can be explained similarly, knowing that most tumours have an inadequate blood supply. For this reason, and because wide differences in behaviour are found among different cell lines[8], the significance of Warburg's findings has been questioned. Nevertheless, there is good evidence[9,10] that there is a fundamental difference in glycolysis of normal and tumour cells.

To test the question critically, systems are needed in which closely related normal and malignant cells can be compared in identical conditions. Ideally, the lines should be grown in vitro, should be derived from the same cell and should have diverged from it very recently. These requirements have been met by the isolation of a stable line of baby hamster kidney cells, designated BHK21, which can be transformed into cells of different morphology by the SE polyoma virus[11,12]. Cells transformed in this way have the properties of tumour cells, whereas the 'untransformed' cells used by us in this study have very low transplantability. Both have a similar growth rate and high cloning efficiency[13]. It is possible to derive equivalent sub-clones of either normal or transformed cells from within a single clonal population and to compare them directly. The lines used in the work recorded here were clones derived simultaneously from the same cell inoculum which was exposed to polyoma virus[14]. A and C were morphologically untransformed and clones Y and Z transformed. A, C and Y are predominantly diploid and Z near tetraploid. The cells were maintained in Eagle's medium with 10 per cent calf serum and 10 per cent tryptose phosphate broth.

Respiration was measured with the Cartesian diver by techniques which have been detailed elsewhere[15]. Glycolysis was measured by estimating the glucose used and lactate produced by 2×10^6 cells in 2 ml. medium in 17 h. Since these cells produce large amounts of lactic acid and pH has a profound effect on glycolysis[5] the medium was heavily buffered. The incubations were performed in 25 ml. conical flasks maintained in a shaking water-bath at 37° C. Table 1 shows comparative values for respiration; if anything it is higher in the transformed strains. Table 2 shows comparative values for glycolysis; it is clear that the transformed cells have

Table 1. Respiration of Transformed and Untransformed Cell Lines Derived from BHK21 after Treatment with Polyoma Virus

Respiration was measured at pH 7·4 in a salt solution in equilibrium with 5 per cent carbon dioxide in air

Exp.	Cell strain and type	Oxygen uptake $\mu M/h/mgDNAP$
1	A (untransformed)	509, 439
	Y (transformed)	451, 553
2	A (untransformed)	467, 505, 550
	Y (transformed)	598, 659, 534

Table 2. Glycolysis of Transformed and Untransformed Cells

Glucose utilization was measured for 17 h at pH 7·4 in a medium in equilibrium with 5 per cent carbon dioxide in nitrogen. (10^9 cells: 0·8 mg DNAP)

Type of cell	Strain	No. of obs.	Glucose uptake $\mu M/h/10^9$ cells (mean $\pm S.D.$)
Untransformed	A	4	336 ± 104
		4	418 ± 57
		4	317 ± 36
	C	3	190
			347 ± 37
Transformed	Y	3	667
		4	574 ± 43
		4	601 ± 56
	Z	3	978
		4	660 ± 80
		4	777 ± 80

greater glycolytic capacity than the untransformed. Enzymatic investigations have been undertaken to reveal the underlying nature of this difference and will be reported elsewhere.

While these findings provide no support for Warburg's view that tumour cells have an irreversible lesion of the respiratory pathways they very strongly support his contention that there is a marked difference in the glycolysis of normal and tumour cells. Before drawing a firm general conclusion to this effect it would be desirable to carry out similar studies on other systems, including some in which a malignant transformation was produced by agents other than viruses. Defendi[16] has described a spontaneously arising variant of BHK21 which (unlike the untransformed cells used in this work) is highly malignant though it has the appearance of an untransformed strain. Some untransformed lines maintained by us for a very long time are also malignant[12,13]. Another untransformed line which has been maintained continuously for very many passages has been found to exhibit a high rate of glycolysis. Its transplantability is being tested, having in mind the possibility of a correlation between transplantability and high glycolytic capacity.

While we feel it would be unwise to draw a general conclusion until we have more information, we consider that the results obtained in this work warrant a reappraisal of the role of glycolysis in carcinogenesis.

M. Broadfoot
P. Walker
J. Paul

Institute of Biochemistry,

I. Macpherson
M. Stoker

Institute of Virology,
University of Glasgow.

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