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INVITED REVIEW

METABOLIC AND MITOTIC CHANGES ASSOCIATED WITH THE FETAL ALCOHOL SYNDROME

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Abstract — In the USA, fetal alcohol syndrome (FAS) is the leading known cause of mental retardation. FAS is estimated to affect 4000 infants yearly in the USA with an additional 7000 children suffering various forms of fetal alcohol effects in the absence of the full syndrome. A comparable incidence would be expected in other industrialized countries, but essentially no data are available from either developing or third world countries. An understanding of the biochemical causes of FAS has been slow to develop, but progress has been made toward a molecular causation theory of FAS. This paper summarizes much of the current work as to the effects of fetal ethanol exposure on mitotic and metabolic parameters as well as ethanol's effect on the cellular signalling pathways thought to regulate these processes. Based upon these studies, it is apparent that exposure of embryonic tissue to ethanol results in decreased growth and that alcohol adversely affects a multitude of cellular functions critical for the growth of the developing organism, including inhibition of protein and DNA synthesis. In addition, ethanol alters the uptake of critical nutrients such as glucose and amino acids and causes changes in several kinase-mediated signal transduction pathways that regulate these biochemical processes.

INTRODUCTION

Fetal alcohol syndrome (FAS) results from *in utero* exposure to ethanol. Though much research has been done in the 25 years since the name FAS was coined, much remains to be accomplished. This paper brings together data on the cellular changes that ethanol elicits in a developing embryo, including ethanol's effect on DNA synthesis, protein synthesis, glucose uptake, amino acid uptake and on the kinase signalling pathways that regulate these processes. Growth inhibition is the most common defect resulting from fetal alcohol exposure (Lochry *et al.*, 1980; Sulik *et al.*, 1981; Pennington *et al.*, 1983; Abel, 1985; Gallo and Weinberg, 1986; Pennington, 1988*a*; Goodlet *et al.*, 1989; Pennington *et al.*, 1995; Shibley and Pennington, 1995) and intrauterine growth retardation is a strong predictor of fetal outcome. Thus, ethanol-induced growth retardation and the molecular mechanism(s) by which the growth retardation occurs have been the focus of much recent work.

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CELL DIVISION/GROWTH

DNA synthesis

Numerous studies have described the effects of ethanol on DNA synthesis in cultured cells. For example, a study (Weston *et al.*, 1994) of ethanol-induced changes in craniofacial growth using embryonic rat palate mesenchymal cells exposed *in vitro* to ethanol (200 mM) for 24 h found a marked reduction in [^{3}H]thymidine incorporation. Adickes *et al.* (1993) measured the rates of DNA synthesis in cardiac myocytes from 1–2-day-old rats exposed to ethanol for either 7 continuous days in culture or only during a 24 h period of hyperplastic growth. [^{3}H]Thymidine incorporation was decreased by ethanol during the first 3 days of exposure, but uptake was not significantly different during the last 4 days. Thus, the data suggest

that the early hyperplastic growth period of these cells is the period of greatest susceptibility to ethanol-induced decrease in DNA synthesis.

Astrocytes cultured from 21-day rat fetuses and grown in primary culture have also been used to assess the effects of ethanol on DNA synthesis and cellular proliferation (Guerri *et al.*, 1990). Astrocytes from animals exposed to ethanol *in utero* showed decreased [^{3}H]thymidine incorporation and decreased cellular proliferation. Cultured cells from control embryos exposed to ethanol *in vitro* experienced a similar degree of inhibition of [^{3}H]-thymidine incorporation (Guerri *et al.*, 1990). Concentrations of ethanol $\leq$50 mM resulted in no change in [^{3}H]thymidine incorporation (Snyder *et al.*, 1992*b*).

Ethanol not only decreases basal DNA synthesis in most cells, but also inhibits growth-factor-stimulated DNA synthesis. Balb/c 3T3 and p6 cells (Balb/c 3T3 cells overexpressing the IGF-I receptor) both showed inhibition of IGF-I-induced proliferation in the presence of 10–150 mM ethanol (Resnicoff *et al.*, 1993). Even at concentrations of IGF-I as high as 60 ng/ml, the ethanol-treated parental Balb/c 3T3 cells showed essentially no growth response to IGF-I. The ethanol-treated p6 cells did respond to IGF-I, but they required increased concentrations of IGF-I to do so and never reached the growth rate of the control p6 cells (Resnicoff *et al.*, 1993).

Epidermal growth factor (EGF)-mediated proliferation has been demonstrated to be inhibited by ethanol in cultured fetal rat hepatocytes (Henderson *et al.*, 1989, 1991). Hepatocyte replication is decreased after 24 h in ethanol-containing media (Henderson *et al.*, 1989, 1991) and DNA synthesis is decreased by ethanol after 20 h (Henderson *et al.*, 1991). In primary rat hepatocyte cultures from adult rats that had been given ethanol-containing liquid diets for 8 weeks, EGF-stimulated DNA synthesis was significantly decreased after 2 days in culture (Bhavani *et al.*, 1993). The inhibition of EGF-mediated DNA synthesis by ethanol became more pronounced with time in culture. Thus, whether cells were treated *in situ* with ethanol or cultured from *in vivo* treated hepatic tissue, EGF-mediated DNA synthesis was significantly decreased.

One recent report did find that NIH 3T3 fibroblasts experienced significantly enhanced insulin-stimulated [^{3}H]thymidine incorporation after incubation in ethanol-containing media (10–150 mM) for 24 h (Tomono and Kiss, 1995). However, the rat astrocyte study discussed above (Snyder *et al.*, 1992*b*) demonstrated that [^{3}H]-thymidine incorporation may not be changed by low doses of ethanol in some cells. However, the majority of studies support the idea that ethanol exposure will decrease the DNA synthesis of the developing embryonic cells.

An enzyme known to be critical for normal cellular division is ornithine decarboxylase (ODC). Quiescent cells that are stimulated to divide show increased levels of ODC activity prior to any measurable increase in cellular DNA, RNA, or protein synthesis (Russell and Snyder, 1968). Also, an increased rate of DNA synthesis has been correlated with increased ODC activity (Thadani, *et al.*, 1977; Janne *et al.*, 1991). Ethanol decreases ODC activity in embryonic chick cells (Sandstrom *et al.*, 1993) suggesting a mechanism by which ethanol inhibits cellular growth. The effects of ethanol on ODC activity and polyamines have recently been reviewed (Shibley *et al.*, 1995) and thus will not be considered further here.

Protein synthesis

Kennedy (1984) proposed an integrating hypothesis to explain the effects of ethanol on the developing fetus. The growth deficiency associated with FAS could be most directly explained, according to Kennedy, by a decrease in protein synthesis. The proposed hypothesis included several citations of ethanol-induced reductions in protein synthesis in fetal tissue (Rawat, 1975, 1976; Brown *et al.*, 1979; Wunderlich *et al.*, 1979; Dreosti *et al.*, 1981). Other studies following Kennedy's review have supported the idea that ethanol exposure reduces protein synthesis in most cells.

Unlike [^{3}H]thymidine incorporation, which serves as a universal indicator of DNA synthesis, protein synthesis rates can be measured using a variety of radiolabelled amino acids (Rawat, 1985; Guerri *et al.*, 1990; Snyder *et al.*, 1992*b*; Siddiq *et al.*, 1993*a*,*b*). Ethanol-exposed rat embryo brain protein synthesis was measured both *in vivo* and *in vitro* by Rawat (1985). Both synthesis rates were significantly reduced in ethanol-exposed embryonic brains. Insulin-stimulated protein synthesis has also been shown to be inhibited by ethanol (Snyder *et al.*, 1992*b*). Additional studies (Siddiq

et al., 1993*b*) demonstrated that ethanol decreased the rate of protein synthesis in the ventricular mitochondria of exposed rats.

Recent reviews by Preedy and Richardson (1994) and Preedy *et al.* (1994) summarize the results of many studies as to the effect of ethanol on cardiac protein synthesis. Protein synthesis in the rat heart was found to be inhibited by ~20% by acute ethanol exposure (Preedy and Peters, 1990; Siddiq *et al.*, 1993*a*). Many studies (Wallin and Mørland, 1987; Renau-Piqueras *et al.*, 1989; Coleman and Cunningham, 1991) have also reported impaired protein synthesis in the liver caused by ethanol, but an *in vivo* study (Donohue *et al.*, 1987) found no changes in the protein synthetic capabilities as the result of either acute or chronic ethanol exposure. Relatively few studies of the effects of ethanol exposure *in utero* on protein synthesis have been undertaken.

CELLULAR METABOLISM

Glucose uptake

The transport of glucose across the plasma membrane of the cell is mediated by a family of facilitative transporter proteins called glucose transporters. An explosion of information has been accruing on these transporters as evidenced alone by the number of reviews appearing in the 1990s. Not only have descriptive reviews been published (Gould and Bell, 1990; Lienhard *et al.*, 1992; Bell *et al.*, 1993; Mueckler, 1994), but reviews on the regulation of glucose transporters (Jones, 1991; Czech *et al.*, 1992; Pessin and Bell, 1992; Merrall *et al.*, 1993; White and McCubrey, 1995) and the similarities between glucose transporters and other transporters (Fischbarg and Vera, 1995) have also appeared. Glucose transporter 1 (glut1) serves as the primary glucose transporter of fetal tissue and cultured cells. Glut1 has been found in all stages, and in nearly all tissues, of early mouse embryos (Hogan *et al.*, 1991; Aghayan *et al.*, 1992).

Fetal glucose levels have been demonstrated to be a significant factor in normal embryonic growth. The rate of transfer of glucose across the placenta increases during embryonic growth spurts (Rosso, 1975). If prolonged maternal hypoglycaemia is induced in rats, intrauterine growth retardation results (Gruppuso *et al.*, 1981; Nitzan, 1981) with a concomitant decrease in embryonic glucose levels. Thus, the limitation of fetal glucose appears to be a significant cause of intrauterine growth retardation. Prolonged maternal fasting of a rat has also been associated with low blood glucose in the fetus (Girard *et al.*, 1977). It may therefore be concluded 'that changes in glucose transport modulation might contribute to the development of asymmetric growth retardation and that the maintenance of normal transporter function and expression in brain may play a role in sparing its growth.' (Simmons *et al.*, 1993). Despite the critical importance of glucose uptake to normal embryonic development, few studies have been published on the modulation of specific glucose transporters by ethanol in the developing embryo.

Regulation of glucose uptake by growth factors and other mitogens occurs by at least three mechanisms: (1) redistribution of existing transporter proteins; (2) increased transporter activity; (3) altered synthesis or degradation of transporter proteins. Ethanol could theoretically affect any, or all, of these processes. Surprisingly, the alteration of glut1 expression by ethanol exposure has received attention mostly in the adult. For example, one recent study reported that rats exposed to ethanol for 4 weeks had higher levels of glut1 protein in their brains, but a decreased level of glut1 mRNA in the brain (Singh *et al.*, 1993). Another study found increased glut1 expression in the liver of rats exposed to ethanol for a month (Hagman *et al.*, 1993). A further study of human lymphocytes monitored glucose uptake after only 4 min of exposure to ethanol. In these cells, ethanol caused a dose-dependent decrease in glucose uptake (Krauss *et al.*, 1994). In the same study, ethanol inhibited glucose uptake in Chinese hamster ovary (CHO) cells overexpressing glut1.

Chronic alcoholic mothers suffer from undernutrition and therefore would be expected to experience impaired glucose levels which might, in turn, lower those in fetal blood. Rats given ethanol-containing diets had fetuses with significantly lower blood glucose levels, although maternal hypoglycaemia did not occur (Singh *et al.*, 1986). This result suggests that ethanol has a direct effect on glucose uptake in fetal tissue. The direct effect of ethanol may exacerbate the decreased fetal glucose levels caused by ethanol-induced maternal undernutrition.

Several studies have reported that maternal ethanol exposure inhibits the uptake of glucose by fetal tissue (Tanaka *et al.*, 1982; Singh *et al.*, 1988, 1989, 1992; Snyder *et al.*, 1992*a*). Ethanol also appears to inhibit glucose uptake by the placenta (Snyder *et al.*, 1986). The mechanism of the ethanol-induced decrease in glucose uptake has received little attention. One study on the effect of prenatal ethanol exposure on glut1 expression in rat brain (Singh *et al.*, 1992) reported a 50% decrease in glut1 mRNA and a direct correlation between the rate of glucose uptake and brain weight. Further studies on the regulation of glucose transport in the developing fetus are therefore warranted.

Amino acid uptake

Similar to the transport of glucose, the transport of amino acids into cells has been extensively studied and reviewed (Guidotti *et al.*, 1978; Lerner, 1985; Van Winkle, 1988, 1993; Christiansen, 1989; Kilberg *et al.*, 1993; McGivan and Pastor-Anglada, 1994). Though a multitude of amino acid transport systems have been identified, system A has received the most attention. System A exhibits broad reactivity toward amino acids with short, polar, or linear side chains. The system is dependent upon, and energized by, Na^+. The alanine analogue, (*N*-methylamino)-α-isobutyric acid (AIB), has been used widely to categorize this system.

Amino acid transport by both placental and embryonic tissue is critical for normal development (Jones and Rolph, 1985). Amino acids in the embryo are used predominantly for protein synthesis and the dysfunction of placental amino acid transport has been linked to intrauterine growth retardation (Moe, 1995). Thus, small for gestational age human babies have lower umbilical veno-arterial concentration differences for most essential amino acids (Bell *et al.*, 1986), suggesting that the placenta is not properly transporting essential amino acids to the growth-retarded fetuses.

A recent report showed that microvillous membrane vesicles of the placental syncytiotrophoblasts transported AIB 63% less in placental vesicles of small for gestational age babies, compared with the vesicles from the placentas of appropriate size for gestational age infants (Yasuda *et al.*, 1990). This finding implicates disruption of system A as a possible cause of ethanol-induced intrauterine growth retardation. Ethanol had little effect on placental AIB uptake in sheep (Fisher *et al.*, 1981*a*), but lowered AIB uptake by 35% in rats (Snyder *et al.*, 1986). In other rat studies, ethanol exposure *in vivo* inhibited placental amino acid uptake (Fisher *et al.*, 1981*a*,*b*; Henderson *et al.*, 1981, 1982*a*,*b*; Lin, 1981). The same effect was seen in a non-human primate model (Fisher *et al.*, 1983).

A study of human placental tissue found that ethanol exposure *in situ* had virtually no effect on amino acid uptake (Schenker *et al.*, 1989). The authors concluded that human placenta is resistant to the effects of ethanol on amino acid transport. More recent data on cultured human trophoblasts repeated the early finding by demonstrating unchanged amino acid uptake in cells pre-treated for 72 h with ethanol (Karl and Fisher, 1994). Though the basal uptake was unaltered, the hormone-stimulated amino acid uptake was significantly decreased by ethanol exposure (Karl and Fisher, 1994). Both insulin- and IGF-I-stimulated, Na^+-dependent AIB uptake were decreased in the ethanol-treated cells. The investigators found insulin and IGF-I binding to the trophoblasts unchanged, suggesting a downstream block in the signal transduction pathway.

SIGNAL TRANSDUCTION

Protein kinase C (PKC)

Although PKC activity has been recognized for less than 20 years (Inoue *et al.*, 1977; Takai *et al.*, 1977), PKC's enzymatic activity has now been determined to arise from at least 12 isoforms and reviews of PKC have been appearing regularly since the original discovery (Nishizuka, 1984; Blumberg, 1988; Kikawa *et al.*, 1989; Stabel and Parker, 1991; Hug and Sarre, 1993; Dekker and Parker, 1994). PKC has been suggested to regulate many cellular parameters. To illustrate, activation of PKC via treatment with a phorbol ester increases glucose transport (Henriksen *et al.*, 1989; Dwivedi *et al.*, 1994) and ODC activity (Groblewski *et al.*, 1992). Furthermore, PKC isoform distribution has been suggested to account for some of the genetic differences in ethanol sensitivities between strains of mice (Balduini *et al.*, 1994). Ethanol's effect on PKC appears to

depend on the duration of ethanol exposure and on the cell type being studied. Suggested molecular targets of ethanol include several of the proteins regulating PKC activity, e.g. phospholipase C (PLC) and G-proteins as well as the PKC protein itself (Hoek and Rubin, 1990). Ethanol has also been suggested to cause a desensitization of PLC via a PKC-dependent mechanism in isolated rat hepatocytes (Hoek and Higashi, 1991). Additional studies also suggest that short-term ethanol exposure causes an increase in PKC activity, but that longer exposure will result in down-regulation of PKC due to the chronic activation of PKC. To illustrate, treatment of LRM55 astroglial cells with ethanol for 30 s induced PKC translocation from the cytosol to the membrane, leading to a 100% increase in membrane PKC activity (Skwidh and Shain, 1990). Other studies have also suggested that short-term ethanol exposure leads to an increased PKC activity (DePetrillo and Liou, 1993; Kharbanda *et al.*, 1993; Sanna *et al.*, 1994). A study designed to explore the effects of chronic ethanol exposure used rats fed an ethanol-containing diet for 25 days (Battaini *et al.*, 1989) as a model. These rats exhibited decreased brain PKC. However, even though chronic ethanol exposure may decrease PKC activity in most cells, work by Messing *et al.* (1991) suggested that, in neurite-derived PC12 cells, ethanol exposure for 2–8 days increased PKC activity. Two PKC isoforms, PKC-ε and PKC-δ, were shown to be elevated by ethanol (Messing *et al.*, 1991) and the increase augmented signal transduction via pathways involving these two PKC isoforms (Roivainen *et al.*, 1995) as well as increasing neurite outgrowth (Roivainen *et al.*, 1993).

cAMP-dependent protein kinase A (PKA)

PKA becomes activated through the binding of cyclic AMP to the kinase regulatory subunit protein. Cyclic AMP synthesis is stimulated by an extracellular hormonal signal being transmitted to adenylate cyclase via a standard G-protein-mediated mechanism. Activation of PKA by changes in cAMP levels appears to cause differential effects. For example, increased cAMP levels have been shown to stimulate (Rozengurt, 1986; Dumont *et al.*, 1989) or to inhibit (Nilsson and Olsson, 1984; Blomhoff *et al.*, 1987; Magnaldo *et al.*, 1989) cellular proliferation in different cells.

Just as the effect of cAMP on growth seems dependent on cell type, the effect of ethanol on cAMP levels also seems dependent on cell type and ethanol dose. Thus, ethanol exposure for 1 h has been reported to increase adenosine receptor-stimulated cAMP levels in a dose-dependent manner in NG108-15 neuroblastoma–glioma hybrid cells (Gordon *et al.*, 1986) and a 5 min exposure of platelet-rich plasma from Sprague–Dawley rats to various concentrations of ethanol increased cAMP levels (Hwang *et al.*, 1987). A recent report on Wistar rat hepatocytes found that 25–50 mM ethanol treatment *in situ* decreased cAMP levels, but that 100–200 mM ethanol actually increased cAMP levels (Nagy, 1994). Forty-eight hour exposure of primary rat hepatocyte cultures to ethanol caused an increase in agonist-stimulated cAMP levels (Nagy, 1994). Glucagon and forskolin exposure both caused a much larger increase in cAMP levels in ethanol-exposed hepatocytes. Treatment with ethanol for periods longer than 16 h of cultured human placental trophoblasts resulted in an increased sensitivity to adrenaline-stimulated increases in cAMP levels (Karl *et al.*, 1994). Receptor-stimulated increases in cAMP levels were inhibited in PC-12 cells exposed to 150 mM ethanol for 4 days (Rabin, 1990; Rabin *et al.*, 1993). Sprague–Dawley rats given ethanol for 6 days had decreased cAMP levels in all brain areas, but the cAMP levels returned to normal when the ethanol was withdrawn (Shen *et al.*, 1983).

The most striking evidence for cell-specific changes in cAMP levels due to ethanol exposure comes from a report by Rabe *et al.* (1990). In two subclones of PC-12 cells, isolated membrane preparations exhibited increased basal and agonist-stimulated cAMP levels after a 5 min ethanol exposure. However, when intact cells were studied, one subclone displayed ethanol-induced inhibition of receptor-stimulated cAMP increases, whereas the other subclone exhibited stimulation. The conclusion from the above study suggests caution: 'The results indicate that extrapolation of the effects of ethanol from one cell type to another, or from *in vitro* to *in vivo* systems, may be complicated by the interaction of ethanol with regulatory processes that influence second messenger systems, and can differ in various types of intact cells.' (Rabe *et al.*, 1990).

In addition to cAMP levels, other components

of the signalling system involved in the activation of PKA have been studied, including PKA itself, G-proteins, and adenylate cyclase. For instance, chronic ethanol exposure has been shown to decrease the receptor-stimulated adenylate cyclase activity (Rabin, 1990, 1995), but rats exposed to ethanol for 8 weeks were found to have increased hepatic adenylate cyclase activity (Blumenthal *et al.*, 1991). When the mechanisms of ethanol-induced changes in adenylate cyclase activity, and hence of cAMP production, were investigated, the same type of disparate results were seen. Chronic ethanol exposure decreased the quantity of inhibitory G-protein (G_i) in rat hepatocytes (Nagy and DeSila, 1992), but enhanced the expression of G_i-proteins in the brains of mice (Wand *et al.*, 1993). In rats with a partial hepatectomy, chronic ethanol exposure inhibited expression of G_s-protein and, therefore, cAMP accumulation (Diehl *et al.*, 1992). Likewise, in PC-12 cells, 7 day ethanol exposure caused a decrease in the membrane levels of G_s (Rabin, 1993).

The effects of ethanol on PKA in a developing embryo have been explored in the chick model. The responsiveness of brain adenylate cyclase to stimulation by prostaglandins was inhibited by ethanol (Pennington, 1988*b*) and cAMP levels were found to be decreased by ethanol exposure (Pennington, 1990). Both basal and cAMP-stimulated autophosphorylation of the regulatory subunit of PKA (RII) were significantly lowered by ethanol exposure (Beeker *et al.*, 1988). The binding of cAMP to RII was also inhibited by ethanol (Pennington, 1988*b*). These studies suggest that ethanol acts by decreasing the ability of RII to bind cAMP which results in the loss of catalytic activity of PKA.

Insulin signalling pathway

The insulin signalling pathway directly involves insulin-dependent tyrosine kinase activity. Following the activation of insulin-dependent tyrosine kinase, the pathway has many branch points. Because of the ability of insulin to regulate cell growth, division and metabolism, numerous studies have examined the involvement of insulin in the early growth of organisms. Work has been done using fetal rats (Akashi *et al.*, 1991; Simmons *et al.*, 1993), fetal mice (Spaventi *et al.*, 1990), *Xenopus* oocytes (Chuang *et al.*, 1993), chick (De Pablo *et al.*, 1982, 1985, 1990; Bassas *et al.*, 1987, 1988, 1989) and sea urchin embryos (Kuno *et al.*, 1994).

Ethanol has varied effects of the cellular response to insulin, which could adversely alter fetal growth. In adults, ethanol has been suggested to cause insulin resistance in peripheral tissue (Boden *et al.*, 1993) and is known to inhibit insulin-induced insulin receptor substrate-1 (IRS-1) phosphorylation (Xu *et al.*, 1995) and IGF-I-induced IRS-1 phosphorylation (Resnicoff *et al.*, 1994) in cultured cells. In another cell model, ethanol added concomitantly with IGF-I inhibited the IGF-I-induced increases in transcription of c-myc, c-fos, and c-jun (Resnicoff *et al.*, 1993). A similar result was observed in rat hepatocytes during liver regeneration, where decreased IRS-1 phosphorylation and decreased phosphoinositol-3 kinase (PI-3) activity occurred due to ethanol exposure (Sasaki and Wands, 1994). Few studies have explored the insulin signalling pathway in fetal tissue. In humans, fetal alcohol exposure may result in insulin resistance in the adult (Barbanti *et al.*, 1987), and fetal ethanol-induced insulin resistance was also detected in an animal model (Gilani and Persaud, 1986; Villarroya and Mampel, 1985).

Thus, even though the effects of ethanol on fetal development have now been documented in many different tissues and cell types, an understanding of the ramifications of these changes will be necessary. Hoek and Rubin (1990) succinctly state the challenge for researchers as follows: 'To go beyond the phenomenology and develop a mechanistic understanding of the actions of ethanol on cellular control processes in different tissue will continue to be a major challenge to investigators in the field of alcoholism.'

CONCLUSIONS AND COMMENTS

Ethanol can adversely affect a multitude of cellular functions associated with fetal development including such functions as protein synthesis, DNA synthesis (thymidine uptake), glucose uptake, and amino acid uptake. The signalling pathways mediated by PKC, PKA, and the insulin-dependent tyrosine kinase are important in regulating these functions and are all affected by ethanol exposure. Thus, though much information has been generated on the kinase pathways

themselves, relatively little is known about the specific molecular effects of ethanol on these kinase pathways. Because each pathway is involved in cell growth and differentiation, further study of ethanol's effect on these pathways in embryonic cells is needed.

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