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# Coxsackie and mumpsvirus infection in a prospective study of acute pancreatitis

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SUMMARY A prospective study of 116 patients with acute pancreatitis included routine screening for evidence of viral infection. Five patients (all female) exhibited significant rising antibody titres to Coxsackie B or mumpsvirus, while none of the remaining 111 patients did. Diarrhoea was a prodromal feature of the pancreatitis in those patients with evidence of viral disease. Screening patients with acute pancreatitis for Coxsackie B and mumpsvirus infections is worthwhile in the identification of aetiological factors and may minimise protracted biliary investigations. The incidence of 'idiopathic' acute pancreatitis in this study was 5·2% (six patients).

Recent prospective studies of acute pancreatitis (Ranson *et al.*, 1974; Imrie and Whyte, 1975) have indicated that the group of patients considered to have 'idiopathic' acute pancreatitis represent no more than 12% of the total cases. The two common aetiological factors associated with acute pancreatitis in over 75% of patients are biliary disease and excess alcohol ingestion (Ranson *et al.*, 1974; Howes *et al.*, 1975; Imrie and Whyte, 1975). Hyperlipoproteinaemia, hyperparathyroidism, steroids, hypothermia, and pancreatic neoplasm are aetiological factors in a small percentage of cases.

It is well recognised that acute pancreatitis may be associated with mumps infection (Joske, 1955; Melin and Ursing, 1958) and the agent responsible for infective hepatitis may occasionally cause accompanying pancreatitis (Lisney, 1943; Joske, 1955; Achord, 1968; Gillespie, 1973; Ham and Fitzpatrick, 1974). Coxsackie B viruses have been reported to cause acute pancreatitis (Kibrick and Benirschke, 1958; Fechner *et al.*, 1963; Murphy and Simmul, 1964; Ursing, 1973) and, in an outbreak of Coxsackie $B_5$ induced aseptic meningitis in children, a positive result was obtained in 31% of those screened for raised amylase in blood and/or urine (Nakao *et al.*, 1964). In addition, it has clearly been shown that Coxsackie B virus infections in animals cause acute pancreatitis (Pappenheimer *et al.*, 1951; Burch *et al.*, 1971; Tsui *et al.*, 1972; Ross *et al.*, 1974).

Recently it has been shown that moderately raised antibody titres to Coxsackie B are present in some patients with pancreatitis but the authors concluded that no case in their series was caused by acute viral infection (Capner *et al.*, 1975). They suggest a selective anamnestic response, or low grade infection, or common determinants between pancreatic and Coxsackie antigens may explain their observations. Similar antibody responses, without definite evidence of acute infection, have been found to *Mycoplasma pneumoniae* (Leinikki *et al.*, 1973) but acute pancreatitis has also been described as a complication of *Mycoplasma pneumoniae* infection (Mardh and Ursing, 1974).

In order to investigate further the small group of 'idiopathic' patients and the role of virus infection in acute pancreatitis, routine screening for viral antibodies was included as part of a prospective study of acute pancreatitis performed in one hospital over a period of two years.

## Methods

One-hundred-and-sixteen patients admitted to Glasgow Royal Infirmary were included in the study. The criteria for diagnosis of acute pancreatitis have already been described (Imrie and Whyte, 1975). Acute and convalescent phase clotted blood samples were assayed for mumps and Coxsackie B virus antibody titres, the acute sample being taken in the first week of illness and the convalescent sample after the second week (Table 1).

All 116 patients were screened for biliary disease, and specifically questioned to obtain an estimate of

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Table 1 *Antibody titres to mumps and Coxsackie B viruses in five cases of acute pancreatitis*

| Case No. | Days from onset of illness | Mumps | | Reciprocal antibody titres to Coxsackie type | | | |
|---|---|---|---|---|---|---|---|
| | | S | V | B1, 2, 6 | B3 | B4 | B5 |
| 1 | 3 | < 16 | < 16 | <16 | 64 | <16 | 128 |
| | 30 | < 16 | < 16 | <16 | 1024 | <16 | 256 |
| 2 | 4 | < 16 | < 16 | <16 | < 16 | <16 | < 16 |
| | 18 | < 16 | < 16 | <16 | 64 | 32 | < 16 |
| 3 | 4 | < 16 | 32 | <16 | < 16 | <16 | < 16 |
| | 27 | < 16 | 128 | <16 | < 16 | <16 | < 16 |
| 4 | 7 | < 16 | < 16 | <16 | < 16 | <16 | < 16 |
| | 18 | 128 | < 16 | <16 | < 16 | <16 | < 16 |
| 5 | 2 | < 16 | < 16 | <16 | < 16 | <16 | 128 |
| | 25 | < 16 | 64 | <16 | < 16 | <16 | 256 |

regular alcohol intake, and of intake immediately before the attack of acute pancreatitis. During and after the illness full metabolic screening for hyperparathyroidism and hyperlipoproteinaemia was performed.

Mumps antibodies were titrated by complement fixation test (CFT), using commercial v & s antigens and allowing fixation to occur for 18 hours at 4°C. using 2 units of complement and 50% end point readings. Reciprocal titres are shown in Table 1.

Antibody titres to Coxsackie B1-6 were assayed by an immunofluorescent (FA) method which has been described previously (McLure *et al.*, 1972). Infected VERO cells were used as substrate, with viral antigen present in approximately 50%, after incubation for 18 hours at 37°C. Replicate spots were placed on microscope slides so that dilutions of serum from 1:2 through 1:256 could be tested on a single slide. Slides were stored at −180°C after air drying and fixing in acetone, before use for titrations. Serum dilutions were made in microtitre 'U' plates. The indirect FA method was used throughout. Incubation of each step was for 30 minutes at 37°C. Rabbit anti-human:FITC coupled serum was purchased from Nordic Pharmaceuticals. This was used at 4 units and had 1·0 mg naphthalene black added per millilitre of working dilution. No non-specific fluorescence was present. Appropriate positive and negative controls were included in every batch of titrations. Fifty per cent end-points were used and reciprocal titres are shown in Table 1. Readings of FA titres were made on a Wild M20 fluorescence microscope fitted with a Balzer FITC interference filter as exciter and a Kodak Wratten 12 gelatin filter as barrier. Each serum was titrated by this method against cells preinfected with Coxsackie viruses B1-6.

## Results

Of the 116 patients studied, five had significant rising antibody titres to mumpsvirus or one or more Coxsackie B viruses, while 111 patients exhibited no rising titres (Table 2). All five patients were female and all Australia antigen negative.

Two patients had rising antibody titres to mumpsvirus and two patients had rising antibody titres to Coxsackie B viruses types B3, 4, or 5. One patient had positive titres to mumps V and Coxsackie B5 (Tables 1, 3). In those patients with rising antibody titres to mumpsvirus, two were positive to V antigen (cases 3 and 5) and one to S antigen (case 4).

Good evidence of biliary disease was present in two of the five patients (cases 1 and 4; Table 3) and none of the five had any evidence of excess alcohol intake, hyperlipoproteinaemia, hyperparathyroidism, or other known aetiological factor. The rises in serum amylase on admission are indicated in Table 3, showing that only one patient did not record a serum amylase level greater than four times the upper limit of normal (normal serum amylase 70-300 IU/l). A marginal rise in both serum amylase and amylase clearance was present in this case after a 72 hour history of excessive vomiting and

Table 2 *Aetiology of acute pancreatitis*

| | |
|---|---|
| Biliary disease/alcohol, etc. | 105 |
| Viral infection + biliary disease | 2 |
| Viral infection alone | 3 |
| No identified cause ('idiopathic') | 6 |
| Total | 116 |

Table 3 *Aetiological factors in five patients reported*

| Case No. | Age (yr) | Prodromal symptoms | Biliary disease | Viral titres | Serum amylase (U/l) |
|---|---|---|---|---|---|
| 1 | 21 | Yes | Yes | Coxsackie $B_{3, 5}$ | 8200 |
| 2 | 32 | Yes | — | Coxsackie $B_{3, 4}$ | 410 |
| 3 | 49 | — | — | Mumps V | 1400 |
| 4 | 80 | Yes | Probable | Mumps S | 3200 |
| 5 | 47 | Yes | — | Coxsackie $B_5$, mumps V | 7600 |

only six hour history of severe acute epigastric pain. Laparotomy was therefore carried out and this confirmed the presence of acute pancreatitis and no other intra-abdominal pathology. Postoperative progress was satisfactory and she was discharged well after two weeks. The remaining four patients were all successfully managed through their acute pancreatitis.

The evidence for biliary disease in one of these five patients was conclusive and cholecystectomy has now been carried out, while in the second patient oral cholecystography has been performed on two occasions and the gall bladder has failed to outline. In view of her age, no further biliary investigation or surgery is proposed.

The presence of a prodromal illness would be compatible with a possible viral aetiology in a case of acute pancreatitis and it is therefore notable that four of the five patients had clearly defined prodromal signs and symptoms, not usually associated with the onset of acute pancreatitis. Diarrhoea preceded the abdominal pain in four patients and in the remaining patient (case 3) no accurate preceding history to her illness was obtained because of amnesia for the period before admission in coma and renal failure.

None of those patients with positive mumps antibody titres exhibited signs of parotid or gonadal involvement. Other stigmata of acute viral infections were not observed.

## Discussion

The finding of significant rising antibody titres in five patients with acute pancreatitis is good evidence of concurrent viral infection. In two of those five, evidence of biliary disease was also present. It is impossible to ascertain the relative roles of gall stones and viral infection in the onset of a particular attack of this disease in an individual patient, and double aetiological factors in a single case are not uncommon (Imrie 1974). The remaining three patients had no other aetiological factor identified after complete investigation.

The finding that three of the nine patients with no putative precipitating factor had concurrent viral infection, and two of the 107 with a known aetiological factor also had evidence of viral infection suggests that a minority of cases of acute pancreatitis are caused by infection with mumps or a Coxsackie B virus.

The exact manner in which the viruses may attack the pancreas is as yet uncertain. Oedema of the papilla of Vater and pancreatic ducts has been suggested in the human (Ursing, 1973) but from the experimental work done in rats and mice (Pappenheimer *et al.*, 1951; Burch *et al.*, 1971; Tsui *et al.*, 1972; Ross *et al.*, 1974) a direct attack on the acinar cell would appear to be the more likely explanation, at least in the experimental animal.

We have concerned ourselves in this study only with patients who exhibited high CF titres to Coxsackie B and mumpsvirus. However, we have noted a similar experience to that of other workers (Leinikki *et al.*, 1973; Capner *et al.*, 1975) regarding moderate CF titre rises to Coxsackie B and *Mycoplasma pneumoniae* occasionally occurring in acute pancreatitis patients. Therefore we support their contentions that a selective anamnestic response may be an accompaniment of acute pancreatitis or that pancreatic antigens (released during acute pancreatitis) may share common determinants with Coxsackie B and mycoplasma pneumoniae antigens.

With regard to the overall role of viral factors in the aetiology of acute pancreatitis, this prospective study has shown that a small percentage of cases may have a viral aetiology of Coxsackie or mumpsvirus grouping. This represents an important proportion of the small group of patients with so-called idiopathic acute pancreatitis.

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## Addendum

Since this paper was submitted we have admitted a 31 year old teetotal male librarian with acute pancreatitis and no prodromal illness. Admission serum amylase was 10 000 U/1 and Coxsackie B2 titres rose from an initial titre of 64 to 128 on the ninth day of illness, representing good evidence of concurrent infection. Full screening for other aetiological factors has been negative and at later follow-up the Coxsackie B2 titre was 32. There have been no late complications to his illness.