INVITED REVIEW

THE TERATOGENIC EFFECTS OF ALCOHOL FOLLOWING EXPOSURE DURING PREGNANCY, AND ITS INFLUENCE ON THE CHROMOSOME CONSTITUTION OF THE PRE-OVULATORY EGG

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Abstract — Much information has emerged over the years concerning the teratogenicity of acute and chronic alcohol exposure during pregnancy. Both alcohol and its primary metabolite, acetaldehyde, are teratogenic. Exposure during pregnancy may lead to fetal alcohol syndrome (FAS), and this is said to occur in a substantial proportion of infants born to mothers who are chronic, heavy daily drinkers. Such infants usually survive to birth but are mentally retarded, often display growth retardation and additionally display a characteristic range of clinical features, principally craniofacial abnormalities and neurological damage. We have recently been interested in the effect of exposure of pregnant female mice to a single high level of alcohol during pregnancy, equivalent to an episode of 'binge' drinking, on the optic nerve, and believe that our findings, which are outlined in the first part of this review, may shed important light on the pathogenesis of some of the ocular features characteristically seen in infants with this syndrome.

What is not generally appreciated, is that exposure to alcohol and other 'spindle-active' substances that have a similar action on the meiotic spindle apparatus during the menstrual cycle before conception can induce chromosome segregation errors in the ovulated oocyte. The successful fertilization of such eggs consequently results in the production of aneuploid embryos, which have a very high chance of being spontaneously aborted during the first trimester of pregnancy. Those relatively few aneuploid conceptuses that survive to term invariably show moderate to severe degrees of mental retardation, craniofacial and other abnormalities, as well as having a significantly reduced life expectancy. The findings from our experimental studies that have been carried out in mice draw attention to important principles which are of general applicability to the situation in the human. These findings, and our conclusions drawn from them, are discussed in detail in the second part of this review.

1. GENERAL OBSERVATIONS ON THE TERATOGENIC EFFECT OF EXPOSURE TO ALCOHOL DURING EARLY PREGNANCY

The teratogenicity of ethyl alcohol (alcohol, ethanol) has been known since antiquity (Warner and Rosett, 1975; Abel, 1984; Plant, 1985), though only over the last 20 years or so has it been fully appreciated that infants born to mothers with a high alcohol intake during pregnancy run the risk of exhibiting a characteristic pattern of craniofacial features which are often associated with growth retardation. According to Streissguth et al. (1984), those individuals with an intake of >30 g/day of absolute alcohol were at greater risk of delivering an infant with an alcohol-related mental and psychomotor deficit than matched controls. At higher levels of intake (>59 g/day) the risk of long-term sequelae was substantially increased. In some studies, the average daily consumption of alcohol during pregnancy was considerably higher (e.g. 140 ± 72 g, mean ± SD, see Spohr and Steinhausen, 1984). While the data in the latter study were not clearly interpretable in terms of a dose-response relationship, a consistent decrease in performance in a range of psychomotor tests was noted with increasing alcohol exposure.

The greatest cause for concern, however, relates to the substantial incidence of brain dysfunction...
seen in these infants (Palmer et al., 1974; Barry and O’Nuallain, 1975; Smith et al., 1976; Clarren et al., 1978; Streissguth et al., 1978; Wisniewski et al., 1983; Pratt, 1984), where narrowing of the head (Tenbrinck and Buchin, 1975), abnormal EEG (Havlicek and Childaeva, 1976) and cortical atrophy (Jones et al., 1974) have all been reported. They also commonly display evidence of pre- and postnatal growth retardation (Kyllerman et al., 1983; Streissguth et al., 1984; Abel and Hannigan, 1995) and are usually moderately mentally retarded with an average IQ of about 65–70, depending on how the sample is collated (see Streissguth et al., 1984). In other studies (Spohr and Steinhausen, 1984), a wider range of IQ scores were reported. In severe cases, this condition can result in the birth of a stillborn infant or the death of the infant during the neonatal period.

This relationship between alcohol consumption during pregnancy and the birth of mentally retarded infants often with an abnormal facial appearance was first highlighted in the late 1960s and early 1970s (Lemoine et al., 1968; Jones and Smith, 1973; Jones et al., 1973) and termed the fetal alcohol syndrome (FAS) (for additional early references, see Hanson et al., 1976; Mulvihill and Yeager, 1976; Clarren and Smith, 1978; Clarren, 1981; Spohr and Steinhausen, 1984; for bibliography of alcohol and reproduction, and fetal alcohol exposure and effects, see Abel, 1982, 1984, 1990; for recent review of the literature, see Abel, 1995). These infants usually have characteristic craniofacial features such as an abnormally long philtrum, a short stubby nose and short palpebral fissures associated with a positive history of maternal alcoholism (for incidence and range of clinical features, see Clarren and Smith, 1978; Spohr and Steinhausen, 1984). Abnormal dermatoglyphic configurations have also been reported in FAS (Qazi et al., 1980).

In an early study, Jones et al. (1974) stated that FAS occurs in 30–45% of infants born to chronic, heavy daily drinkers. A more recent study indicates that the incidence is probably closer to 4.3–4.7% among ‘heavy’ drinkers, depending on how the figures are analysed (Abel, 1995). While the criteria for diagnosing this condition are now more precise than formerly, FAS still represents a substantial cause of perinatal morbidity. The worldwide incidence of this condition is said to be about 0.97 per 1000 live births in the general obstetric population (Abel, 1995), and a similar incidence has been reported in the west coast of Scotland (Beattie et al., 1983).

A recent review of the US and European literature suggested that ‘the major factor associated with FAS is low socio-economic status, rather than racial background. Low socio-economic status and heavy alcohol consumption are both associated with smoking, poor nutrition, poor health, increased stress and use of other drugs, and it is likely that they exacerbate the effects of heavy alcohol intake’ (Abel, 1995). This study further emphasizes that, because criteria for determining ‘heavy drinking’ vary to such a degree between studies, any attempt to make comparisons between them—for example, on the incidence of FAS—is likely to be extremely tentative (for a comprehensive set of guidelines regarding the definition of ‘alcoholism’, see Criteria Committee, National Council on Alcoholism, 1972).

It is unclear at the present time whether alcohol has a single mode of action at the cellular or subcellular level, or the underlying mechanism may vary according to the organ system under examination. Our attempts to analyse the influence of prenatal exposure to ethanol on one component of the visual system using an animal model (see below) represents an example of the approach being taken to investigate this complex problem. It is hoped that this may eventually provide insight into the pathogenesis of the ocular neurological damage sustained in a high proportion of infants with FAS.

a. Experimental studies carried out to investigate the teratogenicity of ethanol

Numerous experimental studies have been carried out over the years using a wide range of animal species to investigate the underlying mechanism of action of ethanol. This agent has, for example, been given to experimental animals by intraperitoneal and intravenous injections, by gavage, in the drinking water or in the diet (Papara-Nicholson and Telford, 1957; Sandor and Amels, 1971; Tze and Lee, 1975; Kronick, 1976; Rosman and Malone, 1976; Chernoff, 1977; Henderson and Shenker, 1977), and all have produced some degree of developmental and/or growth retardation as well as an increased incidence of embryonic losses and perinatal mortality.

During pregnancy, ethanol is metabolized in the...
system have been particularly commonly encoun-
tered following exposure to alcohol (Hammer and
Schiebel, 1981; Goodlett et al., 1989; Miller, 1993).

In the most recent studies, exposure to alcohol at regular intervals during pregnancy in a non-
human primate (Macaca nemestrina) revealed a
dose-related neuroteratogenic deficit in the num-
ber of cerebellar Purkinje cells (Bonthius et al.,
1996), being consistent with the generalized and
permanent alcohol-induced reduction in neuronal
number reported by others. Such an effect had
previously been reported in the hippocampus
(Barnes and Walker, 1980), somatosensory cortex
(Miller and Potempa, 1990), cerebellum (Hamre
and West, 1993), retina (Clarren et al., 1990) and
olfactory bulb (Bonthius et al., 1992). Neuronal
loss has also been induced in areas known to
influence specific behaviour patterns, such as
inducing spatial memory deficits due to loss of
CA1 pyramidal cells in the hippocampus (Good-
lett et al., 1992).

A considerable literature is also now accumu-
lating regarding the effect of exposure of cells in
tissue culture to ethanol. For example, cortical
neurons and glial cells isolated from fetal and
newborn rat brains from pregnancies where the
mother has been exposed to ethanol either before
or during pregnancy have been incubated for
various periods of time in the presence of this
agent. Delayed nerve cell maturation, assessed
using a variety of parameters, was reported (Ledig
et al., 1991; see also Renau-Piqueras et al., 1989;
Guerri et al., 1989). Neural cells maintained in
tissue culture have also been used to explore the
cellular and molecular mechanisms which underlie
ethanol intoxication, tolerance and withdrawal
(Charness et al., 1984). Additional studies have
noted that exposure of a variety of other (non-
neural) cell types to ethanol in tissue culture can
not only interfere with chromosome segregation,
but also induce chromosome damage (Obé and
Ristow, 1979; Obé et al., 1977, 1986; see also
section 2, below).

Culture of rat embryos for 4 h in serum isolated
from individuals shortly after consuming alcohol
giving a serum ethanol level of 115 mg/dl),
followed by 44 h in alcohol-free serum, compared
to controls maintained for 48 h in normal alcohol-
free serum, revealed a teratogenic growth-retard-
ing effect of exposure to this agent. A similar
effect was observed when rat embryos were
incubated in culture medium containing a similar
level of ethanol. Toxic effects of acetaldehyde were only seen at concentrations of 20 ug/ml, in the absence of alcohol (Beck et al., 1984).

b. Influence of prenatal exposure to ethanol on the optic nerve

Because the clinical and experimental literature in relation to the teratogenicity of ethanol is now so well documented (see above), it seemed appropriate here to review only our most recent experimental findings on the effects of prenatal exposure to ethanol on the optic nerve of the mouse, as we believe that this is a model which allows us to study the pathogenesis of the ocular neurological damage characteristically seen in FAS.

It is important to note that the optic nerve is not a true peripheral nerve, but a central tract whose fibres differentiate mainly from the neural retina, which is itself a derivative of the embryonic forebrain (Hamilton and Mossman, 1972). It is formed by neurons of the ganglion cells of the retina as they converge at the optic disc to leave the eyeball near its posterior pole. From here, the nerve runs to the anterolateral horn of the optic chiasma. After the nerve leaves the eyeball, it becomes ensheathed by extensions of the meningeal coverings of the central nervous system. This relationship extends almost throughout the entire length of the nerve with the exception of a small portion at the chiasmatic end where both the dura and arachnoid mater layers are deficient. The relationship between the meningeal coverings of the optic nerve allows the subarachnoid and subdural spaces of the brain to extend around the nerve; consequently any raised intracranial pressure affecting the brain also affects the optic nerve (Williams et al., 1989). It is also the most accessible of the central tracts, and therefore ideal for experimental studies.

The optic nerve has been extensively studied to determine the effects of ethanol on this system (Cohen, 1967; Kennedy and Elliott, 1986; Chan et al., 1991; Pinazo-Duran et al., 1993). Although some morphometric studies have been carried out on the long-term effect of acute prenatal exposure of the optic nerve to ethanol in the mouse (Parson et al., 1993, 1995; Ashwell and Zhang, 1994), no systematic attempt had been made to investigate its effect during the early postnatal period.

i. Methodology employed. Morphometric studies were undertaken using optic nerves isolated from various inbred strains of immature, juvenile and adult mice. The cross-sectional areas of intact optic nerves excluding their meningeal coverings were determined, and a systematic random sampling procedure (Mayhew, 1990) allowed the numbers and diameters of the myelinated nerve fibres present to be established. The distribution of diameters of all the myelinated nerve fibres studied in each optic nerve was then plotted, and an estimate of the total myelinated nerve fibre population in each optic nerve and their density could then be calculated using a ratio technique (Matheson, 1970; Mayhew, 1988; 1990).

ii. Findings from morphometric studies. Of the variables analysed, no significant differences were observed in the cross-sectional areas, total number of nerve fibres present, and nerve fibre density between the left and right optic nerves and between any of the variables analysed between the male and female mice (Dangata et al., 1995). In the rodents that have been studied, the total number of myelinated nerve fibres present in various strains of mice has varied between 66 000 and 94 000 (Gyllensten and Malmfors, 1963; Gyllensten et al., 1966; Dangata et al., 1995) and 117 000 and 120 000 in the rat (Forrester and Peters, 1967; Hughes, 1977), while the maximum fibre diameter in the mouse was just over 1.9 μm (Dangata et al., 1995). The small fibre size diameter seen in rodents compared to that seen in primates, for example, may reflect the fact that rodents are more dependent on senses other than sight for most of their activities.

iii. Influence of alcohol on timing of onset and progression of myelogenesis. No myelinated nerve fibres were observed in the mouse before the fifth day of postnatal life. Once myelination was initiated, however, it progressed very rapidly during the early stage of postnatal development, and slowed thereafter. The peak level of myelination corresponded with the age when the maximum number of myelinated nerve fibres was measured, and occurred at the 16th week of postnatal life (Dangata et al., 1996). It should be noted that in the mouse the eyelids open ~12 days after birth (Findlater et al., 1993), by contrast to the situation in the human where this event occurs during the seventh month of fetal life (Hamilton and Mossman, 1972).
Studies on the timing of onset and progression of myelinogenesis (Dangata and Kaufman, 1997; see also Tennekoon et al., 1977) have also proved to be instructive, in that they have highlighted a particularly subtle effect of prenatal exposure to ethanol, namely that it not only causes a delay in the onset of this process, but also interferes with its normal progression. The spectrum of size distribution of the myelinated nerve fibres was broader in the matched controls that received saline alone compared to the corresponding ethanol-exposed group. With increasing age, the population of small and medium-sized fibres was greater in the experimental group than in the controls, while in the case of the large diameter fibres the reverse was observed (Y. Y. Dangata and M. H. Kaufman, unpublished work).

iv. Possible mode of action of prenatal exposure to alcohol on the optic nerve. The delay in the onset and rate of progression of myelinogenesis in the mouse, together with the reduction in mean myelinated nerve fibre count and density observed suggests that ethanol probably exerts its teratogenic effect either on the glial precursors of oligodendrocytes or on their cytodifferentiation, and that this could result in both a reduction in their number and/or their competence to synthesize myelin. A similar effect was observed in the rat optic nerve following combined pre- and postnatal exposure to ethanol (Phillips, 1989; Phillips et al., 1991; Phillips and Krueger, 1992; see also Samorajski et al., 1986). It was speculated that such an effect might explain the neurological signs and severe visual dysfunction commonly seen in infants diagnosed as having FAS (Phillips et al., 1991; Phillips and Krueger, 1992). A decrease in brain myelin and a delay in brain myelin synthesis have also been reported following exposure of rats to acute doses of ethanol (Lancaster et al., 1982, 1984).

When an attempt was made to determine the long-term effect in the mouse of an acute prenatal exposure to ethanol, no significant difference was observed in the total myelinated axon population before 9 postnatal weeks. However, between 9 and 15 weeks, the alcohol-treated group showed a loss of ~25% of myelinated axons, so that by 15 weeks the alcohol-treated group had significantly fewer myelinated axons; axons of all sizes were lost in the experimental group during this period. This was also reflected in a trend towards a smaller cross-sectional area of the optic nerve (see Parson et al., 1995).

c. Comparison between clinical and experimental findings

Despite obvious differences in total myelinated axon count, and fibre diameter spectrum between the mouse and the human optic nerve, it is clear that the mouse optic nerve is particularly sensitive to exposure to ethanol when this occurs during mid-gestation. In this way, it appears to resemble closely the sensitivity of the human fetal optic nerve to this agent, since optic nerve hypoplasia is one of the most characteristic features of FAS, and is believed to contribute to the poor visual acuity of infants with this condition. Up to 90% of affected children have eye defects, and over half of these have either unilateral or bilateral optic disc hypoplasia, with the optic nerve-head of affected children being significantly smaller than that of normal children (Strömland, 1987; Chan et al., 1991). It should also be emphasized that in some instances hypoplasia of the optic nerve-head may be the only visible sign of alcohol damage to the central nervous system (Pettigrew, 1986). While it has been established that the decrease in area of the optic nerve-head is related to a loss of axons in the nerve, and that this is directly related to a concomitant loss of retinal ganglion cells (Lambert et al., 1987), no similar postmortem studies have been carried out on FAS children to enable analysis of retinal ganglion cells or axon counts in the optic nerve.

Using the same experimental model, which attempts to compare the effect of an acute prenatal exposure to ethanol with that described previously, Parson and Sojitra (1995) investigated whether the axon loss previously reported in the optic nerve was stable and specific to the central nervous system. Optic, tibial and saphenous nerves were isolated from 12- and 23-week-old experimental and matched control mice in order to determine their cross-sectional area, total number of myelinated axons present and axon diameter distribution. The studies confirmed previous findings regarding axonal loss in the experimental group, but noted that no further loss occurred between 15 and 23 weeks. No significant differences were observed, however, in any of the parameters measured in the tibial and saphenous nerves between the experimental and control
groups. The teratogenic effect of ethanol therefore appears to be specific to the central nervous system, with no obvious effect on the peripheral nervous system.

No evidence of a peripheral neuropathy was seen in this 'binge' model, nor have there been instances of peripheral neuropathy reported in cases of FAS, though in one model for this condition subtle differences in the sciatic nerve of rats fed alcohol throughout pregnancy and during the first postnatal week were reported (Baruah and Kinder, 1989). This is in marked contrast to the situation observed in chronic alcoholic subjects, where peripheral neuropathy is one of the commonest clinical manifestations (Victor, 1975; Junrunen, 1980), and generally believed to be due to B vitamin deficiencies (Victor, 1975).

The optic nerve has also been used as a system of choice to study the pre- and postnatal influence of exposure to a wide range of other teratogenic agents: X-irradiation (Colello and Schwab, 1994; Colello et al., 1994), for example, appears to act primarily by preventing myelin formation in the retinofugal pathway. Cycloheximide also interferes with myelination in the CNS, possibly by inhibiting the synthesis of myelin basic protein, thus altering the ability of oligodendrocytes to incorporate membrane components into the CNS myelin sheath (Cullen and Webster, 1989). This contrasts with the proposed mode of action of 5-azacytidine (5-AZ) which it is believed may exert its teratogenic effect by altering gliogenesis in the rat optic nerve (Black and Waxman, 1986; Black et al., 1986). Similar findings were reported following prenatal exposure of rats to cortisol (Bohn and Friedrich, 1982).

The mode of action of teratogens, such as alcohol, on the optic nerve is likely to be extremely complex (see above), and is almost certain to depend on the timing of exposure and dose involved as well as the biochemical nature (i.e. molecular configuration) and properties of the agent under consideration, and may even depend in certain cases on its mode of administration. Clearly, there is still much to be learned concerning the mode of action of ethanol. It is only by pursuing these studies that we may hope to gain insight into the pathogenesis of this agent's effects.

2. OBSERVATIONS ON THE POTENTIAL HAZARD OF EXPOSURE OF PRE-OVULATORY (UNFERTILIZED) OOCYTES TO ALCOHOL

It has long been recognized that ~50–60% of all human spontaneous abortuses have an abnormal karyotype, and that of these about 70% are aneuploid (these are mostly autosomal trisomics with usually one or two more chromosomes than the normal complement, while a significantly smaller proportion are X monosomics with an X0 sex chromosome constitution); a further 20–25% are polyploid, with a triploid: tetraploid ratio of about 3:1 (Boüé and Boué, 1973; Therkelsen et al., 1973). These early findings have been confirmed in all subsequent studies (see, for example, Warburton et al., 1991).

Most conceptuses with an abnormal karyotype are aborted during the first trimester of pregnancy, though very occasionally fetuses with an abnormal karyotype may survive to later stages of gestation, or even to term (Ford, 1975; Epstein, 1986), the most commonly encountered of these neonates being individuals which are trisomic for either chromosome 21, 18 or 13, all of whom, if they survive, show a moderate to severe degree of mental retardation and reduced life expectancy. Embryos with autosomal monosomy (i.e. when only one instead of a pair of autosomes is present), and those with a Y0 sex chromosome constitution, do not usually survive much beyond implantation.

Various experimental studies indicate that the origin of the polyploids (which have multiples of the normal haploid complement) is most likely to be due to the fertilization of postovulatory aged oocytes (Kaufman, 1991, 1995), though this is not usually associated with an increased incidence of chromosome segregation errors (O’Neill and Kaufman, 1988); no age-related effect is observed in this group. The origin of the aneuploids, however, appears to be less well understood. While a relationship clearly exists between maternal age and non-disjunction, the hypotheses that have been proposed to account for this have so far been found to be wanting when tested in experimental animals (see, for example, Henderson and Edwards, 1968; Luthardt et al., 1973; Polani and Jagiello, 1976; Speed, 1977).

Indirect evidence from experimental studies with rodents suggests that exposure to spindle-
active substances (or trisomigen) that interfere with the normal functioning of the meiotic (usually) and, but to a lesser extent, the mitotic apparatus at the time of fertilization or during early cleavage, may induce chromosome segregation errors. If exposure to these substances occurs during the menstrual cycle leading to ovulation, then the ovulated oocyte may as a consequence contain an abnormal chromosome constitution with either one or two more or less chromosomes than the normal complement — a condition termed aneuploidy.

a. General observations on possible mechanisms involved in the induction of aneuploidy

Information regarding the factors that are known to increase the incidence of aneuploidy is remarkably limited (Bond and Chandley, 1983; Epstein, 1986; Dyban and Baranov, 1987; Warburton et al., 1991). While it is clear that there is a relationship between increased maternal age and the incidence of non-disjunction, the underlying mechanism involved has yet to be established. Much has been written about the relationship between maternal age and the birth of infants with Down's syndrome (trisomy 21) (Penrose, 1933; Penrose and Smith, 1966). While the overall incidence of this condition is somewhat in the region of 1 in 2500 of all livebirths, it has been established that the risk in women over the age of 40 is closer to 1 in 20–40, even though possibly up to 65–80% of conceptuses with trisomy 21 are lost at some stage before birth (Creasy and Crolla, 1974; Boué et al., 1981). In the case of D- and E-trisomics, the prenatal losses may be >90% (Alberman and Creasy, 1977).

Even though a similar detrimental effect of maternal ageing appears to be a general phenomenon in mammals, this cannot be the complete story. Down's syndrome, for example, shows a bimodal maternal age distribution; one component (~40% of the affected subjects) belongs to the age-dependent group (mean maternal age = 43 years) and the others (~60% of all affected subjects) comprise the age-independent group (mean maternal age = 28.5 years), where the mean maternal age corresponds to the peak for all births in the population. It has long been recognized that a bimodal distribution with regard to maternal age is also observed in relation to all the other trisomies (Warburton et al., 1991).

b. When does the malsegregation event occur?

Much useful information is now available from the cytogenetic analysis of infants with Down's syndrome in which the parental origin of the extra chromosome 21 has been unequivocally established (Mikkelsen et al., 1976, 1980; Magenis et al., 1977) and from the analysis of infants with sex chromosome anomalies (Race and Sanger, 1969; Sanger et al., 1971a, b). These studies have revealed that, in 77% of cases, the malsegregation event must have occurred in the egg, with malsegregation at meiosis I and II respectively accounting for 66% and 11% of these cases, with the fertilizing sperm being responsible in only 23% of cases (Mikkelsen et al., 1980). In a high proportion of aneuploid conceptuses, the egg, when ovulated, must already have had an extra chromosome present in its complement (Wramsby et al., 1987). In individuals with a 47,XXY sex chromosome constitution, the extra X chromosome present is maternally derived in 67% and paternally derived in 33% of cases, with the majority of chromosome segregation errors arising during the first meiotic division (Race and Sanger, 1969; Sanger et al., 1971a, b; see also Angell et al., 1983, for cytogenetic analysis of human embryos after in-vitro fertilization).

c. What mechanism might interfere with chromosome segregation during the first meiotic division?

Chromosome malsegregation, whether during meiotic or mitotic divisions, can only occur as a consequence of interference with the normal functioning of either the microtubules or microfilaments of the spindle apparatus, or the centromere (kinetochore) region by which the chromosomes are attached to the spindle elements, and which disjoin during anaphase (McIntosh and Landis, 1971; Bond and Chandley, 1983). The cytoskeletal elements may, of course, be polymerized by exposure to a wide range of spindle-active agents with actions on the spindle apparatus similar to those produced by exposure to substances such as colchicine, Colcemid or cytochalasin.

Following exposure to alcohol, some of the microtubules at the periphery of the spindle during anaphase may be displaced from the main body of the spindle apparatus; multipolar spindles are also
commonly encountered, and may be associated with disorganized alignment of the chromosomes (O’Neill and Kaufman, 1989). Chromosome ‘lagging’ is also commonly seen (O’Neill et al., 1989). The underlying effect of these agents might be through their capacity to disrupt the regulation of the changes that normally occur in the concentration of calcium ions in the proximity of the spindle apparatus during cytokinesis. Certainly there are many reports which indicate that acetaldehyde interferes with microtubule integrity and tubulin polymerization (Tuma and Sorrell, 1987; Tuma et al., 1991), and it is possible that this effect on tubulin may account for the induction of chromosome segregation errors observed following exposure to alcohol and other spindle-active substances (Barthelmess, 1970).

d. Are there any spindle-active substances to which females are commonly exposed that should be considered as possible causative agents in those cases of spontaneous abortion in which a chromosome malsegregation event has occurred where no other reasonable explanation is available?

It is particularly relevant to ask whether there are any substances that have spindle-active properties to which females might be exposed during their reproductive life that could account for those cases of spontaneous abortion with chromosome segregation errors that cannot be accounted for by any other known mechanism, such as increased maternal age or the presence of a balanced chromosome translocation in the karyotype of one or other parent.

Exposure to alcohol and/or general anaesthetics, or possibly other agents that have a similar action to them on the components of the spindle apparatus, might be the causative agents in those cases of spontaneous abortion in which chromosome malsegregation occurs where no other reasonable explanation is available (Kaufman, 1985; Kaufman and O’Neill, 1988). Both alcohol and general anaesthetics, even in relatively low dosage, are spindle poisons, and are certainly capable of interfering with normal cell division and chromosome segregation in particular (Kaufman, 1977).

e. Observations in relation to the teratogenic effect of alcohol

Numerous experimental studies have confirmed the long-known clinical and epidemiological observations that alcohol is teratogenic. When exposure is at high levels, particularly during the early stages of pregnancy, this can result in the birth of infants with FAS (for references, see section 1).

It is at present unclear whether ethanol per se, or its primary metabolite, acetaldehyde, is the active teratogen, since both are capable of inducing a wide range of congenital abnormalities if given to pregnant experimental animals at appropriate stages of gestation (for references, see section 1). When pregnant animals are exposed to one or other of these agents during embryogenesis or during early organogenesis, the cytoskeletal disruption is principally confined to those cells which are undergoing morphogenetic shape changes. The neuroepithelial cells, for example, tend to round up, and the resultant interference that occurs with the process of neurulation produces neural tube defects along the embryonic axis (O’Shea and Kaufman, 1981). Similarly, cellular disruption can occur during early cardiogenesis, with the production of cardiac defects (O’Shea and Kaufman, 1981).

f. Clinical findings with regard to the influence of alcohol on the chromosome constitution of individuals

Occasionally infants with FAS have an abnormal karyotype (e.g. Qazi et al., 1978; Gardner et al., 1985; Bingol et al., 1987); in each of these published examples, different chromosomes were involved. In one of these studies (Bingol et al., 1987), five cases of infants with FAS associated with trisomy 21 were described. In these infants, growth deficiency was more pronounced than with FAS alone, and all had congenital heart disease. Trisomy 21 and FAS may occur together, at random, in 1 in 525 000 newborn infants in the USA. In this study, the incidence was 1 in 6600, a 30-fold increase over the chance occurrence of these two conditions together. All of these cases had a chronic alcoholic mother as well as maternal grandmothers, and it was suggested that there might be an increased incidence of trisomy 21 in children with two generations of alcoholic
mothers. This situation can only arise, assuming both parents have a normal chromosome constitution, if one or other gamete is exposed to a mutagenic stimulus (in this instance, exposure may have occurred to alcohol or its breakdown product acetaldehyde, which is known to be mutagenic) during gametogenesis or at the time of conception. It is known that acetaldehyde, for example, can interfere with the functioning of the mitotic/meiotic spindle apparatus, inducing chromosomal aberrations, sister chromatid exchanges and cross-links between DNA strands (Obe and Ristow, 1979; Natarajan and Obe, 1982).

Chromosome aberrations are also seen in alcoholics (De Torok, 1972), and the underlying mechanism may be due to interference by alcohol or acetaldehyde with the functioning of the spindle apparatus (see above). The high degree of constancy of the normal diploid karyotype among all age groups of the nonalcoholic (control) somatic cells is contrasted by the non-specific and unpredictable nature of the cells of the alcoholic subjects, particularly in those with alcohol-connected organic-brain-syndrome. From these patients, 43.7% of the cells had a 2n–1 chromosomal complement, with other cells having 2n–2, 2n+1 and 2n–3 complements, with still many other secondary and minor modes including changes in chromosome structure. In some subjects, only 4.4% of the counted cells carried a normal diploid complement of 46 chromosomes. In the FAS infants with an abnormal karyotype (see above), all of their cells have an identical chromosomal aberration. This contrasts with the situation seen in alcoholics where the majority of their cells have an aberrant and variable chromosome constitution.

g. The findings from experimental studies used to test the hypothesis that exposure of pre-ovulatory oocytes to ethanol is capable of inducing aneuploidy

When ovulated unfertilized mouse eggs are briefly exposed in vitro to a dilute solution of ethanol in tissue culture medium, very high rates of parthenogenetic activation are achieved. Parthenogenesis is defined as the production of an embryo, with or without eventual development into an adult form, from a female gamete in the absence of any contribution from a male gamete. In mammals, this is largely an experimental phenomenon, but it is commonly employed as a reproductive strategy in many forms of animals and plants (for details, see Kaufman, 1983a). Cytogenetic analysis of the first cleavage mitosis of these embryos revealed that between 10 and 20% of the preparations examined displayed evidence of non-disjunction. In ~80% of the latter cases, either one extra chromosome was present or one missing from the expected complement. In the remaining 20% of this group, two extra chromosomes were involved in the malsegregation event (Kaufman, 1982). No evidence was found that specific chromosomes were more likely to be involved in these malsegregation events than other members of the complement (O’Neill and Kaufman, 1989). Parthenogenetic activation per se is not normally associated with non-disjunction, and it is the properties of the agent employed to induce this phenomenon that appear to be the critical factor (Kaufman, 1983a; O’Neill and Kaufman, 1989).

In a follow-up study, recently mated female mice were given a dilute solution of ethanol by mouth, and the chromosome constitution of the fertilized embryos determined at the first cleavage mitosis. Male mice bearing easily recognizable balanced translocation ‘marker’ chromosomes were used in order to establish whether the malsegregation event involved the oocyte- or sperm-derived chromosome set. In all instances where there was evidence of non-disjunction, this involved the oocyte-derived set of chromosomes (Kaufman, 1983a). Since both series of experiments involved the exposure of oocytes or recently fertilized embryos to ethanol during the time that they were completing the second meiotic division, subsequent studies were carried out to investigate whether exposure to similar levels of ethanol in vivo given during the completion of the first meiotic division produced a similar or different result (Kaufman and Bain, 1984). Cytogenetic analysis revealed that any non-disjunction observed was exclusively confined to the oocyte-derived chromosome set. Complementary studies in which unfertilized oocytes and very recently fertilized 1-cell stage embryos were exposed in vivo to the general anaesthetic Avertin produced almost identical results (Kaufman, 1977; Kaufman and Bain, 1984).

These experimental findings have confirmed
that exposure of unfertilized mouse oocytes to spindle-active agents during the first meiotic division can lead to non-disjunction. Since alcohol is the only spindle-active agent which is freely available to women during their reproductive lives, these observations would seem to indicate that it is the most likely causative agent in those spontaneous abortions with aneuploidy where no other factor is involved (Kaufman, 1984, 1985; Kaufman and O’Neill, 1988).

It has been stated that the risk of spontaneous abortion is twice as high in women drinking 1 oz. of absolute alcohol as infrequently as twice per week compared to matched controls (see Kline et al., 1980). These latter authors noted that it was unclear whether this was due to a fetotoxic rather than a teratogenic effect. If it is assumed that the alcohol was just as likely to have been consumed before as after the onset of pregnancy, then the effect could just as easily have been mutagenic on the mother’s germ cells. Mutagenicity tests indicate that ethanol, both by itself but principally via its metabolite acetaldehyde, is mutagenic in both male rodents (Badr and Badr, 1975; Barilyak and Kozachuk, 1981; Hunt, 1987) and female rodents (Alvarez et al., 1980), and is equally likely to be so in the human should exposure occur during appropriate stages of gametogenesis.

**GENERAL CONCLUSIONS**

Despite the fact that the CNS in the prenatal and early postnatal period, and the optic nerve in particular, is extremely sensitive to a wide range of teratogenic stimuli, it is its sensitivity to ethanol which should be the greatest cause for concern: (i) because of its unrestricted availability; (ii) its universal acceptance as a ‘harmless stimulant’. While the risk of exposure to alcohol during pregnancy is now fully accepted in ‘informed’ circles, to date, no appropriate legislation has appeared in the UK, as is in force in many other countries where wine and spirits are freely available to women during their reproductive lives. These observations would seem to indicate that it is the most likely causative agent in those spontaneous abortions with aneuploidy where no other factor is involved (Kaufman, 1984, 1985; Kaufman and O’Neill, 1988).

The author’s experimental findings indicated that the potential hazard of exposure of pre-ovulatory human eggs and, but to a lesser extent, recently fertilized embryos, to alcohol is at least as harmful as exposure to this agent during pregnancy, and consequently this should be an equal cause for concern. This specific topic was discussed in the House of Lords almost 10 years ago (Whaddon, 1987), but little information in this regard has apparently percolated down into the public consciousness in the interim years. The availability of such information should by now be within the public domain, and certainly should be readily available to medical practitioners and ancillary workers whose role it is to advise individuals who wish to maximize their chance of having a successful outcome to their pregnancy.

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**Note.** In a field of study as extensive as this, the publications cited should only be viewed as representative rather than exhaustive, and serve as a guide for those who may wish to pursue individual topics considered in this review in greater detail.

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