aggregation of cells from the marine sponge *Microciona prolifera*. Homophilic binding of an extracellular adhesion proteoglycan mediates aggregation in the presence of Ca^{2+}. Aggregation-inhibiting monoclonal antibodies produced against the purified proteoglycan recognize carbohydrate epitopes (JBC 262 (1987) 5870 – 5877). In addition to uronic acid and *N*-acetylglucosamine these glycans also contain neutral sugars, including fucose. The glycans have been postulated to mediate polyvalent carbohydrate-carbohydrate interactions (JBC 261 (1986) 2853 – 2859). In order to elucidate the molecular mechanism of these interactions we have purified and characterized carbohydrate epitopes from the proteoglycan. One of the oligosaccharides isolated after fragmentation of the glycans was identified as the pyruvylated trisaccharide

$$\text{Pyr}<^{6}_{4}>\text{Gal}\beta1\text{-}4\text{GlcNAc}\beta1\text{-}3\text{Fuc}.$$

The negatively charged pyruvate is essential for the recognition by the blocking antibody and might also provide one of the Ca^{2+} binding sites necessary for the multivalent interaction of the adhesion proteoglycan. Because this structure does not resemble any previously known repeating unit of glycosaminoglycans attempts are being made to localize this structure in the native glycan. The role of the saccharide will be tested in the postulated polyvalent carbohydrate – carbohydrate mediated interactions of the sponge cell adhesion proteoglycan.

S5.7

Identification of a Cell Surface Heparan-Sulfate Proteoglycan Involved in HB-GAM (Heparin-binding Growth-Associated Molecule)-Induced Axon Outgrowth

E. Raulo, R. Nolo and H. Rauvala
Institute of Biotechnology, University of Helsinki, SF-00380 Helsinki, Finland.

HB-GAM (heparin-binding growth-associated molecule) was previously characterized as a neurite outgrowth-promoting protein localized to developing axonal pathways in rat brain. HB-GAM is strongly expressed in perinatal rat brain during the developmental phase of rapid axonal growth and formation of synaptic connections (1,2.).

Our observation, that HB-GAM-guided neurite outgrowth *in vitro* is specifically inhibited by heparin as well as in the presence of heparitinase led us to search for an HB-GAM binding cell surface component that contains heparin-type polysaccharide chains. Baculovirus recombinant HB-GAM was used to construct an affinity column. ^{35}S-sulfate labelled brain cells were homogenized in 50 mM octylglucoside, centrifuged and the supernatant was passed through the column and eluted in rising salt (0.15 – 2 M NaCl). Fractions were counted and treated with various GAG-degrading enzymes and run on SDS-PAGE followed by autoradiography. A 200-300 kDa heparitinase sensitive smear was identified as a cell surface heparan sulfate proteoglycan involved in HB-GAM function.

1. E. Raulo, I. Julkunen, J. Merenmies, R. Pihlaskari, and H. Rauvala (1992). Secretion and biological activities of heparin-binding growth-associated molecule (HB-GAM); Neurite outgrowth-promoting and mitogenic actions of the recombinant and tissue-derived protein. *J. Biol. Chem.* **267**: 11408 – 11416.

2. H. Rauvala, E. Castren, A. Vanhala, R. Nolo, E. Raulo, J. Merenmies and P. Panula (1993). Localization of HB-GAM (heparin-binding growth-associated molecule) to the pathways of developing axonal processes *in vivo* and neurite outgrowth along HB-GAM tracts *in vitro*. Submitted for publication.

S5.8

Chemical Composition of Two Commercial Heparins with Differing Antiproliferative Effect on Smooth Muscle Cells

H. G. Garg, B. T. Thompson, C. G. W. Dahlberg, P. M. Joseph, C. R. Spence and *C. A. Hales*
Pulmonary & Critical Care Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA.

We reported earlier that certain heparin (HP) preparations impair the development of hypoxic pulmonary arterial hypertension (Hales, C. A. *et al.*, ARRD, **128** (1983) 747 – 75; 139 (1989) 736 – 768). This ability of HP appears to be due to an antiproliferative effect on smooth muscle cells (SMC) and may be caused by inhibition of growth factor stimulation of the Na^+/H^+ exchanger, which is a prerequisite to SMC proliferation. The structural or chemical properties necessary to inhibit cell growth and Na^+/H^+ exchange are poorly understood. In this study, we examined the chemical properties of HP preparations from Upjohn (U) and Elkins-Sinn (E). HP (U) inhibits PA smooth muscle cells growth by 18 ± 4% at 1 μg/ml (N = 12; ± SEM) but HP (E) did not (2 ± 2% at 1 μg/ml, P = NS vs control growth without HP, N = 24). The protein contents in HP (U) and HP (E) preparations are 1.5 and 0.17 percent dry weight. Amino acids in HP (U) are: ETSFGAFKH whereas in HP (E) are: SGMFKH. The amounts of glucosamine in HP (U) and HP (E) are 7.12 and 12.2 percent, respectively. The sulfate composition of HP (U) and HP (E) was assayed by infrared assay and no appreciable differences are found. HP (U) does not contain galactosamine, suggesting that it is not contaminated with other proteoglycans. Our results demonstrate that commercial HPs differ in their antiproliferative potency and chemical composition. Differences in glucosamine, protein or galactosamine content but not the degree of sulfation may be important for the antiproliferative effect.

Supported by NIH Grant #HL 36829-1.

S5.9

Heparin-like Compounds Prepared by Chemical Modification of Capsular Polysaccharide From *E. Coli* K5

N. Razi[1], E. Feyzi[1], I. Björk[1], U. Lindahl[1], P. Oreste[2], F. Tursi[2], G. Zoppetti[2], G. Grazioli[3], M. Guerrini[3], A. Naggi[3], G. Torri[3], B. Casu[3]
[1]*Dept. of Medical and Physiol. Chemistry, Univ. of Uppsala, and Swedish Univ. of Agricultural Sci., Uppsala, Sweden;* [2]*Italfarmaco Research Center, Milan, Italy;* [3]*Istituto di Chimica e Biochimica G. Ronzoni, Milan, Italy.*

Capsular polysaccharide from *E. coli* K5, with the structure -[GlcAβ1,4-GlcNAcal,4]$_n$ (Vann *et al.* (1981) *Eur. J. Biochem.*