

## Holoprosencephaly in a Fetal Macaque (*Macaca nemestrina*) Following Weekly Exposure to Ethanol

JOSEPH R. SIEBERT, SUSAN J. ASTLEY, AND  
STERLING K. CLARREN

*Departments of Pathology (J.R.S.) and Pediatrics (S.J.A., S.K.C.), Child Development and Mental Retardation Center (S.K.C.), Regional Primate Center (S.K.C.), University of Washington, Seattle, Washington 98195*

**ABSTRACT** Previous studies in rodents have indicated that the facial changes of fetal alcohol syndrome (FAS) closely resemble those of a mild form of holoprosencephaly. In order to examine this relationship in non-human primates, we evaluated a 133-day gestation macaque (*Macaca nemestrina*) with holoprosencephaly, median cleft lip and palate, and encephalocele. The mother had been given ethanol once per week (1.8 g/kg body weight) from weeks 2 to 19 postconception. Diagnosis of holoprosencephaly was made following ultrasound evaluation for polyhydramnios and delivery of the female fetus by caesarean section. Another fetus of identical age was delivered by caesarean section for use as a control. Both fetuses were studied by anthropometric, gross, radiographic, and histologic techniques. In the fetus exposed to alcohol, no extracranial anomalies were identified and the karyotype was normal. The brain was micrencephalic, with absent olfactory bulbs, tracts, optic nerves and chiasma, fused frontal lobes, and a single, dilated lateral ventricle; a parietooccipital encephalocele consisted of thin, dysplastic cortex bordering the ventricle; the cerebellum was dysplastic and superiorly displaced. Within the craniofacial complex, anophthalmia was bilateral; premaxillary components were absent, palatal shelves separate, the maxillae closeset, and the ethmoid bone small and deformed. Most of these defects are similar to those encountered in humans with holoprosencephaly and support the hypothesis of shared etiologic and pathogenetic relations between the facial anomalies of fetal alcohol syndrome and holoprosencephaly.

Studies in mice indicate that the changes of fetal alcohol syndrome (FAS) closely resemble those associated with mild forms of holoprosencephaly (Sulik and Johnston, '82; Sulik et al., '84). Several authors have indicated that the frontonasal anomalies in human FAS may also be seen as part of the facial dysmorphology associated with holoprosencephaly (Pfeiffer et al., '79; Jellinger et al., '81; Majewski, '81; Ronen and Andrews, '87). While no consensus has been achieved, the issue remains an important one. To identify alcohol as an etiologic agent responsible for holoprosencephaly in humans would broaden the teratogenic range of alcohol and possibly further the understanding of mechanisms responsible for the development of holoprosencephaly.

### CASE REPORT

As part of a continuing study of the teratogenic effect of binge drinking in different periods of gestation, 28 pregnant pigtail macaques (*M. nemestrina*) were randomized to one of four cohorts. Three cohorts received ethanol once per week (1.8 g/kg body weight by nasogastric tube) for the first 3 or 6 weeks of gestation, or for the complete 24-week gestation; the fourth cohort received a sucrose solution that was isocaloric and iso-lemic to the ethanol solution. One mother

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Address reprint requests to Joseph R. Siebert, PhD, Department of Laboratories, Children's Hospital and Medical Center, P.O. Box C-5371, Seattle, WA 98105.

TABLE 1. Comparison of somatic and craniofacial features<sup>1</sup>

Feature	Exposed	Control
Gestational age (days)	133	133
Sex	Female	Male
Body weight (g)	218	292
Crown-rump length (mm)	135	160
Foot length (mm)	53	60
Head length (mm)	53	63
Head width (mm)	40	49
Outer orbital distance (mm)	23	30
Inner canthal distance (mm)	6	7
Brain weight (g)	24	57

<sup>1</sup>Measures obtained after formalin fixation. See text for discussion.

in the 24-week exposure cohort developed polyhydramnios; her peak plasma ethanol concentration was 211 mg/dl, and she had previously given birth to three normal infants. Ultrasound examination in the 18th week demonstrated maldevelopment in the brain of the fetus. Caesarean section was performed at 133 days gestation and the female fetus died shortly thereafter. A karyotype was subsequently normal. An untreated male fetus of identical gestation (and different mother) was later delivered by caesarean section and used as a control. Both animals were studied by anthropometric, gross, radiographic, and histologic techniques.

#### RESULTS

The fetus exposed to alcohol was smaller than the control animal, as evidenced by decreases in body weight, crown-rump length, and foot length; the head was considerably smaller in length and biparietal width, and outer orbital and inner canthal distances were reduced (Table 1). A median cleft lip and palate were evident, the snout was flattened and eyelids asymmetric, and a parietooccipital encephalocele protruded through the calvaria (Figs. 1, 2). Radiographically, the premaxilla and upper central and lateral incisors were absent; the palate was cleft and the ethmoid complex small and deformed; and the medial orbital walls were intact (Fig. 3). A gross dissection of facial tissues revealed absence of the orbicularis oris in the region of the cleft. Histologic examination of the craniofacial complex confirmed the radiographic findings and in addition revealed bilateral anophthalmia; eyelids, conjunctiva, extraocular muscles, glands, and nerves were well-formed and focally edematous (Fig. 4).

The brain of the treated animal was severely microencephalic (Figs. 5, 6; Table 1). The frontal poles were fused and an encephalocele protruded from the convexity; olfactory bulbs, tracts, optic nerves, and chiasma were absent; and the lateral ventricles existed as a large, confluent space which extended to and occasionally ruptured through the cortical surface. In the left temporal pole, a distended subarachnoid space bordered areas of nodular cerebral dysplasia and cortical thinning. A defect in occipital cortex was apparent; the cerebellum and brainstem were displaced superiorly. Microscopic examination showed severe dysplasia of cerebrum and cerebellum.

#### DISCUSSION

To our knowledge, this is the first report of holoprosencephaly and associated changes of the craniofacial complex in a non-human primate. In addition, only two instances of naturally occurring cleft lip and palate in macaques are known (Hendrickx and Prahalada, '86). One pigtail macaque (*M. nemestrina*) was born at the Regional Primate Center, Seattle, WA, in 1983, and although retarded, lived nearly 3 years; the animal had a novel karyotype (42,XY/43,XY, + variable), termed *mosaic variegated trisomy* (Vigfusson et al., '86). A rhesus monkey (*M. mulatta*) with cleft lip and palate was described by Swindler and Merrill ('71) and likewise survived a number of years, serving as a breeder at the Primate Field Station in Medical Lake, WA. Autopsy information was unavailable for the two animals, but we surmise that neither had holoprosencephaly—at least of the severe variety—because of the length of survival and reasonably intact neurologic function.

The present case manifests a rare form of holoprosencephaly associated with anophthalmia, exencephaly (encephalocele), and anterosuperior displacement of posterior fossa tissues. Anophthalmia has been produced experimentally by a variety of agents, including ethanol, and has been reported in some cases of holoprosencephaly (Hogan and Zimmerman, '62; Warkany, '71; Torczynski et al., '77; Cook et al., '87; Siebert et al., '90). The condition represents a failure of optic vesicle outgrowth or, in holoprosencephaly, damage to prechordal mesoderm or anterior neural plate during or possibly prior to gastrulation. The association of holoprosencephaly and exencephaly/anence-

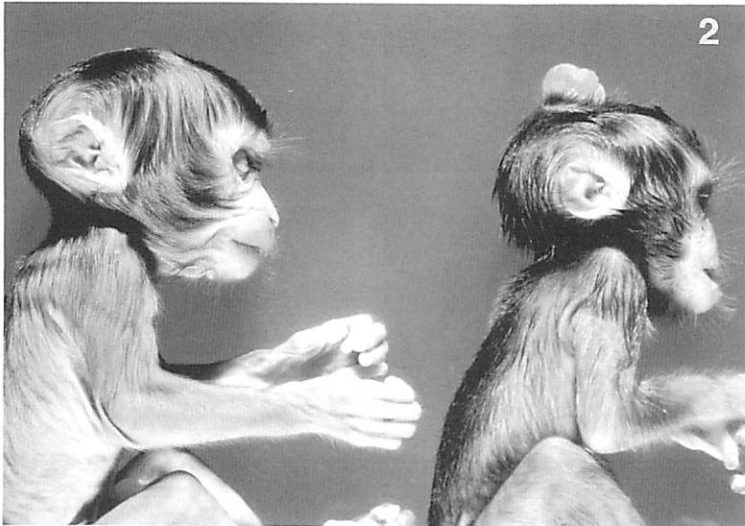
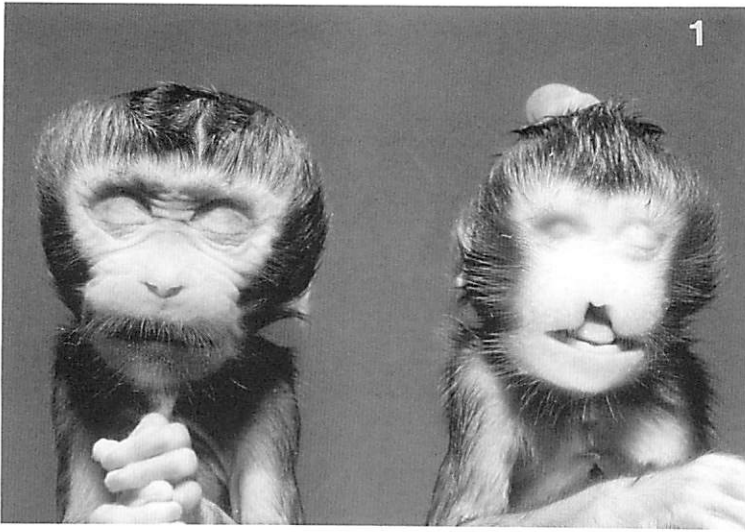


Fig. 1. Frontal view of fetal macaques (*Macaca nemestrina*). Gestational age of control (left) and treated (right) animals was 133 days. Median cleft lip and encephalocele are apparent in treated animal.

Fig. 2. Lateral view of control (left) and treated (right) fetuses. Note encephalocele and flattened snout in the latter animal.

phaly has been reported in a small number of humans, but those individuals had severe deficits in components of the anterior and middle cranial fossae (Lemire et al., '81; Siebert et al., '81). Disruption of posterior fossa tissues probably arose from tethering by the encephalocele during early development. Changes in the craniofacial skeleton of the present case are also considerably milder than those in more severe forms of holoprosencephaly, such as cebocephaly, ethmo-

cephaly, and cyclopia (Kokich et al., '82; Souza et al., '90). Reasons for these differences are unknown, although dosage, time of exposure, nutrition, and genetic predisposition are presumably among the more important factors.

The somatic changes observed in the present case represent a severe reduction in growth that is almost certainly due to the prenatal administration of ethanol. Differences in body size between the treated and

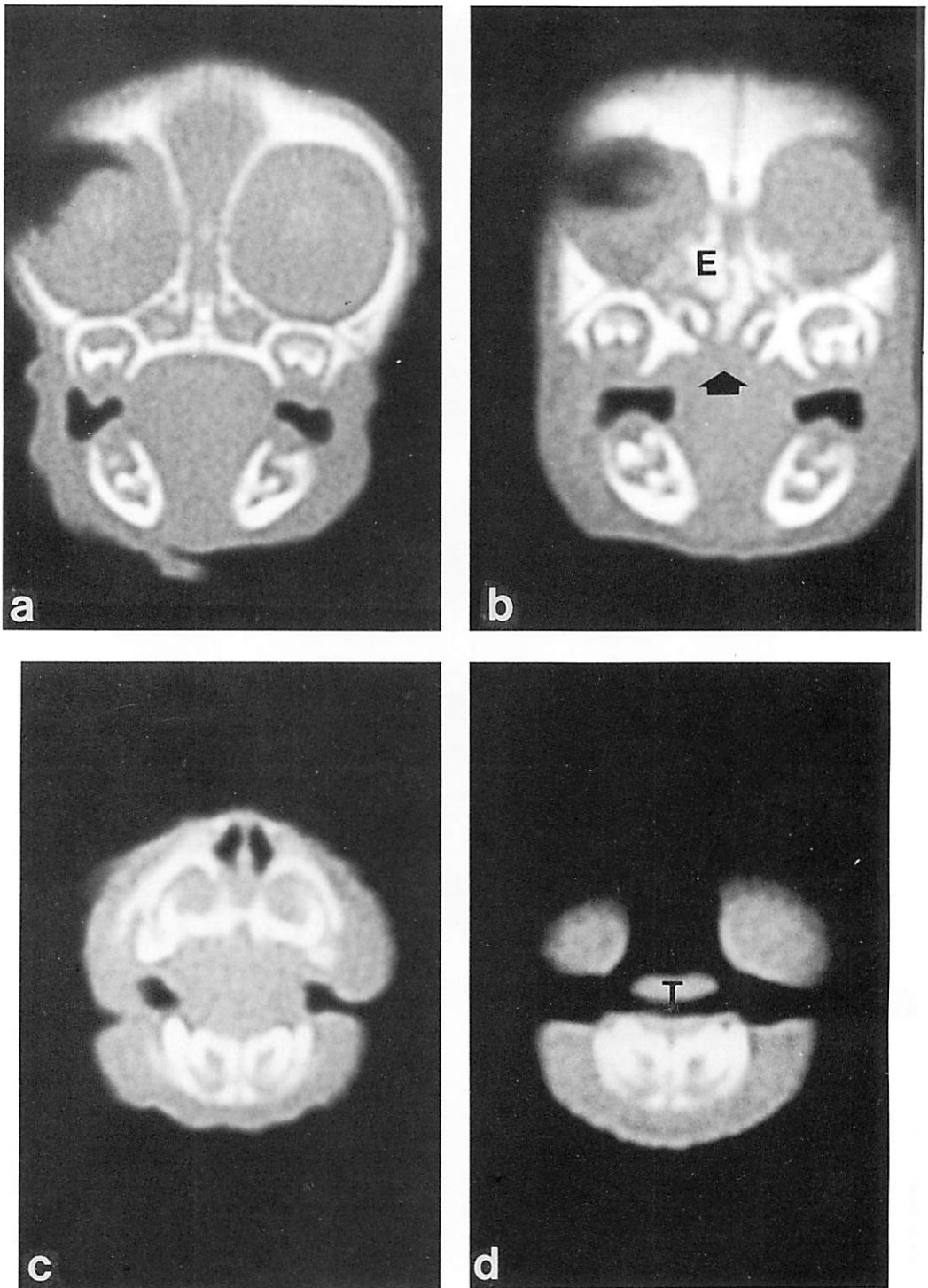


Fig. 3. Computed tomographic scans of control (a,c) and treated (b,d) fetuses. A dysplastic ethmoid bone (E) and cleft palate (arrow) are visible in b; in d, a median cleft lip, with absent central and lateral incisors, is seen above the tongue (T).

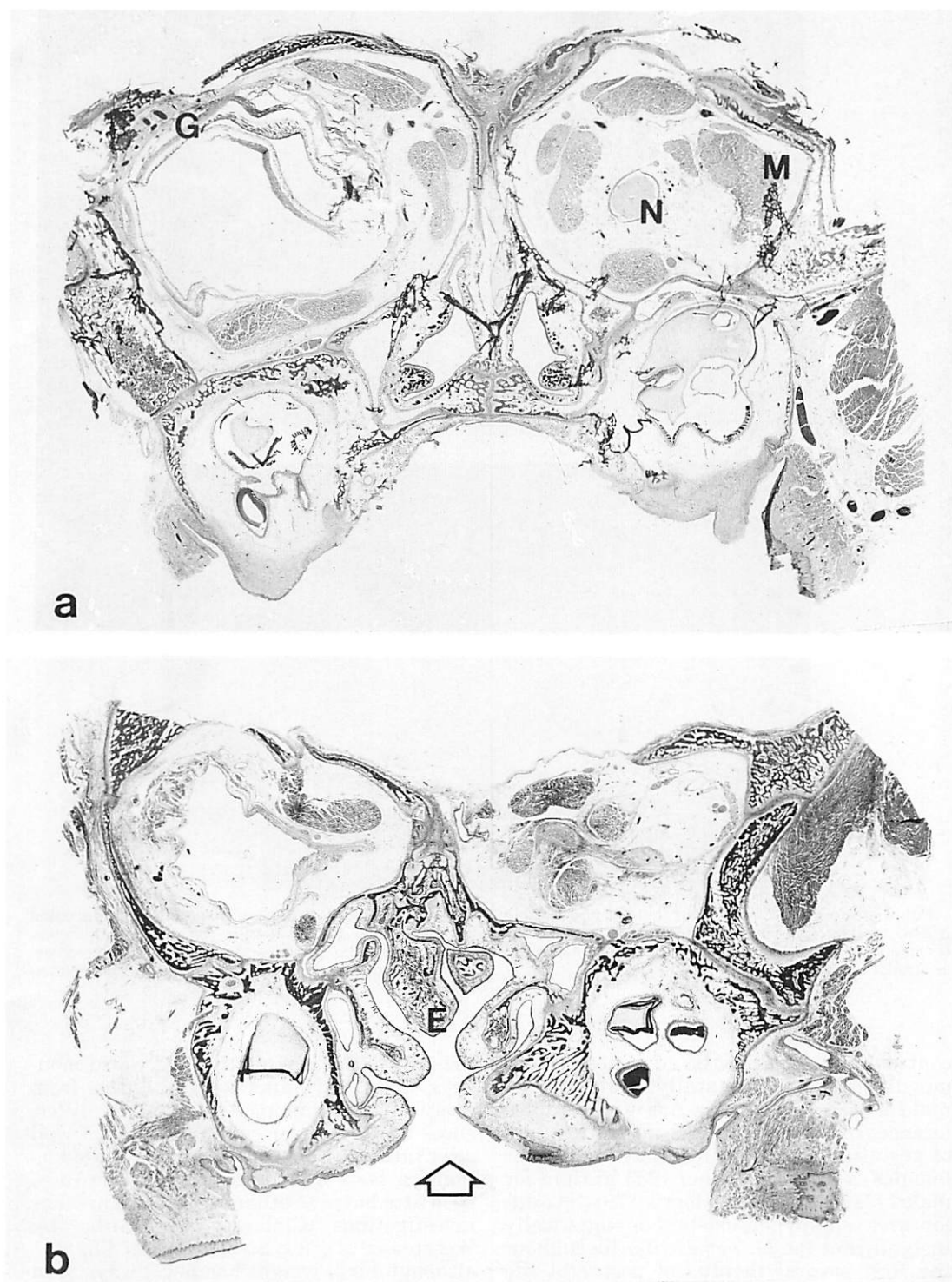


Fig. 4. Microscopic view of the craniofacial complex, sectioned coronally through the orbits, of control (a) and treated (b) animals. In both, orbital cavities are lined by conjunctiva and contain extraocular muscles (M),

nerves (N), and glands (G); serial sections revealed globes in the control animal only. Cleft palate (arrow) and dysplastic ethmoid bone (E) are evident in b. Masson's trichrome stain. Original magnification  $\times 6$ .

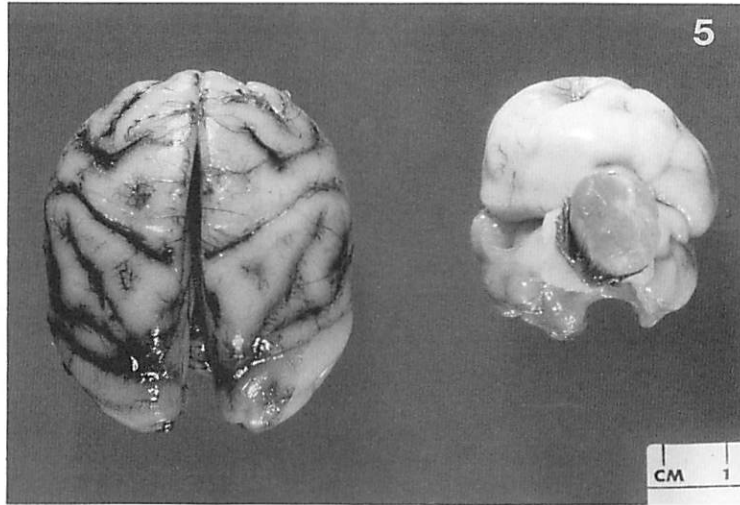


Fig. 5. Superior view of control (left) and treated (right) brains. Note single frontal lobe (top of figure), encephalocele, and occipital defect in the brain exposed to alcohol.

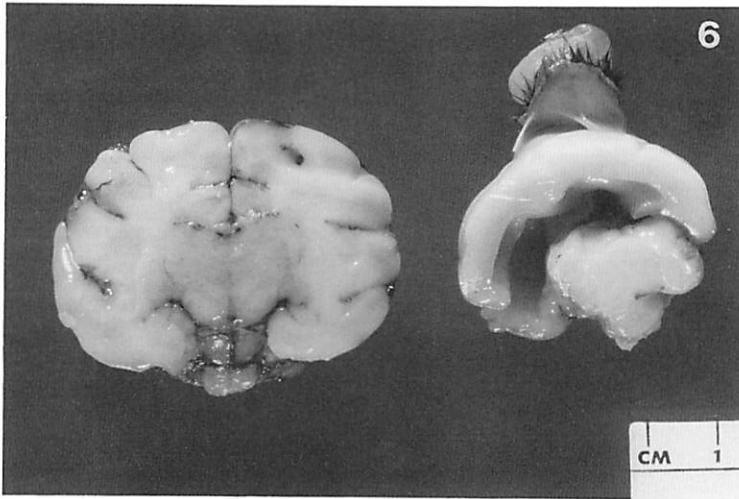


Fig. 6. Coronal sections of control (left) and treated (right) brains. Encephalocele, fused cerebral cortex, single ventricular cavity, and superiorly displaced cerebellum and brainstem are apparent in the treated brain.

control animals cannot be attributed to sexual dimorphism. Prenatally, male and female *M. nemestrina* show no significant differences in linear measures, and at 133 days of gestation, the expected body weight for females is actually higher (333 g) than for males (313 g) (Newell-Morris, '79). Sex differences remain non-existent or statistically insignificant for *M. nemestrina* throughout the first several months of postnatal life (Sirianni and Swindler, '85). This follows

the growth pattern of other old world monkeys, apes, and humans—and differs from most other mammals—in that sex differences fail to become pronounced until well after infancy (Laird, '67; Bogin, '88). Reductions in body and head size at term have been attributed to ethanol exposure in other investigations (Clarren and Smith, '78; Webster et al., '83; Streissguth et al., '85), although birth weight has not always been reduced in macaques exposed to high doses

prenatally (Clarren and Bowden, '82; Bowden et al., '83).

The clinical appearance of the frontonasal complex in mild forms of holoprosencephaly is quite similar to that of FAS. Subtle changes, such as wide inner canthal distance relative to palpebral fissures, short nose relative to midface length, indistinct, elongated philtrum, flat medial midface, and thin vermilion border of the upper lip, as well as the more overt findings of cleft lip and/or palate, are common to both conditions (DeMyer et al., '64; Cohen et al., '71; Jones et al., '73; Clarren and Smith, '78). The midface and anterior cranial base are reduced in both holoprosencephaly and FAS, and associated with choanal narrowing and a hypoplastic ethmoid bone (Johnson, '79; Siebert, '81; Frias et al., '82; Clarren et al., '87). The similarity in phenotypes raises the question of whether the two disorders have a common formative pathway. Put somewhat differently, do subtle anomalies of the frontonasal region (e.g., the facial changes of FAS) represent mild forms of holoprosencephaly? This appears to be the case in the rodent, where the administration of ethanol to pregnant mice produces holoprosencephaly and craniofacial changes equivalent to those in human forms of FAS (Sulik et al., '82; Sulik and Johnston, '83). These observations imply identical or at least overlapping modes of pathogenesis (i.e., Siebert, '83; Siebert et al., '85), although precise mechanisms need to be elucidated.

Previously, diabetes mellitus has been the only non-chromosomal disorder positively associated with holoprosencephaly in humans (Barr et al., '83). Other cases of holoprosencephaly have been associated with maternal alcohol abuse in humans, but only a few have been verified, particularly those reported by Jellinger et al. ('81), Ronen and Andrews ('87), and Bönnemann and Meinecke ('90). In light of the prevalence of FAS, it seems that cases of holoprosencephaly in alcohol-abusing mothers should be more common and easier to document. This is not the case, however. A variety of explanations are possible: It may be that alcohol teratogenesis in non-human primates and humans occurs at highly specific times or dosages. This has been demonstrated in several studies of rodents (Sulik and Johnston, '82; Webster et al., '83; Sulik et al., '85). In addition, Cohen ('89) has pointed out that

the comparative rarity of holoprosencephaly in FAS is to be expected, because holoprosencephaly is itself so rare. According to this view, if the prevalence of holoprosencephaly as a fetal alcohol effect were to increase 100 times, it would be observed in only 1 out of every 100–1,000 cases of FAS.

What are the implications of this study for humans? The model demonstrates that a single bolus of alcohol administered weekly initiates teratogenesis in the macaque. In this regard, the experiment mimics binge drinking as it occurs in the human. A dosage of 1.8 g/kg body weight in the macaque is the equivalent of 7 fluid ounces of whiskey, 23 ounces of wine, or 59 ounces of beer in a 55-kg woman (Dubowski, '76). These observations have obvious ramifications for basic scientists, medical workers, and, of course, for society. Despite the accumulating evidence for alcohol teratogenesis, it nevertheless seems unclear if the substance positively causes holoprosencephaly in humans. Both epidemiologic and individual case studies will therefore be necessary to resolve the issue. In the meantime, the appearance of the disorder in rodents and non-human primates requires that our suspicions remain high.

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