Glutathione (GSH) is present as the most abundant low molecular weight thiol (LMWT) in virtually all mitochondria-bearing eucaryotic cells, often at millimolar concentrations (Meister, 1988). Functions of GSH include roles in DNA and protein synthesis, maintenance of cell membrane integrity, drug and chemical metabolism, and protection from oxidative stress. The role of GSH in normal reproduction and development, as well as its role in protecting against reproductive toxicants, has been studied extensively, but remains poorly understood. Sources of reducing equivalents other than GSH provide unique as well as redundant functions and include the thioredoxin pathway, superoxide dismutase, catalase, and cysteine. The presence of functional redundancy, as well as the ability of many mammalian cells to tolerate substantial decreases in intracellular GSH, has made studies of the specific roles of glutathione in reproductive and developmental toxicity difficult, and results have been discordant. In the feature article, Beck et al. take a novel and important approach to studying the content and distribution of GSH and cysteine in organogenesis-stage embryos, using acetaminophen (APAP) as a modulator of GSH and mercury orange staining to localize LMWT. Their results provide insight on the redox status and capacity of these embryos, including compartmentalization and intracellular distribution.

Previous studies have demonstrated that APAP is toxic to both preimplantation mouse (Laub et al., 2000) and organogenesis-stage rat embryos (Harris et al., 1989) in vitro but not in vivo. One possibility for this difference is that APAP reduced embryonal GSH in vitro but not in vivo. The study by Beck et al. shows that APAP does indeed lower GSH in embryos in vivo, despite its lack of developmental toxicity.

Thus, it appears that embryos at this stage have a substantial buffer of reducing equivalents in vivo, and that either this buffer is not present in vitro, or the degree of GSH depletion achieved in vitro cannot be attained in vivo. These results serve to highlight redox metabolism as a critical factor in assessing developmental effects, especially when comparing in vivo and in vitro results. Equally important is the intracellular distribution of LMWT. Exposure to APAP did not produce a uniform effect on LMWT, but rather the depletion was tissue-specific and may represent loss of cytoplasmic stores in affected tissues. Beck et al. demonstrate that following APAP treatment, some subcellular LMWT remain, possibly in mitochondria. As the authors point out, differences in the localization of LMWT depletion at the subcellular level may explain why APAP is not teratogenic, while ethanol, which depletes mitochondrial GSH, is embryotoxic and teratogenic (Beck, 2000).

The importance of GSH during development has been well established. In mature mouse and hamster oocytes, GSH concentrations are high, comparable to those in hepatocytes (Meister and Andersen, 1983; Perreault et al., 1988). During fertilization, GSH appears to aid in development of the male pronucleus (Calvin et al., 1986; Perreault et al., 1988; Sutovsky and Schatten, 1997), as well as in oocyte spindle formation (Zuelke et al., 1997). Induction of GSH synthesis in bovine (de Matos and Furnus, 2000) or porcine (Abeydeera et al., 1999) oocytes with beta-mercaptoethanol and cysteine during in vitro maturation improved cleavage rates and embryo development. Conversely, reduced GSH levels contributed to the impaired growth of bovine embryos from oocytes matured in high-glucose media (Hashimoto et al., 2000).

Developmental delay is a consistent observation in mammalian preimplantation embryos grown in vitro. Exposure to atmospheric oxygen tension (~20%) is thought to be a major contributing factor (Wright and Bondioni, 1981); indeed, the maternal reproductive tract has been reported to have reduced oxygen (e.g., about 8% in rabbit and hamster oviduct and 5.3 and 3.5%, respectively, in the hamster and rabbit uterus at the time of implantation [Fischer and Bavis- ter, 1993]). Yet, the use of reduced oxygen tension has produced mixed results. Improved blastulation rates have been reported in the mouse (Gardner and Lane, 1996; Orsi...
and Leese, 2001; Quinn and Harlow, 1978), human (Dumoulin et al., 1999) and domestic ruminants (Olson and Seidel, 2000), but others report no improvement with reduced oxygen tension in mice (Legge and Sellens, 1991; Nasr-Esfahani et al., 1992) or sheep (reviewed by Walker et al., 1992). Murine embryos in culture show a two-cell block under some conditions (Goddard and Pratt, 1983), and this has been attributed at least in part to ROS toxicity (Legge and Sellens, 1991). This may be due to impaired ROS protective mechanisms, as the block can be overcome by the addition of reduced glutathione or other antioxidants. Addition of GSH to culture media also overcomes an 8–16 cell block observed in cultured goat embryos (Lee et al., 2000). Transcripts encoding for antioxidant enzymes including γ-glutamylcysteine synthase (GGCS) as well as glutathione peroxidase and superoxide dismutase have been reported to be present in the oviduct, oocyte, and embryo, and are probably stored during oocyte maturation (Guerin et al., 2001). Stover et al. (2001) found GGCS heavy chain mRNA and protein only at the blastocyst stage in untreated embryos. However, GGCS expression could be up-regulated in two-cell stage embryos by treatment with the oxidizing agent tertiary-butyl hydroperoxide, but not with the GSH-depleting agent diethyl maleate. These results demonstrate the presence of GSH as part of an active antioxidant mechanism in the preimplantation embryo.

Glutathione depletion in cultured postimplantation embryos by L-buthionine-S,R-sulfoximine (BSO) (Slott and Hales, 1987) or diamide (Hiranruengchok and Harris, 1993; 1995) results in embryotoxicity. The generation of mice with a targeted disruption in the gene for the heavy subunit of GGCS (Shi et al., 2000) has unequivocally demonstrated that glutathione synthesis is essential for embryogenesis. Embryos homozygous for this disrupted allele had undetectable GSH at gestation day (gd) 7.5, failed to gastrulate and died by gd 8.5. Examination of embryos at gd 6.5 revealed massive apoptosis. Cell lines derived from homozygous mutant blastocysts grew indefinitely in medium with added GSH, even though the intracellular GSH concentrations in mutant cells under these conditions were only ~2% of control levels. These data again demonstrate the apparent reserve of GSH present in the mammalian embryo.

Many teratogens have been hypothesized to act via the generation of free radicals. Some teratogens are thought to be activated by embryonic cytochromes P450, prostaglandin H synthase, and lipoxygenases to electrophilic or free radical reactive intermediates. Examples of such teratogens include the anticonvulsant phenytoin and its derivatives, benzopyrene, and thalidomide (Wells et al., 1997). The teratogenicity of phenytoin is exacerbated by acetaminophen pretreatment (Lum and Wells, 1986), supporting a protective role for glutathione. Species differences in the teratogenicity of thalidomide between rabbits (sensitive) and rats (insensitive) have been linked to higher GSH concentrations in the rat visceral yolk sac (VYS) compared to that of the rabbit, and depletion of VYS GSH by thalidomide only in the rabbit (Hansen et al., 1999). Thalidomide also preferentially depleted GSH in the rabbit embryo compared to the rat embryo. That ROS play a role in thalidomide teratogenesis is further supported by the findings of Parman et al. (1999) that thalidomide causes DNA oxidation and teratogenesis in rabbits but not mice, and that a spin-trapping agent abolishes the effects in rabbits. A role for ROS in diabetic embryopathy has also been proposed (Eriksson and Borg, 1991, 1993), and Trocino et al. (1995) reported that growth retardation and induction of malformations in rat embryos grown in hyperglycemic media could be counteracted by the addition of GSH. Cells from embryos grown in hyperglycemic media had lower GSH content and GGSC activity.

So, it appears that GSH is important for reproduction and development at many if not all stages, and that under normal conditions there is often a surplus of GSH present. However, various stressors can deplete GSH or decrease the ratio of GSH to GSSG. While such effects may result in teratogenesis, experience to date suggests that they are more likely to increase the sensitivity of the embryo to a variety of xenobiotics. Maternal treatment with APAP in the highlighted article by Beck et al. resulted in significant reductions in GSH and cysteine, but the magnitudes of these effects (to about half the level of controls in the worst cases) are probably insufficient to be toxic per se. In order to study interactions with other toxicant exposures, the ability to reduce the GSH status of the embryo predictably in vivo or in vitro is an important tool. Ability to interpret results will also be improved by understanding not just the overall GSH and LMWT content of the embryo, but also their regional distribution. The approach of Beck et al. allows one to deplete glutathione using a nonteratogenic dosage of APAP in vivo, and to follow changes in LMWT in specific regions of the embryo. Their results with mercury orange staining demonstrate that the eutoplacental cone and visceral yolk sac have the highest concentration of LMWT, and these tissues are the closest to the maternal/embryonic interface. Treatment with APAP decreased tissue LMWT, except in the eutoplacental cone, while LMWT increased in exocelomic fluid and in embryonic lumina. Their results further suggest that specific subcellular compartments may selectively retain LMWT in the face of APAP exposure, although the identity of these compartments remains to be determined.

REFERENCES


