Bioscience Reports **4**, *751-756 (1984)*
Printed in Great Britain

Increased glycolysis in ageing cultured human diploid fibroblasts

A. H. BITTLES and N. HARPER

Department of Human Biology, Chelsea College, Manresa Road, London SW3 6LX, U.K.

(Received 13 July 1984)

With increasing population doubling in vitro, human diploid fibroblasts exhibited a highly significant increase in glucose uptake from the growth medium and a corresponding increase in lactate production. The switch to glycolysis occurred prior to the onset of changes in intracellular glucose and lactate concentrations or in the specific activity of the glycolytic regulatory enzyme, pyruvate kinase. It also preceded the morphological alterations held to be characteristic of cellular senescence.

Human diploid cell strains exhibit a characteristic growth cycle and life-span in culture (Hayflick & Moorhead, 1961; Hayflick, 1965), the number of cell doublings achieved being inversely proportional to the age of the original explant donor (Martin et al., 1970; Schneider & Mitsui, 1976). Although glucose is the main energy source for human diploid fibroblasts, relatively little attention has been focussed on the role of glycolysis in cellular energy production. The aim of the current study was to investigate the relative uptake of glucose and its utilization via glycolysis during the in vitro lifespan of a human diploid cell strain.

Materials and Methods

Human diploid embryonic lung fibroblasts, Strain 2002, obtained at early passage, were cultured in Minimal Essential Medium, Glasgow modification, containing penicillin (100 U/l), streptomycin (110 g/l) and supplemented with 10% fetal bovine serum (Flow Labs). Cells were grown routinely in static culture (growth area 175 cm^2) and passaged on reaching confluence using a 1:3 split. The cultures were routinely checked for mycoplasma contamination by a fluorescent DNA staining method (Chen, 1977) and remained mycoplasma-free throughout the study. For the experimental studies, conducted at each fifth cell population doubling (CPD) until cessation of growth, fibroblasts were seeded into roller-culture bottles (growth area 840 cm^2) using a 1:5 split and allowed to grow to early confluence, attained in 5-6 days. Medium samples for glucose and lactate estimations were deproteinized with uranyl acetate and ice-cold $HClO_4$ respectively and stored at -130°C prior to assay. Six replicate assays were run at each CPD for the two growth-medium constituents. For the intracellular measurements, conducted in quadruplicate, cells were washed 3

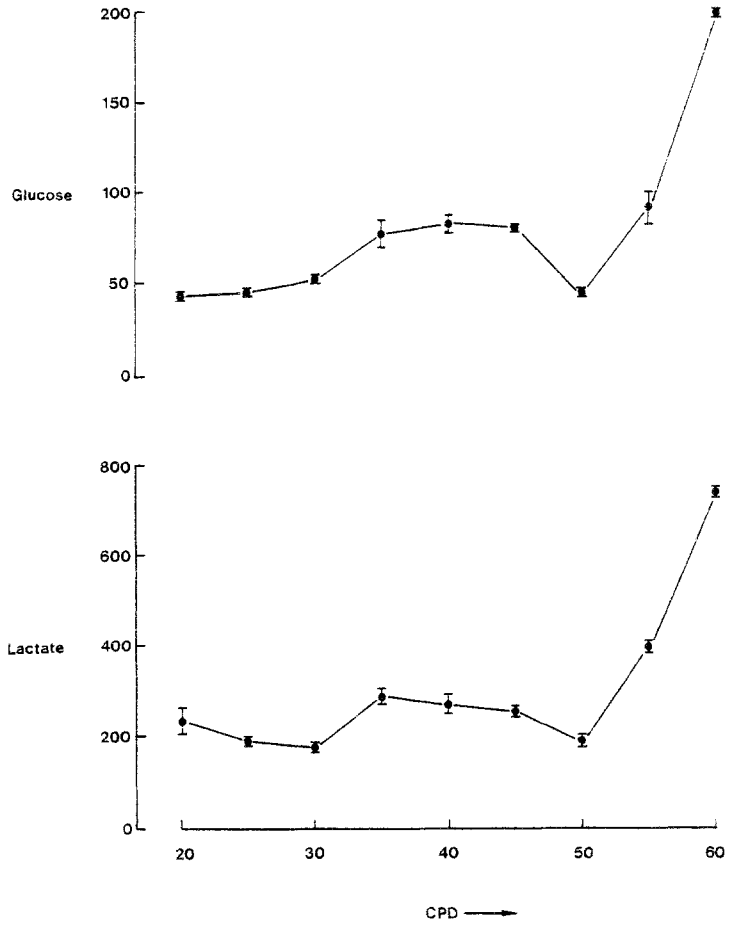

Fig. 1. Intracellular glucose and lactate concentration (nmol/mg protein) measured at each fifth cell population doubling (CPD). Results are plotted as means (n = 4) ± S.E.M.

times with PBS 'A' pre-warmed to 37°C, detached from the growth surface using trypsin/EDTA, washed 3 times with PBS 'A + B + C', resuspended in 0.03 M phosphate buffer, pH 6.8, and disrupted by maximum-amplitude sonication at 0°C for 3 x 10 s. Particulate material was removed by centrifugation at 13 000 **g** for 15-20 min at 4°C.

Glucose and lactate concentrations and pyruvate kinase (PK; EC 2.7.1.40) activity were assayed using kits provided by Boehringer; phosphohexose isomerase (PHI; EC 5.3.1.9) activity was assayed by the Sigma kit technique. Lactate dehydrogenase (LDH; EC 1.1.1.27) activity was determined by the method of Wroblewski & La Du (1955) and protein estimated using the Hartree (1972) method. Linear regressions were fitted to the means of each set of results and the slopes tested for significant difference to zero by 2-tailed t-tests.

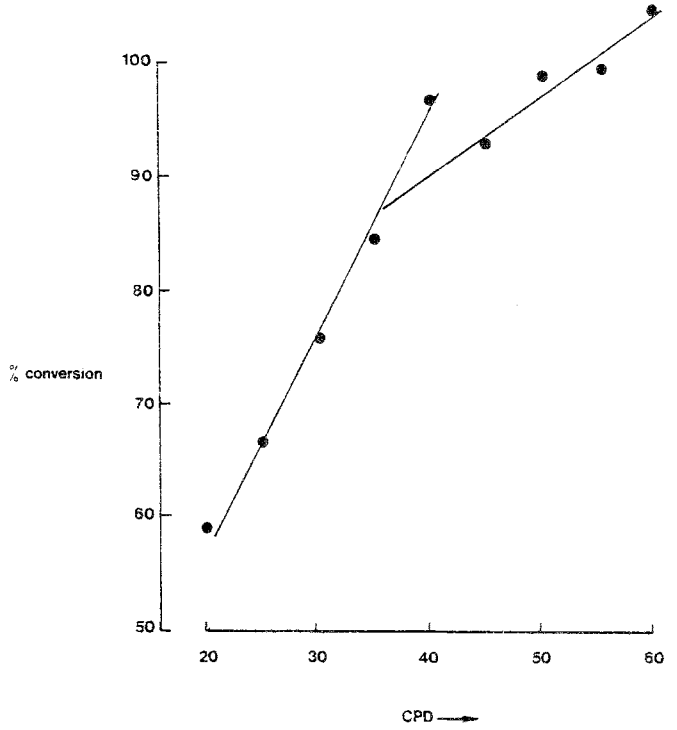

Fig. 2. Mean cellular lactate production at each fifth cell population doubling (CPD), expressed as a percentage of the mean glucose uptake (n = 6).

Results and Discussion

Intracellular glucose and lactate concentrations at each cell population doubling level (CPD) are shown in Fig. 1. From CPD 20 to 40 there were no significant differences in either glucose or lactate levels. A significant overall increase in both glucose and lactate levels was seen between CPD 20 and 60, $0.02 < P < 0.05$ in each case, due to the marked rise in each parameter at CPD 55 and 60. This may well reflect the increased size and volume of the fibroblasts late in their in vitro lifespan (Schneider & Fowlkes, 1976; Greenberg et al., 1977). By comparison, between CPD 20 and 40 glucose uptake from the growth medium gradually decreased, accompanied by a decline in lactate production (Table 1). However the two parameters did not change in parallel. As shown in Fig. 2, glucose taken up by the cells accounted for in terms of lactate production and output showed a highly significant increase, $P < 0.002$. From CPD 45 to 60 both glucose uptake and lactate production increased but again the percentage conversion of glucose to lactate increased independently, although to a lesser degree, $0.02 < P < 0.05$. The slopes of the two plots in Fig. 2, from CPD 20 to 40 and CPD 45 to 60, differed significantly, $P < 0.002$. During the lifespan of the cells no significant changes were seen in the specific activities of either PHI or LDH.

Table 1. Changes in growth-medium glucose and lactate concentrations following in vitro fibroblast culture

Results (μmol/10^6 cells seeded) are the means (n = 6) ± S.E.M. measured at each fifth cell population doubling (CPD)

	Glucose uptake	Lactate release
CPD 20	162.0 ± 3.0	190.6 ± 2.3
25	133.5 ± 6.1	181.1 ± 5.2
30	85.2 ± 2.9	128.6 ± 2.7
35	86.7 ± 3.0	148.0 ± 8.2
40	56.3 ± 2.2	108.9 ± 5.7
45	79.5 ± 2.4	148.6 ± 4.4
50	157.7 ± 5.8	311.4 ± 16.9
55	175.0 ± 3.3	348.1 ± 7.1
60	290.7 ± 10.3	609.9 ± 9.2

The specific activity of PK, a regulatory enzyme of the glycolytic pathway, increased from CPD 20 to 60, $0.002 < P < 0.01$, largely due to its greater activity between CPD 45 and 60 (Fig. 3).

Up to 40% of the glucose utilized by human diploid fibroblasts in culture goes towards energy production, glycolysis being the predominant source of ATP with minor contributions from the citric acid cycle and the pentose pathway (Cristofalo & Kritchevsky, 1966; Condon et al., 1971). Under normal growth conditions, the major role of glucose in the cells is to act as a source of ribose moieties for nucleic acid biosynthesis (Zielke et al., 1976, 1984). Therefore the increasing proportion of glucose utilized via glycolysis during the in vitro lifespan of the cells (Fig. 2) may be of major significance in terms of its consequent reduced availability as a substrate for cellular anabolic reactions.

There is a reduced rate of DNA synthesis in senescent fibroblast cultures (Macieira-Coelho et al., 1966a,b; Choe & Rose, 1974). It is noteworthy that the apparent critical point in the biphasic curve of glucose uptake/lactate production at or near CPD 40 corresponds closely with the proposed transition from growth-phase II to phase III (Macieira-Coelho & Taboury, 1982), characterized by an increase in the number of non-dividing cells and accompanied by the onset of changes in cellular nucleoprotein organization (Puvion-Dutilleul et al., 1982). Thus it seems reasonable to propose that many of the critical biochemical and morphological changes associated with cellular ageing (reviewed by Hayflick, 1980a,b) may be associated with, if not secondary to, the switch to glycolysis which commences early in the cells' in vitro lifespan.

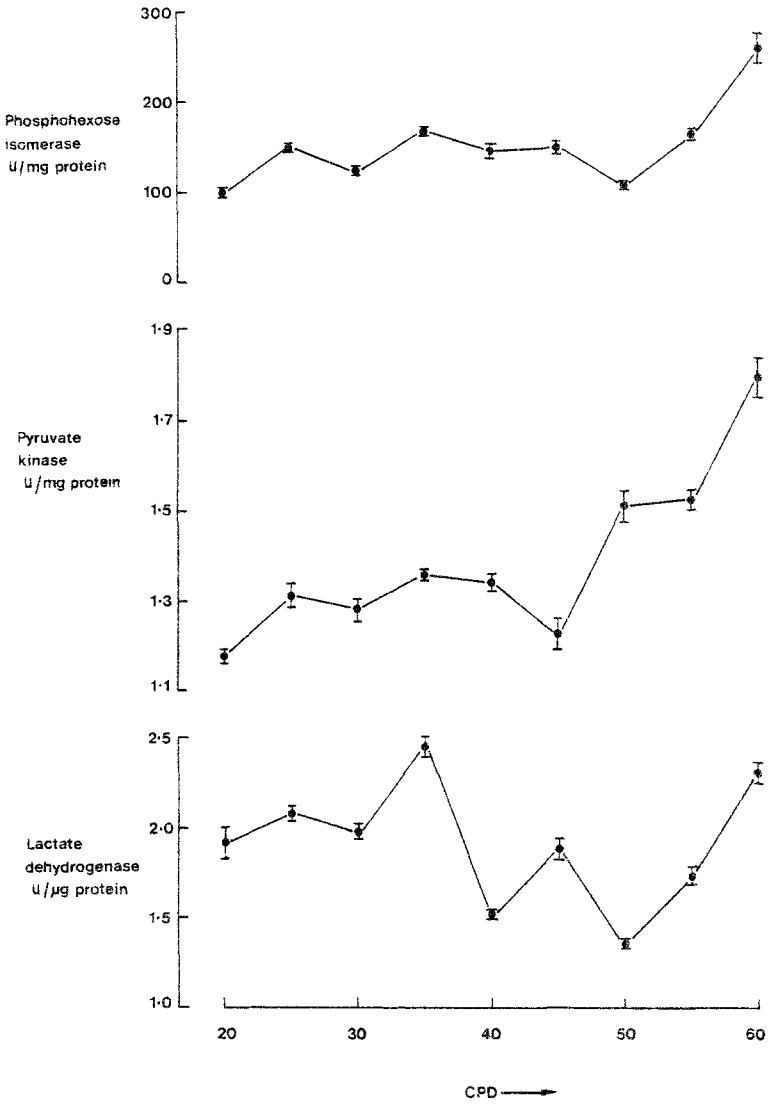

Fig. 3. Specific activities of cellular PHI, PK, and LDH assayed at each fifth cell population doubling (CPD). Results are plotted as means (n = 4) ± S.E.M.

Acknowledgements

We wish to acknowledge the valuable advice and assistance of Dr. U. E. Makov with statistical analyses.

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