time being between 90 and 600 years [as indicated by the 2 σ confidence bands (σ is the standard deviation)]. There is a substantial difference between these results and those for the period extending from 1974 back to 1725 (Fig. 2b). Similar analysis of the data for the Boston–New Hampshire region yields results which also change markedly with the observation period (Fig. 2, c and d). For the period 1725 through 1849 we obtain results for each area (not plotted in Fig. 2) not greatly different from those for 1725 through 1974 (Fig. 2, b and d). In Table 1 the estimates of return periods and probabilities for events of intensity $I \geq$ VI, VII, and VIII are compared for the periods 1725 through 1974 and 1850 through 1974. The actual time intervals between successive earthquakes could vary greatly from those listed in Table 1. To the extent that these results can be compared with those of other studies (which do not include estimations of time variations in the seismic activity, nor do they always consider the same area), there is a general agreement in the estimated return periods (*10*).

Earthquake occurrence rates in other parts of the world have changed with time (*11*). However, these changes have not been permanent; there have been periods of intense activity and periods of relative quiet. It is unlikely that the observed decline in the number of earthquakes in New England since 1825 will continue into the future. Examining Fig. 1b, we notice a marked increase in the seismic energy release since 1940, which could represent the beginning of another episode of increased seismic activity in southern New England. For hazard estimates, then, it is most appropriate to use the return periods and probabilities based on data covering the longest time period. For New England, these are the values for the period 1725 through 1974.

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Synthetic Galactocerebrosides Evoke Myelination-Inhibiting Antibodies

Abstract. *Synthetic galactodihydrocerebrosides with widely different fatty acid components can evoke myelination-inhibiting antibodies in rabbits. Whether these are the only such haptens involved in experimental immunizations of other species or in spontaneous human diseases is not yet known.*

Complement-dependent, central nervous system (CNS)–specific factors associated with immunoglobulins (Ig) of the 7*S* IgG2 type are detectable by their ability to inhibit myelination in unmyelinated CNS cultures or to demyelinate already myelinated cultures (*1–11*). Such factors may be defined as myelination-inhibiting or demyelinating antibodies. They can be detected in cerebrospinal fluid (*12*), and a high proportion of serums (*4, 11*) from patients with multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). They are also present in serums from animals with experimental allergic encephalomyelitis (EAE) induced by whole CNS (*1–3*) tissue or by myelin (*9, 10*) but not in serums from animals with EAE induced by encephalitogenic myelin basic protein (*6–11*).

Both types of antibodies have been evoked in rabbits by cerebrosides extracted from CNS (*13*), but similar cerebrosides did not evoke demyelinating antibody in guinea pigs (*10*) or myelination-inhibiting antibody in rats (*9*). Although both types of antibody in rabbit serums can be absorbed out by cerebrosides (*13*), and although demyelinating antibody in guinea pig serums can be absorbed out by purified myelin (*10*), demyelinating antibody from MS serum can be absorbed out not by myelin but by the nonmyelin pellet (*14*).

Since extracts of CNS designed to yield particular lipids or proteins are difficult if not impossible to prepare with absolute purity, and since animals can react immunologically to trace contaminants not otherwise detectable, the resolution of some of the above discrepancies seemed unlikely unless synthetic materials could be shown to be active.

N-Lignoceroyldihydroglucocerebroside (Glu-24), *N*-lignoceroyldihydrogalactocerebroside (Gal-24), and *N*-palmitoyldihydrogalactocerebroside (Gal-16) were obtained from Miles Laboratories, Inc., Kankakee, Ill. These had been prepared by Miles-Yeda, a commercial adjunct of the Weizmann Institute of Science, Rehovot, Israel, by the procedure of D. Shapiro and were certified as 100 percent pure by thin-layer chromatography.

White New Zealand rabbits (2 to 3 kg) were immunized with a total of 1 ml of

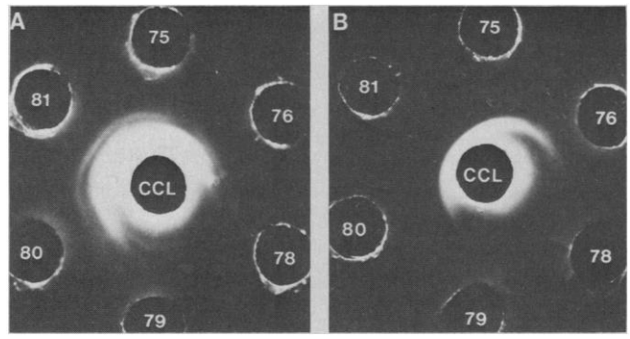

Fig. 1 (A) Immunodiffusion with natural bovine cerebrosides suspended in an alcoholic solution of cholesterol and lecithin (CCL) in the center well against serums from rabbits 75, 76, 78, 79, 80, and 81 obtained on 3/17/76 (42 days after beginning immunization of rabbits 75 and 76 with Gal-24, 21 days after immunizing rabbits 78 and 79 with Glu-24, and 14 days after immunizing rabbits 80 and 81 with Gal-16). Note the multiple lines of immunoprecipitation with antiserums to both galactocerebrosides but not with antiserum to glucocerebroside. (B) Similar immunodiffusion with serums obtained 5 weeks later (4/21/76). Note the persistence of only one line of galactoimmunoprecipitation.

mixture containing 2.5 mg of synthetic cerebroside, 2.5 mg of bovine serum albumin (BSA), and 1 mg of heat-killed *Mycobacterium tuberculosis* in a water-in-oil emulsion injected intradermally in 0.1-ml doses. Booster injections, each containing a total of 1 mg of synthetic cerebroside and 2.5 mg of BSA in a water-in-oil emulsion were given subcutaneously 7 and 10 days later.

Immunodiffusions were performed in 1 percent agarose (Bioware, Wichita, Kan.) in 0.075 percent barbital buffer, *p*H 8.6. The test antigens included the synthetic cerebrosides and natural (bovine) cerebrosides (Applied Sciences Laboratory, State College, Pa.) suspended in cholesterol (C. P grade; Phanstiehl, Waukegan, Ill.) and synthetic L-α-lecithin (Nutritional Biochemical, Cleveland, Ohio) according to the technique of Niedieck (*15*). The antigenic suspension was prepared from a solution containing 2.7 mg of cerebroside, 2.9 mg of cholesterol, and 1.3 mg of lecithin solubilized in 1 ml of hot (60°C) alcohol. On the day of testing one part of this solution was mixed with four parts of water and applied to the agarose 4 hours before the antiserum. The reactants were allowed to diffuse for 2 days, the precipitin patterns were photographed, and then rinsed, stained by Coomassie blue, and dried.

Myelination-inhibition tests were performed on newborn mouse cerebellar tissue as described (*7–9*). Test and control rabbit serums were coded and incorporated into the nutrient medium in 10 percent concentration, along with 10 percent pooled normal guinea pig serum as a source of complement. Cultures were observed (double blind) for 15 to 16 days for signs of myelination, well beyond the 9- to 12-day period when these preparations usually myelinate (*7*). A culture was considered myelinated even if it contained only a single myelinated fiber, in accordance with established criteria (*3*), and a serum was considered as negative for myelination-inhibition if half or more of the cultures exposed to it contained myelinated fibers (*7*).

As shown in Table 1, there is a clear correlation between myelination-inhibiting and precipitating antibodies evoked by either of the two galactocerebrosides. None of the serums obtained prior to immunization reacted in either test, and all of the serums from rabbits immunized with either of the two galactocerebrosides reacted in both tests. The two serums from rabbits immunized with glucocerebrosides did not react in either test, although antibodies to the concomitantly administered BSA were equally readily detectable.

The correlation of myelination-inhibiting antibodies with precipitating antibodies is complicated by the observation of at least two such lines of precipitation in agarose gels after diffusion of the early serums which were tested for myelination-inhibition (Fig. 1A). These lines were specifically inhibited when 0.5 to 1*M* galactose, but not glucose, was added to the gels. Only a single line persisted in the late serums (Fig. 1B), and this line was inhibited by 0.5*M* galactose. It is not likely that different classes of antibodies are involved since absorption of the serums with goat antiserum to rabbit IgM did not change the pattern and since the same pair of lines of precipitation are present in the IgG region on immunoelectrophoresis of the early serums.

Since the physicochemical state of the cerebroside haptenic sites in the various-sized lecithin-cholesterol micelles necessary to achieve these immunodiffusions may be variable, we imagine that the two immunoprecipitates may be related to different antibodies directed against different (opposite?) regions of the galactose portion of the molecule which may be selectively exposed in different micelles (*15, 16*).

A number of independent observations suggests that galactose may be of importance in demyelinating diseases: Galactocerebrosides appear in the plasma in MS and after massive strokes (*17*), galactoproteins are exposed on the surface of the myelin sheath (*18*), and ceramide analogs that inhibit galactocerebrosidase also inhibit myelination (*19*). Although consistent with these observations and with the data showing that glucocerebrosides are relatively small components of myelin as contrasted with galactocerebrosides, the absence of myelination-inhibiting and gel-precipitating antibodies in the serums of rabbits "similarly immunized" with synthetic glucocerebrosides is not conclusive, since glucolipids are weaker haptens and may

Table 1. Comparison of results of tests for myelination-inhibition and immunodiffusion.

Serums			Inhibited myelination*	Immunoprecipitates with cholesterol-lecithin suspension of				
Rabbit No.	Day obtained	Immunizing agent		Natural cerebrosides	Gal-16	Gal-24	Glu-24	BSA
75	0	Gal-24	0 (5/5)	0	0	0	0	0
	20		+ (1/7)	+	+	+	0	+
	42		+ (2/6)	+	+	+	0	+
76	0	Gal-24	0 (5/6)	0	0	0	0	0
	20		+ (1/7)	+	+	+	±	+
	42		+ (0/7)	+	+	+		+
80	0	Gal-16	0 (4/5)	0	0	0	0	0
	14		+ (1/5)	+	+	+	0	+
81	0	Gal-16	0 (3/5)	0	0	0	0	0
	14		+ (0/6)	+	+	+	0	+
78	0	Glu-24	0 (3/5)	0	0	0	0	0
	21		0 (3/5)	0	0	0	0	+
79	0	Glu-24	0 (4/5)	0	0	0	0	0
	21		0 (3/5)	0	0	0	0	+

*In CNS cultures; results are expressed as myelination-inhibiting antibodies present or absent and as a ratio of the number of cultures becoming myelinated to the number of cultures exposed to 10 percent serum.

not have been presented to the rabbits in immunologically optimal conditions.

Our experiments provide conclusive evidence that galactocerebrosides not possibly contaminated with other CNS components can evoke myelination-inhibiting antibodies in rabbits. To date, evocation of demyelinating and myelination-inhibiting antibodies by either natural (*13, 20*) or synthetic cerebrosides has been accomplished only in this species. The minor differences in the molecular structure of cerebrosides from central and peripheral nervous system myelin (*21*) would appear to make the specificity of antibodies to myelin for central or peripheral nervous system cultures (*1, 3, 22*) difficult to explain if cerebroside is the only factor involved.

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Slow Persistent Infection Caused by Visna Virus: Role of Host Restriction

Abstract. *Proviral DNA has been demonstrated by in situ hybridization in foci of cells of a lamb infected with the RNA slow virus visna. A few of these cells also contain the major virion structural antigen p30. This restriction in virus gene expression in the infected animal provides a mechanism for persistence of virus in this chronic infection.*

Visna, an inflammatory and demyelinating disease of the central nervous system (CNS) of sheep, is caused by a virus closely related to RNA type C viruses both in structure (*1*) and in the initial aspects of replication in vitro; in permissively infected cells the genetic information in the RNA genome of the virus is completely transferred to a DNA intermediate or provirus (*2*). Visna is also a prototype of slow and persistent infections (*3*) in which the virus elicits an immune and inflammatory response from its host which fails to eliminate the infectious agent. One of the central issues in understanding chronic virus infections is how a virus like visna is able to persist for periods as long as 8 years in an animal with demonstrable neutralizing antibody (*4, 5*).

We have proposed (*1, 2*) one mechanism for persistence analogous to the lysogenic relationship between viruses and their bacterial hosts; in the infected animal provirus formation occurs as it does in vitro, but later steps in the life cycle of the virus are believed to be repressed in most cells. Virus genetic information is therefore conserved, and virus survives to perpetuate infection because the infected cell is neither detected nor destroyed by the immune surveillance system of the host animal. The important consequences of this argument now amenable to experimental test are: (i) infected cells should harbor viral DNA; but, (ii) because of host restriction, only a small proportion of infected cells should also synthesize viral proteins or progeny at detectable levels.

We have examined these predictions as follows. Viral DNA can be detected and quantitated in cells in tissue sections by hybridization in situ. A restriction in virus gene expression can then be assessed by determining, by immunofluorescence, the proportion of cells that also contain p30, the major structural polypeptide and gene product of the virus. These studies were conducted in an animal model (*6*) congruent in many respects with the natural slow infection, and the postulated temperate relationship of virus and host cell in vivo. Visna virus causes a persistent infection of American lambs in which virus particles are not observed in tissues, and in which virus cannot generally be recovered from tissue homogenates. However, explant cultures uniformly produce virus after a few days in culture, consistent with the notion that virus growth is blocked in vivo, and that this restriction can be relieved in some manner by the conditions of cultivation in vitro.

The nature of the virus host cell relationship in the animal was investigated in sheep choroid plexus (SCP) tissue. We chose this tissue because, in our studies of virus isolation from various tissues, we found that choroid plexus was invariably infected and contained the largest percentage of cells that could be induced to yield virus on explantation (*7*). In this investigation we examined choroid plexus from one fetus killed 2 weeks after virus inoculation. No virus particles were detected by electron microscopy in a sample of the tissue, and infectious virus was not recovered from cell-free homogenates prepared from another portion of the tissue. Virus, however, was isolated from every explant culture established from this particular choroid plexus tissue. Sections were cut from the remaining tissue and fixed for assay of viral DNA and p30.

To detect viral DNA by in situ hybridization (*8, 9*) RNA was removed by digestion with ribonuclease, and the DNA in the tissue section was denatured and hybridized to a virus specific probe (*10*) labeled to high specific activity with [^{3}H]deoxythymidine triphosphate (dTTP). At the end of the reaction, unreacted probe was removed by extensive washing and digestion with single strand specific S1 nuclease, and the tissues were prepared for radioautography. After exposure of 9 to 14 weeks slides were developed and stained.

Most of the cells in the sections from infected tissues had no more than the background of 1 to 2 grains per nucleus seen in uninfected tissues, but in other areas significant hybridization is evident. In these foci we observed discrete collections of grains over the nuclei of cells (Fig. 1). In two independent experiments we found that the number of cells with a significant number of grains over the nucleus constituted 18 percent of the total