The identification of a glutathione S-transferase isoenzyme in fetal rat livers which is not apparent in normal adult rat livers

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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 vary in substate affinity. 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 livers and adult rat livers 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 gels 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 (YaYc) and 2 - 2 (YcYc). Immunodiffusion of adult and fetal GSH S-T's against these two antisera suggested that this identity was incomplete.

The subunit differed from that found in placenta and was not produced when unpurified 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 antigens shared with the known subunits. This is of considerable 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 requiring up to 6 chromatographic steps. 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-carboxyalkyl dipeptide, 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 gel.

CA-GlyGly was synthesised in 72% overall yield. The compound is a weak competitive inhibitor (K_I = 0.18 mM) with a marked pH-dependent inhibitory profile. 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 fractionated 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 K_m's (1.9 and 1.7 mM). Antiserum to this human ACE, raised in rabbits, yielded 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 simple as well 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. Other sources of PAI include endothelial cells, platelets and monocytes/macrophages.

Abnormal fibinolysis is described in patients with chronic liver disease or hepatoma. A human hepatoma 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 I^{125} fibrin plate assay were of the order of 10 fmol tpa/ml harvest fluid. The inhibitor was SDS stable and migrated with M_r 53,000 and 104,000. Plasminogen activators were also secreted in low concentration and migrated at 55,000.

The PAI secreted by the Hep-G2 differs from that of the monocyte-macrophage which binds preferentially to U-PA and has a M_r of 66,000. Modulation of secretion of the Hep-G2 PAI occurs on exposure to dexamethasone, endotoxin and acetylated 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 produce functionally abnormal fibrinogen and procoagulant substances. Coagulation pathways are activated and antifibrinolytic activity appears to be enhanced. In order to investigate the extent of fibrin(ogen)olysis in HCC, the profile of serum fibrin- and fibrinogen related antigens (FRA's) was analysed in 12 such patients.

We used an assay, developed in our laboratory, in which panspecific anti-fibrinogen IgG is bound covalently to activated diazo-phenylthioether paper disc. The resulting probe is able to extract FRA's when incubated in serum samples. The affinity bound FRA's are eluted from the probe and subjected to SDS-PAGE. Densitometric analysis of the gel allows for the proportionation of the various fragments and quantitation is achieved with a radioimmunoassay using labelled fibrin-derived D-dimer and anti-fibrinogen antiserum.

All the patients had elevated levels of FRA's in their serum compared with controls ($p < 0.001$). Furthermore the patients had elevated proportions of fibrinogen derived D-monomer (mean 3.16%) compared with the normal value of 1.36% ($p < 0.001$) and elevated fibrin derived D-dimer (mean 33.99%) compared with the normal value of 29.7% ($p < 02$).

Our data suggest that the activated coagulation pathways seen in patients with HCC may be associated with a parallel activation of the corresponding fibrin(ogen)olytic pathways.

Enzyme studies in dual porphyria

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Dual porphyria is a rare disorder in the haem biosynthetic pathway characterised by the superimposition of the porphyrin excretory profiles of quiescent variegate porphyria on the profiles normally associated with porphyria cutanea tarda.

Uroporphyrinogen decarboxylase and protoporphyrinogen oxidase activities were measured in haemolysates and in Epstein-Barr virus transformed lymphoblasts of patients with dual porphyria as well as control subjects.

Sensitive fluorimetric assays were employed and HPLC was used to separate reaction products in the uroporphyrinogen decarboxylase assay.

The lymphoblast protoporphyrinogen oxidase mean activity was decreased by 45% from 0.82 ± 0.10 to 0.45 ± 0.09 nmol protoporphyrin/mg protein/hour in dual porphyric patients ($p < 0.001$). This enzyme deficiency is also the underlying basis of variegate porphyria. Lymphoblast uroporphyrinogen decarboxylase was significantly reduced (mean 0.084 ± 0.02 u*/mg protein/hour) as compared to control subjects (mean 0.12 ± 0.05 u/mg protein/hour ($p < 0.01$)). In the haemolysates from the 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 excretory profiles found in patients with dual porphyria. Our data suggest that there may be two subpopulations of variegate porphyria, one with normal uroporphyrinogen decarboxylase activity and one with decreased enzyme activity.

*u = nmol 7, 6, 5 and 4 carboxyl 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), near neutral (pI ± 6.5) and acidic (pI < 5.5), have been identified. The organ concentration and cellular distribution of the basic GSH S-T's (ligandins) 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 micrograms of transferase per milligram cytosol protein.