active at near pH 5.0 and showed Mr = 65,000 by sucrose density gradient centrifugation. Gangliosides were preferential substrates, but the others including 4-methylumbelliferyl Neu5Ac were poorly hydrolyzed. The rate of hydrolysis of gangliosides having $\alpha 2 \rightarrow 3$ or $\alpha 2 \rightarrow 8$ sialyl linkage was higher than 2-fold that of $\alpha 2 \rightarrow 6$ linkage. Thiol-modifying 4-hydroxymercuribenzoate (50 $\mu$M) and $Cu^{++}$ (1 mM) caused complete inhibition of the sialidase activity.

### S11.7
### Brain Cytosolic Sialidase: A "Protein Complex" Containing a Catalytic and a Protective Unit

*G. Tettamanti*, L. Di Francesco, A. Chiarini, A. Fiorilli, E. Monti[1], D. Zizioli[1] and B. Venerando
*Department of Medical Chemistry and Biochemistry, University of Milan, Milan, Italy and [1]Department of Biochemistry, University of Brescia, Brescia, Italy.*

Cytosolic sialidase, extracted from pig brain, was purified about 14,000-fold by a new procedure, where the most effective step was FPLC chromatography on a cation column (Mono S). SDS-PAGE of the active fraction showed a single protein band with a molecular weight of 31 KDa. The purified enzyme was active on different sialic acid containing compounds (including ganglioside GD1a), and obeyed, in all cases, regular Michaelis-Menten kinetics. The apparent Km values on MU-NeuAc and GD1a were $2.4 \times 10-4$M and $1.6 \times 10-5$M and Vmax values 80 mU and 100 mU/mg protein, respectively. This enzyme preparation was extremely unstable during storage and freezing/thawing and all the attempts made to preserve the enzyme activity (including the addition of albumin and other proteins) were unsuccessful. We observed that the loss of stability occurred after the last step of purification (FPLC chromatography). The addition of an enzymatically inactive protein, separated from the enzyme during this step, resulted in complete stabilization of the purified sialidase preparation. In addition, this stabilizing protein fraction had the peculiarity to cause a shift of the V/[S] relationship of sialidase action on GDla from a hyperbolic to a sigmoidal-shaped kinetics. The reason of this shift is under investigation. On the basis of these results, we suggest that the cytosolic sialidase occurs in brain as a complex containing a catalitic unit and a "protective" or "stabilizing" unit. This complex seems to resemble the sialidase-$\beta$-galactosidase-carboxypeptidase complex of lysosomal origin.

### S11.8
### Occurrence and Formation of the Lactone Form of Ganglioside in Cerebellar Granule Cells Differentiated in Culture

R. Bassi, L. Riboni and *G. Tettamanti*
*Department of Medical Chemistry and Biochemistry, The Medical School, University of Milan, Milan, Italy.*

The lactone form of ganglioside GD1b (GD1b-lactone) occurs in both human and rodent brain. Intracisternal administration of GD1b to rat is followed by formation of GD1b-lactone in brain but not in liver, suggesting that ganglioside lactonization is an organ-specific metabolic event. The occurrence and metabolic formation of GD1b-lactone was studied in primary cultures of granule cells and astrocytes obtained from rat cerebellum. After direct treatment of both cell types with NaB $[^3H]_4$, GD1b-lactone was detected as the $^3$H-labeled stable nonulosamine containing derivative (GD1b-ol) in cerebellar granule cells but not in astrocytes. In metabolic studies [$^3$H-Gal] GD1b was administered for different pulse times (30 min – 2 h) to both cell types. At all investigated times radiolabelled GM1 was the major metabolic product in neurons and astrocytes. However, after sodium borohydride reduction of the cell pellet, a radioactive band comigrating with, and identified as GD1b-ol was detected in granule cells but not in astrocytes, indicating the formation of GDlb-lactone. It is concluded that cultured neurons but not neuroglial cells from rat cerebellum are capable to produce GD1b-lactone from GD1b. This finding suggests that GD1b lactonization may play a role in some functional expressions of neurons.

### S11.9
### Molecular Mimicry Between Ganglioside and a Bacterium Elicits Guillain-Barré Syndrome

*N. Yuki*[1], T. Taki[2], T. Kasama[2], M. Takahashi[3], K. Saito[3], F. Inagaki[4], S. Handa[2] and T. Miyatake[1]
*Dept. [1]Neurol. and [2]Biochem., Tokyo Medical and Dental University, Bunkyo-ku Tokyo 113, Japan; [3]Tokyo Metropolitan Res. Lab. of Public Health; [4]Tokyo Metropolitan Inst. of Med. Sci.*

We investigated the lipopolysaccharide (LPS) from Penner's serotype 19 (PEN 19) of *Campylobacter jejuni* that had been isolated from a patient with the Guillain-Barré syndrome (GBS). The LPS was extracted from the bacterium and separated by a silica beads column chromatography. One homogeneous fraction showing reactivity with the patient's serum, rabbit anti-$G_{M1}$ antibody, and also cholera toxin, which binds oligosaccharide of $G_{M1}$, was obtained. The purified LPS was found to contain Gal, Glc, GalNAc, NeuAc, GlcN, Hep, KDO and fatty acids (3-hydroxy-myristic acid and palmitic acid) by gas-liquid chromatography-mass spectrometric analysis. Proton 2D-NMR spectroscopy showed the presence of Gal$\beta$1-3GalNAc$\beta$1-4(NeuAc$\alpha$2-3)Gal$\beta$1-structure. We assume that infection by *C. jejuni* (PEN 19) which has the $G_{M1}$-oligosaccharide structure induces autoantibody to $G_{M1}$ ganglioside, and elicits GBS.

### S11.10
### Biosynthesis of Ganglioside Molecular Species Containing C18 or C20 Long Chain Base

*S. Sonnino*, V. Chigorno and M. Valsecchi
*Department of Medical Chemistry and Biochemistry, Medical School, University of Milan, Italy.*

Gangliosides constitute a family of compounds differing in both the oligosaccharide and the ceramide moieties. Gangliosides undergo pronounced compositional changes during nervous system development, suggesting a direct involvement of gangliosides in the differentiation and aging processes of neural cells. These changes regard not only the oligosaccharide moiety, but also the ceramide one, suggesting that the chemical features of the ceramide portion play a relevant role in ganglioside metabolism and functional implications. In particular, of the two main long chain base