R. Arango[1], E. Rodriguez-Arango[1], D. Belenky[1], R. Adar[1], S. Rozenblatt[2] and *N. Sharon*[1]
[1]Dept. Membrane Res. & Biophysics, Weizmann Inst. Science, Rehovot, 76100, [2]Dept. Molecular Microbiol. & Biotechnol. Tel Aviv Univ., Tel Aviv, 69978 Israel.

In the 3-D structure of *Erythrina corallodendron* lectin (ECorL) in complex with a ligand (lactose), the first of its kind for a Gal/GalNAc-specific lectin (Shaanan *et al.*, 1991, Science, **254**, 862), a hydrophobic cavity is present surrounded by Tyr^{108}, Pro^{134} and Trp^{135} not in contact with Gal of the lactose in the combining site. The cavity can accommodate bulky substituents such as acetamido or dansylamido (NDns) at C-2 of the lectin-bound Gal. Molecular modelling suggested that the Dns of MeβGalNDns, which binds the lectin ~300 times stronger than Gal, fits snugly into the cavity. Comparison of the primary sequence of ECorL with that of the homologous peanut agglutinin (PNA), which is Gal specific but does not bind GalNAc or GalNDns, showed that in PNA such a cavity is absent, being filled by two extra amino acids.

We therefore constructed a mutant L2 in which Pro^{134}-Trp^{135} in ECorL was replaced by Ser-Glu-Tyr-Asn (as in PNA), a single point mutant Y108T (also as in PNA), a double mutant L2;Y108T, and another single point mutant W135A. They were expressed in *Escherichia coli* as done for rECorL (Arango *et al.*, 1992, *Eur. J. Biochem.*, **205**, 575) and examined for their specificity by inhibition of hemagglutination and of binding of the lectin to asialofetuin. In the latter assay, Y108T had a specificity similar to rECorL; L2 had the same affinity for Gal as rECorL, and 8 and 15 times lower affinity for GalNAc and MeβGalNDns, respectively. L2;Y108T had a similar affinity for Gal and GalNDns as L2, but twice the affinity for GalNAc. W135A, on the other hand, bound Gal 5 times stronger, and GalNAc and GalNDns 3 and 6 times weaker, respectively, than rECorL.

Our results demonstrate that (a) Trp^{135} contributes to the strong binding of MeβGalNDns to ECorL, whereas Tyr^{108} does not; (b) the region close to Trp^{135} must be free in order to allow the lectin to bind Gal derivatives with bulky substituents at C-2; (c) changing a residue such as Trp^{135}, which is not in direct contact with the Gal ligand, may change the conformation of the combining site of the lectin.

S8.4
The Primary Structure of *Maackia amurensis* Hemagglutinin (MAH)

Y. Konami, C. Ishida, K. Yamamoto, T. Osawa and T. Irimura
Division of Chemical Toxicology and Immunochemistry, Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan.

The presence of a hemagglutinin in *Maackia amurensis* seeds was first described by Boyd *et al.* In our previous paper, we reported the purification and characterization of the hemagglutinin from the seeds of *M. amurensis* (MAH) and found that porcine submaxillary mucin having blood group H activity was a potent hapten inhibitor against the hemagglutinating activity of MAH. We have also determined the primary structures of *Lotus tetragonolobus* lectin (LTA), *Ulex europeus* lectins I and II (UEA-I and II), *Laburnum alpinum* lectin I (LAA-I), *Cytisus scoparius* lectin II (CS-II) and *Cytisus sessilifolius* lectin I (CSA-I) and compared them with those of several other leguminous lectins. Extensive homology was found throughout the stretch of the peptides. Here we show the determination of the complete amino acid sequence of MAH by use of a protein sequencer. We purified MAH by affinity chromatography on a column of Sepharose coupled with fetuin glycopeptides, followed by SP-Toyopearl ion exchange chromatography. After digestion with endoproteinases of Lys-C and Asp-N of MAH, the resulting peptides were purified by reversed phase high performance liquid chromatography and subjected to the sequence analysis. The primary structure of MAH was compared with those of several leguminous lectins already determined. The amino acid sequence of the putative carbohydrate-binding domain of MAH was comparatively different from those of the above described lectins. The study of the precise carbohydrate-binding specificity of MAH by means of immobilized lectin affinity chromatography is in progress.

S8.5
The Fourth Immunoglobulin-like Domain of NCAM Contains a Carbohydrate Recognition Domain for Oligomannosidic Glycans Implicated in Association with L1 and Neurite Outgrowth

R. Korstkorte, M. Schachner, J. P. Magyar, T. Vorherr* and *B. Schmitz*
*Department of Neurobiology, Swiss Federal Institute of Technology, Zurich; and *Hoffman La-Roche, Basel, Switzerland.*

In this study we have identified consensus sequences for C-type carbohydrate recognition domains and plant lectins in the fourth immunoglobulin-like domain of NCAM. In solid phase binding assays we could show that the cell adhesion molecule L1 binds to NCAM through oligomannosidic glycans carried by L1. Since L1 and NCAM are expressed at the cell surface of early postnatal cerebellar neurons and since L1 seems to be the only glycoprotein on cerebellar neurons to carry oligomannosidic glycans, we studied the influence of oligomannosidic glycans added to the culture medium of cerebellar neurons on the morphological appearance of the cells. A strong reduction in neurite outgrowth was observed in the presence of oligomannosidic glycans. Neurite outgrowth was also inhibited in the presence of a synthetic peptide comprising part of NCAM's carbohydrate recognition domain, but not in the presence of other neutral glycans or synthetic peptides. These results implicate oligomannosidic glycan-mediated cis-association between cell surface molecules in important neuronal cell recognition and adhesion processes.

S8.6
Nectadrin (Heat-Stable Antigen) Glycoforms as Ligand for Mouse P-Selectin

M. Sammar, A. Kowitz, D. Vestweber, M. Schachner and P. Altevogt
FSP 7 Tumorimmunology, German Cancer Research Center, Heidelberg, Germany; MPI for Immunobiology, Freiburg, Germany; and Dept. of Neurobiology, ETH, Zurich, Switzerland.

Nectadrin, a GPI-linked cell surface molecule recognized by the novel monoclonal antibody 79 (mab 79), was found to be immunologically identical to the heat-stable antigen. It is a highly glycosylated protein with a polypeptide core of only 30 amino acids. In a previous study we have shown that nectadrin is involved in cell adhesion and that the carbohydrate portion