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Reticuloendothelial system and hepatocyte function in fulminant hepatic failure

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SUMMARY Kupffer cell and hepatocyte function were studied in 36 patients with fulminant hepatic failure by measurement of clearance of ^{125}I microaggregated albumin ($^{125}IMAA$) and galactose, respectively. Both were impaired but there was no correlation with final outcome, although on sequential testing 48 hours later, those patients who survived had a significantly greater improvement in Kupffer cell and hepatocyte function. In six other patients with similar hepatocyte dysfunction but no encephalopathy, Kupffer cell function was not significantly different from that of controls. This is additional support for a possible relation between encephalopathy and damage to Kupffer cell function. The latter was also shown to correlate with renal failure; this is consistent with the suggestion that endotoxaemia is important in the pathogenesis of this complication.

The massive dysfunction of hepatocytes in fulminant hepatic failure has been documented both histologically and by functional measurement such as the galactose clearance capacity.[1] Less is known about Kupffer cell function.[2] The latter is important not only in relation to specific functions of the reticuloendothelial system but also because the degree of hepatocellular damage may be profoundly modified by altering reticuloendothelial function.[3] Blockade or stimulation of the reticuloendothelial system in experimental galactosamine hepatitis results in aggravation or prevention respectively of the liver cell lesion. Other studies in ischaemically induced hepatic necrosis have shown that survival is influenced by changes in reticuloendothelial function[4] which may be related to the failure of the damaged Kupffer cells to clear gut-derived endotoxins from portal venous blood. Systemic endotoxaemia has been implicated in the pathogenesis of renal failure, intravascular coagulation, and gastric mucosal haemorrhages in patients with fulminant hepatic failure.[5] We have assessed reticuloendothelial function in 36 patients with fulminant hepatic failure by measuring the blood clearance of microaggregated iodinated human albumin. At the same time the hepatocyte functional cell mass was determined by a galactose clearance test.

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Methods

PATIENTS

The 36 patients studied (19 women, 17 men; aged 16–65 years) all fulfilled the criteria for fulminant hepatic failure.[6] All had severe hepatic necrosis, considerably raised serum aspartate transaminase levels (3600±SEM 713 IU/l), and a prothrombin time prolonged by 97±8·6 s (mean±SEM). Liver failure was attributed to paracetamol overdose in 24 patients, viral hepatitis in nine (HAV in five, HBV in three, and NANB in one), and halothane associated hepatitis in three. The patients received intensive care as described elsewhere[7] and a daily period of haemodialysis using the polyacrylonitrile membrane (RP6) and /or haemoperfusion through a Haemocoll 100 column (Smith and Nephew Research Ltd), with prostacyclin as a protective agent.[8] All investigations were made before these procedures, before the administration of blood products, and as soon as the patient had shown signs of developing grade IV encephalopathy or on arrival in the Unit if this were already established. Investigations were repeated 48 hours later.

Measurements were made in six other patients (three men, three women; aged 19–39 years) with severe hepatic damage from paracetamol overdose in whom encephalopathy had not developed. Serum aspartate transaminase levels were considerably raised (mean±SEM=3000±220 IU/1) and the pro-

thrombin time prolonged (mean±SEM=49·3±9·2 s). These patients were studied 72–96 hours after the paracetamol overdose, a time comparable with that for patients who did develop encephalopathy.

The control group consisted of volunteer laboratory staff and patients with uncomplicated myocardial infarction with an age range similar to that in the patient group. Informed consent was obtained from all patients or their relatives and from controls before testing.

Microaggregated iodinated human serum albumin (^{125}IMAA) was prepared by the method of Iio and Wagner.[9] After blockade of the thyroid by intravenous injection of sodium iodide, ^{125}IMAA (5 mg/kg body weight) was injected and 2 ml blood samples were taken into EDTA at regular intervals for 20 minutes; 0·5 ml plasma was counted directly using a Hewlett-Packard gamma counter. The half life of ^{125}IMAA in the circulation was calculated by plotting the $\log_{10}$ CPM versus time. For the control subjects the mean half life was 13·7±1·1 min which is similar to that found in other series using the same system.[10] In six patients a low dose (0·5 mg/kg body weight) of ^{125}IMAA was injected. At this dose removal depends more on hepatic blood flow than on Kupffer cell function;[9] removal was similar to that found in controls given the same dose (half life 3·8±0·3 min and 3·5±0·1 min respectively).

The intravenous galactose clearance test was performed by the method of Tengstrom[11]: 350 mg/kg body weight of a 20% (W/V) solution of galactose was given intravenously over a period of up to three minutes. Zero time was taken as the time when half the volume had been given. Venous blood was taken at regular intervals over 40 minutes, and mixed immediately with 5 vol 6% perchloric acid. Galactose concentrations in the protein-free supernatant were estimated enzymatically with galactose dehydrogenase.[12] The half life of galactose was determined by plotting $\log_{10}$ galactose concentration against time. Renal excretion of galactose during the 40 minute test was less than 1% of the total dose (all patients had indwelling catheters). Values for the controls were 11·5±1·4 minutes, which are similar to those reported by other workers.[11]

Statistical analysis was by Student's *t* test or Wilcoxson's rank test. Results are expressed as mean ± standard error of the mean.

Results

^{125}IMAA CLEARANCE CAPACITY

The initial half life (T½) of ^{125}IMAA estimated in 34 patients was significantly longer than that in the control subjects (26·0±1·0 and 13·7±1·1 min, respectively, P<0·001). In the six patients with severe liver dysfunction but no encephalopathy, however, the T½ of 14·1±0·9 min was not significantly different from that of the control values (Fig. 1). There was no significant difference in initial T½ values between those with fulminant hepatic failure who survived and those who died (26·6±1·0 and 24·7±2·1 min, respectively). Similarly, T½ values were not related to the cause of the liver failure. However, the second T½ estimation was significantly improved in the 11 patients who survived and was unchanged in the five patients who died (−6·4±1·9 and 0·0±2·1 min respectively, P<0·05) (Table). In relation to the development of renal failure (plasma creatinine >350

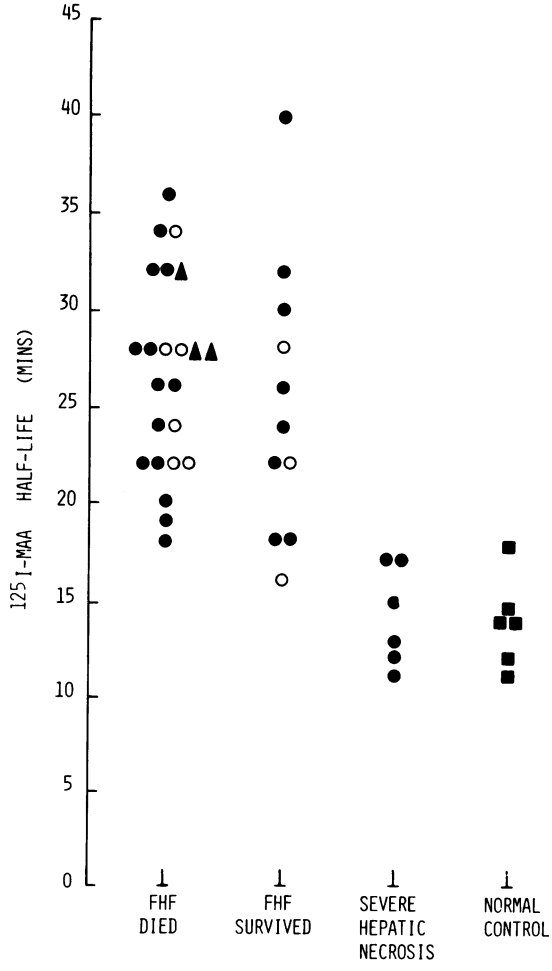


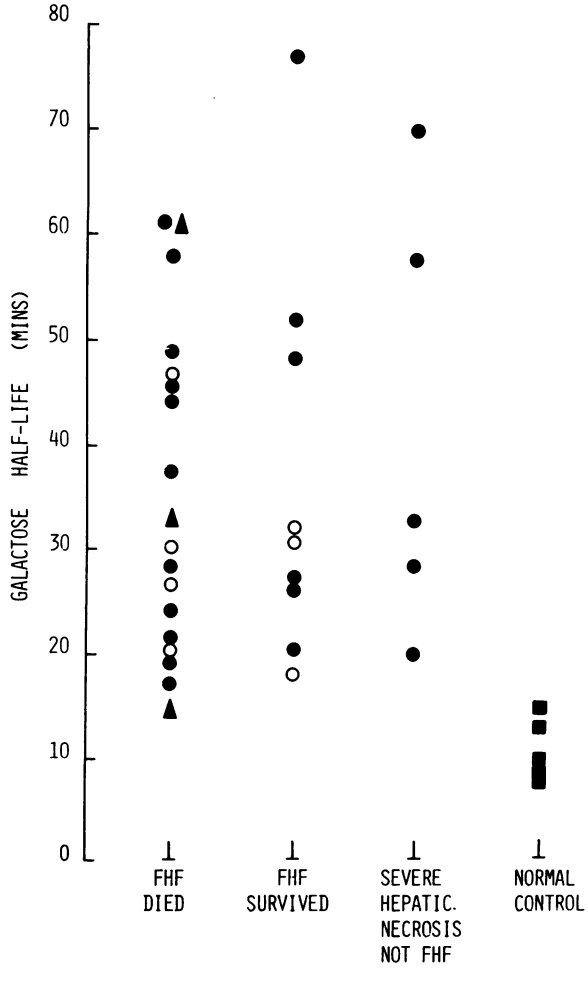
Fig. 1 *^{125}IMAA half life in patients with fulminant hepatic failure and severe hepatic necrosis (● paracetamol overdose, ○ viral hepatitis, ▲ halothane hepatitis), and in normal controls (■).*

Table *Change in ^{125}IMAA and galactose half lives in patients with fulminant hepatic failure over two day period (mean ±SEM)*

Fulminant hepatic failure	*Change in half life over two days*	
	^{125}IMAA	*Galactose*
Survived	−6·4±1·9	−11·3±5·9
N	11	6
Died	0±2·1	+12·0±7·1
N	6	5

P<0·05. Survivors *vs* non-survivors.

mmol/l and urine output <300 ml/24 h), the initial T½ values were significantly more prolonged, reflecting the greater Kupffer cell impairment in patients who eventually developed renal failure than in those who did not (28·9±1·4 and 23·9±0·8 min respectively, P<0·005).



GALACTOSE ELIMINATION CAPACITY

The T½ of galactose measured in 27 patients with fulminant hepatic failure was similar to that in five patients with severe liver dysfunction but no encephalopathy, but both were significantly prolonged compared with control values (36·6±3·1, 42·5±9·3, and 11·5±1·4 min respectively, P<0·001 (Fig. 2).

Patients who survived and those who died had the same degree of impairment of galactose clearance (initial T½ values 37·3±6·3 and 36·3±3·5 min respectively). In the 10 patients in whom a second measurement was made at the same time as the second T½ measurement of ^{125}IMAA (five survivors and five non-survivors) the elimination capacity had increased in those who survived but had decreased in those who eventually died (change in T½ −8·0±3·6 and +12·0±7·1 min respectively, P<0·005) (Table). There was no correlation between the initial degree of impairment of ^{125}IMAA clearance and the degree

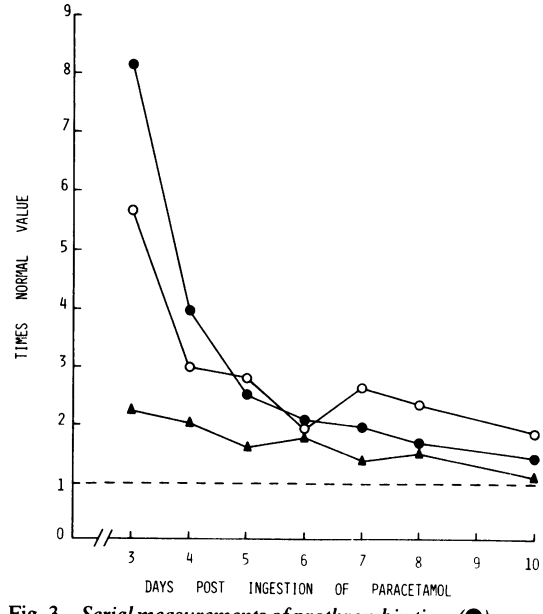


Fig. 3 *Serial measurements of prothrombin time (●), galactose half life (○), and ^{125}IMAA half life (▲) in patients surviving fulminant hepatic failure.*

of reduction in galactose elimination capacity (r=0·38). Other tests performed over subsequent days showed that the ^{125}IMAA clearance and galactose elimination capacity continued to improve in patients who survived, shown by a progressive fall in T½, and returned to normal within 10 days (Fig. 3).

Fig. 2 *Galactose half life in patients with fulminant hepatic failure and severe hepatic necrosis (● paracetamol overdose, ○ viral hepatitis, ▲ halothane hepatitis), and in normal controls (■).*

In contrast with the findings for ^{125}IMAA, the T½ of galactose in patients who developed renal failure was not significantly different from the T½ in those who did not develop renal failure (41·4±5·5 min and 33·0±5·0 min respectively).

Discussion

Although impaired function of the reticuloendothelial system has been reported in cases of acute alcoholic hepatitis and chronic liver disease[13] the considerable impairment shown in fulminant hepatic failure has not been reported before. Kupffer cells usually comprise about 80% of the fixed cells of the reticuloendothelial system.[2] In the absence of any other known mechanism for clearance of microaggregated albumin, the prolonged half life of ^{125}IMAA in these patients is most likely due to Kupffer cell dysfunction. Reduced hepatic blood flow and portosystemic shunting of blood around the Kupffer cells can be excluded as possible causes, as liver blood flow, as assessed from clearance of a lower dose of ^{125}IMAA was normal. Furthermore, in ischaemically induced hepatic necrosis, values for liver blood flow return to normal within 24 hours but reticuloendothelial function remains abnormal.[4] It is more likely that the impairment of reticuloendothelial function is related to the release of cell debris associated with massive hepatocyte destruction. Increased concentrations of circulating immune complexes have been found in fulminant viral hepatitis and these could also cause reticuloendothelial blockade (personal communication). The possible role of endotoxaemia which has already been referred to might also depress reticuloendothelial function.[14]

Ramsoe *et al.* found that the reduction in galactose clearance from the circulation was significantly less in patients with fulminant hepatic failure who survived than in those who eventually died,[1] although there was some overlap between the two groups.[15] On the basis of this and the finding that the rate of liver regeneration assessed by the mitotic index and the frequency of liver cells with interploid DNA is similar in both fulminant and uncomplicated hepatitis,[16] it has been suggested that liver cell destruction is the major determinant of prognosis in acute liver failure, hepatic regeneration being less important. Our findings of a similar prolongation of the initial T½ of galactose in survivors and non-survivors would not support this suggestion. One reason for this difference may be that in our patients galactose was given according to body weight, whereas in other studies a fixed dose was given. Although the proportion of patients with viral hepatitis in our series was smaller than that reported by Ramsoe *et al.* this is unlikely to be the explanation, because the T½ of galactose was similar in all groups. Our results are more consistent with the hypothesis that hepatic regeneration is important in determining the final outcome and is reflected in the greater improvement in T½ for galactose and ^{125}IMAA over 48 hours in patients who ultimately survive. This improvement in hepatocyte function may not necessarily be attributed to newly regenerated cells, but may reflect the return to normal function of viable hepatocytes or Kupffer cells which previously had impaired metabolic function.

The normal reticuloendothelial function found in six patients with severe hepatic necrosis not progressing to encephalopathy is interesting, as experimentally induced hepatic necrosis due to either ischaemia or galactosamine can be influenced by both reticuloendothelial blockade and stimulation.[4 17] An intact reticuloendothelial system may therefore be preventing the entry into, or promoting the removal from the circulation of potential cerebral toxins. The identity of these substances is unclear, although endotoxins have been shown to cause cerebral damage in neonatal cats,[19] and levels were found to correlate with the level of encephalopathy in a patient with Reye's syndrome.[20]

The importance of Kupffer cell function during the course of fulminant hepatic failure is also reflected in the significantly worse reticuloendothelial function found in patients who developed renal failure. This is in accord with the current views on the mechanism of this complication: renal failure in fulminant hepatic failure has been attributed to systemic endotoxaemia[5] which results in reduced renal perfusion.[21] This is likely to be present only when reticuloendothelial function is no longer adequate to prevent the spread of endotoxins from the portal to the systemic circulation.

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