pretreatment with HMD 1 hour prior to administration of 5-HTP. When challenged with apomorphine 3 hours later, these rabbits displayed hyperthermic and behavioral responses indistinguishable from the responses of rabbits that had not been pretreated.

Table 1 shows the comparison between concentrations of 5-HT in the brainstems of animals from all four groups. Concentrations of 5-HT are reduced to approximately 30 percent of normal after pretreatment with *p*CPA at the doses and times described above. Administration of HMD and 5-HTP to animals pretreated with *p*CPA restored concentrations of 5-HT to their normal levels. Elevation of 5-HT by means of HMD and 5-HTP alone produced a 60 percent increase over control levels.

Our results indicate that 5-HT has an essential role in the mediation of the hyperthermic response of rabbits to apomorphine (*7*). This conclusion is based on the finding that, after depletion of cerebral 5-HT stores by *p*CPA, apomorphine is unable to elicit its characteristic increase in rectal temperature. However, after central 5-HT concentrations are restored to normal, apomorphine can again induce a hyperthermia. Although this restored response is dissimilar to the control response in magnitude and duration, the intimate involvement of 5-HT in the hyperthermic response to apomorphine is clearly implicated. The differences between control and restored responses may reflect insufficient replenishment of 5-HT stores in specific brain regions. The uptake of 5-HTP into adrenergic and dopaminergic nerve terminals and subsequent conversion to 5-HT via the action of ubiquitous *l*-aromatic amino acid decarboxylase may contribute to the increase of total 5-HT in the brainstem.

A number of laboratories have reported the failure of 5-HT depletion with *p*CPA to disrupt stereotypic and gnawing behaviors induced by apomorphine in rats (*8*). This is, however, inconsistent with a recent report in which midbrain raphe lesions selectively blocked apomorphine-induced gnawing in rats (*9*). In our study, the behavioral effects of apomorphine in rabbits appear to be independent of central 5-HT, inasmuch as the behavior of animals after injection in all four groups was essentially identical.

Our hypothesis that apomorphine exerts its hyperthermic action via a 5-HT mechanism is consistent with other studies from our laboratory which indicate tryptaminergic neuronal substrates for certain agents that alter thermoregulation (*10*). This is, however, to our knowledge, the first finding of a dopaminergic–5-HT interaction in temperature responses to pharmacologically active agents (*11*).

Table 1. Changes in concentrations of brainstem 5-HT after various pretreatments. Values are expressed as the means ± the standard error of the mean (S.E.M.); *N* indicates the number of animals used.

Prior treatment	*N*	5-HT content (μg/g) ± S.E.M.	Control level (%)
None	9	0.58 ± 0.03	100
*p*CPA	11	.18 ± .03*	31
*p*CPA + HMD + 5-HTP	7	.61 ± .04	105
HMD + 5-HTP	6	.95 ± .06*	164

* $P < .01$, compared to no pretreatment.

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Cerebroside Antibody Inhibits Sulfatide Synthesis and Myelination and Demyelinates in Cord Tissue Cultures

Abstract. *Antiserum to cerebroside was prepared in rabbits by injection of cerebroside together with bovine serum albumin in complete Freund's adjuvant. When applied to cultures of embryo mouse spinal cord at explantation, this antiserum inhibited sulfatide synthesis and myelination; when applied to myelinated cultures it inhibited sulfatide synthesis and produced demyelination. Complement fixation assays also show antibody to cerebroside in serums from rabbits with experimental allergic encephalomyelitis induced by injection of whole white matter. Absorption of such serum with cerebroside abolishes the inhibiting and demyelinating activities.*

Myelinated cultures of mammalian central nervous system (CNS) tissue become demyelinated when exposed to serum from rabbits with experimental allergic encephalomyelitis (EAE serum) induced by injection of whole white matter in complete Freund's adjuvant (CFA) (*1*). Others have shown that such serums contain antibodies of at least two different specificities, namely, antibodies against the encephalitogenic basic protein and cerebroside (*2*, *3*). Seil *et al.* (*4*) have reported that serums from guinea pigs sensitized with myelin basic protein did not demyelinate CNS tissue cultures. However, Yonezawa *et al.* (*5*) reported that demyelination did occur when serums from guinea pigs or rabbits sensitized with myelin basic protein was added to CNS cultures. Bornstein (*6*) has also found that serums from rabbits inoculated with basic protein in CFA are capable of demyelinating cultured mouse spinal cord but that this effect is demonstrated less consistently than with serums from rabbits exposed to whole tissue in CFA. In 1970, Dubois-Dalcq *et al.* (*7*) showed that antiserums to cerebroside produced demyelination in CNS tissue cultures.

Bornstein and Raine (*8*) showed that

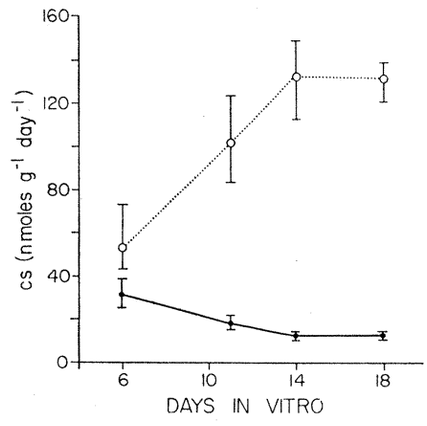

Fig. 1. Cerebroside sulfate (CS) synthesis. The results are expressed in nanomoles of cerebroside sulfate, measured by [^{35}S]sulfate incorporated into cerebroside sulfate during a 24-hour period beginning on the days indicated. Each point is the mean value for four cultures. Control antiserum (open circles) and antiserum to cerebrosides (closed circles) were present as 5 percent of the nutrient medium from the time of explantation.

when cultures of embryo mouse spinal cord are constantly exposed to EAE serum (antiserum to whole white matter) differentiation of oligodendroglia and the formation of myelin are inhibited. Kies *et al.* (*3*) showed that most serums from guinea pigs sensitized with myelin basic protein do not inhibit in vitro myelination in CNS tissue cultures.

The synthesis of cerebroside sulfate (sulfatide) in cultures shows a rapid increase just prior to myelin formation, and cerebroside sulfate synthesis is almost completely inhibited in cultures exposed to EAE serum from the time of explantation (*9*). We now report that rabbit antiserum to cerebroside demyelinates mature cultures and inhibits the formation of myelin and sulfatide synthesis. Antibody to cerebroside is demonstrable in our EAE serum, and absorption of such serum with cerebroside removes the inhibition of sulfatide synthesis, inhibition of myelination, and demyelinating activity.

Tissue cultures were prepared and maintained as described (*9*). Cultures maintained on normal or control nutrient medium developed easily identifiable myelin by day 10 in vitro. For inhibition experiments, the nutrient media contained 5 percent antiserum plus complement. For demyelination, they contained 15 percent antiserum plus complement. Sulfatide synthesis was determined by measuring the rate of [^{35}S]sulfate (Amersham/Searle) incorporation into cerebroside sulfate of the tissue cultures. The preparation of the medium and the processing of the cultures for the incorporation experiments have been described (*9*). The dried cultures were added to 50 μg of dry nonradioactive cerebroside sulfate (*10*) and extracted four times with 300 μl of a mixture of chloroform, methanol, and H_2O (16 : 8 : 1). The four extracts from each culture were pooled and evaporated to dryness under a stream of nitrogen. To each was then added 1.3 ml of lower phase and 0.75 ml of upper phase of a previously prepared partition system, namely, a mixture of chloroform, methanol, and 0.43 percent K_2SO_4 (8 : 4 : 3) (*11*). After mixing (vortex mixer) and brief centrifugation, the upper phase was removed. The lower phase, containing the sulfatide, was washed with three successive changes (0.75 ml) of upper phase to remove free [^{35}S]sulfate. A 1.0-ml portion of the washed lower phase was removed to a glass counting vial and evaporated. The radioactivities of the sulfatide and of portions of feeding medium were counted in Aquasol (*12*) in a Packard Tri-Carb liquid scintillation spectrometer. The residue from the original lipid extraction was dissolved in 72.5 μl of 1*N* NaOH, and the protein content was determined by the method of Lowry *et al.* (*13*), except that copper sulfate was excluded in order to permit measurement of noncollagen protein.

Table 1. Myelination inhibiting activities and titers of antiserums to cerebroside and whole white matter. For myelination studies the test serums were present as 5 percent of the nutrient medium. The results of the myelination test are expressed as the number of cultures myelinated divided by the number of cultures tested. Antibody titers were determined by complement fixation. The X and C designations are those of Rapport and Graf (*15*). Antiserums to cerebroside, R5, R3, and R1, are from individual rabbits. Antiserums to whole white matter, pool 1 and pool 2, are from four rabbits each; Neg., negative.

Source	Antibody titer		Myelinated cultures	Antibody titer to BSA
	X	C		
Antiserum to cerebroside				
R5	1:55	1:17	0/6	1:32
R3	1:37	1:12	0/22	1:35
R3L*	1:29	1:6	0/8	1:35
R3LC†	1:14	Neg.	10/10	1:32
R1‡	Neg.	Neg.	36/36	1:32
Antiserum to whole white matter				
Pool 1	1:55	1:7	0/24	
Pool 2	1:55	1:2	0/10	
Pool 2L*	1:46	1:2	0/8	
Pool 2LC†	1:22	Neg.	10/10	

* Treated with lecithin alone. † Treated with lecithin plus cerebroside to absorb antibody to cerebroside. ‡ Serum from a control rabbit immunized with BSA in CFA.

The demyelination and myelination inhibition tests were carried out as described (*1*, *8*). Rabbits were immunized with cerebroside by a series of four injections at 10-day intervals. The first inoculation material consisted of 1 mg of cerebroside (*10*), 5 mg of bovine serum albumin (BSA), 5 mg of *Mycobacterium tuberculosis* (*14*), 0.5 ml of saline, and 0.5 ml of CFA and was injected into hind foot pads. The second injection had the same composition, except that 1 mg of *M. tuberculosis* was injected subcutaneously on the back. The third and fourth injections contained no *M. tuberculosis* and were administered intramuscularly. The cerebroside, BSA, and *M. tuberculosis* (when present) were sonicated in the saline, Freund's adjuvant was added, and an emulsion was formed. The major bleeding was done 10 days after the fourth injection. Control rabbits were treated in the same way, except that cerebroside was omitted from the injection mixture. Titers of antibody to cerebroside and BSA were assayed by complement fixation (*15*). For the detection of cerebroside antibody, both X and C types were measured in the presence of six 50 percent hemolysis units of complement, and the results are reported as the antiserum dilution giving 50 percent hemolysis.

The antiserums (both to cerebroside and to white matter) were absorbed by adding 1 mg of a mixture of cerebroside and lecithin (1 : 4, by weight) per milliliter of antiserum. The appropriate amount of lipid was added to each tube in a solution of chloroform and methanol (1 : 1), and the solvent was evaporated under nitrogen. Ethanol (3 percent of final volume) was added, and the tube was heated and mixed in a 60°C water bath. Sterile saline (47 percent of final volume) was added to form a suspension and antiserum (50 percent of the final volume) was added. The mixture was gently stirred on a magnetic stirrer for 3 hours at room temperature and overnight in the refrigerator and then centrifuged for 8 hours in a Sorvall refrigerated centrifuge at 11,000 rev/min (20,000*g*); the supernatant was removed. Before applying to

Table 2. Demyelination and inhibition of myelination produced by rabbit serums applied to cultured embryonic mouse spinal cord.

Test serums	Demyelination*	Inhibition of myelination†
Normal	0-0-0-0-0-0-1-0-0-0	0/12
Pooled EAE serum‡	5-3-5-5-2-5-5-5-5-3	12/12
Antiserum to cerebroside	2-2-3-4-5-4-5-4-4-5	12/12
Pooled EAE serum exposed to cerebroside and lecithin	0-0-2-3-2-2-1-1-1-0	0/12
Pooled EAE serum exposed to lecithin	3-3-3-3-2-4-3-3-3-2	12/12
Antiserum to cerebroside exposed to cerebroside and lecithin	1-1-0-0-1-1-1-1-1-2	0/12
Antiserum to cerebroside exposed to lecithin	1-1-0-0-1-1-2-1-2-1	12/12

* Demyelination is expressed on a 0 to 5 basis for each of ten cultures. 0, no change; 1, swelling and fragmentation of myelin sheaths; 2, 25 percent loss of myelin; 3, 50 percent loss of myelin; 4, 75 percent loss of myelin; and 5, total demyelination. † Inhibition is expressed as the number of totally inhibited cultures over the total number exposed from the time of explantation. ‡ Pooled serums from four rabbits inoculated with whole bovine white matter in CFA.

cultures the diluted serums were passed through 0.22-μm Millipore filters to ensure sterility. Antiserums were treated with lecithin alone for controls.

In the presence of antiserum to cerebroside from the time of explantation sulfatide synthesis decreases to very low levels (Fig. 1), following a pattern similar to that with EAE serum (*9*). In the presence of the control rabbit serum the developmental pattern of sulfatide synthesis is normal. As is reported for EAE serum (*16*), antiserum to cerebroside also produces late inhibition of sulfatide synthesis. For late inhibition, cultures were maintained on normal nutrient medium for 12 days and then placed in medium containing 5 percent antiserum to cerebroside for 24 hours followed by a 24-hour incorporation period in the presence of the same medium supplemented with [^{35}S]sulfate. Sulfatide synthesis in the late inhibited cultures was less than 25 percent of that in control cultures.

Myelination inhibition is related to the cerebroside antibody titer in antiserums to cerebroside and whole white matter (Table 1). Absorption of antibody with cerebroside removed at least 60 percent of the X type and essentially all of the C type antibody from both types of serums with the resultant loss of inhibiting activity. When antiserums were treated with lecithin alone, there was no change either in their effect on myelination or in antibody liter. Antibody titers to BSA were measured in the rabbit antiserums to cerebroside and BSA and were unchanged by absorption with cerebroside-lecithin.

An experiment was performed to ascertain the effect of antiserums to cerebroside and whole white matter on sulfatide synthesis with and without absorption of antibody to cerebroside. The cerebroside sulfate synthesis was measured by [^{35}S]sulfate incorporation into cerebroside sulfate during a 24-hour period beginning on day 18 in vitro. Cultures maintained on nutrient medium containing: (i) antiserum to BSA in CFA (the control) synthesized 215 ± 50 nmole g^{-1} day^{-1}; (ii) antiserum to cerebroside and BSA in CFA synthesized less than 10 nmole g^{-1} day^{-1}; (iii) antiserum to cerebroside and BSA absorbed to remove antibody to cerebroside synthesized 125 ± 15 nmole g^{-1} day^{-1}; (iv) antiserum to whole white matter in CFA synthesized 20 nmole g^{-1} day^{-1}; and (v) antiserum to whole white matter in CFA from which the antibody to cerebroside was absorbed synthesized 125 ± 15 nmole g^{-1} day^{-1}. All serums were present as 5 percent of the nutrient medium from the time of explantation. Thus, removal of at least 60 percent of antibody to cerebroside from the active serums leads to sulfatide synthesis approaching that of control.

When antiserums were tested for demyelination the medium containing 15 percent antiserum plus complement was applied to a minimum of six cultures. While serum from a control rabbit (BSA in CFA) produced no demyelination, our antiserums to cerebroside and antiserums to whole white matter consistently demyelinated cultures (Table 2). This activity was abolished in both serums by absorption with cerebroside. When antiserum to cerebroside was absorbed with lecithin alone, the demyelinating activity was also apparently reduced. The significance of this observation is not clear at this time, since myelination inhibiting activity was not affected by this treatment. The fact that demyelination, inhibition of myelination, and inhibition of sulfatide synthesis are not produced by antiserum to whole white matter after absorption of antibody to cerebroside implies that these phenomena are due to the presence of specific antibody to cerebroside.

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