The gill tissues of three mussels of this species had carbon isotope ratios ($\delta^{13}C$) of −51.8, −51.6, and −52.1 per mil, and the mantles of the same mussels had $\delta^{13}C$ values of −57.3, −52.1, and −52.3 per mil, respectively. Such extremely light numbers separate the seep mussels from the other (apparently sulfur-based) animal bacterial symbioses around these seeps, which have $\delta^{13}C$ values between −27 and −35 per mil (*6*). Since, as indicated above, the other symbioses tested do not appear to consume CH_4, the $\delta^{13}C$ values in the mussel may reflect the carbon isotopic composition of thermogenic CH_4 (−45 per mil) in its environment (*16*). The apparent homogeneity of the $\delta^{13}C$ values throughout the seep mussel tissues indicates that the carbon derived from CH_4 is distributed throughout the animal. Since CH_4 oxidation is taking place only in the gills, this implies the translocation of organic carbon derived from CH_4 from the gills to other tissues throughout the animal. The degree to which the $\delta^{13}C$ differs from that of the other organisms from the same environment also suggests that oxidation and incorporation of CH_4 carbon is a major nutritional input for this mussel. The stable carbon isotope data suggest that the $\delta^{13}C$ of animal tissue on the Louisiana slope may be useful for differentiating CH_4-based symbioses from sulfur-based symbioses. However, extrapolation of isotopic ranges between chemosynthetic systems (hydrothermal vent, subduction zones, brine seeps) is highly speculative, since a variety of processes can affect carbon isotope ratios, and CH_4 stable isotope ratios vary widely (*17*).

The question remains: what is the agent responsible for the oxidation? Optical and transmission electron microscopy (*18*) reveal the presence of abundant intracellular coccoid bacteria in vacuoles within the gills (Fig. 2a). Stacks of intracytoplasmic membranes, typical of type I methanotrophs (*16*), are visible in many of these bacteria (Fig. 2b). Type I methanotrophs have the ribulose monophosphate cycle and incorporate carbon from CH_4 into organic compounds (*16*). These symbionts are very close to the gill surface, which would facilitate CH_4 uptake from the seawater.

This symbiosis between a methanotrophic bacteria and an animal host is potentially able to derive a large fraction of its energetic and carbon needs from the consumption of the reduced single carbon compound CH_4 (*19*). This form of symbiosis may well be found in other vent and seep mussel species.

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19. Although many sulfur-fueled symbioses (lucinid clams excepted) are reputed to be rather repulsive food items because of their high sulfide contents, one of us (J.J.C.) has sampled this mussel and finds its flesh to be quite sweet and delicious.

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Effects of Alcohol on the Generation and Migration of Cerebral Cortical Neurons

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Prenatal exposure to alcohol produces many developmental defects of the central nervous system, such as microcephaly, mental retardation, motor dysfunction, and cognitive deficiencies. Therefore, the generation of neurons in the cerebral cortex was examined in the offspring of female rats fed a diet containing ethanol. Prenatal exposure to ethanol delayed and extended the period during which cortical neurons were generated, reduced the number of neurons in the mature cortex with the same time of origin, and altered the distribution of neurons generated on a particular day. Thus, the proliferation and migration of cortical neurons are profoundly affected by in utero exposure to ethanol.

ALCOHOL IS A POTENT TERATOGENIC substance, and as such it is the prime cause of mental retardation in the Western world (*1*). It is estimated that as many as 2% of all babies born alive are afflicted with fetal alcohol effects (*2*). Prenatal exposure to alcohol affects the development of various systems, including the musculoskeletal, cardiovascular, and peripheral and central nervous systems. Many of these defects are neurological and indicate abnormal development of the cerebral cortex, particularly motor areas. Such abnormalities include hypotonia, language and coordinative difficulties, and deficiencies in cognitive and fine motor skills (*1, 3*). Moreover, anatomical studies of humans and rodents prenatally exposed to ethanol show that their brains are smaller and contain ectopic cell clusters (*1, 4, 5*), both of which suggest that neuronal development is altered by ethanol. My study provides evidence that in the earliest stages of neuronal ontogeny—that is, neuronal proliferation and migration—cortical neurons are affected profoundly by prenatal exposure to ethanol.

The time of origin of cortical neurons was determined with [^{3}H]thymidine autoradiography in rats prenatally exposed to ethanol and in control rats. Pregnant hooded rats were fed one of two protein-enriched liquid diets from gestational day (GD) 6 to the day of birth, which was GD 23 for ethanol-exposed rats and GD 22 for control

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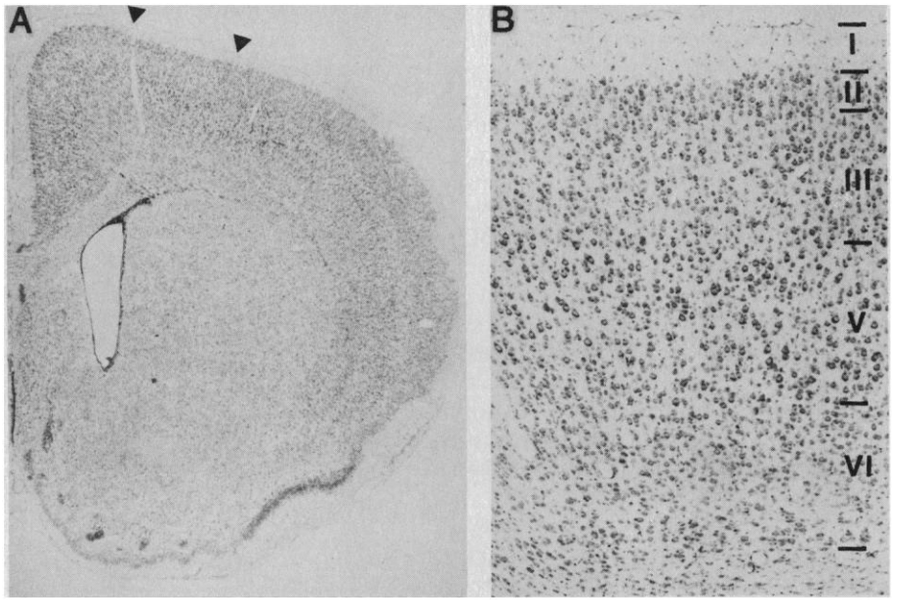

Fig. 1. Area 4, primary motor cortex, is an agranular field (A, between arrowheads) located on the dorsal surface of the frontal cortex. (B) The five layers composing area 4 are denoted by Roman numerals which increase from the pial surface to the white matter. Each layer can be distinguished by the size of the constituent neurons and by the relative cell packing density. Tissue was stained with cresyl violet. Magnifications: (A) ×10; (B) ×40.

rats (*6*). Ethanol constituted 6.7% of the total volume of the alcohol-containing diet and provided 37% of total caloric intake (*7*). A comparable rate of consumption for pregnant women would be 280 ml of alcohol per day, which is the equivalent of twelve 12-ounce cans of beer each day (*8*). In the control diet, dextrins and maltose were substituted for the ethanol so that the two diets were isocaloric. On each day during the period from GD 12 to GD 23, two females per dietary group were injected with [^{3}H]thymidine (*9*). After birth, all pups were cross-fostered to surrogate mothers that had not been fed ethanol. From each litter, four 21-day-old pups were anesthetized and killed by transcardial perfusion of 4.0% paraformaldehyde in 0.10*M* phosphate buffer. Each brain was embedded in paraffin and cut into a series of 10-μm-thick coronal sections. Sections were processed by standard autoradiographic techniques (*10, 11*) and stained with cresyl violet. Autoradiographs were analyzed by light microscopy, and the distribution of heavily labeled neurons (*10, 12, 13*) in motor cortex (*14*) (Fig. 1) was charted.

Prenatal exposure to ethanol had multiple actions. It altered the duration of the generation period, the number of neurons generated on a specific day, and the distribution of neurons with a particular time of origin.

In control rats, neurons in the motor cortex were generated as early as GD 13, and cortical neurogenesis was completed by GD 21 (Fig. 2). On the other hand, in rats exposed to ethanol, the first cortical neurons were generated on GD 14, and the last as late as GD 23. Thus, prenatal exposure to ethanol delayed the generation period by 1 day and extended it by 2 days.

Overall, an injection of [^{3}H]thymidine during the period from GD 13 to GD 19, when generation was most active, labeled fewer neurons in rats prenatally exposed to ethanol than in control rats (Fig. 2). The differences between the numbers of neurons generated at each age during this period in ethanol-fed and control groups were statistically significant ($P < 0.01$, t test for independent samples). After GD 19, however, there was an anomalous late surge in the generation of neurons in rats exposed to ethanol.

The decrease in labeling between GD 13 and GD 19 occurred despite an increase in the packing density of neurons in the motor cortex. The mean total number of neurons in a 200-μm-wide strip of motor cortex (an index of cell packing density) was greater in ethanol-exposed rats than in control rats (Table 1) ($P < 0.01$). This 14% increase in cell packing density was matched by a mean 13% decrease in the weight and volume of the cerebrum, a difference that was similar in all areas of neocortex (*5, 15*). Taken together, these data suggest that the numbers of neurons in the cortices of ethanol-fed and control rats are comparable. Thus, the changes in the generation of neurons over time indicate that the reduction between GD 13 and GD 19 is compensated by the transient increase after GD 19.

In control animals, the temporal change in the distribution of cortical neurons conformed to the classic inside-to-outside pattern of generation (*12, 16, 17*). Accordingly, neurons generated early resided in deep cortex and those generated late were in superficial laminae (Fig. 3). For example, neurons originating between GD 13 and GD 15 or on GD 16 or GD 17 were located in deep cortex—layer VI and layer V, respectively—and neurons generated on GD 20 or GD 21 resided in superficial cortex—layers II and III. Ethanol, however, markedly altered this pattern of neuronal generation. During the early stages, neurons generated on a particular day remained in a tangential band much as they did in control animals. The timing of the generation of the early neurons was only slightly altered by the ethanol exposure. Neurons generated on GD 14 to GD 16 or on GD 17 migrated to positions in layers VI or V, respectively. The generation of neurons in superficial layers, however, did not follow an orderly pattern. Although a few neurons generated on GD 20 migrated to their "correct" position in layer II, many additional neurons were gen-

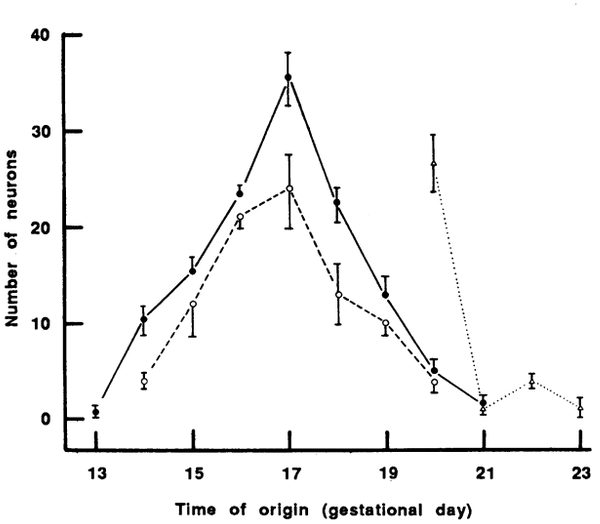

Fig. 2. Generation of cortical neurons. The number of neurons in a 200-μm-wide strip of motor cortex that was heavily labeled by an injection of [^{3}H]thymidine on one day during the period from GD 13 to GD 23 are plotted against the day of generation. Each value represents the mean of ten strips from four animals. Most of the neurons were generated before GD 20. The solid line describes neurons in control animals and the dashed line, appropriately distributed neurons from animals exposed to ethanol. Note the late surge in abnormally distributed neurons in the rats exposed to ethanol (dotted line).

Table 1. The number of neurons in each cortical layer. Each value was compiled from four 200-μm-wide radial strips of cortex from each of ten animals. Data are expressed as the mean ± SD.

Layer	Number of neurons		
	Ethanol	Control	Change (%)
II	72 ± 4	76 ± 11	−5.3 ± 2.1
III	135 ±13	115 ± 5	+17.4 ± 1.8*
IV	58 ± 3	50 ± 8	+16.0 ± 2.6*
V	186 ± 7	162 ± 11	+14.8 ± 2.4*
VI	241 ±15	203 ± 7	+18.7 ± 3.2*
Total	692 ± 8	606 ± 8	+14.2 ± 2.3*

*$P < 0.01$, t test for independent samples.

erated on GD 20, and they were distributed in layers V and VI. Furthermore, the few neurons generated on GD's 21, 22, or 23 resided in the white matter or in layer VI (Fig. 4) instead of taking a position in layer II as they do in control animals. This abnormal distribution of late-generated neurons in deep cortex was matched by alterations in the cell packing density of cortical layers (Table 1). Despite an overall increase in cell packing density for neurons in the cortex, the cell packing density was decreased in layer II and was increased in layers III to VI.

Although the mechanism by which ethanol affects neuronal development remains unclear, this study provides some insights. The delay in the generation period and the alterations in the numbers of cortical neurons generated on a particular day, in conjunction with evidence that ethanol reduces the syntheses of DNA, RNA, and proteins (*18*), indicate that neuronal proliferation is affected by prenatal exposure to ethanol. This interpretation may be confounded by the unknown role of ethanol-induced and naturally occurring cell death. That is, the decrease in the numbers of neurons generated between GD 13 and GD 19 may result from the death of neurons before or after their migration. The occurrence of neuronal death, however, does not account for the late burst of neuronal proliferation.

Studies of normal cortical ontogeny show that the orderly inside-to-outside generation of neurons establishes an orderly inside-to-outside migratory schedule (*12, 17, 19*). Therefore, the evidence of neurons generated on GD 20 in deep cortical laminae, coupled with the finding of a few, deeply disposed neurons generated on GD's 21, 22, and 23, suggest that ethanol produces defects in neuronal migration. Such defects may be caused by abnormalities or premature breakdown in the glial scaffolding that guides neuronal migration, the withdrawal of growth factors or recognition molecules, or a disruption of neuron-glial or neuron-neuronal interactions required for proper migration. Nevertheless, these ontogenetic defects in conjunction with documented errors in cortical differentiation (*20*) probably lead to the structural and connectional abnormalities (*5, 20, 21*) underlying symptoms such as mental retardation and motor dysfunction that are characteristic of children with fetal alcohol effects.

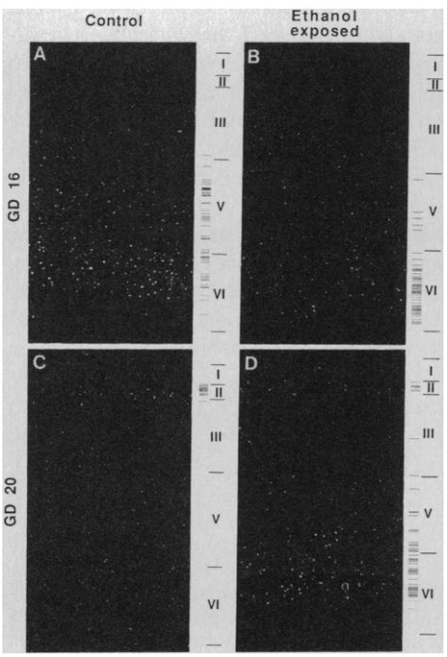

Fig. 3 (left). Dark-field autoradiographs depicting the effect of prenatal exposure to ethanol on the distribution of neurons generated on GD 16 or on GD 20. The lines to the right of each micrograph are a graphic representation of the radial distribution of heavily labeled neurons. In control rats, neurons generated on GD 16 (A) resided in layers V and VI of the mature motor cortex. In rats exposed to ethanol, most neurons that had been generated on GD 16 (C) were in layer VI. Unlike the control animals, in which neurons generated on GD 20 were exclusively in layer II (B), in rats prenatally exposed to ethanol, neurons generated on GD 20 were not confined to a single tangential band of cortex (D). While some neurons generated on GD 20 in ethanol-exposed rats resided in layer II, most were located in deep cortex; ×19.

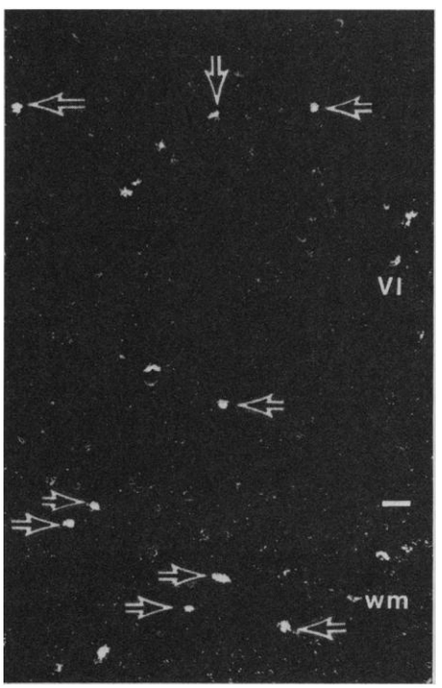

Fig. 4 (right). In rats exposed to ethanol, neurons generated on GD 20 (arrows) were abnormally distributed in layer VI and in the white matter (WM); ×80.

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7. The mean (±SD) consumption of the ethanol diet for all animals was 17.1 (± 1.4) g of ethanol per kilogram of body weight per day or 83 ml of liquid ethanol diet each day. The mean blood ethanol content in four pregnant females on GD 17 was 171 (± 20) mg of ethanol per deciliter.
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Bacterivory: A Novel Feeding Mode for Asteroid Larvae

RICHARD B. RIVKIN, ISIDRO BOSCH, JOHN S. PEARSE, EVELYN J. LESSARD

Planktotrophic larvae that occur beneath the annual sea ice in the Antarctic assimilate organic solutes and preferentially ingest bacteria, whereas they actively exclude phytoplankton. In regions where phytoplankton biomass is temporally limited by light or nutrient concentrations, the growth and development of planktotrophic larvae may not be directly coupled to phytoplankton production.

ECHINODERM LARVAE HAVE A COSMOPOLITAN distribution (*1*). They can ingest phytoplankton, bacteria, and detritus (*2–4*) as well as directly absorb dissolved organic solutes (*5, 6*). However, laboratory studies show that microalgae are the preferred food source for larvae of some tropical and temperate echinoderm species (*2, 3*). Food availability influences larval survivorship, and it has been suggested that low phytoplankton biomass, typical of some oceanic areas, may be insufficient to support the rapid growth and development of planktotrophic echinoderm larvae (*7, 8*). The tendency for increased brooding and lecithotrophic development at high latitudes (*1, 9*) has been attributed to the short growing season that results in low phytoplankton production and the slow developmental rates of the larvae (*10*). These conditions are thus unfavorable for planktotrophic larvae if they depend on phytoplankton as a primary food source. Echinoderms are a major component of the benthos in the Antarctic (*11*). Their planktonic larvae were first described almost 80 years ago (*12*), yet their reproduction and development have been examined only recently (*13*). In this report we describe the nutrition and feeding behavior of the larvae of the antarctic asteroid *Porania antarctica*. These larvae assimilated amino acids and glucose and ingested exclusively bacteria; they actively excluded as a food source all of the algal species that we tested. Field-collected larvae of other species also ingested bacteria as a source of food. These results suggest that the development of feeding larvae in the light-limited environment under the annual sea ice (*14, 15*) is not coupled directly to phytoplankton production.

Experiments were carried out in McMurdo Sound, Antarctica, during the 1985 austral spring (*16*). Ambient concentrations of amino acids and glucose but not of thymidine (TdR) were directly assimilated by *P. antarctica* (*17*). Assimilation rates of the amino acid mixture and glucose were significantly greater than that of leucine and glutamic acid (Table 1). These larvae were not treated with antibiotics (*6*). However, (i) the absence of direct [^{3}H]TdR uptake by the larvae (or gut-associated bacteria) and (ii) the negligible amount of filter-retained radioactivity (0.22 μm) (Table 1) imply that the washing procedure with sterile, filtered seawater removed whatever bacteria that may have adhered to the surface of the larvae (*18*). Thus the larvae mediated the uptake of these radioactive organic solutes.

Although particulate material was routinely observed within the gut of bipinnaria and brachiolaria larvae of *P. antarctica* that were fed bacterized algal cultures, the material did not exhibit chlorophyll a autofluorescence when examined by epifluorescence microscopy, and chlorophyll a was not detected in acetone extracts of the larvae. The ingestion of bacteria (bacterivory) and algae from natural populations and from bacterized cultures representing three classes of algae was assessed by dual labeling with [^{3}H]TdR and ^{14}C-labeled bicarbonate (*19*). Larvae of *P. antarctica* ingested ^{3}H-labeled particles exclusively. Particles labeled with ^{14}C were not ingested even at algal concentrations up to 35 μg of chlorophyll a per liter (Table 2). Clearance rates for *P. antarctica* that were reared in the laboratory and fed bacterized algal cultures were <0.01 μl per larva per hour and 43 to 70 μl per larva per hour for ^{14}C-labeled and ^{3}H-labeled particles, respectively. When *P. antarctica* fed on natural planktonic populations, clear-

Table 1. Incorporation of ^{3}H-labeled organic solutes by larvae of *P. antarctica*. L-Leucine (2, 3, 4, 5-^{3}H), L-glutamic acid (3, 4-^{3}H), the amino acid mixture (algal protein hydrolysate contained 8% L-alanine, 7% L-arginine, 8% L-aspartic acid, 12.5% L-glutamic acid, 4% glycine, 1.5% L-histidine, 5% L-isoleucine, 14.0% L-leucine, 6% L-lysine, 8% L-phenylalanine, 5% L-proline, 4% L-serine, 5% L-threonine, 4% L-tyrosine, and 8% L-valine), and D-glucose (6-^{3}H) were added at about 1.0 μCi ml^{-1} and thymidine (methyl-^{3}H) at about 0.1 μCi ml^{-1} final activity. Details of the experiment are in (*17*). The in situ concentrations of amino acids and glucose were not measured; however, previous studies (*30, 31*) have reported 0.1 to 5.0 μmol of dissolved free amino acids per liter. Glucose concentrations of 0.01 to 1.0 μmol liter^{-1} have been reported in the Sargasso Sea [R. E. Vaccaro, S. E. Hick, H. W. Jannasch, F. L. Carey, *Limnol. Oceanogr.* **13**, 356 (1968)]. Assimilation rates were calculated on the assumption that amino acid and glucose concentrations were 1.0 μmol liter^{-1} and 0.01 μmol liter^{-1}, respectively. Since minimum concentrations were used in these calculations, assimilation rates are conservative estimates. NS, not significant; radioactivity in larvae was not significantly greater than background radiation.

Substrate	Solute assimilation		Filter retained†
	Disintegrations per minute per larva*	Picogram per larva per hour	Disintegrations per minute per milliliter
L-Leucine	121,299 ± 15.6%, n = 8	31.5 ± 4.9	59
L-Glutamic acid	13,430 ± 13.5%, n = 9	19.8 ± 2.6	48
Amino acid mixture	100,783 ± 7.8%, n = 8	720 ± 56	52
D-Glucose	123,420 ± 13.4%, n = 9	238 ± 31	49
Thymidine	53 ± 18.2%, n = 9	NS	58

*Radioactivity accumulated by *P. antarctica* during a 4-hour incubation. Each value is the mean ± coefficient of variation. Background radioactivity was about 45 dpm. †Radioactivity in the 0.22-μm to 64-μm filtrate of the larval suspension. This represents potential bacterial contamination within the incubation container. Details in (*17*).

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