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Teratogenic Actions of Ethanol in the Mouse: A Minireview

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BECKER, H. C., J. L. DIAZ-GRANADOS AND C. L. RANDALL. *Teratogenic actions of ethanol in the mouse: A minireview.* PHARMACOL BIOCHEM BEHAV **55**(4) 501–513, 1996.—The deleterious effects of prenatal ethanol exposure have been extensively documented in clinical and experimental studies. This paper provides an overview of work conducted with mice to examine the myriad of adverse consequences that result from embryonic/fetal exposure to ethanol. All of the hallmark features of the clinical fetal alcohol syndrome have been demonstrated in mice, including prenatal and postnatal growth retardation, structural malformations and behavioral abnormalities associated with central nervous system dysfunction. As expected, the severity and profile of effects is related to both dosage level and timing of exposure. In addition, these effects have been demonstrated following acute and chronic exposure, with a variety of routes of administration employed. Furthermore, a number of strains have been used in these studies and the variant response (susceptibility) to the teratogenic actions of ethanol exhibited among different mouse strains support the notion that genetic factors govern, at least in part, vulnerability to these effects of ethanol. More recent studies using mouse models have focused on examining potential mechanisms underlying the full spectrum of ethanol's teratogenic actions. **Copyright © 1996 Elsevier Science Inc.**

Ethanol teratogenesis Mouse Fetal alcohol syndrome Animal models

THE teratogenic properties of ethanol have been firmly established in the clinical and experimental literature [for reviews see (79,109)]. Research with animals has significantly contributed to our current knowledge about the wide array of deleterious effects of ethanol on fetal growth and development (12). In particular, animal studies have provided unequivocal evidence of the teratogenic potential of ethanol by controlling for numerous confounding factors that are commonly associated with chronic alcohol use, such as malnutrition, poor environmental conditions, disease, smoking and other drug use. In addition, experimental studies conducted with laboratory animals have allowed for rigorous control over variables such as dose and pattern of ethanol consumption and duration of exposure. This control has led to a better understanding of the relationship between fetal ethanol exposure and teratogenic consequences. Furthermore, animal studies have begun to evaluate the relative roles of maternal variables, environmental setting and genetic factors that govern susceptibility to prenatal ethanol effects (5,110).

In addition, the use of animal models has allowed for the investigation of mechanisms underlying the teratogenic actions of ethanol (87,98,123). For example, studies have begun to elucidate perturbations in brain systems that may mediate observed behavioral abnormalities. Similarly, animal models have been used to examine biochemical events underlying the structural (morphologic) teratogenic effects of ethanol. Such studies would be obviously limited, if not unfeasible, in human subjects. Of course, the value of these investigations aimed at elucidating etiologic factors in ethanol-related embryopathology is the hope that results will contribute to the development of clinically relevant therapeutic interventions or prevention strategies.

Finally, the general validity of animal models is underscored by the fact that most of the deleterious effects of prenatal ethanol exposure observed in animals have been identified following maternal ethanol exposure (blood ethanol levels) that approximate typical levels of exposure reported in pregnant alcohol-consuming women (31). Moreover, as observed clinically, the adverse effects of in utero ethanol exposure in animal models have been shown to exist on a continuum,

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ranging from gross morphological defects at one extreme to more subtle cognitive/behavioral dysfunction at the other. These dysfunction effects have established ethanol as a behavioral teratogen. Importantly, animal research has demonstrated that the particular constellation of resultant defects is related to a variety of factors including the dose of exposure, pattern of exposure (and corresponding blood ethanol levels) and the timing of the pharmacologic insult as it relates to stage of embryonic/fetal development (5,79,109).

Although the teratogenic effects of ethanol have been demonstrated in different species including fish, chickens, mice, rats, guinea pigs, ferrets, dogs, pigs and subhuman primates, rodents have been the most commonly studied species (116). In this regard, it is interesting that the mouse has been the primary species used for investigations involving high ethanol exposure-related morphologic damage, whereas the rat has been the primary subject used in studies addressing the behavioral teratogenic actions of ethanol. However, more recent studies have shown that the mouse model may exhibit the full spectrum of prenatal ethanol effects, ranging from dysmorphology to behavioral deficits in the absence of physical anomalies and as such may best model the clinical condition (11). A growing body of literature has emerged using mouse models in investigations involving several levels of analyses including molecular, biochemical, physiological and behavioral (4). This report will focus on work conducted with mice investigating the morphological and neurobehavioral teratogenic actions of ethanol.

This paper is not intended to provide an exhaustive review of all work that has been conducted investigating the teratogenic effects of ethanol in mice. Rather, it represents a selective overview of studies demonstrating the range of effects of ethanol on the developing mouse embryo/fetus, with particular emphasis on outcomes related to exposure at specific discrete periods of embryonic and fetal development.

The gestation period for the mouse is approximately 19 days (range = 18–20 days). Detailed information on the prominent features of mouse embryonic and fetal development may be found in reviews by Rugh (94) and Theiler (108). Although the last human trimester equivalent corresponds to the first 1–2 weeks of postnatal life in the mouse (particularly with regard to brain development), very few mouse studies have examined the effects of ethanol delivered to neonatal mouse pups in a controlled fashion. Some innovative experimental strategies have been employed to examine the consequences of third-trimester-equivalent ethanol exposure in mice (77). However, given the general paucity of such studies involving postnatal ethanol exposure in mice, studies reviewed in this report have been restricted to those involving prenatal exposure to ethanol.

The teratogenic actions of ethanol have been documented in mice following acute and chronic gestational exposure. For uniformity in reporting study results, the day of conception has been designated as gestation day 0 (GD 0) in this review. In addition, for organizational purposes, studies involving acute exposure (1 or 2 days) are described separately from those employing chronic gestational exposure (3 or more days). Furthermore, the effects of acute ethanol exposure on the developing embryo and fetus are discussed within the framework of three stages of embryonic development: the preorganogenic, organogenic and postorganogenic periods (116). This division of the gestation period is somewhat arbitrary. Nevertheless, this represents a convenient strategy because each stage of pregnancy encompasses distinct aspects of embryonic/fetal growth and development; furthermore, these stages of pregnancy may not be equally vulnerable to the damaging effects of ethanol.

Studies involving chronic gestational ethanol exposure have been partitioned into those focusing on morphologic development and growth, behavioral effects and immune system function. In many cases, several endpoint measures were reported. Thus, the categorization of these studies was determined by the most salient features of the results.

ACUTE ETHANOL EXPOSURE

Preorganogenic Period

The preorganogenic period of embryonic development in mice roughly constitutes the first 6 days of gestation (from fertilization to implantation). This period corresponds to approximately the first 2 weeks of human pregnancy. This stage of embryogenesis is initiated by fertilization and the resultant formation of a zygote (fertilized ovum). The conceptus then enters a period of cleavage, marked by a rapid succession of mitotic cell divisions in which the size of the embryo does not change, but the size of the cleavage cells (blastomeres) decreases with each division. By the third day of gestation, the conceptus appears as a morula, or solid spherical mass of blastomeres. By the fourth day of development, some of the blastomeres become internalized while others form an enveloping layer. This marks the stage of blastulation, or the formation of a blastocyst (blastula). The blastocyst is characterized as a hollow spherical body with an outer layer of cells (blastoderm) surrounding a cavity (blastocoele). The enveloping cells become the trophoblast layer that is critical in the attachment of the embryo to the uterine wall (implantation). Implantation begins approximately 4.5 days following fertilization and is complete by the sixth day of gestation. As the embryo is being implanted in the uterine wall, the blastocyst undergoes the process of gastrulation. During gastrulation three primary germ layers are formed: the endoderm, mesoderm and ectoderm. The inner cell mass nearest to the blastocoele splits off to form the endoderm, and the remaining cells form the ectoderm. The mesoderm is the last germinal cell layer to be formed in the mouse embryo.

The effects of ethanol during the preimplantation phase of pregnancy has not been extensively studied possibly because the preorganogenic stage of pregnancy has been sometimes viewed as being the least sensitive to the teratogenic effects of ethanol because exposure during this period typically results in spontaneous abortion or resorption of the embryo. However, this result may be also considered the most extreme adverse effect of gestational ethanol exposure because it culminates in embryonic death and pregnancy termination. In one study, MF1 mice were treated with approximately 5.8 g/kg ethanol (IP) on GD 1, GD 2, GD 3, GD 4, GD 5, or GD 6 (73). Interestingly, ethanol did not influence litter size (implantation), but there was a marked increase in prenatal mortality (resorptions). Furthermore, ethanol exposure during this period of gestation produced malformations in 80–100% of the viable fetuses. Along with this increased incidence of structural anomalies, ethanol exposure on each of the first 6 days of gestation was associated with increased placental weight, decreased umbilical cord length and decreased fetal weight at GD 15. Although it is somewhat surprising that such early exposure is capable of producing a number of malformations (craniofacial, eye, urogenital, limb) in the affected offspring, these results suggest that exposure to ethanol during the preimplantation stage of pregnancy can be teratogenic

TABLE 1

SUMMARY OF EFFECTS OF ACUTE PRENATAL EXPOSURE ON MORPHOLOGIC DEVELOPMENT IN MICE

Type of Malformation	Ethanol Administration	Mouse Strain	Critical Period	References
Craniofacial	IP	C57BL/6J, B6D2/J, CD-1, CF-1, MF1	GD7–GD9	15, 23, 35, 59, 60, 61, 71, 74, 75, 104, 105, 106, 118, 119
Brain	IP	C57BL/6J, LACA, TO, CD-1, CF-1, B6D2, MF1	GD7–GD8	2, 3, 15, 23, 59, 74, 75, 76, 97, 99, 100, 107, 118, 119
Ocular	IP	C57BL/6J, B6D2, MF1	GD7–GD10	1, 24, 78, 119
Cardiovascular	IP	C57BL/6J, CD-1	GD8–GD10	15, 27, 116, 117
Urogenital	IP, IG	C57BL/6J, B6D2/J, CD-1, CF-1	GD9–GD10	15, 16, 23, 40, 47, 51, 61, 80, 81, 82, 83, 84, 85, 90
Skeletal (limb)	IP, IG	C57BL/6J, B6D2, BALB/c, CD-1, CF-1, MF1	GD9–GD11	15, 23, 47, 51, 58, 61, 74, 75, 80, 81, 82, 83, 84, 85, 90, 103, 118, 119, 129

and embryolethal. In contrast to these results, another study found that ethanol exposure (2.5 and 5.0 g/kg, IG) on GD 6 did not have a negative impact on pregnancy outcome in C3H/He mice (63). This discrepancy in results may be related to different variables including mouse strain and route of ethanol administration. Moreover, in vitro ethanol exposure of cultured mouse blastocysts was found to enhance the development and subsequent success rate for implantation of the embryos (in utero), an effect thought to be related to ethanol-induced increase of intracellular calcium levels (101,102). The general ramifications of this finding are yet to be fully understood.

Organogenic Period

The organogenic period of development in the mouse includes GD 7–14, which corresponds to weeks 3–8 in human pregnancy. The period of organogenesis is marked by the progressive subdivision of the germinal layers, resulting in the rudiments of organ differentiation, which could represent a particularly sensitive stage of pregnancy to teratogenic insult. In most studies, ethanol administration during this period of pregnancy resulted in dose-dependent increases in prenatal mortality (resorptions), decreased fetal growth and an increased incidence of skeletal and organ malformations. Within this general period of embryonic/fetal development, the temporal sequence of developmental events is unique for each major organ system. Thus, although exposure to ethanol during this phase of development results in a myriad of morphological anomalies, including craniofacial, brain, cardiac, urogenital and skeletal defects, the specific profile of anomalies is dependent on the time of ethanol insult. A summarization of studies investigating the effects of acute prenatal ethanol exposure on morphologic development in mice is presented in Table 1.

GD 7 exposure. Exposure to ethanol during the early phase of gastrulation (GD 7) results in an increase in prenatal mortality and in a number of craniofacial and brain abnormalities. In C57BL/6J mice, administration of 2.9 g/kg ethanol twice (4 h apart) resulted in craniofacial defects that were remarkably similar to the facial features of children diagnosed with fetal alcohol syndrome (106). These defects include small eyes with short palpebral fissures, maxillary and mandibular hypoplasia (which contributes to a hypoplastic midface), small nose and a long upper lip with a deficient philtrum (105,106,118,119). Using scanning electron microscopy, a closer examination of the developmental events related to these craniofacial defects revealed that within 24 h of ethanol treatment early embryos (in the 4–6 somite stage) showed a decrease in the size of the neural plate, particularly in the forebrain region (105). Analyses at later embryonic stages have demonstrated closely set olfactory placodes, with resultant deficiencies in the medial nasal prominences (which contribute to the development of the philtrum, alveolar ridge containing the upper incisors and the anterior portion of the hard palate).

Ethanol exposure during this period of embryonic development also resulted in a number of eye and brain malformations. The ocular anomalies observed in C57BL/6J mice are similar to those reported in children with fetal alcohol syndrome (24,119). In particular, the deficiency of the anterior portion of the neural plate electron microscopy resulted in defects of the optic sulci and optic vesicles, which were manifested as a number of ocular defects in older embryos including anophthalmia, microphthalmia and corneal and lenticular anomalies (24). In addition, ethanol exposure on GD 7 altered development of the optic nerve, as shown by a reduction in the number of axons and by a deficiency in myelination (1). Interestingly, later developmental exposure to ethanol (GD 10) resulted in a significant reduction in myelinated axons in the optic nerve, but this was only evident in adult offspring (78). Thus, this deficiency resulting from GD 10 ethanol exposure may be due to late occurring atrophy rather than to ganglion cell hypoplasia (78).

The brain abnormalities resulting from ethanol exposure on GD 7 have been primarily demonstrated as microcephaly, exencephaly (due to incomplete closure of the neural tube) and deficiencies in a number of ventromedial structures (incomplete/abnormal development). These structures include the cerebral hemispheres, striatum, olfactory bulbs, limbic structures (septum, hippocampus), corpus callosum and lateral ventricles (97,107,118,119). Another reported consequence of midline brain hypoplasia related to ethanol exposure on GD 7 is reduced number of luteinizing hormone releasing hormone (LHRH) neurons, as assessed by immunohistochemical analysis of GD 18 C57BL/6J fetal brains (99). However, a subsequent study demonstrated no alteration in the number of neurons expressing LHRH mRNA, suggesting that ethanol treatment of GD 7 may not result in death of such neurons but rather may interfere with LHRH peptide biosynthesis (100). Interestingly, when acute ethanol treatment occurred at a time coinciding with neurogenesis and migration of LHRH neurons (GD 10–11), the number of neurons expressing LHRH mRNA was significantly decreased (100).

In addition, neurochemical analysis of GD 18 fetuses following GD 7 ethanol exposure revealed a significant loss of cholinergic neurons identified by a monoclonal antibody for choline acetyl transferase. The deficit in cholinergic containing neurons was particularly evident in midbrain structures (e.g., medial septum). In contrast, no consistent changes were observed in catecholamine or serotonergic neurons in the midbrain or hindbrain, as identified by polyclonal antibodies for tyrosine hydroxylase and 5-hydroxytryptamine, respectively (97). These results support the notion that medial facial dysmorphologies produced by ethanol exposure on GD 7 in the C57BL/6J mouse (which are similar to those associated with the clinical fetal alcohol syndrome) are reflective of brain abnormalities (107).

Other mouse strains including CD-1 (15), QS (118), C3H/He (63) and B6D2/J hybrid (61) were much less sensitive to the teratogenic actions of ethanol when administered on GD 7 (relative to effects observed following exposure to ethanol at later stages of development). In one study, however, histological and morphometric analyses of 7.5-day-old CD-1 embryos revealed alterations in morphogenetic movements associated with the gastrulation process, some of which may be later manifested as craniofacial defects (71).

GD 8 exposure. Ethanol exposure on GD 8 results in a variety of malformations in C57BL/6J and other mouse strains, although the pattern of anomalies is somewhat different from that produced following GD 7 exposure. For example, in C57BL/6J mice, the incidence of ocular anomalies is reduced (24,119). In addition, the profile of facial dysmorphologies is primarily characterized by deficiencies in maxillary prominences that lead to frontonasal dysplasia and midface clefting (104). Hence, craniofacial defects observed in C57BL/6J mice following GD 8 ethanol exposure included micrognathia, low-set ears, short philtrum, cleft palate and cleft lip (59,60, 118,119). Many of these facial features in mice have been noted in clinical cases of fetal alcohol syndrome. Furthermore, these craniofacial anomalies produced by ethanol exposure on GD 8 may result from effects on the development and migration of neural crest cells (59,60). More specifically, within 12 h of ethanol treatment, increased cell death was noted at the rim of the anterior neural plate. Neural crest cells form the ectomesenchymal cell populations that significantly contribute to the development of the face. Accordingly, the pattern of cell death appeared to relate pathogenetically to the observed craniofacial malformations (exencephaly, maxillary hypoplasia, cleft palate and cleft lip, median facial deficiencies). In addition, excessive programmed cell death was noted at the otic placodes (which form the sensory cells of the inner ear and neurons of the VIII cranial nerve), epibranchial placodes (which contribute to cranial sensory ganglia), regions of the rhombencephalon corresponding to cranial nerve nuclei, sensory and motor trigeminal nuclei and olfactory placodes. As such, these defects may underlie a number of sensorimotor deficits commonly observed in the fetal alcohol syndrome patient, such as sensorineural hearing loss and feeding and language deficiencies (109).

A similar constellation of craniofacial anomalies have been documented in other strains of mice following ethanol exposure on GD 8. These include CD-1 (15,35), CF-1 (23), MF1 (74,75), and B6D2/J hybrid (61). Brain abnormalities, primarily neural tube defects and exencephaly, have been documented in C57BL/6J, CD-1, CF-1, MF1, TO, and LACA mice (2,3,15,23,59,74–76,118). In contrast, BALB/c, CBA/h, C3H/He and QS mouse strains were relatively resistant to these teratogenic actions of ethanol (63,103,118).

Ethanol exposure on GD 8 also resulted in a number of cardiac defects. For example, within 12 h of initial ethanol treatment, abnormalities and a reduced size in the cardiac tube were observed in C57BL/6J mouse embryos (27). By GD 12–13, abnormalities of the atrioventricular canals were noted, as was a lack of closure of the ventricular septum. In the GD 18 fetus, ventricular septal defects and anomalies of great vessels (aortic arches) were identified (27,117). These cardiovascular anomalies have been also observed in C57BL/6J mice following exposure on GD 9 and to a lesser extent on GD 10 (117). In addition, cardiac defects have been documented in CD-1 mice following ethanol exposure on GD 8 (15). Ethanol insult to cranial neural crest cells may, at least in part, result in these cardiac malformations (27,116). Finally, ethanol exposure on GD 8 has been reported to produce skeletal anomalies in some strains of mice, including CD-1 (15), CF-1 (23), MF1 (75), and BALB/c (103). These skeletal malformations primarily involved the vertebrae, sternum and ribs.

GD 9–10 exposure. Acute ethanol exposure on GD 9 or GD 10 results in a number of urogenital and limb anomalies. Urogenital defects typically have been hydronephrosis and/or hydroureter. These renal malformations have been reported in several mouse strains, including C57BL/6J (16,40,47,51,80–85,90), CD-1 (15), CF-1 (23) and B6D2/J hybrid (61). In one study, the early pathogenetic events underlying ethanol-induced urogenital defects were investigated in C57BL/6J mice given 2.9 g/kg ethanol (IP) twice (4 h apart) on GD 9 (40). Within 12 h of ethanol treatment, excessive cell death (as assessed by vital staining of whole embryos with Nile blue sulfate) was observed in selective populations (mesonephric and premigratory neural crest cells). These cell populations play a critical role in the development of the renal system. Later changes were followed in GD 13–17 fetuses using scanning electron microscopy. Ureteral alterations were first detected in GD 16 fetuses, and hydronephrosis was first detected in GD 17 fetuses. Examination of fetuses on GD 18 revealed that 41% showed hydronephrosis and/or hydroureter. In another study, vesicoureteral reflux (the retrograde passage of urine from the bladder into the kidney) was examined as a possible mechanism contributing to the ethanol-induced hydronephrotic condition (16). Pregnant C57BL/6J mice were given 5.8 g/kg ethanol (IG) on GD 9. Examination of GD 18 fetuses revealed that although vesicoureteral reflux and hydronephrosis occurred independently the incidence of reflux appeared to be related to the severity of hydronephrosis.

Limb malformations have primarily involved the forelimbs, with ectrodactyly (missing digits) most commonly observed. Polydactyly (additional digits), syndactyly (fused digits) and clinodactyly (deflected digits) have been also noted. Following ethanol exposure on GD 9 or GD 10, these limb defects have been documented in different mouse strains, including C57BL/6J (47,51,58,80–85,90,118,119,129), CD-1 (15), MF1 (74), and B6D2/J hybrid (61). Exposure to ethanol on GD 11 generally resulted in a lower incidence in limb anomalies (15,61,74,118). In one study, increased cell death was noted within 4 h of ethanol treatment in the developing limb buds of C57BL/6J mouse embryos on GD 9 (58). Cell death was particularly evident in two regions: the apical ectodermal ridge and proximal mesenchymal cells (the former region is especially involved in the normal outgrowth of the limb buds). Moreover, the temporospatial pattern of cell death appeared to be pathogenetically related to the types of limb defects that are seen in GD 18 fetuses.

GD 12–14 exposure. The incidence of ethanol-induced malformations is much lower when exposure is restricted to GD

12–14, although some studies have demonstrated the existence of both soft tissue and skeletal anomalies in CF-1 mice (23) and BALB/c mice (103). Interestingly, some mouse strains, including the outbred Swiss Webster (129) and QS (118), the inbred C3H/He (63) and CBA/c (103), and mice selectively bred for increased sensitivity (long-sleep, LS) and decreased sensitivity (short-sleep, SS) to ethanol narcosis (51) are relatively resistant to the teratogenic actions of ethanol when the agent is administered during the organogenic period of development. This result suggests that genetic factors represent important determinants in governing susceptibility to ethanol teratogenesis.

Postorganogenic Period

Following the period of organogenesis, the formed organ rudiments enter a period of growth and histological differentiation. This postorganogenic period in the mouse encompasses GD 15–19 (or birth). This period roughly corresponds to weeks 9–26 in human pregnancy. During this stage of development, the organ systems grow in size and volume and becomes mature with regard to function. This developmental stage also corresponds to a period of intense central nervous system development. Accordingly, exposure to ethanol during this phase of development does not typically result in gross morphological damage but rather in growth retardation and brain structural anomalies that are manifested as behavioral abnormalities. Unfortunately, very few studies have investigated the effects of acute ethanol exposure during the latter part of pregnancy in mice. In one study, ethanol exposure on GD 15 or GD 18 resulted in a dose-dependent decrease in fetal body weight and a decrease in brain DNA synthesis (23). In addition, young offspring from the same study exhibited delayed development of neonatal reflexive behaviors. In a more recent study, ethanol (6 g/kg) administered on GD 16 or GD 17 resulted in decreased fetal body weight and premature birth in C57BL/6 mice (95). A lower dose of ethanol (4 g/kg) given on GD 15, however, did not have a similar effect (96). Most of the work aimed at examining the effects of late gestational ethanol exposure in mice has involved chronic treatment regimens.

CHRONIC ALCOHOL EXPOSURE

Growth and Morphologic Development Studies

Whereas acute ethanol exposure typically results in partial expression of the fetal alcohol syndrome (with the abnormalities being unique to the period of exposure), it is perhaps not surprising that chronic ethanol exposure throughout pregnancy results in a wide variety of effects ranging from structural defects to growth retardation (collectively characteristic of the fetal alcohol syndrome). Nevertheless, a few studies have demonstrated that skeletal and major organ system malformations predominate when chronic exposure is restricted to the organogenic period of pregnancy (17,45,91,92), and exposure during the postorganogenic period of pregnancy is primarily associated with retarded growth (39,65). A summarization of experimental reports pertaining to the effects of chronic prenatal ethanol exposure on growth and morphologic development in mice is presented in Table 2.

Studies examining the effects of chronic ethanol exposure on growth and morphologic development in mice have employed different strains, including C57BL/6J (17,18,22,45,91,92), C57BL/6cr (20,65,68), DBA/2J (45), DBA/1J (14), BALB/c (20), C3H/He (89), C3H/lg (21,22), CBA/J (21,22), Swiss Webster (45,127,128), CD-1 (45), HS (39), B6D2 hybrids (113) and LS and SS mice (48,50). In most of these studies, ethanol was chronically administered to pregnant mice in liquid diets, with 15–30% of the calories provided by ethanol. The intragastric (14,48,50) and intraperitoneal (45) routes of administration have also been used. In many instances, control groups received a treatment that was isocaloric to the ethanol treatment, although this was not always the case (14,45). Pair-feeding techniques have been employed in the majority of liquid diet studies, with some exceptions (20–22,127,128). In two studies examining perinatal mortality and growth retardation, offspring were cross fostered to minimize postnatal influences (39,65). Some of the early work in this area involved delivering ethanol to pregnant mice in their drinking water (26,28,38,124–126). However, results from these studies are difficult to interpret because of the lack of proper nutritional controls, males were commonly treated along with females (potentially confounding teratogenic effects with possible mutagenic effects) and in many instances alcohol treatment was continued following parturition. Hence, results from these studies are not included in this report.

Increased perinatal mortality has been reported following chronic ethanol exposure during mid-pregnancy (17,45,91,92), late pregnancy (39,66) or throughout gestation (21,22,48,50,68,89). In one study, neonatal mortality at birth was greatest when ethanol exposure was terminated 24 h prior to parturition than when exposure was continued beyond delivery. This finding suggests that ethanol withdrawal may play some role in this pregnancy outcome measure (66).

Growth retardation as a result of gestational ethanol exposure has been demonstrated in a number of studies employing a wide variety of mouse strains (17,21,22,39,45,48,50,65,66,68,89,127). In many cases, the effect was dose related (21,50,68,89) and observed following different exposure patterns, although exposure during the late portion of pregnancy (GD 12–17) appeared to be most critical for this pregnancy outcome measure (39,65). Although in many instances growth retardation was evident at birth, in some studies decreased body weight in offspring did not become evident until adolescence and extended into adulthood (65,66,68). In fact, whereas reduced birthweight was dependent on ethanol exposure occurring within 24 h of birth, the late onset growth deficit (occurring during adolescence and extending into adulthood) was observed regardless of whether birthweight was influenced by gestational ethanol exposure (66). This periadolescent growth retardation was not related to deficits in caloric intake (66), and use of a cross-fostering technique minimized the possibility that this effect was due to other postnatal factors (65). These data suggest that prenatal ethanol exposure in mice results in intrauterine and postnatal growth retardation, which are hallmark features of fetal alcohol syndrome and fetal alcohol exposure.

A number of studies have demonstrated that chronic prenatal ethanol exposure results in a myriad of birth defects (Table 2). Most organ system and skeletal defects have resulted from chronic gestational ethanol exposure that encompassed the critical period of organogenesis. For example, ocular defects primarily consisted of anophthalmia and microphthalmia following exposure during mid-pregnancy (45,89,91,92). Cardiac malformations included ventricular septal defects and abnormalities of large vessels such as the aorta. These defects were primarily observed following mid-gestational ethanol exposure (21,45,89,91,92). Urogenital defects also were observed following exposure during the organogenic period of fetal development. These malformations included hydronephrosis,

TABLE 2

SUMMARY OF EFFECTS OF CHRONIC PRENATAL ETHANOL EXPOSURE ON GROWTH AND MORPHOLOGIC DEVELOPMENT IN MICE

Teratogenic Effect	Ethanol Administration	Mouse Strain	Critical Period	References
Perinatal Mortality	IP, IG, Liquid diet	C57BL/6J, C57BL/6cr, CBA/J, C3H/1g, C3H/He, DBA/2J, CD-1, SW, HS, LS/SS	throughout pregnancy	17, 21, 22, 39, 45, 48, 49, 49, 50, 66, 70, 89, 91, 92
Growth Retardation	IP, IG, Liquid diet	C57BL/6J, C57BL/6cr, CBA/J, C3H/1g, C3H/He, DBA/2J, CD-1, SW, HS, LS/SS	late-pregnancy (GD 12–17)	17, 18, 21, 22, 39, 45, 48, 50, 65, 66, 70, 89, 91, 92, 127
Brain Defects	IG, Liquid diet	C57BL/6, B6D2F2, CBA/J, C3H/1g, C3H/He, DBA/1J, BALB/c, B6D2, SW	throughout pregnancy	14, 20, 21, 22, 89, 91, 92, 111, 112, 127, 128
Ocular Defects	IP, Liquid diet	C57BL/6J, C3H/He, DBA/2J, CD-1, SW	mid-pregnancy (GD 4–12)	45, 89, 91, 92
Cardiovascular Defects	IP, Liquid diet	C57BL/6J, C3H/1g, C3H/He, DBA/2J, CD-1, SW	mid-pregnancy (GD 4–12)	21, 45, 89, 91, 92
Urogenital Defects	IP, Liquid diet	C57BL/6J, C3H/He, DBA/2J, CD-1, SW	mid-pregnancy (GD 4–10)	17, 45, 89, 91, 92
Skeletal (Limb) Defects	IP, Liquid diet	C57BL/6J, C3H/1g, DBA/2J, CBA/J, CD-1, SW	mid-pregnancy (GD 4–12)	22, 45, 89, 91, 92

hydroureter, undescended testes and delayed sexual maturation (17,45,89,91,92). Skeletal malformations primarily included defects of the forelimbs and resulted from mid-pregnancy ethanol exposure (22,45,89,91,92).

In contrast with other organ systems, the brain is unique because it is one of the first to develop and the last to fully mature (120). Thus, the brain appears to be sensitive to the teratogenic actions of ethanol throughout its development, which encompasses the entire gestational period and extends into early postnatal life in rodents (122). Brain abnormalities in mouse models have been observed following ethanol exposure during mid-pregnancy, late pregnancy and throughout gestation. These brain abnormalities have included exencephaly (21,91,92), hydrocephaly (89,91,92), microcephaly (14), dilated ventricles (22), altered membrane phospholipid content (111) and structural defects in a number of brain regions (20, 127,128). Of course, of clinical significance is whether such brain structural defects are manifested as functional deficits. The next section reviews some of the recent results demonstrating behavioral deficiencies obtained in mice following gestational ethanol exposure.

Behavioral Studies

Although the behavioral consequences of prenatal ethanol exposure have been primarily documented in the rat, recent studies have shown that the mouse model is also sensitive to the behavioral teratogenic actions of ethanol. In fact, a number of mouse strains is sensitive to the behavioral effects of prenatal ethanol exposure and to the morphological teratogenic actions of higher doses of ethanol. Thus, depending on the level of ethanol exposure, the mouse species appears to be sensitive to a wide range of prenatal ethanol effects. It is this appealing feature that has led to the suggestion that the mouse model may be particularly useful because it may more closely approximate the clinical condition (11). Although not as extensive in nature, the behavioral teratogenic consequences of ethanol observed in mouse models are, for the most part, similar to those reported in rats (5). A number of studies have documented brain defects that result from acute gestational ethanol exposure in mice. Unfortunately, very few studies have attempted to link these brain defects to noted behavioral abnormalities. In fact, most of the work demonstrating behavioral teratogenic effects has involved the chronic administration of ethanol to pregnant mice, although behavioral abnormalities have been demonstrated following acute gestational ethanol exposure (32). A summary of studies investigating the behavioral teratogenic effects of ethanol in mice is presented in Table 3.

Regulatory behavior and locomotor activity studies. As demonstrated in rats, prenatal ethanol exposure can alter the capacity of mouse neonates to thermoregulate (49). In addition, ethanol-exposed C57BL/6 mouse pups moved further along a thermal gradient to maintain body temperature equivalent to that of controls (43). This finding suggests that the ethanol-exposed pups behaviorally compensated for the greater heat loss they experienced during the testing session.

One of the most common behavioral effects observed following prenatal ethanol exposure is an alteration in spontaneous locomotor activity. Increased open-field activity in ethanol-exposed offspring as opposed to controls has been demonstrated in C57BL/6 mice (9,86). However, this effect may differ as a function of genotype. That is, in another inbred mouse strain (C3H/He), prenatal ethanol exposure resulted in decreased locomotor activity as compared with controls (7). As with rat models, the magnitude, direction and durability of the effects as the offspring mature depend on a host of other variables including sex, ethanol dosage and testing parameters (5).

Learning and memory studies. Perhaps the most devastating consequence of prenatal ethanol exposure is compromised mental abilities. In recent years, a number of studies using mice have demonstrated deficits in performance in different appetitive and aversive learning and memory paradigms. For example, deficits in passive avoidance (9,11) and shuttle avoidance (11,86) learning have been demonstrated in mice prenatally exposed to ethanol. In another study, prenatal ethanol exposure did not alter latency re-entering a shock-

TABLE 3

SUMMARY OF EFFECTS OF CHRONIC PRENATAL ETHANOL EXPOSURE ON BEHAVIORAL PERFORMANCE IN MICE

Type of Malformation	Ethanol Administration	Mouse Strain	Critical Period	Ethanol Effect	References
Thermoregulation	Liquid diet	C57BL/6cr, LS/SS	GD 5–18	deficit	43, 44, 49
Locomotor activity	Liquid diet	C3H/He, C57BL/6cr	GD 5–18	decreased/ increased	7, 9, 86
Avoidance learning	Liquid diet	C57BL/6cr	GD 5–18	deficit	9, 11, 68, 86
Operant performance	Liquid diet	C57BL/6cr	GD 5–17; GD 5–10; GD 12–17	deficit	41, 42, 64, 69
Spatial learning	IG, Liquid diet	B6D2F2, C57B1/6J	GD 5–17	mixed results	32, 112
Sexually dimorphic behaviors	Liquid diet	C3H/He, C57B1/6J	GD 7–17	mixed results	10, 56
Responsiveness to EtOH challenge	Liquid diet	C3H/He, C57B1/6J	GD 7–15	mixed results	6, 7, 29, 46, 64, 88
Sensitivity to pharmacologic challenge	Liquid diet	C3H/He, C57B1/6J	GD 7–15	mixed results	7, 13, 41, 115

associated compartment when testing was conducted 24 h after the initial footshock experience in adult offspring (68). That is, there was no apparent effect on retention of a learned passive-avoidance task. However, the adult prenatal ethanol-exposed C57BL/6 offspring were significantly more susceptible to the memory disrupting effects of electroconvulsive shock (ECS) than were controls, particularly when the ECS was administered 45 s following the initial footshock. This result suggests that prenatal ethanol exposure may result in deficient memory processing, as reflected by an increased susceptibility to memory-interference procedures (68).

In addition, prenatal ethanol exposure can produce impaired performance in a number of operant conditioning situations. For example, in comparison with controls, acquisition to learn the response requirements in a fixed-ratio (FR) schedule of reinforcement was retarded in prenatal ethanol-exposed mice (64). This effect was greatest when the response requirements were high (e.g., FR 100) (41,42). Moreover, the deficit in FR responding was demonstrated when in utero ethanol exposure was restricted to late (GD 12–17) but not earlier (GD 5–10) gestational treatment (69). Deficits in operant behavior have also been demonstrated when the response requirements for reinforcement are low. For example, prenatal ethanol exposure impaired performance in a differential reinforcement of response omission schedule (42). In this situation, reinforcement was contingent on the mouse refraining from responding until a cue was presented that signaled the availability of reinforcement. Impaired performance exhibited by prenatal ethanol-exposed offspring in many of these tasks has been attributed to deficits in response inhibition and in reduced efficacy of reward (5).

Permanence of behavioral abnormalities. Prenatal ethanol exposure adversely impacts different behavioral functions that are critical to the survival of neonates and to the mature animal. In fact, prenatal ethanol exposure alters behavioral responses associated with thermoregulation in mice as early as 3 days of age (43). Some of the behavioral teratogenic effects of ethanol are transient, with the deficits waning as the animal matures (5). However, in cases where deficits are transient, the apparent "normalization" in function that comes with maturity may in fact reflect the development of compensatory strategies designed to cope with and overcome the disabilities. Dysfunctional behaviors may then later reemerge when these compensatory mechanisms break down with age and/or the need for these compensatory systems compromise the ability to perform more complex functions later in life (5,93). For example, although prenatal ethanol exposure has not been reported to impair performance in spatial learning tasks in young adults (70–90 days of age), severe deficits were observed in these offspring when testing was conducted in aged mice (12–24 months old) (32,112). Other behavioral abnormalities in mouse offspring prenatally exposed to ethanol can also be be quite durable (enduring into adulthood). For example, deficits in avoidance learning (11,86) and impaired performance in operant tasks (41,42,69) have been reported in adult prenatal ethanol-exposed mice.

In addition, behavioral deficits that would otherwise not be readily apparent may become "unmasked" when the complexity or demands of the testing situation increase or when testing is conducted under more stressful or drug challenged states. For instance, impaired operant performance in adult mice prenatally exposed to ethanol was most apparent when the response requirements were most demanding (41). In addition, the ability to defend body temperature in the face of environmental (thermal) challenge was impaired in adult pre-

natal ethanol-exposed mice (44). As another example, memory of a learned behavioral task was more fragile in prenatal ethanol-exposed offspring when the mice were experimentally challenged with a memory-disruptive procedure (68). Similarly, behavioral abnormalities were noted in adult prenatal ethanol-exposed mice when testing was conducted following different drug challenges (7).

Responsiveness to pharmacologic challenges. Psychopharmacologic assessment of ethanol-exposed offspring has proved to be a valuable tool in studying the behavioral teratogenic properties of ethanol (5,57). A number of studies have focused on whether prenatal ethanol exposure alters later responsiveness to the drug. Unfortunately, as in the literature involving rat studies, many of these findings are equivocal, with the direction and magnitude of effects depending on numerous experimental parameters. For example, prenatal ethanol exposure increased ethanol intake in preference tests in young C3H/He mouse offspring. However, the effect was transient, with differences between prenatal treatment groups diminishing as the offspring matured (88). Prenatal ethanol exposure was reported to not significantly influence ethanol-induced hypothermia (46) and hypnosis (46,88) responses in adult offspring. In addition, prenatal ethanol-exposed adult offspring did not differ from controls in sensitivity to the acute ataxic effects of ethanol or GABAergic modulation of this ethanol response (29). In contrast, offspring prenatally exposed to ethanol exhibited greater sensitivity than did control offspring to the low-dose locomotor stimulant properties of ethanol (7). This effect, however, differed as a function of sex, age and dose of ethanol. In another study, adult C57BL/6 mice prenatally exposed to ethanol were less sensitive than were control offspring to ethanol's disruptive effects on operant responding (64). Furthermore, in a drug discrimination paradigm, Middaugh and Ayers (64) reported that adult mice exposed prenatally to ethanol exhibited reduced sensitivity to the discriminative stimulus properties of ethanol. That is, following discrimination training (1 g/kg ethanol vs. water), ethanol-exposed mice were less able to discriminate lower doses of ethanol from placebo than were control offspring (64). Although not many studies have focused on subsequent sensitivity to the chronic effects of ethanol, in one study prenatal ethanol exposure retarded the development of tolerance to the motor incoordinating actions of ethanol in adult mice (6). Interestingly, development of ethanol dependence was not apparently influenced by prenatal ethanol exposure (Becker, unpublished data).

Studies have also examined behavioral sensitivity to different drug challenges, particularly those targeting brain monoamine systems. For example, adult male mice prenatally exposed to ethanol exhibited reduced sensitivity to the dopamine receptor agonist apomorphine, particularly its ability to attenuate ethanol-stimulated locomotor activity (13). Similarly, reduced sensitivity to the locomotor suppressant effects of the alpha-2 adrenergic agonist clonidine given alone and with a stimulant dose of ethanol was observed in adult mice prenatally exposed to ethanol in comparison with control offspring (115). In contrast, prenatal ethanol-exposed adult mice exhibited enhanced sensitivity to the ability of the catecholamine synthesis inhibitor alpha-methyl-*p*-tyrosine to attenuate the stimulant effects of ethanol (7). In another study, sensitivity to the disruptive effects of amphetamine on operant responding under a progressive-ratio schedule of reinforcement was enhanced in prenatal ethanol-exposed adult mice in comparison with controls (41). In general, these studies have demonstrated altered behavioral responding when prenatal ethanol-exposed offspring are tested under pharmacologically challenged states and some indirect support for alterations in brain neurochemical systems.

Possible mechanisms underlying behavioral deficits. For the most part, a relatively small number of studies using mice have focused on neuroanatomical, neurochemical and neuroendocrinological consequences of prenatal ethanol exposure, particularly as they relate to observed functional (behavioral) deficits. Several studies have demonstrated a general reduction in brain weight and structural anomalies of different brain regions following acute and chronic gestational ethanol exposure. Presumably, these brain abnormalities may underlie behavioral deficits; however, such correlative analyses have not been typically conducted. In one recent study, B6D2F2 mice prenatally exposed to ethanol showed reduced depth (thickness) of the occipital cortex but not frontal or parietal cortex (112). This finding may relate to the reported deleterious effects of prenatal ethanol exposure on optic nerve development (1,78). Furthermore, although the Wainwright et al. (112) study demonstrated that prenatal ethanol exposure may have adverse effects on selective neuron populations, no relationship was observed between the cortical defect and performance in a Morris maze task.

Neurochemical studies have primarily focused on brain monoamine systems, although alterations in other systems (e.g., cholinergic) have also been noted (97,129). Previous studies have demonstrated decreased levels of serotonin and unaltered levels of dopamine and norepinephrine in prenatal ethanol-exposed mice (33,34,62). However, it is difficult to interpret these results because nutritional controls were not included in the study design and neurochemical analyses were conducted on whole brain samples, possibly masking more specific regional differences. A more recent study found no effect of prenatal ethanol exposure on concentrations of dopamine or its intermediate metabolite, dihydroxyphenylacetic acid, in striatum and nucleus accumbens in adult C57BL/6 mouse offspring (67). In addition, prenatal ethanol exposure did not apparently influence the binding parameters of striatal D1 and D2 dopamine receptors in either young or adult offspring (19). Thus, at the present time, it is difficult to reconcile the apparent lack of effect of prenatal ethanol exposure on brain dopamine systems in the face of reported altered behavioral sensitivity to dopaminergic agents (7,13,41). Clearly, further investigation is needed to address these discrepancies.

A few studies have examined neuroendocrine function in mice prenatally exposed to ethanol. In particular, studies have addressed effects of prenatal ethanol exposure on hypothalamic–pituitary–gonadal (HPG) axis function. For example, C57BL/6 female ethanol-exposed offspring exhibited delayed sexual maturation (18). However, this effect was probably more reflective of a general developmental retardation than of reproductive system dysfunction because these female offspring were capable of becoming pregnant and maintaining full-term pregnancies (8). Interestingly, the data suggested that offspring of ethanol-treated mothers that did not consume ethanol themselves during their own pregnancy still had a tendency to have offspring of lower birth weight. Furthermore, if mothers prenatally exposed to ethanol did consume alcohol during their own pregnancy, the impact on fetal weight suppression was even greater than expected for in utero ethanol exposure alone. These results suggest a pernicious carryover effect of prenatal ethanol exposure on reproductive function that is evident even when the mothers abstain from alcohol consumption during their own pregnancy (8). Perturbation of the HPG axis in males may be inferred from studies demon-

strating alterations in male sexual behavior (114) and in sexually dimorphic patterns of nonreproductive behavior. As an example of this latter case, adult male C3H/He mice prenatally exposed to ethanol exhibited a demasculinized pattern of scent marking behavior as compared with control offspring (56). In contrast, prenatal ethanol exposure did not influence the sexually dimorphic differences in saccharin preference (10).

Studies on Immune Function

In recent years, a number of studies have demonstrated immune dysfunction in mouse offspring prenatally exposed to ethanol (52). For example, prenatal ethanol exposure results in a reduction in the number of thymocytes (cells in the thymus that play an important role in the body's defense against infection) and a marked reduction in T-lymphocyte responses to mitogens in fetal and neonatal mice (36,37). Moreover, deficits in immune function have been demonstrated in adult ethanol-exposed mouse offspring including long-lasting suppression of cellular immunity as measured by contact hyperresponsivity and local graft vs. host responses (53).

Furthermore, Gottesfeld et al. (53,55) conducted a series of studies aimed at examining the possibility that prenatal ethanol exposure may interfere with normal immune function by altering autonomic nervous innervation of lymphoid organs. Mice exposed to alcohol in utero displayed persistent suppression of cell-mediated immunity and selective neurochemical changes in lymphoid organs, including enhanced norepinephrine turnover, lower tissue levels of norepinephrine and reduced number of beta-adrenoceptor binding sites (53,55). Because these noradrenergic changes were observed in spleen and thymus tissue but not in heart tissue, these data suggest that the neurochemical effects were organ specific and not related to a generalized effect on the sympathetic system. Furthermore, histofluorescent studies have indicated that the alteration in sympathetic innervation of lymphoid tissue is not due to structural deficits (number of sympathetic axons projecting and terminating on lymphoid organs) but most likely due to some perturbation in noradrenergic synaptic transmission. In addition, mice prenatally exposed to ethanol also displayed a marked enhancement of nerve growth factor (NGF) binding activity, particularly in the thymus (55). Because sympathetic neurons require NGF for their development and maintenance and because NGF may play a role in modulating immune responses, these data suggest that ethanol may disrupt the effects of NGF on noradrenergic modulation of immune function.

Interestingly, these deficits in immune function and neurochemical alterations in lymphoid tissue were also observed in mice exposed to ethanol through their mothers' milk (54). This finding is particularly significant because blood ethanol levels in the suckling pups are typically lower following lactational exposure to ethanol than to in utero ethanol exposure. Hence, these results suggest that the nascent immune and nervous systems may be especially sensitive to these teratogenic actions of ethanol during the critical period of early postnatal development in mice.

BENEFITS AND LIMITATIONS OF THE MOUSE MODEL

As with any model system, there are benefits and limitations associated with its use and extrapolation to the clinical condition. Many of the advantages associated with mouse models of ethanol teratogenicity have already been described. Most notably, research has accumulated that demonstrates that many mouse strains are sensitive to the classic (morphologic) and behavioral teratogenic actions of ethanol. In contrast, the rat species has been used primarily to demonstrate behavioral teratogenic properties of ethanol. Although there are some exceptions (121), for the most part, when higher doses of ethanol have been administered to induce dysmorphogenesis, pregnant rats were either severely malnourished or aborted their pregnancies, which obviates the ability to evaluate frequency and severity of ethanol-induced malformations [(109); Riley, personal communication). Thus, the mouse species appears well suited for investigations focusing on the full spectrum of deleterious consequences of prenatal ethanol exposure. In this regard, the mouse model may be particularly useful in allowing for the examination of potential factors that influence vulnerability to different features of the clinical syndrome, which does not minimize the important contributions of rat studies but does indicate how mouse studies may complement such existing information.

Another important benefit of mouse models is related to the readily available wealth of genetic information on these animals. In particular, there is a large literature demonstrating differential sensitivity to different pharmacologic properties of ethanol among different mouse genotypes (25). Similarly, it is generally established that genetic factors and a host of environmental variables influence vulnerability to the deleterious effects of prenatal ethanol exposure (109). Thus, use of different mouse strains allows for the partitioning of these different biologic (genetic) and environmental forces that interact and ultimately govern susceptibility to the damaging effects of ethanol exposure on fetal growth and development.

As indicated throughout this review, numerous inbred and outbred mouse strains have been examined, and variability in sensitivity to the deleterious effects of prenatal ethanol exposure among genotypes has been demonstrated. In many cases, however, differences between mouse strains were noted by comparisons across different studies (and thus, different procedural settings). In fact, few studies have been designed specifically to contrast sensitivity to the teratogenic properties of ethanol among different mouse strains. In these limited number of studies, inbred strains were generally more sensitive than outbred mice (45,118). Furthermore, among inbred strains, differences in sensitivity to the teratogenic actions of ethanol have been attributed to differences in maternal metabolic capacity (22). Although not firmly established, genotype most likely also influences fetal tissue sensitivity to ethanol (103). However, the identification of an "ideal" mouse strain for studies on the teratogenic actions of ethanol is highly unlikely. As a basic principle in teratology, differential vulnerability/sensitivity to the teratogenic actions of an agent among different species/strains will differ depending on the embryopathic endpoint of interest (72). Accordingly, it is not surprising that the order of sensitivities among mouse genotypes differ for ethanol-induced embryolethality, malformations, growth suppression and behavioral abnormalities.

Furthermore, it is unlikely that sensitivity (or insensitivity) to other pharmacologic properties of ethanol directly relates to susceptibility to the general (and specific) teratogenic actions of the drug. Mice selectively bred for increased or decreased sensitivity to the hypnotic effects of ethanol (L-S and S-S mice, respectively) do not differ in their susceptibility to ethanol-induced malformations, and in fact both selected lines are quite resistant to this teratogenic outcome compared with other strains (50). Nevertheless, the large amount of information known about the mouse genome makes this species particularly attractive for studies aimed at examining genetic

contributions to susceptibility and vulnerability to ethanol teratogenicity. In addition to numerous inbred strains of mice, other genetic mouse models (e.g., recombinant inbred, congenic and selectively bred strains) are available but have yet to be exploited in teratogenic studies involving ethanol. Such studies have the potential of providing important insight about molecular mechanisms that contribute to (mediate) the adverse effects of ethanol on the developing embryo/fetus. Thus, this is an area of research that is certainly deserving of, and undoubtedly will receive, more experimental attention in the future.

Aside from these benefits of mouse models for studies on the teratogenic actions of ethanol, there are some limitations associated with using this species for these studies. In particular, the small size of the mouse presents a number of practical limitations and challenges. For example, although intragastric infusion and artificial rearing techniques have been developed for the neonatal rat, it would be difficult to employ such procedures in the neonatal mouse. This difficulty is important because the first 2 weeks of postnatal life in the mouse (and rat) corresponds with the last trimester of pregnancy in humans (30,120). Moreover, this developmental period corresponds with dynamic changes in brain growth, and it is during this brain growth spurt that the central nervous system is especially vulnerable to structural and functional adverse effects of ethanol. Thus, the mouse model has not been used to study these consequences of early postnatal exposure to ethanol. However, new techniques are being developed to administer ethanol in a relatively noninvasive manner to mouse neonates (77).

Another limitation related to the small size of the mouse is difficulty in conducting studies on brain function. Neuroanatomical resolution of ethanol-induced perturbations (e.g., morphological, neurochemical) is more difficult to achieve in mice, given the relatively small size of the mouse brain, which may be why brain studies aimed at elucidating mechanisms underlying functional (behavioral) abnormalities following prenatal ethanol exposure have lagged behind in mouse studies compared with work conducted using rats. In addition, the behavioral repetoire (particularly instinctive behaviors) are not identical for rats and mice. Furthermore, similar behavior deficits observed in both species may not be mediated by the same brain (dys)functions. Nevertheless, as technological advances are made, new approaches should be available to conduct similar studies on brain structure–function relationships following prenatal ethanol exposure by using mouse models.

SUMMARY

The deleterious effects of prenatal ethanol exposure have been extensively documented in studies using mice. All of the hallmark features of the clinical fetal alcohol syndrome have been demonstrated in mice, including prenatal and postnatal growth retardation, structural malformations and behavioral abnormalities associated with central nervous system dysfunction. As expected, the severity and profile of effects is related to both dosage level and timing of exposure. In addition, these effects have been demonstrated following acute and chronic exposure, with a variety of routes of administration employed.

Although most of the prenatal ethanol studies using mice have focused on skeletal and major organ system malformations, the behavioral teratogenic effects of ethanol have also been demonstrated in mice. The fact that mouse models of ethanol teratogenicity have demonstrated sensitivity to the full spectrum of effects (ranging from overt structural anomalies to behavioral deficits) is particularly appealing because it provides additional validation of such models in approximating the clinical condition. Furthermore, given the plethora of mouse genotypes available for study, mouse models appear to be critically useful in examining the role of genetics in ethanol teratogenesis. A number of strains have been used in the studies cited in this review, and the variant response (susceptibility) to the teratogenic actions of ethanol exhibited among different mouse strains support the notion that genetic factors may play an important role in governing, at least in part, vulnerability to these effects of ethanol. These strain differences in response to prenatal ethanol exposure may be useful in studies evaluating potential mechanisms of action.

Mouse models have recently been employed to study potential mechanisms underlying the classic (structural) teratogenic actions of ethanol. Much of this work has been reviewed elsewhere (87,98). However, there is a general paucity of studies using mice aimed at examining neuroanatomical, neurochemical and neuroendocrinological consequences of prenatal ethanol exposure, particularly because such brain perturbations may underlie observed functional (behavioral) deficits apparently because much of the work detailing the behavioral teratogenic properties of ethanol has been conducted with rats. In addition, technical difficulties related to the small size of the mouse brain have also hampered work in this area. Nevertheless, this is an area where additional investigation is certainly warranted, so that the same species can be used for descriptive, functional and mechanistic studies involving prenatal ethanol exposure.

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