speed supernatant from mouse liver on *Q*-Sepharose followed by reconstitution experiments allowed us to identify three inactive fractions eluting at 170 mM, 300 mM and 430 mM NaCl. Hydroxylase activity could only be restored by mixing all three fractions. The fractions eluting at 170 mM and 430mM NaCl were shown to contain cytochrome b_5 reductase and cytochrome b_5, the remaining fraction to contain the CMP-Neu5Ac specific monooxygenase. The hydroxylase activity in this latter fraction could be reconstituted by addition of either detergent-solubilised mouse liver microsomes or purified microsomal cytochrome b_5 and cytochrome b_5 reductase.

Attempts to identify the molecular forms of cytochrome b_5 and cytochrome b_5 reductase in high speed supernatants revealed that the amphiphilic forms of both electron carriers predominate. Surprisingly, reconstitution of the above mentioned monooxygenase fraction with the purified forms of both electron carriers revealed that the hydroxylase interacts preferentially with the soluble proteins. Addition of detergent, however, enhanced the electron transfer from the amphiphilic forms, suggesting that cytochrome b_5 is rendered more accessible to the monooxygenase. The significance of these results for the situation *in vivo* remains open.

1. Shaw, L., Schneckenburger, P., Carlsen, J., Christiansen, K. and Schauer, R. (1992) *Eur. J. Biochem.*, **206**, 269–277.
2. Kozutsumi, Y., Kawano, T., Kawasaki, H., Suzuki, K., Yamakawa, T. and Suzuki, A. (1991) *J. Biochem.*, **110**, 429–435.

S18.11

Potential Regulatory Role of Sialic Acid Modifications in Cell Adhesion Mediated by Murine Sialoadhesin as a Macrophage-Restricted Cell Adhesion Molecule

*S. Kelm**, A. Pelz*, R. Schauer* and P. R. Crocker[+]
**Biochemisches Institut der Universität Kiel, Olshausenstraße 40, W-2300 Kiel, Germany; [+]ICRF Laboratories, Institute of Molecular Medicine, University of Oxford, OX3 9DU, UK.*

Murine sialoadhesin has been characterized as a stromal macrophage-restricted receptor of 185 kD which can bind to Neu5Acα2→3Galβ1→3GalNAc on cell surface glycoconjugates. The tissue specific distribution of sialoadhesin in bone marrow, the marginal zone of spleen and the subcapsular sinus and cortex of lymph nodes indicates a potential role of this receptor in cellular trafficking. Furthermore, binding assays using either iodinated purified receptor or macrophages induced to express sialoadhesin demonstrated that the receptor preferentially bound to granulocytes isolated from inflammatory sites, blood or bone marrow when compared to other hematopoietic cells which might interact with sialoadhesin expressing macrophages *in vivo*. This suggested that sialoadhesin plays a role in development and trafficking of granulocytes at different stages of their life span.

In the mouse modified sialic acids like Neu5,9Ac_2 and Neu5Gc have been found in tissue specific distribution. In various assays using cells and glycoconjugates containing appropriate structures we could demonstrate that sialoadhesin cannot bind to oligosaccharide structures containing Neu5,9Ac_2 or Neu5Gc as sialic acids. These results suggest that in the mouse modifications of sialic acid are important regulatory elements in cellular interactions mediated by sialoadhesin. The implications of characteristic features of the metabolic pathways leading to these sialic acid modifications on the regulation will be discussed.

S18.12

Characterisation of S-Adenosylmethionine: Sialate 8-*O*-Methyltransferase from Gonads of the Starfish *Asterias rubens*

A. de Freese, L. Shaw, G. Reuter and R. Schauer
Biochemisches Institut, Christian-Albrechts-Universität, Olshausenstr. 40, D-2300 Kiel 1, FRG.

The echinodermata are among the most primitive animals possessing sialoglycoconjugates. In addition to *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycoloylneuraminic acid (Neu5Gc) the starfish *Asterias rubens* contains *O*-methylated sialic acids, mainly in the form of *N*-glycoloyl-8-*O*-methylneuraminic acid (Neu5Gc8Me). Previous studies (1,2) demonstrated that the methylation of Neu5Gc in homogenates of whole starfish takes place at the level of glycoconjugate-bound sialic acid. In this work, we have further investigated the 8-*O*-methylation of Neu5Gc using cell free extracts of gonads of *Asterias rubens* fractionated by centrifugation.

After incubation of supernatant and particulate fractions with *S*-adenosyl-[^{14}C]-methionine, total protein was precipitated with trichloroacetic acid (10%) and washed to remove non-bound radioactivity. Protein-associated radiolabelled sialic acids were hydrolyzed with HCl (pH 1, 80°C, 1 h). Analysis of the hydrolysates by TLC revealed the presence of radioactivity comigrating with the standard Neu5Gc8Me only in incubations with the particulate tissue fraction. This shows that radioactivity had been transferred to endogenous glycoprotein-bound Neu5Gc and that the methyltransferase activity is in the particulate fraction. Methylation of free Neu5Gc was not detected in either tissue fraction. Addition of excess glycoconjugate-bound Neu5Gc in the form of horse erythrocyte ghosts (99% Neu5Gc) increased the incorporation of radioactivity significantly, giving the basis for a new sensitive test for the 8-*O*-methyltransferase.

(1) R. Schauer and M. Wember (1985) in: Glycoconjugates, Proc. VIIth Int. Symposium (E. A. Davidson, J. C. Williams, N. M. Di Ferrante, eds), Vol I Praeger, New York, 264–265.
(2) A. A. Bergwerff, S. H. D. Hulleman, J. P. Kamerling, J. F. G. Vliegenthart, L. Shaw, G. Reuter and R. Schauer (1992) *Biochimie* **74**, 25–38.

S18.13

A Novel Family of Gangliosides, "KDN-Gangliosides". Their Structures, Distribution, Sensitive Probes, and Biosynthesis

Y. Song, T. Terada, T. Angata, A. Kanamori, *K. Kitajima*, S. Inoue[1], Y. Muto, T. Kasama[2], S. Handa[2] and Y. Inoue
Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Hongo-7, Tokyo 113; [1]School of Pharmaceutical Sciences, Showa University, Hatanodai-1, Tokyo 142; [2]Department of Biochemistry, Faculty of Medicine, Tokyo Medical and Dental University, Yushima, Tokyo 113, Japan.

In 1991 we demonstrated for the first time the occurrence of a KDN-containing glycosphingolipid ("KDN-ganglioside") in rainbow trout sperm and its complete structure was