123 ± 8 mm Hg; SHR: 177 ± 5 mm Hg). Blood was drawn on Potassium EDTA (final concentration 2 mg/ml) after overnight fasting from abdominal artery.

The insulin level was quantitated by standard radioimmunoassay (POLATOM, Poland). The proportion between glycosylated and total haemoglobin was tested spectrophotometrically based on different affinity glycosylated and normal haemoglobin to cation – exchange resin. Mann – Whitney rank sum test of unpaired data were used for analysis of differences between SHR and WKY. $P<0.05$ are considered significant.

The insulin amount of hypertensive rats plasma (10.2 ± 2.6μlU/ml, No. = 7) was lower compared with normotensive rats material (17.9 ± 4.7 μlU/ml, No. = 7). However the percentage of glycosylated haemoglobin in SHR red blood cells (3.5 ± 0.5%, No. = 6) was compared to WKY probes (3.7 ± 1.0%, No. = 6).

No differences observed in glycosylated haemoglobin differences between SHR and WKY can be explained by compensatory process as an insulin resistance in genetically induced hypertension.

S15.30

Expression of Laminin, Tenascin and Fibronectin by Cultures of Skin Fibroblasts from Patients with Tuberous Sclerosis

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Tuberous sclerosis (TS) is a genetic disorder which affects cellular migration, proliferation and differentiation, causing a variety of neurological and skin lesions. Large, slowly dividing dendritic cells in fibroblast cultures of TS skin lesions have previously been described. It has also been reported that affected tissue from TS patients contains high levels of carbohydrates associated with glycoproteins, especially fibronectin. In order to understand the biological consequences of such changes in the extracellular matrix (ECM), we are purifying and further analysing the glycans of fibronectin from conditioned medium of skin fibroblasts of TS patients and normal individuals. This involves gelatin affinity and anion-exchange liquid chromatography, and also high pH anion-exchange chromatography (Dionex). Our immunocytochemical studies have confirmed that laminin is not expressed at significant levels in skin fibroblasts from normal individuals, however we find strong staining with anti-laminin in TS skin fibroblasts. TS fibroblasts also show increased and abnormal staining with anti-tenascin compared to normal fibroblasts.

However, anti-fibronectin staining shows similar levels of immunoreactivity, with only slight changes in distribution between normal and diseased cells. Staining with lectins and anti-leu7 (for the expression of HNK-1 carbohydrate epitope) on these glycoproteins and fibroblasts are also under investigation. Alterations in distribution and structure of these glycoproteins may cause functional disruption in their binding and interactions with cells and ECM macromolecules. Studies of these changes may contribute to the understanding of the mechanisms involved in the aetiology of hardened tissues of TS.

S15.31

Monosaccharide Composition of Haptoglobin in Liver Disease: Fucose/Sialic Acid Ratio is the Best Discriminator of Alcoholic and Non-Alcoholic Disease

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The glycosylation of many serum proteins such as α_1 acid glycoprotein, α_1 antitrypsin, haptoglobin and transferrin is affected by alcoholic liver disease (ALD). Desialylation, increased branching and fucosylation have all been reported. Previously, we have shown that there are large increases in the concentrations of fucose and *N*-acetyl glucosamine (GlcNAc) in alcoholic cirrhosis (*Biochem. Soc. Trans.*, 21: 214S, 1993). In order to investigate this further, we have determined the monosaccharide content of haptoglobin (Hp) isolated from patients with different liver diseases, a group of alcohol abusers (AA) and healthy individuals. Hp was isolated by affinity chromatography using an anti-Hp antibody. Protein content and the purity of the extracted Hp were established by rocket electrophoresis and SDS-PAGE respectively. Aliquots of the purified Hp were subjected to acid hydrolysis in 0.1 M trifluoroacetic acid (TFA) at 80°C for 1 hour to release *N*-acetyl neuraminic acid (NANA) and 2 M TFA at 100°C for 5 hours to release the neutral sugars. Monosaccharides were separated by high pH anion exchange chromatography (HPAEC) using a Carbopac PA100 column eluted with a 50 – 150 mM sodium acetate gradient in 100 mM NaOH for NANA, and 15 mM isocratic NaOH for the neutral sugars. Separated monosaccharides were detected by a pulsed amperometric detector. Fucose and GlcNAc levels were elevated in Hp from all disease groups when compared with Hp from the "healthy" group (Mann – Whitney, $P<0.05$). The highest fucose levels were found in alcoholic cirrhosis and these were significantly higher ($P<0.05$) than those obtained in the non-ALD and AA groups. The NANA content of Hp from the alcoholic cirrhosis group was significantly lower ($P<0.05$) than that of Hp from the other groups. The best discriminator between the ALD and non-ALD groups was the ratio of fucose/sialic acid; a high value being associated with ALD and a lower value being associated with non-ALD or AA.