Activity of enzymes of energy metabolism in single human preimplantation embryos

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A method was developed to measure the activities of enzymes in extracts from single human preimplantation embryos. The method permits the analysis of two enzymes plus appropriate controls in an extract from a single embryo, and was used to investigate the control of energy metabolism during the development of human embryos from the two-cell to the blastocyst stage. Hexokinase (HK), 6-phosphofructokinase (PFK), pyruvate kinase (PK), fructose-1,6-diphosphate aldolase (ALD), glucose phosphate isomerase (GPI), lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6PDH) and 2-oxoglutarate dehydrogenase (ODH) were all detectable, whereas glycogen phosphorylase (GP) was not. The enzyme activities of ODH, PFK, LDH, PK, GPI and G6PDH, averaged over all stages of development from the two-cell to blastocyst stage (days 2–6 after insemination), were 3.5, 6.6, 15, 69, 73 and 87 times greater than HK, respectively. The activity of ALD was very similar to that of HK. The activities of ALD, GPI, PFK, PK and LDH showed no significant variation with stage of development, although the activity of GPI fell significantly from the four–eight cell to the eight–sixteen cell stage (P < 0.05). HK activity decreased from the two–eight cell to the eight–sixteen cell (P < 0.05), and increased significantly from the eight–sixteen cell to the blastocyst stage (P < 0.01). The overall relationship between hexokinase activity and stage approached significance (P = 0.059, one-way analysis of variance). The activity of G6PDH decreased significantly with development (P < 0.001, one way analysis of variance). The data suggest that hexokinase plays a key role in the control of glucose utilization in the early human embryo, although a role for PFK is not ruled out. The fact that GP could not be detected suggests that the breakdown of glycogen contributes very little to the energy metabolism of the human preimplantation embryo. The absolute and relative enzyme activities in the human embryo are comparable to those in adult tissues, although that of G6PDH is very high and that of GP is low. The activities of the enzymes are discussed in relation to the changes in pyruvate and glucose uptake and the production of lactate, during early human development.

Introduction

Preimplantation mammalian embryos generate ATP largely via the uptake and metabolism of exogenous nutrients present in oviduct and uterine fluids in vivo, and in culture media in vitro (Biggers and Stern, 1973; Wales, 1975; Leese, 1991). The early developmental stages are characterized by low metabolic activity and the oxidation of substrates such as pyruvate and lactate. Glucose may be toxic at this time (Chatot et al., 1989), although this effect depends on the media (Lawitts and Biggers, 1992). The main features of the later stages, especially the blastocyst, are an increase in metabolic activity and a switch to the consumption of glucose, some or all of which is converted to lactate (Leese and Barton, 1984; Gardner and Leese, 1986, 1988; Hardy et al., 1989; Gott et al., 1990; Brison and Leese, 1991). The switch in energy substrate preference suggests a block to glucose utilization during the early developmental stages. This paper is concerned with the enzymatic basis of the control of energy metabolism in early human embryos.

Two experimental approaches are commonly used to investigate the control of metabolism. The first is the measurement of maximal enzyme activities in vitro; the second is the determination of metabolite concentrations and calculation of mass action ratios (Newsholme and Start, 1973). Both approaches have been used to elucidate the biochemical basis of the block to glucose utilization during early development in mouse embryos. Key enzymes of energy metabolism have been measured, including 6-phosphofructokinase (PFK) (Brinster, 1971) and hexokinase (HK) (Brinster, 1968a;
Hooper and Leese, 1989). The activity of 6-phosphofructokinase is greater than that of hexokinase, and shows no significant variation with embryo development. By comparison, hexokinase increases from the eight-cell to the blastocyst stage, in parallel with the rise in glucose uptake at this time. However, the activity of HK is about four times greater than the consumption of glucose at each stage of development (Gardner and Leese, 1986; Hooper and Leese, 1989).

Barbehenn et al. (1974, 1978) used enzymatic cycling techniques to measure the concentrations of key metabolites in single mouse embryos. The embryos, at a variety of developmental stages, were cultured in substrate-free media for 60 min. and glucose or glucose and pyruvate were then added. The effects of this treatment on metabolite concentrations suggested that the metabolic block to glucose utilization resided at the level of 6-phosphofructokinase, although roles for hexokinase and the transport of glucose into the embryo were not ruled out; this conclusion was also reached by Gardner and Leese (1988). Nutrient uptake in the early human embryo differs both qualitatively and quantitatively from that in the mouse. Significant amounts of glucose are taken up by the human embryo during the early stages of development, and the subsequent fall in pyruvate and rise in glucose uptake is less dramatic. If the difference in volume of the embryos in the two species is taken into account, the uptake of glucose is similar, but pyruvate consumption is approximately four times greater in the human than in the mouse embryo (Hardy, 1992; K. Hardy, unpublished observation). Chi et al. (1988) measured the activity of 17 metabolic enzymes in human and mouse oocytes and found that the activities differed considerably between the two species. Data on the enzyme content of human preimplantation embryos were not presented.

We have measured the maximal activities of nine enzymes of energy metabolism in single spare human embryos at different stages of development. The choice of which enzymes to measure, although constrained by the availability of surplus human embryos, was made on the following basis. Glycogen phosphorylase (GP) and 6-phosphofructokinase were chosen since they are considered to provide a quantitative index of the maximum flux of glycolysis from glycogen (Newsholme et al., 1980; Newsholme and Crabtree, 1986). Hexokinase and 2-oxoglutarate dehydrogenase (ODH) are considered to indicate the maximum flux of glycolysis from glucose and the tricarboxylic acid cycle (TCA cycle), respectively (Newsholme et al., 1980; Newsholme and Crabtree, 1986). Pyruvate kinase (PK) is a further glycolytic enzyme which is considered potentially rate-limiting because it catalyses a non-equilibrium reaction. Glucose phosphate isomerase (GPI), fructose-1,6-diphosphate aldolase (ALD) and lactate dehydrogenase (LDH), although regarded as non-regulatory, provide examples of enzymes that may catalyse near-to equilibrium reactions in glycolysis. Moreover, GPI has been measured in human embryos by West et al. (1989). Finally, glucose-6-phosphate dehydrogenase (G6PDH), an X-chromosome linked enzyme, was included, which may indicate the potential activity of the pentose phosphate pathway which is responsible for generating NADPH and ribose moieties for biosynthetic processes.

Materials and Methods

Embryo collection

Ethical permission for the work was obtained from the UK Interim Licensing Authority (ILA), now the Human Fertilization and Embryology Authority (HFEA), and from the Ethics Committees of the collaborating institutions. Donated, surplus human embryos were obtained from patients undergoing IVF treatment at the Royal Postgraduate Medical School, Hammersmith Hospital, London. The superovulation procedure involved pituitary–gonadal suppression and an LHRR agonist (Buserelin: Hoechst, Hounsfield, Middlesex, UK), prior to and during superovulation with human menopausal gonadotrophin (hMG; Pergonal: Serono, Welwyn Garden City, Herts., UK) (Hilliard et al., 1985; Rutherford et al., 1988). Ovulation was induced with 10 000 iu hCG (Pregnyl: Organon, Cambridge, UK). Oocytes were retrieved 36–36 h later, and were preincubated for 4–8 h and inseminated (day 0), as described by Hillier et al. (1984). After 15–18 h the oocytes were checked for the presence of pronuclei to confirm fertilization (day 1).

Embryos were cultured in 1 ml of either T6 medium (Quinn et al., 1982) or Earle's balanced salt solution (Gibco, Paisley, UK), under a gas phase of 5% O2:5% CO2:90% N2. Both media were supplemented with 10% heat-inactivated maternal serum, and in the case of the Earle's, with 25 mmol sodium bicarbonate 1−1, 0.47 mmol pyruvate 1−1 and antibiotics. On day 2 (40–42 h after insemination) each embryo was examined, graded morphologically (Conaghan et al., 1993) and the best two or three selected for embryo transfer on day 2 or 3.

After obtaining the patients' consent, any remaining cumulus cells were removed from the surplus embryos by gentle suction through a narrow bore pipette. The embryos were then cultured in the original 1 ml of medium or in 20 ml drops of the same medium under paraffin oil.

Enzyme extraction

Human embryos were removed from culture at a variety of developmental stages, scored and placed singly in 2.5 ml of an extraction medium in a 5 ml microcapillary (Drummond Scientific, UK), the ends of which were sealed with paraffilm. The medium served to extract, solubilize and preserve the enzymes; its composition was based on that used by Chi et al. (1988) and contained 50 mmol K2HPO4 1−1, 25% glycerol, 0.5 mmol EDTA 1−1, 5 mmol β-mercaptoethanol 1−1 (BDH, Merck Ltd. Poole), 2 mg BSA ml−1 (ICN Biomedicals, High Wycombe) and 0.5% Triton X-100, at pH 7.5. Samples were frozen immediately and stored at −70°C before analysis. The zona pellucida remained intact. The presence of the zona has been shown not to limit enzyme activity measured in vitro (Brinster, 1965, 1966).

Enzyme assay technique

To measure enzyme activity in single human embryos it was necessary to miniaturize the conventional methods of analysis in which NADH or NADPH are generated or consumed in coupled reactions. The method developed was based on the
ultramicrofluorescence techniques of Mroz and Lechene (1980),

Enzyme assays were carried out in a total volume of 1 μl or
1.5 μl in sealed 5 μl microcapillaries, by adding 0.5 μl or 1.0 μl of
thawed embryo extract to 0.5 μl of reaction mixture. Enzyme
activity, measured as the rate of appearance or disappearance of
NADH or the appearance of NADPH, was monitored using a
fluorescent inverted microscope with MPV compact 2 photometer
and photomultiplier attachments (Leica, Milton Keynes). There
was a linear relationship between NAD(P)H concentration and
arbitrary units of fluorescence in the range 0–2 mmol l⁻¹.

Changes in fluorescence owing to nonspecific oxidation of
NADH, or reduction of NAD(P)⁺ were accounted for by
including controls for each enzyme assay, in which the substrate
was omitted. For enzyme reactions in which NAD⁺ or NADP⁺
were reduced, there was no significant increase in fluorescence in
the absence of substrate. For reactions involving NADH oxidation, a small fall in fluorescence was usually observed in
the absence of substrate, and was therefore subtracted from the
rate for each enzyme in the presence of substrate. Reaction rates
were recorded for a minimum of 1 h and were linear during the
period over which measurements were made.

The composition of the enzyme reaction mixtures are given
below:

**Hexokinase** ([EC 2.7.1.1](Hooper and Leese, 1989)): 100 mmol
triethanolamine 1⁻¹, 5 mmol MgCl₂ 1⁻¹, 5 mmol ATP 1⁻¹,
1.5 mmol NADP⁺ 1⁻¹, 1 mmol glucose 1⁻¹ (BDH), 5 U
glucose-6-phosphate dehydrogenase ml⁻¹ ([EC 1.1.1.49]), pH 7.6.

**6-Phosphofructokinase** ([EC 2.7.1.11](modified from Brinster,
1971)): 100 mmol Tris-HCl 1⁻¹ (BDH), 100 mmol KCl 1⁻¹
(BDH), 5 mmol MgCl₂ 1⁻¹, 1 mmol EDTA 1⁻¹, 10 mmol
fructose-6-phosphate 1⁻¹, 10 mmol ATP 1⁻¹, 5 mmol NADH
1⁻¹, 1 mmol dithiothreitol 1⁻¹, 10 μg aldolase ml⁻¹ ([EC
4.1.2.13]), 10 μg triosephosphate isomerase ml⁻¹ ([EC 5.3.1.1]),
170 U glycerol-3-phosphate dehydrogenase mg⁻¹ ([EC 1.1.1.8]),
ph 8.0.

**Pyruvate kinase** ([EC 2.7.1.40](Chi et al., 1988)): 50 mmol
imidazole–HCl 1⁻¹ (Boehringer Mannheim, Lewes), 0.1 mol KCl
1⁻¹, 3 mmol MgCl₂ 1⁻¹, 1 mmol ADP 1⁻¹, 10 mmol 5'-AMP
1⁻¹, 1 mmol phosphoenolpyruvate 1⁻¹, 500 μmol NADH 1⁻¹,
2 μg lactate dehydrogenase ml⁻¹ ([EC 1.1.1.27]), pH 7.0.

**Fructose-1,6-diphosphate aldolase** ([EC 4.1.2.13](Bergmeyer,
1983)): 94 mmol triethanolamine 1⁻¹, 0.2 mmol NADH 1⁻¹,
1.1 mmol fructose-1,6-diphosphate 1⁻¹, 170 U glycerol-3-phosphate
derhydrogenase mg⁻¹ ([EC 1.1.1.8]), 5000 U triosephosphate
isomerase mg⁻¹ ([EC 5.3.1.1]), pH 7.6.

**Glucose phosphate isomerase** ([EC 5.3.1.9](Bergmeyer, 1983)): 85 mmol triethanolamine 1⁻¹, 1.14 mmol fructose-6-phosphate
1⁻¹, 6.8 mmol MgCl₂ 1⁻¹, 0.39 mmol NADP⁺ 1⁻¹, 140 U
glucose-6-phosphate dehydrogenase mg⁻¹ ([EC 1.1.1.49]), pH 7.6.

**Lactate dehydrogenase** ([EC 1.1.1.27](modified from Brinster,
1965)): 70 mmol KH₂PO₄ 1⁻¹, 0.4 mmol K₃HPO₄ 1⁻¹, 4 mmol
NADH 1⁻¹, 8 mmol pyruvate 1⁻¹, 7.8 mmol NaHCO₃ 1⁻¹
(BDH), pH 7.6.

**Glucose-6-phosphate dehydrogenase** ([EC 1.1.1.49]): 86 mmol
triethanolamine 1⁻¹, 1 mmol glucose-6-phosphate 1⁻¹,
0.5 mmol NADP⁺ 1⁻¹, 7 mmol MgCl₂ 1⁻¹, pH 7.6.

**2-Oxoglutarate dehydrogenase** ([EC 1.2.4.2] (Cooney et al.,
1981)): 250 mmol mannitol 1⁻¹ (BDH), 10 mmol KPO₄ 1⁻¹,
100 mmol MgCl₂ 1⁻¹, 5 mmol MgCl₂ 1⁻¹, 1 mmol dithiothreitol
1⁻¹, 0.05% Triton X-100, 2 mmol NAD⁺ 1⁻¹, 0.6 mmol
coenzyme A 1⁻¹, 10 mmol 2-oxoglutarate 1⁻¹, pH 7.4.

**Glycogen phosphorylase** ([EC 2.4.1.1] (Chi et al., 1988)): 50 mmol
imidazole–HCl 1⁻¹, 0.4% glycogen, 1 μmol glucose-1,6-diphosphate
1⁻¹, 1 mmol NADP⁺ 1⁻¹, 3 μg phosphoglucomutase
ml⁻¹ ([EC 1.5.4.2]). 2 μl glucose-6-phosphate dehydrogenase
ml⁻¹ ([EC 1.1.1.49]), 1 mmol AMP 1⁻¹, 0.5 mmol MgCl₂ 1⁻¹,
20 mmol K₂HPO₄ 1⁻¹, pH 7.0.

Auxiliary enzymes and nicotinamide nucleotides were obtained
from Boehringer Mannheim (Lewes); all other chemicals were from Sigma Chemical Company (Poole) or Fisons
(Loughborough) unless otherwise stated.

**Embryo quality**

All the embryos were normally fertilized as confirmed by the
presence of two pronuclei on day 1. Arrested embryos were
eliminated from the study. On days 2–6 after insemination, the
embryos were at the following stages: two–four cell (day 2); four–eight cell (day 3); eight–cell–morula (day 4); morula–blastocyst (day 5); morula–late blastocyst (day 6). The fol-
lowing criteria were used to classify the embryos into grades
1–5: grade 1 embryos had the expected number of blastomeres
for the time of transfer, which were uniform and intact, with no
extracellular fragmentation; grade 2 embryos exhibited one of the
following: slow development, asymmetrical blastomeres, or
fragmentation; grade 3 embryos had one degenerate blastome-
re, with the remaining cells intact; grade 4 embryos had at
least one blastomere intact; and grade 5 embryos had no intact
cells (Conaghan et al., 1993). Embryos were randomly assigned
to each enzyme assay. The embryos used in this study were
classified as follows: grade 1 (7%), grade 1.5 (14%), grade 2
(47%), grade 2.5 (19%), grade 3 (8%), grade 3.5 (4%), grade
4–4.5 (2%). For each enzyme assayed, 77–87.5% (mean 87%) of
the embryos were of grade 2.5 or above. The small numbers of
embryos available precluded any further analysis of enzyme
activity versus grade.

**Expression of results**

Enzyme activities were expressed as nmol substrate
consumed per embryo h⁻¹ at 20°C. The statistical significance
of the results was analysed using Student’s t test and one-way
analysis of variance. Student’s t test was used to analyse for
differences between mean enzyme activities on separate days
of development. One-way analysis of variance was used to
test for an overall relationship between enzyme activity and
developmental stage.

Previously published values for enzyme activities in human
oocytes at 25°C, 35°C or 37°C were compared with those
obtained in the present study for human embryos at 20°C, by assuming a Q10 of 2; i.e. that for every rise in 10°C, enzyme activity was doubled.

The dry mass of a human embryo was estimated as 181 ng on the basis of values obtained for single mouse embryos (Turner et al., 1992) and by assuming that the human embryo has a volume five times greater than that of the mouse. This was calculated from the diameter of mouse and human embryos, at the eight-cell to morula stage, which were 75.3 ± 3.4 μm (n = 10) and 129.5 ± 7.0 μm (n = 5), respectively (K. Hardy and K. L. Martin, unpublished observations). The estimated value agrees well with that of 175 ng obtained for morphologically normal oocytes by Chi et al. (1988).

Results

Comparison of enzyme activities

All of the enzymes assayed were detected except glycogen phosphorylase. The maximum activity of each enzyme averaged over all stages of development from the two-cell to the blastocyst stage (day 2 to day 6 after insemination) is shown (Table 1; Fig. 1).

Table 1. Maximal enzyme activities in single human preimplantation embryos averaged over all stages of development from the two-cell to blastocyst stage (days 2–6 after insemination)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mean maximal activity (nmol substrate consumed per embryo h⁻¹ ± SEM)</th>
<th>Number of embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>0.0464 ± 0.0059</td>
<td>36</td>
</tr>
<tr>
<td>6-Phosphofructokinase</td>
<td>0.313 ± 0.061</td>
<td>21</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>3.22 ± 0.36</td>
<td>28</td>
</tr>
<tr>
<td>Fructose-1,6-diphosphate aldolase</td>
<td>0.0425 ± 0.0074</td>
<td>18</td>
</tr>
<tr>
<td>Glucose phosphate isomerase</td>
<td>3.38 ± 0.40</td>
<td>18</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>0.698 ± 0.079</td>
<td>29</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>4.04 ± 0.31</td>
<td>27</td>
</tr>
<tr>
<td>2-Oxoglutarate dehydrogenase</td>
<td>0.162 ± 0.059</td>
<td>10</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>not detectable</td>
<td>8</td>
</tr>
</tbody>
</table>

Of the eight enzymes detected, hexokinase and fructose-1,6-diphosphate aldolase had the lowest activities. Pyruvate kinase, glucose phosphate isomerase and glucose-6-phosphate dehydrogenase had the highest maximum activities with values 69, 73 and 87 times greater than those of hexokinase, respectively. Enzymes with an intermediate range of activity were 2-oxoglutarate dehydrogenase, 6-phosphofructokinase and lactate dehydrogenase, the activities of which were 3.5, 6.6 and 15 times greater than hexokinase, respectively.

The enzyme activities, averaged over all stages of development, are compared with those measured by others on human oocytes (Fig. 2). Data for GPI measured in five human embryos by West et al. (1989) are also included. The average enzyme activities measured in single human embryos and those reported for human oocytes are of the same order of magnitude.

Fig. 1. Maximum enzyme activities in single human preimplantation embryos averaged over all stages of development from the two-cell to blastocyst stage (day 2 to day 6 after insemination). Values are nmol substrate consumed per embryo h⁻¹ ± SEM. The number of determinations for each enzyme is shown in Table 1. Glycogen phosphorylase was not detectable. HK: hexokinase; PFK: 6-phosphofructokinase; PK: pyruvate kinase; ALD: fructose-1,6-diphosphate aldolase; GPI: glucose phosphate isomerase; LDH: lactate dehydrogenase; G6PDH: glucose-6-phosphate dehydrogenase.

Variation of enzyme activity with stage of development

No significant variation with stage of development was seen for the enzymes 6-phosphofructokinase, pyruvate kinase, fructose-1,6-diphosphate aldolase, glucose phosphate isomerase and lactate dehydrogenase (Figs 3b,c, 4a–c), although the activity of GPI decreased from the four–eight cell to the eight–sixteen cell stage (P < 0.05). The maximum activity of hexokinase decreased from the two–eight cell to the eight–sixteen cell stage (P < 0.05), and increased significantly from the eight–sixteen cell to the blastocyst stage of development (P < 0.01; Fig. 3a). The overall relationship between hexokinase activity and stage approached significance (P = 0.059, one-way analysis of variance). Glucose-6-phosphate dehydrogenase activity decreased significantly with stage of development (P < 0.001, one-way analysis of variance; Fig. 5).
Discussion

This is the first report of the developmental profile of enzymes concerned with energy metabolism in human preimplantation embryos. The method used permits the analysis of two enzymes, with appropriate controls, on an extract from a single embryo. It has the further advantage of enabling enzyme activity to be measured directly, without the use of enzymatic cycling techniques (Lowry and Passoneau, 1972), which are time-consuming and technically very demanding.

The absolute enzyme activities, expressed in terms of cell dry mass, compared well with those in adult mammalian tissues; heart, liver, kidney, lung, fat, brain and skeletal muscle (Lowry and Passoneau, 1964; Shonk and Boxer, 1964; Shonk et al., 1964; Crabtree and Newsholme, 1972; Budohoski et al., 1982; Blomstrand et al., 1983, 1986; Lowry et al., 1983; Board et al., 1990). This was especially the case for hexokinase, 6-phosphofructokinase, pyruvate kinase, fructose-1,6-diphosphate aldolase, glucose phosphate isomerase, lactate dehydrogenase and 2-oxoglutarate dehydrogenase. In contrast, the activity of glucose-6-phosphate dehydrogenase in the embryos was extremely high, and that of glycogen phosphorylase low. When the enzyme activities for human embryos were expressed relative to hexokinase, the pattern for PFK, PK, ALD, GPI, LDH and ODH was similar to that found in the adult tissues listed above. The exceptions were again, G6PDH, the relative activity of which was very high and GP, which was low.

Potential exogenous and endogenous energy sources available to the human preimplantation embryo include glucose, pyruvate, lactate, glycogen, amino acids and lipids (Leese, 1991). The uptake of exogenous nutrients, glucose and pyruvate, and the formation of lactate, have been measured in single human embryos cultured in vitro (Hardy et al., 1989; Gott et al., 1990). During the early stages of development, pyruvate is consumed preferentially, before a significant increase in glucose uptake

Fig. 2. Maximum activities of enzymes in single human preimplantation embryos averaged over all stages of development from the two-cell to blastocyst stage (day 2 to day 6 after insemination) (■), compared with previously published values for human oocytes and a mean value for GPI in five preimplantation embryos* (□). Enzyme activities are expressed as a percentage of the values obtained for human preimplantation embryos, in Table 1 and Fig. 1. HK: hexokinase; PFK: 6-phosphofructokinase; PK: pyruvate kinase; GPI: glucose phosphate isomerase; LDH: lactate dehydrogenase; G6PDH: glucose-6-phosphate dehydrogenase. *Tsutsumi et al. (1990); †Chi et al. (1988); ‡West et al. (1989); †Brinster (1968b).

Fig. 3. Variation in enzyme activity with embryo development of: (a) hexokinase, (b) 6-phosphofructokinase and (c) pyruvate kinase. Enzyme activities were measured in extracts from single human preimplantation embryos. Values are means of an average of seven determinations for each stage of development (range 3–15), for total numbers assayed for each enzyme see Table 1, and are expressed as nmol substrate consumed per embryo h\(^{-1}\) ± SEM. The activity of hexokinase decreased from the four–eight cell to the eight–sixteen cell stage (P < 0.05)*, before increasing significantly from the eight–sixteen cell to the blastocyst stage (P < 0.01†), the variation of activity with development approached significance (P = 0.059, one-way analysis of variance).
occurs between the morula and blastocyst stage, on days 3.5–5.5 after insemination.

The results reported here suggest that hexokinase may be important in regulating the metabolism of glucose in the human preimplantation embryo. Activities of the other two potentially rate-limiting enzymes in the glycolytic pathway, 6-phosphofructokinase and pyruvate kinase, averaged over all stages of development, were 6.6 and 70 times greater than hexokinase, respectively. In addition, the significant rise in hexokinase from the eight–sixteen cell to blastocyst stage (Fig. 3a) parallels the increase in glucose uptake (Hardy et al., 1989), although the activity of hexokinase is greater than the uptake of glucose at each stage of development, by a factor of 2–6. The corresponding values for PFK and PK are 13–39 and 134–403, respectively, depending on which developmental stage is taken for comparison. However, a regulatory role for 6-phosphofructokinase is still likely, as is the case in the mouse embryo (Barbehenn et al., 1974, 1978), as the allosteric properties of this enzyme are not exhibited at the assay pH of 8.0.

The average and relative enzyme activities in human preimplantation embryos reported here compare well with those in human oocytes. This is especially the case for glucose phosphate isomerase (West et al., 1989) and pyruvate kinase (Chi et al., 1988). Hexokinase and 6-phosphofructokinase activities are 4.5 times greater in embryos than in oocytes, suggesting that of the glycolytic enzymes, they may become most important in the regulation of glucose metabolism after fertilization.

Total lactate production steadily increases with development in the human embryo, from 43.6 pmol per embryo h\(^{-1}\) on day 2.5 after insemination to 95.4 pmol per embryo h\(^{-1}\) on day 5.5 (Gott et al., 1990). Wales et al. (1987) reported a maximum value of 36.6 pmol per embryo h\(^{-1}\) for lactate production from glucose, by the human blastocyst. The activity of LDH is 6–7 times greater than the total rate of lactate production, and 8–28 times greater than the potential contribution to lactate formation from the aerobic glycolysis of exogenous glucose (Wales et al., 1987; Gott et al., 1990). The activity of LDH in mouse preimplantation embryos is
similarly far greater than the amount required to account for lactate production (Brinster, 1965; Epstein et al., 1969).

The activities of ALD and GPI in the human embryo are similar to those in the mouse (Epstein et al., 1969; West et al., 1986, 1989), if the fivefold difference in volume is taken into consideration. However, the activities of G6PDH, HK and PFK are higher in human than in mouse embryos (Brinster, 1966, 1968a, 1971; Epstein et al., 1969; Leese, 1987; Hooper and Leese, 1989) and cannot be accounted for in this way. The reverse is true for LDH which is 9–86 times greater in mice (Brinster, 1965; Epstein et al., 1969).

It has been suggested that the breakdown of glycogen may contribute to the production of lactate in the human embryo (Gott et al., 1990). However, glycogen phosphorylase could not be detected, indicating that glucose is the main carbohydrate substrate for lactate production. The activity of GP in human oocytes is also extremely low, 0.095 pmol per oocyte h−1 (Chi et al., 1988). A comparison of the activities of enzymes involved in both glycogen synthesis and degradation indicate that GP is the rate-limiting enzyme of glycogen breakdown, and that net synthesis is favoured in both human and mouse oocytes (Chi et al., 1988), and in mouse preimplantation embryos (Hsieh et al., 1979). The fact that GP cannot be detected suggests that this may also apply to the human embryo, although further investigations are required.

The activity of glucose-6-phosphate dehydrogenase reported here fell significantly with development. G6PDH catalyses a reaction considered to be far from equilibrium and is the rate-limiting step of the pentose phosphate pathway in liver and adipose tissue (Newsholme and Leech, 1981). The activity in the human embryo suggests that the pathway has a potentially high activity, which decreases with time after fertilization. The activities of this enzyme are far greater than those required to support the pentose phosphate pathway, which has been shown in the rabbit and mouse embryo to represent less than 15% of glucose consumed (Fridhandler, 1961; O’Fallon and Wright, 1986). Even if all the exogenous glucose consumed by the human embryo was converted to glucose-6-phosphate, and subsequently metabolized by this pathway, the activity of G6PDH would still be greater, by a factor of 168–505.

Enzymes detected in the embryo up to the four–eight cell stage will be proteins synthesized from the mRNA inherited from the oocyte, as the embryonic genome is not activated until this time (Braude et al., 1988; Tesarik, 1988). The fall in G6PDH activity with development, which also occurs in the mouse embryo (Brinster, 1966; Leese, 1987), probably reflects the net degradation of enzyme coded for by the oocyte mRNA. The high activity of G6PDH in the human preimplantation embryo may therefore be due to the previous requirements of the oocyte; indeed, the activity measured in human oocytes is also high (Chi et al., 1988), although it is only half of that found in the embryo. However, the questionable quality of the unfertilized oocytes used by Chi et al. (1988), which had been incubated with spermatozoa for 3–4 days, may account for this difference.

The oxidative metabolism of pyruvate represents a large potential source of energy for the human embryo. 2-Oxoglutarate dehydrogenase is a multienzyme complex and one of the potentially rate-limiting enzymes of the TCA cycle, together with citrate synthase, isocitrate dehydrogenase and succinate dehydrogenase (Newsholme and Leech, 1981). Barbehenn et al. (1974, 1978) suggested that an enzyme between 2-oxoglutarate and malate, might play a role in regulating the metabolism of glucose in the mouse preimplantation embryo. If such a step was rate-limiting, it could account for the high concentration of citrate found in the embryo (Barbehenn et al., 1978). Citrate inhibits 6-phosphofructokinase allosterically, and so may contribute to the block to glucose utilization in the early preimplantation stages.

The activity of ODH has been shown to equal the TCA cycle flux in muscles (Newsholme et al., 1980), rat heart (Randle et al., 1970; Cooney et al., 1981), and the hearts and mammary glands of ruminants and non-ruminants (Read et al., 1977). However, in rat lymphocytes, ODH activity was found to be greater than the TCA flux (Ardawi and Newsholme, 1982). The activity of ODH in the human embryo is comparable to the values found in adult tissues (Blomstrand et al., 1983, 1986). Assuming that all the pyruvate consumed by the human embryo enters the TCA cycle, ODH is 4–6 times greater than that required to account for the oxidative flux of this nutrient.

In conclusion we have presented novel information on the phenotype of the early human embryo at the biochemical level. This information is essential to our understanding of the regulation of metabolic pathways and their integration with development, and is a prerequisite for the genetic analysis of these phenomena. Moreover, defining the control of metabolism in human embryos could enable improvements in embryo culture techniques to be made on a rational as opposed to an empirical basis.

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