The carbohydrate moieties in glyco*proteins* influence uptake and distribution and provide protection against enzymatic proteolysis. To investigate the influence of glycosylation on the conformation, metabolic stability, and pharmacological properties of *peptides* we have prepared the derivatives **1 – 4** of the antidiuretic hormone vasopressin.

The glycopeptides **2** and **3** were prepared from N^{α}-Fmoc-3-O-(Ac_4-β-D-Gal)-Ser-OPfp by solid phase synthesis and purified by reversed phase HPLC (29 and 20% yields, respectively). Conformational studies using ^{1}H-NMR spectroscopy showed that **1 – 3** populate similar conformations in H_2O, which are independent of glycosylation and include a type II β-turn for the $Cys^6 - Gly^9$ segment. We also found that glycosylation (compounds **2** and **3**) abolished the antidiuretic effect of **1** and **4**, *but* resulted in a significant increase in metabolic stability towards rat intestinal juice and in transport over the intestinal wall. Furthermore, in rats the doses of **2** and **3** available in plasma after oral administration increased 20 – 30 fold as compared to the drug DDAVP (**1**).

S7.10

Influence of Amino Sugar Analogues on the Activation of Lymphocytes

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Human lymphocytes were prepared according to a method described by Boyum (1). We synthesized different radiolabelled amino sugar analogues (2). Their phosphorylation in a lymphocyte homogenate could be shown by chromatographic analysis. The uptake of the nonphysiological precursor *N*-propanoyl-D-mannosamine into the cytosolic fraction of lymphocytes is three times higher compared to *N*-acetyl-D-glucosamine. Similar results could be obtained for the incorporation into the glycoprotein or glyco-sphingolipid fraction, respectively. Induction of proliferation of a crude lymphocyte population could be achieved by use of the nontoxic *N*-acyl-D-mannosamines, especially by the *N*-propanoyl- and *N*-pentanoyl-derivatives. Using the respective glucosamine analogues only a week stimulation could be measured. The increase of ^{3}H-TdR incorporation was similar to that observed using Con A, which is shown to be toxic *in vivo*.

(1) Boyum, A. *Nature*, **204**, 793 – 794 (1964).
(2) Kayser, H. *et al.*, *J. Biol. Chem.*, **267**, 16934 – 16938 (1992).

S7.11

Isolation and Structural Determination of High-Molecular-Weight Glycan Units Present in Egg Cortical Granular-Derived Glycoproteins of *Bufo japonicus* and *Xenopus laevis*

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Cortical alveoli (or granules) are known to be specialized secretory vesicles found in the peripheral cytoplasm of mature eggs of almost entire animal species including sea urchin and human. Extensive investigations from our research groups have established that cortical alveoli of the eggs of teleost fishes contain carbohydrate-rich unique glycoproteins. Recently, we introduced the term 'hyosophorin' as a collective name to describe the family of cortical alveolar glycoprotein molecules. The criteria for defining glycoproteins in eggs and oocytes as 'hyosophorin' are: [1] Golgi-derived secretory vesicular, i.e. cortical alveolar (or granular) component; [2] carbohydrate-rich glycoproteins (about 85% of the total mass is carbohydrate); [3] high-molecular-weight form (H-hyosophorin) present in the unfertilized eggs is made up of the repeating glycopeptide unit(s) (L-hyosophorin); [4] proteolyzed into the tandem repeat unit(s) upon egg activation or fertilization; [5] during a certain stage of early embryogenesis, a part of the L-hyosophorin undergoes deglycosylation and an apo-L-hyosophorin is considered to play an important role in further development. Novel carbohydrate-rich sialoglycoproteins that we have tentatively identified as hyosophorin were isolated from the fertilized and unfertilized eggs of the amphibian species, *Bufo japonicus* and *Xenopus laevis*. In this study, the structure of the carbohydrate and peptide portions of the purified glycoproteins was studied. The glycoprotein isolated from *Bufo* was found to have a bulky multi-antennary *N*-linked glycan closely similar to the structure of *Oryzias* hyosophorin recently elucidated (1). The present results represent the first isolation and structural analysis of hyosophorin other than fish.

(1) T. Taguchi, A. Seko, K. Kitajima, S. Inoue, T. Iwamatsu, R. A. Wallace, K.-H. Khoo, H. R. Morris, A. Dell and Y. Inoue (1993) **In *the 12th International Symposium on Glycoconjugates.***

S7.12

Effect of Autoxidative Glycation on α-Crystallin

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Alteration of eye glycoconjugates (e.g. collagen, crystallin) underly the ocular complications of diabetes mellitus. In previous studies we showed crosslinking of corneal collagen type I following incubation with glucose and transition metal ions (Cu, Fe). The inhibitory effects of catalase and the chelator diethylenetriamine-pentaacetic acid (DTPA) support a mechanism involving hydrogen peroxide generated by metal catalyzed autoxidation of glucose and glycated collagen (1). In recent experiments, SDS-PAGE analysis of bovine α-crystallin incubated with 100 mM glucose + 10 μM $CuSO_4$, 37°, 1 – 7 days, showed time-dependent increases of a 43 kDa band. This observation, which is consistent with the formation of a crosslinked dimer of crystallin, was accompanied by increases in fluorescence (exc. 340 nm, em. 420 nm), in UV absorbance at 330 nm, and in carbonyl content. Since control experiments showed that the reaction requires glucose but proceeds in the absence of added $CuSO_4$, the crystallin preparation was subjected to electron probe X-ray microanalysis which showed the presence of endogenous bound Cu. Following removal of virtually all the contaminating Cu using Chelex 100 resin, we showed