#### S1.37

# The Effect of Heparegen and *D*-Penicillamine on the Catabolism of Glycoconjugates in the Stomach and Liver of Rats Intoxication with Ethanol

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The aim of our report is to evaluate the influence of ethanol intoxication on the catabolism of glycoconjugates in the stomach mucosa and liver. The influence of Heparegen and *D*-Penicillamine on glycoconjugate catabolism was also investigated. The catabolism of glycoconjugates was evaluated on the basis of determination the specific activity of exoglycosidases (*N*-acetyl- $\beta$ -hexosaminidase,  $\alpha$ -mannosidase and  $\beta$ -galactosidase) in homogenates of gastric mucosa and liver. It seems that ethanol, Heparegen and *D*-Penicillamine alone increase the catabolism of glycoconjugates in the gastric mucosa and liver. Heparegen when given simultaneously with ethanol prevents an increase in catabolism of glycoconjugates in the liver but has no effects on the gastric tissue.

#### S1.38

## Solubilization and Characterization of the Yeast Dehydrodolichyl Pyrophosphate Synthase

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When the membrane preparation of Saccharomyces carlsbergensis was incubated with the radiolabelled isopentenyl pyrophosphate in the presence of farnesyl pyrophosphate and Mg<sup>2+</sup>, both the phosphorylated and free long chain polyprenols were formed. The reaction was inhibited by EDTA and heavy metal cations. A series of nonionic detergents were studied to solubilize the prenyltransferase. The enzyme completely lost its activity in the presence of 0.1% of Triton X-100. n-Octyl- $\beta$ -(D)glucopyranoside at the concentrations of 0.25-0.5% (10-15 mM) was used to solubilize the prenyltransferase. Both the membrane-bound enzyme and the solubilizate possessed a broad pH optimum shifted to the alkaline pH values. The temperature optimum of the solubilizate was somewhat lower than that of the membrane preparation obviously due to significantly inferior thermostability of the solubilized enzyme in comparison with the membrane-bound one. The phosphorylated reaction products formed in the presence of the membrane preparation did not differ in their composition from the yeast dolichol synthesized in vivo. The non-phosphorylated polyprenols were formed during incubation with the membranes but not the solubilized enzyme. The composition of the polyprenols was also coincident with that of yeast dolichol, and the individual C<sub>80</sub>-homolog of the mixture being polyprenol but not dolichol as judged by adsorption HPLC. The results are discussed in connection to the terminal stages of dolichol biosynthesis.

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# Solubilization and Characterization of Dolichyl Pyrophosphate Phosphatase From Yeasts

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*N*-Glycosidic linkages are formed by the transfer of glycosylated "pro-oligosaccharides" from Dol-PP to polypeptide acceptors. Presumably, the pyrophosphate form of the glycosyl carrier lipid is discharged as *N*-glycosylation occurs. Dol-P would then be regenerated by the action of a Dol-PP phosphatase. Therefore, this phosphatase plays an important role in reforming Dol-P to initiate the synthesis of a new oligosaccharide lipid intermediate and to maintain the *N*-glycosylation process.

To investigate Dol-PP phosphatase in yeast  $[\beta^{-33}P]$ -Dol-PP has been prepared chemically. Yeast membrane preparations catalyze the enzymatic release of  ${}^{33}P_i$ , from exogenous  $[\beta^{-33}P]$ -Dol-PP. Subcellular distribution studies indicate that the enzyme is localized in the microsomal fraction and vacuoles. A substantial fraction of the Dol-PP phosphatase activity can be solubilized by treating yeast microsomes with Triton X-100 at protein to detergent ratio 1:1 (w/w). The solubilized Dol-PP phosphatase activity: 1) is optimal at pH 8; 2) is inhibited by divalent cations and stimulated by EDTA; 3) is stable at 50°C. Procedures for yeast membrane fractionation and the solubilization of Dol-PP phosphatase activity from microsomes are presented.

#### S1.40

### Isolation and Characterization of Arabinosyl- and Ribosyl-1-Monophosphopolyprenols from Mycobacterium Smegmatis

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A family of monoglycosylated polyprenyl phosphates was isolated from exponentially growing Mycobacterium smegmatis. Arabinose, ribose, mannose and glucose were identified as the major glycosyl residues within the endogenous polyprenyl-P-sugar pool. The isoprenoid nature of the lipid component was established by 'H-NMR spectroscopy. Fast atom bombardment/mass spectrometry analysis demonstrated the predominance of the C50 decaprenyl-P-analogs with much smaller amounts of the C35 octahydroheptaprenyl-P-products; these contained either pentosyl or hexosyl units. The identification of decaprenyl-Parabinose in mycobacteria implies that it is an intermediate in the biosynthesis of the arabinan component of cell wall arabinogalactan and lipoarabinomannan. Ethambutol, a powerful antituberculosis drug known to inhibit arabinan biosynthesis, appeared to result in the accumulation of the decaprenyl-P-arabinose with a corresponding decrease in the cellular levels of polyprenyl-P-ribose. Conversely, ageing of cultures resulted in an accumulation of the polyprenyl-Pribose and a decrease in polyprenyl-P-arabinose, all pointing