

Amylase activity in human bile

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SUMMARY The mean amylase level in 42 human bile samples was 154 IU/l and there was no significant difference in the amylase activity of 32 paired serum and bile samples. Estimation of the amylase thermostability of bile showed it to be similar to that of serum. This suggests that the amylase activity in bile may have filtered through the liver from the hepatic circulation rather than refluxed from the pancreatic duct. The presence of amylase in human bile provides further evidence that the liver might have a role in the regulation of serum amylase.

While there is evidence that the amylase activity in human serum is related to hepatocellular dysfunction (Bhutta and Rahman, 1971) it is controversial whether there is a hepatic production of amylase (Janowitz and Dreiling, 1959; Nothman and Callow, 1971; Gamklou and Scherstén, 1972; Taussig *et al.*, 1974; Skude, 1975; Otsuki *et al.*, 1976). In dogs (Nothman and Callow, 1971) and rats (Arnold and Rutter, 1963) the liver has been shown to play an important role in amylase production with canine bile containing approximately one quarter of the activity of normal serum (Singh *et al.*, 1970). Until recently the main difficulty in confirming or refuting a hepatic origin for amylase in man has been the difficulty in identification of amylase isoenzymes (Warshaw *et al.*, 1976).

Using the technique of thermostability (Donaldson *et al.*, 1977), it has been suggested that only 44% of the amylase activity in normal human serum appears to have originated from pancreas and salivary glands. The tissue(s) of origin of the remaining 56%, which is a heat stable amylase, is not established. However, intestinal mucosal amylase in the rat is known to be thermostable (Alpers and Solin, 1970). Human bile was examined to see if it contained amylase activity, and whether it had a pattern of thermostability similar to normal serum.

Methods

Bile samples (2-3 ml) were collected from patients

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undergoing elective biliary surgery, who had no clinical evidence of hepatic disease and with normal liver function tests (bilirubin, AST, ALT, LDH, total protein, albumin, alkaline phosphatase, and γ GT). At laparotomy the liver was macroscopically normal. The samples were collected by direct needle puncture and aspiration, after opening the peritoneal cavity before manipulating the gallbladder or common bile duct. Patients with a fibrosed and contracted gallbladder were excluded, as were patients with an empyema or mucocoele of the gallbladder. The gallbladder samples were taken to facilitate removal of the organ and the common bile duct samples collected at needle aspiration for identification of the duct before performing operative duct cholangiography. Before collection, the common bile duct was occluded distally by digital pressure to avoid possible contamination by pancreatic duct and duodenal contents. Bile was collected from 42 patients and a preoperative serum sample was available in 32 of these patients. The amylase activity was estimated using the Phadebas amylase test (Pharmacia Ltd.) with a normal serum range of 80-300 IU/l. In 20 of the samples of bile, the amylase thermostability was also assessed using a previously described method (Donaldson *et al.*, 1977).

Results

Amylase activity was present in all samples of bile. The mean amylase content of the 42 samples of bile was 154 (SEM 16) IU/l. The mean preoperative serum amylase in the 32 patients was 179 (SEM 15) IU/l. There was no significant difference between the amylase activity in the 32 paired samples of bile and serum ($p > 0.1$), assessed either by parametric or non-parametric tests. The mean amylase thermo-

lability in bile was 47% (SEM 4.5), which was similar to the thermolability of normal serum.

Discussion

The possible relationship between the liver and amylase found in the serum has been extensively investigated in animals. Arnold and Rutter (1963) showed that the liver synthesised most of the amylase found in serum. Deficiency of the essential amino acid tryptophan, diminished amylase production, and administration of puromycin, a specific inhibitor of protein synthesis, caused a cessation of amylase production by the liver. In the dog, the liver appears to produce amylase (Nothman and Callow, 1971), and, in the baboon, Duane *et al.* (1972) showed that the liver was a major site of amylase destruction.

In contrast, there is little direct evidence for the existence of a hepatic amylase in man, although it has been suggested for many years that the serum amylase level is altered in patients with liver disease (Janowitz and Dreiling, 1959; Bhutta and Rahman, 1971). The identification of a hepatic isoamylase in man would give conclusive evidence for its existence.

Many workers have attempted to identify the isoenzymes of human amylase using electrophoresis, electrofocusing, or immunodiffusion techniques. In some cases only zones of activity consistent with salivary origin were identified in the serum (Benjamin and Kenny, 1974; Taussig *et al.*, 1974; Otsuki *et al.*, 1976). Other workers found additional zones of amylase activity, the origin of which was unclear (Harada *et al.*, 1974; Skude, 1975; Takeuchi *et al.*, 1975). Joseph *et al.* (1966) isolated a possible hepatic isoamylase and Warshaw *et al.* (1976) found an additional faint zone of amylase activity in one-third of normal people, which was prominent in 67% of patients with hyperamylasaemia and liver disease. The pancreas may also be able to manufacture an S-type isoamylase (Shimamura *et al.*, 1975).

Bile performs both an excretory and digestive function and, although human bile has been extensively investigated (Chenderovitch, 1973), little reference has been made to its amylase activity. Previous investigations with regard to the amylase content of bile have involved small numbers of samples with no details regarding the types of patient studied, methods of collection, or whether safeguards were taken to avoid reflux of pancreatic juice up the common bile duct. One report (Skude, 1975) examined only a single sample of bile and another (Warshaw *et al.*, 1976) investigated eight samples of bile, three of which contained no amylase activity.

In the 42 samples of bile examined, there was a similar amount of amylase activity found in human bile and normal serum. Furthermore, the amylase activity was 47% thermolabile, which is almost identical with the percentage of serum activity. In this study particular care was taken during the bile collection to prevent reflux of amylase from pancreatic duct. In view of the very high amylase activity of 120 000 IU/l in pure pancreatic juice, which is >99% thermolabile (Donaldson *et al.*, 1977) any reflux of minute amounts of pancreatic juice would considerably increase the total amount of amylase activity in the bile and its percentage thermolability.

The similarity in the total and percentage amylase thermolability in both bile and serum might suggest that the amylase activity in the bile had freely filtered from the blood flowing through the liver rather than refluxing from the pancreatic duct.

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