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7. The electrodes were made by inserting two diamel-coated Nichrome wires (40 μm in diameter: tip separation, 20 to 100 μm) into a short hypodermic steel needle. Careful positioning in the muscle optimized the isolation of motor unit potentials of discriminable wave form and amplitude that were clearly isolated from background activities, even in strong or brisk contractions. In order to minimize mechanical disturbances, a 15-cm loop of the flexible nichrome wires connected the electrode to the high-input-impedance differential amplifier.
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10. For extracting the twitch of a motor unit, the analog signal of the interosseous muscle myogram was averaged by a Nicolet model 1074 digital computer (words of 9 bits; bin width, 200 μsec) during steady voluntary contractions just exceeding the threshold of the chosen unit (fatigue effects were avoided). The spike of the motor unit initiated the averaging cycle through an electronic window trigger, except when the same unit fired in the interval from 90 msec before to 90 msec after any selected spike. This conditional rejection prevented distortion of the spike-averaged twitch by mechanical superposition of contractions of the same unit (*11*). Activities of other motor units sometimes influenced the relaxation profile of the twitch, but this was minimized by averaging as many as 512 samples.
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Fetal Alcohol Syndrome: Embryogenesis in a Mouse Model

Abstract. *When two small doses of ethanol were administered to pregnant mice during the gastrulation stage of embryogenesis, the embryos developed craniofacial malformations closely resembling those seen in the human fetal alcohol syndrome. Striking histological changes appeared in the developing brain (neuroectoderm) within 24 hours of exposure. Decreased development of the neural plate and its derivatives apparently accounts for the craniofacial malformations. The critical exposure period is equivalent to the third week in human pregnancy.*

Maternal consumption of ethanol severely affects the mental ability and facial appearance of at least one in 750 infants born in the United States (*1*). Ethanol consumption is associated not only with abnormal live births but also with an increased incidence of stillbirths and a tenfold increase in perinatal mortality (*2*).

In 1968 Lemoine *et al.* (*3*) described some characteristic defects in the offspring of alcoholics. However, it was not until 1973 that the pattern of malformations was more completely described and the term "fetal alcohol syndrome" (FAS) coined (*4*). There followed considerable interest in the teratogenicity of ethanol. Although clinical, behavioral, and epidemiological studies in humans and experimental studies in laboratory animals have shown that ethanol adversely affects development, critical exposure periods and underlying developmental alterations have not been clearly identified.

Major features of FAS are intrauterine and postnatal growth retardation, microcephaly, central nervous system dysfunctions (including mental retardation and hyperactivity), and craniofacial dysmorphology. Although many disorders feature mental and growth deficiency, Clarren and Smith (*5*) state that "it is the facial similarities among the children with the syndrome that unite them into a discernible entity" (Fig. 1, A and B). Typical of the facial characteristics are a narrow forehead, a flat midface, narrow palpebral fissures (eyelid openings), a short nose, a long upper lip with a narrow vermilion border, and a diminished or absent infranasal depression (philtrum).

Most experimental (*6*) and clinical studies have focused on the consequences of long-term exposure to ethanol. However, studies of mice by Webster and co-workers (*7, 8*) indicate that brief exposure on gestational day 7 or 8 is sufficient to produce severe facial malformations. Our study was designed to examine further the effects of brief ethanol exposure and the mechanisms underlying ethanol-induced abnormal development.

Procedures utilized by Webster and co-workers (*7, 8*) were modified in accordance with the suggestion (*9*) that two small doses administered intraperitoneally 4 hours apart on gestational day 7 might be more effective than a single larger dose. Female C57BL/6J mice were examined for vaginal plugs after being placed with males for 1 hour between 9:00 and 10:00 a.m. The day of plug detection was designated gestational day 0. The females received intraperitoneal doses of 25 percent ethanol (0.015 ml per gram of body weight) at 7 days 0 hours (10:00 a.m.) and again at 7 days 4 hours. Vehicle-treated controls were injected according to the same regimen.

Blood ethanol concentrations, as determined by a very accurate gas chromatographic technique (*10*), peaked at 193 to 215 mg per 100 ml of blood 20 to 25 minutes after each injection and then fell to about 30 mg within 4 hours. The behavioral alterations seen during the first 1½ hours after each injection were similar to the lethargy and ataxia observed in humans with comparable blood ethanol levels. Food and water consumption were normal the first night after ethanol administration.

We examined 72 live fetuses from ten litters for malformations 7 days after the injections (14 days after fertilization). The incidence of embryonic resorption in these litters was 18 percent, compared to 10 percent in the control litters. Females treated with the same doses only a few hours later had a much higher incidence of resorptions. These early embryonic deaths may be related to interference with heart development.

The most easily identifiable malformations involved the eyes. Thirty of the live fetuses had eye malformations, including coloboma of the iris, microphthalmia, and apparent anophthalmia (Fig. 1C). The right eye was affected more frequently and more severely. As in human FAS (Fig. 1, A and B), primary growth deficiency of the eye was reflected in shortened palpebral fissures. Short palpebral fissures are one of the most important diagnostic signs in FAS (*5*). Structural alterations of the eye and microphthalmia have also been seen in clinical cases (*11*).

The C57BL/6J mouse has a genetic predisposition for the types of eye malformations induced by ethanol exposure; a 12 percent incidence of microphthalmia, anophthalmia, and other eye defects was observed in our controls. Thus the ethanol-treated mice manifested a multifactorial threshold phenomenon. However, ocular anomalies have also been noted in other strains of mice chronically exposed to ethanol—including strains that do not have a high incidence of spontaneous ocular malformations (*12*).

Nine of the 30 live fetuses with eye

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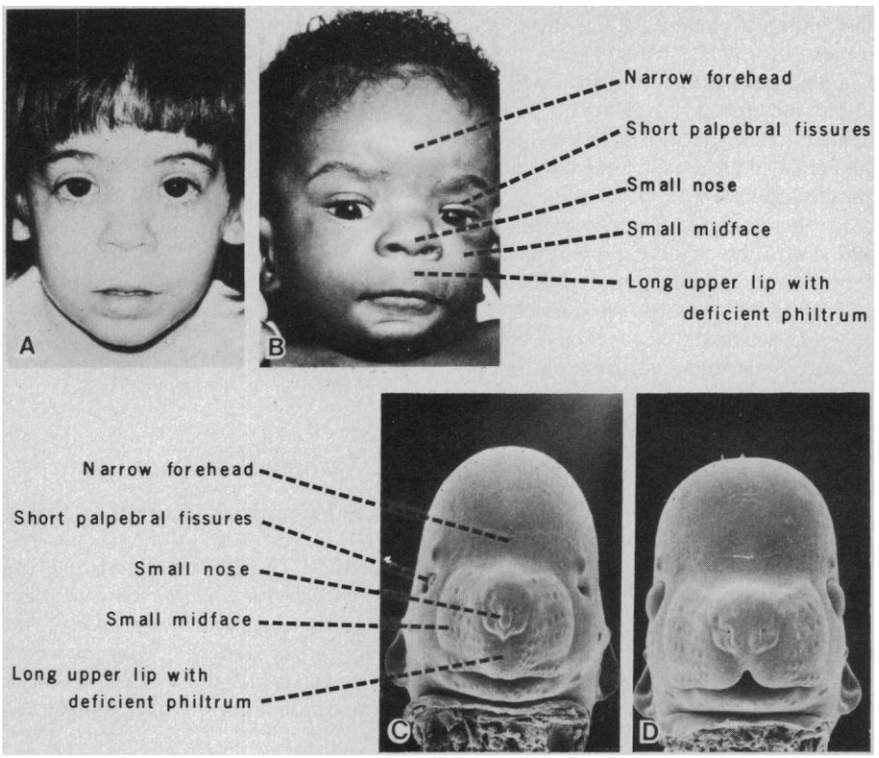

Fig. 1. Children with FAS (A and B) and 14-day-old mouse fetuses (×11.5) from ethanol-treated (C) and control (D) mothers. Both children had thin vermilion upper lip borders and were microcephalic, and the child in (B) had small corneas. The affected mouse fetus also had small eyes and was microcephalic. [Figure 1A reprinted from (*7*) with permission; Fig. 1B courtesy of R. S. Wilroy, Jr., M.D.]

defects also had abnormal nasal and upper lip regions; deficiencies were obvious in areas derived from the embryonic frontonasal prominence and its derivatives, in particular the medial nasal processes (*13*). A midline notch is present in the normally developing rodent upper lip, probably as the result of relatively large maxillary processes extending below the philtral area (Fig. 1D). Along with the frontonasal prominence and lateral nasal processes, the medial nasal processes contribute to the developing nose. The medial nasal processes form the columella, the philtrum, the portion of the dentoalveolar ridge containing the upper incisors, and the anterior portion of the hard palate. As shown in Fig. 1, C and D, hair follicles on the right and left maxillary processes were closer to the midline in the affected than in the normal mouse fetus. In embryos with deficient medial nasal process derivatives, the maxillary processes may meet at the midline to form a long upper lip.

We propose that in human FAS, the absent or diminished philtrum, the long upper lip, and the thin vermilion lip border result from the developmental alterations noted in this animal model. A deficiency in the medial nasal processes results in a reduced or absent philtrum, convergence of the maxillary processes, and a thin lip border. The small nose apparently results from deficiencies in the frontonasal prominence and the medial nasal processes.

We noted an overall decrease in embryo size 24 hours after ethanol exposure. Reduction in the size of the brain was particularly noticeable (Fig. 2, A and B). Examination with a scanning electron microscope revealed extensive bleb formation on the neuroepithelium (Fig. 2, C and D). The neuroepithelial blebs were also seen in 1-μm sections viewed under a light microscope (Fig. 2, E and F). The blebs contained cytoplasmic and nuclear components and usually extended from the neuroepithelium, rather than having settled there as extrinsic debris. Other tissues were affected less severely. In all tissues mitotic figures were prevalent, and no pycnotic nuclei were observed.

Two of 72 embryos examined on gestational day 14 were exencephalic or anencephalic, conditions which presumably result from the neuroepithelial alterations noted above. Anencephaly has been reported in children born to women who abuse alcohol, and other neural tube closure defects have been reported in children with FAS. Deficiencies in neural plate development at early stages would lead not only to abnormal brain morphogenesis but also to deficient eye formation, since the eyes develop at later stages as evaginations from the neural epithelium.

The presence of a narrow forebrain results in closely set olfactory placodes

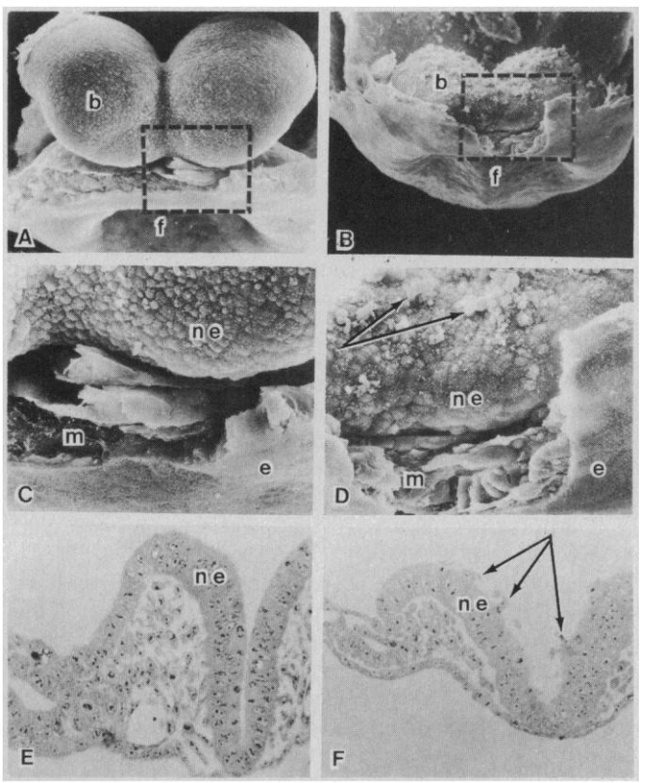

Fig. 2. (A to D) Scanning electron micrographs illustrating developmental differences between control (A and C) and experimental (B and D) embryos on gestational day 8. The outlined areas in (A) and (B) are shown at higher magnification (×495) in (C) and (D). In the treated embryo, blebs (arrows) stud the neuroepithelium (*ne*) of the brain (*b*). The mesoderm (*m*) and endoderm (*e*) appear relatively normal (*f*, foregut). (E and F) Micrographs of frontal sections through the brain of control (E) and treated (F) embryos, showing marked neuroepithelial differences, including prominent blebs (arrows) (×170). Changes in other tissues are less apparent.

and underdeveloped medial nasal processes (*14*). This was observed with scanning electron microscopy in 10- and 11-day-old embryos from ethanol-treated mothers. The inferior portions of the medial nasal processes were particularly diminished in size.

A remarkable finding of this study is that short-term ethanol exposure affects neural plate development at such an early gestational stage. At the time of ethanol exposure on day 7, the mouse embryos were undergoing gastrulation, or formation of the mesoderm. The mesoderm is responsible for induction and maintenance of the neuroepithelium. Although pronounced histological changes were noted only in the neuroepithelium, it is possible that disruption of the mesoderm was at least partially responsible for the neural deficiencies.

The striking teratogenic effect of ethanol at very early developmental stages provides excellent opportunities for studying the cellular and molecular mechanisms of ethanol teratogenesis, since very few cell types are present. Also, since mouse embryos can be grown in vitro at these stages, analysis of cellular activity and determination of direct effects of ethanol or its metabolites are possible.

In conclusion, ethanol, one of the most prevalent human teratogens, has a major effect in the mouse at a time corresponding to the third week of human gestation. Many women are not aware of their pregnancy at this stage. Those who are aware may not realize that social or binge drinking so early in pregnancy may be as deleterious to the embryo as constant heavy drinking. Results of a study by Hanson *et al.* (*15*) indicate a significant relation between alcohol consumption in the month preceding pregnancy recognition and FAS-type abnormalities. Further epidemiological studies in humans are needed to confirm or deny the existence of a critical period during which major features of FAS are determined.

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The Stroop Effect: Brain Potentials Localize the Source of Interference

Abstract. *The P300 component of the event-related brain potential was used in conjunction with reaction time to identify the locus of interference on the Stroop color-word test. Whereas response time varied with the congruence between the stimulus word and the color in which it was printed, the duration of stimulus processing, as indexed by P300 latency, remained constant. The results indicate that response competition is the primary source of Stroop interference.*

When a stimulus provides irrelevant as well as relevant cues, research latency is often affected by the degree of congruence between the cues. A classic example of interference from stimuli that provide conflicting cues is demonstrated by the Stroop color-word test (*1*). In the standard Stroop test, the time required to name the ink color in which a word is printed is increased if the word spells a conflicting color name (for example, the word *blue* printed in red ink). Explanations for the Stroop effect differ according to whether the source of interference from the irrelevant cue is attributed to stages of stimulus encoding (*2*) or response production (*3, 4*).

Numerous studies have been conducted with the aim of disentangling stimulus and response effects on Stroop performance. A confounding of possible effects of the conflicting cue on stimulus and response processes has made it difficult to distinguish between perceptual and response conflict models of Stroop interference through the use of behavioral measures alone. This difficulty could be surmounted, however, if a procedure were available for directly measuring the duration of a subset of the component processes that contribute to the total duration of the reaction time (RT) (*5*). There is convincing evidence that the latency of the P300 component of the human event-related brain potential (ERP) provides such a measure (*6*): P300 latency seems to index the duration of stimulus-evaluation processes and to be independent of the time involved in response production (*7, 8*). In this study, concurrent measures of RT and P300 latency identified response competition as the primary source of the Stroop interference effect.

Twelve male subjects (*9*) performed a discrete-trials version of the Stroop task, in which each stimulus was the word *red*, *blue*, or *town* printed in either red or blue ink. There were thus three categories of stimuli: incongruent (for example, the word *red* in blue ink), congruent (for example, the word *red* in red ink), and neutral (for example, the word *town* in red ink) (*10*). The six stimuli were presented with equal probabilities in a random sequence in blocks of 80 trials. Slides containing the stimuli were presented for 200 msec at the rate of one every 2 to 4 seconds. The experimental room was dimly lit, and external sounds were masked with continuous low-level white noise delivered through earphones.

In one condition, subjects were instructed to name the color of ink (*11*), and in a second condition, to read the word. The word-relevant condition was included to maintain the association between the printed word and its name and thereby prevent possible attentuation of interference with practice (*1, 12*). This condition also served to assess the extent to which the faster responses consistently observed in the word-naming task (*1, 13, 14*) are attributable to a reduction in stimulus-processing time.

Subjects responded vocally in both conditions (*15*). After practice, the two conditions were presented four times each in an alternating sequence, with the

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