necessary or even desirable for any of the vast number of social and nonsocial animals that perform the same feats of navigation, but lack a symbolic language (*16*).

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9. Bees try to use *any* stimulus directly above them as if it is a part of the sky. A zenith sun has no azimuth, while a zenith sky pattern has only two possible interpretations. This explains the conflict between Frisch's report (*2*, p. 402) that bees treat small sources of white, polarized light as the sun, and the experiments of W. Edrich and O. von Helversen [*J. Comp. Physiol.* **109**, 309 (1976)], for which bees used white, polarized *zenith* stimuli as part of the sky. The difference between Frisch's finding (*1*) that bees need at least 10° to 15° of blue sky in order to orient to polarized light, and those of Edrich and Helversen in which bees oriented well to far smaller spots, is also explained. The natural sky used by Frisch made the data fall behind the 15° boundary of sky and sun in Fig. 1, while Edrich and Helversen's fall to the right of the 20 to 30 percent UV boundary. Bidirectional dances reported by others (*1*, *15*) probably result from using stimuli near the boundary of sun and sky. Color-opponent interneurons with these characteristics have been found in bees by J. Kien and R. Menzel [*J. Comp. Physiol.* **133**, 35 (1977)], and could be the innate releasing mechanism.
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13. Bees also appear to use a fourth rule. When shown an E-vector orientation that does not exist at the elevation chosen (Fig. 2), they still perform consistently oriented dances. We do not yet know how this rule works since the dance orientation does not seem to be predicted by any geometrical theory [for example, K. Kirschfeld and M. Lindauer, *Z. Naturforsch.* **30c**, 88 (1975)] of polarization orientation in bees. Indeed, this ability on the part of bees suggests that they may not use the Rayleigh scattering relationships at all (*8*, *15*).
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16. We thank R. Alexander, R. Dahl, K. Schenk, D. Thompson, E. Tyner, and H. Wildman for technical assistance and C. G. Gould, R. M. Shapley, W. G. Quinn, and especially D. R. Griffin, for valuable discussions. Supported in part by NSF grant BNS 76-01653 to J.L.G.

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Ethanol Embryotoxicity: Direct Effects on Mammalian Embryos in vitro

Abstract. *Exposure to ethanol retards growth and differentiation in cultured rat embryos during organogenesis. The development of untreated embryos is indistinguishable from growth in utero. These data suggest that the hypoplastic features of children born to chronically alcoholic mothers are due, at least in part, to a direct action of ethanol, which causes reduced embryonic cellular proliferation early in gestation.*

Excessive use of alcoholic beverages results in a variety of medical, psychological, and sociological disruptions that identify alcoholism as one of modern society's major problems. Since a characteristic pattern of congenital malformations associated with the offspring of alcoholic mothers was described (*1*), attention has been focused on the toxic effects of alcohol consumption in pregnancy (*2*). This fetal alcohol syndrome (FAS) has now been observed in more than 200 infants, and the relationship to chronic alcoholism is well established (*3*). However, the means by which FAS is produced are uncertain at present. It is not known if the developmental anomalies are the result of a direct action of ethanol or its metabolites on embryonic tissue, or if they are the product of altered maternal function, or a combination of such factors. In addition, it is not known if there is a sensitive period of gestation during which alcohol may exert teratogenic effects or if prolonged heavy drinking before pregnancy is a prerequisite for the complete FAS.

We are currently evaluating the embryotoxic potential of environmental agents in cultured rat embryos during a major portion of the organogenesis period. The culture system supports embryonic growth and differentiation indistinguishable from that in utero. Organogenesis is thought to be the interval of greatest embryonic sensitivity to environmental factors, and congenital malformations are most likely to be the result of teratogenic insult over this period. Our studies have shown that in embryos cultured in the presence of ethanol, both differentiation and growth were retarded as a function of dosage, but no gross alterations in morphogenesis were induced. To our knowledge, this is the first unequivocal demonstration of a direct action of ethanol on the developing mammalian embryo, without the confounding factors of altered maternal function, nutrition, or metabolism.

Our experiments were designed to investigate the development of embryos continuously exposed, during organogenesis, to ethanol at concentrations of 150 or 300 mg of ethanol per 100 ml of culture medium (*4*). Conceptuses were explanted from outbred rats (Charles River) during the afternoon of the tenth day of pregnancy (embryonic age, $9^1/_2$ days) (*5*). All operations were carried out aseptically, and no antibiotics were used throughout the study. Embryos within the yolk sac and amnion were dissected free of maternal decidua and Reichert's membrane, the ectoplacental cone being left intact. Two conceptuses were cultured in 4 ml of medium (*6*) contained in 30-ml serum bottles. During culture, bottles were kept in gentle motion by use of a roller apparatus (*6*), and the temperature was maintained at 37°C for the 48-hour culture period. The oxygen concentration in the gas phase of the bottles was increased from an initial 5 percent O_2 to 20 percent O_2 at 17 hours, and 40 percent O_2 at 26 hours (5 percent CO_2 at all times, the balance N_2). At least two conceptuses from each rat were randomly assigned to 300 mg of alcohol per 100 ml, 150 mg of alcohol per 100 ml, and control bottles. Alcohol was added to the medium at the beginning of the culture from a stock solution of ethanol which was at a concentration such that the osmolarity of the serum (305 mosmole/liter) was maintained (*7*). Control bottles received the same volumes of Hanks basic buffered salt solution isosmolar to the serum.

At the end of the culture, embryos and their associated membranes were examined, measured, photographed, and frozen for subsequent biochemical analysis. To estimate differentiation and abnormal organogenesis, we have devised a com-

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Table 1. Effect of ethanol on the in vitro growth of 9½-day rat conceptuses, given as means ± standard errors.

Growth after 48 hours of culture	Control culture (N = 18)	Ethanol per 100 ml of culture medium	
		150 mg (N = 11)	300 mg (N = 13)
Embryo			
Crown-rump length (mm)	4.54 ± 0.08	4.29 ± 0.12	3.78 ± 0.10*†
Head length (mm)	2.26 ± 0.04	2.15 ± 0.05	1.84 ± 0.07*†
Number of somites	29.2 ± 0.23	28.6 ± 0.37	26.6 ± 0.51*†
Total DNA (μg)	33.4 ± 1.68	31.9 ± 1.81	22.7 ± 2.13*†
Total protein (μg)	333.3 ± 13.5	295.4 ± 16.7	223.4 ± 17.1*†
Yolk sac			
Diameter (mm)	5.07 ± 0.08	5.05 ± 0.16	4.75 ± 0.14
Total DNA (μg)	8.58 ± 0.28	9.04 ± 0.71	8.61 ± 0.55
Total protein (μg)	169.8 ± 5.88	166.0 ± 14.4	151.2 ± 9.37
Placenta			
Total DNA (μg)	4.02 ± 0.36	4.68 ± 0.76	3.71 ± 0.50
Total protein (μg)	60.3 ± 7.70	96.8 ± 19.2	59.8 ± 14.6

*Significantly different from control values and from 150 mg/100 ml values (pairwise Mann-Whitney U test, $P < .01$). †Significant dose response (Jonckheere's test, $P < .01$).

prehensive morphological scoring system to grade the development of the yolk sac, placenta, and embryonic organ primordia according to observable morphologic features (*8*). This system makes it possible to determine embryonic development with an accuracy equivalent to ± 2 hours of gestation. Total protein and DNA contents (*9*) were measured after the tissues were homogenized by sonication.

Over the 48-hour period from embryonic age 9½ days to 11½ days, the rat embryo develops from the early neurula stage with 0 to 3 somites to the tail bud stage with 28 to 30 somites (Fig. 1). This period is equivalent to approximately 10 days of human embryonic development, from 20 days to 30 days of gestation. Tissues become extensively segregated into the primordia of the neural, sensory, cardiac, circulatory, and hepatic organs. Within this culture system, the growth of embryos in vitro was indistinguishable from growth during the equivalent period in vivo (*10*). Growth of the ectoplacental cone was severely reduced in vitro; nevertheless, a vigorous, functional, chorioallantoic placental circulation was established in cultured conceptuses.

Embryos cultured in the presence of ethanol showed a marked reduction in growth. The embryonic growth measures of length from crown to rump, total DNA, and total protein contents were significantly reduced in the 300-mg alcohol group, with a significant dose-response trend in all cases (Table 1). Growth measures for the yolk sac and placenta were not affected by the presence of alcohol. Not only was embryonic growth reduced, but differentiation was also retarded as a function of dosage. The morphological scores were 41.6 ± 0.4 for control embryos, 40.7 ± 0.3 for the 150-mg group, and 38.4 ± 0.8 (significantly different from control, $P < .02$) for the 300-mg group. Retarded development was also indicated by the dose-dependent reduction of the mean number of somites developed. Observations detected microcephalic growth of treated embryos, illustrated by reduced head lengths (Table 1). No gross structural defects were observed in either treated or control embryos.

Comparing the growth and development measures of embryos in the 300-mg group with equivalent measures for control cultures, we estimated that treated embryos were retarded by 5 to 7 hours of gestation. This value is consistent, whether based on morphological, mensural, or biochemical variables. From total DNA concentrations, we have calculated the cell contents and kinetics of cultured embryos (*11*). Embryos treated with 300 mg of alcohol per 100 ml have a deficiency of about 8.9×10^5 cells, compared with control embryos. This result is consistent with the calculated 5- to 7-hour retardation, which represents approximately two-thirds of the cell cycle time at this stage of gestation (*11*). The ratios of total DNA to total protein contents were not significantly affected by ethanol treatment, which suggests that cell size was not altered.

Investigations in our laboratories have demonstrated that the culture of rat embryos, according to this method, can be a sensitive system to detect developmental malformations. For example, dimethadione, the major metabolite of the anticonvulsant trimethadione, a known rodent and human teratogen (*12*), induced abnormalities of neural tube closure, cardiogenesis, mesoderm segmentation, cephalocaudal flexion, and brain stem development at concentrations of 2.5 to 10 m*M* (*13*). (By comparison, 300 mg of ethanol in 100 ml is a 33 m*M* solution.) In contrast, no gross defects were observed in embryos cultured in the presence of ethanol. However, developmental retardation during gestation, as seen in this study, is consistent with the major manifestations of FAS. The most frequent phenotypic features of FAS (prenatal and postnatal growth deficiencies, microcephaly, short palpebral fissures, mandibular and midfacial growth reduc-

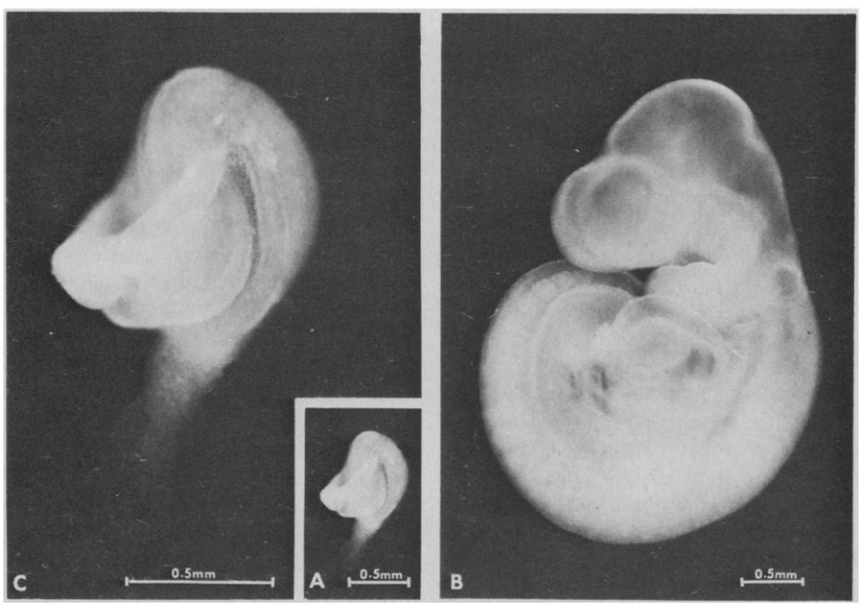

Fig. 1. Rat embryos at age 9½ days (A) and after 48 hours of culture (B). (A) and (B) are at the same magnification and show the extensive growth over the culture period. (C) A 9½-day embryo at ×2.5 greater magnification to illustrate the relative lack of differentiated tissue at this stage.

tions) are hypoplastic (*3*). Our observations suggest that these structural deficiencies may be the result of reduced cellular proliferation in the organogenesis phase, due to a direct action of ethanol (*14*). Clinical correlation of head size at birth with subsequent brain function has suggested that microcephaly is strongly related to mental retardation (*15*). Since we observed microcephalic growth in this study, the mental retardation seen in both fully and partially expressed FAS (*16*) may be the result of a direct inhibition by ethanol of neural growth early in gestation.

Our demonstration of ethanol-induced developmental retardation suggests that FAS may not be the result of maternally produced metabolites or altered maternal function. Whether the embryotoxic agent is ethanol itself or some other species produced by embryonic metabolism of ethanol is not yet clear. Current evidence, however, shows that embryos at this stage of gestation do not possess any ethanol-oxidizing or alcohol dehydrogenase activities (*17*). Although our results demonstrate that continuous exposure to high levels of ethanol exerts a direct toxic action on the developing embryo, the effects of short-term ethanol exposure have yet to be determined.

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Water, Protein Folding, and the Genetic Code

Abstract. *The absolute affinities of amino acid side chains for solvent water closely match their relative distributions between the surface and the interior of native proteins and are associated with a remarkable bias in the genetic code.*

Many processes of biological "recognition" require the stripping away (at least in part) of solvent water from interacting groups. The mutual affinities therefore reflect in part the ease with which they can be removed from solvent water, in addition to any specific forces of attraction or repulsion that may be present. We now report the free-energy changes associated with the removal of side chains of common amino acids from solvent water. These changes resemble the relative distributions of the amino acids between the surfaces and the interiors of native globular proteins, and are associated with a sharp bias in the genetic code.

The affinity of a compound for watery surroundings can be expressed quantitatively in terms of its free energy of transfer from the dilute vapor phase, in which intermolecular forces are virtually absent, to an aqueous solution so dilute that solute-solute interactions can be neglected. Results obtained for many compounds suggest that this measure of the

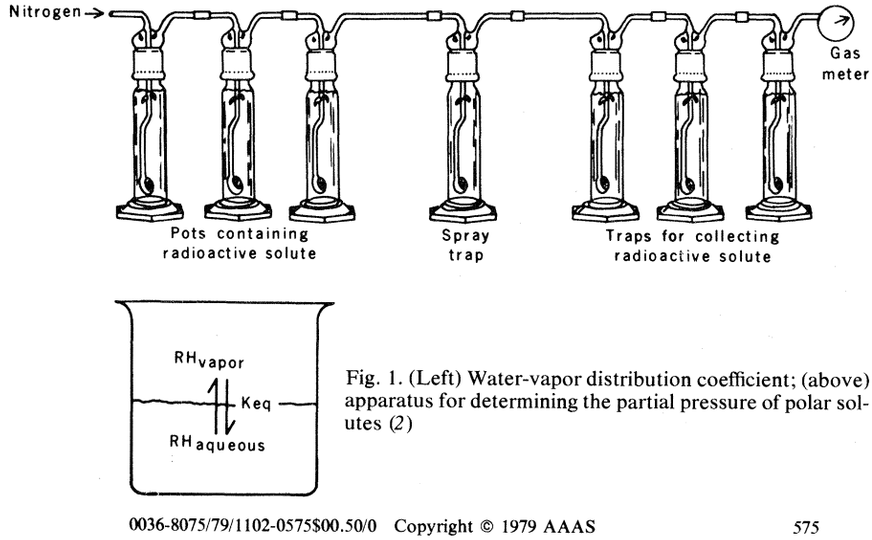

Fig. 1. (Left) Water-vapor distribution coefficient; (above) apparatus for determining the partial pressure of polar solutes (*2*)

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