Enzymes of purine salvage and catabolism in the mouse preimplantation embryo measured by high performance liquid chromatography

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The activities of five enzymes involved in purine salvage and catabolism—hypoxanthine phosphoribosyl transferase (HPRT), adenine phosphoribosyl transferase (APRT), adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP) and guanase—were measured in mouse embryo extracts, from the one-cell to the blastocyst stage. Xanthine oxidase activity was not detected. The analyses were performed using high performance liquid chromatography and the enzymes showed different patterns of activity during development. Activities of HPRT, APRT and PNP were low before morula formation, and then increased until the blastocyst stage. ADA and guanase showed high activities after fertilization; guanase activity decreased sharply after the two-cell stage and ADA activity decreased sharply after the morula stage. Blastocyst formation was accompanied by a further decline in activity of both enzymes. The methods used may be suitable for measuring these enzymes in single human embryos, or in biopsies derived from them.

Introduction

Cells are supplied with purine nucleotides either via de novo synthesis or by salvage of nucleotides and bases derived mainly from the breakdown of RNA (Murray, 1971; Zollner, 1982). Enzymes of particular interest in purine salvage and degradation are: adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), guanase, hypoxanthine phosphoribosyltransferase (HPRT), adenine phosphoribosyltransferase (APRT) and xanthine oxidase (XO) (Fig. 1).

ADA (EC 3.5.4.4) catalyses the irreversible hydrolytic deamination of adenosine to inosine and ammonia. Different forms of the enzyme are found in birds and mammals (Brady and O'Donovan, 1965; Ma and Fisher, 1968; Daddonna and Kelley, 1977; Vetteranta and Raivio, 1988). In the mouse fetus, the activity shows a stage-specific increase, which continues in most organs after birth (Lee, 1973).

In 1988, Benson and Monk used a biochemical radiolabelled microassay to investigate whether adenosine deaminase was present in mouse preimplantation embryos. The technique was based on a three-step reaction, where the final product measured was inosine monophosphate (IMP):

\[
\text{ADA} \rightarrow \text{PNP} \rightarrow \text{HPRT} \rightarrow \text{adenosine}\rightarrow\text{inosine}\rightarrow\text{hypoxanthine}\rightarrow\text{IMP}
\]

The existence of this pathway in the mouse preimplantation embryo was demonstrated, but it was not possible to measure the activity of the enzyme of interest, ADA.

PNP (EC 2.4.2.1) catalyses the degradation of guanine and hypoxanthine nucleosides and deoxyanalogues to their corresponding bases. Although the reaction is reversible, base formation is favoured because intracellular phosphate concentrations normally exceed those of ribose or deoxyribose phosphate. This enzyme has not previously been measured in preimplantation mouse embryos.

Guanase (EC 3.5.4.3) catalyses the formation of xanthine using guanine as the substrate. This enzyme was first measured in unfertilized mouse oocytes and zygotes by Epstein et al. (1971), and was shown to be high at about the time of fertilization, with a sharp decline after the two-cell stage.

HPRT (EC 2.4.2.8) is an X-linked enzyme which catalyses the conversion of hypoxanthine and guanine to their monophosphate nucleotides, IMP and GMP, respectively. The enzyme has been studied in preimplantation mouse embryos (Epstein, 1970; Kratzer and Garth, 1978; Harper and Monk, 1983; Kratzer, 1983; Monk, 1987; Downs and Dow, 1991), together with the autosomal enzyme, APRT (EC 2.4.2.7), which is responsible for the conversion of adenine to AMP. The results showed that the activities of both enzymes were low and maternally controlled until the four-cell stage; there was then a rapid increase in activity after the eight-cell stage until the formation of the blastocyst.

Xanthine oxidase (XO: EC 1.2.3.2) catalyses the oxidation of hypoxanthine to uric acid in a two-step reaction, in which xanthine is the metabolic intermediate. This enzyme can also act as a dehydrogenase (XDH: EC 1.2.1.37), when NAD+ is used as the electron receptor instead of oxygen (Fridovich and Handler, 1962; Battelli et al., 1973; Wajner and Harkness, 1989). Uric acid is the endproduct of purine degradation in humans, whereas in adult rodents, uric acid is converted to allantoin by...
uricase. Studies on the catabolic enzymes in mouse fetal tissues showed that xanthine oxidase activity is very low or undetectable until birth. Fifteen days after gestation, xanthine oxidase is present in organs such as the liver, kidney or intestine, but in very low amounts (Lee, 1973).

Some of the above enzymes are associated with genetic diseases of the immune and the nervous systems, such as the Lesch-Nyhan syndrome (HPRT-deficiency: Harkness et al., 1988; Allsop and Watts, 1990; Davidson et al., 1991), severe combined immunodeficiency (ADA-deficiency: Giblett et al., 1972; Thompson and Seegmiller, 1980), and T-cell immunodeficiency (PNP-deficiency: Seegmiller et al., 1979; Simmonds, 1987). Natural substrates of these enzymes, such as adenosine, are physiologically active and can inhibit tissue growth (Karnofsky and Accon, 1961; Henderson and Scott, 1980; Knudsen and Elmer, 1987); other substrates such as hypoxanthine are involved in free radical formation, which may be deleterious for embryo development (Loutradis et al., 1987; Nasr-Esfahani et al., 1991); inhibition of development of mouse zygotes by inosine and adenosine has also been reported by Thomson Ten Broeck (1968) and Nurredin et al. (1990).

There has been no systematic comparison of the activities of the key enzymes of purine turnover throughout preimplantation development. We therefore measured the activities of ADA, PNP, guanase, HPRT and APRT in small groups of mouse embryos from the one-cell to the blastocyst stages using high performance liquid chromatography.

It was hoped that the assays developed might find clinical application in diagnosing human genetic disorders at the preimplantation embryo level.

**Materials and Methods**

**Animals and cultures**

Female mice, 5–8 weeks old, from the inbred, F1 hybrid strain CBA/Ca × C57BL/6 were superovulated between 12:00 and 13:00 h with 5 iu pregnant mares' serum gonadotrophin (PMSG) (Folligon: Intervet, Cambridge, UK), followed 48 h later by 5 iu human chorionic gonadotrophin (hCG) (Chorulon: Intervet), and placed with males of the same strain. Mating was confirmed on the following morning (day 1) by the presence of a vaginal plug. On day 1 or day 2, the oviducts were removed, dissected free from fat and washed several times in medium M2 (Quinn et al., 1982). The cumulus oophorus surrounding zygotes on day 1 (embryos removed 20–22 h after hCG) was dispersed with M2 containing hyaluronidase (1 mg ml⁻¹) (Sigma Chemical Co, Poole, Dorset). Embryos on day 2 were recovered 44–46 h after hCG by flushing the oviducts with M2. After the washes in M2, the embryos were rinsed in M16 (Whittingham, 1971) and cultured in 2–5 µl droplets of M16, in 35 mm plastic sterile dishes (Nunl, Gibco, Life Technologies, Paisley), under paraffin oil (Analar grade; BDH, Poole, Dorset). Each drop contained between 20 and 150 embryos. Cultures were maintained for up to 4 days in a humidified atmosphere of 5% CO₂ in air, at 37°C. The ionic salts, glucose and phenol red for preparation of culture media were supplied by BDH; Hepes, Ultrot grade for preparation of the medium M2 was from Calbiochem Corp (La Jolla, CA); D/L-lactic acid (sodium salt), sodium pyruvate and penicillin were supplied by Sigma Chemical Co.

**Enzyme extraction procedure**

Embryos were extracted by a modification of the method of Leese et al. (1991). Groups of between 1 and 20 embryos, depending on the enzyme being assayed, were removed from culture at the four-cell, eight-cell, morula, early blastocyst and hatching blastocyst stages, and rinsed quickly in 3 ml of the buffer used for extraction. The embryos were transferred with a glass pipette into a 1.5 µl drop of extraction mixture, placed on a siliconized microscope slide, and immediately taken up into a 2 µl glass microcap (Drummond Scientific, London). The volume of buffer transferred with the embryos was the minimum possible, and did not affect the final volume in the microcap. The ends of the microcap were sealed with paraffin, and stored at –80°C, in most cases for not more than 2 weeks. Before being analysed, the extracts were thawed and expelled.

![Figure 1](image-url)
onto a siliconized slide under a drop of oil (paraffin or mineral). The freezing and thawing process was carried out only once because for some of the enzymes (e.g. HPRT), repeated freezing and thawing resulted in a loss of enzyme activity. One microlitre of the embryo extract was mixed with 2 µl incubation mixture on the microscope slide. The reaction mixture was then taken up into a 5 µl microcap which was sealed with paraffin and incubated on a warm plate at 37°C.

Controls were run containing 1 µl of embryo extract mixed with 2 µl reaction buffer without substrate. A second control was carried out in which 2 µl reaction mixture containing the substrate was mixed with 1 µl of the buffer used for extraction. These controls served to check the stability of the compounds in the incubation mixtures and to confirm that non-enzyme-catalysed reactions did not take place.

**HPRT and APRT.** HPRT and APRT activities were measured simultaneously in the same extracts by determining the amounts of IMP and AMP produced after incubation for 3 h. The reactions were linear for at least 3 h. For each determination, two to six embryos were extracted in Tris/Mg<sup>2+</sup> buffer, pH 7.4, containing 275 mmol Tris 1<sup>−1</sup>, 25 mmol MgCl<sub>2</sub> 1<sup>−1</sup> and 0.5 mg BSA ml<sup>−1</sup> (ICN Biomedicals, Cleveland, OH). The same buffer was used for incubation but with the addition of 50 µmol adenine 1<sup>−1</sup> and 50 µmol hypoxanthine 1<sup>−1</sup>, as substrates for APRT and HPRT, respectively, and 1 mmol PRPP 1<sup>−1</sup> as the ribose donor (Leese et al., 1991). The purines were dissolved in 0.2 mol KOH 1<sup>−1</sup> before dilution in the Tris buffer.

**ADA.** ADA activity was estimated as the amount of inosine formed by embryo extracts incubated with 750 µmol adenosine 1<sup>−1</sup> for 1 h. Owing to the high activity shown by this enzyme, only one or two embryos were required for each assay. The extraction mixture was 0.1 mol sodium phosphate buffer 1<sup>−1</sup>, pH 7.4, made with 0.2 mol Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 1<sup>−1</sup> and 0.2 mol NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O 1<sup>−1</sup> (pH adjusted with 0.2 mol HCl 1<sup>−1</sup>), and supplemented with 0.5 mg BSA ml<sup>−1</sup>. For enzyme activity determinations, the substrate adenosine was added after being dissolved in 0.2 mol KOH 1<sup>−1</sup>. The maximum enzyme activity was estimated for a variety of adenine concentrations in the range of 0.1–3 mmol 1<sup>−1</sup>. The reaction was linear for at least 3 h.

**PNP.** PNP activity was measured as the amount of hypoxanthine formed by embryo extracts incubated with 3 mmol inosine 1<sup>−1</sup> for 70 min. Maximal activity was estimated in the range 0.1–5 mmol inosine 1<sup>−1</sup>. The extraction mixture was the same as that used for the ADA assay. For the two-cell, four-cell and eight-cell stages, 20 embryos were used per assay. Ten to fifteen embryos were extracted for the one-cell stage and for the early and late blastocyst stages. The reaction was linear for at least 3 h.

**Xanthine oxidase.** Xanthine oxidase activity was measured as the formation of xanthine or uric acid, using hypoxanthine or xanthine as substrates, respectively. Incubations were carried out for between 1 and 7 h. For each sample, 20 embryos at the hatching blastocyst stage (120 h after hCG) were analysed.

Two different extraction buffers were used, both having a pH of 7.9–8.0: (a) 0.01 mol KH<sub>2</sub>PO<sub>4</sub> 1<sup>−1</sup> plus 0.2 mol NaOH 1<sup>−1</sup> plus 0.2 mmol EDTA 1<sup>−1</sup>, and (b) 1.5 mol Tris·HCl 1<sup>−1</sup> plus 0.02% (v/v) mercaptoethanol. Embryo extracts were incubated in buffer supplemented with 0.05 mmol xanthine 1<sup>−1</sup> or hypoxanthine as substrates; those extracted in Tris buffer were incubated in the same buffer without mercaptoethanol, at a substrate concentration of 0.1 mmol 1<sup>−1</sup>. The possibility that the enzyme existed in the dehydrogenase form was tested in groups of embryos extracted in Tris buffer, by adding 100 mmol NAD<sup>+</sup> 1<sup>−1</sup> to serve as the electron acceptor.

**Guanase.** Guanase activity was measured as the amount of xanthine formed after incubation for 1 h with 0.2 mmol guanine 1<sup>−1</sup>. Groups of two to seven embryos were extracted per sample in the extraction buffer used for the ADA and PNP assays. The reaction was linear for at least 2 h.

Preliminary experiments showed that the addition of purines in 0.2 mol KOH 1<sup>−1</sup> did not alter the pH of the incubation buffers.

Hypoxanthine, xanthine, adenine, guanine, adenosine, inosine, uric acid, IMP and PRPP were supplied by Sigma Chemical Co.; NAD, NADH, AMP, ADP and ATP were supplied by Boehringer Mannheim GmbH (Mannheim).

**High performance liquid chromatography (HPLC) analysis.**

HPLC analysis was carried out on a Kontron 400 series system (Kontron Instruments, Watford, Herts) consisting of a solvent pump, an autosampler injector, a silica packed column, a variable wavelength UV detector and a computer system MS-DOS. Reverse-phase chromatography was performed in an isocratic mode at room temperature.

Two types of column were used, which differed in internal diameter. For the analysis of HPRT, APRT and xanthine oxidase, the column used was a Hypersil, 5 ODS (25 cm × 2 mm) (Shandon, Runcorn, Cheshire) with a mobile phase of 25 mmol NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 1<sup>−1</sup>, pH 4.5. The ADA, PNP and guanase assays were done with a Hypersil 5 ODS (25 cm × 4.6 mm) column, using a pre-minicolumn (1 cm × 4.6 mm) as a filter (Jones Chromatography, Macclesfield) with a mobile phase of 25 mmol NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 1<sup>−1</sup>, pH 4.5, with the addition of 1.5% and 1% methanol (Analar grade), for ADA and PNP, respectively. The elution buffers were prepared with double-distilled, deionized water and filtered through a 0.4 µm membrane filter immediately before use. The columns were cleaned with Analar methanol after approximately 50 samples had been run. Elution was performed at a flow rate of 0.3 ml min<sup>−1</sup> with the detector set at 254 nm and an absorbance sensitivity of 0.002 AFS (absorbance full scale).

Samples for HPLC analysis were made by adding 3 µl reaction mixture to 27 µl elution buffer; the injected volume was 20 µl.

The representative chromatograms for the (a) ADA, (b) PNP and (c) guanase assays are shown in Fig. 2.

Peak identification was performed by comparing retention times with those of freshly prepared standards and, in some cases, the addition of more of the substance to be identified. Further confirmation was obtained by setting the UV detector.
to different wavelengths, including the one representing the highest absorption of the standards.

Statistical analysis

Statistical analysis of the data was performed by using analysis of variance (one-way), and Newman–Keul’s multiple-range test.

Results

HPRT activity was very low during the first two cleavage divisions (0.2–0.6 pmol embryo$^{-1}$ h$^{-1}$), but showed a sevenfold increase by the morula stage ($P < 0.05$) (Fig. 3). Blastocyst formation was accompanied by a further increase in HPRT activity to values of $13 \pm 2.6$ pmol embryo$^{-1}$ h$^{-1}$ on day 4 and $11 \pm 4$ pmol on day 5, when the embryos were hatching from the zona pellucida.

APRT activity showed a pattern qualitatively similar to that of HPRT, but with lower values, such that by the later stages of preimplantation development, the activities were almost ten times lower (Fig. 4). There was a non-significant decline in APRT activity during the first cleavage division, and activity rose sharply after the two-cell stage ($P < 0.05$). In contrast to HPRT, APRT activity increased before and after blastulation, until the hatching blastocyst stage.

ADA activity was extremely high in comparison with the other enzymes measured (Fig. 5). The average value between fertilization and the eight-cell stage was approximately $135$ pmol embryo$^{-1}$ h$^{-1}$ (maximum $147$ pmol for the one-cell stage and $121$ pmol for the two-cell stage) ($P > 0.05$). A sharp decline in ADA activity was found late on day 3; almost 50% of the enzyme activity was lost during the first 10 h of blastulation, so that by the time blastocyst expansion had occurred, only 20% of the activity remained ($36 \pm 9$ pmol embryo$^{-1}$ h$^{-1}$) ($P < 0.05$). A further small decrease was shown just before the normal time of implantation (with a value of $27 \pm 2.7$ pmol embryo$^{-1}$ h$^{-1}$) at the hatching blastocyst stage. Although a dramatic decrease was observed during the preimplantation period, the lowest value for ADA, at the hatching blastocyst stage, was still above the highest activity of all the other enzymes measured ($P < 0.05$).

PnP activity increased by 11 times between fertilization and the two-cell stage (Fig. 6). This was followed by an equivalent decline between the two-cell and the four-cell stages ($P < 0.05$). Between the four-cell and eight-cell stages,
Fig. 4. Activity of adenosine phosphoribosyltransferase (APRT) in mouse preimplantation embryos, estimated as adenosine monophosphate (AMP) production. The values represent means ± SEM. The number of determinations (n) for each stage was: unfertilized egg (uf, day 1) n = 5; one-cell (1c, day 1) n = 7; two-cell (2c, day 2) n = 8; four-cell (4c, late day 2) n = 5; eight-cell (8c, day 3) n = 9; early blastocyst (eb, day 4) n = 9; hatching blastocyst (hb, day 5) n = 12. Each determination corresponded to at least two and a maximum of ten embryos.

Fig. 5. Activity of adenosine deaminase (ADA) in mouse preimplantation embryos, estimated as inosine production. The values represent means ± SEM. The number of determinations (n) for each stage was: one-cell (1c, day 1) n = 14; two-cell (2c, day 2) n = 14; four-cell (4c, late day 2) n = 6; eight-cell (8c, day 3) n = 12; compacted morula (cm, late day 3) n = 4; early blastocyst (eb, day 4) n = 8; two groups of mid-blastocysts (mb, late day 4) n = 13 and 4; hatching blastocyst (hb, day 5) n = 14. Each determination corresponded to one or two embryos.

Fig. 6. Activity of purine nucleoside phosphorylase (PNP) in mouse preimplantation embryos, estimated as hypoxanthine production. The values represent means ± SEM. The number of determinations (n) for each stage was: unfertilized egg (uf, day 1) n = 3; one-cell (1c, day 1) n = 10; two-cell (2c, day 2) n = 5; four-cell (4c, late day 2) n = 3; eight-cell (8c, day 3) n = 6; early blastocyst (eb, day 4) n = 11; hatching blastocyst (hb, day 5) n = 12. Each determination corresponded to at least ten and a maximum of 20 embryos.

Fig. 7. Activity of guanase in mouse preimplantation embryos, estimated as xanthine production. The values represent the means ± SEM. The number of determinations (n) for each stage was: one-cell (1c, day 1) n = 7; two-cell (2c, day 2) n = 4; four-cell (4c, late day 2) n = 5; eight-cell (8c, day 3) n = 4; early blastocyst (eb, day 4) n = 5. Each determination corresponded to at least two and a maximum of seven embryos.

Guanase activity (Fig. 7) increased from the one-cell to two-cell stage, and remained at this value until the four-cell stage, after which it started to decline. The most marked decrease occurred between the compacted morula and...
byproducts. Incubated present, acid. Incubation successful. Extracts, recovered enzyme the High activities, assayed. (Fig. 200 100

![Graph showing enzyme activities](image)

Enzyme activities (pmol embryo\(^{-1}\) h\(^{-1}\))

Time (h) after hCG

Fig. 8. Activities of adenosine deaminase (ADA), adenosine phosphoryltransferase (APRT), hypoxanthine phosphoryltransferase (HPRT), purine nucleoside phosphorylase (PNP) and guanase during mouse preimplantation development, for comparison. (□) Guanase; (○) ADA; (□) APRT; (⊗) HPRT and (■) PNP.

blastocyst stages (\(P < 0.05\)). The values ranged between 9 pmol embryo\(^{-1}\) h\(^{-1}\) and 30 pmol embryo\(^{-1}\) h\(^{-1}\), for the blastocyst and the two-cell stages, respectively.

Attempts to measure xanthine oxidase activity were unsuccessful. Various combinations of number of embryos and incubation time were tested; the highest number of embryos extracted per sample was 20, and the maximum time they were incubated was 7 h. Uric acid could not be detected in any of the samples assayed. Samples assayed in the presence of NAD\(^+\) resulted in very complex chromatograms, with many peaks, probably corresponding to NAD\(^+\) and NADH, or their byproducts. However, there was no peak corresponding to uric acid. It was therefore concluded that, if the enzyme was present, its activity could not be measured with the analytical system used.

Discussion

High performance liquid chromatography was used to measure the activity of five key enzymes involved in purine salvage and catabolism in mouse preimplantation embryos. A further enzyme tested, xanthine oxidase, was not detectable.

For all the enzymes tested, there was no significant variation between the activities in cultured embryos and those freshly recovered from the reproductive tract at the equivalent developmental stages. As with all experiments of enzymes in cell extracts, caution should be exercised in assuming that the activities correspond precisely to those present in vivo. However, with this proviso, the developmental profiles of the enzymes fell into two distinct groups. Adenosine deaminase and guanase showed high activity during the early developmental stages, with a sharp decline following the formation of the morula. By contrast, HPRT, APRT and PNP activities were initially low, but increased sharply after morula formation (Fig. 8).

The high activity of ADA observed after fertilization may result from extensive biosynthesis during oocyte maturation. Most maternally derived transcripts then decline shortly after the onset of embryonic genome expression, at the two-cell stage in mice (Schultz, 1986). The ADA activity present until the eight-cell stage is likely to be mainly maternally controlled, as there is a decline in activity, following the switch to the embryonic genome. Indeed, Harper and Monk (1983) and Kratzer (1983) have provided evidence that before the stage of major expression of the embryonic genome, the HPRT activity detected is maternally derived. If ADA is maternally derived before the eight-cell stage, the question arises as to why it shows such a high activity compared with HPRT.

A possible explanation is that ADA has a protective role against adenosine toxicity, a function that has been shown in adult tissues (Henderson and Scott, 1980). In preliminary experiments, it has been shown that adenosine strongly inhibits mouse preimplantation development. At a concentration of 0.1 mmol l\(^{-1}\), only 8% of the embryos formed blastocysts; with 0.05 mmol l\(^{-1}\), the proportion reaching the blastocyst stage was increased to 33% (Alexiou, unpublished data).

Progressive inhibition of mouse preimplantation development, at adenosine concentrations of 10\(^{-5}\)–10\(^{-2}\) mol l\(^{-1}\), has also been reported by Thomson Ten Broeck (1968). In addition, Nureddin et al. (1990) showed that 30 µmol adenosine 1\(^{-1}\) or 30 µmol inosine 1\(^{-1}\) inhibit development of zygotes from randomly bred mice. Moreover, increasing amounts of adenosine deaminase, expressed at the implantation sites of rat, mouse and human fetuses (Vetteranta and Raivio, 1986; Krudsen et al., 1988, 1991; Hong et al., 1991), have been postulated to have a protective role against adenosine-induced damage.

Using a radiolabel assay, Benson and Monk (1988) showed the existence of a pathway leading from adenosine to inosine and then to hypoxanthine in the mouse preimplantation embryo. However, they did not measure ADA activity directly. The study reported here showed that ADA activity can readily be measured in single mouse embryos by HPLC analysis. ADA is deficient in severe combined immunodeficiency (SCID) in humans and it is likely that HPLC could be applied to human embryos, and to single blastomers derived from them. HPLC analysis has already been used to measure HPRT (the activity of which in mouse embryos is much lower than ADA) in single human blastocysts (Leese et al., 1991).

The developmental patterns of HPRT and APRT are in agreement with those reported by Epstein (1970) and Monk (1987). The absolute values are similar to those measured by Epstein, but considerably higher than those reported by Monk. This is probably because the substrate concentrations in the study reported here were high enough to allow the assay to proceed at a maximal rate. This does not seem to be possible in the radiolabel assay of Monk (1987), at least in the case of HPRT. In preliminary work done by us and as reported by Banholzer and Grobner (1983) and Loffler and Grobner (1983), the Michaelis constant for APRT is in the range of 1.4–2.9 µmol l\(^{-1}\), and for HPRT about 8 µmol l\(^{-1}\). The final concentration of hypoxanthine used by Monk (1987) for the HPRT assay was only 10 µmol l\(^{-1}\), a concentration that would not allow the reaction to proceed at its maximal rate.

We suggested that xanthine is the main end product of purine metabolism during preimplantation development in
mice (Alexiou and Leese, 1992). The absence of uric acid from the excretory products leads to the question as to whether xanthine oxidase, the enzyme responsible for the conversion of hypoxanthine to xanthine and of xanthine to uric acid, is present in preimplantation mouse embryos. This question is of interest because of the possible involvement of this enzyme in the formation of oxygen free radicals, which have damaging effects on early mouse embryos (Loutradis et al., 1987; Nasr-Esfahani and Johnson, 1991; Legge and Sellsens, 1991; Noda et al., 1991). Furthermore, we showed (Alexiou and Leese, 1992) that embryos incubated with hypoxanthine produce increasing amounts of xanthine, in a stage-dependent manner. This observation supports the possibility that xanthine oxidase may be present in early mouse embryos in an active form. However, we were unable to detect xanthine oxidase activity in mouse embryos at the blastocyst stage, the stage corresponding to the maximal excretion of xanthine. Lee (1973), who could not detect xanthine oxidase in murine fetal tissues, suggested that the lack of this enzyme and of uricase during embryonic stages may be a reflection of the pattern of nitrogen metabolism (Fisher, 1967).

It is more likely that guanase is responsible for xanthine excretion from exogenous hypoxanthine, since we have shown that xanthine is excreted by mouse embryos in response to guanine treatment (Alexiou and Leese, 1992). In support of this proposition is the high activity of guanase found in the study reported here, and reported by Epstein et al. (1971). However, the significance of such a high activity of a catabolic enzyme, in a period when nucleotide turnover is likely to be rapid, is difficult to explain. As with ADA, guanase seems to be the product of extensive biosynthesis during oogenesis, when the enzyme may fulfill a protective role for the embryo. Thus nucleotides and bases that cannot be completely recycled, owing to insufficient activity of the salvage enzymes, could be broken down by guanase, avoiding the potentially deleterious xanthine oxidase route.

Evidence for the presence of an active purine nucleoside phosphorylase, which catalyzes the reversible conversion of nucleosides to their corresponding bases, during mouse preimplantation development, was provided by Benson and Monk (1988). In the study reported here, the enzyme activity was estimated by conversion of inosine to hypoxanthine. Although it is difficult to explain the significance of the peak in enzyme activity seen at the two-cell stage, the overall pattern from the one-cell stage to the blastocyst was similar to that of HPRT, and might represent a coordination in the activities of the two enzymes which would enhance the activity of the salvage pathway.

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