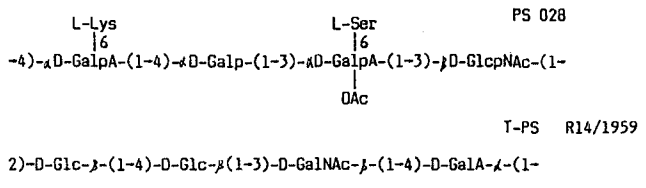

The epitope specificity of both antigens was studied by use of rabbit polyclonal *O* and R-specific antisera, as well as synthetic antigens — partial structures of P. mirabilis 028 and R14/1959 lipopolysaccharides.

S19.11
Different Distribution of Glycosphingolipids in Mouse and Rabbit Skeletal Muscle Demonstrated by Biochemical and Immunohistological Analyses

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Glycosphingolipids (GSLs) are ubiquitous components of the outer surface of animal cell membranes. Their localization suggests that they are involved in several membrane-mediated phenomena. In this study we describe the structural characterization of GSLs from mouse and rabbit skeletal muscle by biochemical analyses and their localization in muscle tissue using immunohistochemical methods.

Neutral GSLs of rabbit muscle showed a simple TLC pattern expressing mainly monohexosylceramide (MHC). In addition to MHC, lactosylceramide, lacto-*N-neo*tetraosyl ceramide, globoside and Forssman-GSL were detected in the neutral GSL fraction of mouse muscle by TLC-immunostaining (overlay technique) with specific monoclonal and polyclonal antibodies.

The major ganglioside in both species was G_{M3}. Muscle gangliosides of rabbit are characterized by the absence of Neu5Gc, expressing exclusively G_{M3} (Neu5Ac), whereas G_{M3} (Neu5Ac) and G_{M3} (Neu5Gc) were found in a 1.85 : 1 ratio in mouse muscle. Sialyl lacto-*N-neo*tetraosyl ceramide and sialyl lacto-*N-nor*hexaosyl ceramide were found in both animals in relatively high concentrations. Furthermore, minor amounts of the ganglio-series gangliosides G_{M1}, G_{D1a}, G_{D1b} and G_{T1b} were detected in muscles of both species. GSL expression, determined by chromatographic analysis and TLC-immunostaining, could be confirmed immunohistochemically by examining frozen sections of skeletal muscle samples from both species.

The results provide the basis for the investigation of specific GSLs that might modulate membrane protein function in muscle.

S19.12
Improved Separation of Gangliosides on High Performance Thin-Layer Chromatography Plates by Automated Multiple Development

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Gangliosides are membrane-bound glycosphingolipids (GSLs), containing one or more sialic acid residues. Analytical and preparative high performance thin-layer chromatography (HPTLC) are widely used for separation and identification as well as for isolation and purification of individual GSLs. In this study an improved method for the separation of gangliosides on HPTLC-plates by automated multiple development (AMD) is described, that permits high-resolution separation of complex ganglioside mixtures.

The theoretical goal of increasing separation of bands by decreasing solvent strength was achieved by three consecutive runs in the same solvent but of a polarity lower than normally used for single chromatography. The AMD equipment (Camag) consists of a development unit, a control unit and a vacuum pump. All solvents contained 2 mM $CaCl_2$ and threefold runs were performed for 55 min each, interrupted by oil pump vacuum drying for 10 min, respectively.

The three compound solvent mixture chloroform (C)/methanol (M)/water (W) (120/85/20, by vol) has found wide application for ganglioside separation on HPTLC plates. Improved separation of highly complex monosialoganglioside mixtures, isolated from murine YAC-1 lymphoma cells and human granulocytes, was obtained by threefold chromatography in C/M/W (120/85/12, by vol). Improved separation of the disialoganglioside fraction of YAC-1, consisting of about 20 different yet unknown compounds with chromatographic behaviour between reference G_{D1a} and G_{T1b}, was obtained by threefold chromatography in C/M/W (120/85/16, by vol). A further advantage of the AMD technique is an excellent separation of polysialogangliosides, isolated from embryonic chicken brain containing up to six sialic acids, which was attained by three runs in C/M/W (120/85/22, by vol).

The described technique offers a suitable tool for analytical as well as preparative HPTLC.

S19.13
Unusual Neutral Glycosphingolipids Obtained from Marine Animals

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In a series of structural studies on neutral glycosphingolipids (GSLs) of marine invertebrates, some novel and unusual GSLs were found in shellfish, chiton and sponge.

Total lipids were extracted from fresh tissues of marine invertebrates with chloroform-methanol and subjected to Folch's partition. Lipids obtained in Folch's lower layer were fractionated into neutral lipids, glycolipids and phospholipids on a silicic acid column. The glycolipid fraction was further fractionated by repeated Iatrobeads column chromatography. The structure of each glycolipid isolated was determined using FAB/MS, NMR, chemical analysis and GC/MS of the degradation products.

The following unusual GSLs were discovered: 1) two kinds of GSLs with an octasaccharide chain containing four fucose residues (two pairs of Fucα1-2Fuc) and Fucα1-3GlcNAcβ1-4Glc linkage from the testes of the shellfish *Turbo cornutus* (this GSL is specific to the testes and not found in ovary and visceral tissues); 2) GSLs with a hexasaccharide chain containing rhamnose or 3-*O*-Me-fucose in the sugar chain terminal from the viscera of the chiton *Halocynthia roretzi*; 3) three GSLs with pyruvated galactose in the sugar chain terminal and some fucolipids, e.g., Fucα1-6Galβ1-1Cer and Fucα1-6-(Fucα1-3)Galβ1-4Glcβ1-1Cer, from the sponge *Halichondria japonica*.