PLASMA ENZYME AND ISOENZYME CHANGES DURING PERFUSION OF THE ISOLATED PIG LIVER

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SUMMARY

1. Changes in the perfusate activities of aspartate transaminase, lactate dehydrogenase, its 'LD-5' component, sorbitol dehydrogenase, ornithine carbamoyltransferase, and the isoenzyme patterns of lactate dehydrogenase and aspartate transaminase, were investigated in eleven perfusions of pig liver with human blood in order to assess liver cell damage during perfusion.

2. The aspartate transaminase values were a sensitive indicator of liver damage provided that, as was usually the case, the degree of haemolysis was small. Appearance on electrophoresis of the mitochondrial isoenzyme of aspartate transaminase indicated severe liver damage.

3. Measurement of sorbitol dehydrogenase activity was also shown to be a sensitive index of liver cell damage, and had the advantage that haemolysis did not interfere.

4. Measurement of total lactate dehydrogenase activity was unreliable as this largely reflected the degree of haemolysis rather than liver cell damage. However, the isoenzyme pattern of lactate dehydrogenase on electrophoresis distinguished liver cell damage from haemolysis. The chemical determination of 'LD-5' was not a sensitive index of pig liver damage as this fraction is found only in low concentration in pig liver.

5. Ornithine carbamoyltransferase was also found not to be a sensitive marker of liver cell damage.

Key words: enzyme tests, isoenzymes, liver function tests, pig liver perfusion.

Perfusion of the isolated pig liver with human blood has been used to study liver physiology, and as a form of temporary hepatic support in patients with acute liver failure (Parbhoo,
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Adequate assessment of the different functions of the perfused liver remains a major problem. Blood flow, vascular resistance, general appearance and bile flow remain the best simple guides to a well perfused liver. A variety of biochemical 'liver function tests' have been carried out to evaluate aspects of the viability of the isolated pig liver but not all have been helpful.

The estimation of plasma enzymes commonly used for detecting cytolysis, aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LD), and also alkaline phosphatase (ALP) for detecting cholestasis, has not solved the problems (Abouna, Ashcroft, Hull, Hodson, Kirkley & Walder, 1969; Ham, Pirola & Elmslie, 1969; Jablonski, Douglas, Gordon, Owen & Watts, 1971). Others (Hickman, Saunders, Simson & Terblanche, 1971) have suggested that a wider spectrum of enzyme analysis would yield more information. Since any increase in plasma enzymes may come from the pig liver, from erythrocytes in the perfusate, or from the patient in the case of clinical liver perfusion (or may be the result of a diminished rate of disposal of enzymes), the results of unselective enzyme assays do not necessarily reflect damage to the isolated liver. Increased enzyme activity in the perfusate may reflect haemolysis which is an inevitable consequence of extracorporeal circulation. The determination of selected enzymes and isoenzymes may overcome these problems.

Aspartate transaminase and lactate dehydrogenase were measured as they are used extensively; although both enzymes are present in erythrocytes, in clinical practice this rarely matters as haemolysis is not a problem. The LD-5 fraction, the predominant LD isoenzyme of human liver, was measured separately, and isoenzyme patterns of LD and AST gave additional information. To exclude interference due to haemolysis, sorbitol dehydrogenase (SD) and ornithine carbamoyltransferase (OCT) were measured, both of which may be regarded for practical purposes as absent from erythrocytes; these determinations are less often needed clinically.

We report enzyme and isoenzyme changes during two perfusions with the liver excluded, and during eleven extracorporeal pig liver perfusions over a 4 h period.

MATERIALS AND METHODS

The technique of isolated pig liver perfusion using fresh heparinized human blood was the same as that reported for clinical perfusion except that patient connections were omitted (Parbhoo et al., 1971). The liver was flushed before, during and after its removal from the pig, with 3 litres of an electrolyte–dextran solution (Chalstrey & Parbhoo, 1971) at 48°C. The perfusion circuit is shown in Fig. 1; for the anhepatic perfusions, the liver was excluded from the circuit and replaced by a simple junction.

Total plasma enzyme assays were carried out using the following methods: LD by the method of Wroblewski & La Due (1955) as described by King (1965); AST by the modified method of Karmen (1955) with the concentration of l-aspartate being doubled (King, 1965); SD by the method of Gerlach & Schurmeyer (1960); and OCT as described by Ceriotti & Gazzaniga (1967). The first three enzymes were measured at 35°C on the LKB reaction rate analyser (LKB Instruments Ltd, Croydon, Surrey, U.K.) with the reagent and sample volumes halved; OCT was assayed manually. The enzyme activity is reported in international units per litre (i.u./l) with LD, AST and SD results corrected to 25°C. The temperature correction factors (35°C to 25°C), which we determined using human liver enzyme, were 0·51, 0·52 and
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FIG. 1. Circuit diagram of extracorporeal liver perfusion. BT, bubble trap; HA, hepatic artery; IVC, inferior vena cava; PV, portal vein.

0.42 respectively. Ornithine carbamoyltransferase results are reported at 37°C. To measure LD-5, DEAE-Sephadex was used to adsorb the other LD fractions leaving LD-5 (Richterich, Schafroth & Aebi, 1963); we found it necessary to use four times the recommended amount of DEAE-Sephadex (Buttery, 1972). The LD-5 activity was then determined by the same method as for total LD. The LD isoenzyme separation on cellulose acetate was carried out by the method of Myers & Van Remortel (1968), and AST isoenzymes were separated employing the technique of Romel & La Mancusa (1965), but using Cellogel (Chemitron, Milan) instead of cellulose acetate (Baron & Buttery, 1972).

RESULTS

Table 1 shows the activities of LD, AST, ALT, SD and OCT in normal liver, erythrocytes and plasma of man and pig (Buttery, 1972).

Release of enzymes during the anhepatic and liver perfusions are presented in Figs. 2(a)–2(e). Alanine transaminase was not measured in the perfusate because of the relatively low liver activity (Jablonski et al., 1971). During two anhepatic perfusions the mean SD showed no marked change (Fig. 2a), AST rose from 13 i.u./l to 17 i.u./l (Fig. 2b), LD from 131 i.u./l to 333 i.u./l (Fig. 2c), and OCT rose from 1.3 i.u./l to 2.1 i.u./l (Fig. 2e).

On the basis of the values for SD, as this enzyme is specific for liver cells, the results were plotted into three groups of perfusions, A, B and C (Fig. 2a). Marked increases in SD were
noted in two perfusions (group A), the mean level at 4 h being 270 i.u./l. In three perfusions (group B) the SD increased to 70 i.u./l at 1 h but changed little thereafter. The six perfusions in group C showed small increases in SD during perfusion. The increase of AST was similar to that of SD in the three groups (Fig. 2b). Mean levels of 88 i.u./l and 415 i.u./l were recorded at 4 h in group C and group A perfusions, the initial value being 14 i.u./l and 10 i.u./l respectively. The changes in LD showed a wide scatter, and those in group C were similar to those in the anhepatic perfusions (Fig. 2c). Analysis of LD-5 showed increases of between two and five times initial values at 4 h, group A and C perfusions showing similar values (Fig. 2d). Very little OCT activity was found in the perfusate, but the pattern of change is similar to the changes seen with SD and AST (Fig. 2e).

Five isoenzymes of LD in the plasma and liver extract of man and pig were separated by electrophoresis. Each pig isoenzyme moves slightly faster than the corresponding human isoenzyme. This difference in mobility makes it possible to distinguish porcine from human LD isoenzymes (Fig. 3). In our system the mobility of pig LD-4 and LD-5 is usually similar to that of human LD-3 and LD-4. Because of this, it is often difficult to distinguish these bands for the pig and normally one sees only eight bands during an isolated perfusion: five fractions from human and three from pig. The predominant isoenzyme in pig liver is LD-3, the apparent order of activity being LD-3 > LD-2 > LD-4 > LD-1 > LD-5.

The serial LD isoenzyme pattern changes in the perfusate during a typical perfusion are seen in Fig. 4. The first sample shows the five LD bands of human plasma. By the second hour of perfusion three additional bands have appeared, these being pig LD-I, 2 and 3. These bands become denser during the course of perfusion with pig LD-I showing the highest concentration. In this perfusion human LD-3 and pig LD-4 can be distinguished, though in many cases they merge, forming a wide band (Fig. 3).

Both human and pig hepatic AST can be separated by electrophoresis into a mitochondrial cathodic band and a cytoplasmic anodic band (Fig. 5). The erythrocytes have a single anodic band. The human anodic fraction moves faster than the pig anodic fraction and these can therefore be distinguished from each other. Human and pig cathodic fractions display a similar electrophoretic mobility. Fig. 6 shows the AST isoenzyme pattern during a group A perfusion.
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FIG. 2. Changes in enzymes during anhepatic and isolated pig liver perfusion: (a) sorbitol dehydrogenase; (b) aspartate transaminase; (c) lactate dehydrogenase; (d) 'LD-5'; (e) ornithine carbamoyltransferase. ●, Group A; ○, group B; □, group C; ---, anhepatic perfusion.
It is not possible to detect low isoenzyme activity in the priming blood with this technique. However, subsequent samples all show an anodic band. In some samples a faint cathodic band was also seen. The anodic band was seen in six perfusions at 1 h, and 2 h onwards for the remaining five perfusions. The cathodic band was faintly visible in only three perfusions.

**DISCUSSION**

The changes in AST during isolated liver perfusion using fresh blood and a low circuit haemolysis rate (mean initial plasma haemoglobin 90 mg/l and a 4 h level of 320 mg/l) may be interpreted as due to liver damage; liver biopsy and an intrahepatic temperature probe were avoided during perfusion. The rate of haemolysis was similar in perfusions with or without the liver. The damage mainly released cytoplasmic AST: severe damage leading to release of mitochondrial (cathodic) AST was rare. However, Abouna et al. (1969) found the interpretation of aspartate and alanine transaminases difficult due to the appreciable degree of haemolysis that occurred. The variable results of other workers (Zimmerman, Fischer, Braun, Rehfeld, Bannert, Dierkesmann & Seitz, 1970; Ham et al., 1969; Jablonski et al., 1971) can be ascribed to their use of stored or expired human blood or dilute pig blood for perfusion.

Sorbitol dehydrogenase appears to be a sensitive index of active liver damage. Erythrocytes and normal plasma have very low levels of SD; hence any increased activity of this enzyme during perfusion indicates hepatic release, and isoenzyme separation of SD is therefore unnecessary. Perfusions in groups A and B show an increase in the first sample taken after commencing perfusion. This probably reflects damage to the liver before perfusion due to the operative manipulation and cold ischaemic preservation. In group B there was little change in SD after the initial rise; the rise in group A indicates continuing liver damage. The grouping of perfusions according to the SD results is supported by the finding of similar patterns of other enzyme changes, in particular AST.

The changes in LD and SD levels were similar, but individual serial perfusion LD values
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**Fig. 4**

Fig. 4. Hourly changes in lactate dehydrogenase isoenzymes during an isolated pig liver perfusion.

**Fig. 6**

Fig. 6. Hourly changes in aspartate transaminase isoenzymes for a perfusion with significant enzyme changes.

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showed erratic changes due to the sensitive interference by erythrocyte LD from variable haemolysis. Preliminary work has shown that during liver perfusion there is a continuous removal of LD into the perihepatic fluid (fluid oozing from surface of liver, and hepatic lymph).

The LD-5 fraction showed small increases during perfusion (Fig. 2d) even in group A which had high levels of SD and AST; unlike many other mammalian livers, the pig liver has a low concentration of LD-5.

The isoenzyme patterns of LD and AST allow one to distinguish between increases in enzyme due to haemolysis from that due to liver damage. Haemolysis is manifested by increased density of the bands for human, while liver damage causes appearance of additional (pig) bands. Isoenzyme analyses are of value in distinguishing pig from human enzymes during clinical perfusion (Buttery, Parbhoo & Baron, 1971; Baron & Buttery, 1972).

Increased serum activity of OCT has been claimed to be a sensitive marker of liver cell damage in clinical hepatitis (Reichard, 1961). However, the enzyme changes here have been small and because of this and the poor precision of the method, estimation of OCT has no place in the investigation of liver perfusion.

Like Abouna et al. (1969) and Jablonski et al. (1971), we found little change in alkaline phosphatase levels during liver perfusion and consider that cholestasis does not occur. We have shown (D. N. Baron & S. P. Parbhoo, unpublished work) that complete cholestasis in a pig, by ligation of the common bile duct, caused a rise in its serum alkaline phosphatase from 18 to 50 KA units/dl over 24 h, while the AST rose from 12 i.u./l to a peak of 110 i.u./l at 48 h. Both enzyme values returned to normal at about 5 days.

In conclusion, serial perfusate aspartate transaminase activities are a good indicator of cytolysis in the isolated perfused pig liver; sorbitol dehydrogenase may be used if there is haemolysis.

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REFERENCES


