

British Association for the Study of the Liver

The 1991 Spring Meeting of the British Association for the Study of the Liver was held at the Hammersmith Hospital, London on 21-22 February 1991. The 18 abstracts selected for presentation by the Programme Committee are printed below.

Effect of total hepatectomy with and without orthotopic liver transplantation on systemic haemodynamics and oxygen transport variables in fulminant hepatic failure

P M HARRISON, J A WENDON, A E S GIMSON, J O'GRADY, K C TAN, ROGER WILLIAMS (*Institute of Liver Studies, King's College Hospital and School of Medicine and Dentistry, London*) Patients with fulminant hepatic failure (FHF), irrespective of aetiology, have a hyperdynamic circulation. In addition, tissue oxygen consumption becomes dependent upon oxygen delivery as the ability to extract oxygen is reduced. In this prospective study we have investigated the changes in these circulatory abnormalities after orthotopic liver transplantation in 11 patients with FHF. The systemic vascular resistance index rose from 638 (195) immediately before transplant to 1144 (292) dyn·second/cm⁵·m² afterwards ($p < 0.001$), associated with an increase in mean arterial pressure from 70 (13) to 90 (12) mm Hg ($p < 0.01$), despite a fall in cardiac index from 6.8 (1.2) to 5.4 (1.7) l/minute ($p < 0.05$). Although oxygen delivery remained constant at 753 ml/minute/m², oxygen consumption rose from 91 (25) to 136 (22) ml/minute/m² ($p < 0.01$) because of an increase in the oxygen extraction ratio from 12 (1) to 19 (4%) ($p < 0.001$).

Although these changes might be attributable to the rapid removal of vasoactive substances by the grafted liver, the same changes were seen in two other patients studied immediately after total hepatectomy, performed as an emergency procedure before delayed liver grafting.

On the basis of these findings, it is postulated that the failing liver is producing substances which lead to vasodilatation and impairment of microcirculatory blood flow.

Role of endothelium derived nitric oxide in the control of hepatic arterial vascular tone

R T MATHIE, B ALEXANDER, V RALEVIC, G BURNSTOCK (*Department of Surgery, Royal Postgraduate Medical School; Department of Surgery, King's College School of Medicine; and Department of Anatomy, University College, London*) The recent identification of endothelially derived relaxing factor (EDRF) as nitric oxide (NO) has transformed our broad understanding of vasodilatory mechanisms in the cardiovascular system. Adenosine 5'-triphosphate (ATP) is thought to induce vasodilatation predominantly via EDRF released by the activation of a purinergic receptor located on the vascular endothelium.¹ In this study, the role of NO in ATP induced vasodilatation of the hepatic arterial (HA) vascular bed was investigated in an *in vitro* perfused rabbit liver model.

Livers of 16 New Zealand white rabbits were perfused with Krebs-Bülbring buffer via both

the hepatic artery and the portal vein, at constant flows of 24 ml/minute/100 g (140 mm Hg perfusion pressure) and 76 ml/minute/100 g (10 mm Hg perfusion pressure) respectively. ATP and the endothelium dependent vasodilator acetylcholine (ACh) produced dose related dilatations of the HA bed which were significantly antagonised by the NO inactivator methylene blue (MeB, 10 µmol/l) and by the L-arginine: NO pathway inhibitors N-monomethyl-L-arginine (L-NMMA, 100 µmol/l) and N-nitro-L-arginine methyl ester (L-NOARGme, 100 µmol/l). Responses to the endothelium independent vasodilators adenosine and sodium nitroprusside were not inhibited by MeB, L-NMMA, or L-NOARGme.

These results support the view that NO is the mediator of vasodilatation resulting from activation of an endothelial ATP purinoceptor, and indicate that NO may have an important role in the control of HA vascular tone.

1 Palmer RMJ, *et al. Nature* 1987; 327: 524.

2 Burnstock G, Kennedy C. *Prog Pharmacol* 1986; 6: 111.

Control of hepatocyte proliferation by hepatocyte growth factor

A J MARKER, M BUSHFIELD, S PALMER, G W GOULD, T NAKAMURA (*Department of Pathology and Biochemistry, University of Glasgow and Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan*) Hepatocyte growth factor (HGF) is the most potent known mitogen for hepatocytes in primary culture and is thought to have an important role in stimulating liver cell proliferation during liver regeneration. We have attempted to characterise the intracellular signalling systems through which HGF induces hepatocytes to proliferate. Using either freshly isolated or cultured rat hepatocytes, we have examined the effects of HGF on hepatocyte adenylate cyclase, phospho-inositide metabolism, and protein phosphorylation. HGF induced the concentration dependent stimulation of DNA synthesis in cultured hepatocytes with an EC₅₀ of 6 ng/ml. HGF had no significant effect on the stimulation of adenylate cyclase by forskolin (100 µmol/l) or glucagon (10⁻⁹ to 10⁻⁶ mol/l). Moreover, HGF had no effect on the intracellular value of IP₃ or [3-H] inositol phosphates in freshly isolated and cultured hepatocytes. However, incubation of freshly isolated hepatocytes with HGF (12 ng/ml) resulted in an increase in the phosphorylation of an unidentified protein of approximately 44 000 mr. HGF also induced a time dependent increase in protein phosphorylation in cultured hepatocytes. Using anti-phosphotyrosine antibodies we have demonstrated that HGF induces an increase in tyrosine phosphorylation in cultured hepatocytes. These results indicate that the HGF receptor may belong to the family of growth factor receptor tyrosine kinases which includes EGF, PDGF, and insulin.

Perisinusoidal (ITO) cell proliferation and phenotypic modulation in acute liver injury

S J JOHNSON, J E HINES, A D BURT (*Division of Pathology, School of Pathological Sciences, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne*) Perisinusoidal (ITO) cells (PSCs) are considered the principal source of extracellular matrix proteins during hepatic fibrogenesis. Previous *in vitro* studies have shown that in response to certain peptide growth factors, PSCs proliferate and may become activated. Activated PSCs show altered expression of cytoskeletal proteins and contain the alpha isoform of actin, a protein characteristic of myofibroblasts. In the present study we have investigated the *in vivo* response of PSCs to acute liver injury.

Perivenular necrosis was induced in Wistar rats using a single intragastric bolus of carbon tetrachloride. Liver tissue was obtained on days 0 (control), 1, 2, 3, 4, 7, and 10; bromodeoxyuridine (BrdU) was administered one hour before death. PSCs were identified by immunohistochemistry using a monoclonal anti-desmin antibody; PSCs in S phase were detected using a double labelling method for the simultaneous demonstration of desmin and incorporated BrdU. Expression of alpha actin was investigated using immunohistochemistry.

Quantitative analysis showed expansion of the desmin positive PSC population in perivenular zones, reaching a peak on day 3; these cells were larger and exhibited more intense desmin immunoreactivity than PSCs in control animals. Proliferating PSCs could be identified on day 1 with a peak in the labelling index on day 2 (18.7 (0.9)%). In parallel with the increase in desmin positive PSCs, there was accumulation of morphologically similar cells expressing alpha actin. These results suggest that PSCs proliferate and may undergo phenotypic modulation in response to experimental acute liver injury.

Kupffer cells release a 95 kD metalloproteinase with degradative activity against gelatin

P J WINWOOD, P S KOWALSKI-SAUNDERS, M J P ARTHUR (*Department of Medicine II, University of Southampton*) Degradation of the normal liver matrix (comprised of type IV collagen, laminin, and proteoglycans) results in hepatic lipocyte activation and promotes liver fibrosis. We have previously reported release of a 72 kD type IV collagenase-gelatinase by lipocytes and now report identification of a 95 kD gelatinase released by rat Kupffer cells.

Kupffer cells were prepared from rat liver by pronase/collagenase perfusion and purified by density centrifugation and centrifugal elutriation. Release of gelatinase activity was assessed by gelatin substrate SDS-PAGE and degradation of ¹⁴C-gelatin.

By substrate gel analysis, Kupffer cells release of 95 kD gelatin degrading activity which was inhibited by EDTA but not by thiol or serine proteinase inhibitors. The proteinase

did not degrade casein. Aminophenylmercuric acetate (an activator of pro-metalloproteinases), increased detectable gelatinase activity in conditioned media from 2.81 to 5.23 mU/ μ g DNA/24 hours. Exposure of Kupffer cells to phorbol myristate acetate increased release of total gelatinase activity to 9.5 mU/ μ g DNA/24 hours ($p < 0.05$, $n = 5$).

These results indicate that Kupffer cells release a 95 kD latent gelatinase (identical to monocyte-derived type IV collagenase/gelatinase), with increased release after Kupffer cell activation. This enzyme has the potential to degrade normal liver matrix and may be important in liver injury and fibrosis.

Activity and subcellular distribution of hepatic phosphatidate phosphohydrolase (EC3.1.3.4) in alcoholic fatty liver

K J SIMPSON, S VENKATESAN, D N BRINDLEY, T J PETERS (*Division of Clinical Cell Biology, MRC Clinical Research Centre, Harrow, and AHFMR Lipid Group, Edmonton, Canada and Clinical Biochemistry, King's College SMD, London*) The pathogenesis of alcoholic fatty liver remains obscure but enhanced triacylglycerol synthesis has been implicated. Phosphatidate phosphohydrolase (PAH) catalyses the conversion of phosphatidate to diacylglycerol and is the rate limiting step in triacylglycerol synthesis. Under the influence of fatty acids, soluble PAH interacts with the endoplasmic reticulum, where its activity is expressed, and this has been proposed as an important control in triacylglycerol synthesis.

The activity and distribution of hepatic PAH was assayed, for the first time, with and without oleate, in control subjects and patients with alcoholic liver disease. The specific activity of PAH in total homogenate, cytosolic, and membranous compartments was similar in patients and controls. Significantly more PAH was associated with the membranous fraction in patients with moderate/severe fatty liver (26.1% (6.9), $n = 11$, mean (SD), $p < 0.05$) than patients with mild fatty liver (18.9% (4.8), $n = 9$) and controls (19.4% (5.5), $n = 8$). Oleate produced a shift in activity from the cytosol to the membranous fraction in all patients studied. However, the translocation of enzyme activity was significantly reduced in patients with moderate/severe fatty liver (55.4% (9.8), $n = 11$, $p < 0.05$) compared with patients with mild fatty liver (69.0% (12.3), $n = 9$) and controls (77.1% (20.0), $n = 8$).

PAH in human liver is, therefore, predominantly cytosolic in location and oleate shifts it to the membranous compartment. The increased membrane associated PAH may, therefore, explain the enhanced triacylglycerol synthesis in alcoholic fatty liver.

Sandostatin in the long term management of portal hypertension – a preliminary prospective randomised controlled clinical trial

S A JENKINS, S ELLENBOGEN, J N BAXTER, M CRITCHLEY, J S GRIME, R SHIELDS (*University Departments of Surgery and Nuclear Medicine, Royal Liverpool Hospital, Liverpool*) Since sandostatin (SMS) administration results in a sustained decrease in portal pressure, we have examined its efficacy as an adjuvant to injection sclerotherapy (IS) in the long term management of portal hypertension.

Approximately three weeks after their first variceal bleed, 32 cirrhotic patients were readmitted to hospital for a thorough investiga-

tion of the severity of their liver disease, including assessment of reticuloendothelial system (RES) activity (single photon emission computed tomography), hepatocyte function (aminopyrine breath test), and wedge hepatic venous pressure (WHVP). Sixteen patients were randomised to IS and SMS and 16 to IS alone. The efficacy of the two treatments were evaluated six months later.

In patients receiving IS and SMS compared with those receiving IS alone there were significant reductions in mortality (1/16 *v* 5/16) $p = 0.434$ (Fisher's exact test), number of variceal rebleeds (0 *v* 16) $p < 0.001$, and number of sclerotherapy sessions required to obliterate the varices (40 *v* 89) $p < 0.05$. Combined IS and SMS also resulted in a sustained decrease in WHVP (25.1 (1.8) to 19.2 (1.2) mm Hg $p < 0.002$) but significantly stimulated hepatic RES activity (19.8 (1.3) to 29.8 (1.5); % injected dose 99m Tc sulphur colloid) and hepatocyte function (1.5 (0.2) to 3.7 (0.6) % cumulative excretion 14 C₂). No significant changes in WHVP, RES activity, or hepatocyte function were observed in the IS group.

These results suggest that SMS may be a valuable adjuvant to IS in the long term management of portal hypertension.

Chromosome allele loss in hepatocellular carcinoma

S DING, J DOOLEY, J DELHANTY, L BOWLES, C WOOD, N HABIB (*Liver Unit, Royal Free Hospital School of Medicine, Department of Surgery, Hammersmith Hospital, Department of Genetics and Biometry, University College London, London*) The aim of this study was to identify consistent changes in DNA (allele loss) in hepatocellular carcinoma (HCC) which might represent tumour suppressor gene loss. We have prepared DNA from tumour and non-tumour materials from 19 patients with HCC (hepatitis B virus (HBV) positive: 8; HBV negative: 7; fibrolamellar: 4). Southern analysis was performed with 29 restriction fragment length polymorphism probes assigned to chromosomes 1, 2, 3, 4, 5, 7, 9, 11, 12, 13, 14, 16, 17, 18, and 20, and the pattern of banding in tumour DNA compared with that in non-tumour DNA. Non-tumour DNA from four of the seven patients with HBV negative HCC had an informative (heterozygous) pattern with probe Lambda MS8 (5q35-qter), and in all four there was loss of an allele (band) in tumour DNA. No loss was found with this probe in either HBV positive or fibrolamellar HCC. A probe near the locus of p53 tumour suppressor gene on 17p13 showed allele loss in three out of four informative HBV negative HCC and five out of five informative HBV positive HCC. Screening with other probes has shown no consistent allele loss in all three types of HCC yet.

In conclusion, this study suggests that the possible tumour suppressor gene loss on chromosomes 5 and 17 might play a role in the development of HCC.

Mutational activation of ras oncogenes in human hepatocarcinogenesis

C CHALLEN, K GUO, D CAVANAGH, M F BASSENDINE (*Department of Medicine, The University of Newcastle upon Tyne*) The ras proto-oncogene family consists of three closely related genes, Kras, Hras, and Nras which can become activated by point mutations in the coding

regions particularly in codons 12 and 61. In rodents, ras activation has been reported in both spontaneous and chemically induced hepatocellular carcinoma (HCC). The aim of this study was to look for mutational activation in codons 12 and 61 of Kras, Hras, and Nras genes in human HCC.

DNA was extracted from HCC tissue ($n = 21$) and corresponding non-malignant tissue ($n = 10$), and amplified using the polymerase chain reaction (PCR) with oligonucleotide primers flanking the ras coding sequence of interest. Some 10–20 μ l of the amplified product was denatured, slot blotted onto nylon filters, and hybridised with oligonucleotides specific for point mutations at codons 12 and 61 of each ras gene using the tetramethylammonium chloride method.

One of 21 HCC's had a mutation at codon 12 of the Kras gene, which resulted in replacement of glycine (GGT) with serine (AGT); another had a mutation of codon 61 (CAACAT). In both cases the corresponding non-malignant tissue had the normal codon, suggesting mutational activation of Kras was involved in transformation to malignancy in two of 21 (10%). Three tumours had mutations at codon 61 of the Nras gene, all resulted in replacement of glutamine with leucine (CTA). In one patient the same mutation was found in the corresponding cirrhotic liver suggesting that mutational activation of Nras was an early event in multistage hepatocarcinogenesis.

In contrast to cholangiocarcinoma, where a high prevalence of ras gene activation has been reported, we have found that such mutational activation occurs in a minority (25%) of cases of HCC.

Protective role of antiandrogens in experimental hepatocellular cancer

M A PARSONS, G R RODGERS, S TEJURA, P MINGLETON (*Department of Pathology, University of Sheffield Medical School*) The effects of the antiandrogens flutamide (FLUT) and cyproterone acetate (CYP) were studied in female SUAH Wistar rats given diethylnitrosamine (DENA). In this model, as in human hepatocellular cancer (HCC), raised hepatic androgen receptor (AR) and reduced oestrogen receptor (ER) values accompany liver tumour formation. Female rats (12/group) were fed DENA (5 mg/100 ml water) for 16 weeks, with FLUT or CYP (1.5 mg/100 g food) for 16 or 20 weeks (controls: DENA alone/untreated). DENA alone induced neoplastic nodules (NN) or HCC, or both, in all rats at 16/20 weeks; at 16 weeks FLUT/CYP reduced tumour incidence to 58% and 45% (χ^2 , $p < 0.05$) respectively; at 20 weeks FLUT/CYP had no effect on the 100% tumour incidence. At 16 and 20 weeks DENA raised liver AR (fmol/mg cytosol protein) $p < 0.001$, but FLUT/CYP inhibited this rise (16 weeks control: 0.39 (0.01); DENA: 5.40 (1.28); DENA+FLUT: 1.95 (0.34); DENA+CYP: 0.98 (0.13), $p < 0.02$); 20 weeks control: 0.28 (0.12); DENA: 5.11 (1.15); DENA+FLUT: 1.62 (0.38); DENA+CYP: 0.72 (0.18), $p < 0.02$). At 16 weeks DENA+FLUT or CYP reduced ER (control: 19.7 (0.27); DENA: 12.95 (3.15); DENA+FLUT: 12.4 (3.2); DENA+CYP: 8.64 (2.17), $p < 0.05$).

Antiandrogens seem to delay liver tumour formation but do not affect progression of established tumours (despite partial reversal of AR changes); this has possible implications for humans at high risk of developing liver cancer.

$\gamma\delta$ +T clones from liver biopsy specimens of children with autoimmune chronic active hepatitis and primary sclerosing cholangitis are cytotoxic to human liver target cells

L WEN, M PEAKMAN, A P MOWAT, G MIELI-VERGANI, D VERGANI (*Departments of Immunology and Child Health, King's College Hospital, London*) Autoimmune chronic active hepatitis (ACAH) and primary sclerosing cholangitis (PSC) are liver diseases of unknown aetiology but with an autoimmune pathogenesis. To characterise the T lymphocytes infiltrating the liver, we established T cell clones from needle biopsy specimens of three children with ACAH and two with PSC by limiting dilution in the presence of recombinant interleukin 2. Sixty two T cells clones were obtained from the children with PSC and 60 from the children with ACAH. Fifty one clones expressed $\gamma\delta$ receptor, 41 from the PSC (37 CD4-/CD8- and 4 CD8+) and 10 from ACAH (all CD4-/CD8-). Forty six clones expressed the $\alpha\beta$ receptor and were either CD4+ or CD8+. Thirty $\gamma\delta$ +T cell clones (19 from PSC and 11 from ACAH) were tested against liver and non-liver specific targets and showed increased non-MHC restricted cytotoxicity to human hepatoma (Alexander) cells (median 22%), lower cytotoxicity to the NK sensitive target (K562) cells (median 14%), but no cytotoxicity to tumour cells derived from human vulva (A431, median 7%). None of 25 $\alpha\beta$ +T cell clones tested showed cytotoxicity to any of the targets.

We conclude that $\gamma\delta$ +T but not $\alpha\beta$ +T lymphocytes may be directly involved in the liver damage both in PSC and ACAH.

T cell antigen receptor V γ gene usage of T cell clones derived from liver biopsy specimens of children with autoimmune liver disease.

L WEN, A DEMAINE, S WONG, M HIBBERD, A P MOWAT, G MIELI-VERGANI, D VERGANI (*Departments of Medicine, Immunology and Child Health, King's College Hospital, London*) T cells recognise antigens via one of two T cell antigen receptors (TCR- $\alpha\beta$ or $\gamma\delta$). Each α , β , γ , or δ chain is composed of a variable (V) and a constant (C) region. It has been suggested that in certain autoimmune diseases there is preferential utilisation of certain TCR-V genes. We have previously shown that peripheral TCR- $\gamma\delta$ bearing T cells are raised in children with primary sclerosing cholangitis (PSC) and autoimmune chronic active hepatitis (CAH), and may be important in the pathogenesis of these diseases. We have investigated the TCR-V γ gene usage in 19 $\gamma\delta$ -T cell clones with cytotoxic activity derived from five liver biopsy specimens of children with PSC (n=2) and CAH (n=3). Total cytoplasmic RNA was isolated from 1×10^6 cloned cells and used to synthesise cDNA using oligodeoxyribonucleotides and avian myeloblastosis virus reverse transcriptase. Polymerase chain reaction was performed on the cDNA using oligonucleotide primers specific for either TCR-V γ 1, V γ 2, or V γ 3 subgroups; the V γ 4 subgroup was deemed to be present in the absence of V γ 1-3. The amplified products were analysed by electrophoresis in 1% agarose. Six of 19 clones utilised V γ 1, 4 V γ 2, 9 V γ 4, and none expressed V γ 3. This distribution differs from that found in normal subjects where 60% of $\gamma\delta$ T cells express V γ 1, 10%V γ 2, 10%V γ 3 and <10%V γ 4.

These preliminary results suggest that TCR-V γ 4 gene usage may be important in the pathogenesis of autoimmune liver diseases.

Oligonucleotide DNA typing analysis of the HLA DRB3 locus in patients with primary sclerosing cholangitis (PSC)

W Z MEHAL, P B WORDSWORTH, J L BELL, K A FLEMING, R W CHAPMAN (*Department of Gastroenterology, Institute of Molecular Medicine, and Nuffield Department of Pathology, John Radcliffe Hospital, Oxford*) The DRB3 locus in the HLA DR cluster encodes antigens responsible for the DRw52 serological specificity, and has three known alleles: DRB3*0101, DRB3*0201, and DRB3*0301 (the respective serological types being DRw52a, DRw52b, and DRw52c). Prochazka *et al* have recently reported a 100% incidence of DRw52a in their population of 29 patients with PSC, and on this basis propose that the product of the DRB3 locus is of importance in determining the susceptibility to developing PSC.

We have typed 23 patients with PSC at the DRB3 locus by amplifying genomic DNA with primers specific for the DRB loci, followed by probing the amplified product with P³² labelled oligonucleotides which distinguish between the three possible alleles. In our population 15 of 23 (65%) were DRw52a, 8 of 23 (35%) were DRw52b, and 2 of 23 (9%) were DRw52c. Two of the patients did not encode any of the DRw52 subtypes.

Our observed increased incidence of DRw52a above controls (35%) is expected because of the previously reported increased incidence of DR3 in the PSC population and the known linkage disequilibrium between DR3 and DRw52a (80% of DR3 individuals being DRw52a).

In conclusion, we do not believe that HLA DRB3 is a specific disease susceptibility locus for PSC.

1 Prochazka EJ, Terasaki PI, Sin Park M, Goldstein LI, Busuttill RW. Association of primary sclerosing cholangitis with HLA-DRw52a. *N Engl J Med* 1990; 322: 1842-4.

HLA DR-DQ haplotypes in primary sclerosing cholangitis

J M FARRANT, D G DOHERTY, P T DONALDSON, K I WELSH, A L W F EDDLESTON, ROGER WILLIAMS (*Institute of Liver Studies and Department of Child Health, King's College Hospital and School of Medicine and Dentistry, London and Molecular Immunogenetics Laboratory, Guy's Hospital, London*) Primary sclerosing cholangitis (PSC) is associated with the HLA A1-B8-DR3 haplotype while DR4 has a protective effect. The aim of this study was to characterise the HLA class II association in PSC in more detail by typing at the DRB and DQB loci.

Sixty one northern European white patients with PSC and 130 controls were studied. DR typing was performed serologically and by restriction fragment length polymorphism analysis. DQB alleles were identified using the polymerase chain reaction and sequence specific oligonucleotide probes. The TaqI-DRB band representing DRw52a/c was found in only 71% of the patients. Two DR-DQ haplotypes were associated with PSC; DRw17-DQG*0201 (28/60, (47%) v 26/128 (20%), p<0.0005) and DRw13-DQB*0603 (15/60 (25%) v 6/128 (5%), p<0.0005). Only four patients were heterozygous with respect to these two haplotypes. As expected, the DR4 antigen was less frequent in

patients than in controls (p<0.001) but both of the DQ alleles in linkage disequilibrium with DR4 were reduced (NS).

In conclusion: (1) DRw52a is not a universal finding in PSC; (2) at least 2 DR-DQ haplotypes are associated with PSC; (3) these may act independently in contributing to susceptibility or they may share an amino acid alteration in the peptides encoded; (4) the protective effect of the two DR4-DQ haplotypes is more likely to reside near the DR locus than near DQ.

Serological response and detection of viraemia in acute hepatitis C virus infection

S DOURAKIS, J BROWN, P KARAYIANNIS, U KUMAR, J MONJARDINO, H THOMAS (*Academic Department of Medicine, St Mary's Hospital Medical School, London*) The serological response during acute hepatitis C virus (HCV) infection was examined by enzyme linked immunosorbant assay (ELISA) in sequential serum samples from 13 haemophiliacs after their first exposure to factor VIII concentrates contaminated with HCV. The commercially available C100-3 peptide and a new 22 kD recombinant protein (P22) encoded by the nucleocapsid region of the viral genome were used for antibody detection, whilst a nested polymerase chain reaction (PCR) method was used for the detection of viraemia. In addition, two sporadic cases of acute HCV infection were studied.

The results showed that seroconversion to the C100-3 antigen occurred in only one third of the patients within 12 weeks of disease onset, but all of the patients had a diagnostic serological response to P22 during this phase of the disease. The new test was positive in both the sporadic cases (a death due to subacute hepatic necrosis and an acute non-A, non-B hepatitis of pregnancy) at a time when the commercially available test was negative.

Although PCR offers a sensitive method for the detection of recent HCV infection, the complex methodology makes it unsuitable for diagnostic laboratories. The new ELISA test with P22 may therefore have a useful diagnostic role in acute disease.

Geographical heterogeneity of hepatitis C virus (HCV) infection in autoimmune chronic hepatitis

M LENZI, P J JOHNSON, I G MCFARLANE, H SMITH, D VERGANI, F BIANCHI, ROGER WILLIAMS (*Universita di Bologna, Istituto di Clinica Medica, Bologna and Institute of Liver Studies and Department of Immunology, King's College Hospital, London*) Reports from Spain, Italy, and the UK have documented the frequent detection of antibodies against HCV using the Ortho HCV ELISA in autoimmune chronic active hepatitis (ACAH) patients. A UK study suggested that many positive results may be false positives. We now report a collaborative study employing UK and Italian ACAH sera using a new anti-HCV assay (United Biomedical Inc (UBI)) and based entirely on synthetic HCV peptides. Among 47 Italian patients with type 1 ACAH (ANA or SMA positive, or both), 28 (60%) were positive in the Ortho ELISA and 25 of these were (strongly) positive by UBI (14 confirmed by RIBA). By contrast, of the 25 UK patients, 15 (60%) were Ortho ELISA positive but only two were (weakly) positive on the UBI assay. Most (88%) of 35 Italian patients but none of 10 UK patients with type 2 ACAH (anti-LKM1 posi-

tive) were very strongly positive in the UBI assay.

Since prospective studies from Italy suggest that patients with chronic parenterally acquired hepatitis do not develop autoantibodies (SMA, ANA, or LKM1), we conclude that either (i) autoantibodies are a specific response to sporadically acquired HCV infection or (ii) patients with 'true' ACAH have an enhanced antibody response to HCV, an infection frequently acquired in Italy.

Hepatitis C in patients with chronic liver disease

R SALLIE, C TIBBS, A RAYNER, G ALEXANDER, ROGER WILLIAMS (*Institute of Liver Studies, King's College Hospital, Denmark Hill, London*) Although antibodies to hepatitis C (HCV) have been found in a substantial proportion of patients with cryptogenic cirrhosis (CC), auto-immune chronic active hepatitis (ACAH), and hepatocellular carcinoma (HCC), the true prevalence of active HCV infection in patients with chronic liver disease is unknown and the importance of an anti-HCV test is uncertain. We used reverse transcription (rT) followed by nested polymerase chain reaction (nPCR), using two sets of nested primers, to amplify HCV RNA extracted from formalin fixed wax embedded liver tissue obtained from 60 consecutive patients undergoing orthotopic liver

transplantation (OLT). The mRNA coding for gamma glutamyl transpeptidase (GGT) was amplified as an internal control for both the rT and nPCR steps. Overall, 10 patients were positive for HCV RNA. Of the 18 transplanted for either CC or non-A, non-B hepatitis, eight were positive and two of these had HCC. One of three patients transplanted for ACAH, and one of nine with HBV were positive for HCV RNA. The HCV RNA positive patient with ACAH also had coexistent HCC found at hepatectomy. In five patients positive for HCV before OLT HCV RNA was detected in follow up biopsy specimens.

In this preliminary study we conclude that: (i) HCV PCR is feasible from formalin fixed tissue; (ii) HCV infection seems common in our highly selected group of patients with CC or non-A, non-B hepatitis; and (iii) HCV recurs in the grafted liver after OLT.

Alcohol consumption, prognosis and outcome in paracetamol overdose

G P BRAY, C MOWAT, D F MUIR, ROGER WILLIAMS (*Institute of Liver Studies, King's College Hospital and School of Medicine and Dentistry, Denmark Hill, London*) Although case reports suggest that chronic alcohol consumption enhances hepatotoxicity after paracetamol overdose (POD), the level of intake at which

this may occur and its effect on outcome and prognosis is unknown. In the present study we studied 79 patients after severe POD and stratified them into two groups according to weekly alcohol consumption – less than or more than 21 units for men and 14 for women (n=49 and 30, Royal College of Physicians (RCP) guidelines). Endpoints were death from FHF and incidence of adverse prognostic features (1) raised serum creatinine on admission, (2) acidosis pH<7.30 on admission, (3) serum creatinine >300 µmol/l associated with a prothrombin time >100 seconds and grade 3/4 encephalopathy, (4) peak prothrombin time>180 seconds. Survival was worse if patients had been consuming more than RCP guidelines for alcohol intake before overdose (33.3% v 65.9%, p<0.01) despite similar time to presentation and amount of paracetamol ingested. Serum creatinine on admission was higher in those who had been drinking more than these limits (median 207 v 138 µmol/l, p=0.027) and admission acidosis was more common (30% v 12.2%, p=0.05). All patients died who had either of the two adverse prognostic markers and drank more than RCP guidelines.

In conclusion, alcohol intake above 21 units per week for men and 14 for women is an adverse prognostic feature in severe paracetamol overdose. The more severe course of the disease is partly due to increased nephrotoxicity.