Mouse monoclonal antibodies Ak 735, 20-1, 2-1B and 2-2B reacted in similar fashion with the compounds tested. A rough estimate of the binding constant for 20-1 (Kaff $\approx 10^{-12}$ M^{-1}) indicated high affinity.

Of the antibodies against *N*-propionylated MenB polysaccharide, the mouse monoclonal NP-4 and mouse polyclonal 106-6 reacted also with human embryonal glycopeptides. The binding was inhibited with bacteriophage endosialidase. The 'switch variant' human monoclonal antibodies 5E1 and t5E1 (IgM and IgG) reacted identically, and no differences were observed when performing the experiment at +25, +37 or +40°C. In newborn rat brain immunoblots they revealed identical smears in the high molecular weight region, corresponding to mobility of *N*-CAM.

The observed binding characteristics are in accordance with the presence of long conformational polysialosyl epitopes in human embryonal brain. This is supported by the result, that endosialidase treatment of the glycopeptide totally abolished the binding to NP-4. Whether this causes problems in vaccination remains to be evaluated. The 106-6 antibody which had a high titre against the *N*-propionylated MenB polysaccharide is also the only antibody bactericidic for MenB, although the other mouse polyclonal 109-3 was also protective in mice. This may also reflect different avidities of the antisera.

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S18.8
CMP-NeuNAc: Polyα2→8Sialosyl Sialyltransferase Activity in Human Neuroblastoma

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Polymers of α2→8NeuNAc (polysialic acid) were found on human neuroblastoma cells (1) and other human tumors (2). It has been proposed that the longer polymers may relate to the metastatic properties of neuroblastoma (3). Therefore it was of interest to study the activity of polysialyltransferase in neuroblastoma cells, particularly the enzyme involved in the synthesis of the long polymers. A method which detected polymers of 10 or longer sialyl residues was used to demonstrate the activity of polysialyltransferase. The method was based on an immunoaffinity column prepared with a purified antibody to polysialic acid. The immunoaffinity column was characterized with rat brain extracts which synthesized polysialic acid from CMP-[^{14}C]NeuNAc and endogenous substrate (4). An extract from human neuroblastoma, CHP-134 cells synthesized polysialic acid in polymers of 10 or more sialyl residues as detected by the immunoaffinity column. The time course of the synthesis from CMP-[^{14}C]NeuNAc and endogenous substrate, and other parameters showed some differences from those of rat brain. These studies lead to the suggestion that the embryonic and tumor polysialyltransferases may be different and that a family of polysialyltransferases may exist. Supported by NIH RO1 CA 52526 and The Children's Hospital/Weizmann Institute Program.

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S18.9
Studies on CMP-Neu4ac Hydroxylation in Pig Submandibular Gland

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Our work on the CMP-Neu5Ac hydroxylase (EC 1.14.99.18) in high-speed supernatants of pig submandibular glands suggests that the mechanism of CMP-Neu5Gc formation is similar to that in mouse liver. In common with the hydroxylase from mouse liver the enzyme from pig submandibular glands was sensitive to increased ionic strength (I_{50}: 100 mM NaCl) and activated by the addition of Triton X-100. An anti-(rat-cytochrome b_5) antibody was a potent inhibitor of the hydroxylase, while the addition of purified cytochrome b_5 from mouse liver gave rise to a dramatic increase in the specific activity of the hydroxylase from 0.9 to 105 pmol/min/mg protein. Therefore, cytochrome b_5 is also involved in CMP-Neu5Ac hydroxylation in pig submandibular glands.

The hydroxylase from pig submandibular glands could be activated by microsomes from pig submandibular glands, pig liver and mouse liver in the presence of Triton X-100. On addition of pig liver microsomes (20 μg protein per assay) + Triton X-100 (1.3%) the specific activity increased from 0.9 to 151.4 pmol/min/mg protein. This high specific activity should make an isolation of the CMP-Neu5Ac hydroxylase from pig submandibular glands possible. Fractionation of high-speed supernatants from the glands on Cibacron Blue 3GA-agarose columns gave approximately a 5-fold increase in specific activity, the recovery being about 30%. This purification step was particularly effective, as it led to a complete separation of the hydroxylase from the salivary mucins.

S18.10
Interaction of CMP-Neu5ac Hydroxylase with Cytosolic and Microsomal Forms of Cytochrome b_5 and Cytochrome b_5 Reductase

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As previously reported, cytochrome b_5 takes part in the hydroxylation of CMP-Neu5Ac to CMP-Neu5Gc in mouse liver [1,2]. By analogy to other cytochrome b_5-dependent enzyme systems, the participation of a CMP-Neu5Ac specific monooxygenase and an NADH-cytochrome b_5 reductase in the hydroxylase system was proposed. Recent work in our group now clearly demonstrates the involvement of the reductase in this enzyme system. Chromatography of a high