significant inhibition of all the parameters mentioned above by the addition of DTPA, or desferoxamine (1 mM) to the incubation mixture. Catalase also caused nearly 50% decrease in fluorescence; the other parameters could not be assessed due to catalase interference. The crystallin alterations observed and the inhibitory effects of transition metal complexing agents and of catalase support the hypothesis of free-radical mediated autoxidative glycation of crystallin during the formation of diabetic cataracts.

K. V. Chace, R. Carubelli, R. E. Nordquist, *Arch. Biochem. Biophys.*, **288**, 473 – 480 (1991).

This research was supported in part by an unrestricted grant from Research to Prevent Blindness, Inc.

S7.13

Phytanyl and Mono-Branched Lipid Substrates for Dolichyl Phosphate Mannose Synthetase and β-Mannosyltransferase

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Dolichylphosphate mannose (Dol-P-Man) synthetase and β-mannosyltransferase are enzymes involved in glycoconjugate biosynthesis and possess a putatively conserved dolichol binding site. In order to probe the interaction between the enzymes and the dolichol chain, lipid phosphates varying in length and extent of branching have been tested as substrates in crude microsomal preparations from *Saccharomyces cerevisiae* and lysates of recombinant *Escherichia coli.*

In assays for Dol-P-Man synthetase, it was found that a fully saturated lipid, phytanyl (3,7,11,15-tetramethylhexadecanyl) phosphate, was utilised at 60 – 70% of the efficiency of the natural dolichyl lipid, whereas addition of (S)-3-methyloctadecanyl phosphate, which is of similar length to the phytanyl analogue but with only one branch, resulted in approximately 25% of the incorporation. Incubations with the unbranched tetradecanyl phosphate and the short, doubly branched (R)- and (S)-dihydrocitronellyl (3,7-dimethyloctanyl) phosphates exhibited levels of activity just above or similar to incubations with no exogenous acceptor. Analysis of the saccharide component of the [^{3}H]-mannosylated lipid-linked material from microsomal incubations indicated that the major product was the singly mannosylated lipid phosphate.

In similar studies with β-mannosyltransferase it was concluded that phytanylpyrophosphoryl *N,N'*-diacetylchitobiose functions as a mannosyl acceptor[1]. Current studies on recombinant enzyme seek to increase the yield of trisaccharyl lipid product. It is concluded that only the presence of an α-saturated isoprene unit in lipid phosphates is essential for their status as substrates for dolichylphosphate mannose synthetase and that transfer of mannose occurs even if only branching at C-3 is present.

[1]Flitsch, S. L., Pinches, H. L., Taylor, J. P. and Turner, N. J. (1992) *J. Chem. Soc., Perkin Trans.*, **1**, 2089 – 2094.

S7.14

On the Occurrence of Complex Glycosphingolipids in Digestive Tracts of Dogs and Rabbits

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Rabbit and dog small intestines were delipidated by extraction with chloroform/methanol and the residues were acetylated in formamide/pyridine/acetic ·anhydride. The peracetylated glycolipids were extracted with chloroform and separated by means of Sephadex LH-20 and Sephadex LH-60 chromatography. The fractions obtained were subjected to endoglycoceramidase digestion and analysed further for the presence of free saccharides and free lipids. The ceramide species were identified by fast atom bombardment mass spectrometry. The saccharides were separated by high pH ion exchange chromatography, and after derivatization with 7-amino-4-methylcoumarin (fluorescent probe) by Bio Gel P-6 gel filtration. The results indicated the existence of series of highly polar, large molecular weight (several thousand daltons) glycosphingolipids in both analyzed tissues.

S7.15

Glycosphingolipids are Fourfold Increased in Platelet Membranes from Thrombocytopenic Rats

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Cell surface carbohydrates are distributed among glycoproteins (GPs) and glycosphingolipids (GSLs). Biological significance of this distribution is largely unknown. Addressing this problem requires knowledge of absolute contents of cell membrane carbohydrates of GPs and GSLs in cells of different types, functions, sizes, degrees of maturation and stages of development. For this purpose we elaborated a method that allows for quantitative determination in a single sample (amounting to at least 0.5 mg of cells or cell membranes) of carbohydrates (by HPAEC with PAD, separately in GPs and GSLs), protein, cholesterol, lipid P, and sphingoid base. GSLs, in the acetate form, were separated from sphingolipids by chromatography on Florisil (T. Saito, S. Hakomori, *J. Lipid Res.* 1971, 12: 257-259). We used this method to study carbohydrates of rat platelets produced under physiological and thrombocytopenic conditions when platelets are released to the bloodstream prematurely. In plasma membranes from thrombocytopenic platelets (that are larger than the "normal" ones) the GSL:Gp ratio increased over threefold on the basis of sphingoid base determination and fourfold on the basis of carbohydrate analysis. Carbohydrate compositions of GPs of "normal" and thrombocytopenic platelet membranes were similar. Those of GSLs were changed in that in addition to Glc other monosaccharides (Fuc, GalN, GlcN and Gal), previously barely detectable, were now present in the hydrolysates of GSL fractions. Our results suggest that an elevated GSL:Gp ratio in the cells may be a sign of an incomplete maturation or development as proposed by one of us (J. Kościelak, *Glycoconjugate J.* 1986, **3**: 95 – 108).