Table 1. *Effect of gossypol, FCCP and iodoacetate on Lettre cell metabolism*

Treatment (A): Lettre cells were incubated without or with (−)gossypol at the concentrations shown for 30 min at 37°C. Treatment (B) was similar to (A), but with the addition of FCCP, iodoacetate or gossypol at the concentrations shown. Results are normalized as percentage of control where no additions = 100% and are the mean ± S.E. of three experiments, except in the case of iodoacetate which is from four experiments: $*P < 0.02$ and $**P < 0.001$.

| Treatment | ATP | ADP | $P_i$ | Lactate | [ATP]/([ADP]·[$P_i$]) |
|---|---|---|---|---|---|
| (A) | | | | | |
| 9 μM-Gossypol | 103±3 | 75±15 | 93±5 | 101±2 | 101±17 |
| 24 μM-Gossypol | 82±7 | 80±17 | 114±10 | 104±6 | 73±21 |
| 47 μM-Gossypol | 24±21 | 86±29 | 159±23 | 107±4 | 13±10* |
| 72 μM-Gossypol | 3±1 | 107±36 | 157±5 | 105±3 | 3±1** |
| 94 μM-Gossypol | 7±2 | 108±20 | 162±7 | 106±5 | 6±1** |
| (B) | | | | | |
| 47 μM-Gossypol | 14±6 | 156±24 | 169±13 | 100±1 | 4±2** |
| 2 μM-FCCP | 9±4 | 136±20 | 186±5 | 97±2 | 4±1** |
| 5 mM-Iodoacetate | 9±2 | 130±22 | 93±13 | 88±3* | 9±3** |

Untreated Lettre cells normally had an ATP value of 300 nmol/$10^8$ cells and an ADP/(ADP+ATP) ratio of between 0.1 and 0.15. We found that greater than 35 μM (20 μg/ml) of (−)gossypol at 37°C lowered [ATP], and increased intracellular [$P_i$] by about 60%, while [ADP] was not significantly altered. The [AMP] was also raised, but this peak could not be resolved from other monophosphate peaks including IMP on our h.p.l.c. column. Intracellular [lactate] remained unchanged by gossypol during the period of incubation (Table 1). Essentially the same effect was seen after 5 min incubation except that intracellular [$P_i$] was not raised. The glycolytic inhibitor iodoacetate (5 mM) also rapidly depressed [ATP] without affecting [ADP] and significantly reduced intracellular [lactate], but did not alter the intracellular [$P_i$] during 30 min at 37°C (Table 1).

Gossypol (47 μM) also caused a rapid stimulation of oxygen uptake by Lettre cells (89 ng atoms O/min per $10^8$ cells to 209 ng atoms O/min per $10^8$ cells). This effect was very similar to that seen when Lettre cells were incubated with 2 μM of the mitochondrial uncoupler *p*-trifluoromethoxyphenylhydrazone (FCCP). FCCP affected the levels of adenine nucleotides and intracellular [$P_i$], without altering intracellular [lactate] in a similar manner to that observed for gossypol (Table 1).

Gossypol (47 μM) did not affect the phosphorylation potential of freshly isolated human erythrocytes (cells without mitochondria) following 30 min incubation at 37°C. In contrast, iodoacetate (5 mM) lowered the phosphorylation potential 80% and also reduced intracellular [lactate] (results not shown).

These results are not consistent with gossypol acting as a glycolytic inhibitor, but rather as having an uncoupler-like effect on the cell leading to subsequent cell lysis and death. The predominantly glycolytic nature of tumour cells, even under conditions of high oxygen tension (Eigenbrodt *et al.*, 1985), would be likely to make tumours less sensitive than host tissue to gossypol cytotoxicity. Thus these results do not correlate with the proposal that gossypol has a role in chemotherapy.

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## Age-dependent growth inhibition of human diploid fibroblasts by 2-deoxyglucose

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A wide variety of biochemical and morphological changes have been reported during the replication *in vitro* of human diploid fibroblasts (HDF) (reviewed in Hayflick, 1980*a*, *b*). It is probable that the majority of these observations represents the effect(s) of cellular ageing, as opposed to a primary cause or causes. However, recent evidence indicates the existence of a highly significant shift to glycolysis with increasing cell population doubling (CPD) which may be of central importance in the ageing process (Bittles & Harper, 1984). To further investigate this phenomenon, HDF were cultured at five stages during their lifespan *in vitro* in the presence of the glycolytic inhibitor 2-deoxyglucose and the relative cell yields at each of the CPD were determined.

Abbreviations used: HDF, human diploid fibroblasts; CPD, cell population doubling.

Table 1. *Daily cell yield in the presence of 2-deoxyglucose (10 mM) at increasing cell population doubling levels*

Data are presented as means ± S.E.M. ($n = 10$). Abbreviation: n.d., not determined.

| | CPD | Cell yield (μg of protein/well) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day in culture... | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 23 | 1.85 ± 0.05 | 3.61 ± 0.19 | 5.40 ± 0.06 | 6.47 ± 0.02 | 7.45 ± 0.05 | 7.49 ± 0.04 | 7.62 ± 0.06 |
| | 33 | 1.46 ± 0.06 | 2.40 ± 0.03 | 3.73 ± 0.08 | 5.16 ± 0.07 | 6.37 ± 0.06 | 6.97 ± 0.03 | 6.93 ± 0.05 |
| | 43 | 1.62 ± 0.04 | 2.26 ± 0.03 | 3.30 ± 0.13 | 4.35 ± 0.06 | 6.03 ± 0.04 | 6.54 ± 0.05 | 6.83 ± 0.04 |
| | 53 | 0.89 ± 0.02 | 0.80 ± 0.03 | 0.82 ± 0.08 | 1.23 ± 0.04 | 1.94 ± 0.05 | 2.41 ± 0.07 | 2.84 ± 0.09 |
| | 61 | 0.39 ± 0.01 | 0.40 ± 0.01 | 0.37 ± 0.02 | 0.31 ± 0.02 | 0.67 ± 0.02 | 1.09 ± 0.04 | n.d. |
| 2-Deoxyglucose | 23 | 1.34 ± 0.03 | 1.48 ± 0.05 | 1.71 ± 0.12 | 1.53 ± 0.03 | 1.71 ± 0.10 | 2.33 ± 0.12 | 2.51 ± 0.75 |
| | 33 | 1.16 ± 0.04 | 1.19 ± 0.03 | 0.77 ± 0.02 | 0.75 ± 0.04 | 0.86 ± 0.05 | 0.86 ± 0.07 | 0.94 ± 0.05 |
| | 43 | 1.27 ± 0.05 | 0.70 ± 0.03 | 0.52 ± 0.05 | 0.35 ± 0.03 | 0.28 ± 0.04 | 0.55 ± 0.02 | 0.36 ± 0.03 |
| | 53 | 0.92 ± 0.02 | 0.58 ± 0.03 | 0.50 ± 0.07 | 0.63 ± 0.03 | 0.81 ± 0.02 | 0.69 ± 0.02 | 0.69 ± 0.06 |
| | 61 | 0.46 ± 0.01 | 0.41 ± 0.01 | 0.37 ± 0.01 | 0.32 ± 0.02 | 0.52 ± 0.01 | 0.82 ± 0.04 | n.d. |

Human embryonic lung fibroblasts, strain 2002 (Flow Labs), were routinely cultured in minimal essential medium (Glasgow modification) containing penicillin (100 units/l), streptomycin (110 μg/l) and supplemented with 10% (v/v) fetal bovine serum (Gibco BRL). The lifespan of this cell strain previously was determined as 60 ± 3 CPD. For the growth studies, conducted at CPD 23, 33, 43, 53 and 61, HDF were seeded into microtitre plates (Nunc) at a density of approximately $2.7 \times 10^3$ cells per well. Filter-sterilized 2-deoxyglucose (Sigma; final concentrations 1, 3 and 10 mM) was added to the growth medium immediately before commencement of the cultures. Cell yields were estimated directly in the microtitre plates as total cell protein, by solubilization with Triton X-100 and a Coomassie Brilliant Blue dye-binding method (Bio-Rad), daily over a 1 week incubation period. All assays were conducted as tenfold replicates, with seven plates used per CPD.

Markedly different growth patterns were observed in the control cells (no 2-deoxyglucose) with increasing CPD (Table 1). From CPD 23 to 43 there was a slight lengthening of the initial leg phase before onset of the logarithmic phase of growth; however, by CPD 53 the lag phase had extended to 3 days and by CPD 61 to 4 days. This was paralleled by significant, age-related reductions in total cell yield. Between CPD 23 and 43 the cell yield, measured on day 7, declined by only 10.4%, but by CPD 53 the decrease was 62.7% and 85.4% by CPD 61 (measured on day 6.)

Incorporation of 2-deoxyglucose (10 mM) into the growth medium significantly reduced cell yields in the younger cultures: by 67.1% (CPD 23), 86.4% (CPD 33) and 94.7% (CPD 43). Despite the poor growth characteristics of the older cells a marked inhibitory effect was still apparent at CPD 53 (75.7%) and CPD 61 (24.8%). Similar, but less pronounced, patterns of inhibition were obtained at the lower concentrations (1 mM and 3 mM) of 2-deoxyglucose tested (results not shown).

A decline in growth potential after prolonged culture *in vitro* is a consistent feature of many different human cell types (reviewed in Bittles & Sambuy, 1986). Attention has been drawn to an apparent critical point in HDF cultures, at approximately CPD 45, beyond which there are increased numbers of non-dividing cells (Macieira-Coelho & Taboury, 1982), the onset of changes in nucleoprotein organization (Puvion-Dutilleul *et al.*, 1982) and eventually, altered organelle structure and function (Johnson, 1984). The marked increase in cellular sensitivity to 2-deoxyglucose between CPD 23 and 43 in the present study confirms the previous observation of an early shift to glycolysis in HDF (Bittles & Harper, 1984). Significantly, this enhanced utilization of the glycolytic pathway both precedes and appears independent of the alterations in mitochondrial structure (Ghadiminejad *et al.*, 1987) and enzyme activity (Harper *et al.*, 1987) characteristic of cells at advanced CPD.

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## The uptake of iron by hepatocytes is not coincident with transferrin endocytosis

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The mechanism of iron accumulation by hepatocytes remains unresolved despite many studies in recent years to biochemically disect the endocytic pathway. Successive studies in rapidly proliferating transformed cell lines have shown that iron-loaded transferrin is recognized and internalized by specific transferrin receptors and that iron is released to the cell in the acid mileau of the endosome (Morgan, 1981; Huebers & Finch, 1987). However, similar studies in hepatocytes have been notable for conflicting evidence both for the unequivocal presence of cell surface

Abbreviation used: TBS, Tris-buffered saline.