teratocarcinoma F9 cells. Northern blot analysis showed that, following 4 – 5 days' exposure of F9 cells to RA(1 μM), there was a 8 – 10-fold increase of β-1,4-galactosyltransferase(β-1,4-GalT) mRNA level. In contrast, actin mRNA level was slightly increased at one and a half times during the same interval.

The rate of β-1,4-GalT and actin gene transcription, assayed by nuclear run-on assays, remained unchanged in F9 cells exposed to RA. The half-life of β-1,4-GalT mRNA, determined by pulse-chase with [3H] uridine, was greatly prolonged in RA-treated F9 cells as compared with untreated cells. These results suggests that RA can up-regulate β-1,4-GalT expression in F9 cells at a posttranscriptional level by stabilizing its mRNA.

S13.17

Carbohydrate Analysis of Invertase from Wild Type and Mutant Strains of *Aspergillus nidulans*

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A. nidulans is of interest to biotechnologists because it can be grown on simple media, secretes glycoproteins efficiently and is well studied genetically. The potential of this organism for expression of heterologous glycoproteins has made it important to understand more clearly the relationship between glycosylation and secretion and to attempt to engineer a glycoprotein pattern similar to that in animals. As part of this study we are investigating the changes in glycosylation of the model glycoprotein invertase in mutants prepared by a ^{3}[H]-mannose-suicide technique. Induced invertase, from wild type and mutants, was secreted into a culture filtrate and partly purified by batch DEAE-Sephadex, DEAE Mem-Sep cartridge, followed by gel filtration on Superose 12 and Superdex columns. Determination of the monosaccharide content of the enzyme was by HPAE-PAD. The enzyme was found to be heavily glycosylated (>50%), with mannose and galactose being the main constituents. In the mutants examined the mannose and galactose content was reduced by 60% and 40% respectively. The purified invertase gave a broad band staining for protein running in the region of 80 kD on SDS PAGE, this reacted positively for terminally bound mannose using the lectin GNA. The deglycosylated material ran as a narrow band at 65 kD. This deglycosylated material is being used to raise antibody. The complex glycan structures released from the enzyme are being analysed.

S13.18

Effects of LDL on Protein Glycosylation in Platelet Precursors

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The platelet is derived from a bone marrow precursor cell, the megakaryocyte. These cells produce the surface glycoproteins which mediate the platelet's involvement in adhesion and aggregation. Changes in the complement of platelet glycoproteins cause functional disorders. There is good evidence that risk factors of coronary heart disease such as dietary fat and cholesterol induce changes in platelet function.

The collection and purification of sufficient quantities of megakaryocytes for experimental purposes poses severe difficulties. Therefore an immortal cell line, RPM, which is an analogue of an immature form of rat megakaryocyte is being used. RPM cells are being incubated with purified human LDL and changes in the surface glycans of the cell detected by isotopic labelling with radioactive *N*-acetylglucosamine. This is incorporated both directly and as *N*-acetylgalactosamine and *N*-acetylneuraminic acid after enzymatic modification in the cell. Levels of glycosylation of both protein and lipid fractions of the cells and the level of cell sialylation can then be measured. Further characterisation of changes in the surface glycosylation involves monosaccharide analyses, elution profiles and composition of both *N*- and *O*-linked oligosaccharides present using high pH anion exchange chromatography in conjunction with pulsed amperometric detection. Present work indicates that cells incubated with LDL for 24 and 48 hours possess a lower level of surface sialylation, 87% ± 9% and 62% ± 9%, respectively, compared to control levels.

S13.19

Specific Inhibitor and Stimulator of Glycosphingolipid Biosynthesis

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The addition of a glycosphingolipid (GSL) to a cell or membrane preparation may produce a misleading effect if the site of action is already occupied by endogenous GSL. Moreover, the production of an effect by exogenous GSL may not signify a physiological role for this lipid since it might simply react with the effector in a nonphysiological way. From these reasons, there was a need to establish the physiological significance of the effect by depleting cellular GSLs specifically. The role of endogenous GSL has been assessed recently because of the availability of D-PDMP (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol) and L-PDMP [reviewed in ref. 1]. The former compound inhibits the synthesis of GlcCer by suppressing the activity of UDP-glucose:ceramide glucosyltransferase, resulting extensive depletion of GSLs derived from glucosylceramide (GlcCer). Moreover, D-PDMP enhances the cellular levels of ceramide and sphingoid base. In contrast, L-PDMP increases the cellular levels of GSLs, especially lactosylceramide [2].

The stimulatory effect of L-PDMP and its homologs having different length of acylchain have been further examined in metabolically labelled ([^{3}H]Gal) B16 melanoma and human fibroblast cells. We have synthesized various analogs of PDMP which possess different types of amines instead of morpholine ring, and have assessed their effects on GlcCer synthase activity. Among them the piperidine analog (D-isomer) exhibited the most potent inhibitory activity.

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