D. tertiolecta and 100 ppb for *E. gracilis* did not inhibit growth; *C. reinhardtii* was only slightly inhibited at a PCB concentration of 100 ppb.

Data on the amounts of PCB's in natural waters are limited. The low solubility of PCB's in water and their high solubility in lipids indicate that organisms, including algae, will accumulate PCB's from water, and that analysis for PCB's in water is an inadequate criterion for evaluating water quality (*2*). Thus, although PCB's were present in various marine organisms in Scotland, none were detected in the water (*16*). Nevertheless, high concentrations of PCB's in water may occur; PCB's freshly discharged into a Florida river reached concentrations as high as 275 ppb (*5*), well above the concentration that was lethal to the sensitive organisms in our experiments.

Selective inhibition of sensitive phytoplankton species by PCB's, DDT, and other stable pollutants in the environment may alter the species composition of natural algal communities (*10, 17*). Such effects at the base of aquatic or estuarine food webs could profoundly affect higher organisms as well.

JERRY L. MOSSER
NICHOLAS S. FISHER
TZU-CHIU TENG
CHARLES F. WURSTER

Marine Sciences Research Center,
State University of New York,
Stony Brook 11790

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11. *Thalassiosira pseudonana* (strain 3H), formerly *Cyclotella nana* [G. R. Hasle and B. R. Heimdahl, *Nova Hedwigia* **31**, 559 (1970)], was obtained from C. S. Hegre of the National Marine Water Quality Laboratory, Environmental Protection Agency, West Kingston, R.I.; *S. costatum* (clone "Skel") and *D. tertiolecta* (clone "Dun") were obtained from R. R. L. Guillard, Woods Hole Oceanographic Institution, Woods Hole, Mass.; and *E. gracilis* (strain *z*) and *C. reinhardtii* (strain 89) were obtained from H. Lyman and D. O'Kane, respectively, of the Division of Biological Sciences of the State University of New York at Stony Brook. For the culture of *T. pseudonana* and *D. tertiolecta*, Instant Ocean (Aquarium Systems, Eastlake, Ohio), adjusted to a salinity of 25 parts per thousand, replaced natural seawater in what was otherwise half-strength f-1 medium of R. R. L. Guillard and J. H. Ryther [*Can. J. Microbiol.* **8**, 229 (1962)]. *Skeletonema costatum* was grown in a medium prepared with water from Long Island Sound. The $NaHCO_3$ concentration was adjusted to 200 mg/liter in both media. Culture media were sterilized by filtration through 0.45- and 0.22-μm membrane filters (Millipore Corporation, Bedford, Mass.). All cultures were grown axenically.
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13 September 1971; revised 13 October 1971 ■

Sulfatide Synthesis: Inhibition by Experimental Allergic Encephalomyelitis Serum

Abstract. *The rate of ^{35}S incorporation into cerebroside sulfate in cultures of embryo mouse spinal cord shows a rapid acceleration at the time of myelin formation. Exposure of cultures to dilute serum from rabbits with experimental allergic encephalomyelitis results in almost complete inhibition of sulfatide synthesis. Within 24 hours after replacement of inhibiting medium with normal medium there is an increase in sulfatide synthesis followed by myelination.*

Incorporation of [^{35}S]sulfate into galactocerebroside to form sulfatide has been used for studying the formation and turnover of myelin and other membranous structures of the nervous system (*1*). Such studies indicate that in the developing rat brain there is a peak incorporation of sulfate to form sulfatide when the rat is about 18 to 20 days of age, the time of maximum myelination. It was further shown by McKhann and Ho (*2*) that the patterns of incorporation of [^{35}S]sulfate into sulfatide both in vivo and by brain homogenate in vitro are similar to the developmental pattern of activity of galactocerebroside sulfotransferase, measured in vitro.

Myelin first appears in cultures of spinal cord tissue of the embryo mouse on about day 8 to day 10 in vitro (*3*). By day 14 or day 15 in vitro the majority of cord cultures have an apparently normal complement of neurons, glia, and myelinated axons. When these cultures are constantly exposed to dilute serum from rabbits with experimental allergic encephalomyelitis (EAE), in the presence of complement, the differentiation of the oligodendroglia and the formation of myelin are totally inhibited. However, differentiation of neuroglia and primary myelination promptly follow the removal of EAE serum (*3*).

We now report that the developmental pattern of sulfatide synthesis in embryonic mouse spinal cord, in relation to myelination, is comparable to that in rat brain. In addition, a marked depression of sulfatide synthesis occurs during EAE serum inhibition with a prompt increase of sulfate incorporation when the nutrient medium containing dilute EAE serum is replaced by normal nutrient medium.

Tissue cultures of 13- to 14-day-old embryo mouse spinal cord were prepared and maintained as described (*3*). All cultures were derived from the same litter (called sister cultures) of CD Swiss mice (Charles River) and each cover slip bore two fragments of spinal cord. At the time of explantation, half of the cultures were fed and subsequently maintained on normal nutrient medium, and half were fed and subsequently maintained on nutri-

ent medium containing 3 percent EAE serum plus complement (EAESC). This amount of EAESC inhibits completely the formation of myelin. Sister cultures maintained on normal medium developed normally with myelinated axons (*3*).

In our experiment cultures maintained on normal nutrient medium developed easily identifiable myelin after 10 days in vitro, while the inhibited cultures, maintained on nutrient medium containing 3 percent EAESC showed no myelination. After 14 days in vitro, a group of the inhibited cultures were drained of inhibiting medium, washed in two changes of Simm's balanced salt solution (BSS), and subsequently maintained on normal nutrient medium. These cultures (which are referred to hereafter as disinhibited) began to develop visible myelin 4 days later.

The rate of synthesis of sulfatides was determined by measuring the rate of [^{35}S]sulfate (*4*) incorporation into cerebroside sulfate of the tissue cultures. Culture feeding medium containing $4.0 \times 10^{-4} M$ sulfate with and without EAESC was prepared to contain approximately 1.2×10^{9} count/min per micromole of sulfate. The feeding medium was drained at specific times (Fig. 1) from groups of the sister cultures and replaced with 50 μ of radioactive feeding medium. After a 24-hour incorporation period, a portion of the feeding medium was taken to determine its exact ^{35}S activity to correct for dilution by the residual nonradioactive medium. The cover slips bearing the cultured fragments were washed in two changes of BSS for 5 minutes each, drained for 10 seconds, frozen by immersion for 30 seconds in Freon 12 chilled in a bath of Dry Ice and acetone, and dried at reduced pressure at −25°C. The dried cultures were peeled from the collagen substrate and placed in small tubes containing 50 μg of dry nonradioactive cerebroside sulfate (*5*). Lipid was extracted from the dried tissue with a mixture of chloroform, methanol, and H_2O (16 : 8 : 1) (*6*). To each tube was added 200 μl of the solvent mixture, and the tubes were stoppered and allowed to stand overnight in the refrigerator. The solvent was removed, and the residue was further extracted three times with 116 μl of solvent each time. Pooled extracts from each sample were evaporated to dryness under a stream of nitrogen in glass-stoppered

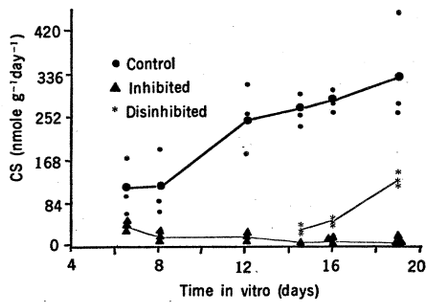

Fig. 1. The rate of ^{35}S incorporation into cerebroside sulfate (CS). The results are expressed in nanomoles of [^{35}S]sulfate incorporated in cerebroside sulfate during a 24-hour period, beginning on the days indicated. Each point represents incorporation by one culture (one cover slip bearing two fragments), and the lines were drawn through the mean values for each day. The actual activity of ^{35}S incorporated per culture was as follows: control, 2000 to 10,000 count/min; inhibited, 75 to 2000 count/min; and disinhibited, 850 to 4500 count/min. The amount of [^{35}S]sulfate incorporated was calculated from the determined ^{35}S specific activity in the feeding medium. All radioactivity measurements were made at the same time, and thus no correction for ^{35}S decay was necessary. The amount of protein per culture ranged from 16 to 34 μg.

test tubes. To each tube was then added 2.0 ml of lower phase and 0.4 ml of upper phase of a previously prepared partition system, namely, chloroform, methanol, and 0.74 percent KCl (8 : 4 : 3) (*7*). The tubes were shaken and centrifuged for a short time; the upper phase was then removed. The lower phase, containing the sulfatide, was washed with four successive changes of 0.4 ml each of upper phase to remove nonincorporated [^{35}S]sulfate. A 1.5-ml portion of the washed lower phase was removed to a fresh tube and evaporated to dryness under nitrogen. The residue was dissolved in 50.7 μl of a mixture of chloroform and methanol (1 : 1) and 38.9 μl of this were applied to silica gel G thin-layer plates. After chromatographic development in a chloroform, acetone, methanol, acetic acid, and water system (5 : 2 : 1.5 : 0.74) (*2*), the cerebroside sulfate spot, visualized by iodine adsorption, was scraped from the plate and eluted with methanol; the radioactivity was then counted in Bray's solution (*8*) in a Packard Tri-carb liquid scintillation spectrometer. No detectable radioactivity could be found on the thin-layer chromatography plate outside of the sulfatide region. The residue from the original lipid extraction was dissolved in 72.5 μl of 1*N* NaOH and the protein content was determined by modification of the method of Lowry *et al.* (*9*) for noncollagen protein (*10*).

In normal cultures the rate of sulfatide synthesis increases with the number of days in vitro, with the sharpest increase occurring between day 8 and day 12, just before the appearance of myelin (Fig. 1). Cultures exposed to EAESC showed no myelination, and the rate of sulfatide synthesis was extremely low and decreased with days in vitro. When inhibited 14-day cultures were disinhibited, sulfatide synthesis more than tripled during the first 24 hours and then increased at a rate comparable to that in the control cultures at the time of myelination.

In cultures of normal mouse embryo spinal cord, the increase of sulfatide synthesis in relation to myelination is similar to that reported for rat brain (*2*). The results with the inhibited and disinhibited cultures show that EAESC does not simply inhibit the formation of myelin but also prevents the synthesis of at least one myelin component. The inhibition of sulfatide synthesis may reflect an inhibition of the synthesis of precursor cerebrosides or sulfate activation. Since, in the EAESC-inhibited cultures, no differentiated oligodendroglia cells can be identified while neurons appear normal except for the absence of myelin (*3*), our results suggest that the major site of cerebroside synthesis or sulfatide synthesis or both is in the oligodendroglia cells. On day 12 in vitro, when myelin is easily detectable in control cultures, the rate of synthesis of sulfatides in the inhibited cultures is about 7 percent of that in the control cultures. On day 19, the rate of sulfatide synthesis in the inhibited cultures is about 2 percent of that in normal cultures. These data are consistent with a close association between the capacity of the tissue to incorporate sulfate into lipid and the formation of myelin.

JUNE M. FRY
GERARD M. LEHRER
Department of Neurology, Division of Neurochemistry, Mount Sinai School of Medicine of the City University of New York, New York 10029
MURRAY B. BORNSTEIN
Saul R. Korey Department of Neurology, Albert Einstein College of Medicine of Yeshiva University, New York 10461

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27 July 1971; revised 24 September 1971

Antibody-Dependent Lymphoid Cell–Mediated Cytotoxicity: No Requirement for Thymus-Derived Lymphocytes

Abstract. *The capacity of lymphoid cells from nonsensitized mice to lyse antibody-coated target erythrocytes in vitro does not require the presence of thymus-derived or thymus-dependent lymphocytes. Thus, spleen cells from thymus-deprived mice and spleen cell populations from which thymus-dependent lymphocytes had been removed were fully competent to mediate destruction of antibody-coated target cells. However, prior treatment of spleen cell populations with antibody to κ chains diminished this function, suggesting a role for bone marrow–derived lymphocytes.*

Lymphoid cell–mediated cytotoxicity has been studied as a model for allograft and tumor immunity. Several models of cell-mediated destruction of target cells in vitro have been described (*1*). In one instance, lymphocytes from sensitized donors cause the lysis of target cells. In this model, thymus-derived or thymus-dependent (T) lymphocytes are required for cell destruction (*2*).

By contrast lymphoid cells from nonsensitized donors lyse antibody-coated target cells (antibody-dependent cell-mediated cytotoxicity) (*3*) or lyse target cells in the presence of phytohemagglutinin (*4*). We now report that antibody-dependent lymphoid cell–mediated cytotoxicity does not require the presence of T lymphocytes. Thus, spleen cells from nonsensitized, thymus-deprived mice and spleen cell populations from which T lymphocytes have been removed by lysis with antiserum to θ (an antigen on the surface of T lymphocytes) together with complement are fully competent to mediate this reaction. In addition, prior treatment of spleen cell populations with antibodies to immunoglobulin light chains of the κ class diminishes their capacity to lyse antibody-coated target cells.

Table 1. Capacity of spleen cells from thymus-deprived mice to mediate antibody-dependent cytotoxicity.

Cells ($\times 10^6$)	Anti-serum	Lysis (%) Exp. 1	Lysis (%) Exp. 2
		Intact	
5	+	74.1	61.2
2.5	+	57.7	
1.25	+	40.9	
5	−	7.5	7.1
		Thymus-deprived	
5	+	83.1	82.2
2.5	+	72.7	
1.25	+	57.4	
5	−	8.8	8.3
0	+	12.6	

The ability of lymphoid cells to effect antibody-dependent cell-mediated cytotoxicity was evaluated by the release of radioactivity from antibody-sensitized burro erythrocytes labeled with ^{51}Cr. A lymphoid cell suspension (1 ml; 1.25 to 5×10^6 cells) in tissue culture medium (*5*) was mixed with ^{51}Cr-labeled (*6*) burro erythrocytes (0.5 ml; 2×10^5 cells) and with heat-inactivated, guinea pig antiserum to burro erythrocytes (25 μl of a 1:2 dilution) (*7*); the final dilution was 1 : 130. Controls without added lymphocytes and others without added antiserum were always included in the experiments. Unlabeled sheep erythrocytes (3×10^7; 0.1 ml) were added to all mixtures to prevent spontaneous lysis of the target cells (*6*). The reaction mixtures were incubated in duplicate tubes at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 for 24 hours, and the total radioactivity was measured. After centrifugation for 10 minutes at 1000 rev/min, the radioactivity of a measured portion of supernatant was determined. Cell lysis was expressed as the percentage of the total radioactivity released from cells into the supernatant.

Thymus-deprived mice are deficient in T lymphocytes (*8*). Therefore the effectiveness of a spleen cell population from CBA mice that had been thymectomized, lethally irradiated, and reconstituted with syngeneic bone marrow cells (*9*), was compared with that of spleen cells of normal CBA mice (Table 1). The spleen cells from thymus-deprived animals were no less capable of mediating cytotoxicity than were cells from normal mice. Indeed, they were somewhat more effective than cells from normal mice. This strongly suggests that T lymphocytes are not necessary for this type of lymphocyte-mediated cytotoxicity.

Another approach to the analysis of the relative role of T lymphocytes is to remove such cells from lymphoid cell populations of normal animals through immune cytolysis with antiserum to θ (anti-θ) (*10*). Spleen cell suspensions were prepared from 8-week-old male BALB/c mice. Lymphocytes bearing the θ antigen were removed from the population as follows: Fifty million cells were incubated with 0.2 ml of undiluted AKR antiserum to θ · C3H (*11*), in a total of 1.0 ml of tissue culture medium for 15 minutes at 4°C. The cells were layered on fetal calf serum (FCS) and centrifuged to remove unbound antiserum; the sedimented cells were incubated in 1.0 ml of medium containing a 1:8 dilution of guinea pig complement (*12*) for 30 minutes at 37°C. The cells were then washed four times by centrifugation through FCS (as described above) and enumerated. Control populations were treated in an identical fashion, except that antiserum was omitted. As a measure of the effectiveness of depletion, the number of θ-positive lymphocytes remaining after the above procedures was estimated as follows. Portions of the lymphoid cells were labeled with ^{51}Cr

Table 2. Effect of treatment of mouse spleen cells with anti-θ and complement (C) on their capacity to mediate antibody-dependent cytotoxicity.

Spleen cells: Prior treatment	Spleen cells: θ-Positive remaining (%)	Spleen cells: No. ($\times 10^6$)	Lysis (%)
	Experiment 1		
Medium + C	20	5	76.3
		2.5	61.2
		1.25	41.8
Anti-θ + C	0	5	74.9
		2.5	57.9
		1.25	41.7
	Experiment 2		
Medium + C	30.9	5	41.6
Anti-θ + C	0	5	40.1