The glutathione S-transferases (GSH S-T's) are an important family of drug metabolising enzymes. GSH S-T isoenzymes are dimeric proteins made up of different combinations of a series of subunits. Isoenzymes are defined by their subunit composition. We have investigated the subunit expression of GSH S-T isoenzymes in fetal rat livers since differences in expression could have important teratological implications. The GSH S-T subunit composition of fetal rat liver and adult rat liver were investigated by means of affinity chromatography followed by polyacrylamide gel electrophoresis in sodium dodecylsulphate (SDS-PAGE).

In normal adult rat liver there are four major subunits. In the rat fetal liver we identified a fifth subunit and hence isoenzyme(s) not apparent in the adult liver. The fetal subunit had an approximate molecular mass of 25 500 daltons, gave two bands of pI 8.0 and 8.5 on isoelectric focusing and a densitometric scan of the subunits on SDS-PAGE gel revealed that it accounted for approximately 26% of fetal liver GSH S-T. 'Western blots' revealed that the subunit reacted to antibodies raised in rabbits against adult rat liver GSH S-T's 1-2 (YcYc) and 2-2 (YcYc). Immunodiffusion of adult and fetal GSH S-T's two antisera suggested that this identity was incomplete. The subunit differed from that found in placenta and was not produced when unperfused adult rat liver cytosol was subjected to conditions favouring hydrolysis. Our studies suggest that the additional band seen on SDS-PAGE analysis of GSH S-T's from fetal livers represents a fetal isoenzyme bearing antigen shared with the known subunits. This is of considerable new interest in view of the significant restriction in the capacity of the fetus to metabolise certain xenobiotics.

Purification of human lung and kidney angiotensin converting enzyme (ACE) by a novel affinity technique

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Sarcoidosis and tuberculosis accounted for 68% of 116 ante-mortem liver biopsies with hepatic granulomata at Groote Schuur Hospital. The differential diagnosis of granulomata due to these two conditions is often difficult. Increased serum ACE activity favours sarcoidosis. Relatively crude studies have suggested that ACE is present in abundance in hepatic granulomata due to sarcoidosis but not in those due to tuberculosis.

Immunohistochemical staining for ACE may thus prove a useful adjunct in differentiating between these conditions. Such studies have been limited by the low recoveries of pure ACE from methods recommended for histochemical studies. We report a new purification scheme for human ACE requiring only 1 chromatographic step. The method depends on the use of a previously undescribed ACE competitive inhibitor CA-GlyGly a N-earboxyalkyl peptide, related to enalapril. CA-GlyGly was first synthesised in our laboratory. When bound covalently to an agarose gel CA-GlyGly binds ACE. Bound ACE can be released simply by changing the pH of the eluting buffer.

CA-GlyGly was synthesised in 72% overall yield. The compound is a weak competitive inhibitor (K = 0.18 mM) and a mixed pH-dependent inhibition profile type. 2.4 umol CA-GlyGly/ml gel was bound to the agarose. Human lung and kidney were homogenised, solubilised with detergent ( Triton X-100) and incubated with ammonium sulphate. The crude ACE-containing extract was applied to the affinity column and pure ACE was obtained by raising the pH of the eluting buffer.

This single chromatographic step yielded lung and kidney ACE in electrophoretically pure form with high specific activities (104 and 88 Units/mg respectively) and low Ks (1.9 and 1.7 mM). Anti-serum to this enzyme inactivated it yielding a single band against lung and kidney starting material on immunodiffusion and immuno-electrophoresis.

The CA-GlyGly affinity technique represents a considerable improvement in terms of both yield and specific activity and is as simple as readily reproducible.

Hepatoma cell line plasminogen activator inhibitors

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Plasminogen activators are serine proteases which convert the proenzyme plasminogen into plasmin and thus regulate fibrinolysis. Inhibitors to plasminogen activators (PAI) appear to originate from the liver and are secreted at a basal level and as acute phase reactants. PAI include endothelial cells, platelets and mononuclear/macrophages.

Abnormal fibrinolysis is described in patients with chronic liver disease or hepatoma. A human heptoma cell line, Hep-G2, which synthesizes and secretes several components of the fibrinolytic system was examined for its ability to secrete PAI in vitro.

Fast acting inhibitors to both Urokinase (U-PA) and Tissue Plasminogen Activator (t-PA) were noted in both harvest fluids and cell lysates from confluent cultures of Hep-G2 cells. These inhibitors were stable at pH 3 and lost activity at 45°C. Inhibitory levels tested in a 1 fibrin plate assay were of the order of 10 fmol tpa/ml harvest fluid. The inhibitor was SDS stable and migrated with Mr 53,000,104,000. Plasminogen activators were also detected in low concentration and migrated at 55,000.

The inhibitor secreted by the Hep-G2 differs from that of the monocoyte-macrophage which binds preferentially to U-PA and has a Mr of 66,000. Modulation of secretion of the Hep-G2 PAI occurs on exposure to dexamethasone, endotoxin and acetylelated LDL.

The inhibitor has been partially purified by affinity chromatography with Con A Sepharose and PMSF-Urokinase coupled to cyanogen bromide activated Sepharose.

The spectrum of serum fibrin- and fibrinogen related antigens in patients with primary hepatocellular carcinoma

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Patients with hepatocellular carcinoma (HCC) may present functionally abnormal fibrinogen and pro- coagulant substances. Coagulation pathways are activated and antifibrinolytic activity appears to be enhanced. In this study we have analysed the extent of fibrinogen/fibrinolytic activity in the serum of dual porphyric patients (p < 0.001). This enzyme deficiency is also the underlying basis for variegate porphyria. Lymphoblast uroporphyrinogen decarboxylase activity was significantly decreased (mean 0.084 ± 0.02 nmol/mg protein/hour) as compared to normal controls (mean 0.10 ± 0.09 nmol/mg protein/hour) (p < 0.001). In the haemolysates from patients with dual porphyria there was a similar 27% decrease in the mean uroporphyrinogen decarboxylase activity. However, the mean enzyme activity of five patients with classic variegate porphyria was not significantly less than that of the normal population.

The reported decreases in activity of these two enzymes are consistent with the abnormal porphyrin secretory profiles found in patients with dual porphyria. Our data suggest that there may be two sub-populations of variegate porphyria, one with normal uroporphyrinogen decarboxylase activity and one with decreased enzyme activity.

α = nmol 7, 6, 5 and 4 carbonyl porphyrinogens

Tissue distribution of human basic and near-neutral glutathione S-transferase

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The glutathione S-transferases (GSH S-T's) play an important role in the metabolism of xenobiotics. Three groups of human GSH S-T, basic (pI > 7.5), neutral (pI 6.5 to 7) and near-neutral (pI < 5.5), have been identified. The organ concentration and cellular distribution of the basic GSH S-T's (lipidants) are well documented but less is known about the concentration and distribution of neutral and acidic transferases. Our previous work suggested that GSH S-T's were subject to interorgan and interindividual variation. This might explain inter-individual and organ differences in susceptibility to drug toxicity and chemical carcinogenesis.

The concentrations of basic and near-neutral transferases were measured by radial immunodiffusion in 18 organs from 8 victims of motor vehicle accidents. Basic GSH S-T's were present in all 8 individuals.

Neutral transferase was present only in 3 of the 8. Table I lists the concentration of basic transferases (mean ± SD) and that, where present, of the near-neutral transferase (mean and range). Concentrations are expressed as micromols of transferase per milligram cytoxic protein.