83 QUANTITATIVE HISTOCHEMISTRY:A MEANS OF DETERMINING THE SITE OF INITIAL HEPATOCYTE DAMAGE IN CIRRHOSIS?

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Cirrhosis is the endstage of liver disease of whatever etiology. Once established,it may be impossible to determine the site of the initial lesion. We have investigated whether periportal area (PPA) & perivenular zones(PVZ) can be identified within cirrhotic nodules by determining hepatocyte specific enzyme activities. Quantitative histochemistry(QH) was used to determine the activity of the microsomal enzymes glucose 6 phosphatase(GP),which has highest activity in the PPA,and NADPH diaphorase (ND),highest in the PVZ.Results in 5 normal rats & 2 histologically normal child livers where compared with those obtained in 5 rats with CCl_4 induced cirrhosis & 3 children with extrahepatic biliary atresia (EHBA),the former being initial PVZ damage & the latter PPA.Both in normal rat & human liver, highest GP activity was detected in the PPA (PPA:PVZ mean ratio 1.7 & 1.8 respectively),while highest ND activity was found in the PVZ(PPA:PVZ 0.5 & 0.85).In CCl_4 induced cirrhosis,GP activity was greater at the centre of the nodule than at its periphery(centre:periphery 1.8) while ND was higher at its periphery (ratio 0.58),suggesting that the centre of the nodule is of periportal and the periphery of perivenular origin. In contrast,the reverse was found in EHBA,with a centre:periphery ratio of 0.6 for GP & 1.3 for ND. Our data indicate that the enzymatic activity in the cells at the periphery of the cirrhotic nodule are similar to that in the cells initially injured.Although adaptive changes may account for these observations, QH determination of microsomal enzymes may identify the site of initial damage in cryptogenic cirrhosis.

84 THE DISTRIBUTION OF ENZYME ACTIVITIES IN CIRRHOTIC LIVERS OF DEVELOPING RATS SUGGESTS A SIMILAR METABOLIC ROLE FOR THE PERIPHERY OF THE REGENERATING NODULES & ZONE 3 OF THE NORMAL LIVER ACINUS.

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To assess whether the development of experimental cirrhosis was associated with changes in the intralobular distribution of enzyme activities, we have used microspectrophotometry to quantitate in periportal (PPA) and centrilobular areas (CLA) of the liver succinate dehydrogenase (SDH),glutamate dehydrogenase (GDH), acid phosphatase (AP),glucose-6 phosphatase (GP) and NADPH diaphorase (ND).Rats were treated with phenobarbitone (PB) 30mg/kg/day from day 4 and CCl_4 0.5mg/kg twice weekly from day 8 to 62 and developed centrilobular necrosis followed by fibrous tissue deposition from day 25 and cirrhosis by 45 days. During drug exposure,all enzymes showed decreased activity,particularly in the CLA,with SDH and GP falling by 85-90%, AP and GDH by 30-50% and ND by 10%. In the PPA , AP and ND showed little change, SDH and GDH decreased by 10-30% and GP by 40%. Although cirrhosis persisted after both drugs were stopped and enzymatic activity returned to normal, the distribution had changed, with the cells adjacent to the vascular fibrous septa at the periphery of the regenerating nodules expressing activities similar to the cells in the CLA of the controls, while enzyme activity in the centre of the nodules were similar to the PPA of the controls. Further application of this technique may elucidate metabolic and pharmacological complications of both experimental and clinical cirrhosis.

85 Evaluation of a novel liver function test in pediatric liver disease

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A novel liver function test was developed using formation kinetics of the metabolite monoethylglycinexylidide (MEGX) after i.v. bolus injection of lidocain (1 mg/kg). Formation of MEGX is based upon a cyt. P-450 dependent N-deethylation step. The MEGX test was performed (1,2) using a fluorescence polarisation immunoassay as described perviously. 38 patients aged 0.5-24 y have been tested so far. They suffered from biliary atresia (BA) (n=6), chronic hepatitis (LC) (n=11) and hepatic based metabolic disease (HMD) (n=12). Children with normal liver function 1-4 years after liver transplantation (LTX) completed this series (n=9). In addition, 11 normal subjects (NORM) (mean age 23 y) were tested. Results are given in ug/l (15 min values).

	LC	BA	HMD	LTX	NORM
median	43	19	37	95	75
16 perc	<11	<11	<11	71	36
84 perc	79	40	71	117	105

Patients with MEGX serum concentrations <11 ug/l did not survive 4 weeks unless they were transplanted. In cirrhotic patients MEGX serum concentrations between 12 and 50 ug/l indicated severe hepatic impairment, whereas concentrations >90 ug/l were not associated with clinically significant hepatic dysfunction. Adverse effects of the test were not observed so far. According to our preliminary findings this test gives valuable prognostic information in pediatric liver disease.

Ref. 1) Oellerich, M. et al., J. Clin. Chem. Clin. Biochem. 25, 845, 1987;
2) Burdelski, M. et al., Transpl. Proc. 19, 3838, 1987.

86 SERUM HYALURONIC ACID (HA) - A NEW TEST OF LIVER FUNCTION?

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Hyaluronic acid (HA) is cleared from the circulation specifically by hepatic endothelial cells. To assess whether measurement of serum HA may give information about hepatic function not provided by conventional liver function tests, we have measured serum HA in 48 infants who presented with cholestatic liver disease in the first 6 months of life (25 extrahepatic biliary atresia [EHBA],13 alpha-1 antitrypsin deficiency [α1-ATD],10 idiopathic hepatitis of infancy [IHI] and 51 age-matched healthy infants.)

Serum HA was raised above the ULN (100µg/l) at presentation in 80% of infants with EHBA (351.7 ± 225.5), 79% with α1-ATD (155.0 ± 67.6) and 80% with IHI (152.2 ± 48.1). There was no correlation between serum HA and AST, total bilirubin,gammaglutamyl transferase or albumin. At one year follow-up,19 patients still had raised HA and, of these, 95% (18/19) had clinical and/or histological evidence of cirrhosis. Of the 29 patients who had normal HA at 1 year,none had cirrhosis,although 21 had abnormal AST or GGT.

While serum HA is raised in cholestatic infants at presentation, it remains high only in those who develop cirrhosis. The lack of correlation between serum HA and conventional tests of liver function suggests HA may be a new measure of hepatic function, possibly related to endothelial cell function.

87 FORMULA WITH REDUCED PROTEIN CONTENT IN TERM INFANTS DURING WEANING: RESPONSES ON GROWTH AND NITROGEN METABOLISM.

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20 healthy term infants between 4 and 6 months of age were randomly assigned to either a low protein formula (LP) containing 1.3 g protein/dl or a high protein formula (HP) containing 1.8 g/dl. Both were isocaloric (72 kcal/dl) and had a whey-casein ratio of 50:50. 10 control infants were breast-fed (BF). The mean protein intakes were: 1.9, 2.6 and 1.3 g/kg/d respectively. The mean concentrations of serum urea were 2.8 (LP), 4.1 (HP) and 2.2 mmol/l (BF) at 6 months (HP vs BF $p<0.001$). The urine excretion of nitrogen were similar in the BF and LP groups, being 75 and 81 mg/kg/d. In the HP-group nitrogen excretion was markedly higher, 138 mg/kg/d. Plasma concentrations of albumin, prealbumin and transferrin were normal and similar among the groups. The concentrations of methionine, threonine, leucine, isoleucine, valine, tyrosine and phenylalanine were significantly elevated in the infants in the HP-group when compared to the BF-group. Weight gain was significantly higher in the HP-group 22.8 ± 1.7 g/kg/week when compared to the LP- and BF-groups, 19.9 ± 3.9 and 18.0 ± 4.3 ($p<0.01$) respectively. These data indicate that a decreased protein intake from a whey adapted formula during weaning results in many indices of protein metabolism and growth more similar to those found in breast-fed infants than when conventional high protein formulas are used.

88 INFLUENCE OF FOOD RESTRICTION ON URINARY EXCRETION OF MODIFIED RNA CATABOLITES AND OF 3-METHYLHISTIDINE IN RELATION TO NITROGEN BALANCE IN GROWING RATS

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Our aim is the development of noninvasive methods for assessing the nutritional and metabolic status. For this purpose we developed HPLC methods to selectively measure the turnover of rRNA (from urinary pseudouridine, Ψ), tRNA (from N^2,N^2-dimethylguanosine, m_2^2G) and mRNA (from 7-methylguanine, m^7Gua). Preliminary data from preterm infants suggested that any kind of general stress (hunger, infection, artificial ventilation) increases excretion rates of Ψ, m_2^2G, m^7Gua as well as that of 3-methylhistidine (m^3His; from actin and myosin breakdown). We therefore studied the excretion of Ψ, m^7Gua and m^3His in growing rats (age 41-46 days) fed either a control diet ad libitum or 1/2 and 1/4 respectively of the amounts consumed by the control animals. In control animals N retention was ~1.4 g/kg/d throughout the 5-day period studied. With half the food intake of the controls, N retention briefly fell to zero but returned to near-normal values (~1 g/kg/d) thereafter. Animals given 1/4 of the normal food intake had a negative N retention throughout. Ψ and m^7Gua excretion increased within a day in these animals (by 45 and 24 %, respectively) and fell to values at or below normal after ~3 days, whereas m^3His excretion increased by 50 % on the second day and remained on this level. We conclude that there was a rapid transient breakdown of ribosomes and mRNA followed by a longer lasting breakdown of actin + myosin in food restriction. Measurement of modified RNA catabolites in urine may therefore represent a means to assess rapid changes in metabolic state, while m^3His excretion and N balance may be more appropriate for monitoring longer lasting deficiencies.