A CLINICAL AND EXPERIMENTAL STUDY OF INTRAPERITONEAL ANTIPROTEASE THERAPY IN ACUTE PANCREATITIS

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A thesis submitted to the University of Glasgow for the degree of Doctor of Medicine.

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To my family and friends.

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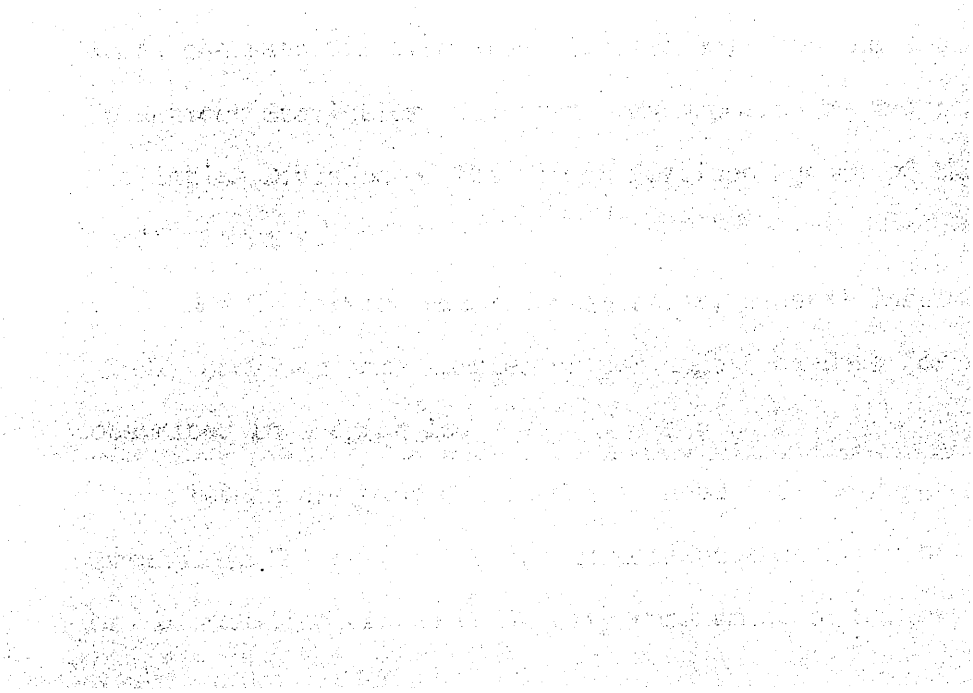

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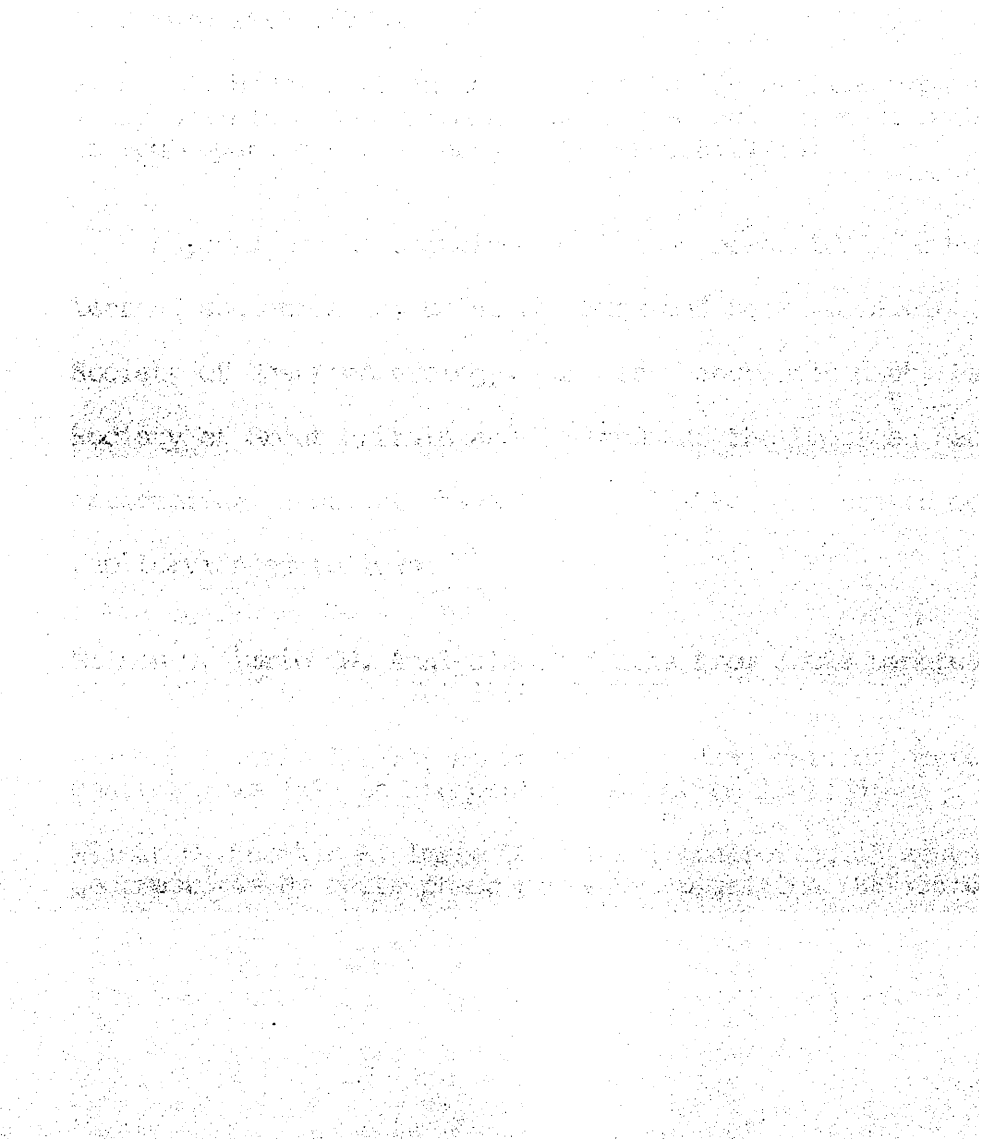

DECLARATION OF WORK PUBLISHED

At the time of submission of this thesis 4 papers had been published:

Wilson C, Imrie CW. Deaths from acute pancreatitis: why do we miss the diagnosis so frequently? Int J Pancreatol 1988;3:273-82.

Wilson C, Imrie CW, Carter DC. Fatal acute pancreatitis. Gut 1988;29:782-8.

Wilson C. Artefactual body wall ecchymosis in acute pancreatitis. Br J Surg 1988;75:704.

Wilson C, Heads A, Shenkin A, Imrie CW. C-reactive protein, antiproteases and complement factors as objective markers of severity in acute pancreatitis. Br J Surg 1989;76:177-81.

Aspects of the contained work have been widely presented at learned societies including the Surgical Research Society, British Society of Gastroenterology, European Pancreatic Club, Pancreatic Society of Great Britain and Ireland and the American Pancreatic Association. Abstracts have been published or accepted for publication as follows:

Wilson C, Imrie CW. Analysis of deaths from acute pancreatitis: disturbing findings. Gut 1986;27:A230.

Wilson C, Imrie CW. Why do we miss the diagnosis of acute pancreatitis (AP) so frequently? Digestion 1986;35:63.

Wilson C, Shenkin A, Imrie CW. Serial monitoring of acute pancreatitis by acute phase proteins. Digestion 1987;38:64.

Wilson C, Shenkin A, Imrie CW, Carter DC. Peritoneal fluid enzymes and antiproteases in acute pancreatitis. Br J Surg 1987;74:1165.

Wilson C, Shenkin A, Imrie CW, Value of C-reactive protein and antiproteases in the objective monitoring of acute pancreatitis. Gut 1988;29:A269.

Wilson C, Murray GD, Imrie CW, Carter DC. Intraperitoneal antiprotease therapy in experimental acute pancreatitis. Gut 1988;29:A270.

Heath DI, Wilson C, Imrie CW. Assessment and monitoring of acute pancreatitis by the APACHE II scoring system. Digestion 1988;40:85.

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Wilson C, Heath DI, Shenkin A, Imrie CW. Biochemical studies of peritoneal exudates and pseudocyst fluid. Pancreas 1988;3:633.

Wilson C, Heath DI, Shenkin A, Imrie CW. Biochemical studies of peritoneal exudates and pseudocyst fluid. Gut 1989;30:000.

STATEMENT OF COLLABORATION

The clinical study investigating intraperitoneal antiprotease therapy with aprotinin was originally proposed by Mr. M. J. McMahon, Senior Lecturer in Surgery, General Infirmary, Leeds. The form of the final protocol was agreed with others including Mr. C. W. Imrie, Consultant Surgeon, Royal Infirmary, Glasgow, Mr. M. Larvin, Research Registrar, General Infirmary, Leeds, Mr. D. Lees, Medical Services Manager for Bayer UK Ltd. in addition to myself.

The study has been conducted within hospitals in both the Glasgow and Leeds areas to ensure sufficient recruitment of patients. This thesis details my analysis of 160 patients assessed personally and entered into the study between February 1986 and August 1987.

All the remaining study designs, including the biochemical studies and the studies of antiprotease therapy in experimental pancreatitis, were constructed by myself.

All the sampling and testing of blood and peritoneal fluid was performed by myself, except where otherwise indicated.

Ms. M. Anderson RGN, RSCN assisted in the collation of data in chapters 2 and 8. Mr. D. I. Heath, Reseach Registrar, Royal Infirmary assisted in the collation of data in chapter 8. The data in chapter 9 were analysed with the assistance of Mr. G. D. Murray, Statistician, University Department of Surgery, Royal Infirmary.

The entire contents of this thesis have been typed by myself using Wordstar 2000 on an Amstrad PC1640. The figures were prepared by myself using a statistical programme written in the University

Department of Surgery, on an Apple 2e personal computer and Hewlett Packard plotter. Artwork was prepared by Ms. J. McDonald of the Medical Illustration Department, Royal Infirmary.

All the references cited in the text have been read by myself.

SUMMARY

Peritoneal exudate appears to be toxic in experimental pancreatitis, possibly as a result of overwhelming of its antiprotease defences by proteolytic enzymes released from the pancreas. Removal of this exudate by peritoneal lavage has been a uniformly successful therapy. In contrast, the role of the protease-antiprotease balance in peritoneal exudate in human acute pancreatitis, and its relationship with the early "shock-like" illness which may complicate severe attacks, has not been clearly defined and the efficacy of peritoneal lavage is unproven.

This thesis has addressed 3 main issues. The nature and extent of the problem presented by acute pancreatitis has been investigated, as have means for monitoring and predicting the severity of the illness. The major part of the thesis has examined aspects of the protease-antiprotease balance in the peritoneal exudate that complicates severe acute pancreatitis, and the efficacy of treatment by the administration of intraperitoneal antiproteases in experimental pancreatitis and in man.

Incidence and mortality

The incidence and mortality trends of acute pancreatitis in Scotland have been reviewed between 1961 and 1985 using data from the Scottish Hospital In-patient Statistics. Over this 25 year period the number of patients discharged with a diagnosis of acute pancreatitis increased 11-fold in males and 4-fold in females and may be largely

explained by an increased frequency of diagnosis (diagnostic rate), due to greater clinical awareness and more frequent diagnostic testing. The absolute mortality rate has increased slightly although, because of the marked increase in incidence, the case mortality rate has fallen from 17.8% to 5.6%.

The incidence and mortality trends were also examined in Glasgow Royal Infirmary between 1974 and 1984, over which period the diagnostic rate was thought to have been unchanged. Despite a 23% fall in the catchment population the annual number of admissions remained relatively constant, suggesting a true increase in local incidence.

Amongst the fatal attacks, 42% had the diagnosis first made at post mortem, the fall in overall mortality from 14.9% to 10.8% reflecting a reduction in the numbers first diagnosed at post mortem. Mortality amongst patients diagnosed in life was unchanged at 9% throughout, thus when the effect of an increased diagnostic rate is excluded, improvements in therapy do not appear to have influenced the mortality rate.

Prediction of outcome

Investigation of invasive or expensive therapies requires that patients with severe pancreatitis and at risk of a complicated attack, are identified accurately, early in their illness. There is also a need to develop means to objectively monitor the course of the illness and any response to therapy, which might aid their management.

Simple clinical assessment was shown to have the highest overall accuracy in predicting outcome but, even 48 hours post-admission, detected under 50% of those developing complications. Multiple factor scoring systems provided a useful prediction of outcome, particularly for attacks associated with alcohol abuse, but provided a "once only" prediction and failed to allow for sequential monitoring of the course of an attack.

Sequential monitoring of 5 different complement factors was of no value in discriminating complicated from uncomplicated attacks. Serum antiproteases demonstrated a falling $alpha_2$macroglobulin concentration and a rising $alpha_1$antiprotease concentration in complicated attacks, both providing useful discrimination. C-reactive protein provided better discrimination but peak levels ($\geqslant$210mg/l on the 2nd, 3rd or 4th day), while providing equivalent accuracy to the multiple factor scoring systems incurred a similar delay. C-reactive protein concentrations fell with clinical improvement, persistently high levels ($\geqslant$120mg/l on day 7) indicating an increased risk of complication.

The APACHE II scoring system provided a prediction of outcome within a few hours of admission. The peak score recorded in the first 3 days provided greater accuracy, equivalent to the multiple factor scoring systems, but incurred a similar delay. The APACHE II was objective, reproducible and appeared to reflect improvement or deterioration in the patient's clinical condition and may permit sequential monitoring of the course of the illness.

None of the scoring systems or factors examined was able to

predict complicated attacks earlier and more accurately than multiple factor scoring systems. C-reactive protein and APACHE II both appear to be of value, C-reactive protein for its simplicity and both for objective monitoring during the course of an attack.

Intraperitoneal antiprotease therapy

Retrograde intraductal injection of sodium taurocholate produced a severe pancreatitis in rats, death usually occurring within 24 hours. Only a small proportion of patients die of such a fulminant, rapidly fatal illness but it is this early "shock-like" illness which therapy aims to ameliorate and, therefore, this model appeared an appropriate one to study.

Saturation of the peritoneal antiproteases by proteases has been shown to be associated with the development of shock and death in experimental pancreatitis possibly by the activation of complement, kininogens and zymogens with release of vasoactive peptides, including kinins and histamine, which may escape into the circulation to exert a systemic effect.

Saturation of the peritoneal antiproteases was demonstrated by the presence of free proteolytic activity in 40-50% of rats one hour after the induction of pancreatitis and in 60% of control rats at death. Intraperitoneal therapy comprising aspiration of the exudate, a single peritoneal lavage and instillation of an antiprotease or similar inactive solution, one hour after the induction of pancreatitis was found to prolong the median survival suggesting that the continued presence of exudate was deleterious.

Fresh frozen plasma instilled into the peritoneal cavity after the lavage was associated with the longest survival and thus appeared to confer additional benefit, perhaps because of its antiprotease ($alpha_2$macroglobulin and $alpha_1$antiprotease) content. Instillation of the antiprotease aprotinin, although possessing a much greater trypsin binding capacity, just failed to significantly improve survival compared with the control group and was no better than instilling saline alone. The nature of the antiprotease administered, rather than its trypsin binding capacity, may be of most importance.

Study of the peritoneal exudates from patients with acute pancreatitis confirmed proteolytic enzyme activation and reduced trypsin binding capacities indicating a degree of saturation of their antiproteases. Three patients had marked reduction of their trypsin binding capacities, 2 dying of fulminant acute pancreatitis soon after sampling and in whom saturation of the antiprotease defences may have occurred. Thus similar, although less marked changes in the protease-antiprotease balance were observed in man.

Of 160 patients studied prospectively in a clinical trial, 51 fulfilled the inclusion and exclusion criteria and were randomised to receive standard therapy alone (26), or with additional intraperitoneal antiprotease therapy (25) comprising peritoneal aspiration, a single saline lavage and instillation of aprotinin (5×10^6KIU on 2 occasions 8 hours apart). Intraperitoneal therapy with aprotinin was found to markedly reduce peritoneal tryptic amidase activity during the initial 8 hours of treatment. In this preliminary report intraperitoneal therapy with aprotinin produced no benefit in

terms of the incidence of death or major complication. There was a suggestion of a trend towards earlier clinical improvement, with a reduced incidence of shock and early death from fulminant acute pancreatitis in the treated group but this requires confirmation in a larger group of patients.

PART 1.

INTRODUCTION AND AIMS

CHAPTER 1. ACUTE PANCREATITIS.

General introduction

Acute pancreatitis appears to be increasing in incidence but the case mortality rate has remained unchanged over the past 30 years at around 20%[69,357] suggesting that improvements in therapy introduced during this period may have failed to influence the outcome.

Typically 2 phases of mortality are recognised in acute pancreatitis. Death may occur early, within the first week, resulting from a "shock-like" illness complicated by respiratory, cardiovascular and renal failure. Trapnell found that 45 (59%) of his patients died during this period, 33 within the first 48 hours, the majority (67%) having oedematous pancreatitis at post mortem[355]. Thus a considerable proportion of those dying appeared to do so in the presence of a seemingly mild, recoverable, pancreatic lesion. Patients surviving this early phase may succumb later in the course of the attack, due to the local complications resulting from pancreatic necrosis and sepsis.

"Toxic factors" released into the circulation, from the inflamed pancreas or from the commonly associated peritoneal exudate, are postulated to be the cause of the early "shock-like" illness. Therapeutic strategies which have been put forward to influence this phase of the illness are numerous and have generally failed to show benefit when subjected to controlled clinical trial.

There is a need to increase understanding of the mechanisms

underlying this early systemic toxicity and to develop means of specifically treating this phase of the illness. This thesis examines the role of the protease-antiprotease balance in pancreatitis exudate as a cause of the toxicity and investigates a novel therapeutic approach comprising removal of the exudate by aspiration and a single peritoneal lavage, followed by intraperitoneal instillation of an exogenous antiprotease solution.

Accurate identification of patients with severe acute pancreatitis early in their attack is another priority, so that resources, including any potentially invasive therapies, may be directed towards these patients.

Acute pancreatitis is defined and its diagnosis and clinical features are described in the first part of this thesis. The pathogenesis of acute pancreatitis and its complications are reviewed, with particular reference to the role of the proteolytic enzymes and evidence for the pathogenicity of peritoneal exudate. Aspects of therapy including severity prediction and disease monitoring are reviewed and the past experience of peritoneal lavage and antiprotease therapy are critically assessed.

Historical aspects

The word pancreas derives from the Greek "pan" meaning all, and "kreas" meaning flesh or meat. The name has been attributed to Rufus of Ephesus (circa 100 AD) although the first description of the organ can be traced to Herophilus of Chalcedon (circa 300 BC).

The function of the pancreas was, for many years, unclear.

Niccolo Massa (1536) considered the pancreas to be a pad "upon which the mouth of the stomach rests lest it touch the hard surface of the vertebrae without a buffer between"[333]. Vesalius (1514-1564) described the pancreas as a glandulous organ but the first description of the pancreatic duct, thus establishing the glandular nature of the organ, came from Johann Wirsung of Padua in 1642. The accessory duct was described by Giovanni Santorini in 1724 and both ducts have come to be known eponymously. In the 19th century Bernard first established the importance of the pancreatic secretion in digestion[55].

Pancreatitis was induced experimentally in dogs by Bernard in 1855 by injecting bile and other substances into the pancreatic duct[36]. Alcohol as an aetiological factor in pancreatitis in man was first highlighted in 1878 by Friedreich's description of "drunkard's pancreas"[115]. The description of another mechanism of production of pancreatitis was provided in 1901 by Opie who reported a gallstone obstructing the ampulla of Vater, thus creating a common channel with reflux of bile into the pancreatic duct[266]. However, despite much effort since then we are little closer to understanding the initiating factor(s) in this and other forms of acute pancreatitis.

The exocrine pancreas

Embryology

The pancreas is formed from the gut endoderm by the fusion of two separate primordia (Fig. 1). The dorsal primordium arises first from the floor of the duodenum, proximal to the smaller, ventral

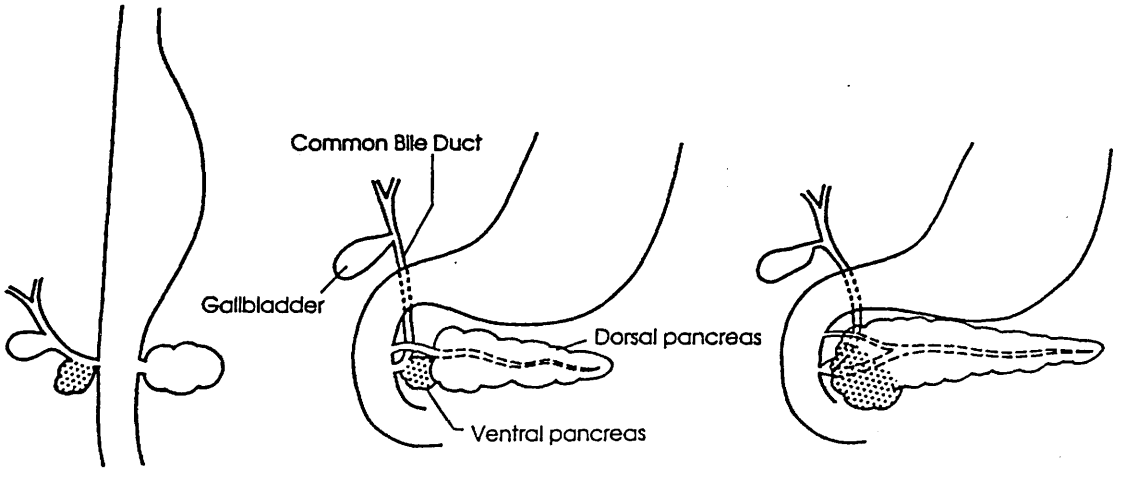


FIGURE 1.

Diagram showing stages of pancreatic development: formation of the dorsal and ventral primordia, rotation of the termination of the hepatic diverticulum and ventral pancreas to the right where it fuses with the dorsal pancreas to form the adult gland.

primordium. The ventral primordium arises from the base of the hepatic diverticulum, accounting for the common opening of the main pancreatic duct and bile duct in the adult[333].

Growth changes within the duodenum cause the ventral pancreas and hepatic diverticulum to swing round to the right where fusion between the two primordia occurs, the ventral pancreas forming the uncinate process and inferior portion of the head, and the dorsal pancreas the remainder of the adult gland. After fusion of the two primordia their ducts fuse, the main pancreatic duct (Wirsung) arising from the duct of the ventral pancreas and the distal portion of the duct of the dorsal pancreas. The proximal portion of the dorsal pancreatic duct enters the duodenum separately as the accessory duct (Santorini).

Structure

The acinus is the secretory unit of the pancreas, each comprising a group of pyramidal-shaped acinar cells around a lumen lined by flattened centro-acinar cells, into which the acinar cells discharge their secretory granules when stimulated to secrete (Fig. 2). The pancreas is composed of multiple lobules, each made up of numerous acini draining into intercalated ducts. These ducts unite to form intralobular ducts which join interlobular ducts running in the connective tissue between lobules. Finally the interlobular ducts join the main pancreatic duct which drains via the ampulla of Vater into the duodenum.

Pancreatic secretion

The pancreatic secretion comprises two separate secretory processes - enzyme secretion and electrolyte secretion. Secretion is primarily under hormonal control although, as in gastric secretion, there is a neural component (cephalic phase) mediated by the vagus. Secretin liberated from the duodenal mucosa stimulates secretion of a watery, alkaline fluid from the ductular cells. Pancreozymin, also released from the duodenal mucosa, stimulates secretion of an enzyme-rich fluid by the discharge of zymogen granules from acinar cells.

Pancreatic enzymes

The enzymes produced by the pancreatic acinar cells play a major role in intraluminal digestion. The pancreatic enzymes are all hydrolases and are classed according to their substrate molecule: amylase - starch, proteolytic enzymes - proteins, lipolytic enzymes - fats and nucleolytic enzymes - nucleic acids (Table 1). Pancreatic enzymes make up between 80 and 85% of the protein content of pancreatic juice[79].

Definition and classification

Fitz, in 1889, was the first to attempt to classify acute pancreatitis describing haemorrhagic, suppurative and gangrenous forms[106]. Various classifications were subsequently proposed but the first unified attempt at classification followed an international symposium at Marseille in 1963[317].

Pancreatic inflammatory diseases were classified into 4 categories: acute pancreatitis, acute relapsing pancreatitis, chronic relapsing pancreatitis and chronic pancreatitis[317]. The two acute forms - acute and relapsing acute pancreatitis were considered to be associated with clinical and biological restitution of the gland, if the primary cause or factors had been eliminated whereas in the chronic forms, anatomical and/or functional damage persisted. The Marseille classification has been helpful in the study of pancreatitis but problems remained, one example being the difficulty in practice of distinguishing acute relapsing from chronic relapsing pancreatitis.

The classification of acute pancreatitis has been updated more recently at symposia in Cambridge and Marseille, during 1983 and 1984 respectively[319,331]. Both classifications were similar with regard to their clinical definition of acute pancreatitis but the second Marseille symposium again stressed the morphological changes within the pancreas in mild and severe attacks, although such information is not readily available in clinical practice and is seldom sought in these patients[25]. Thus the clinical definition of acute pancreatitis, as suggested by the Cambridge group[319], which can be applied at the time of diagnosis, would appear to be the most useful in clinical practice and is the one adopted in this thesis.

TABLE 1.

The human pancreatic enzymes.

Protein	Activator	Substrate	Function
Alpha-amylase	-	Starch	Hydrolysis of alpha 1,4 glycosidic bonds
Lipase	-	Tri-glycerides	Hydrolysis of C_1 and C_3 glycerol ester bonds
Carboxyl ester hydrolase	-	Cholesterol esters	Hydrolysis of esters of water soluble short chain fatty acids
Ribonuclease	-	RNA	Hydrolysis of phosphate ester bonds in RNA
Deoxyribonuclease	-	DNA	Hydrolysis of phosphate ester bonds in DNA
Trypsinogen (Trypsin)	Entero-kinase	Proteins	Hydrolysis of peptide bonds at arginine and lysine
Chymotrypsinogen (Chymotrypsin)	Trypsin	Proteins	Hydrolysis of peptide bonds at amino acids with aromatic side chains
Proelastase (Elastase)	Trypsin	Elastin	Hydrolysis of peptide bonds at amino acids with aliphatic side chains
Procarboxy-peptidase A (Carboxypeptidase A)	Trypsin	Proteins	Hydrolysis of peptide bonds at C terminal amino acids with aromatic or branched aliphatic side chains
Procarboxy-peptidase B (Carboxypeptidase B)	Trypsin	Proteins	Hydrolysis of peptide bonds at C terminal amino acids with basic side chains
Prophospholipase A_2 (Phospholipase A_2)	Trypsin	Phospho-lipids	Hydrolysis of fatty acid esters at 2-position of phospholipids
Kallikreinogen (Kallikrein)	Trypsin	Kininogens	Cleavage of kininogen to active kinin

Clinical features

"Acute pancreatitis is the most terrible of all the calamities that occur in connection with the abdominal viscera. The suddenness of its onset, the illimitable agony which accompanies it, and the mortality attendant upon it, all render it the most formidable of catastrophies" (Moynihan, 1925).

Moynihan went on to describe the characteristic distribution of the pain "of fiercest intensity in the epigastrium, but it is felt also in the back and often both loins". He also described the collapse and shock that may occur in acute pancreatitis and considered that vomiting was almost invariably present in these patients[244] although this classical picture was observed by Pollock in only 50% of cases[279].

The vast majority of patients present with abdominal pain, the proportion ranging from 84%[165] to 98%[40]. Some attacks may be painless, one study reporting this in 7 (15%) of 47 patients[296] but, as such cases may not be diagnosed in life, its true prevalence is difficult to estimate. The abdominal pain is epigastric in around 60% of cases[8,65,305], but may be right hypochondrial in 20%[40,108] or, more rarely, maximal in the left hypochondrium or in the lower abdomen[40,65,108]. Radiation of pain to the back was described in around half the patients[40,305], in 40% of cases[8,65,165] and in as few as 28%[108]. Vomiting was a frequent association found in between 53%[165] and 90% of patients[279].

Although there are no specific clinical features which can be considered pathognamonic of acute pancreatitis, the presence of body wall staining is suggestive. Both Cullen's sign[75] and Grey Turner's sign[135] are rare, having been reported in between 1%[8,165,279] and 3%

of attacks[81]. These signs are seldom present on admission of the patient to hospital and are not generally of value in establishing the diagnosis. Therefore, although most patients present with a fairly characteristic clinical picture, this is rarely specific enough to allow a confident diagnosis to be made.

Diagnosis

Acute pancreatitis can be diagnosed definitively only at laparotomy or post mortem. As most patients recover without recourse to surgery the diagnosis is, in practice, based on an abnormal pancreatic enzyme elevation in the presence of a compatible clinical picture. Most widely used for its diagnosis is estimation of amylase.

Amylase

A "starch-splitting ferment" in the blood was described by Magendie in 1846[95] and its value in the diagnosis of acute pancreatitis was first reported in 1929[95]. Serum amylase measurement has become the corner-stone of diagnosis but elevated levels do not necessarily prove the diagnosis of acute pancreatitis, neither do normal levels exclude it[84].

Hyperamylasaemia is thought to correlate with the presence of acute pancreatitis in between 75% and 80% of cases[315], although many other conditions may also be associated with hyperamylasaemia[166,315]. The amylase activity selected as the diagnostic cut-off level for acute pancreatitis influences this figure, the higher the level set, the fewer non-pancreatic causes of hyperamylasaemia will be

erroneously included.

With the advent of the Phadebas amylase test (Pharmacia Diagnostics, Uppsala, Sweden) most recent British series have quoted their amylase results in international units (IU/l). In the UK the diagnostic cut-off levels have varied between 1,000IU/l[350], 1,200IU/l[159] and 2,000IU/l[245], (normal range 70-300IU/l).

Amylase isoenzymes

Amylase comprises 2 principle isoenzymes, pancreatic (p-type) isoamylase and salivary (s-type) isoamylase, each composed of several slightly different subunits in terms of their chromatographic mobility[315]. The s-type is the predominant fraction in normal serum while the p-type predominates in urine. Knowledge of the isoamylase profile has been reported to provide information which could change the clinical diagnosis in between 20% and 40% of cases with hyperamylasaemia[181,375]. In contrast, amongst patients with clinically suspected acute pancreatitis 97% had p-type isoamylase elevation confirmed[174].

Urinary amylase

Urinary amylase excretion has been considered to be a more sensitive index of acute pancreatitis than serum amylase estimations[323], rising earlier, higher and persisting for longer[315]. Hyperamylasuria, like hyperamylasaemia, is not necessarily diagnostic of acute pancreatitis, nor does a normal urinary amylase exclude its presence[84].

Amylase-creatinine clearance ratio

The increased renal clearance of amylase by the kidney in acute pancreatitis was put forward as a more specific diagnostic test and expressed as the ratio of the amylase clearance to the creatinine clearance, has become known as the amylase creatinine clearance ratio (ACCR)[212]. This finding was attributed to a direct effect on the kidney, either on glomerular permeability or tubular reabsorbtion[370], others implicating a reversible renal tubular defect[167]. The specificity of the ACCR has been questioned more recently[85,194] and it is rarely employed.

Lipase

Lipase has been considered a more specific test of pancreatic disease than amylase[35,370] but like amylase, a number of other acute abdominal conditions may give rise to lipase elevations[84]. The elevations of both amylase and lipase parallel each other in the majority of cases[35,213]. Others consider that lipase is more closely correlated to pancreatic p-type isoamylase and that either test is more specific than the total amylase[182].

Trypsin

Trypsin may be measured by a radioimmunoassay, the bulk of the immunoreactivity in serum representing trypsinogen and a small proportion trypsin complexed to circulating inhibitors such as $alpha_1$antiprotease[49] (trypsin does not retain its immunoreactivity when complexed to $alpha_2$macroglobulin). Immunoreactive trypsin was

markedly raised in acute pancreatitis[49,191,240] and remained elevated for longer than urinary amylase[240]. At present the test is more expensive and time consuming to perform than the tests for either amylase or lipase, and is not in routine clinical use.

Radiology

Acute pancreatitis may be associated with various non-specific appearances on X-ray including the "sentinel loop" and the "colon cut-off sign". Either may suggest the diagnosis of acute pancreatitis, but are themselves not diagnostic[84]. Localised duodenal ileus may be of more diagnostic value but may be a feature of other inflammatory conditions such as acute cholecystitis[140].

The diagnosis can be made on both ultrasound and computed tomography by the demonstration of pancreatic enlargement, perhaps with loss of density, a surrounding halo or more distant inflammatory infiltration.

Incidence and mortality

"The incidence of acute pancreatitis probably reflects the thoroughness with which the disease is sought as well as the criteria which are accepted to define its existence" (McMahon, 1977).

In the early part of this century acute pancreatitis was infrequently diagnosed, and then only with certainty at laparotomy or post mortem. With the advent of serum amylase testing, permitting for the first time accurate non-operative diagnosis in life, the reported incidence of the condition increased[258] and has continued to rise.

Trapnell and Duncan examined the incidence and mortality

trends in Bristol over a 20 year period, finding an average annual incidence of 53.8 cases per million population for the period 1961 to 1967 with a mortality of 9 per million population[357]. Between 1968 and 1979 the annual incidence had risen to a mean of 73 cases per million population with a mean annual death rate of 15.7 per million population[69]. Thus while the incidence and mortality had both increased, the overall case mortality rate had remained unchanged at 19.6% between 1968 and 1979 compared with 20.5% for the preceding 20 years[69,357].

Patients diagnosed in life comprise only a proportion of the the total number with the condition. A further group are those first diagnosed at post mortem, having been unsuspected and/or undiagnosed during life. The number falling into this category has been reported at between 22%[53] and 86%[378] of all pancreatitis deaths, with a mean incidence of 41.6% in 7 series reviewed (Table 2). The size of this group will depend, amongst other factors, on the proportion of patients dying in hospital who are submitted to post mortem examination.

A final group have unsuspected acute pancreatitis and remain undiagnosed, either surviving, or dying without a post mortem. More widespread diagnostic testing will identify more of these patients, resulting in an increased diagnostic rate and an apparent increase in incidence. Many will have mild pancreatitis and thus be less likely to die and their inclusion in epidemiological studies will effect a lowering of the case mortality rate. This can be illustrated in 3 published series from Glasgow Royal Infirmary which point to an

TABLE 2.

Reported proportion of deaths from acute pancreatitis undiagnosed before post mortem.

Authors	Deaths	Undiagnosed before post mortem
Trapnell (1966)	76	24 (32%)
Whalen et al. (1971)	50	43 (86%)
Read et al. (1976)	10	4 (40%)
Bourke (1977)	54	22 (41%)
Peterson and Brooks (1979)	40	17 (43%)
Buggy and Nostrant (1983)	32	7 (22%)
Corfield et al. (1985)	125	44 (35%)
Totals	387	161 (42%)

increased diagnostic rate being at least partly responsible for the increasing incidence and falling mortality rates (Table 3).

Other factors influencing the mortality include the patient's age and underlying aetiology[155,157,165,290,355]. Attacks due to gallstones are associated with a higher mortality than attacks due to alcohol[157,165] but whether this simply reflects the patients' greater age is unclear. A higher mortality has been recorded in postoperative pancreatitis and pancreatitis secondary to trauma[155,160,292,381] and also in attacks in which no aetiological factor is identified[69,165,235]. Whether the patients in this latter category are simply older and/or sicker and, therefore, less likely to be investigated, is not known. The first attack of acute pancreatitis is thought by some to be more likely to be fatal than the second or subsequent attack[292,322,357], although others have been unable to confirm this[69].

Pathology

The histological changes of acute pancreatitis are characterised by pancreatic parenchymal destruction, necrosis of blood vessels with haemorrhage, fat necrosis and an accompanying inflammatory reaction[142].

Investigating the initial lesion in human acute pancreatitis Foulis found two distinct patterns of necrosis[109]. In alcohol or gallstone-associated pancreatitis there was ductal inflammation and periductal necrosis suggesting the initiating factors may have been "duct-borne"[109]. In contrast other patients demonstrated only

TABLE 3.

Reported incidence and mortality from acute pancreatitis at Glasgow Royal Infirmary (1960-1977).

Type of study	Years	Number of cases	Average number of cases/year	Mortality	Reference
Retrospective	1960-70	140*	14	21.4%	Imrie, 1974
Prospective	1971-72+	78	39	11.5%	Imrie and Whyte, 1975
Prospective	1974-77#	161	54	8.7%	Imrie et al. 1978

* 51 cases recorded within last 2 years of study.
+ coincided with introduction of Phadebas amylase test.
# study over 34 month period.

perilobular necrosis, without ductal inflammation, the pancreatitis in these cases having followed an episode of shock. This pattern of necrosis has also been observed in patients developing pancreatitis following cardiac surgery[101] and appears to have an ischaemic basis.

Kloppel and co-workers in a review of 367 autopsy cases and 3 surgical specimens came to entirely different conclusions regarding the initial lesion[177]. Using immunocytochemical techniques the peripheral acinar cells within a lobule neighbouring an area of fat necrosis were found to be depleted of enzymes, whereas the more central acinar cells retained their enzyme content. No major differences were apparent between pancreatitis of varying aetiologies and the fact that the ducts appeared intact, seemed to indicate the initial lesion was the discharge of enzymes by peripheral acinar cells (perhaps by basolateral release) into the surrounding interstitial tissue[177].

Aetiological factors

Epidemiological studies have provided evidence of an association between acute pancreatitis and a number of conditions or factors, some of which are now considered to be causally related or aetiological factors. The list of associated conditions is extensive and has been thoroughly reviewed by Durr[84]. I propose only to deal with the two conditions most widely encountered, namely biliary tract disease and alcohol abuse, which together account for the majority of cases of acute pancreatitis world-wide. In a cumulative review of

3836 patients these two conditions were considered to have accounted for 68% of attacks[84], the next most important group being those in whom no definite aetiological factor could be identified, accounting for 22% of attacks[84].

Gallstones

The close correlation between gallstones and acute pancreatitis is evident from post mortem studies such as that of Bell where the incidence of gallstones in 8540 unselected post mortems was 21.9% compared with 56.2% in post mortems of 178 patients with acute pancreatitis[32]. The incidence of acute pancreatitis in unselected post mortems ranged between 0.14 and 1.3%[84], whereas in patients with gallstones the incidence was 4.7%[186].

Gallstone pancreatitis is predominantly a disease of women. In 5 series reviewed the mean female incidence was 72%, ranging from 58% to 83%[4,89,157,172,340]. The incidence of gallstone pancreatitis increases with advancing age as does the incidence of gallstones which, in every age group, were commoner in women than in men[32]. In contrast, Imrie found that this female predominance disappeared in patients over 60 years of age[157].

The description of a gallstone obstructing the ampulla, permitting reflux of bile into the pancreatic duct, was first made in 1901[266]. Recent work has shown a frequent association with gallstone migration through the ampulla of Vater, gallstones having been recovered from the faeces after an attack in between 84%[171] and 94%[2] of patients, compared with 11% and 8% of control cases respectively.

Gallstones in these patients are typically smaller and more numerous than in controls without pancreatitis and their cystic ducts are generally wider[12,225], factors which favour gallstone migration. The observation that 75% of these patients have ampullary obstruction by gallstones if operated on within 2 days of the onset of symptoms, falling to 45% after 2 days and 25% after 4 days provides strong evidence for the theory of transient ampullary obstruction arising secondary to gallstone migration[4].

Alcohol-associated pancreatitis

Acute alcoholic pancreatitis tends to occur in young men, often in their early to mid-thirties, who have been drinking steadily for 8 to 10 years[151]. There is some debate in the literature as to whether alcohol ever causes acute pancreatitis in the absence of pre-existing chronic lesions, many believing that acute pancreatitis in an alcoholic is a manifestation of pre-existing chronic pancreatitis[344]. Commonly these "acute" attacks do recur and after several years it is clear that many of these patients do have chronic pancreatitis[9].

The effect of ethanol on the pancreas has been extensively studied in the past but the mechanisms involved in the pathogenesis of the pancreatitis are still unclear and conflicting. Ethanol has a variable effect on the intact animal depending on species and the route of administration[127]. Intravenous administration of alcohol inhibits basal as well as secretin and cholecystokinin-pancreozymin stimulated pancreatic secretion[353]. Ingestion of alcohol on the other hand stimulates the release of secretin[342], promoting pancreatic

secretion. Alcohol has been shown to increase sphincter of Oddi pressure[239,278], thus the combined effect of alcohol ingestion may be secretory stimulation and outflow obstruction. In more recent studies in humans, however, intragastric alcohol was found to decrease both peak and basal sphincter of Oddi pressure as assessed by endoscopic manometry[367].

Evidence from a 3-dimensional reconstruction of the pancreatic ductal system has implicated the deposition and subsequent calcification of protein plugs, within the smaller pancreatic ducts, as the earliest lesion in chronic pancreatitis[249]. Alcoholic men have a higher protein content in pancreatic juice than normal men[313] with reduced concentrations of bicarbonate and citrate[318]. Increased concentrations of calcium, total protein and trypsin were found in another group following complete recovery from an "acute" attack of alcohol-induced pancreatitis[62]. Therefore, in the earliest stages of chronic pancreatitis the environment exists for the formation of protein plugs. The major protein in the pancreatic precipitates is a degradation product of trypsinogen[104] suggesting premature proteolytic enzyme activation occurs and is an important factor in man.

Pathogenesis

The molecular events leading to the initiation of acute pancreatitis in man have not been fully elucidated. A large and diverse number of conditions have been considered to be aetiological factors but it has not been possible to devise a unifying theory to

account for the initiation of pancreatitis in all. It seems likely that the disease which we recognise clinically, biochemically and histologically as acute pancreatitis may be a common response of the gland to a variety of different insults.

The concept of autodigestion by the digestive enzymes was first proposed in 1896 by Chiari and has dominated our thinking about the causation of pancreatic necrosis ever since[254]. Activation of the pancreatic proteolytic enzymes has been demonstrated in pancreatic tissue[123,200], pancreatic juice[122,361] and most convincingly in pancreatitis exudate[17,261-3] by the presence of protease-inhibitor complexes. Others consider that phospholipase A_2 alone, or with lysolecithin which it metabolises from lecithin in the bile, may be responsible for the necrotizing process and systemic features such as shock and the adult respiratory distress syndrome[72,254,325].

In health several factors act to prevent premature enzyme activation within the pancreas. Firstly, all potentially harmful pancreatic enzymes are synthesised as inactive precursors or zymogens. Secondly these proenzymes are stored in the acinar cell within zymogen granules, isolated from the cytoplasm and other intracellular organelles by a phospholipid membrane. Thirdly, the acinar cells synthesise an inhibitor, pancreatic secretory trypsin inhibitor (PSTI), which prevents premature activation of trypsin within the acinar cells and ductal system. Finally, trypsinogen is first activated when it comes in contact with enterokinase in the duodenum, trypsin subsequently activating all the other zymogens. Breakdown or overwhelming of any one of these lines of defence might

lead to premature pancreatic enzyme activation and autodigestion.

In the initiation of acute pancreatitis it has been assumed that pancreatic enzyme activation occurs outside cells within the ducts or in the interstitial tissues. Theories which have sought to explain this have included bile reflux, pancreatic ductal obstruction/secretion and duodenal reflux, all of which are possible sequelae of gallstone migration with transient ampullary obstruction.

Bile reflux

Reflux of bile into the pancreatic duct was observed to have occurred at the post mortem of a patient with acute haemorrhagic pancreatitis[266]. The patient had a small calculus impacted at the ampulla of Vater occluding the common orifice and forming a continuous closed channel between the common bile duct and pancreatic duct. Opie found evidence of a common channel in 89% of 100 specimens examined[267] but in only 30% did he consider that an impacted gallstone would permit bile reflux[268]. Mann and Giordano examining 200 specimens considered that only 3.5% would permit reflux of bile[232]. Other workers have shown reflux is possible in between 54% and 66% of cases[57,150].

Patients undergoing cholecystectomy for gallstone pancreatitis demonstrate reflux of contrast into the pancreatic duct at cholangiography in 62%[12] and 67% of cases[171], compared to between 15% and 17% of controls, thus these patients may have papillary abnormalities, even in the absence of an impacted gallstone.

Papillary abnormalities have also been demonstrated manometrically, during ceruletide infusion, in patients with gallstone pancreatitis undergoing cholecystectomy[77].

Against this theory is the evidence that pancreatic duct pressures are usually higher than bile duct pressures[73,239,272] and that at physiological pressures bile is not damaging to the pancreas[96,297,380].

Pancreatic ductal obstruction/secretion

Simple ductal ligation produces atrophy of the pancreatic exocrine tissue without pancreatitis[285] but extensive pancreatic oedema results from concurrently stimulating the gland with secretin[281,283,285]. Ductal obstruction alone appears insufficient for the production of haemorrhagic pancreatitis[283] but ischaemia, by temporary occlusion of the main pancreatic artery, was found to transform pancreatic oedema into necrosis[238,282].

Ductal obstruction with stimulation of the gland could conceivably occur due to an obstructing gallstone occluding the pancreatic duct following a meal. Although evidence of such a causal relationship is lacking, there is circumstantial evidence of pancreatitis often coming on within an hour or two of a large meal[92,238,301,330].

Both this and the bile reflux theory fail to explain activation of the pancreatic enzymes in the presence of inhibitors such as PSTI. Rich and Duff proposed that rupture of the duct/acinar system occurred and that enzyme activation occurred in the interstitial

tissues[301] but no plausible theories exist to explain this[137].

Duodenal reflux

Pancreatic enzymes are normally activated by enterokinase in the duodenum and it is possible that reflux of duodenal contents may cause their intraductal activation. Duodenal juice also includes activated pancreatic enzymes, bile acids and lysolecithin which, when infused into the pancreatic duct, are all capable of inducing pancreatitis experimentally. This theory has gained ground, not least because acute pancreatitis can be induced in experimental animals by the creation of a closed duodenal loop. Reflux of duodenal contents into the pancreatic duct results from overdistension of the closed loop and pancreatitis can be prevented by ligation of the pancreatic ducts[56,218,276]. Whether this occurs in man is unknown, the sphincter of Oddi and the pressure gradient between the pancreatic duct and duodenum serving as a defence mechanism, although the increased incidence of pancreatitis following Polya compared with Bilroth I gastrectomy[314,369] has been attributed to this mechanism.

Other theories of enzyme activation

Two recently described experimental models have demonstrated that pancreatic enzyme activation by lysosomal hydrolases may in fact occur inside the acinar cell. Severe haemorrhagic pancreatitis can be induced in young female mice by feeding a choline deficient,

ethionine supplemented diet[215]. Pancreatitis may also be induced in rats by the administration of supramaximal doses of the cholecystokinin-pancreozymin analogue caerulin[192]. In each model large vacuoles, containing both pancreatic digestive and lysosomal enzymes, develop within acinar cells. Thus it may also be possible that the release of activated pancreatic enzymes in acute pancreatitis occurs as a result of pancreatic acinar cell necrosis and is not necessarily the cause. The relevance of these models, however, to human disease is not clear.

Complications

The complications of acute pancreatitis can be divided into local and systemic according to whether they affect the pancreas or more distant organs or systems. Local complications result from the effects of pancreatic necrosis. Systemic effects are thought to be mediated indirectly, by pancreatic enzymes, kinins, peptides and perhaps other toxic products released from the pancreas and peritoneal exudate.

Local complications: necrosis and abscess

Our knowledge of the incidence and natural history of pancreatic necrosis is increasing with the advent of contrast-enhanced CT scanning and this is helping to define more clearly the indications for surgical intervention. Non-enhancement of pancreatic tissue following a rapid intravenous bolus of contrast media appears to indicate non-perfusing tissue, which has or will

become necrotic. These findings can be demonstrated early in the course of an attack of pancreatitis[176].

Infection of the necrotic pancreatic and peripancreatic tissue may supervene early, often within the first week of illness[30]. Patients with infected necrotic tissue have a poorer prognosis than when the necrosis is sterile, with mortalities of 38% and 9% respectively[30]. Infection will, over a period of time, form pus and eventually a pancreatic abscess will result. This has, in the past, been a feared complication of acute pancreatitis with a mortality when unrecognised or untreated approaching 100%[356]. Trapnell found an incidence of pancreatic abscess of 5.3% from a study of 581 patients and others have found a similar incidence ranging between 4.7%[82] and 6%[112]. Pancreatic abscesses typically manifest in the second or third week of the illness but can present much later, 3 of Trapnell's cases presenting 6 months after the initial attack[356]. Pancreatic abscesses typically present with an abdominal mass associated with fever, tachycardia, and a leukocytosis.

Necrotic pancreatic tissue may also give rise to bleeding from the pancreatic bed which can be life-threatening and call for urgent surgical intervention[343]. The presence of necrotic tissue, perhaps by compromising the arterial blood supply to the colon, may also give rise to a number of colonic complications including stricture, fistula formation or frank colonic necrosis[1,188,310].

Pseudocysts

The term pseudocyst is probably best reserved for the mature,

chronic pseudocyst, a localised collection of pancreatic juice usually confined to the retroperitoneal area and characterised by possessing a fibrous wall with no epithelial lining. Many of these cysts communicate with the main pancreatic duct or one of its branches and presumably result from necrosis and breakdown of a small area of the adjacent gland and duct.

Ultrasound was not available to Trapnell who reported pseudocysts developing in 3% of 581 patients with acute pancreatitis[356]. A higher incidence of 10% was reported in two more recent American series[46,112]. The majority of pseudocysts presented in the second, third or fourth week of illness but, as with abscess, could present much later. The majority presented with a mass and 5 (28%) resolved spontaneously without surgery. The earlier the pseudocyst developed the higher the associated mortality[356].

It has been estimated that complications arise in 33% of pseudocysts[316]. Pseudocysts may rupture acutely or chronically, usually into an adjacent viscus or cavity. Rupture has been estimated to occur in 11% of cases and as a consequence the pseudocyst may resolve, produce a cystoenteric fistula or, if it ruptures into the peritoneal or pleural cavities, may give rise to pancreatic ascites or a pleural effusion respectively[316]. Pseudocysts may become secondarily infected[45]. Haemorrhage may occur secondary to rupture of the pseudocyst or by erosion of an adjacent or contained visceral artery and is associated with a mortality of 61%[316]. By increasing in size, it may compress adjacent structures and giving rise to bile duct obstruction[46,118] or intestinal obstruction.

Systemic complications: Shock

Shock has been considered the major cause of early death in acute pancreatitis[329,341,355]. Hypovolaemia, in addition to other cardiovascular factors, is thought to contribute to the shock.

Hypovolaemia

Plasma volume deficits in a canine experimental pancreatitis model, calculated by the change in the haematocrit, have been estimated to peak at 38%[59]. Intravascular volume measured in another study fell 19% after 2 hours and 30% six hours after the induction of pancreatitis[11]. It was calculated that in a 70kg man with comparable pancreatitis, the intravascular volume deficit would be nearly 2 litres during the first 6 hours of the illness[11]. A significant proportion of the plasma volume loss was fluid exuded from the pancreas, accumulating in the peritoneal cavity[60]. Because of the retroperitoneal position of the pancreas in man, much of the fluid would be sequestered in the peripancreatic and retroperitoneal tissues. In addition there may be a more widespread capillary leak with loss of fluid into the extravascular space in tissues remote from the pancreas, including the lungs[345] and subcutaneous tissues. The proposed mechanism for this fluid leak is altered capillary permeability, mediated directly or indirectly by vasoactive agents released from the pancreas or peritoneal exudate[94,346].

Thal and co-workers demonstrated vasoactive substances to be present in the blood of dogs with acute pancreatitis and suggested

that these might be kinins (bradykinin or kallidin) or related peptides[349]. These vasoactive substances were present in increased amounts in patients with acute pancreatitis and appeared to correlate with clinical status[349] and may contribute to the hypotension, pain and increased capillary permeability[280].

Because of the rapid action of kinin degrading enzymes (kininases) in the circulation, kinins are thought to have a predominantly local effect in mediating inflammation. These defences can be overwhelmed by infusing increasing amounts of trypsin to dogs. This results in consumption of $alpha_2$macroglobulin and, following saturation of this inhibitor, decreasing amounts of kininogen were found (indicating bradykinin release) associated with a simultaneous drop in blood pressure[19].

Such a degree of systemic trypsin release is likely to be a rare occurrence in human pancreatitis. Kinin activation has, however, been demonstrated in the peritoneal exudate in human acute pancreatitis[202]. Sustained absorption of kinins from the exudate could deplete or overcome the serum kininases and may contribute to the shock and altered vessel permeability which characterise the early systemic toxicity of pancreatitis.

Myocardial depression

The existence of a myocardial depressant factor, thought to be a peptide with a molecular weight of between 800 and 1000 daltons, has been demonstrated in the plasma of humans and experimental animals in various forms of shock and also in acute

pancreatitis[209,217].

Haemodynamic studies in man have appeared to confirm myocardial depression in patients with severe acute pancreatitis[80,163]. Others were unable to prove this conclusively, considering rather that patients with severe acute pancreatitis have a high output, low resistance picture resembling that seen in sepsis[47]. Cobo and co-workers considered that depressed myocardial function alone did not account for the hypotension in patients with acute pancreatitis, rather that myocardial depression compromised cardiac compensation for the significantly diminished vascular tone[63].

Renal failure

Acute renal failure may complicate acute pancreatitis in up to 6% of patients and is usually a consequence of shock[23]. Acute tubular necrosis appears to be the most common form of renal failure although renal cortical necrosis may occur occasionally[59]. The mortality associated with this complication is typically high ranging between 69% and 100%[23,31,156]. While hypovolaemia and/or hypotension have been almost constant factors in its pathogenesis studies on dogs and in man have also shown increased renal vascular resistance suggesting there may be release of a vasopressor[113,377].

Respiratory complications

Respiratory complications are frequently associated with acute pancreatitis and in one study were thought to be the most significant factor contributing to death[300]. Clinical evidence of respiratory

failure was usually not obvious and only 9 patients (11%) had any radiographic evidence of respiratory complications[291]. The most severe form of lung involvement in acute pancreatitis comprises pulmonary oedema, atelectasis and diffuse pulmonary infiltrates which may give rise to the adult respiratory distress syndrome. In one study diffuse pulmonary involvement occurred in 18% of patients and was associated with a 56% mortality[162]. Possible causes of the lung lesion are physical factors such as aspiration, fluid overload, hypoproteinaemia, shock, diffuse intravascular coagulation and fat embolism[221]. The various pancreatic enzymes have also been implicated, in particular phospholipase A_2, which may have a specific action on pulmonary surfactant. Complement breakdown products and kinins released in acute pancreatitis may produce capillary permeability changes and kinins may, in addition, give rise to pulmonary venous hypertension thus exacerbating the increased vessel permeability[221].

Coagulopathy

Disseminated intravascular coagulation may occur as a rare complication but in several of the reported cases there were factors other than acute pancreatitis which might have been responsible for its occurrence[136,190]. Experimental studies in dogs suggest that a similar coagulopathy may be induced by the infusion of trypsin but not by infusion of lipase or phospholipase[190].

In a sequential study of coagulation parameters in 25 patients with acute pancreatitis mild changes suggesting a hypercoagulable

state were found, only one patient showing features of disseminated intravascular coagulation[247]. Another study found no consistent pattern of change in the various coagulation parameters (prothrombin time, partial thromboplastin time and thrombin time)[293]. Alterations in coagulation were greatest in severe attacks and because early coagulation measurements could be correlated with respiratory, renal and hepatic dysfunction, they suggested that enzyme-related intravascular coagulation might be implicated in their pathogenesis[293].

Deposits of fibrin material, thought to derive from intravascular coagulation, have been demonstrated in the glomerular capillaries from the kidneys of patients with pancreatitis and may be an important factor in the pathogenesis of the renal complications[141].

Unusual Complications

Among other complications attributed to systemic enzyme release are subcutaneous fat necrosis[39] and osteolytic lesions in bones due to intramedullary fat necrosis[169,324]. Psychotic disturbances characterised by disorientation, confusion, delirium, delusions or hallucinations may be seen in acute pancreatitis and while these may simply reflect alcohol withdrawal in alcoholic pancreatitis, there appears to exist a distinct entity of pancreatic encephalopathy associated with increased levels of lipase in the cerebrospinal fluid[98].

The serum antiprotease defences

In several conditions other than acute pancreatitis, including sepsis, trauma and shock, proteolytic enzymes may be released into the circulation. In many cases this serves as a defence mechanism, the proteases activating cascade systems such as the complement, haemostatic and kinin systems mediating bacterial opsonization and killing, coagulation and inflammation. These effects may be harmful to the host and activation of these cascade systems is regulated and the body protected against damage from proteolytic enzymes by a group of proteins in the bloodstream known as the antiproteases. There are 7 major antiproteases in serum (Table 4), 3 of these antithrombin III, alpha$_2$antiplasmin and C1 inhibitor are concerned mainly with the haemostatic, fibrinolytic and complement systems respectively, and will not be dealt with further.

Alpha$_2$macroglobulin

Alpha$_2$macroglobulin is a large glycoprotein molecule consisting of 2 pairs of identical subunits. Because of its large size the molecule tends to be retained within the intravascular compartment. Biologically it is the most polyvalent inhibitor, binding proteases irreversibly, but depending on the protease this can be by a 2 : 1 protease to inhibitor ratio or a 1 : 1 ratio as in the case of plasmin and thrombin[102]. Each of the 2 subunits of alpha$_2$macroglobulin contains a specific amino acid sequence termed the "bait region" which is highly susceptible to proteolytic cleavage. Cleavage of this region leads to a conformational change

TABLE 4.

The major serum antiproteases.

Antiprotease	Mol. wt.	Enzymes inhibited	Site of synthesis
$Alpha_2$macroglobulin	725,000	Endopeptidases	Liver, Macrophages, Fibroblasts, Adherent lung cells
$Alpha_1$antiprotease	55,000	Serine proteases	Liver
Antichymotrypsin	68,000	Chymotrypsin, Cathepsin G	Liver
Inter-alpha-trypsin inhibitor	160,000	Trypsin, Plasmin, Chymotrypsin, Cathepsin G	Liver
C_1 inhibitor	104,000	$C\bar{1}$, $C\bar{1}\bar{s}$, $C\bar{1}\bar{r}$, Factors XIa, XIIa, Kallikrein, Plasmin	Liver
Antithrombin III	65,000	Thrombin, Factors IXa, Xa, XIa, XIIa, Plasmin, Trypsin	Liver
$Alpha_2$antiplasmin	70,000	Plasmin, Trypsin, Kallikrein, Thrombin, Chymotrypsin, Factors Xa, XIa	Liver

within the molecule which results in irreversible binding of the enzyme but without blocking its active site[26,102]. This has been termed the "trap hypothesis"[26]. The protease is trapped by the molecule but its active site remains open, explaining the characteristic persistence of proteolytic activity possessed by the alpha$_2$macroglobulin-protease complex. This phenomenon was first recognised in 1962 and was designated the trypsin-protein esterase[147]. Protein-bound trypsin was found to maintain its proteolytic and esterolytic activity within the complex yet was resistant to inactivation by various trypsin inhibitors[147]. This protein was shown in 1964 to be alpha$_2$macroglobulin[237].

The half life of alpha$_2$macroglobulin in its free form in the bloodstream is around 5.5 days[352]. Following protease binding the conformational change in the molecule leads to its rapid clearance from the circulation with a half life of 8 minutes in dogs[260] and in man of 9 and 12 minutes for complexes with trypsin and elastase respectively[264]. In dogs the complexes are largely cleared by cells of the reticulo-endothelial system in the liver, spleen and bone marrow[260]. Clearance is thought to be accomplished by binding of the complexes to cellular receptors and rapid internalisation by endocytosis[154].

Levels of alpha$_2$macroglobulin are usually very stable although low levels have been reported in septic shock, following major bone surgery and during streptokinase infusion[202]. Despite large scale searches no deficiency states have been reported[207], suggesting that this inhibitor may have an essential physiological role.

$Alpha_1$antiprotease

$Alpha_1$antiprotease is a much smaller, more abundant molecule than $alpha_2$macroglobulin. $Alpha_1$antiprotease is an acute phase reactant, levels rising in response to illness, injury or trauma. Genetic variants of the molecule are recognised, deficiency states being associated with pulmonary emphysema and liver disease.

$Alpha_1$antiprotease was originally named $alpha_1$antitrypsin due to its ability to inhibit trypsin but despite accounting for 90% of the serum antitrypsin activity, it is a relatively poor inhibitor of trypsin. $Alpha_1$antiprotease possesses inhibitory activity against a number of proteases including chymotrypsin, kallikrein, renin, urokinase, plasmin and possibly thrombin but of greatest clinical importance appears to be the inhibition of neutrophil elastase and collagenase[352]. Complex formation with proteases leads to complete inactivation of their proteolytic and esterolytic activities[207]. Proteases are bound with a molar ratio of 1 : 1[352].

Being a relatively small molecule it is able to pass freely from capillaries into tissue fluid, some 60% being located in the extravascular compartment. It is thought to act as a "carrier protein", transporting proteases from the extravascular to the intravascular compartment where the protease may be passed to $alpha_2$macroglobulin prior to its clearance from the circulation[202]. This interchange of proteases has been demonstrated by the infusion of $alpha_1$antiprotease-trypsin complexes, which in the presence of $alpha_2$macroglobulin, dissociate with transfer of the protease to

alpha$_2$macroglobulin[27,264].

Complexes of alpha$_1$antiprotease and trypsin in the circulation have a half life of about 3.5 hours and therefore persist much longer in the circulation than alpha$_2$macroglobulin-trypsin complexes[264]. By this "carrier" mechanism proteases may be transported more rapidly from the tissues and extravascular compartment to the reticulo-endothelial system for degradation[352].

Alpha$_1$antichymotrypsin

Alpha$_1$antichymotrypsin also acts as an acute phase reactant, exhibiting a very rapid increase in response to injury, similar to that seen for C-reactive protein[207]. The affinity of alpha$_1$antichymotrypsin for chymotrypsin is not strong enough to compete favourably in vivo with alpha$_1$antiprotease, which is present in much higher concentrations[207] and it contributes little to the total antiprotease capacity.

Inter-alpha-trypsin inhibitor

The concentration of inter-alpha-trypsin inhibitor in serum is low and it contributes only about 3% of the trypsin inhibitory capacity of serum[207]. Furthermore, the complexes formed with proteases are loose and in vivo it probably does not act as a physiological inhibitor[202].

Pancreatic secretory trypsin inhibitor

PSTI is a trypsin-specific inhibitor produced in the pancreatic

acinar cells and present in the pancreatic exocrine secretion. Small amounts are normally detectable in serum (<10ug/l) although much higher levels have been recorded in acute pancreatitis[88]. Available evidence suggests that PSTI acts purely as a local inhibitor in the pancreatic juice as, in the presence of the serum antiproteases $alpha_2$macroglobulin and $alpha_1$antiprotease, its complex with trypsin is immediately broken[22].

Serum antiproteases in acute pancreatitis

Alterations in the levels of the antiproteases have been demonstrated in the sera of patients with acute pancreatitis. Levels of $alpha_2$macroglobulin fall during the course of an attack, particularly during severe attacks[133,204,228], presumably reflecting its consumption as its synthesis does not increase in response to illness. In one study the lowest levels in severe attacks occurred 5 days after admission[228] but could remain low for 14 days[204]. Levels of inter-alpha-trypsin inhibitor were usually low early in the attack returning towards normal in severe attacks after 4 days[204]. $Alpha_1$antiprotease and $alpha_1$antichymotrypsin levels rose in acute pancreatitis and were considered to provide useful discrimination between mild and severe attacks[133,204,228].

Immunoassays provide no information on the amount of free, uncomplexed antiprotease available for binding. A functional assay can be performed to investigate the overall capacity of serum to bind a protease, usually trypsin. The trypsin binding assay was first studied in patients in 1972 and while levels of $alpha_2$macroglobulin

were slightly lower in acute pancreatitis, there was a marked reduction in the ability of their sera to bind trypsin compared to controls[6]. This was attributed to the presence of an enzyme bound to alpha$_2$macrogloblin but both trypsin and chymotrypsin were ruled out[6].

More recently the serum trypsin binding capacity has been shown to increase during the illness, more than doubling within 2 days of admission[133]. Alpha$_1$antiprotease accounts for around 90% of the serum trypsin inhibitory capacity and as its levels rise, due to the acute phase response, this appears a more plausible explanation. Others have shown that, in addition to the fall in the alpha$_2$macroglobulin levels in acute pancreatitis, the remaining alpha$_2$macroglobulin has a reduced trypsin binding capacity indicating the presence of alpha$_2$macroglobulin-protease complexes[204]. Thus the net effect is a fall in the available alpha$_2$macroglobulin and an increase in alpha$_1$antiprotease available for protease binding.

Free proteolytic activity has never been demonstrated in the serum of patients with acute pancreatitis but amidase and esterase activity has been found, presumably due to alpha$_2$macroglobulin bound protease[251,365,383]. The esterase in Worthington and Cuschieri's study was not identified definitively but kallikreins and trypsin were considered likely[383]. Active trypsin has been demonstrated complexed to alpha$_2$macroglobulin[49,365] and immunoreactive trypsin in complex with alpha$_1$antiprotease[42,43,49].

The residual proteolytic activity attributed to circulating alpha$_2$macroglobulin-protease complexes may by degrading peptide hormones produce hypocalcaemia[50,148] or shock[100], may activate other

zymogens or the coagulation cascade[302] and may have an immunosuppressive effect[83], although their rapid clearance from the circulation[260,264] would tend to exclude their playing a major role.

Toxicity of the peritoneal fluid

Experimental pancreatitis and severe acute pancreatitis in man are both associated with early formation of a peritoneal exudate. This comprises an enzyme and protein-rich fluid, much of which is presumably exuded from the pancreas. The fluid is typically "haemorrhagic" or dark coloured due to the presence of the pigment methaemalbumin[126]. The exudate has been shown to possess toxic properties, at least in experimental pancreatitis, which are summarised in Table 5.

The balance between the release of activated proteolytic enzymes from the pancreas and the adequacy of the peritoneal antiprotease defences has been considered a central factor in this toxicity, saturation of the peritoneal antiproteases being closely associated with the onset of shock in canine experimental pancreatitis. Following the induction of pancreatitis in dogs using the closed duodenal loop model, large amounts of trypsinogen were demonstrated first in the exudate and later in lymph and plasma. Alpha$_2$macroglobulin complexes with trypsin-like activity appeared early in the exudate and later trypsin-alpha$_1$antiprotease complexes[261,262]. The proteases were preferentially bound by the

TABLE 5.

Toxicity of peritoneal fluid: experimental evidence.

Effect	Route of administration	Suspected toxic factor	Reference
Hypotension	IV to dogs (0.03ml)	Histamine releasing (large molecule)	Amundsen et al. 1968
	IV to dogs (50ml)	Kinin forming ? kallikrein	Satake et al. 1973
	IP to rats	Histamine releasing	Lankisch et al. 1979
	IV to pigs	Histamine releasing	Traverso et al. 1983
Increased vascular permeability	Intradermally to puppies	Pancreatic enzymes	Takada et al. 1976
Haemo-concentration	IP to rats	?	Ellison et al. 1981
	IP to rats	Small molecule <1000 daltons	Cooperman 1982
Reduced cell respiration	Liver cell culture	?	Pappas et al. 1982
Toxicity/death	IP to mice	?	Frey et al. 1982
	IP to mice	?	Satake et al. 1985
	IP to mice	PGE_1	Farias et al. 1985

alpha$_2$macroglobulin. During the course of the pancreatitis the binding capacity of the alpha$_2$macroglobulin was steadily reduced and when exhausted all the trypsinogen in the exudate was rapidly activated and complexed by the remaining alpha$_1$antiprotease. On saturation of alpha$_2$macroglobulin the animals developed irreversible shock from which they ultimately died[261,262].

Overwhelming of the antiprotease defences occurred relatively late in this slowly evolving model of pancreatitis, whereas, in the pancreatitis induced by retrograde injection of bile there was a rapid release of active pancreatic enzymes (trypsin, chymotrypsin and elastase) and early saturation of both alpha$_2$macroglobulin and alpha$_1$antiprotease with the appearance of free proteolytic activity in the peritoneal exudate[263]. This was associated with an early, dramatic fall in the blood pressure which recovered again to normal levels, before falling once more, just prior to death[263]. The protease-antiprotease complexes appeared in the exudate and in lymph but could not be detected in venous blood indicating a short half-life in the circulation. Local release of vasoactive peptides such as bradykinin and histamine in the peritoneal cavity was considered a possible explanation for the hypotension.

High degrees of alpha$_2$macroglobulin saturation (averaging 65%) have also been demonstrated in the peritoneal exudate from patients with severe acute pancreatitis suggesting that this mechanism might also be important in man[17]. Complete saturation of peritoneal alpha$_2$macroglobulin has been found although the alpha$_1$antiprotease saturation did not exceed 25%[20]. Details, however, of the patients'

clinical state and, in particular, whether they were shocked was not provided[20].

Possible mechanisms of toxicity

Proteases complexed to alpha$_2$macroglobulin retain esterolytic activity and also proteolytic activity against low molecular weight substrates, although the importance of this to the toxicity of the peritoneal exudate is not clear. Alternatively, as saturation of alpha$_2$macroglobulin and the other antiproteases has a close temporal relationship with the onset of shock, it may be the occurrence of uncontrolled proteolysis within the peritoneal cavity which is important.

Overwhelming of the antiprotease defences both in vitro and in pancreatitis exudate has been shown to be associated with complement breakdown and kinin release[19,20,202], the products or breakdown products of which may be absorbed across the peritoneum into the circulation contributing to the systemic toxicity. Kinins such as bradykinin cause vasodilatation, increased capillary permeability, invasion of leukocytes and pain. C3a and C5a are biologically active fragments which may result from cleavage of complement factors by trypsin. They are leukotactic, can cause histamine release from mast cells, contract smooth muscle and increase capillary permeability. Alternatively, both phospholipase A_2 and chymotrypsin, activated by trypsin or possibly by trypsin-alpha$_2$macroglobulin complexes[302], could cause histamine release from mast cells and thus explain the histamine releasing factor in the pancreatitis exudate[10,198].

Mayer and co-workers have demonstrated in dogs that although the bulk of the enzymes released in pancreatitis pool within the peritoneal cavity, absorption from the exudate does not appear to contribute to the serum enzyme levels[236]. If peritoneal exudate does contribute to the systemic toxicity, it would need to be by small molecular weight intermediates such as kinins, complement breakdown products, histamine or other vasoactive peptides.

Others believe transperitoneal absorption to be important[374]. Proteases absorbed into the circulation would be quickly complexed and cleared by the antiproteases but phospholipase A_2, for which there are no natural inhibitors, might mediate toxicity directly.

Several workers have commented that the exudate is more toxic the earlier it is sampled[10,198,346]. Proteases within the exudate undoubtably derive from the pancreas itself but the source of the antiprotease molecules is unknown. They may, in part, derive from the pancreas but may also pass across the peritoneum in response to the peritonitis induced by the irritant pancreatic exudate. It is possible, therefore, that early in the attack there is a relative excess of proteases over antiproteases which may explain why the "early" exudate is more toxic.

Therefore, there does appear to be evidence of toxicity associated with pancreatic exudate, at least in experimental pancreatitis, which may contribute to the early "shock-like" state. The exact mechanism of the toxicity is not clear, nor is the nature of the toxic substance(s) which may be of high molecular weight[104,259] or low molecular weight[68], and which may be heat

resistant[68] or heat labile[259]. Nevertheless this has fuelled interest in peritoneal lavage as a therapy for acute pancreatitis, both in experimental pancreatitis and in man, and has provided the rationale for this thesis.

Prediction of severity

The pancreas is an inaccessible organ and diseases of the pancreas have, in the past, frequently been difficult to diagnose and stage the severity or extent of. Newer imaging techniques such as contrast-enhanced computed tomography have overcome some of these problems, providing both visualisation of the gland's morphology and an assessment of the viability of the pancreatic parenchyma. Such investigations are expensive and often reserved for those patients who are objectively identified as having a severe attack of pancreatitis or who are slow to settle.

Clinical factors

The most basic method of judging severity is clinical assessment by an experienced clinician. On admission clinical assessment, while having a 100% accuracy in predicting mild attacks, correctly predicted only 39% of severe attacks[226]. After 24 hours the accuracy had improved, 73% of the severe attacks being correctly predicted and rising to 83% after 48 hours[226]. Therefore, clinical assessment alone early in the course of the attack often fails to identify those patients who will develop a severe, complicated illness and although the accuracy improves the longer the patient

remains in hospital, it remains a subjective assessment.

Other clinical factors which have been suggested as useful indicators of severity include the presence of shock[108,165,322], pyrexia[165,322], increased respiratory rate[165,306], dyspnoea[24], respiratory distress[322], tetany[279], abdominal mass[165,306,355], ascites[24,306], ileus[355] and body wall staining[81,165].

Laboratory factors

Acute pancreatitis is a uniquely biochemical disease and since the first report of an association between hypocalcaemia and pancreatic necrosis[90] there has been much interest in the use of laboratory parameters as predictors of outcome.

Amylase

The magnitude of the amylase elevation has been found to bear no relationship to the subsequent clinical course[91,279,290,355] whereas others have found it to be inversely related to the severity of the attack[5,322]. Persistent elevation of the serum amylase may be an indication of pseudocyst formation[364].

Calcium

The prognostic significance of low serum calcium levels in acute pancreatitis have been confirmed by several groups[165,214,279,290,322,329,355]. The mechanism of the hypocalcaemia has been attributed to the binding of calcium in areas of fat necrosis[91,279]. Others have suggested the hypocalcaemia may be due to

increased serum glucagon levels in acute pancreatitis[270], parathyroid insufficiency[66,224,303] or to a failure of bone to respond to parathyroid hormone, perhaps because of shock and reduced tissue perfusion[146]. Parathyroid hormone degradation leading to loss of biological activity may occur in acute pancreatitis due to circulating $alpha_2$macroglobulin-protease complexes, proteolysis of parathyroid hormone by sera from patients with acute pancreatitis having been demonstrated in vitro[50,148].

Much of the apparent hypocalcaemia associated with acute pancreatitis is due to hypoalbuminaemia which reduces the measured calcium by reducing the protein bound fraction. When the serum values are corrected for this only about 11% of all the results will show "true" hypocalcaemia of varying degree[158].

Other factors

Other factors which have been suggested as indicators of a severe attack include haemoconcentration[134], a low or falling haematocrit[257,305,355], leukocytosis[348,355], hyperglycaemia[279], methaemalbumin[91,125,195,256,382], fibrinogen[355], complement factors[52,110,199], serum antiproteases[52,228], C-reactive protein[52,233], deoxyribonuclease 1[185], ribonuclease[372,373], cyclic AMP[328] and the absolute lymphocyte count[61].

Multiple factor prediction of severity

In 1974 Ranson and co-workers reported a study of 43 objective clinical, biochemical and haematological criteria in 100 attacks of

pancreatitis[290]. Thirteen factors were shown to be of significant prognostic value from which 11 were finally selected[290]. For patients with fewer than 3 signs positive the risk of death or major complication was small at 2.5% and 11% respectively[290]. By contrast if 3 or more signs were positive, as they were in 21 patients, 62% died and an additional 33% were seriously ill[290]. These findings have been confirmed in subsequent prospective analyses[289,292]. Various modifications of the Ranson multiple factor scoring system have been described, one being that described by Imrie and co-workers[159].

Modifications have subsequently been made to improve prognostic accuracy in patients with gallstone-associated pancreatitis[269,286] but their usefulness may be limited by the fact that the aetiology of the attack is not always known early in the course of the illness. Multivariate linear discriminant and multiple regression analyses may also be used to increase precision and accuracy in the prognostic assessment[288] but the complexity of such methods limits their use in daily clinical practice[287].

Diagnostic peritoneal lavage

The presence of haemorrhagic peritoneal fluid in patients with severe acute pancreatitis has been recognised for many years and diagnostic paracentesis with examination of the fluid for colour and amylase activity was first suggested in 1950[170]. Prediction of the outcome of an attack of acute pancreatitis by paracentesis and peritoneal lavage has been described more recently from Leeds, the presence of more than 10mls of free fluid or a dark haemorrhagic

fluid indicating a severe attack[226,277]. Following the aspiration of any free fluid one litre of warm saline was instilled into the peritoneal cavity and, after turning the patient on each side to circulate the fluid, a dark straw coloured or brown return fluid was also found to indicate a severe attack[277]. Biochemical analysis of the return fluid for total protein, albumin and aspartate aminotransferase also provided good discrimination between mild and severe attacks[227,277]. Diagnostic peritoneal lavage has the advantage of speed but is invasive and associated with a small morbidity, due most often to inadvertent visceral puncture. In a recent study which reported on 253 catheter placements there were 2 episodes of visceral puncture, an incidence of 0.8%[70].

Diagnostic imaging

Both ultrasound and computed tomography are invaluable in the diagnosis of established pancreatic pseudocysts, abscess and necrosis. Both modalities have been studied early in the course of an attack to determine their value in identifying cases likely to have a complicated clinical course.

Ultrasound

McKay and co-workers found that the appearance of the pancreas on early ultrasound examination did not correlate significantly with objective assessment of severity by multiple factor scoring although patients subsequently developing pseudocysts all had early localised pancreatic swelling[220]. Generalised swelling of the pancreas was

frequently seen but appeared to be of no major clinical importance[220]. Furthermore in 22 patients (26%) the pancreas could not be visualised reducing the value of this technique, which is itself very observer dependent.

Computed tomography

Computed tomography appears to be a valuable tool for predicting outcome in acute pancreatitis. Ranson and co-workers have graded the appearances of the pancreas on computed tomography by analysing the extent of pancreatic and peripancreatic inflammation and also on the presence and number of peripancreatic fluid collections[294]. The higher the grade the greater the risk of pancreatic abscess ensuing. Similar results were obtained in another study of 51 patients in which computed tomography was found to be superior to ultrasound where, because of bowel gas or obesity, 55% of examinations were inadequate[255].

Perhaps of more practical importance is the information obtained at contrast-enhanced computed tomography. In this technique after an initial scan a bolus of contrast material was injected intravenously and the scan repeated again. When compared with the initial scan the pancreas normally shows enhancement and this can be measured, decreased or absent enhancement suggesting pancreatic necrosis. This was recorded in 8 of 28 consecutive patients in one study, all 8 patients having necrosis confirmed at subsequent laparotomy[176].

Other illness scoring systems

There has been increasing interest in defining and applying scoring systems to various disease states, most notably to trauma victims[16] and to septic surgical patients[93,338]. An alternative approach has been described by Cullen and co-workers whereby the severity of an illness is assessed and monitored by the frequency with which certain treatments and/or procedures are performed for each patient - the therapeutic intervention scoring system[74]. Its value may be diminished by differences in nursing or medical practice between different centres and even within one centre at different times[243].

Knaus and co-workers in 1981 described a scoring system based on measuring the degree of abnormality of numerous physiological variables (acute physiology score) and coupled this with a grading reflecting the patient's previous health[178]. This was known as the acute physiology and chronic health evaluation (APACHE) classification system. The initial description was based on 34 physiological variables, each having a score attributed to it, depending on the degree of abnormality from a defined normal range. Higher APACHE scores were shown to be associated with an increased mortality[178]. Calculation of the APACHE score was, however, complex and prompted simplifications by other groups[210]. Knaus and colleagues subsequently modified the APACHE scoring system using the 12 most important and commonly recorded laboratory and physiological variables in addition to the age weighting and a shortened chronic health evaluation - the APACHE II scoring system[179]. An increasing

score was found to correlate closely with the risk of subsequent death[180]. They considered that the physiological data when studied over a period of time would distinguish patients who were responding to therapy from those who were not[179]. If applicable to patients with acute pancreatitis, it may permit stratification of risk early in their illness, sequential monitoring during the course of their illness and an objective assessment of any response to therapy.

Treatment: general and specific therapies

The therapy of acute pancreatitis is largely empirical since the effectiveness of many aspects of its treatment has not been demonstrated by controlled clinical trial[193]. The basic principles of therapy consist of relief of pain by analgesics and "putting the pancreas at rest" by allowing no oral intake. This may include nasogastric suction routinely or, more commonly, for persistent vomiting, ileus or gastric distension. Fluids are provided intravenously and the patient monitored for hypoxia and adequacy of the urine output. These principles have evolved over the past 30 years and there is some evidence that they may have contributed to a reduction in deaths from acute renal failure and respiratory insufficiency, particularly amongst elderly patients[156].

Inhibition of exocrine secretion

Attempts to influence the outcome of pancreatitis by inhibiting pancreatic secretion are summarised in Table 6.

Preservation of the microcirculation

Vascular factors appear to influence progression from oedematous to haemorrhagic pancreatitis[131,132]. Treatment with heparin, fibrinolysin - a streptokinase preparation, sympathectomy or dextran 40 all prevented development of haemorrhagic pancreatitis in dogs and reduced mortality[132,384]. In a small double-blind study in man, treatment with dextran 40 was associated with a trend towards improvement, but no significant differences with respect to mortality or major complication rate[28].

Prostaglandins and anti-inflammatory agents

The steroid hormone cortisone has been investigated in experimental pancreatitis, no benefit being found[33]. Steroids have been implicated as a possible aetiological agent in acute pancreatitis[229] and have no present role.

Increased levels of prostaglandins have been demonstrated in the exudate and in the pancreatic venous blood of dogs with experimental acute pancreatitis[129]. Indomethacin blocks prostaglandin production in vivo and prolongs mean survival in rats with experimental pancreatitis[196]. In man indomethacin significantly reduced pain and the number of opiate injections required but too few patients were studied to compare the outcome[87].

Others have shown that prostaglandin PGE_2 may be protective in diet-induced acute pancreatitis in mice, perhaps by a membrane stabilising effect[64,231,336]. In a preliminary study in man, the

treated group demonstrated less elevation of several cell membrane marker enzymes suggesting an effect on membrane stability[336]. An assessment of this therapy on mortality and other clinically relevant endpoints is awaited.

Antibiotics

Ampicillin has been studied in controlled clinical trials in patients with mild and moderate pancreatitis, no improvement in outcome having been demonstrated[71,105,152].

Peritoneal lavage

Peritoneal lavage improves survival in dogs with experimental pancreatitis[295,304,307]. Peritoneal lavage was also found to prolong survival and reduce lethality in rats with experimental pancreatitis[197]. Lavage for 24 hours was less effective than lavage continued for 48 hours but hypothermic lavage was no more effective than normothermic lavage. Lavage commenced early, within 30 minutes of the induction of pancreatitis, was more effective than treatment starting 6 hours later[197].

Peritoneal lavage in man

Lavage of the peritoneal cavity in acute pancreatitis was first reported by Wall in 1965[368]. Lavage was associated with clinical improvement in all 3 patients, 2 of whom eventually recovered[368]. Six further patients were reported the following year, the author considering that the mortality had been improved by therapy[128]. Five

further patients were reported the following year, most showing a dramatic improvement in their clinical condition following lavage[41]. Four further patients were reported in 1970 with only one death, peritoneal lavage in these patients being associated with gradual improvement in their clinical condition, correction of their fluid and electrolyte deficits especially of hypocalcaemia, and a fall in their serum methaemalbumin[124].

Following these anecdotal reports of successful treatment by peritoneal lavage and the evidence of clear benefit in experimental pancreatitis, two studies put the view more firmly that this was indeed an important advance. In the first study 24 patients with severe pancreatitis (6 having laparotomy) underwent peritoneal lavage and were compared with 79 control patients, 18 of whom had laparotomy[289]. Lavage resulted in "immediate clinical improvement" and no lavaged patients died within the first 10 days of their illness. Of the non-lavaged patients 45% of the deaths occurred within the first 10 days, usually from cardiovascular or respiratory failure[289]. Closer examination of the data reveals little difference in the mortality rates amongst those not having surgery, but peritoneal lavage appeared to eliminate early mortality amongst those having surgery. However, the two groups undergoing surgery were not comparable in that those patients subsequently undergoing peritoneal lavage only had an early diagnostic laparotomy with placement of dialysis catheters whereas the group who were not lavaged usually had more extensive surgery. Thus the early improvement in the mortality rate amongst the lavaged patients is perhaps not unexpected as the

two groups were clearly not equivalent. Furthermore, despite the early improvement reported the overall mortality in the two groups of patients was identical, explained by the larger number of late peripancreatic abscesses amongst the lavaged group[289]. Despite the apparent weakness of their data the authors stated that "peritoneal lavage is a highly effective adjunct to the treatment for early complications of severe acute pancreatitis and dramatically reduces early mortality"[289].

The first attempt at a prospective, randomised, controlled trial of peritoneal lavage was reported in 1980[339]. Of 70 patients with alcoholic pancreatitis judged to be severe on a simple scoring system, 36 were randomised to receive standard therapy and 34 to have, in addition, peritoneal lavage for 24 hours. Of the lavaged group 29 patients (85%) had improved within 24 hours whereas of the control group only 13 patients (36%) had improved. Seventeen of the control group were then crossed over to receive treatment by peritoneal lavage, 14 of them showing subsequent improvement[339]. A total of 14 patients died; of the 5 dying in the lavage group only one died of respiratory insufficiency compared with 6 of the 9 dying in the control group. Overall, of 51 patients treated by peritoneal lavage, either initially or secondarily, having failed on standard therapy, only 8 deaths occurred compared to 6 deaths amongst the remaining 19 patients treated by all other methods. The authors made a strong plea in support of peritoneal lavage stating that "the results appear to offer vigorous support for the use of peritoneal dialysis in the management of severe, life threatening, acute

alcoholic pancreatitis"[339]. Because so many patients were removed from the control group at 24 hours the results are difficult to interpret and firm conclusions regarding the efficacy of peritoneal lavage cannot be drawn from this data.

The first properly controlled, randomised study of peritoneal lavage was reported from the UK in 1982; 23 patients were randomised to receive either peritoneal lavage for 72 hours (9) or no lavage (14)[67]. Three deaths and 3 major complications occurred in the 9 patients receiving lavage compared to 7 deaths and 3 major complications amongst the control patients, no statistically significant difference being demonstrated[67].

A large multicentre study was subsequently undertaken in the UK based at Leeds, Bristol and Glasgow, the results of which were reported in 1985[235]. Ninety-one patients with severe acute pancreatitis were selected from 428 attacks studied, 45 being randomised to receive peritoneal lavage and 46 standard therapy. The death rate in the lavaged patients was 27% compared with 28% for the control patients and the incidence of major complication was 38% and 35% respectively. No difference was found in the timing of death, 6 of the lavaged patients and 8 of the control patients dying early (within 7 days)[235].

A Swedish group studying a smaller number of patients in a randomised, controlled fashion reached similar conclusions, there being slightly more deaths (4) in the 19 lavaged patients compared to the controls[153].

Alcohol was the predominant aetiological factor found in the

trials supporting lavage, accounting for 53%[289] and 100%[339] of cases compared with 26% in the recent British trial[235]. It is possible that such patients are more likely to respond to lavage but this is not supported by the Swedish study where 56% were alcohol abusers[153].

It has also been claimed that lavage may reduce abdominal pain and improve the patient's clinical condition, indeed this was the major claim made in the early anecdotal reports of success with this technique. The only group to attempt to evaluate this found no difference whether the patient was lavaged or not[153].

Criticisms have been made of the UK multicentre study of peritoneal lavage, particularly with respect to the delayed institution of therapy and the failure to separate the different aetiological sub-groups[273]. Furthermore, 34 of the 46 patients randomised to standard therapy had a diagnostic peritoneal lavage prior to randomisation, to assess the severity of the attack[226]. Removing the peritoneal exudate, which in experimental studies is most toxic early in the attack, may alone have been therapeutic and have obscured any differences between the groups. The same criticisms apply to the recent Swedish study where both groups of patients had their exudate drained and a lavage with 500mls of saline[153].

Although Stone and Fabian's study may be criticised because of the cross-over of control patients to the lavage group[339], the early response of the lavage group to therapy was striking and suggests that in alcohol-associated pancreatitis, peritoneal lavage may be beneficial, at least in the short-term. Further studies in patients with severe acute pancreatitis comparing peritoneal lavage against a

control group undergoing no peritoneal intervention appear to be justified and strenuous attempts should be made to ensure early randomisation and instigation of therapy.

Antiprotease therapy

Activation of the proteolytic enzymes, particularly trypsin, has been considered for many years to be central to the initiation of pancreatitis, autodigestion and the systemic manifestations of the disease. This concept of pancreatitis as a "enzymatic disease" has lead to the study of various anti-enzyme preparations directed particularly against trypsin.

Soybean trypsin inhibitor

Soybean trypsin inhibitor given to dogs with bile-induced pancreatitis appeared to counteract the shock and elevation in proteolytic activity observed in the controls[309] although a larger study found no benefit[149].

Antifibrinolytic agents

Antifibrinolytic agents inhibit the action of plasmin and, in high concentration, also the action of trypsin. Neither epsilon-aminocaproic acid nor p-aminomethylbenzoic acid (PAMBA) reduced the mortality of experimental pancreatitis in rats[193]. No beneficial effect was found when epsilon-aminocaproic acid was studied in man[183].

Aprotinin

A polypeptide inhibiting trypsin and kallikrein was discovered by Kraut and co-workers in bovine lymph nodes[117] and independently by Kunitz and Northrop in bovine pancreas[189]. This inhibitor, now known as aprotinin, was subsequently found in other bovine organs including lung and parotid gland and is prepared commercially from these sources by Bayer (Leverkusen, West Germany) and marketed as Trasylol. Aprotinin was introduced as a therapy for acute pancreatitis in the 1950's[143] and has been extensively studied since then.

One of the first reports of successful therapy with intravenous aprotinin reduced the mortality of pancreatitis in rats from 37% in controls, to 10%[107]. Subsequent studies were conducted mainly in dogs. Aprotinin was found to be effective in reducing mortality in pancreatitis induced by the retrograde injection of bile/trypsin mixtures[138,219,335], incubated blood and activated pancreatic juice[252], and by bile alone[173]. Larger doses were found to be more effective as was commencement of therapy immediately following induction of pancreatitis, rather than delaying for 4 hours[173]. No mortality was observed in another study when therapy was commenced within 6 hours of the induction of pancreatitis whereas delays of 9 and 12 hours were associated with mortality rates of 25% and 75% respectively[161].

In contrast to the studies of aprotinin therapy in experimental animals, where in most cases an appropriate control group had been studied and appropriate end-points such as mortality or survival time had been reported, in many of the early studies in man the reports

had been anecdotal, based on subjective assessments and/or the measurement of irrelevant parameters[14,145,219,250].

The earliest controlled trials found no benefit from therapy but had studied insufficient numbers (particularly of ill patients) to assess clinically important end-points such as mortality or major morbidity. Furthermore, many of these early trials utilised doses of aprotinin which were later considered inappropriately low[15,334,358].

Interest in aprotinin was rekindled with the publication in 1974 of a controlled study in which aprotinin therapy was found to have reduced the mortality rate from 25% in the placebo treated group to 7.5% in the treated group[359]. The initial dose of aprotinin used in this study was 200,000KIU on diagnosis followed by 200,000KIU 6 hourly for 5 days. Particular value was demonstrated for aprotinin treatment in the elderly in whom therapy abolished the usual trend for mortality to rise with increasing age[359]. Criticisms have been raised, particularly with respect to the reported mortality of 25% in the control group, this being much higher than the mortality rate of around 10% prevalent in other UK centres at that time[156]. Two further large scale controlled studies were initiated using a similar dosage regime, each finding no benefit in terms of mortality[159,245], or in the rate of recovery or incidence of complications[246]. This regime also failed to prevent activation of the kallikrein-kinin system casting doubt on its efficacy in vivo[332]. Even very large doses of aprotinin (4,000,000KIU over 4 hours immediately after diagnosis) failed to show any beneficial effect (Imrie CW, personal communication).

Intravenous antiprotease therapy with aprotinin has no clinically useful effect in man, although apparently efficacious in canine pancreatitis. The possible reasons for this have become clearer and may include the timing of therapy in relation to the onset of the pancreatitis and that pancreatitis induced experimentally in animals is basically different from the human disease and in some way more amenable to antiprotease therapy. More recent evidence points to there also being important interspecies variation with respect to the inhibitory effect of aprotinin. In vitro work has shown that while aprotinin at a concentration of 3μmol/l is protective against trypsin-induced kininogen consumption and complement breakdown in dogs, in humans a 20-fold higher concentration was needed to achieve comparable results in serum and about 5-fold in plasma (the difference between serum and plasma was considered to be due to a potentiating effect of the heparin in plasma)[18,203,205]. Therefore, pronounced species differences have been confirmed and as the doses of aprotinin recommended in humans had been calculated from those successful in canine pancreatitis, the ineffectiveness of aprotinin in human pancreatitis was perhaps not surprising[203].

Intraperitoneal aprotinin therapy

Intraperitoneal administration has been studied in rats with taurocholate-induced pancreatitis. No difference was found in the mortality rate, nor in gross or microscopic pancreatic damage, despite increasing amounts of intraperitoneal aprotinin given in 3

divided doses to a maximum of 1500KIU/100g body weight per day[34]. The addition of aprotinin to peritoneal lavage fluid in another series of experiments in rats conferred no additional benefit when compared to lavage with a standard dialysate[197]. Intraperitoneal administration of aprotinin to mice with diet-induced pancreatitis, at a dose of 10,000KIU or 100,000KIU/kg, 8 hourly for 48 hours influenced neither the mortality nor the biochemical or morphological parameters studied[230].

This therapeutic approach, at first glance, does not appear particularly promising. Only one study in man has addressed this question comparing 2 hourly cycles of peritoneal lavage with 2 litres of a standard dialysate to a dialysate containing aprotinin 500,000KIU. No significant differences emerged with respect to mortality or clinical outcome although the mortality and number of complications were less in the aprotinin treated group, suggesting a trend in favour of this approach[21].

Other antiproteases

Bacterial protease inhibitors including leupeptin, pepstatin, chymostatin and antipain are known to have a broad spectrum of activity and in mice with diet-induced pancreatitis produced a distinct attenuation of the severity of the pancreatitis[216]. A prolongation of survival was also shown in rats when treated with intravenous leupeptin, but not with aprotinin[168]. These molecules, being small oligopeptides, are able to enter and complete the inhibition of the protease-alpha$_2$macroglobulin complex[148], but they

are toxic and their efficacy has not been studied in man.

A large molecular weight trypsin inhibitor from human urine has also been isolated and has improved survival in dogs and rats with pancreatitis[265].

Administration of natural serum antiproteases in the form of fresh frozen plasma given intravenously, has been investigated in an uncontrolled study in man. A low mortality rate of 3.7% was reported but the lack of a control group and the classification of their patients by a previously unreported severity scoring system makes their results difficult to interpret[76].

Synthetic antiproteases

Gabexate mesilate (Foy) was the first to be studied in detail and the results have been reported mainly in the Japanese literature. A comparative trial of Foy and aprotinin in a large multicentre study found that an excellent or good clinical result occurred in 71% of the Foy treated group and in 44% of the aprotinin treated group[347]. This trial, however, was not double blinded nor was there any standardisation of the dose of aprotinin given. Furthermore the assessment of efficacy appeared highly subjective and too few patients with severe disease had been studied, the mortality amongst the entire group of 150 patients being only 4 (2.6%).

In vitro studies of the effectiveness of both Foy and aprotinin in preventing trypsin-induced kinin and complement activation in human and canine sera found that aprotinin but not Foy conferred protection[206]. In dogs infused with trypsin intravenously, aprotinin

but not Foy was effective in preventing the development of shock[206].

A newer analogue of Foy, nafamostat mesilate was introduced in 1977. It appears to be a much more potent inhibitor of pancreatic proteases than Foy and reduced the mortality of rat pancreatitis in a dose dependent manner[164]. The results of a full scale study in man are awaited.

Inhibition of other pancreatic enzymes

The phospholipase A_2 inhibitor procaine hydrochloride was found to reduce the mortality in rats with experimental pancreatitis[7]. Another similar inhibitor xylocaine was found to improve the mortality in piglets when infused at the induction of pancreatitis but did not influence the degree of pancreatic damage[326]. Uncontrolled studies have suggested that another phospholipase A_2 inhibitor calcium disodium edetate may have a beneficial effect in human acute pancreatitis[362] but reports of controlled clinical studies are awaited.

Aims of the current study

This thesis addresses 3 main issues. Firstly, the size of the problem presented by acute pancreatitis and the trends in incidence and mortality are examined, both nationally in Scotland, and within a single centre.

Secondly, means for more rapid and accurate prediction of outcome from an attack and for objectively monitoring the course of

the attack are examined by sequentially measuring single laboratory factors including complement factors, antiproteases and C-reactive protein and by comparing clinical assessment, the multiple factor scoring systems and the APACHE II system.

The major portion of this thesis examines aspects of the protease-antiprotease balance in peritoneal exudate, both in experimental pancreatitis in the rat and in man. Peritoneal exudate appears to be toxic in experimental pancreatitis, possibly as a result of overwhelming of the antiprotease defences, and its removal by peritoneal lavage has been uniformly successful. In contrast, the role of the protease-antiprotease balance in peritoneal exudate in man, and its relationship with the early "shock-like" illness which may complicate severe acute pancreatitis, has not been clearly defined. Although there are reports of successful peritoneal lavage in man, properly controlled trials have shown no clear benefit in terms of survival. Both recent studies suffered from flaws in methodology which may explain the failure to demonstrate any difference between the groups[153,235].

The adequacy of the peritoneal antiprotease defences are examined in an experimental pancreatitis model in the rat and also in human acute pancreatitis. A novel therapeutic approach is also described comprising removal of the exudate by aspiration and a single peritoneal lavage, followed by instillation of an antiprotease solution to the peritoneal cavity. The efficacy of this simple treatment is examined both in the rat model and in a controlled, clinical study in man using the antiprotease aprotinin.

PART 2.

INCIDENCE AND MORTALITY

CHAPTER 2. CHANGING PATTERNS OF INCIDENCE AND MORTALITY FROM ACUTE PANCREATITIS IN SCOTLAND, 1961-1985.

Introduction

Acute pancreatitis appears to be becoming increasingly common but few studies have examined the changing incidence and mortality trends. Studies from Bristol are the most comprehensive and over the 18 year period 1950-1967 demonstrated an increasing incidence over the first 6 years of the study and a second peak of incidence between 1961 and 1964[357]. The earlier increase was attributed to wider application of amylase testing and the later increase to a locally heightened interest in the condition, thus both increases were considered to be due to improvements in diagnosis rather than to an absolute change in incidence[357].

The study of the Bristol population has recently been updated by another group using identical diagnostic criteria. They found that the incidence of acute pancreatitis had increased by 26% over the previous two decades but that throughout this 30 year period, the overall case mortality rate had remained unchanged at around 20%[69,357].

The aims of the present study were to document the incidence and mortality from acute pancreatitis in Scotland and to examine any emerging trends.

Methods

Data on the discharge diagnosis of all patients admitted to hospital in Scotland have been recorded since 1961 as the Scottish Hospital In-Patient Statistics. Details of the number of patients with a primary discharge diagnosis of acute pancreatitis (ICD Code 577.0) have been obtained for each year between 1961 and 1985 (the last year for which complete figures were available) from the Scottish Health Service Common Services Agency (Information and Statistics Division, Edinburgh). The computer print-out provided details of the total number of patients and the number in whom a fatal outcome was recorded each year, with a breakdown of their age, sex and health board area. No information was available from this source on the diagnostic criteria used, on the underlying aetiology or whether the attack was recurrent.

Details of the estimated population of Scotland for each year of the study have been obtained from published data[299].

Results

Overall population trends

Throughout the 25 year period studied the estimated population of Scotland remained fairly stable with a mean population of 5,201,900 $\pm$ 28,600 (Fig. 3). The population peaked at 5,240,800 during the 5 year period 1971-1975 and has declined gradually but progressively since then, the lowest level of 5,136,500 having been recorded in 1985.

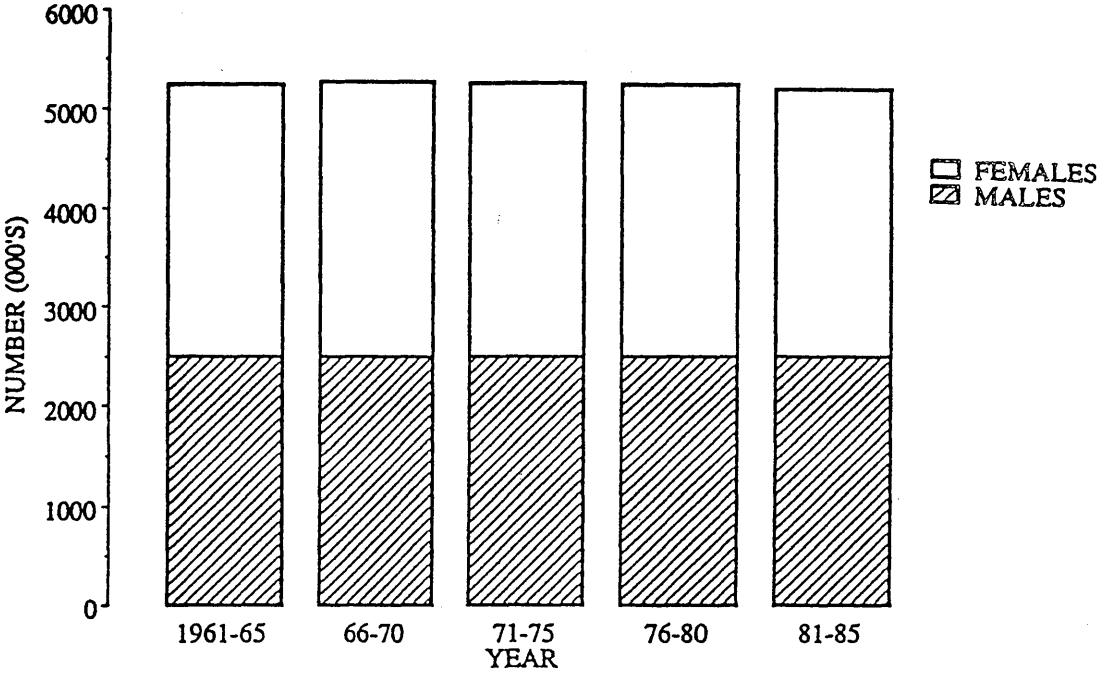


FIGURE 3.

Estimated population of Scotland over successive 5 year periods, 1961-1985. (Registrar General Scotland. Annual Report, 1986).

Incidence and mortality trends

The annual number of discharges from acute pancreatitis recorded rose steadily in both sexes throughout the 1960s but the largest increase occurred during the 6 year period after 1971 (Fig. 4). The increase in the numbers of discharges recorded has tended to plateau since 1977, particularly amongst females, the number of discharges in males continuing to increase, albeit at a slower rate. Since the mid 1970s the diagnosis of acute pancreatitis has been recorded more frequently in males than in females.

Discharges from acute pancreatitis in males have increased almost 11 fold since 1961 from 69 cases/year to 750 cases/year in 1985. In women the incidence has increased over 4 fold in the same period from 112 cases/year to 484 cases/year in 1985 (Fig. 4).

The number of deaths recorded from acute pancreatitis has not shown a corresponding change, doubling in males from 15 cases/year in 1961 to 30 cases/year in 1985 and increasing only slightly in women, from 29 cases/year to 37 cases/year in 1985 (Fig. 4). Taken in conjunction with the marked increase in the number of discharges recorded this has resulted in a dramatic fall in the overall case mortality rate in both sexes (Table 7).

A marked increase in the recorded number of discharges has been witnessed amongst adult males of all ages, particularly amongst young adult males between 20 and 39 years of age (Fig. 5). In females the major increase was observed amongst elderly women over 60 years of age (Fig. 6), smaller increases being observed amongst young and middle-aged adult females.

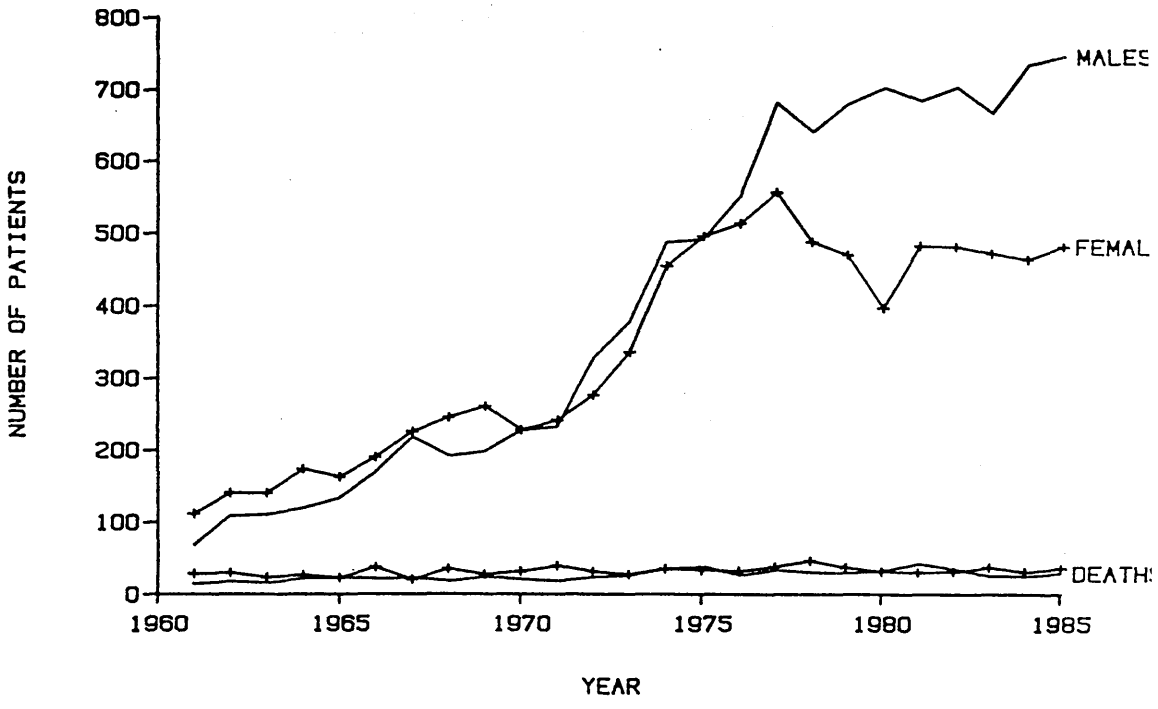


FIGURE 4.

Annual incidence and mortality from acute pancreatitis in Scotland by recorded discharge diagnosis, 1961-1985.

TABLE 7.

Case mortality rate from acute pancreatitis in Scotland, 1961-1985.

	Males		Females		Total	
Year	Number	Deaths	Number	Deaths	Number	Deaths
1961-65	543	94 (17.3%)	731	133 (18.2%)	1274	227 (17.8%)
1966-70	1009	109 (10.8%)	1156	155 (13.4%)	2165	264 (12.2%)
1971-75	1924	144 (7.5%)	1811	170 (9.4%)	3735	314 (8.4%)
1976-80	3265	155 (4.7%)	2431	189 (7.8%)	5696	344 (6%)
1981-85	3550	161 (4.5%)	2391	170 (7.1%)	5941	331 (5.6%)

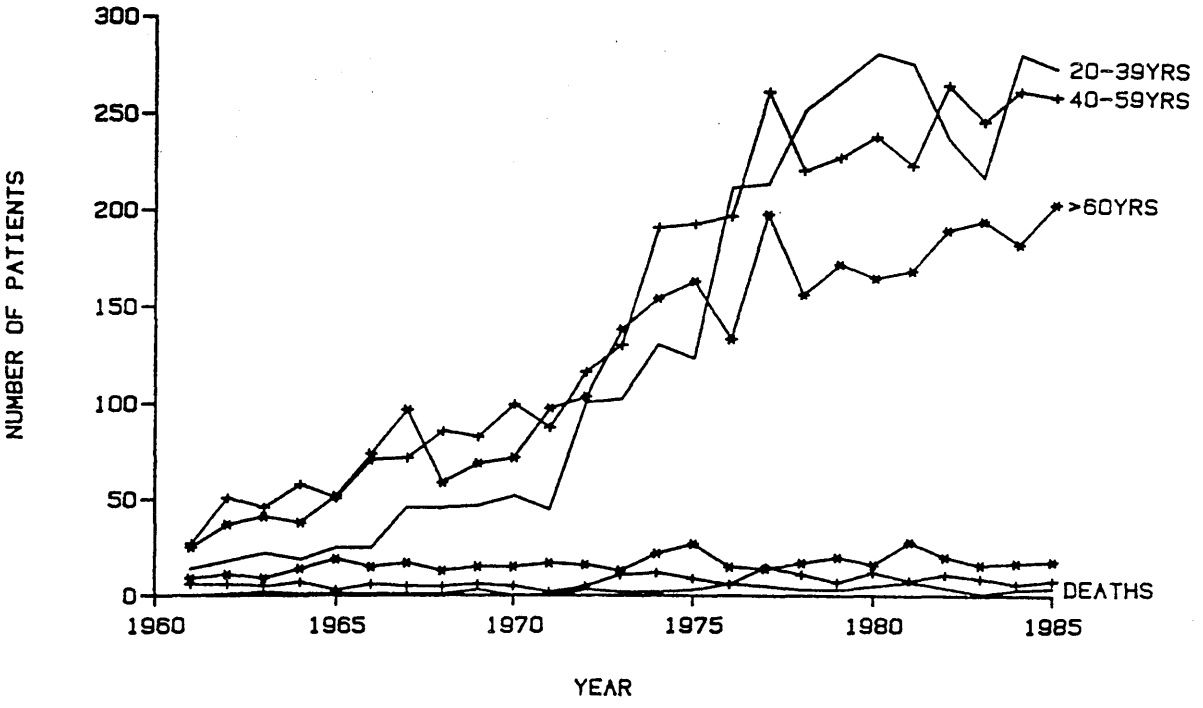


FIGURE 5.

Annual incidence and mortality from acute pancreatitis in males analysed according to age group. (Excludes patients <20 years old)

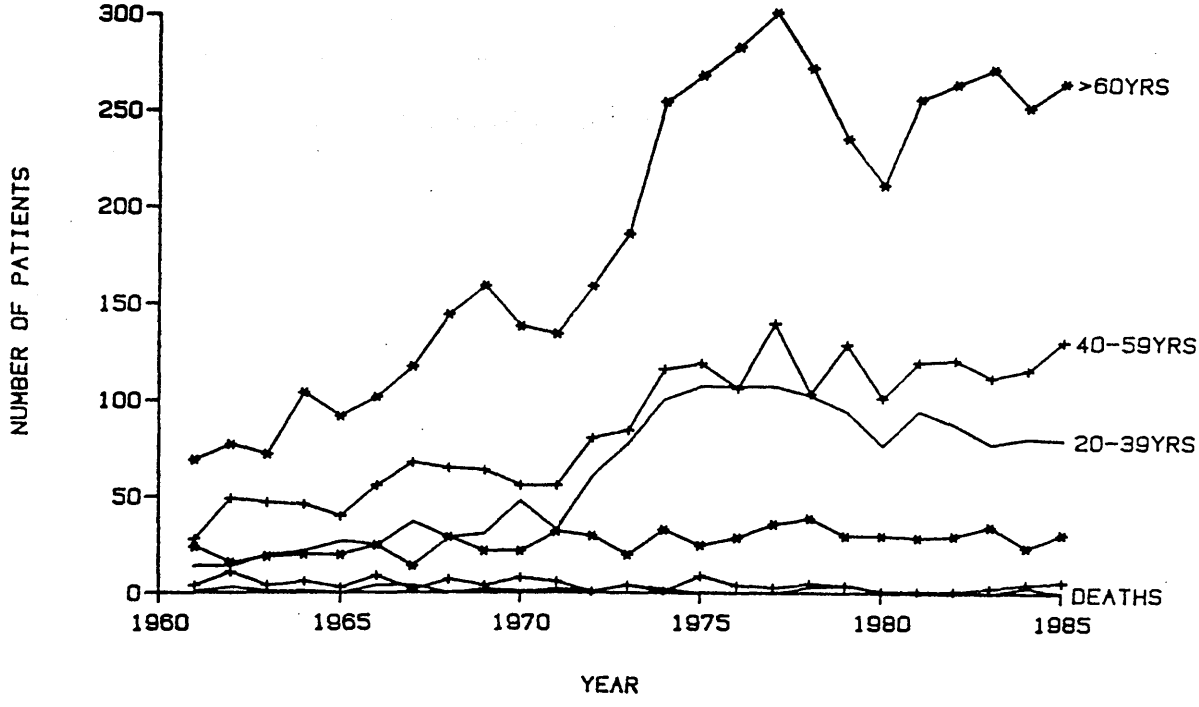


FIGURE 6.

Annual incidence and mortality from acute pancreatitis in females analysed according to age group. (Excludes patients <20 years old)

Regional variations

Examination of the number of discharges recorded in each health board area during successive 5 year periods reveals wide regional differences, the greatest increase (813%) occurring within Argyll and Clyde (Table 8). In contrast Dumfries and Galloway recorded only a 243% increase in successive 5 year periods over the 25 years of the study against an average increase nationally of 471% (Table 8). The regional differences over successive 5 year periods are shown graphically in Figure 7. The largest increases in the number of discharges were recorded in the health boards of the "central belt" area of Scotland, the more remote areas of Highland, Borders and Dumfries and Galloway showing the smallest increases.

The mortality recorded in each of the 12 major health board regions is documented in Table 9. The overall case mortality rates recorded in each region ranged widely during the period 1961-1965, from 12% in Lothian to 31% recorded in Ayrshire and Arran, with a national average of 18%. In the most recent 5 year period the case mortality rates were more comparable, although remaining persistently higher within Ayrshire and Arran at 11%, against a national average of 5.5%.

Discussion

An apparent increase in the incidence of acute pancreatitis may be inferred by the increasing number of discharges recorded in the Scottish Hospitals In-patient Statistics. This increase in incidence

TABLE 8.

Incidence and percentage increase of acute pancreatitis discharges recorded in the 12 major health boards, 1961-1985.

	Years				
	1961-65	1966-70	1971-75	1975-80	1981-85
Health Board	No.	No. (%)	No. (%)	No. (%)	No. (%)
Argyll & Clyde	70	108 (154%)	236 (337%)	433 (618%)	569 (813%)
Forth Valley	37	113 (305%)	141 (381%)	230 (622%)	280 (757%)
Lanarkshire	87	122 (140%)	303 (348%)	443 (509%)	568 (653%)
Fife	73	142 (194%)	243 (333%)	436 (597%)	456 (625%)
Greater Glasgow	254	442 (174%)	876 (345%)	1429 (563%)	1480 (583%)
Tayside	117	223 (191%)	376 (321%)	482 (412%)	505 (432%)
Lothian	211	392 (186%)	528 (250%)	807 (382%)	806 (382%)
Ayrshire & Arran	68	93 (137%)	200 (294%)	274 (403%)	259 (381%)
Borders	40	34 (85%)	68 (170%)	62 (155%)	122 (305%)
Highland	56	71 (127%)	111 (198%)	157 (280%)	168 (300%)
Grampian	181	316 (175%)	453 (250%)	524 (290%)	508 (281%)
Dumfries & Galloway	42	54 (129%)	88 (209%)	117 (278%)	102 (243%)
Totals	1236	2110 (171%)	3623 (293%)	5394 (436%)	5823 (471%)

No. = number of patients.
(%) = % change in incidence compared with 1961-65 (100%).

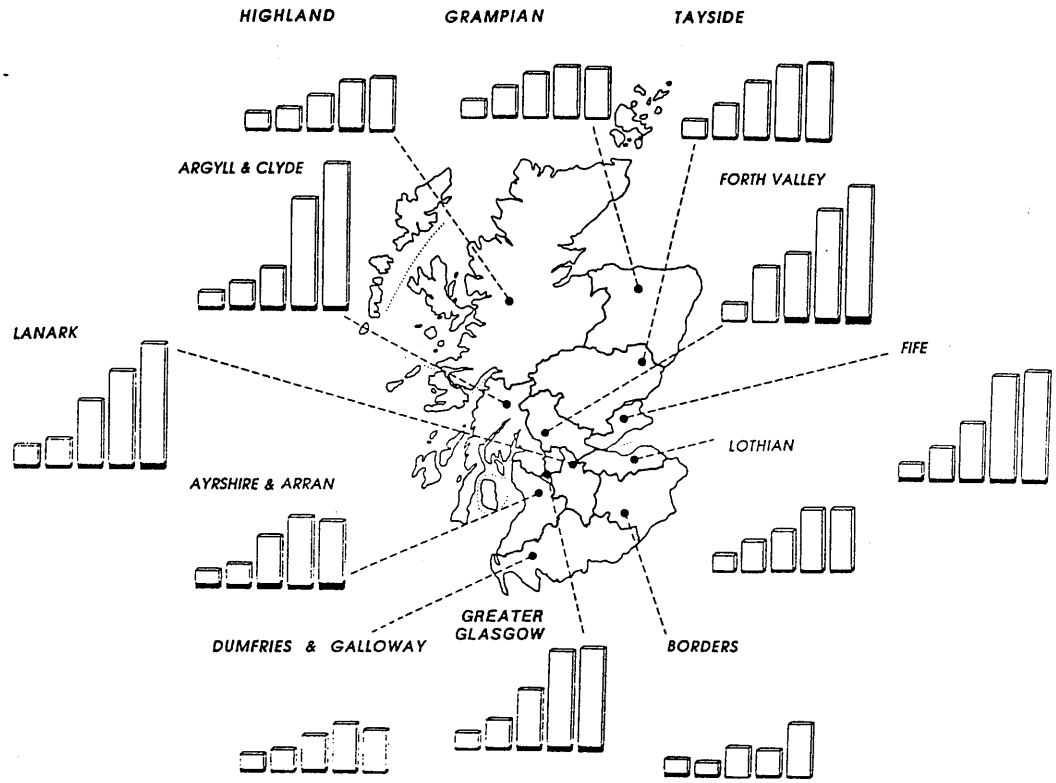


FIGURE 7.

Variation in incidence and mortality from acute pancreatitis by recorded discharge diagnosis in the 12 major health board regions of Scotland. The bars represent successive 5 year periods and are expressed as the percentage increase over the period 1961-5, the shaded areas denoting the fatal cases. The analysis excludes Orkney, Shetland and the Western Isles health boards as too few cases were recorded in each to permit meaningful analysis of the incidence and mortality trends.

TABLE 9.

Changing case mortality rates from acute pancreatitis recorded in the 12 major health boards, 1961-1985.

	Years				
	1961-65	1966-70	1971-75	1976-80	1981-85
Health Board	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Borders	10 (25%)	8 (23.5%)	3 (4.5%)	7 (11%)	4 (3%)
Argyll & Clyde	17 (24%)	21 (19.5%)	20 (8.5%)	23 (5%)	23 (4%)
Fife	21 (29%)	17 (12%)	19 (8%)	21 (5%)	20 (4.5%)
Dumfries/Galloway	8 (19%)	6 (11%)	10 (11%)	8 (7%)	5 (5%)
Tayside	19 (16%)	25 (11%)	29 (8%)	29 (6%)	27 (5%)
Grampian	24 (13%)	41 (13%)	30 (6.5%)	36 (7%	24 (5%)
Lothian	25 (12%)	32 (8%)	38 (7%)	42 (5%)	38 (5%)
Lanarkshire	22 (25%)	20 (16.5%)	26 (8.5%)	28 (6%)	34 (6%)
Greater Glasgow	36 (14%)	54 (12%)	76 (9%)	85 (6%)	90 (6%)
Forth Valley	5 (13.5%)	4 (3.5%)	14 (10%)	13 (6%)	17 (6%)
Highland	11 (19.5%)	11 (15.5%)	14 (12.5%)	17 (11%)	12 (7%)
Ayrshire & Arran	21 (31%)	20 (21.5%)	26 (13%)	19 (7%)	29 (11%)
Totals	219 (18%)	259 (12%)	305 (8.5%)	328 (6%)	323 (5.5%)

No. = number of deaths recorded.
(%) = mortality rate.

has been observed in both sexes during the study and may be attributed to many different factors.

Improvements in the accuracy of diagnosis seem likely to account for the bulk of this increase. In the early years of the study the diagnosis of acute pancreatitis was often made with confidence only at laparotomy or post mortem. Biochemical tests for amylase were, at that time, characteristically complicated, time consuming and infrequently utilised. It is interesting to note that the largest increase in the annual number of discharges occurred after 1971 coinciding with the introduction of the Phadebas Test (Pharmacia Diagnostics, Uppsala, Sweden), a simple, reproducible assay for amylase. Nowadays the serum amylase activity is measured on almost all patients presenting to hospital with upper abdominal pain and it is likely that this has led to the diagnosis being established in a greater proportion of the patients presenting with acute pancreatitis.

The increase in the numbers of discharges recorded with acute pancreatitis was clearly rising prior to 1971. Although acute pancreatitis has been recognised as a clinical entity since the late 19th century, throughout the early and mid 20th century there had been little reference to the condition either in the standard surgical textbooks of the time or in learned journals[222]. The increasing interest and awareness of the condition witnessed in the UK over the past 20 to 30 years appears to date from several important publications around that time, including those of Pollock[279] and subsequently of Trapnell[355]. Both authors reviewed and

analysed large series of patients highlighting aspects of clinical presentation, aetiology, complications and management. These and other publications of that time have stimulated numerous publications on all aspects of the disease and increasing awareness of the condition appears likely to have been an additional factor contributing to the increasing numbers diagnosed.

The Scottish Hospitals In-Patient Statistics are dependent upon the accuracy and quality of the data input supplied by doctors and clerical staff in many hospitals within the 15 health boards, each with differing facilities and procedures for recording and processing such data. Computerisation might be expected to improve data collection but one recent survey has highlighted deficiencies that may remain within this system. The incidence of acute pancreatitis in the Grampian region of Scotland was investigated for the years 1983-1985 by examining the record of amylase examinations carried out in the single regional laboratory performing these assays. Only 53% of the total number of cases identified in the study had been recorded in the hospital's computer linked diagnostic index[351]. If representative of other health boards in Scotland, this may have profound implications for the accuracy of disease reporting nationally and the number of cases reported here may be underestimating the true incidence of the disease in the hospital population by up to 100%.

The incidence of acute pancreatitis continued to increase in males throughout the late 1970s and early 1980s, whereas in females the incidence appears to have levelled out. This differential effect

would tend to be against an overall change in the diagnostic rate of acute pancreatitis which might be expected to affect the incidence in both sexes equally. It is clear that alcohol consumption and abuse is increasing in our population, and in Scotland there appears to be a greater persistence of youthful, heavy drinking into early middle age[327] which may be contributing to the continuing increase in numbers of discharges from pancreatitis amongst males. Recurrent attacks requiring repeated admissions are more common in association with alcoholic pancreatitis and may also be contributing to this increasing incidence.

The regional differences in the increasing numbers of discharges recorded are more difficult to explain. Predominantly rural areas with less easily accessible in-patient hospital care may have a greater proportion of the patients with mild attacks treated at home (where the diagnosis may not be made and will not be recorded) than might be the case in urban areas. This might account for the lower increase in the numbers of discharges in Dumfries and Galloway, Grampian, Highland and Borders. Differences in life-style, particularly with respect to the frequency, extent and nature of alcohol abuse in the young, often socially deprived populations in the urban areas of the central belt of Scotland may also explain some of these differences. Unfortunately the Scottish Hospital In-Patients Statistics do not classify the attacks according to aetiology which might aid this analysis.

Despite the marked increase in numbers of discharges from acute pancreatitis since 1961, the number of deaths recorded have changed

little throughout the 25 year period leading to a marked reduction in the overall case mortality rate. The mortality rate was consistently higher in females than in males throughout the period of the study. In both sexes the mortality occurs predominantly amongst patients in the over 60 year age group. In females this age group accounted for 56% of the discharges and 81% of the deaths. Discharges from acute pancreatitis in males were recorded more frequently amongst the younger age groups and were associated with a low mortality. The over 60 year age group accounted for only 32% of the attacks and 61% of the deaths and thus explains the lower overall case mortality rate observed in males.

While improved treatment of acute pancreatitis may have contributed to the falling case mortality rate, despite the marked increase in the apparent incidence of the condition, a more plausible explanation may be that the nature of the disease recorded has itself changed. If, as seems likely, more cases of pancreatitis have been diagnosed because of increased awareness and more widespread diagnostic testing leading to an increased diagnostic rate, it is probable that many more mild cases (which previously may have been overlooked) have been diagnosed. Patients with severe pancreatitis dying of their illness or developing a complication in the early part of the study are more likely to have been correctly diagnosed, many having come to surgery or post mortem. Thus the net effect of diagnosing more mild cases who are less likely to die would be that the proportion of the total cases dying (case mortality rate) will fall, even if treatment has remained unchanged.

The case mortality rates which varied widely between different health boards during the early years of the study have become more uniform, although remaining slightly higher within Highland and Ayrshire and Arran. The reasons for these differences are not apparent from this data, although Thompson has suggested that the higher diagnostic laparotomy rate may explain some of the increased mortality within the Highland Health Board[350].

Subject to the possible drawbacks mentioned above regarding the overall accuracy of the figures, the national statistics presented here provide a broad overview of the disease and its apparently changing incidence in our society. A closer examination of these factors in a smaller, more carefully defined population must be undertaken to investigate in detail the patterns of the illness, mode of death and to determine whether changes in treatment may have influenced the mortality rate of the condition.

CHAPTER 3. EXAMINATION OF DEATHS FROM ACUTE PANCREATITIS AT GLASGOW ROYAL INFIRMARY, 1974-1984.

Introduction

In a mainly retrospective review of acute pancreatitis from Glasgow Royal Infirmary over the decade to 1970, Imrie reported an overall case mortality rate of 21.4%[155]. A recent review from Bristol of the decade to 1979 found a case mortality rate of 19.6%[69], a figure little changed from that of the preceding two decades[357]. In contrast, in prospective therapeutic trials in Glasgow, mortality from acute pancreatitis fell from 11.5% in 1971-2[157] to 8.7% in 1974-7[159]. Prospective studies, however, give an incomplete picture of the true incidence and mortality of acute pancreatitis by excluding patients who present atypically and those not diagnosed in life.

The past decade has seen many changes in the management of acute pancreatitis and its complications but it is unclear whether these have influenced the mode and timing of death or the overall mortality. A review of all deaths from acute pancreatitis at Glasgow Royal Infirmary between 1974 and 1984 has, therefore, been undertaken, during which time a series of prospective therapeutic trials were being conducted.

Methods

Patients admitted to Glasgow Royal Infirmary with a diagnosis of acute pancreatitis have been documented prospectively since 1971. Between the 1st of January 1974 and the 31st of December 1984, 817 such patients were documented, many of whom have been the subject of prospective therapeutic trials[38,156,157,159,235,269]. A fatal outcome was recorded in 73 (9%) of these patients. Examination of the Hospital Activity Analysis (ICD Code 577.0) for these years revealed that a total of 975 patients with a recorded diagnosis of acute pancreatitis had been treated over this time, including 53 patients who who were first diagnosed at post mortem. The 73 deaths documented prospectively and the 53 patients first diagnosed at post mortem give a total of 126 deaths for an overall mortality rate of 12.9%. The diagnosis in these 126 patients was confirmed at post mortem in 99 (79%), laparotomy in 10 (8%) and in the remainder on a clinical presentation and course of illness consistent with the diagnosis of acute pancreatitis and a serum amylase above 1200IU/l (normal range 70-300IU/l).

Catchment population

The estimated catchment population of Glasgow Royal Infirmary was 243,763 in 1975, falling to 212,506 in 1978 with the opening of a new district general hospital adjacent to the eastern boundary in 1977. The catchment population has continued to decline to 187,136 in 1984, an overall fall of 23% since 1975 (Greater Glasgow Health Board, Information Services).

Aetiology of acute pancreatitis

Gallstones were considered to be the underlying cause of acute pancreatitis when they were recovered at post mortem or laparotomy, and in 2 patients on radiological evidence (intravenous cholangiography - 1, plain X-ray - 1). Alcohol was incriminated in patients admitting to, or suspected of drinking an excessive amount[157]. Patients with other recognised causes of acute pancreatitis have been studied as a group (see below) as have those in whom no definite aetiological factor could be implicated. Table 10 details the age and sex of the groups and the proportion first diagnosed at post mortem.

Pancreatic morphology

Pancreatic morphology was classified according to the description of the organ at laparotomy or post mortem supplemented by histological data where available. Pancreatic pseudocyst and abscess were defined as a pancreatic or peripancreatic collection of fluid or pus respectively. Black pancreatic and peripancreatic tissue either apparent superficially or on sectioning of the gland was diagnosed as necrosis but included in this group were patients with extensive pan-lobular parenchymal destruction on microscopy. The remaining patients with less severe parenchymal destruction were labelled as having had acute haemorrhagic pancreatitis when haemorrhage was apparent on the surface, or on sectioning of the gland, and as having had acute interstitial pancreatitis when the pancreas was inflamed

TABLE 10.

Aetiology of fatal attacks of acute pancreatitis in relation to patient age and sex and proportion of patients first diagnosed at post mortem.

Aetiology	Number	Male:Female	Mean age (yrs)	Proportion undiagnosed before post mortem (%)
Gallstones	*39 (30%)	19:20	68	33%
Alcohol	20 (15%)	16:4	48	40%
Other causes	23 (17%)	12:11	62	74%
Unknnown causes	50 (38%)	21:29	66	42%
Totals	*132	68:64	63	42%

* 6 patients with >1 aetiological factor.

with only fat necrosis and focal parenchymal necrosis on histology.

Analysis of deaths

Death was categorised as "pancreatic" when it appeared to have been a direct consequence of acute pancreatitis and this included some patients in whom events such as myocardial infarction or pulmonary embolism complicated severe acute pancreatitis. Where death appeared to be a consequence of another unrelated medical condition (eg. liver failure or hypothermia) or where pre-existent medical disease was a major contributing factor to death (eg. ischaemic heart disease, carcinoma or chronic obstructive airways disease) such deaths were categorised as "medical". Early deaths were defined as those occurring within 7 days of admission and late deaths, as those occurring after this time.

Statistics

Statistical analysis was conducted by Fisher's exact test and by the Student's 't' test.

Results

The annual incidence and mortality of acute pancreatitis and the number of patients first diagnosed at post mortem are shown in Figure 8. Mortality is seen to decrease throughout the 11 year period. The overall mortality rate for the first half of the study (1st January 1974 to the 30th June 1979) was 14.9% compared with

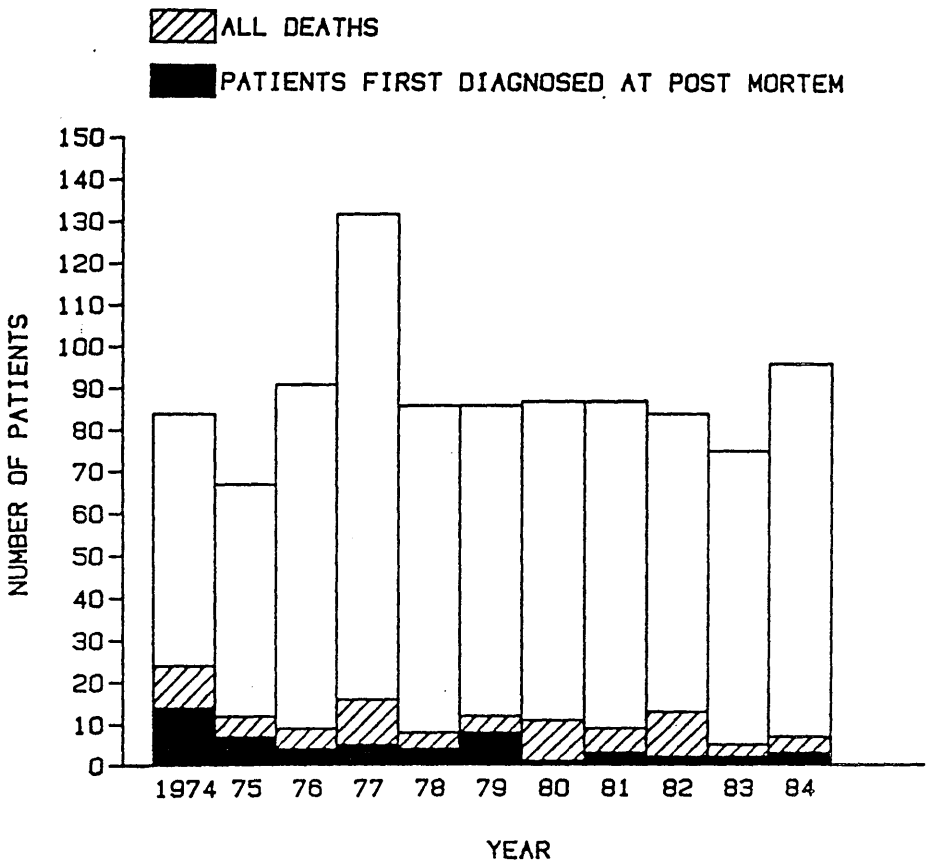


FIGURE 8.

Annual incidence and mortality of acute pancreatitis at Glasgow Royal Infirmary and numbers first diagnosed at post mortem.

10.8% for the latter half. The mortality rates for the 73 patients documented prospectively were 8.9% and 9% respectively and the fall in overall mortality is explained by a reduction in the number of patients who were first diagnosed at post mortem, 38 (72%) of whom were recorded in the first half of the study.

Sixty-eight (93%) of the 73 patients documented prospectively were first diagnosed on clinical and biochemical grounds and the remainder at laparotomy. Of the 53 patients first diagnosed at post mortem 36 (68%) had presented atypically with known or suspected medical conditions, 32 (60%) of them to physicians rather than surgeons. No particular clinical syndrome emerged as being most commonly associated with undiagnosed pancreatitis but rather these patients had widely varying presentations which suggested neurological disease in 6, respiratory disease in 6 and cardiac failure, liver failure and ketoacidosis each in 5 patients. In 10 (19%) of these undiagnosed patients, pancreatitis was an unsuspected cause of postoperative deterioration (see below). Only 7 (13%) of these 53 patients had presented with abdominal pain and a serum amylase level had been measured in only 5 (9%) of them.

The majority of the patients dying from acute pancreatitis had presented directly to the medical and surgical units of this hospital, only seven patients (6%) being secondary referrals from other hospitals.

Gallstone pancreatitis

Thirty-nine patients (30%) had acute pancreatitis associated

with the presence of gallstones. Six patients also had other potential aetiological factors, namely alcohol abuse (3), recent cardiopulmonary by-pass (1), biliary surgery (1) and hypothermia (1). Figure 9 shows the distribution of gallstones in the biliary tree and Table 11 the relationship between the distribution of gallstones and the pancreatic morphology found at laparotomy or post mortem. Severe pancreatic destruction (abscess or necrosis) was present in 21 patients (54%) and was found more commonly in the 23 patients (59%) with calculi in the extrahepatic ducts than in the remainder in whom calculi were confined to the gallbladder. Table 12 details the 21 patients with severe pancreatic destruction, 16 (76%) of whom were diagnosed in life. Gallstones impacted at the ampulla of Vater were found in only two patients (both elderly females). Both died of septicaemia associated with severe cholangitis as did one other patient with gallstones throughout the biliary tree.

Nine patients (23%) had experienced previous attacks of acute pancreatitis. Seven presented in the early half of the study, three having been discharged only 6, 7 and 12 days prior to their final fatal attack. Two patients who had previously undergone cholecystectomy developed recurrent acute pancreatitis in association with persistent or recurrent calculi.

Alcohol-associated pancreatitis

Twenty patients had a history of chronic alcohol abuse. Three of these patients (mentioned above) had gallstones. Sixteen (80%) were male and patients with alcohol-associated acute pancreatitis

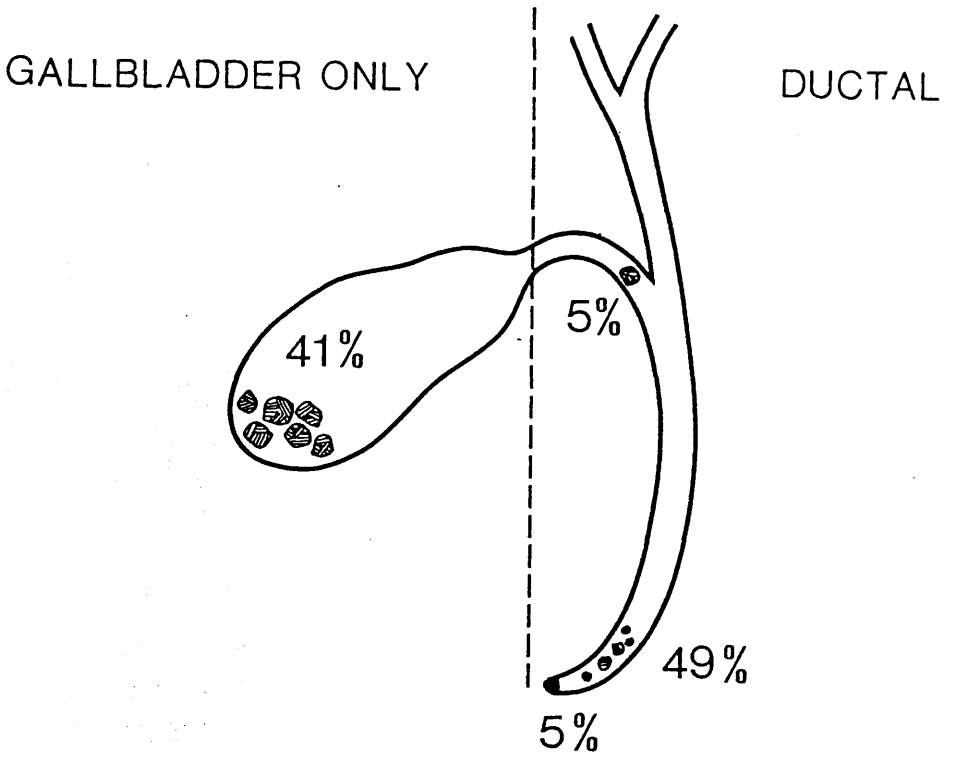


FIGURE 9.

Distribution of gallstones in the biliary tree in the 39 patients with fatal gallstone pancreatitis.

TABLE 11.

Relationship between pancreatic morphology and the distribution of gallstones in the biliary tree in 39 patients with fatal gallstone pancreatitis.

Pancreatitis morphology	Ductal calculi (n = 23)	Calculi in gall bladder only (n = 16)
Interstitial pancreatitis	4 (17%)	4 (25%)
Haemorrhagic pancreatitis	2 (9%)	3 (19%)
Pancreatic necrosis	7 (30%)	3 (19%)
Pancreatic abscess	8 (35%)	3 (19%)
Pseudocyst	1 (4%)	2 (12%)
Unknown	1 (4%)	1 (6%)

No significant difference between groups (Fisher's exact test).

TABLE 12.

Incidence of abscess and necrosis within each aetiological group in patients first diagnosed in life and at post mortem.

	Diagnosed in life		Diagnosed at post mortem		
Aetiology	Necrosis	Abscess	Necrosis	Abscess	Totals
Gallstones	7 (27%)	9 (35%)	3 (23%)	2 (15%)	21 (54%)
Alcohol	6 (50%)	2 (17%)	2 (25%)	1 (12.5%)	11 (55%)
Other	1 (17%)	0	8 (47%)	2 (12%)	11 (48%)
Unknown	4 (14%)	3 (10%)	6 (29%)	0	13 (26%)

were significantly younger than those with acute pancreatitis attributable to other causes (mean age 48 years vs 66 years, $p < 0.001$). Eleven patients (55%) had severe pancreatic destruction (Table 12). Seven patients had alcoholic liver disease consisting of cirrhosis in 5 and acute alcoholic hepatitis in 2; four died in frank hepatic failure. Five patients (25%) had a history of previous pancreatitis, three of whom had required surgery (pseudocyst drainage - 2, abscess drainage - 1).

Other identified aetiological factors.

Twenty-three patients had other recognised aetiological factors implicated. Four patients had hypothermia, all of whom were elderly (mean age 90 years) and 3 of whom were female. One had gallstones and acute pancreatitis may have preceded the hypothermia. Eight patients developed acute pancreatitis following upper abdominal surgery. Three had undergone gastrectomy (2 with splenectomy), 2 had undergone splenectomy and 3 had undergone biliary or pancreatic surgery. Three patients developed acute pancreatitis following cardiopulmonary by-pass and 2 following translumbar aortography. Only one of the patients with post-operative pancreatitis was diagnosed in life having been re-explored for suspected anastomotic leakage following gastrectomy.

Four patient had acute pancreatitis in association with pancreatic carcinoma (primary pancreatic - 3, metastatic from bronchus - 1). Two other patients developed acute pancreatitis associated with fulminant viral hepatitis and iatrogenic

intravascular haemolysis respectively. Eleven patients (48%) had severe pancreatic destruction (Table 12) and only 3 patients (those having undergone biliary or pancreatic surgery) had a history of previous attacks.

Unknown aetiology

The 50 patients (38%) in whom no definite aetiological factor could be implicated (despite laparotomy or post mortem in all but 13) comprise the largest single group. Possible aetiologies were thought to include ischaemia/hypotension in 6, septicaemia in 3 and steroid therapy in 2. Only 13 (26%) had severe pancreatic destruction (Table 12) and none had a history of previous attacks of acute pancreatitis.

Surgery

Surgery for complications was performed in 23 patients (18%) during their final illness, 10 requiring multiple operations (Table 13). Six of the seven patients having surgery for abscess had a post mortem examination and 5 were found to have a residual abscess and/or necrosis. Thirteen patients having surgery had gallstones. In six of these patients gallstones were not eradicated at surgery. In 4 patients gallstones were not identified at surgery, despite multiple operations in two of these patients. Only one of these patients had been treated during the latter half of the study.

Analysis of deaths

Early "pancreatic" deaths were often associated with fulminant

acute pancreatitis. Such deaths were most commonly seen in older patients with no identifiable aetiological factors but were also common in younger patients in whom alcohol was implicated (Figure 10). Late "pancreatic" deaths, usually due to abscess and/or necrosis with sepsis and multi-organ failure, were most common in patients with a gallstone aetiology. Early and late "medical" deaths were most often seen in patients with acute pancreatitis due to other identified aetiological factors. Significant underlying medical disease was present in 71% and 86% of the early and late "medical" deaths compared with 46% and 42% of the early and late "pancreatic" deaths respectively.

Overall 56% of the deaths were considered directly attributable to acute pancreatitis and 32% to "medical" causes. Of the 73 patients diagnosed in life, 60% died of "pancreatic" causes and 16% of "medical" causes compared with 49% and 51% respectively of those first diagnosed at post-mortem (Table 14). In 17 patients (23%) diagnosed in life there were no laparotomy or post mortem data. Nine of these 17 patients (53%) died within the first 7 days predominantly of cardiopulmonary problems, 5 of whom had underlying cardiovascular or respiratory disease. Of the 8 patients dying after 7 days, 5 succumbed from renal failure, 3 of these on a background of pre-existing chronic renal failure.

Sixty-two patients (49%) died within the first 7 days of their illness comprising 57% of the patients first diagnosed at post mortem and 44% of those first diagnosed in life. The pancreatic morphology and the time to death in the 2 groups is shown in Table 15. Patients

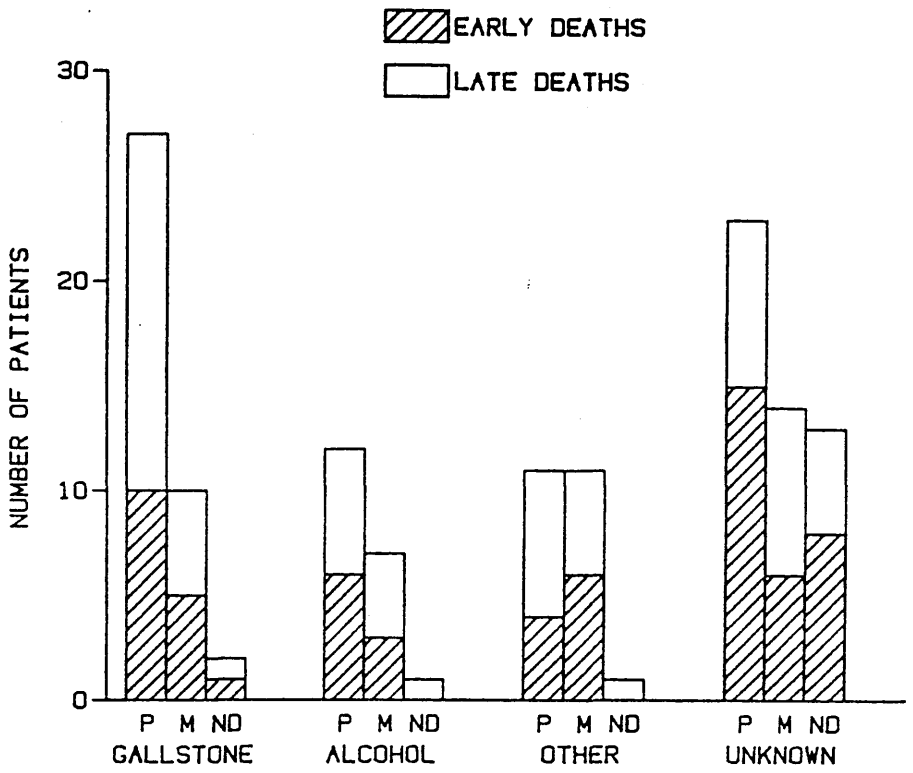


FIGURE 10.

Analysis of cause and timing of death within each aetiological group. P = pancreatic, M = medical, ND = no post mortem or laparotomy data (see text).

TABLE 14.

Analysis of the cause of death in patients diagnosed in life and at post mortem.

	Diagnosed in life				Not diagnosed until post mortem		
	Time to death (days)	Pancreatic	Medical	No data	Time to death (days)	Pancreatic	Medical
Aetiology:							
Gallstone	16(1-129)	20(77%)	4(15%)	2(5%)	6(1-28)	7(54%)	6(46%)
Alcohol	5(1-129)	9(75%)	2(17%)	1(8%)	6(1-34)	3(37%)	5(63%)
Other	11(5-136)	2(33%)	3(50%)	1(17%)	12(1-37)	9(53%)	8(47%)
Unknown	7(2-56)	13(45%)	3(10%)	13(45%)	5(1-48)	10(48%)	11(52%)
Totals		44(60%)	12(16%)	17(23%)		29(49%)	30(51%)

Time to death = median (range).
Includes 6 patients with > 1 aetiological factor.

TABLE 15.

Comparison of the time to death and the pancreatic morphology in patients diagnosed in life and at post mortem.

Pancreatitis morphology	Diagnosed in life	Time to death (days)	Not diagnosed until post mortem	Time to death (days)	Total
Interstitial	9 (12%)	4(1-138)	13 (25%)	6(1-33)	22 (17%)
Haemorrhagic	10 (14%)	5(2-26)	20 (38%)	7(1-48)	30 (24%)
Necrosis	17 (23%)	5(1-58)	15 (29%)	4(1-37)	32 (25%)
Abscess	13 (18%)	31(8-129)	5 (9%)	10(2-17)	18 (14%)
Pseudocyst	7 (10%)	40(33-140)	-	-	7 (6%)
Unknown	17 (23%)	6(2-71)	-	-	17 (13%)
Totals	73		53		126

Time to death = median (range).

first diagnosed at post mortem tended to have less severe degrees of pancreatic parenchymal destruction. Abscesses and pseudocysts typically occur later in the course of the illness and patients developing these complications survived longer. No relationship was found between the severity of pancreatic damage and time to death.

Examination of the mode and timing of death in the first and second periods of the study revealed that the proportion of late "medical" deaths had fallen from 30% to 12% ($p < 0.05$) reflecting the reduction in numbers of patients first diagnosed at post mortem. The proportion of early "pancreatic" deaths rose slightly during the second period of the study from 12.5% to 19% and the patients tended to be older (mean age 65 $\pm$ 17 years vs 62 $\pm$ 14 years), although neither difference was significant.

Discussion

Throughout the eleven year period reviewed the numbers of patients treated for acute pancreatitis each year has remained relatively constant. Given the 23% reduction in the hospital district catchment population that has occurred over this time, the local incidence of acute pancreatitis has probably risen and this would be in agreement with reports from elsewhere in the UK[69,78,357]. Whether this reflects a real increase in the local incidence of acute pancreatitis or whether more patients are being diagnosed because of increased investigative zeal is unknown. Interest in acute pancreatitis at this hospital predated the period of this current

study by several years, these patients having been actively sought and documented prospectively since 1971[157]. A real increase in the local incidence is therefore thought the more likely explanation.

Overall mortality has fallen reflecting a reduction in the numbers of patients first diagnosed at post mortem and this may be partly explained by a concomitant drop in the hospital's post mortem rate. The proportion of pancreatitis deaths that were undiagnosed before post mortem fell from 51% in the first part of the study to 29%, a fall of 43%. During these periods the hospital's post mortem rate has fallen from 29% of all deaths in the first part of the study to 20% of all deaths latterly, a fall of 31%. Our incidence figures and mortality rates obviously fail to take account of all patients with acute pancreatitis who are undiagnosed in life. Although presumably only a proportion of such patients die, improvements in diagnosis leading to increased numbers being diagnosed in life might also contribute to a reduction in the numbers of patients first diagnosed at post mortem.

It is disappointing that the mortality of patients diagnosed in life and documented prospectively has remained unchanged at around 9%. Deaths from gallstone pancreatitis in patients studied prospectively have, however, fallen by 47% from 17 in the first half of the study to 9. This is partly due to a reduction in the number of fatal recurrent attacks (7 vs only 2 in the latter half of the study) and particularly to the abolition of early fatal recurrence. Elimination of these deaths argues in favour of the policy of early cholecystectomy for gallstone pancreatitis which we[269] and others

have favoured[172,209,354], rather than readmitting these patients for surgery 6 to 12 weeks after their original attack.

The fall in the number of deaths attributed to gallstone pancreatitis may also be due to the more successful eradication of gallstones at operation in the latter half of the study. The aim of surgery for gallstone pancreatitis is clearance of all calculi from the biliary tree. In the early years of the study this aim was clearly not achieved. Failure to eradicate gallstones was due to cholecystostomy being performed without operative cholangiography in one case and to a choledochoduodenostomy being performed in another after failure of supraduodenal exploration. In four other patients gallstones were not identified at operation (abscess drainage - 2, pseudocyst drainage - 1 and laparotomy for haemorrhage - 1). Gallstones giving rise to acute pancreatitis are often small, can be difficult to diagnose by standard imaging techniques and as shown here, may be overlooked at laparotomy.

Finally, the fall in the number of deaths associated with gallstone pancreatitis may also reflect a decrease in the incidence of this diagnosis in our practice, for reasons which are not clear. In the early years of this study gallstones accounted for 52% of first attacks of primary acute pancreatitis[159]. Since 1979 gallstones have been incriminated in only 41% of first attacks whereas the incidence of attacks of unknown aetiology has increased from 13% to 22%[159].

The continued presence of calculi in the extrahepatic ducts appears to be associated with more severe pancreatic destruction

(abscess or necrosis) than when gallstones were confined to the gallbladder at the time of laparotomy or post mortem. It is conceivable that earlier clearance of these calculi by operation or endoscopic sphincterotomy would have favourably altered the outcome in some of these patients. Immediate operation to clear obstructing gallstones from the ampulla and biliary tree before irreversible pancreatic necrosis develops has been proposed by Acosta and co-workers[3].

This approach, although supported by Stone's group[340], has not gained favour and an increased mortality has been reported in association with immediate operation[172,354]. The advanced age of the patients subsequently dying of gallstone pancreatitis (mean age 68 years) also militates against a policy of immediate surgery, although it may be tolerated by younger patients. Endoscopic sphincterotomy appears to be a safe alternative in this situation[308,312] and may well be beneficial[253] although its application in fulminant cases awaits full evaluation.

Five (25%) of the patients with alcohol-associated acute pancreatitis had liver cirrhosis, a similar incidence to a large American series where alcohol was a much commoner cause of acute pancreatitis, accounting for 68% of patients overall[301]. Although the incidence of alcohol-associated acute pancreatitis increased from 12% in the period between 1960 and 1970[155] to 26% between 1971 and 1972[157], since 1974 the incidence has remained at around 32% of attacks[159] and currently stands at 34%. The number of deaths overall from alcohol-associated acute pancreatitis has fallen between the

first and second periods of the study, although when only those patients diagnosed in life are considered the proportions remain unchanged.

Acute pancreatitis due to other identified aetiologies was infrequently diagnosed before post-mortem, particularly pancreatitis developing postoperatively. It is likely that diagnosis of these patients in life would have altered management and perhaps the outcome in some. In 1978 better surveillance of patients undergoing "at risk" procedures was recommended[160] and only two deaths from postoperative pancreatitis were recorded in the latter half of the study.

Ischaemia was thought to explain the acute pancreatitis in at least some of the patients in whom no other aetiological factor could be identified[371]. This was the largest group of patients and the high percentage with an unidentified aetiology is surprising, although similar to the recent Bristol experience[69]. Attention has often focused in the past on a comparison of outcome in gallstone and alcohol-associated acute pancreatitis while the idiopathic aetiological group is regularly shown to have the highest mortality[69,159,165,235]. Improved identification of aetiology both during life and at post mortem is desirable. Faecal sieving and ERCP will increase the detection of gallstones and routine viral screening or blood alcohol levels on admission might improve the diagnostic rate further. There is also need for a meticulous post mortem protocol to avoid overlooking small calculi in the common bile duct or ampulla and rarer associations such as parathyroid adenomas.

Death was considered to be due to "medical" causes in 51% of the 53 patients first diagnosed at post mortem. In a retrospective study such as this it can be difficult to determine the actual contribution of the acute pancreatitis to the patient's death and in some it is possible that pancreatitis may have occurred as a result of terminal organ failure. In only 7 patients (13%) was the pancreatitis mild on the pathologist's assessment and considered to be an incidental finding at post mortem. In the remainder pancreatitis was considered to be either the major factor or a significant additional factor contributing to death and in the majority of these patients pancreatitis was thought to account for the patients' presenting symptoms.

The preponderance of "pancreatic" deaths among the patients diagnosed in life, particularly in those with a gallstone or alcohol aetiology, suggests scope for further improvements in management. Many patients continue to die early of fulminant acute pancreatitis, despite seemingly adequate medical treatment. These patients presented in a variety of ways, some with an overtly severe attack, others with an apparently mild attack, subsequently deteriorating, some quite rapidly after admission. Prognostic factor scoring systems may aid in the identification of such attacks, although the delay of up to 48 hours before obtaining an answer may diminish their value in practice. There is a need for more rapid and accurate identification of such patients.

Early operation with pancreatic resection has been suggested for fulminant acute pancreatitis and in a prospective comparison with

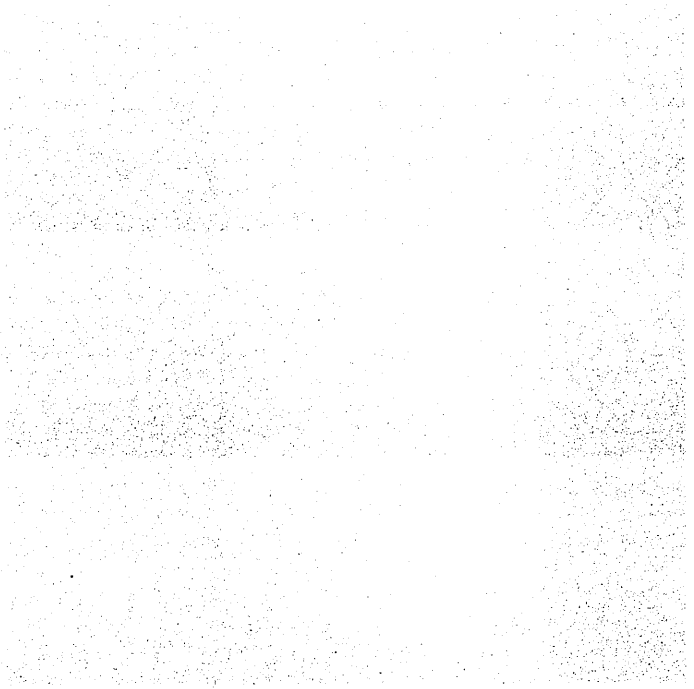
peritoneal lavage (lavage cannulas having been placed at laparotomy) patients undergoing primary resection fared best, with mortality rates of 22% and 47% respectively[175]. These figures were achieved in a predominantly young, male alcoholic patient population with a mean age of 37 years. Emergency pancreatic resection is associated with a much higher mortality elsewhere. Our patient population tends to be much older (mean age 63 years) and both patients in the current series undergoing pancreatic resection for fulminant acute pancreatitis died.

Patients surviving the early phase of the illness may succumb later due to the delayed effects of pancreatic necrosis or abscess. The limitations of the traditional surgical approaches to the management of these problems are highlighted by the frequent need for re-operation and the incidence of residual abscess and/or necrosis at post mortem. Newer developments in imaging techniques and the promising preliminary results of the recently described techniques of postoperative lavage[29] or open packing[48] following digital debridement and necrosectomy for pancreatic necrosis or abscess await full evaluation.

A proportion of patients may benefit from improved surgical treatment, but a dramatic overall improvement in the mortality rate is unlikely to follow. One third of the patients reported here died of medical causes and it was considered that surgical treatment had little or nothing to offer them. Other patients who might benefit from surgery were usually old or had concurrent medical disease and such patients often do poorly after surgery even with intensive care

therapy.

The nihilistic attitude has not much to commend it but a realisation of the task ahead is sensible. More careful surveillance in diagnostic terms, improved speed and accuracy in predicting severe attacks and the application of early ERCP and endoscopic sphincterotomy in gallstone pancreatitis[253] as well as a vigorous surgical policy to infected necrotizing pancreatitis[29,48] must be the way ahead. Meanwhile we seek to increase understanding of the mechanisms responsible for the early "shock-like" illness characteristic of severe acute pancreatitis and continue to search for the panacea of an effective medical therapy.



PART 3.

PATIENTS AND METHODS

CHAPTER 4. A CLINICAL STUDY OF INTRAPERITONEAL APROTININ THERAPY IN HUMAN ACUTE PANCREATITIS

Introduction

The balance of experimental evidence supports the view that the peritoneal exudate in acute pancreatitis possesses some toxic properties. Whatever the exact mechanism of toxicity, its removal might be expected to be therapeutic and in experimental pancreatitis, there is much evidence that this is the case. In man the evidence is conflicting, two earlier studies suggested benefit from peritoneal lavage in predominantly alcoholic patient populations[289,339], whereas the recent studies from the UK and Sweden have failed to confirm this[153,235].

Both recent studies examining peritoneal lavage suffered from drawbacks in their methodology. The majority of the control group in the UK study and all the control patients in the Swedish study undergoing peritoneal cannulation, aspiration of the exudate and a single peritoneal washout. Thus in both studies the control group had been "treated" by removal of the exudate which, according to experimental evidence, is most toxic early in the attack. This may have obscured any difference in outcome between the control group and the group who went on to have cycled peritoneal lavage. A further trial of peritoneal therapy seems warranted on these grounds alone.

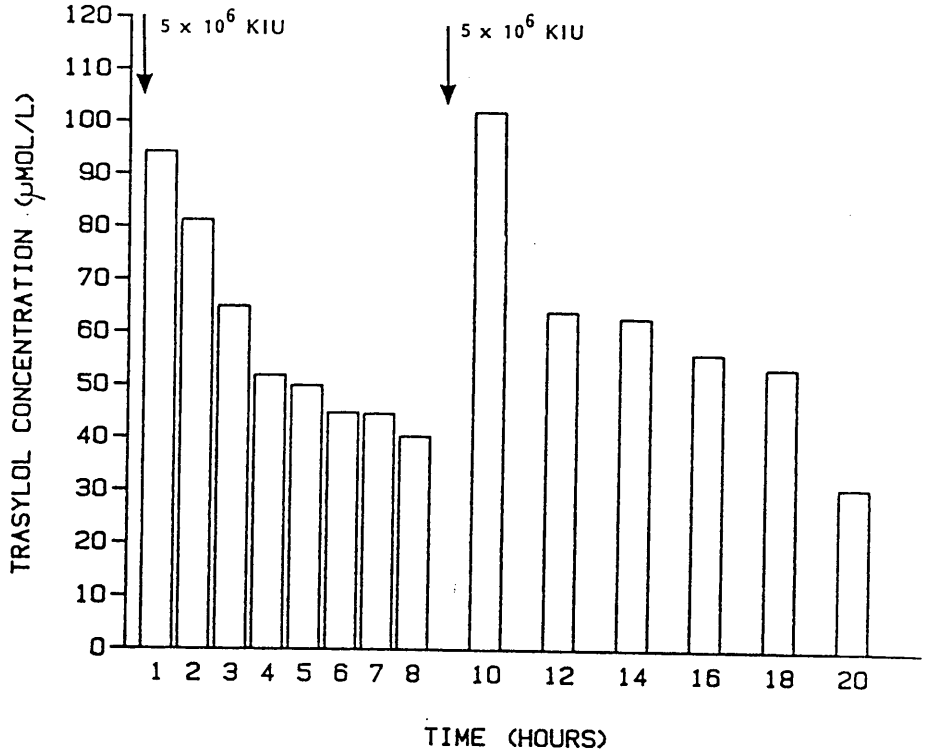
The addition of the antiprotease aprotinin to the dialysate has been examined in one study in which both groups of patients underwent cycled peritoneal lavage. No significant differences emerged between

the groups, although the mortality and number of complications was lower in the aprotinin treated group[21]. The present study was set up to examine whether a simplified regime of intraperitoneal antiprotease administration would improve the outcome and, in particular, decrease the incidence of early systemic complications compared to patients receiving standard therapy alone.

Intraperitoneal aprotinin

Given intravenously to dogs with bile-induced pancreatitis levels of aprotinin achieved in the peritoneal cavity were only about 1% of the initial plasma concentration[22]. To achieve therapeutic levels of aprotinin within the peritoneal cavity, sufficient to inhibit trypsin mediated effects, necessitates direct peritoneal administration[22].

In a pilot study 3 patients received a total dose of 14 $x10^6$KIU of aprotinin, administered 3 hourly in divided doses of 4, 3, 3, 2, 2 $x10^6$KIU respectively per administration. This regime produced intraperitoneal aprotinin levels of $>10\mu mol/l$, considered to be within the therapeutic range[22]. Similar studies performed in Leeds provided equivalent therapeutic levels in the peritoneal cavity, maintained for 20 hours using a simpler regime consisting of 2 separate instillations of 5 $x10^6$KIU aprotinin, each in one litre of 0.9% saline, given 8 hours apart (Larvin M, personal communication) (Fig. 11). It is this regime which has been investigated in the current study.



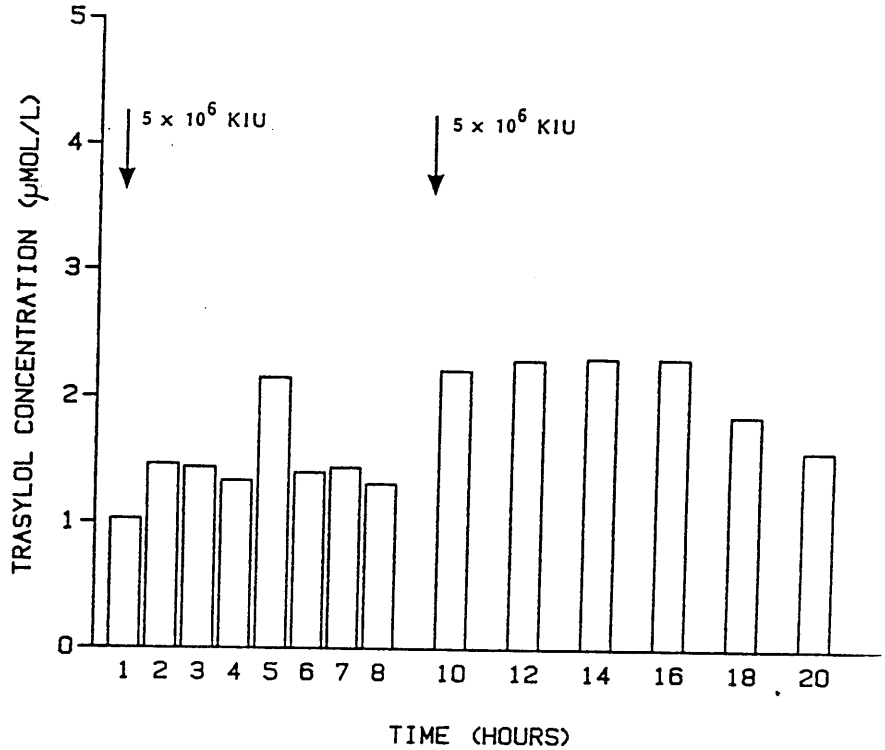


FIGURE 11.

(a) Concentrations of aprotinin in peritoneal fluid in 5 patients during intraperitoneal therapy with aprotinin. Concentrations are shown during the first 20 hours of therapy comprising 2 intraperitoneal instillations of aprotinin (5 x 10^6KIU in one litre of 0.9% saline) given 8 hours apart.

(b) Corresponding concentrations of aprotinin in plasma in 5 patients during intraperitoneal therapy with aprotinin (as above).

Statistical aspects

The vast majority of controlled trials of therapy in human acute pancreatitis have failed to show any benefit in terms of clinically important end-points such as the incidence of death or major complication. Although this may reflect the ineffectiveness of the therapies tested, many studies have lacked sufficient statistical power for the investigators to have confidence in the negative outcomes reported. This has been due to the inclusion of too few patients in the studies and/or that the frequency of clinically important end-points in the untreated control group was too low to permit detection of a difference having occurred[337].

Consideration of the type I and type II error rates allows prediction of the required sample sizes[111,385]. In the recent study of peritoneal lavage, 30% of all patients referred to the study died or developed a major life-threatening complication[235]. Assuming a similar spectrum of disease, to detect a 50% reduction in mortality and major morbidity in the treatment group, a total of 242 patients would need to be studied to provide an 80% power of detection, rising to 90% were 322 patients to be studied.

Selection of a subset of patients with an increased incidence of complications would permit the study of fewer patients, but to recruit sufficient numbers of such patients within a reasonable period of time necessitates a multicentre study design. The current study was set up in conjunction with Messrs. M. J. McMahon and M. Larvin from the General Infirmary, Leeds and with the support of Mr. D. Lee, Medical Services Manager for Bayer UK Ltd. The trial

commenced in February 1986. This chapter details the trial protocol and analyses the patients I have personally entered into the study.

Study design

All patients referred to the study were assessed by an experienced registrar (based in either Glasgow or Leeds) as soon as possible after their admission to hospital. All patients were documented using a standard trial proforma and had their clinical course and outcome recorded.

Only those patients considered to have moderate or severe pancreatitis, on the basis of the initial clinical examination, and fulfilling the other inclusion and exclusion criteria (see below) were entered into the formal study. The formal study examined the incidence of death and major complications in patients randomised to receive standard therapy alone compared with those randomised to receive additional intraperitoneal therapy with aprotinin.

Methods

Recruitment and documentation

Consultant surgeons at the Royal Infirmary, Glasgow and 5 nearby general hospitals were contacted at the end of 1985 with details of the proposed study and were invited to participate. In each hospital a comprehensive trial protocol was submitted to the local ethical committee and authorisation obtained prior to commencement of the study.

Patients admitted with acute pancreatitis to any of the 6 participating hospitals remained under the care of the admitting consultant surgeon but, in addition, were referred for consideration of their inclusion in the study, as soon as possible after their admission to hospital. To this end, I was available to be contacted 24 hours a day by a radiopaging device via the switchboard operators at Glasgow Royal Infirmary.

The patients referred were usually seen within a few hours of my being contacted. A standard proforma was completed for all patients detailing their current and past medical history and inquiring of possible aetiological factors. The assessments made by the admitting doctors were recorded as were my own findings on examination.

Inclusion Criteria:

Diagnosis of acute pancreatitis

Patients were considered to have acute pancreatitis and to be suitable for inclusion in the study based on the presence of a serum amylase greater than 1200IU/l (normal range 70-300IU/l) with a compatible clinical picture. Patients in whom pancreatitis had been diagnosed at laparotomy were documented but not considered for inclusion in the formal study.

Severity of the attack

The patients underwent a clinical assessment when first seen, the severity of the attack being recorded as mild, moderate or

severe. This was repeated at 24 hours and 48 hours post-admission. The assessment of mild disease was based on the following criteria:-

(a) The patient appeared generally well.

(b) The patient had no pain, or only mild discomfort.

(c) The patient had only minimal abdominal tenderness.

Patients with mild pancreatitis were considered to be at low risk of developing complications and were not considered for inclusion in the formal study. Severe attacks comprised those patients judged to be clinically unwell and considered to be at risk of death or developing a major complication. Attacks were judged as moderate when they appeared to fall between these two definitions. All attacks judged to be moderate or severe were considered for randomisation into the formal study.

Patients judged to have a mild attack on the initial assessment but who subsequently deteriorated could be re-allocated into the moderate or severe categories and thus be randomised into the formal clinical study, providing this decision was made within 72 hours of the onset of their attack.

Age

Only patients aged over 16 and under 85 years of age were considered for inclusion in the study. Pancreatitis in children is rare and usually mild. In the very elderly the outcome of an attack often depends on factors such as their chronic health state and on the presence of other underlying illnesses and was considered

unlikely to be influenced by intraperitoneal therapy.

Delays in referral

Patients were only randomised when the delay between the onset of symptoms and assessment was less than 72 hours as commencement of therapy after this time was considered less likely to be beneficial.

Exclusion criteria

The presence of multiple abdominal scars is a contraindication to percutaneous introduction of a peritoneal lavage cannula and such patients were excluded from consideration for the formal study. Similarly intraperitoneal therapy was considered inappropriate during pregnancy and following recent abdominal surgery.

Because of the slight risk of anaphylaxis in patients who had previously received aprotinin, such patients were also excluded from consideration.

Randomisation

Patients fulfilling the inclusion and exclusion criteria were randomised by opening a consecutively numbered sealed envelope containing the randomisation details. These envelopes had been prepared in advance by Bayer UK Ltd. to provide a random allocation of equal numbers of patients into both the intraperitoneal therapy and standard therapy groups.

Standard therapy

Simple guidelines were distributed to each clinician and to every ward involved in the management of these patients and a copy filed in each patient's case record to standardise their investigation and routine care within each of the 6 participating hospitals.

All patients received intravenous fluid as crystalloid solutions (typically 1 litre 5% dextrose : 500ml 0.9% saline, 4 hourly), infused more rapidly or supplemented with human plasma protein fraction (albumin) as a plasma expander where deemed appropriate. The use of fresh frozen plasma (containing clotting factors and active antiproteases) was limited to instances of proven coagulopathy.

Nasogastric suction was advised for the more severely ill patients and in less severe attacks was left to the clinician's discretion. The pulse, blood pressure, axillary temperature and respirations were charted regularly every 4 to 6 hours. Bladder catheterisation was recommended with measurement of the hourly urine volumes for the first 24 to 48 hours, or until the patient was stable. The fluid balance was recorded daily for the first 7 days or until discharge if sooner. Measurement of the central venous pressure was suggested for those with clinically severe pancreatitis.

Parenteral analgesia was provided as intramuscular pethidine, typically 100mg every 3 to 4 hours as required. Other agents such as H_2 receptor antagonists, steroids or antibiotics were not administered except for precise indications.

Routine blood tests including urea, electrolytes, glucose, amylase, calcium, liver function tests and a full blood count were checked daily. Arterial blood gases were measured every 12 hours initially, and thereafter as indicated clinically. Oxygen was provided by mask if the pO_2 fell below 70 mmHg. Blood samples were also withdrawn daily, centrifuged at 4°C and the serum stored at -20°C for subsequent assay of C-reactive protein and antiproteases.

Details of the patients clinical condition, recordings, results of laboratory investigations and treatment were recorded on the trial proforma each day for the first 7 days. All the patients studied at the Royal Infirmary were examined personally each day as were the majority of the patients in Stobhill Hospital and the Southern General Hospital. Elsewhere the patients were examined daily for the first 3 days and then every 2 to 3 days until their discharge. In addition to the clinical assessment of severity, an objective assessment of severity was made based on the established multiple factor scoring systems, to permit later comparison of the groups. The severity assessments are presented and discussed in chapter 7.

Intraperitoneal therapy

All patients referred to the study received standard therapy as outlined above. Patients randomised to the treatment group underwent, subject to written informed consent having been obtained, peritoneal cannulation and intraperitoneal therapy with aprotinin.

Using sterile technique the skin and linea alba 4cm below the umbilicus were anaesthetised with 2% lignocaine and a peritoneal

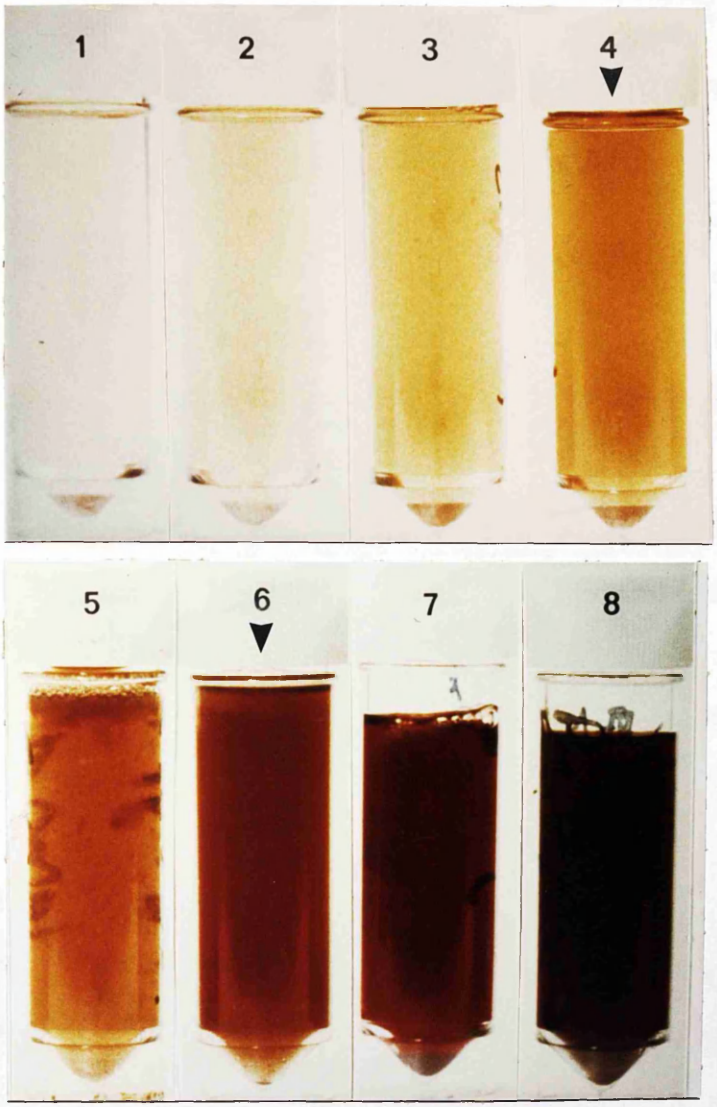
dialysis cannula (FG 8, Kimal Scientific Products Ltd., Uxbridge, UK) was introduced into the peritoneal cavity and its tip directed towards the pelvis.

Any free peritoneal exudate was aspirated into a syringe and then a litre of 0.9% saline solution was instilled. The patient was tilted head down and then turned on each side to circulate the fluid. After 5 minutes the fluid was allowed to drain out.

One litre of aprotinin solution (700mg of aprotinin in 0.9% saline, equivalent to 5 $x10^6$KIU) was then instilled and the patient was tilted and turned as before to circulate the fluid. The catheter was clamped and the fluid left in the peritoneal cavity for 8 hours before being drained off. A further litre of aprotinin solution (5 x 10^6KIU) was then instilled into the peritoneal cavity and the dialysis cannula removed leaving the aprotinin solution in situ. Thereafter these patients were managed exactly as all the other patients, with close monitoring of their clinical course and outcome.

The volume of any exudate aspirated at the initial cannulation was recorded and the colour compared with a standard colour chart (Fig. 12)[226]. A sample was sent to the bacteriology department for an immediate Gram film to exclude the presence of bacteria. The volume and colour of the lavage fluids and aprotinin instillations drained from the peritoneal cavity were also recorded. Samples of all fluids withdrawn and the tip of the peritoneal dialysis cannula were routinely cultured for organisms. Aliquots of all the samples were centrifuged at 4°C and the supernatants separated and stored at -20°C. Biochemical analyses of the protein and pancreatic enzyme

LAVAGE FLUID



FREE FLUID

FIGURE 12.

Leeds colour chart for identification of severity of acute pancreatitis by diagnostic peritoneal lavage. Arrows point to the lightest colour of the lavage return fluid or free peritoneal fluid for acceptance of a severe prediction. (McMahon MJ et al., Br J Surg 1980; 67:22-5. Reproduced by permission of the publishers, Butterworth & Co. (Publishers).

contents of all the fluid samples were performed, the results of which are presented and discussed in chapters 10 and 11.

Determination of aetiology

Each patient had a chest and plain abdominal radiograph performed on admission to hospital. During the admission an ultrasound examination of the liver, pancreas and biliary tree was performed on each patient.

The aetiology of the attack was determined based on the history of the patient's alcohol intake obtained either from the patient or from the relatives and on the presence or absence of gallstones on ultrasound examination, cholecystography or cholangiography, at laparotomy or at post mortem. Patients in whom the aetiology remained obscure often underwent further investigations including fasting blood lipids, viral screen and endoscopic retrograde cholangiopancreatography or had pancreatitis attributed to a recognised antecedent. The remaining patients in whom no aetiological factor could be identified were studied together as a group of "unknown aetiology".

Classification of outcome

Death or the development of a major complication were the primary end-points by which the effectiveness of therapy was judged. In addition any short-term effect of therapy which might not be reflected in the eventual outcome was investigated by assessment of the patient's clinical condition, by documenting the changes in

physiological and laboratory parameters and by recording the analgesic requirements.

Major complications were defined as follows:

1. pancreatic necrosis - necrotic pancreatic and/or peripancreatic tissue recovered at laparotomy or post mortem.
2. pancreatic abscess - a pancreatic or peripancreatic collection where pus was the predominant feature.
3. pancreatic pseudocyst - a pancreatic or peripancreatic fluid collection of greater than 5cm diameter, diagnosed on ultrasound or at laparotomy.
4. acute renal failure - urine volume of less than 400ml/24 hours with a rising blood urea and no response to 24 hours of intravenous fluid therapy.
5. acute respiratory insufficiency - arterial pO_2 less than 60 mmHg requiring ventilation or oxygen therapy by mask for 5 or more consecutive days.
6. myocardial infarction - consistent changes on serial ECG records accompanied by appropriate enzyme changes or when demonstrated at post mortem.
7. left ventricular failure - radiologically demonstrated pulmonary oedema supported by a raised pulmonary capillary pressure where diagnostic doubt exists.
8. septicaemia - a positive blood culture with a pathogen in a patient with an appropriate clinical course.

Attacks were classified as complicated if the patient died or developed a major complication. In addition to the above, minor complications were also recorded including acute confusional states, delirium tremens and pleural effusions. Note was also made of whether the patient appeared to be "slow to settle" from the acute attack, this being defined as either requiring a prolonged convalescence or requiring parenteral analgesia for more than 5 days following admission.

The duration of the hospital stay was recorded, particular note being made as to whether this was prolonged due to a complication having developed, due to cholecystectomy being performed during the index admission, or whether this was due to "social" reasons.

Statistics

Statistical analyses were performed by Fisher's exact test, Chi square test, and Mann-Whitney U test.

Results

During the 20 month period February 1986 to September 1987, 160 attacks of acute pancreatitis occurring in 151 patients were referred to the study and documented (Appendix 1). The attacks occurred in 98 males and 62 females with mean ages of 48.7 years and 58.5 years ($p < 0.001$) respectively.

The majority of the patients had been referred from the Royal Infirmary (89 patients), the remainder having been referred as follows: Stobhill General Hospital - 34 patients, Monklands District

General Hospital - 14 patients, Victoria Infirmary - 8 patients, Law Hospital - 8 patients and the Southern General Hospital - 7 patients.

Clinical features

Pain was the commonest presenting feature being recorded in all but 4 patients, 2 of whom presented in coma and 2 with a confusional state. Of the remaining attacks, 128 (82%) were associated with severe pain, 25 (16%) with moderate pain and in 3 instances the patients reported only mild pain. The duration of the pain prior to admission ranged between 30 minutes and 120 hours with a median duration of 9 hours. The pain radiated to the back in 93 (60%) of the attacks and 132 attacks (85%) were associated with vomiting. Five patients (3%) developed abdominal wall bruising, 3 of these patients subsequently died and the other 2 had attacks complicated by respiratory insufficiency, one of whom required a period of ventilation. One further patient developed body wall bruising on day 11 which was subsequently shown to be artefactual (self-inflicted).

Clinical assessment

The delay from admission to their initial assessment ranged between 1 hour and 60 hours, with a median delay of 5 hours. In 12 patients this delay exceeded 24 hours due to delays in confirming the diagnosis in 8 instances and to delays in referring the patient for assessment in 4.

Based on the initial clinical assessment, 104 attacks (65%)

were considered to be mild and were not randomised. Seven of these patients were reassigned the following morning (6 - moderate, 1 - severe) and ultimately 97 attacks (61%) were considered mild and were not, therefore, randomised.

Twelve further patients were excluded, 4 on the basis of a non-diagnostic serum amylase concentration, 3 on the basis of age (too old - 2, too young - 1), 2 because of recent abdominal surgery (diagnostic laparotomy), one because of delayed presentation of more than 72 hours from the onset of his attack and one because he had previously received aprotinin. The final patient had established acute renal failure on a background of recently diagnosed multiple myeloma and when first seen had just suffered a cardiovascular collapse. Randomisation and the possibility of intraperitoneal therapy was considered inappropriate under these circumstances and she was excluded. The remaining 51 attacks (32%) met all the inclusion and exclusion criteria and were randomised. These patients are examined in detail in chapter 12.

Thus 3 groups of patients have been considered here: those excluded because they were considered to have mild disease on clinical examination (97 patients), those excluded because of other criteria (12 patients) and those meeting all the criteria and who were entered into the formal study and randomised (51 patients) (Appendix 1).

Aetiology

Gallstones were documented in 59 attacks (37%) and were the

commonest aetiological factor identified. Alcohol abuse was implicated in 50 attacks (31%) and in 6 other attacks (4%) gallstones and alcohol were both implicated. In 13 attacks (8%) other recognised antecedents were diagnosed. Hyperlipidaemia was diagnosed in 4 patients, associated with alcohol abuse in 3 and in one was due to familial hypercholesterolaemia. Two patients developed acute pancreatitis following blunt abdominal trauma, one following an assault and one following a road traffic accident. Two patients developed pancreatitis following ERCP. In the remaining patients acute pancreatitis followed an operation for total hip replacement in one, a viral illness (with rising titres to coxsackie B_2) in one, commencement of steroid therapy in one, hypothermia in one and was associated with carcinoma of the pancreas in one.

No definite aetiological factor could be implicated in the remaining 32 attacks (20%). All except 2 of these patients, who died without post mortem permission having been granted, had at least an abdominal ultrasound performed to exclude the presence of gallstones.

The aetiology of the attack in relation to patient age and sex is shown in table 16. Table 17 details the categorisation of the patients into trial groupings in relation to the aetiology of their attack. Table 18 analyses the aetiologies of the attacks in relation to the referring hospital.

Recurrent attacks

Recurrent attacks were most commonly seen amongst those with pancreatitis secondary to alcohol abuse, 18 (36%) giving a history of

TABLE 17.

Categorisation of patients by trial groupings in relation to the recorded aetiology.

	Trial grouping			
	Not randomised			
Aetiology	Mild attack	Other exclusions	Randomised	Totals
Gallstones	40 (41%)	-	19 (37%)	59 (37%)
Alcohol	31 (32%)	5 (42%)	14 (27%)	50 (31%)
Gallstones /alcohol	5 (5%)	-	1 (2%)	6 (4%)
Other	5 (5%)	3 (25%)	5 (10%)	13 (8%)
Unknown	16 (16%)	4 (33%)	12 (24%)	32 (20%)

TABLE 18.

Analysis of the recorded aetiologies in relation to the referring hospital.

Hospital	Gallstones	Alcohol	Gallstones /alcohol	Other	Unknown
Royal Infirmary	24 (30%)	36 (40%)	3 (3%)	12 (13%)	14 (16%)
Stobhill Hospital	16 (47%)	7 (21%)	2 (6%)	-	9 (26%)
Monklands DGH	5 (36%)	5 (36%)	-	-	4 (29%)
Law Hospital	5 (62.5%)	-	-	-	3 (37.5%)
Victoria Infirmary	4 (50%)	1 (12.5%)	-	1 (12.5%)	2 (25%)
Southern General	5 (71%)	1 (14%)	1 (14%)	-	-

previous attacks. Five of these patients were admitted more than once during the period of the study, one having been admitted on 3 different occasions. In 6 (10%) of the patients in whom gallstones were identified there was a history of a previous attack, 4 within 4 months of their first attack and in the other 2 within 10 months and 2 years respectively. Four (12.5%) of the patients in whom no definite aetiological factor was identified had a history of previous attacks. Two of these patients each had one previous attack and 2 others had respectively 3 and 12 previous attacks. One of the patients with hyperlipidaemia in association with alcohol abuse also had a single previous attack.

Therefore, 29 patients had a history of previous attacks of acute pancreatitis and 9 of the total of 160 attacks documented were due to second or subsequent admissions during the actual study period. The recurrent attacks were often mild. Only 3 patients (10%) had a complicated recurrent attack (necrosis - 1, pseudocyst - 1, respiratory insufficiency - 1) with no deaths.

Outcome: deaths

A fatal outcome was recorded in 13 attacks (8.1%) overall and these patients are analysed in table 19. Nine of these deaths occurred amongst the 51 patients randomised into the formal study, the other 4 occurring amongst those excluded on the basis of age over 85 years (in 2), delayed presentation in one and on the presence of acute renal failure and cardiovascular collapse on initial assessment in one.

TABLE 19.

Analysis of the age, previous medical history, mode and timing of death in the fatal attacks of acute pancreatitis.

Patient	Aetiology	Age	Cause of death	Time of death (day)	Underlying illnesses
005	Unknown	65	fulminant pancreatitis	3	IHD, COAD, RHD
009	Gallstones	62	necrosis/sepsis/MOF	50	PVD, COAD, hypertension
043	Post-ERCP	86	fulminant pancreatitis /acute renal failure	3	frail
044*	Gallstones	69	fulminant pancreatitis DIC	2	-
045*	Alcohol	55	fulminant pancreatitis /necrosis	9	hypertension
055*	Trauma	73	necrosis/sepsis	19	hypertension
057	Steroids	80	acute renal failure /septicaemia	10	myeloma, IHD, cardiac failure
076*	Unknown	57	necrosis/sepsis	64	-
088*	Hypothermia	83	hypothermia/cardiac failure/bronchopneumonia	4	hypothermia, IHD
094	Unknown	85	sudden death	5	aortic aneurysm
106*	Gallstones	56	necrosis/sepsis/MOF	38	-
151*	Alcohol	29	fulminant pancreatitis /necrosis	2	fatty liver
156*	Gallstones /alcohol	66	fulminant pancreatitis /necrosis	4	-

KEY:

* post mortem performed, MOF - multiple organ failure, DIC - disseminated intravascular coagulation, PVD - peripheral vascular disease, COAD - chronic obstructive airways disease, IHD - ischaemic heart disease, RHD - rheumatic heart disease.

Seven deaths occurred early, within the first week of illness, 6 of them occurring within the first 4 days of admission. Shock associated with fulminant pancreatitis was the usual mechanism of death in these patients. One further patient mentioned above who had been excluded from the formal study because of age was known to have a large abdominal aortic aneurysm but had been turned down for surgery on the basis of poor general health. His clinical presentation during this admission supported a diagnosis of either acute pancreatitis or a leaking aneurysm although his sudden demise on his 5th day post-admission favoured the latter diagnosis. Unfortunately permission for a post mortem examination was declined by the relatives and the diagnosis of acute pancreatitis was never substantiated.

The other 6 patients died at varying times between 9 days and 64 days post-admission. Two patients dying on days 9 and 10 respectively both had a fulminant, shock-like illness with renal failure, one undergoing sub-total pancreatic resection prior to his death. The remaining patients succumbed of the late effects of pancreatic necrosis and sepsis despite surgical treatment in 3 of them (necrosectomy/pancreatic debridement in 2, pseudocyst drainage in one).

Outcome: major complications

Twenty-six patients developed major complications and survived. Eleven patients developed local pancreatic complications (pseudocysts - 7, abscess - 2, pancreatic necrosis - 2). Twelve patients developed

respiratory insufficiency and 3 septicaemia.

Pseudocysts

Pseudocysts were the commonest pancreatic complication encountered and followed pancreatitis of unknown aetiology in 3 patients, pancreatitis associated with alcohol abuse in 3 and followed gallstone pancreatitis in one. In all cases the pseudocyst was evident clinically or on ultrasound examination prior to the patient's discharge from hospital.

In 4 patients the pseudocyst resolved spontaneously on sequential ultrasound examination, the other 3 requiring formal surgical drainage. One of these patients was operated on early (day 21) because of diagnostic uncertainty on ultrasound, the appearances having been considered compatible with the presence of necrotic retroperitoneal lymph nodes perhaps secondary to tumour. A pseudocyst was found at operation, containing much necrotic debris, and was satisfactorily treated by external drainage. In the other two cases the pseudocysts were drained by cystogastrostomy 2 months after the acute attack.

Abscesses

Two patients developed pancreatic abscesses, in both cases the diagnosis only becoming apparent following discharge from hospital. A pancreatic complication had been suspected in one during his initial hospital stay because of a palpable epigastric mass although ultrasound examination at that time had failed to visualise any

abnormality other than pancreatic swelling. He was readmitted 4 months later because of a persistent mass associated with weight loss and malaise. At operation he was found to have a small residual abscess adjacent to the pancreatic head and this responded satisfactorily to drainage.

The second patient had been considered to have a mild attack of pancreatitis when first seen and had not been included in the formal study. She was slow to settle after her initial attack but was able to be discharged home on day 35. She started vomiting at home and when readmitted 2 weeks later was found to have a mass. At subsequent laparotomy an abscess was drained and cholecystectomy performed.

Necrosis

Two patients developed pancreatic necrosis and required surgery on day 6 and on day 40 respectively. The first patient was treated by distal pancreatectomy, requiring in addition a transverse colectomy because of involvement of the colon in the necrotizing process. His postoperative course was prolonged and complicated by a pancreatic fistula which eventually settled on conservative treatment. He was finally discharged on day 58 but subsequently developed jaundice due to common bile duct obstruction which has since necessitated further surgery.

The second patient developed evidence of a peripancreatic fluid collection and at laparotomy was found to have extensive pancreatic and peripancreatic necrosis. She was treated by debridement of the necrotic tissue on day 40 but further debridement and drainage of pus

was required on days 70 and 87. She subsequently developed a colonic fistula and required transverse colectomy and formation of stomas on day 102. After a protracted convalescence she was finally discharged on day 284.

Respiratory insufficiency

Twelve patients developed respiratory complications, 2 of whom required assisted ventilation for periods of 12 and 13 days respectively. In one of these patients this had followed diagnostic laparotomy.

The remaining 10 patients developed acute respiratory insufficiency with a pO_2 of less than 60 mmHg and requiring O_2 therapy by mask for 5 or more consecutive days (mean 8 days). Five of these patients had co-existing pleural effusions but none of these 12 patients developed other local pancreatic complications on serial ultrasound examination. Two of these patients had been considered to have mild pancreatitis when first seen after their admission and accordingly had not been randomised into the formal study.

Other complications

Three patients, 2 with gallstone pancreatitis and one with pancreatitis of unknown aetiology, developed septicaemia. The latter patient in whom no definite aetiology could be identified had gallstones suspected on biochemical criteria, but not proven. One of these patients was considered to have mild pancreatitis when first seen and accordingly had not been randomised. He also developed

severe left ventricular failure during his hospital stay, although myocardial infarction was not confirmed on either electrocardiogram or cardiac enzymes.

A total of 39 attacks (24.4%) had a complicated outcome resulting in death or a life-threatening complication from which the patient recovered. There were no significant differences in the median delays between the onset of the attack and admission, in patients with uncomplicated, complicated or fatal attacks. There appeared to be no important differences in the incidence of complicated attacks between patients referred from the 6 participating hospitals (Table 20). The lowest proportion of complicated attacks occurred in the patients where a gallstone or alcohol aetiology had been implicated (Table 21). The highest incidence of death and complications was found amongst patients with pancreatitis secondary to other identified aetiological factors.

Uncomplicated outcome

The remaining patients were considered to have had a mild attack of acute pancreatitis. Some manifested a clinically severe, although ultimately uncomplicated attack as has been defined above. Fifteen patients were recorded as "settling slowly" from their attack.

Two patients had recurrent attacks of acute pancreatitis during their index admission, one in association with gallstones, the other with pancreatitis of unknown aetiology, although she had undergone cholecystectomy with common bile duct exploration 3 years previously

TABLE 21.

Outcome and complications in relation to the recorded aetiology.

	Aetiology of attack				
Outcome	Gallstones	Alcohol	Gallstones /alcohol	Other	Unknown
Uncomplicated	45 (76%)	43 (86%)	4 (67%)	6 (46%)	23 (72%)
Complicated	11 (19%)	5 (10%)	1 (17%)	3 (23%)	6 (19%)
Pseudocyst	1	3	-	-	3
Abscess	1	-	-	-	1
Necrosis	-	1	-	1	-
Respiratory	7	1	1	2	1
Septicaemia	2	-	-	-	1
Fatal	3 (5%)	2 (4%)	1 (17%)	4 (31%)	3 (9%)

and on the current admission had biochemical abnormalities typical of a gallstone-associated attack.

Delirium tremens was recorded in 3 patients with alcohol associated pancreatitis in the absence of other complications. Two other women manifested a similar acute confusional state although alcohol abuse could not be incriminated with certainty in either case. Two patients developed small peripancreatic fluid collections (<5cm) on ultrasound examination. In both cases the pancreatitis was associated with alcohol abuse and in both cases resolution occurred on serial ultrasound scanning. One diabetic on insulin developed ketoacidosis but had an otherwise uncomplicated attack.

Two other patients were admitted with abdominal pain and hyperamylasaemia in associated with the presence of cysts, both in association with alcohol abuse. One patient had developed a pseudocyst following an earlier attack of pancreatitis, the second had multiple cysts in the head of his pancreas and was subsequently shown on ERCP to have features of chronic pancreatitis. In both instances, as the cysts appeared to have preceded the monitored episode of acute pancreatitis, they were not considered as complications.

The outcome of the attack in relation to the initial and final categorisation into trial groups is shown in table 22. A complicated outcome was recorded in only 4 patients (4%) considered at 48 hours to have a mild attack.

TABLE 22.

Outcome of the attack in relation to the initial and final categorisation of patients into trial groupings.

Category	Total patients	Outcome of attack: Uncomplicated	Complicated: Survived	Complicated: Fatal	Total % complicated
On admission					
Not randomised: Mild	104	96	7	1	8%
Other exclusions	12	5	3	4	58%
Randomised	44	20	16	8	55%
Final					
Not randomised: Mild	97	93	4	-	4%
Other exclusions	12	5	3	4	58%
Randomised	51	23	19	9	55%

Discussion

The estimated catchment populations of the 6 participating hospitals ranged from 152,418 to 224,295 although the number of patients referred from each varied by up to 10-fold. While this may be partly explained by a varying incidence of the disease within each individual hospital's catchment population, the likely explanation was variation in the pattern of referral, some hospitals referring only a small proportion of patients. Referral was often dependent upon a large number of resident "on-call" medical staff who individually may have encountered a suitable patient only infrequently. Despite regular reminders and communication with my colleagues in the 6 participating hospitals it was clear that this system of referral of patients was less than perfect. The referral system appeared to work best when emergency patients were admitted to one or two wards or areas in a given hospital, as was the case in the Royal Infirmary, Stobhill Hospital and Monklands District General Hospital.

There was no evidence that only severe cases were being referred (Table 20) and thus the study population was considered to be representative of acute pancreatitis in this area of the West of Scotland.

The patient populations studied in the current trial included some affluent suburban areas and also rural areas although the majority of patients derived from the housing schemes and industrialised, urban areas of northern and eastern Glasgow. The pattern of aetiological factors varied from hospital to hospital,

that of Monklands District General Hospital most closely resembling the Royal Infirmary, where the incidence of alcohol-associated attacks recorded was greatest. Elsewhere gallstone disease was the aetiological factor most commonly implicated.

Alcohol and gallstone-associated pancreatitis had the lowest mortality rates, half that recorded in patients with pancreatitis of unknown aetiology. The highest mortality rate was found amongst the patients with other identified aetiological factors, almost a third of whom died. It should be noted that these particular patients were often old, frail or had significant underlying medical problems.

Pancreatitis secondary to alcohol abuse and attacks of unknown aetiology were most often complicated by the development of a pseudocyst. Gallstone pancreatitis on the other hand was most often complicated by respiratory insufficiency. Such patients were often elderly and overweight, factors which may have contributed to both the presence of gallstones and the development of hypoxia.

Septicaemia alone appeared a weaker criterion upon which to judge an attack as severe but nevertheless 2 of the 3 patients developing septicaemia had prolonged admissions associated with a clinically severe illness. The other patient had a transient septicaemia associated with the presence of jaundice on admission, settling quickly thereafter. In all cases the septicaemia was associated with a biochemical picture of biliary obstruction and was thought to represent a cholangitis.

Recurrent attacks most often occurred in association with alcohol abuse. Six patients had recurrent attacks associated with

gallstones. Three were awaiting surgery and one had declined surgery following her initial attack. Fortunately the recurrent attacks were usually mild and no deaths occurred, although deaths amongst such patients have been noted previously (Chapter 3).

The categorisation of patients into the 3 trial groups on the basis of a clinical assessment appeared to be successful, wrongly predicting as mild only 4 attacks with an ultimately complicated outcome. Most patients with acute pancreatitis recover without complication and if the majority of patients with mild pancreatitis can be identified accurately soon after admission, they can be excluded from clinical trials leaving a study group with smaller numbers but a much higher complication rate. This is particularly important where therapy is invasive as in the present study where patients randomised to the treatment limb undergo peritoneal cannulation.

Diagnostic peritoneal lavage is an alternative means of predicting the likely outcome early in the course of an attack and was used for the majority of the study patients in the recent UK trial of formal peritoneal lavage[235]. It is an invasive test and removal of the peritoneal fluid by aspiration and a single lavage may itself be therapeutic. It was the aim of the current study protocol that the control group should have no peritoneal intervention so that any effect of intraperitoneal therapy with aprotinin could be more clearly attributed to that particular therapeutic modality.

Multiple factor scoring systems are non-invasive but often entail a delay of 48 hours before an answer is obtained. Therapy for

acute pancreatitis should ideally be commenced as soon as possible after diagnosis and therefore the decision to randomise or not was based primarily on clinical assessment of the severity. The assessment as described here has been shown to provide an acceptable compromise, accurately selecting a group of patients at higher risk of complication, for investigation by controlled clinical trial. A formal analysis of the accuracy of clinical assessment in comparison with the standard multiple factor scoring systems is detailed in chapter 7.

CHAPTER 5. EXAMINATION OF PANCREATIC ENZYMES AND ANTIPROTEASES IN PERITONEAL EXUDATES AND FLUID SAMPLES - EXPERIMENTAL METHODS

Introduction

The aims of this study were to describe and validate the biochemical methods used to determine the pancreatic enzyme activities, protein and antiprotease contents and the trypsin inhibitory capacity of peritoneal exudate and fluid samples following intraperitoneal therapy.

Methods

Samples of peritoneal exudate were obtained from rats with experimental acute pancreatitis (chapter 9) and from humans with acute pancreatitis and other gastrointestinal emergencies (chapter 10). Additional samples of peritoneal fluid were obtained from patients with acute pancreatitis randomised to receive intraperitoneal antiprotease therapy, following peritoneal lavage with a single litre of saline and 8 hours after the initial instillation of aprotinin (chapters 4 and 11). Samples of pseudocyst fluid were also obtained at the time of cystogastrostomy or percutaneous cyst drainage (chapter 10).

In all cases the samples were centrifuged at $4^{o}C$ and the supernatants separated and deep frozen, within one hour of sampling. Aliquots were stored at $-20^{o}C$ until assay. Samples for protein, albumin, $alpha_2$macroglobulin, $alpha_1$antiprotease, amylase and lipase

measurement were stored for up to 3 months prior to assay, those for protease activity were usually assayed within one month.

The following determinations were carried out in the Department of Pathological Biochemistry at the Royal Infirmary. Total protein and albumin were measured by standard techniques on a Hitachi 704 Analyser using the biuret and bromcresol green methods respectively. Amylase was measured by the Phadebas test (Pharmacia Diagnostics, Uppsala, Sweden). Samples for $alpha_2$macroglobulin and $alpha_1$antiprotease assay were diluted with 4% polyethylene glycol 6000 in 0.9% saline and measured by a conventional rate turbidimetric immunoassay on a Baker Encore Centrifugal Analyser, using appropriate anti-human antibodies and standards (Atlantic Antibodies, Winnersh, Berkshire, UK). The normal ranges in serum ($alpha_2$macroglobulin 1.1 - 3.9g/l, $alpha_1$antiprotease 1.3 - 3.2g/l) had been established on samples from 100 normal controls. The remaining analyses were performed personally in the laboratories of the University Department of Surgery.

Lipase

Lipase activity was measured utilising the Lipase UV System (Boehringer Mannheim, West Germany). Aliquots of 50µl of sample were added to 1.25ml of reagent solution, mixed and poured into a cuvette. The decrease in turbidity at 365nm over a 5 minute period was followed in a spectrophotometer (Pye Unicam, SP6-550 UV/VIS) at 25°C. Prior to analysing each batch of samples the absorbance change of the lipase standard was determined to permit subsequent calculation of

the lipase activities. As the lipase activities recorded in peritoneal exudate were high the samples were pre-diluted with 0.9% saline, as recommended by the manufacturers, to bring the measured absorbance change within scale. The between batch coefficient of variation for the assay was 5.7% at a mean lipase activity of 16870u/l.

Tryptic amidase activity

Tryptic amidase activity was measured by Trypsin test kit (Boehringer Mannheim, West Germany), a colorimetric method utilising a low molecular weight chromogenic substrate benzoylarginine-p-nitroanilide (BAPNA), first described by Erlanger and co-workers[97]. BAPNA is a highly sensitive and specific substrate for trypsin. Cleavage of an amide bond by trypsin results in the liberation of p-nitroaniline and the resultant absorbance change is measured at 405nm. Tryptic amidase activity reports free trypsin and also trypsin-alpha$_2$macroglobulin complexes, in which trypsin retains activity against low molecular weight substrates such as BAPNA. Plasmin also hydrolyses BAPNA, at a very much slower rate than trypsin, and thrombin only extremely slowly[116].

Sequential dilutions of trypsin were freshly prepared in 0.9% saline to give a range of concentrations between 1µg/ml and 50µg/ml (Bovine trypsin, ethanol precipitate, T-8003, Sigma Chemical Co., St. Louis, USA. The same batch of trypsin, Lot No. 25F-8120, was used for all studies reported in this thesis). An aliquot of 100µl of trypsin solution at each trypsin concentration was added to 1.1ml of the

BAPNA substrate/tris buffer solution which had been pre-incubated at 25°C for 5 minutes, rotamixed and then poured into a cuvette. A blank was prepared substituting 100µl of 0.9% saline for the trypsin solution. Absorbance was recorded in a spectrophotometer (Pye Unicam, SP6-550 UV/VIS) before and after incubation at 25°C for one hour. By subtracting the absorbance change in the blank, the net absorbance change was calculated for each trypsin concentration, permitting construction of a standard curve. Stability of the standard trypsin solution (50µg/ml) was examined by assaying tryptic amidase activity in 3 samples before and after storage for one month at -20°C.

Peritoneal exudate samples were studied in 100µl aliquots. Some samples in which the measured absorbance at the beginning of the test was "off-scale" were repeated after dilution of the sample with 0.9% saline. Stability of the tryptic amidase activity in peritoneal exudates was examined in 8 samples assayed before and after storage for 6 months at -20°C. The between batch coefficient of variation for this assay was 3.3% at a trypsin concentration of 50µg/ml.

Free proteolytic activity

The fibrin plate assay is an established method for studying plasminogen activator activity in euglobulin fractions prepared from plasma. The euglobulin fractions are resuspended in buffer and applied to a plasminogen-rich fibrin plate, the area of fibrinolysis after 18 hours incubation reflecting the amount of enzymatically converted substrate and thus quantifying the plasminogen activator

activity[13]. Heating the fibrin plate or utilising a plasminogen-free fibrinogen in the preparation of the plate prevents this application and the plate becomes suitable for the assay of free plasmin or other proteolytic enzymes. In the present study the free proteolytic activity of peritoneal exudate samples was assessed by examining for fibrinolysis on a plasminogen-free fibrin plate as described by Wendt and co-workers[376].

Fibrin is a high molecular weight protein substrate resistant to attack by alpha$_2$macroglobulin bound protease. The finding of fibrinolysis in the peritoneal exudate samples would, therefore, be indicative of the presence of free proteolytic activity rather than alpha$_2$macroglobulin bound protease. This assay is not specific for trypsin but was used as an indicator of whether activated proteolytic enzymes (trypsin being the major pancreatic proteolytic enzyme) had overcome the peritoneal antiprotease defences.

The fibrin plates were prepared using 9cm plastic petri dishes to which was added a solution of bovine plasminogen-free fibrinogen (Poviet BV, Holland supplied by Organon Tecknica, Cambridge, UK) in Owren's buffer (Na diethylbarbitone - 11.756g, Na Cl - 14.7g, molar HCl - 43mls, made up to 2 litres with distilled water and adjusted to pH 7.35 with HCl). The fibrinogen was clotted by mixing with 25 NIH units of bovine thrombin (Fibriquik Thrombin Reagent, General Diagnostics, USA) in 0.5mls of Owren's buffer and the fibrin plate was allowed to clot on a level surface at room temperature for one hour prior to use. The samples for analysis were applied by automatic pipette in 20µl aliquots to the surface of each of the 4 quadrants of

the plate. Fibrinolytic activity was assessed after an 18 hour overnight incubation at 37°C by measuring 2 perpendicular diameters of the lysis zone surrounding each sample application and the lysis area calculated.

An initial experiment using 20µl applications of a freshly prepared, bovine trypsin solution (50µg/ml in 1mmol $CaCl_2$ and 1mmol HCl/litre of distilled water) investigated the effect of varying the volume (10 or 20mls) and concentration (between 0.2% and 2%) of fibrinogen used in preparation of the fibrin plate.

Using standard fibrin plates (10mls of 0.2% fibrinogen) the areas of lysis resulting from the application of varying concentrations of bovine trypsin solution (5µg/ml to 50µg/ml, prepared as above) were determined, permitting construction of a standard curve. The sensitivity of the technique was examined by assaying concentrations of bovine trypsin solution down to 1µg/ml. The stability of the standard trypsin solution (50µg/ml) was examined by measuring the fibrinolysis in 9 samples before and after storage for one month at -20°C.

Studies on peritoneal exudates utilised duplicate 20µl samples with bovine trypsin solution (50µg/ml, prepared as above) as a control. The between batch coefficient of variation for this assay was 11% at a trypsin concentration of 50µg/ml.

Trypsin binding capacity

The trypsin binding capacities of the peritoneal exudates were measured to obtain a functional assessment of the overall

antiprotease reserve available for protease binding. This technique was first described in 1966 using BAPNA and casein as indicators of esterolytic and proteolytic activities respectively. The trypsin binding capacity was determined by adding increasing increments of trypsin to a solution containing 50µl of serum. The point at which the added trypsin exceeded the trypsin binding capacity of serum was indicated by a marked increase in the esterolytic and proteolytic activities, similar to the activities obtained for trypsin alone without the addition of serum[120]. A subsequent report described p-toluene-sulphonyl-arginine methyl ester (TAME) as a suitable indicator of proteolytic activity[6]. In the current study their methodology was modified, utilising the fibrin plate assay, as described above, to detect the occurrence of free proteolytic activity once the antiprotease reserve had been overcome by the added trypsin.

Fibrin plates (10mls of 0.5% fibrinogen) were prepared as described above. Increments, usually of 10 or 20µg of freshly prepared bovine trypsin solution (1mg/ml in 5mmol $CaCl_2$ and 5mmol HCl/litre of distilled water), were added to a series of plastic test tubes containing 100µl of peritoneal exudate to provide a range of trypsin concentrations of between 0 and 160µg/100µl of exudate. The volume in each test tube was made up to 1ml by adding tris buffer (Trizma - 6.06g, concentrated HCl - 1.236ml, made up to one litre with distilled water and adjusted to pH 7.4 with HCl). The test tubes were then rotamixed and incubated at room temperature (21°C) for 15 minutes. Aliquots of 20µl of the exudate/buffer solution containing

increasing concentrations of trypsin were applied sequentially by automatic pipette to the surface of a fibrin plate. After an 18 hour overnight incubation at 37°C the plates were read. The trypsin concentration at which lysis first appeared on the fibrin plate was considered to represent the point at which the trypsin binding capacity of the sample had been exceeded (Fig. 13).

The trypsin binding capacities of the 4 solutions used for intraperitoneal therapy (chapter 9) were also determined in a series of experiments, substituting 100µl of each test solution for the peritoneal exudate. The 4 solutions examined were as follows :- 0.9% saline, human plasma protein solution[+] (predominantly albumin, 45g/l, and devoid of antiproteases and other active plasma factors), fresh frozen plasma[*] and aprotinin (5000KIU/ml 0.9% saline). The same unit of fresh frozen plasma was used for all studies reported in this thesis. Aliquots of 20µl of the test solution/buffer solution containing increasing concentrations of trypsin, ranging from 0 to 450µg/100µl of test solution, were applied to the surface of a fibrin plate, incubated for 18 hours and read as above. Duplicate samples of 100µl of each concentration of trypsin were also added to 1.1ml of BAPNA substrate/tris buffer solution, which had been pre-incubated at 25°C for 5 minutes, and the absorbance changes recorded over one hour as detailed earlier.

[+]Scottish National Blood Transfusion Service Protein Fractionation Centre, Edinburgh.

[*]Glasgow and West of Scotland Blood Transfusion Service, Law Hospital, Carluke.

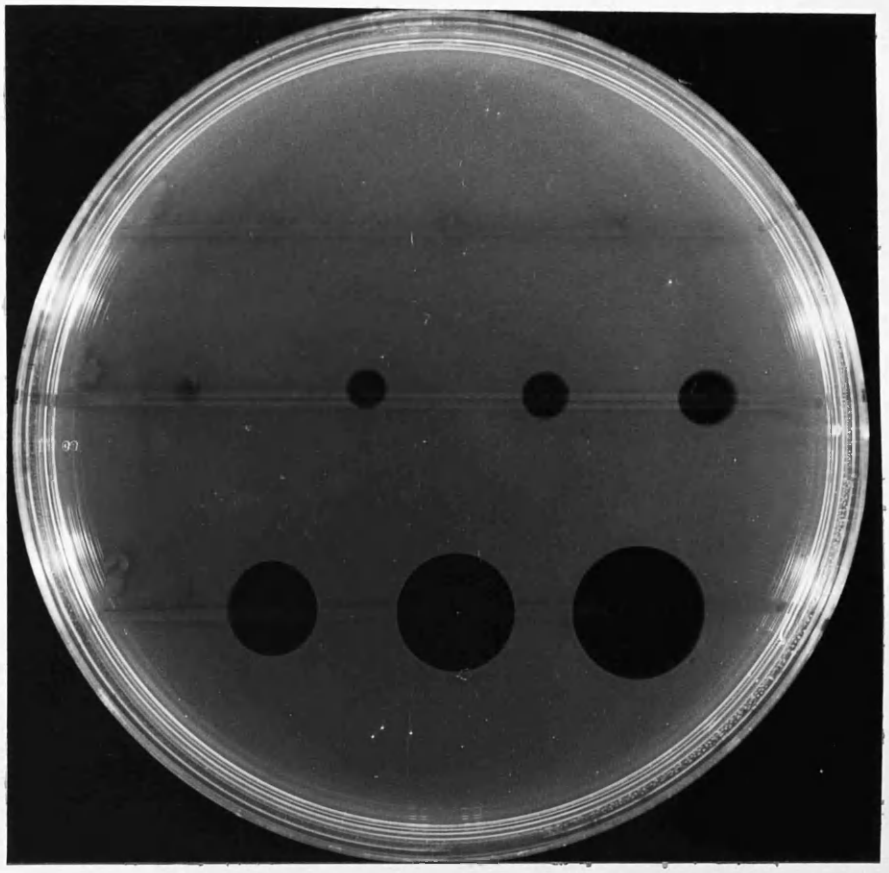


FIGURE 13.

Example of fibrin plate assay for the trypsin binding capacity. Increasing concentrations of trypsin/100ul of sample are applied sequentially to the surface of the plate. Lysis first appears at the 4th application of trypsin corresponding to a trypsin binding capacity of 40ug trypsin/100ul. Concentrations exceeding the binding capacity are seen to give rise to progressively greater lysis areas.

Statistics

The results are expressed as means ± s.e.m. Differences between paired samples were analysed using the Wilcoxon rank sum test.

Results

Peritoneal exudate had the characteristics of slightly dilute serum and no difficulties were encountered with the assays of proteins, antiproteases, amylase and lipase, other than the requirement for pre-dilution of the samples prior to enzyme assay, because of the high activities recorded.

BAPNA-splitting activity

A standard curve was constructed to permit comparison of the absorbance change with the equivalent bovine trypsin concentration in μg/ml (Fig. 14). This produced a linear plot (r = 0.999). BAPNA was confirmed to be a highly sensitive substrate detecting trypsin concentrations as low as 1μg/ml.

Difficulties were encountered with haemorrhagic peritoneal exudate samples obtained from patients with the most severe attacks of acute pancreatitis and from rats with experimental acute pancreatitis. Despite pre-dilution of the samples the absorbance often remained "off scale" at the wavelength of 405nm required for the measurement of p-nitroaniline and tryptic amidase activity could not, therefore, be assayed in such samples.

Trypsin 50μg/ml prepared in 0.9% saline showed no significant

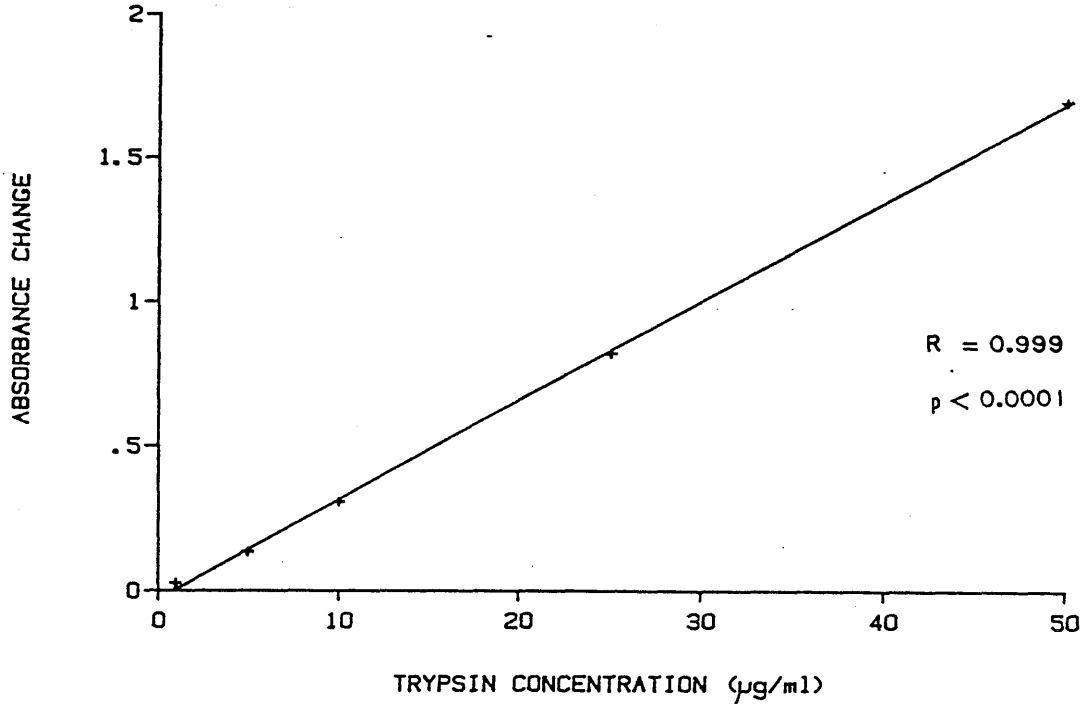


FIGURE 14.

Standard plot of BAPNA-splitting activity (tryptic amidase) against increasing trypsin concentrations. Activity is expressed as the change in absorbance units A_{405nm}/hour.

loss of activity after storage for one month at -20°C (1.71 ± 0.023 vs 1.66 ± 0.025, p = 0.42). Eight peritoneal exudate samples assayed before and after 6 months storage at -20°C showed an average 17% loss of activity (0.117 ± 0.055 vs 0.097 ± 0.038, p = 0.73).

Free proteolytic activity

The technique described by Wendt and co-workers utilised a 2% solution of fibrinogen for preparation of the fibrin plates[376]. Using 10mls of solution instead of 20mls and a lower concentration of fibrinogen resulted in a larger areas of lysis, although associated with some asymmetry of the circular lysis zones (Table 23).

A 0.2% fibrinogen solution was adopted as the standard for the fibrin plates and this increased the sensitivity of the technique, trypsin concentrations as low as 2μg/ml being detected, resulting in areas of partial thickness lysis of less than 4mm diameter. Increasing the trypsin concentrations between 5 and 50μg/ml produced a linear relationship with the lysis area and allowed a standard curve to be plotted (r = 0.994) (Fig. 15).

Trypsin 50μg/ml in 1mmol $CaCl_2$ and 1mmol HCl/litre of distilled water showed no significant loss of fibrinolytic activity during storage for one month at -20°C (mean lysis diameter 18.56 ± 0.212 vs 18.17 ± 0.486, p = 0.26).

Trypsin binding capacity

Duplicate assays and assays repeated on successive days demonstrated the test to be reproducible, providing a measure of the

TABLE 23.

Effect of varying volume and concentration of fibrinogen used in the preparation of the fibrin plates on lysis area.

Fibrinogen concentration	Volume	Lysis zone diameters (mm)	Mean diameter	Area (mm^2)
2% fibrinogen	20mls	10x10, 10x10 10x10, 10x10	10	78.5
2% fibrinogen	10mls	10x10, 10x10 10x10, 10x10	10	78.5
1% fibrinogen	10mls	11x11, 11x11 12x12, 12x12	11.5	104
0.5% fibrinogen	10mls	14x15, 14x14 15x15, 16x17	15	177
0.25% fibrinogen	10mls	18x17, 18x17 18x17, 19x19	17.9	251
0.2% fibrinogen	10mls	18x18, 19x18 19x18, 20x18	18.5	269

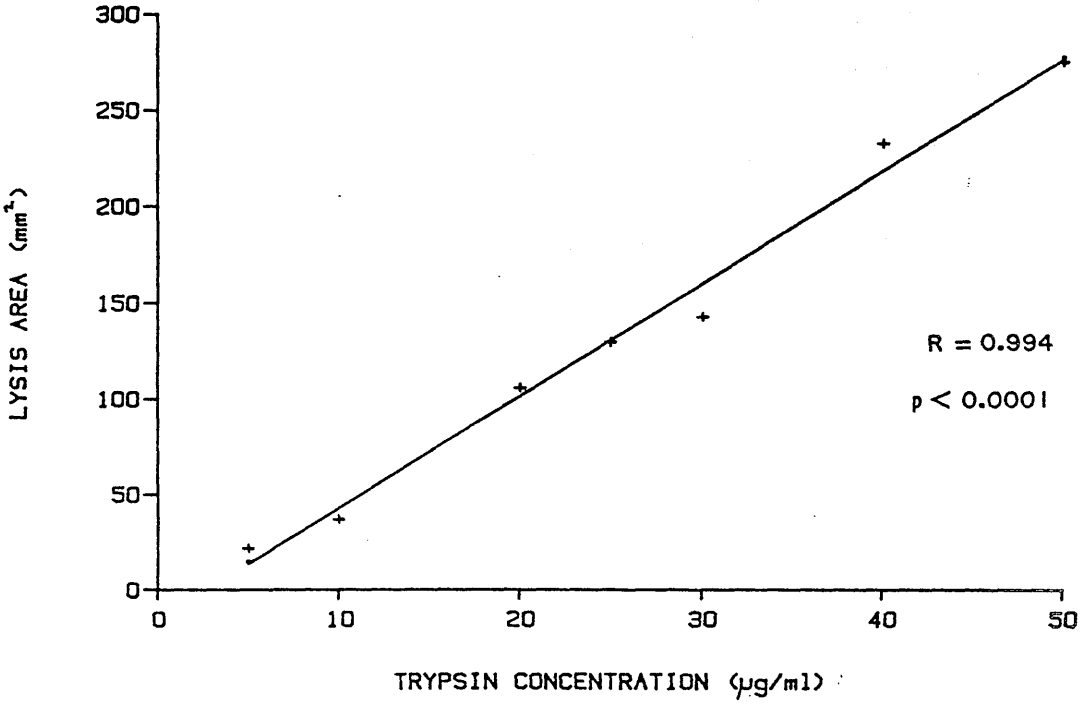


FIGURE 15.

Standard plot of fibrin plate lysis area against increasing trypsin concentrations.

trypsin binding capacity of the peritoneal exudates within the 10 - 20μg increments of trypsin/100μl of sample studied. The results of these assays are detailed in chapter 10.

Examining the solutions used for intraperitoneal therapy, both fibrinolytic activity and tryptic amidase activity were found after the addition of 5μg of trypsin to 100μl of either saline or human plasma protein solution (Fig. 16). Adding increasing increments up to 80μg of trypsin per 100μl of fresh frozen plasma produced no fibrinolysis after overnight incubation. Fibrinolysis was first recorded on the fibrin plate after the addition of 100μg of trypsin to 100μl of human fresh frozen plasma. Monitoring tryptic amidase activity, the absorbance change increased progressively on the addition of 5μg and 10μg increments of trypsin until 20μg of trypsin had been added after which the increase in absorbance change recorded tended to level out. The absorbance change again increased sharply with the addition of 100μg of trypsin indicating that the inhibitory capacity had been overcome at this point (Fig. 16).

Aprotinin solution demonstrated the greatest trypsin binding capacity and was able to inhibit 400μg of added trypsin, the binding capacity being overcome on both the fibrin plate assay and by the appearance of tryptic amidase activity only after the addition of 450μg of trypsin to 100μl of aprotinin solution (Fig. 16). The trypsin binding capacities of the 4 test solutions were, therefore, shown to be identical using either the fibrin plate assay or tryptic amidase activity.

Aprotinin completely inhibited the tryptic amidase activity of

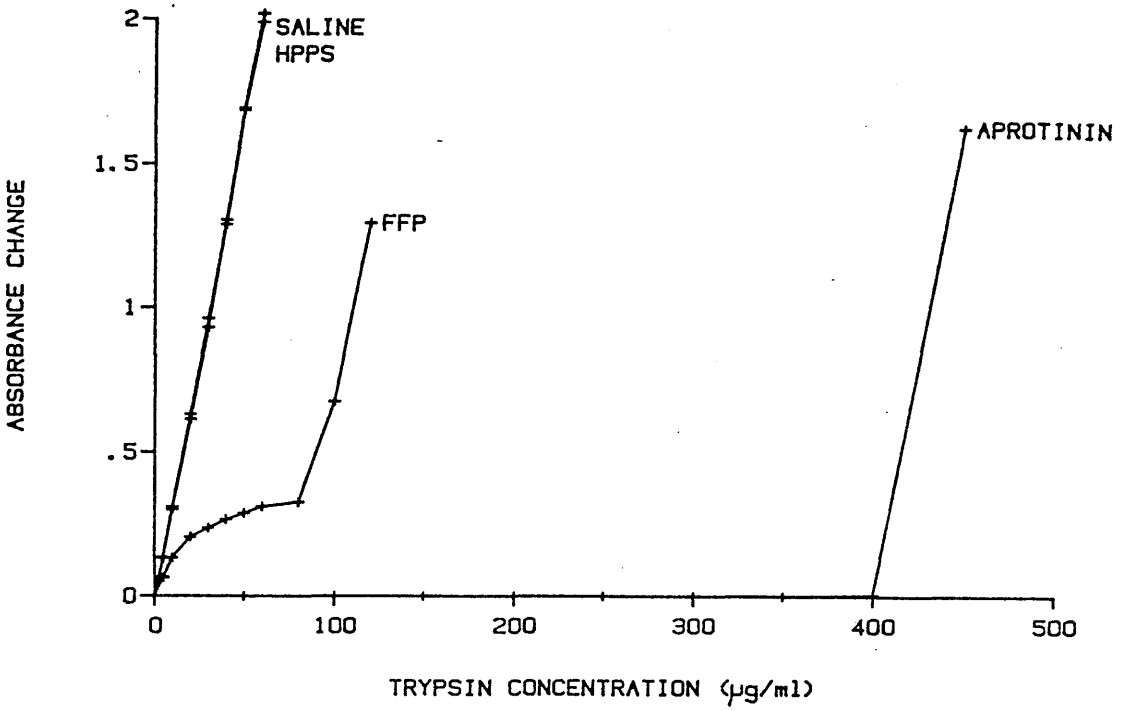


FIGURE 16.

Trypsin binding capacity of the 4 test solutions according to their BAPNA-splitting activity on addition of bovine trypsin. Activity is expressed as the change in absorbance units A_{405nm}/hour. No measurable trypsin binding capacity was demonstrated for either saline or HPPS (human plasma protein solution). The binding capacity of FFP (fresh frozen plasma) was exceeded after 80ug trypsin/100ul and for aprotinin after the addition of 400ug trypsin.

the added trypsin until its binding capacity was overcome, unlike fresh frozen plasma where the tryptic amidase activity of trypsin was shown to persist, presumably within the $alpha_2$macroglobulin complex, prior to the other antiprotease molecules being overwhelmed when fibrinolytic activity became evident.

PART 4.

PREDICTION OF OUTCOME

When you can measure what you are speaking about, and can express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind.

(Lord Kelvin, 1824-1907).

CHAPTER 6. C-REACTIVE PROTEIN, ANTIPROTEASES AND COMPLEMENT FACTORS AS OBJECTIVE MARKERS OF SEVERITY IN ACUTE PANCREATITIS

Introduction

Laboratory prediction of severity in acute pancreatitis has become accepted since 1974 following Ranson's first description of a scoring system utilising 11 objective factors, each with significant prognostic value[290]. Imrie and co-workers subsequently published a simplified scoring system, modified for use in UK patients and based on 9 factors[159].

Multiple factor scoring systems reflect respiratory, renal, hepatic, and haematological disturbance in the body and provide some clinically useful information, but may be influenced by the patient's pre-admission state and the aetiology of the attack. The greater age of the patients and the early, transient liver dysfunction commonly associated with gallstone pancreatitis results in such patients consistently scoring higher than an alcohol-associated attack of equivalent severity. This has lead to modifications being made to both major scoring systems; Ranson now describes two different scoring systems, one for gallstone and one for non-gallstone attacks[286] while the Glasgow system, which has been simplified to include only 8 factors[269] remains applicable to both common aetiologies of acute pancreatitis[69].

Multiple factor scoring systems have been widely used in the study of patients with acute pancreatitis, providing stratification of the risk of death or major morbidity and comparison of patients

between different centres. Their complexity, however, probably limits their routine use in daily clinical practice. Neither system can be applied sequentially to monitor the course of the attack which might usefully assess the response to therapy or provide warning of a developing late complication.

Other possibly useful indicators of severity in acute pancreatitis include complement factors[20,52,110,199], and the major serum antiproteases alpha$_2$macroglobulin and alpha$_1$antiprotease[52,228]. The value of C-reactive protein (CRP) in acute pancreatitis was first suggested from Leeds in 1984[233] and subsequently by others[52].

A comparative study examining the ability of multiple factor scoring systems and these individual biochemical factors to predict outcome early in the course of the illness has not been reported, although a persistently high CRP concentration at the end of the first week of the illness appears to identify patients at risk of developing a late complication[233].

The aims of the current study were to examine prospectively the value of sequential measurement of five complement factors, the two major antiproteases and the non-specific inflammatory marker CRP firstly, as early predictors of outcome in acute pancreatitis relative to clinical assessment and the multiple factor scoring systems[269,290], and secondly in monitoring the course of the disease.

Methods

Acute pancreatitis was diagnosed initially in all patients on the basis of a serum amylase >1200IU/l (normal range 70-300IU/l,

Phadebas, Pharmacia Diagnostics AB, Uppsala, Sweden) and a compatible clinical picture[157].

The patients studied fell into two groups; 30 patients had complement factors assayed and 72 patients had antiproteases and CRP assayed. The first 50 patients having antiproteases and CRP measured were studied consecutively, the remaining 22 patients were selected for study from the next 60 patients admitted, having been judged as having moderate or severe pancreatitis on the basis of a clinical assessment on admission (chapter 4).

Clinical assessment was repeated at 24 and 48 hours, those judged to have severe pancreatitis on assessment being considered at risk of death or developing a complication (see below).

The details of the patients' age and sex, aetiologies and complications are shown in table 24. Complicated attacks of acute pancreatitis were defined as resulting in death or a local pancreatic complication: abscess, pseudocyst or necrosis (defined as necrotic pancreatic and/or peripancreatic tissue recovered at laparotomy or post mortem). Nine additional patients manifested acute respiratory insufficiency defined as a $pO2 < 60mmHg$, requiring O_2 by mask for more than 5 consecutive days (mean 8 days) and were included. Each had a prolonged, clinically severe illness with a mean hospital stay of 20 days although ultrasound scanning in each failed to demonstrate the development of local pancreatic complications.

Multiple factor scoring systems

Laboratory data was collected prospectively from all patients

TABLE 24.

Comparison of age, sex, aetiology and outcome from acute pancreatitis in patients having measurement of complement factors or C-reactive protein and antiproteases.

	Complement factors (n = 30)	C-reactive protein & antiproteases (n = 72)
Sex	21 M : 9 F	47 M : 25 F
Mean age (years)	49.3 ± 3	52.2 ± 2.1
Aetiology: gallstones	14 (47%)	24 (33%)
alcohol	10 (33%)	23 (32%)
other	2 (7%)*	8 (11%)†
unknown	4 (13%)	15 (21%)
gallstones/alcohol	-	2 (3%)
Mean prognostic factor score: Glasgow (8 factors)	2.3 ± 0.36	2.1 ± 0.22
Ranson (11 factors)	2.7 ± 0.43	2.8 ± 0.28
Complicated attacks:	9 (30%)	25 (35%)
fulminant pancreatitis	1	5
necrosis	4	7
pseudocyst	1	4
respiratory insufficiency	3	9
Fatal	2 (7%)	10 (14%)

* familial hyperlipidaemia - 2.
† hyperlipidaemia/alcohol - 2, familial hyperlipidaemia - 1, viral - 1, trauma - 1, steroids - 1, ERCP - 1, hypothermia - 1.

to permit scoring of both the Ranson and Glasgow multiple factor systems[269,290] (Table 27). As the aetiology of the attack was not usually apparent on admission the original Ranson scoring system has been used throughout.

Experimental methods

Blood samples were taken on the day of admission (day 1) and each morning thereafter until day 8 or until discharge if sooner. Samples were centrifuged and the supernatants frozen within 60 minutes of venepuncture.

Complement factors were measured on plasma samples stored at $-60^{0}C$ until analysis. C3 and C4 were measured by radial immunodiffusion (Behring Diagnostics, Hounslow, Middlesex, UK) and factor B by immunodiffusion using an in-house prepared agar plate containing anti-human properidin factor B (Atlantic Antibodies, Winnersh, Berkshire, UK). Clq and C3d were measured on a Beckman ICS nephelometer using anti-human Clq (Atlantic Antibodies) and anti-human C3d (Dako Ltd., High Wycombe, Buckinghamshire, UK) according to the method of Vergani and co-workers[366].

Antiproteases and CRP were measured on serum samples stored at $-20^{0}C$ prior to analysis. $Alpha_2$macroglobulin and $alpha_1$antiprotease were measured by turbidimetric immunoassay (Atlantic Antibodies). CRP was measured by a fluorescence polarisation immunoassay (Abbot TDX, Abbot Laboratories Ltd., Wokingham, Berkshire, UK).

Normal complement and acute phase protein ranges were established on 30 and 100 normal controls respectively.

Statistics

Results are expressed as means ± s.e.m. Statistical analysis was performed by the Mann-Whitney U test. The diagnostic performance of the various tests and scoring systems have been analysed by sensitivity, specificity, predictive value and efficiency[119].

Results

Complement factors

Complement factors C3 and factor B concentrations showed an early fall followed by a sustained rise during the course of the illness in both mild and complicated attacks, factor B rising above the normal range in both groups of patients (Figs. 17,18). The individual results showed a wide scatter and no difference emerged between mild and complicated attacks. C4 concentrations showed no significant changes in either mild or complicated attacks through the course of the illness (Fig. 19). Clq levels fell during the attack, falling below the normal range in complicated attacks, but these differences failed to reach statistical significance (Fig.20). C3d levels (a product of C3 activation) were increased in 33% of patients at some time during their illness, thus indicating complement activation, but this could not be related to the severity of the attack judged clinically or on multiple factor scoring (Fig. 21).

Antiproteases

$Alpha_2$macroglobulin levels fell during the attack with levels

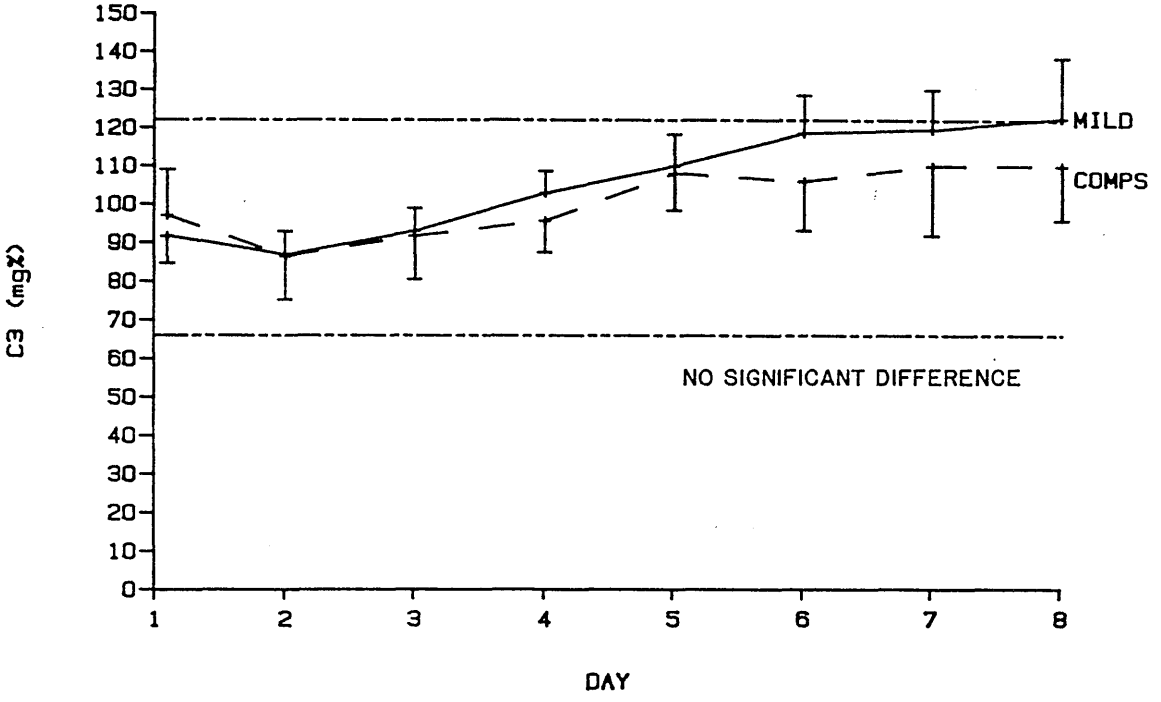


FIGURE 17.

Sequential changes of complement factor C3 during the course of acute pancreatitis in 21 patients with an uncomplicated outcome (mild) and 9 with a complicated outcome (comps).

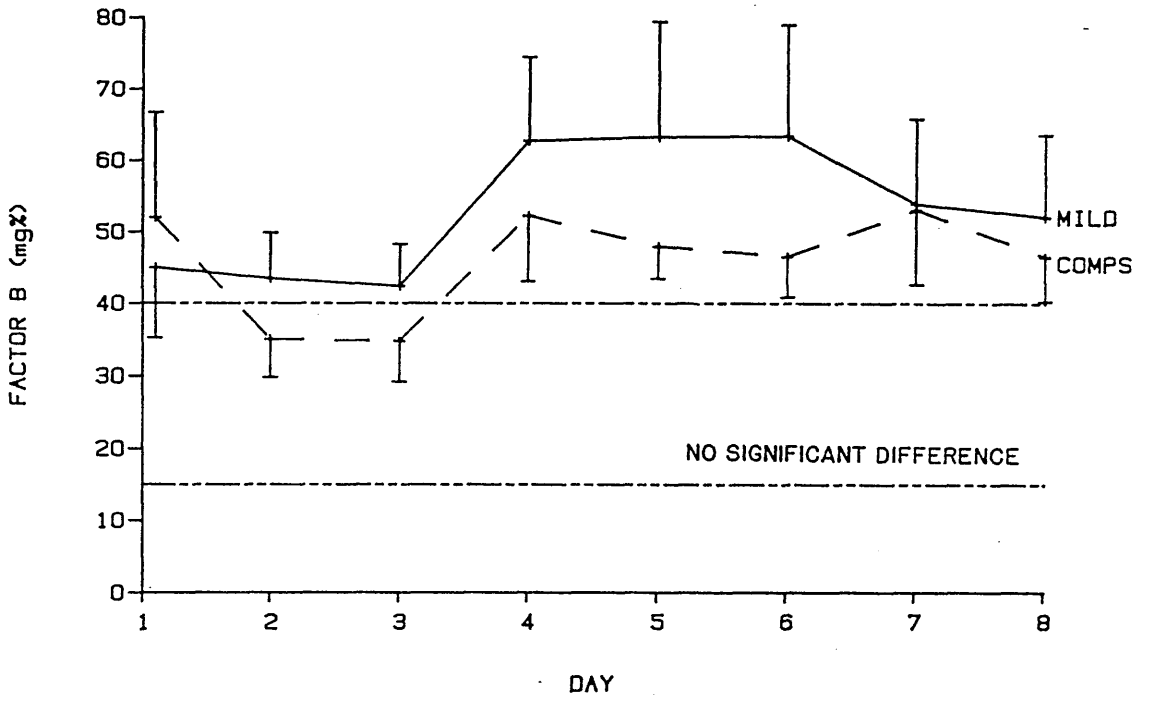


FIGURE 18.

Sequential changes of complement factor B during the course of acute pancreatitis in 21 patients with an uncomplicated outcome (mild) and 9 with a complicated outcome (comps).

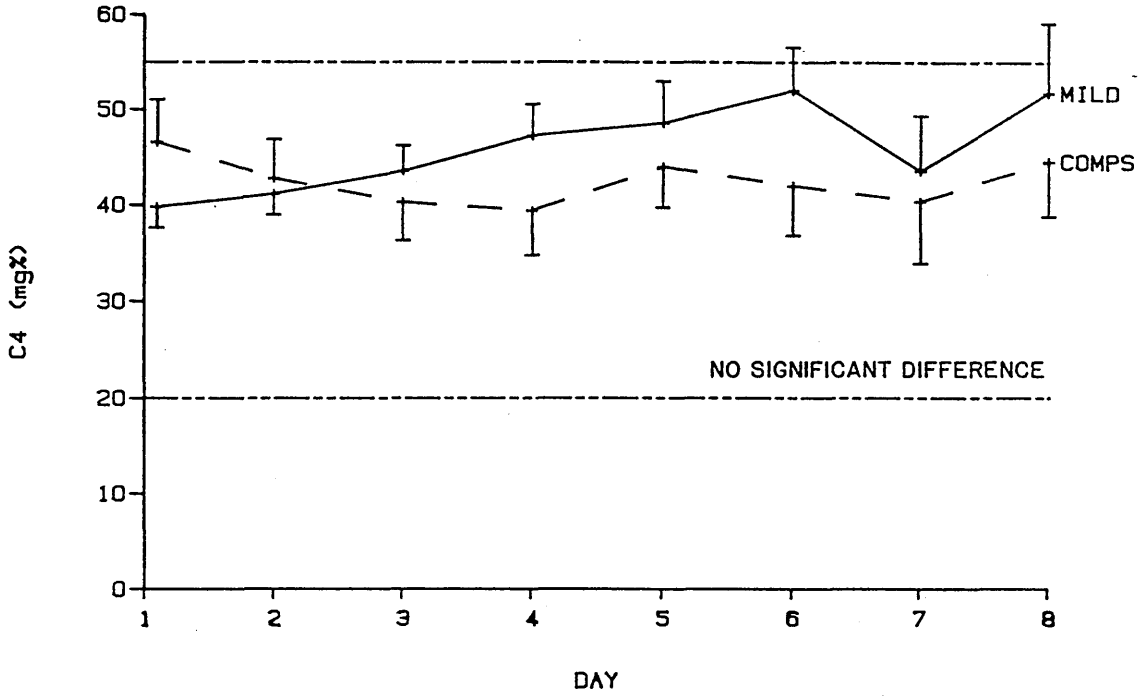


FIGURE 19.

Sequential changes of complement factor C4 during the course of acute pancreatitis in 21 patient with an uncomplicated outcome (mild) and 9 with a complicated outcome (comps).

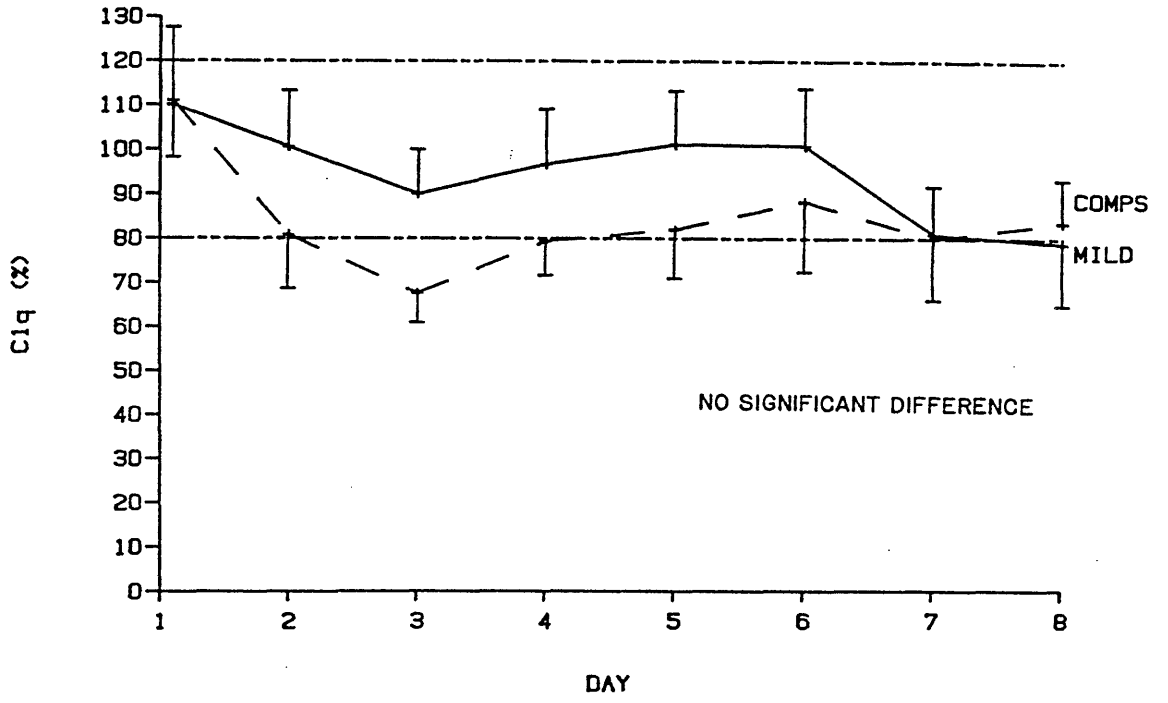


FIGURE 20.

Sequential changes of complement factor C1q during the course of acute pancreatitis in 21 patients with an uncomplicated outcome (mild) and 9 with a complicated outcome (comps).

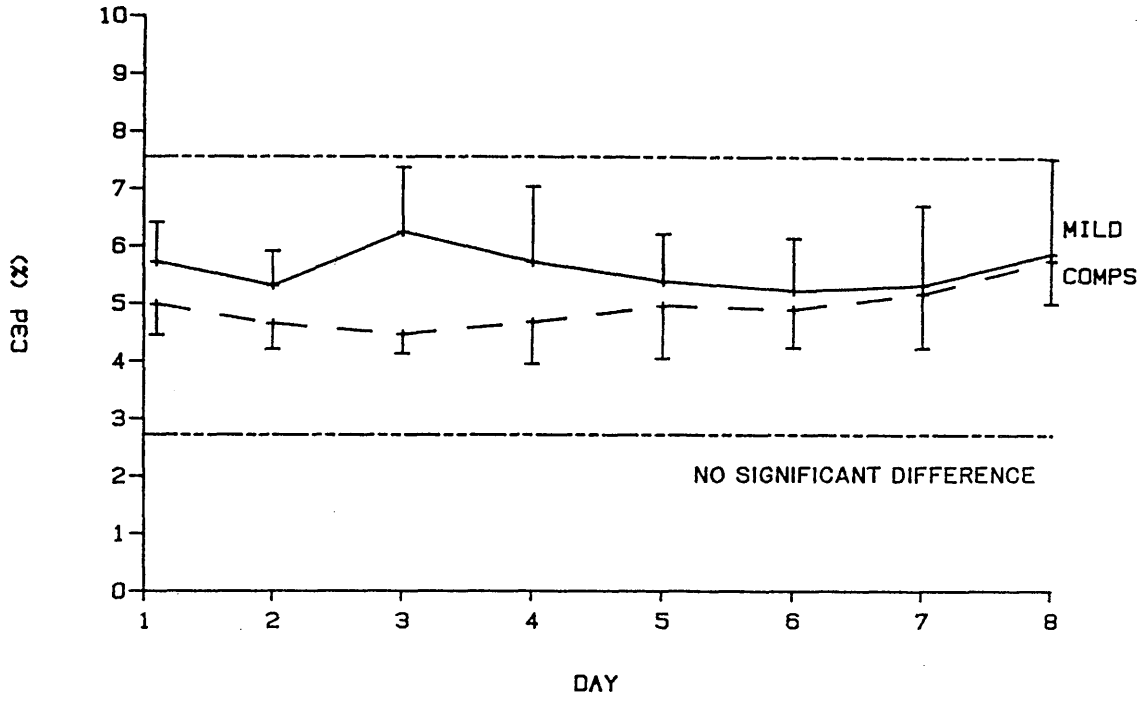


FIGURE 21.

Sequential changes of complement factor C3d during the course of acute pancreatitis in 21 patients with an uncomplicated outcome (mild) and 9 with a complicated outcome (comps).

significantly lower in complicated attacks between days 3 and 8 (Fig. 22). Alpha$_1$antiprotease levels rose during the attack in both mild and complicated attacks with levels significantly higher in complicated attacks between days 4 and 8 (Fig. 23).

C-reactive protein

CRP levels provided the best discrimination, with levels markedly higher and persisting for longer in those with complicated attacks, these differences being highly significant from day 2 (the morning after admission) to day 8 (Fig. 24). CRP, therefore, was shown to be the best of all the factors tested for sequential monitoring of acute pancreatitis.

Eight of the 11 patients subsequently developing pancreatic necrosis or pseudocyst had a peak CRP concentration of $>$300mg/l. However, the mean CRP concentration did not distinguish these 11 patients (6 of whom ultimately required surgery) from 9 patients with acute respiratory insufficiency, all of whom had a severe illness but settled without surgery (Fig. 25). CRP levels did not predict death in 3 elderly patients dying of shock on the second day of admission.

The CRP concentration providing the best discrimination (combining the highest sensitivity and specificity) was determined both for the peak CRP (occurring between the 2nd and 4th day of illness) and for the concentration at the end of the first week. The best discrimination was found with a peak CRP of $\geqslant$210mg/l and, at the end of the first week of $\geqslant$120mg/l (Figs. 26,27). Applying these levels to the data permitted comparison of the prognostic value of

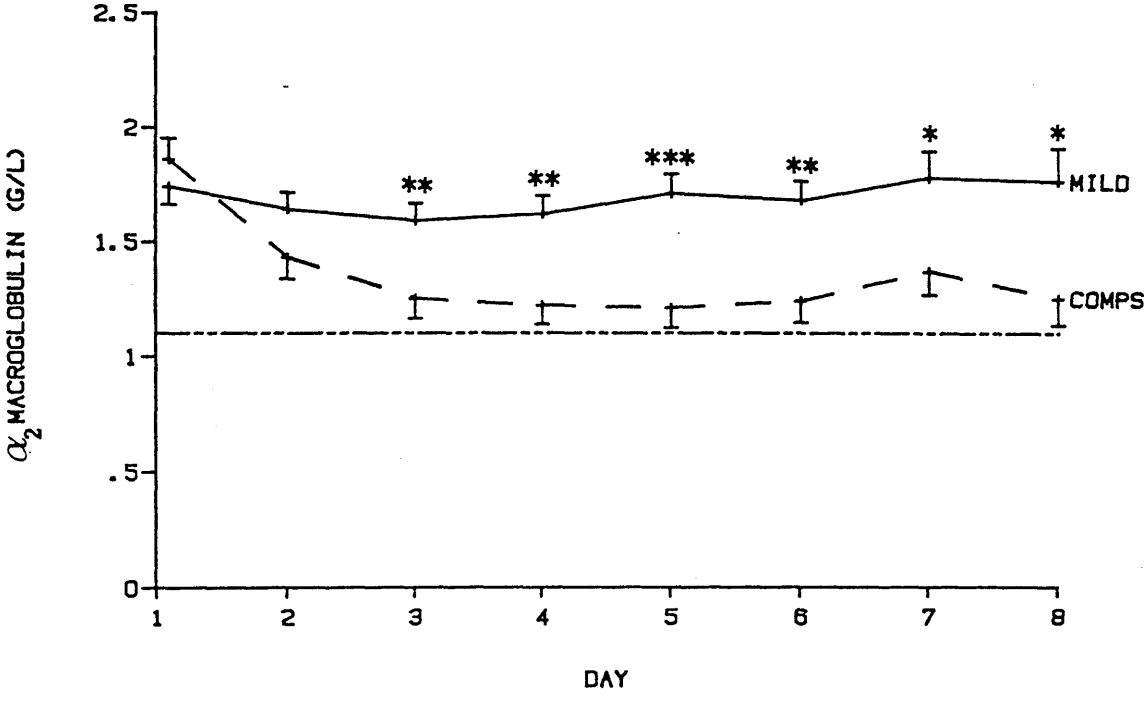


FIGURE 22.

Sequential changes of alpha$_2$macroglobulin during the course of acute pancreatitis in 47 patients with an uncomplicated outcome (mild) and in 25 with a complicated outcome (comps), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

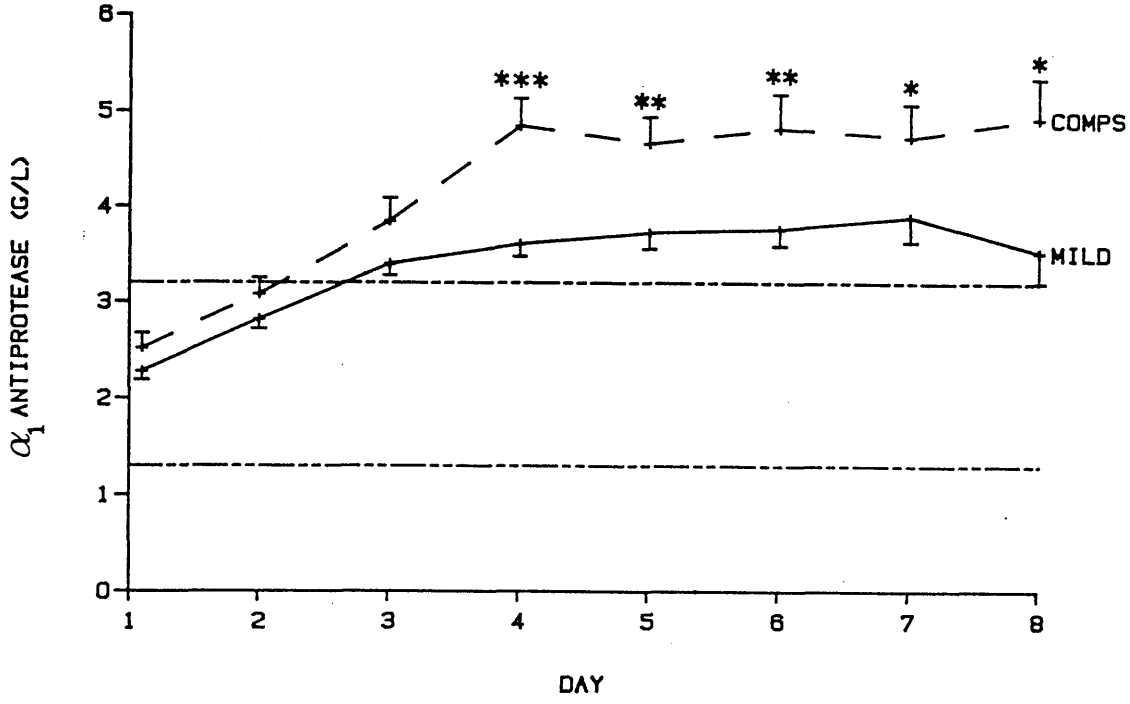


FIGURE 23.

Sequential changes of alpha$_1$antiprotease during the course of acute pancreatitis in 47 patients with an uncomplicated outcome (mild) and in 25 with a complicated outcome (comps), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

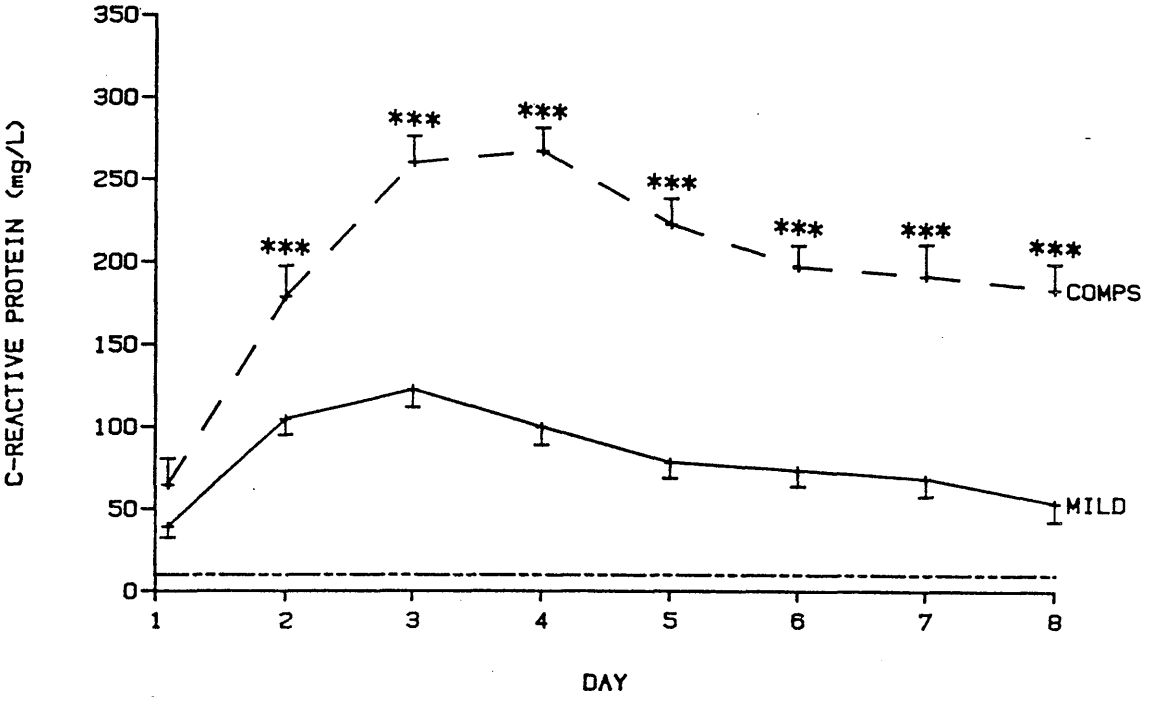


FIGURE 24.

Sequential changes of C-reactive protein during the course of acute pancreatitis in 47 patients with an uncomplicated outcome (mild) and in 25 with a complicated outcome (comps),
*** p <0.001.

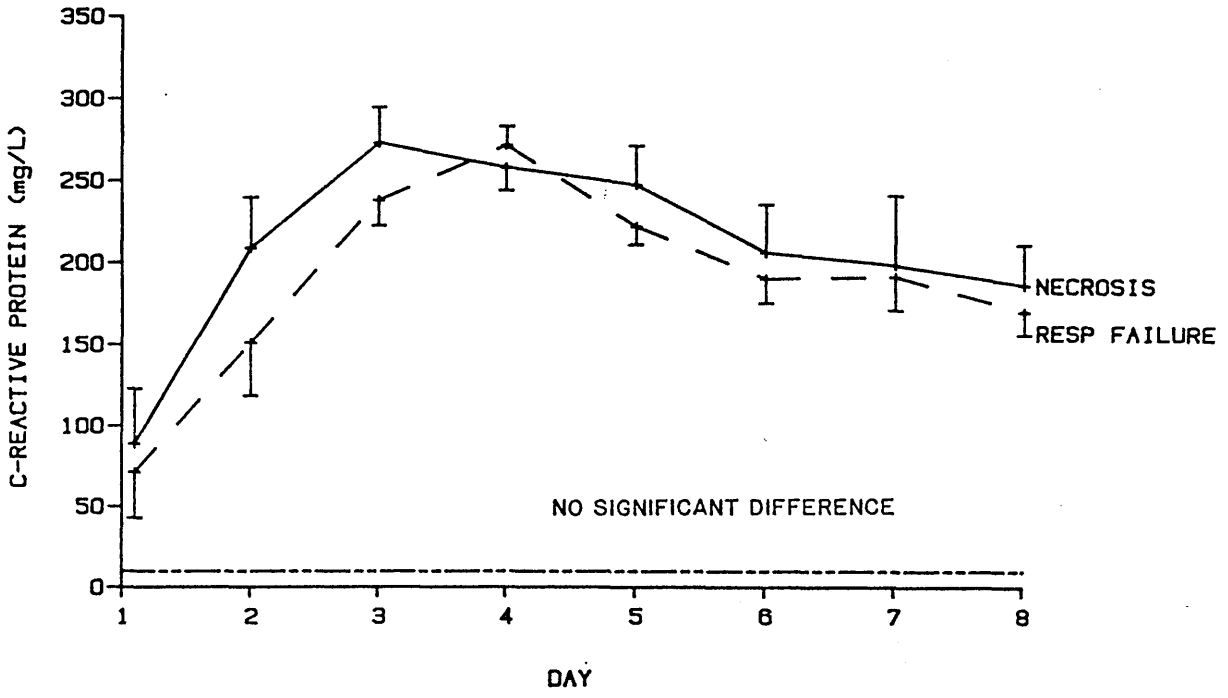


FIGURE 25.

Sequential changes of C-reactive protein in 11 patients developing local pancreatic complications (necrosis) and 9 developing acute respiratory insufficiency (resp fail).

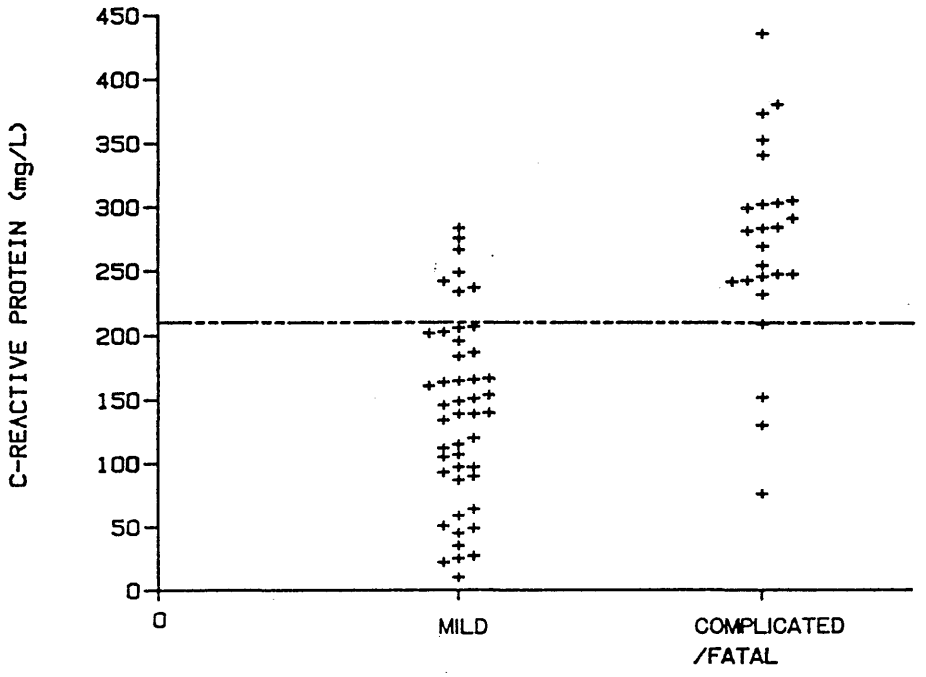


FIGURE 26.

Scattergram showing peak C-reactive protein concentrations (during day 2 - 4) in patients with an uncomplicated outcome (mild) and those with a complicated or fatal outcome.

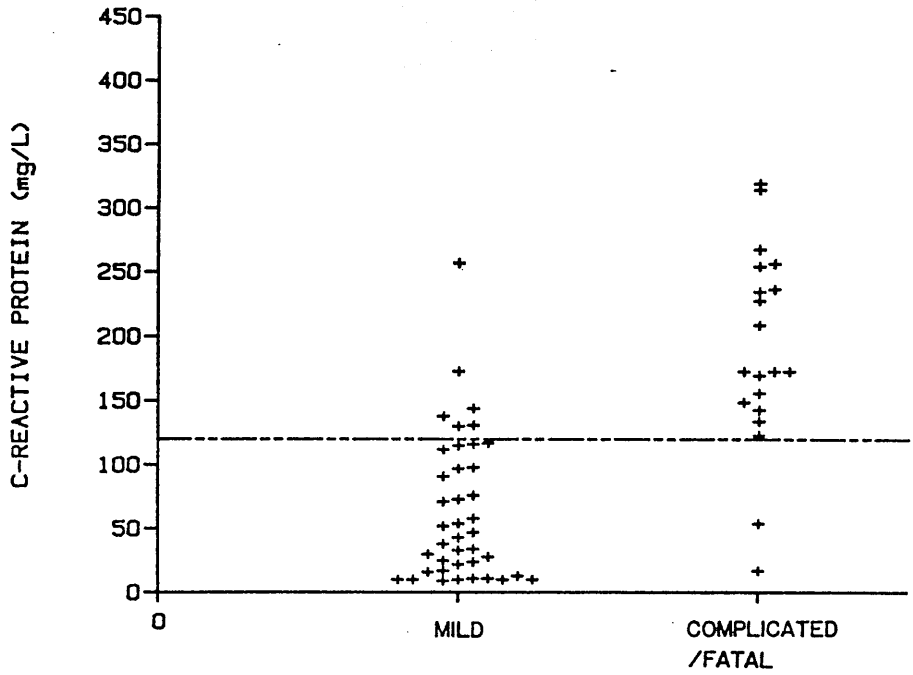


FIGURE 27.

Scattergram showing the C-reactive protein concentration on day 7 in patients with an uncomplicated outcome (mild) and those with a complicated or fatal outcome.

CRP measurement with clinical assessment and the multiple factor scoring systems.

Table 25 shows both the peak and seven day CRP concentrations to be of similar accuracy to either the Ranson or Glasgow multiple factor scoring systems. When the aetiology of the attack was considered, CRP measurement appeared to be slightly better than either of the multiple factor scoring systems for attacks associated with gallstones but slightly less good for alcohol-associated attacks (Table 26).

Although the proportion of attacks correctly predicted by clinical assessment approached that of the multiple factor scoring systems, closer examination of the data reveals that while almost all mild attacks were identified correctly, fewer than half the patients with complicated attacks were correctly predicted, even at 48 hours.

Discussion

Clinical assessment of the severity and likely outcome of acute pancreatitis early in the course of the attack is notoriously unreliable. The accuracy of clinical assessment appears to improve with time and one study suggested that 83% of severe attacks were correctly predicted 48 hours after admission[226]. Our own data have demonstrated that while all patients subsequently dying were correctly identified as having a severe attack at 48 hours, the overall accuracy in predicting complicated attacks was poor at 46%. Clinical assessment, although an essential component of the overall monitoring and management of such patients, remains imprecise,

TABLE 25.

Comparison of the "peak" and "7th day" C-reactive protein concentrations with prediction of outcome by clinical assessment and multiple factor scoring systems.

Parameter	Sensitivity	Specificity	Predictive value +ve	Predictive value -ve	% Correct
Peak CRP* (≥210mg/l)	83%	85%	74%	91%	85%
7th day CRP (≥120mg/l)	90%	85%	75%	94%	87%
Glasgow score (8 factors)	76%	83%	70%	87%	81%
Ranson score (11 factors)	88%	79%	69%	93%	82%
Clinical assessment (on admission)	37.5%	96%	82%	75%	76%
Clinical assessment (at 48 hours)	46%	98%	92%	78%	80%

KEY:

* Peak = highest concentration on 2nd, 3rd or 4th day.

Sensitivity = % of all complicated attacks predicted correctly by test.
Specificity = % of all mild attacks predicted correctly by test.
PV +ve = % complicated attacks among all predicted severe by test.
PV -ve = % mild attacks among all predicted mild by test.
% Correct = % correctly classified.

TABLE 26.

Comparison of C-reactive protein concentration and multiple factor scoring systems in gallstone and alcohol-associated attacks.

Parameter	Sensitivity	Specificity	Predictive value +ve	Predictive value -ve	% Correct
Gallstone					
Peak CRP (≥210mg/l)	100%	79%	81%	92%	87.5%
7 Day CRP (≥120mg/l)	100%	100%	100%	100%	100%
Glasgow score	80%	71%	67%	83%	75%
Ranson score	90%	71%	69%	91%	79%
C/A on admission*	33%	86%	60%	67%	65%
C/A at 48 hours	44.5%	93%	80%	72%	74%
Alcohol					
Peak CRP (≥210mg/l)	100%	87%	75%	100%	90%
7 Day CRP (≥120mg/l)	67%	86%	100%	88%	82%
Glasgow score	67%	100%	100%	92%	93%
Ranson score	83%	96%	83%	96%	93%
C/A on admission	20%	100%	100%	84.5%	85%
C/A at 48 hours	20%	100%	100%	84.5%	85%

* C/A - clinical assessment.

KEY as for table 24.

subjective and non-reproducible and the search continues for an ideal marker of severity in this condition.

Complement factors showed no relationship to the severity of an attack and their measurement was therefore discontinued after 30 patients had been analysed. Other workers have found complement levels, particularly of C3, to be of value in the prediction of outcome in acute pancreatitis[20,52,110,199]. Whicher and co-workers found, as in the current study, a wide scatter of results and no apparent relationship between the presence or absence of complement activation (as indicated by an elevated C3d concentration) and severity of the attack[379].

The most promising of the complement factors studied was C1q. This is the first component of the classical complement pathway and its apparent consumption in acute pancreatitis is difficult to explain. Complement activation in acute pancreatitis is thought to occur by a direct attack on C3 by trypsin[242] or, as Foulis and co-workers have suggested, by the alternative complement pathway[110]. Neither should lead to consumption of the early factors of the classical complement pathway. CRP binds at sites of injury within the body and once complexed is a potent activator of the classical complement pathway beginning with C1q[274]. The fall in C1q which occurs in the more severe cases may, therefore, be associated with this mechanism.

Sequential measurement of the antiproteases appeared more promising, particularly alpha$_2$macroglobulin which showed useful discrimination between mild and complicated attacks.

Alpha$_2$macroglobulin is most probably the key intravascular antiprotease. It binds irreversibly with activated proteolytic enzymes leading to a conformational change within the antiprotease molecule and its rapid uptake by the macrophages of the reticulo-endothelial system. Alpha$_2$macroglobulin does not act as an acute phase reactant since its synthesis does not increase in response to injury. The low levels of alpha$_2$macroglobulin seen in the most severe attacks of acute pancreatitis suggest its consumption and may reflect the amount of proteolytic enzyme activation occurring. A proportion of the measured alpha$_2$macroglobulin in serum is in a complexed form and unavailable for protease binding[204]. The importance of low levels of this antiprotease is as yet unclear, but may have clinical relevance.

Alpha$_1$antiprotease acts as an acute phase reactant, levels rising severalfold during the illness. The net effect of this is that the overall capacity of serum to bind trypsin and other activated proteolytic enzymes increases during the course of the attack[133]. The binding between alpha$_1$antiprotease and trypsin is reversible, this complex appearing to dissociate, the trypsin being taken up by alpha$_2$macroglobulin prior to its clearance from the body[264]. Therefore, although the total trypsin binding capacity of serum may increase, the fall in alpha$_2$macroglobulin may be a rate limiting step to the clearance of proteases from the circulation. Clearance of trypsin-alpha$_2$macroglobulin complexes by the reticulo-endothelial system may also be depressed in severe acute pancreatitis[201] and as these complexes retain proteolytic activity[100,148,302], this might be

pathogenic in man.

A recent controlled clinical trial has studied the therapeutic benefit of administration of fresh frozen plasma in patients with acute pancreatitis. This was able to maintain alpha$_2$macroglobulin levels in treated patients but produced no clear evidence of a beneficial effect on outcome, although too few patients with severe illness had been included to confirm this statistically[208].

In the current study the CRP concentration provided the best discrimination of all the factors investigated. CRP is a protein of uncertain role in the body. It acts as a non-specific inflammatory marker and monitoring has been shown to be of clinical value in a number of conditions[274]. CRP is usually present in low concentrations in the bloodstream. It is synthesised in hepatocytes, elevated serum levels being detected about 8 hours after injury and reaching a peak some 24-48 hours later. The delay until peak levels are attained probably accounts for its failure to detect early death in 3 patients in this study.

Buchler and co-workers, on the basis of a rather limited study of 35 patients, have suggested that a CRP concentration of >100mg/l correctly predicts pancreatic necrosis in 95% of cases[52], these patients having necrosis confirmed at subsequent laparotomy. In the current study this level would result in 57 (79%) of the 72 patients reported here having "pancreatic necrosis" predicted. While a cut-off level of 210mg/l in the current study (more than twice that described by Buchler's group) identifies a group of patients with severe pancreatitis, it was clearly not specific for the presence of

pancreatic necrosis. While a number of the patients in the severe group may have had unsuspected pancreatic necrosis manifesting only as acute respiratory insufficiency, the clinical improvement which occurred without recourse to surgery suggests that Buchler's group may be operating unnecessarily in some patients.

The assay for CRP is quick and simple and the current study has demonstrated that measurement of CRP is at least as accurate as either of the multiple factor scoring systems and probably better for gallstone related attacks, which presently account for over 50% of patients in the UK. In practice a peak CRP of 300mg/l and/or persistent elevation at the end of the first week, as was originally shown by the Leeds group[233], provides useful warning of the development of a local pancreatic complication. Such a test would clearly be of clinical value in selecting patients for investigation by expensive imaging techniques such as contrast-enhanced CT scanning, to select those patients who might usefully be monitored in an intensive care unit or those in whom parenteral nutrition may be indicated if the attack appears to be slow in settling. The complexity of the multiple factor scoring systems has probably limited their routine use and measurement of a single factor, such as CRP, appears more likely to be adopted into daily clinical practice.

CHAPTER 7. COMPARATIVE STUDY OF PREDICTION OF SEVERITY IN ACUTE PANCREATITIS BY CLINICAL ASSESSMENT, MULTIPLE FACTOR SCORING SYSTEMS AND DIAGNOSTIC PERITONEAL LAVAGE.

Introduction

While the majority of attacks of acute pancreatitis are mild and resolve quickly following admission to hospital, some 20% of attacks give rise to complications which may be fatal, necessitate surgery or merely prolong the hospital stay. Some of these patients will be admitted with an overtly severe illness but others may appear to have a mild attack on admission only to deteriorate later despite full supportive therapy. A further group may appear to have a mild attack which appears to settle but are subsequently found, perhaps after discharge, to have developed a complication such as a pancreatic abscess or pseudocyst.

Attempts have been made to identify these patients early in the course of their illness, the hope being that earlier, more aggressive treatment of this subgroup might reduce the mortality rate. The aim of the current study was to evaluate clinical assessment and the objective severity scoring systems in the 160 patients documented in the current trial, to determine their value in identifying such patients.

Methods

Clinical assessment

The results of the initial clinical assessment were recorded in

the patient's proforma and this assessment was repeated on the next two mornings (approximately 24 and 48 hours after admission)(chapter 4). This assessment was purely subjective and was made without reference to the biochemistry or other available laboratory results.

The assessment of an attack as severe took into account the presence or absence of shock, the adequacy of the peripheral perfusion and urine output, the presence of respiratory distress and on the degree of abdominal tenderness, distension and ileus. Patients were also considered to have severe acute pancreatitis if they developed body wall staining. In addition to the overall clinical assessment the presence or absence of each of these individual factors was recorded in every patient at their initial assessment.

Multiple factor scoring systems

Biochemical, haematological and the appropriate clinical data were recorded prospectively on all the patients studied to permit scoring of the Glasgow[269], Ranson[290], modified Ranson (for gallstone-associated attacks)[287] and Bank and Wise scoring systems[24]. The details of the prognostic factors comprising each of the systems are detailed in tables 27 and 28. The equivalent SI. and local units used for scoring the Ranson scoring systems are detailed below table 27. The enzyme units used for LDH and SGOT in the Ranson scoring systems were approximately equivalent to LDH = 600U/l and AST = 100U/l used for the original Glasgow classification[159] (Imrie CW, personal communication) although the most recent modification of this scoring system, as used here, raised the AST/ALT cut-off to >200 U/l[269].

TABLE 27.

Objective prognostic factors comprising the Ranson and Glasgow multiple factor scoring systems.

Ranson	*Modified Ranson	Glasgow
On admission:	On admission:	Within 48 hours:
Age >55 years	Age >70 years	Albumin <32 g/l
WCC >16000/mm^3	WCC >18000/mm^3	WCC >15000/mm^3
LDH >350 IU/l	LDH >400 IU/l	LDH >600 U/l
SGOT >250 SFU%	SGOT >250 SFU%	AST/ALT >200 U/l
Glucose >200mg%	Glucose >220mg%	Glucose >10mmol/l
		Urea >16mmol/l
Within 48 hours:	Within 48 hours:	Calcium <2mmol/l
		pO_2 <60mmHg
Hct fall >10%	Hct fall >10%	
BUN rise >5mg%	BUN rise >2mg%	
Calcium <8mg%	Calcium <8mg%	
pO_2 <60mmHg	Base deficit >5mEq/l	
Base deficit >4mEq/l	Fluid sequestration >4000ml	
Fluid sequestration >6000ml		

Any scoring system ≥3 factors positive = severe disease.

Key and conversion to equivalent SI. or local units:

* Modified Ranson, only for gallstone associated attacks; WCC = white cell count; LDH = lactate dehydrogenase, 350 IU/l equivalent to 600 U/l, 400 IU/l equivalent to 700 U/l; SGOT = AST, 250 SFU equivalent to 120 U/l; Glucose 200mg% equivalent to 10mmol/l, 220mg% equivalent to 11mmol/l; Hct = haematocrit; BUN = blood urea nitrogen, 5mg% equivalent to 0.9mmol/l, 2mg% equivalent to 0.4mmol/l; Calcium 8mg% equivalent to 2mmol/l.

TABLE 28.

Prognostic signs comprising the clinical criteria of Bank and Wise.

System	Prognostic signs
Cardiac	Shock, tachycardia >130/min, arrhythmia, ECG changes.
Pulmonary	Dyspnoea, rales, pO_2 <60mmHg, ARDS.
Renal	Urine output <50ml/hour, rising BUN and/or creatinine.
Metabolic	Low or falling calcium, pH, albumin.
Haematological	Falling haematocrit, DIC (low platelets, split products).
Neurological	Irritability, confusion, localising signs
Haemorrhagic disease	On signs or peritoneal tap.
Tense distension	Severe ileus, fluid ++.

≥ 1 factor positive = severe (potentially lethal) disease.

KEY:

ARDS = adult respiratory distress syndrome; DIC = disseminated intravascular coagulation.

The time at which the laboratory results routinely became available to the ward or when clinical criteria were first considered positive was recorded to permit calculation of the delay from admission to the prediction of severity being made.

Diagnostic peritoneal lavage

Of the 25 patients randomised to receive intraperitoneal therapy with aprotinin, one refused to consent to peritoneal cannulation. Two other patients were excluded because of atypical findings on peritoneal aspiration. One was discovered to have a haemoperitoneum and at laparotomy was found to have had a pancreatic transection secondary to trauma. The other patient had foul smelling haemorrhagic peritoneal exudate and at laparotomy was found to have extensive small bowel infarction due to mesenteric venous thrombosis.

Diagnostic peritoneal lavage was performed in one additional patient who presented over 72 hours after the onset of pain and who was, therefore, excluded from the formal trial. Thus 23 patients underwent diagnostic peritoneal lavage and were available for analysis.

After peritoneal cannulation the colour and volume of any peritoneal exudate and the return fluid following a single litre saline lavage were recorded, the colour having been compared against a standard colour chart[226](Fig. 11). Severe pancreatitis was predicted if at least 20mls of exudate was present on aspiration, if the exudate was dark coloured (6 or more on the colour chart), or if the return fluid was darker than mid-straw colour (4 or more on the

colour chart)[226,234]. The delay from admission to the prediction of severity was recorded.

Outcome of attacks

Complicated attacks of acute pancreatitis were defined as those causing death or a major complication (chapter 4).

Patients

The aetiology of the attacks was determined as outlined in chapter 4. Of the 160 attacks documented, 2 were excluded from consideration here because of an alternative diagnosis made on diagnostic peritoneal lavage. One further patient was excluded as death was considered more likely to be due to rupture of an abdominal aortic aneurysm, although this was not confirmed as permission for post mortem was not granted by the family.

Clinical assessment and the objective severity scoring systems have been analysed in 157 patients overall. Twelve of these patients had a fatal outcome recorded and a further 26 had a complicated clinical outcome but survived. In the remaining 119 patients the attack ran a mild, uncomplicated course.

Statistical analysis

Statistical analysis was conducted by Fisher's exact test, Chi square test and the Mann-Whitney U test. The diagnostic performance of the scoring systems have been analysed as in chapter 6[119].

Results

Clinical assessment

Clinical assessment at 24 and 48 hours provided the highest overall accuracy of all the methods tested (Table 29). On the initial clinical assessment only two of the patients with an uncomplicated outcome had been incorrectly predicted to have a severe attack, and by 24 and 48 hours none of the patients with an uncomplicated outcome were wrongly predicted as severe.

On admission only 13 of the 38 patients with a fatal or complicated outcome were correctly identified by clinical assessment, rising to 18 patients at 24 hours and, therefore, the sensitivity of clinical assessment was low. Of the 20 patients with a complicated outcome incorrectly predicted at 24 hours, 10 had been predicted as moderate and 10 as having a mild attack. The complications recorded in these patients comprised respiratory insufficiency in 10, pseudocysts in 5, abscesses in 2, pancreatic necrosis in 2 and septicaemia in one. Amongst these 20 patients, gallstones were the commonest underlying aetiological factor accounting for 8 attacks.

Clinical assessment on admission failed to predict a complicated outcome in 4 patients who subsequently died although by 24 and 48 hours only one of the patients dying had been incorrectly identified.

Table 30 details the incidence and discriminatory value of the individual clinical factors recorded on admission, many being recorded only infrequently amongst the patients with an ultimately complicated or fatal outcome.

TABLE 29.

Analysis of clinical assessment, multiple factor scoring systems and diagnostic peritoneal lavage in the prediction of outcome from acute pancreatitis.

	Sensitivity	Specificity	Predictive value +ve	Predictive value -ve	% Correct
Clinical assessment:					
On admission	34%	98%	87%	83%	83%
At 24 hours	47%	100%	100%	85.5%	87%
At 48 hours	44%	100%	100%	85.5%	87%
Glasgow	71%	88%	66%	90.5%	84%
Ranson	87%	70.5%	48.5%	94%	74.5%
Modified Ranson*	81.5%	79%	55%	93%	80%
Bank & Wise	100%	12%	26.5%	100%	33%
Diagnostic peritoneal lavage	67%	62.5%	77%	50%	65%

KEY:

* modified for attacks associated with gallstones + standard Ranson score for other aetiological factors.

Sensitivity = % of all complicated attacks predicted correctly by test.
Specificity = % of all mild attacks predicted correctly by test.
PV +ve = % complicated attacks among all predicted severe by test.
PV -ve = % mild attacks among all predicted mild by test.
% Correct = % correctly classified.

TABLE 30.

Comparison of the value of individual clinical factors recorded at the initial assessment in predicting the outcome from acute pancreatitis.

Clinical parameter	Fatal or complicated outcome	Uncomplicated outcome	p value*
Pulse rate >120/min	2	0	NS
Systolic BP <100mmHg	3	1	p <0.05
Temperature >38°C	3	1	p <0.05
Respiratory rate >30/min	3	1	p <0.05
Peripheral shutdown	14	1	p <0.001
Clammy skin	18	9	p <0.001
Peritonism/ abdominal distension	13	4	p <0.001
Absent bowel sounds	8	2	p <0.001
Body wall staining	1	0	NS

* Chi square or Fisher's exact test as appropriate.

Glasgow scoring system

Fourteen patients with an uncomplicated outcome were wrongly predicted as having a severe attack, gallstones being the underlying aetiological factor in 11 (79%) of these attacks. Amongst the 11 cases with a complicated outcome incorrectly predicted as mild were 4 patients developing a pseudocyst, 4 developing respiratory insufficiency, 2 developing septicaemia and one a pancreatic abscess. All 12 patients with a fatal outcome were correctly predicted before death.

Original Ranson system

Thirty-five patients with an uncomplicated outcome were wrongly predicted as having a severe attack, gallstones accounting for 24 (69%) of these attacks. Only 5 patients with a complicated outcome were wrongly predicted as mild, the attacks being complicated by a pseudocyst in 2 patients, respiratory insufficiency in 2, septicaemia in one and an abscess in one. All 12 patients with a fatal outcome were correctly predicted before death.

Modified Ranson system

When the modified Ranson scoring system is substituted for those attacks associated with gallstones, the overall accuracy of the system improved (Table 29). Twenty-five attacks with an uncomplicated outcome were wrongly predicted as severe, including 14 attacks associated with gallstones. Seven patients with a complicated outcome

were wrongly predicted as mild, respiratory insufficiency developing in 4 attacks, pseudocysts in 2 attacks and septicaemia in one. All 12 fatal attacks were correctly predicted before death.

Bank and Wise system

This performed poorly in the patients studied. Although all with a complicated outcome were correctly predicted as severe, so too were 105 of the 119 patients with an uncomplicated outcome. This resulted in poor specificity and low overall accuracy (Table 29).

Diagnostic peritoneal lavage

Three of the 8 patients with an uncomplicated outcome were wrongly predicted as severe. These attacks were all associated with alcohol abuse and had been judged as severe based on the presence of 28mls of haemorrhagic free fluid in one patient, and on the presence of dark straw coloured return fluid (colour chart nos. 4 and 5 respectively) after a single litre saline peritoneal lavage in the other 2 patients.

Five of the 15 patients with a fatal or complicated outcome were wrongly predicted as mild. One of these patients dying of pancreatitis secondary to hypothermia, had no free fluid on peritoneal cannulation. Of the others predicted as having mild pancreatitis, 3 developed respiratory insufficiency and one a pseudocyst, the aetiologies underlying the attacks being attributed to gallstones (2), unknown (1) and hyperlipidaemia (1).

Comparison between alcohol and gallstones

Table 31 compares multiple factor scoring systems and diagnostic peritoneal lavage in attacks associated with either alcohol or gallstones. The original Ranson system performed best for attacks associated with alcohol abuse whereas either the Glasgow or modified Ranson systems appeared best for gallstone-associated attacks. Multiple factor scoring systems performed better overall for attacks associated with alcohol abuse. Diagnostic peritoneal lavage appeared to perform less well for alcohol-associated attacks although this may partly reflect the smaller number of patients studied.

Delay to prediction.

The overall delay to the prediction of severity by the multiple factor scoring systems was always greater than for diagnostic peritoneal lavage, although for patients who ultimately died, these differences were not significant. The analysis of the delays in prediction have been detailed in table 32. The differences between the Glasgow and Ranson scoring systems were minor, 14 and 13 patients respectively had their prediction of severity delayed over 48 hours post-admission.

The Bank and Wise system provided an answer sooner than either the Glasgow or Ranson scoring systems, both in attacks associated with a complicated or fatal outcome, but in attacks with an uncomplicated outcome there were no significant differences. Only 9 patients had their prediction of severity delayed beyond 48 hours.

TABLE 31.

Comparison of multiple factor scoring systems and diagnostic peritoneal lavage for prediction of outcome in gallstone and alcohol-associated attacks of acute pancreatitis.

	Sensitivity	Specificity	Predictive value +ve	Predictive value -ve	% Correct
Gallstone					
Glasgow	64%	78%	47%	88%	75%
Ranson	86%	52%	35%	92%	60%
Modified Ranson	71%	70%	42%	89%	70%
Diagnostic peritoneal lavage	50%	100%	100%	60%	71.5%
Alcohol					
Glasgow	57%	98%	80%	93%	92%
Ranson	86%	98%	86%	98%	96%
Diagnostic peritoneal lavage	100%	25%	62.5%	100%	67%

KEY as for table 29.

TABLE 32.

Delay from admission to prediction of severity by multiple factor scoring systems and by diagnostic peritoneal lavage.

	Delay to prediction (hours) by outcome of attack			
	Mild attacks	Complicated attacks	Fatal attacks	All attacks
Glasgow	22 (2-60)	28 (6-56)	26 (9-72)	24 (2-72)*
Ranson	24 (0-60)	27.5 (2-56)	22.5 (1-72)	24 (0-72)*
Modified Ranson	26.5 (0-60)	28.5 (6-56)	22.5 (1-72)	27 (0-72)*
Bank and Wise	24 (0-60)	12 (0-48)	7.5 (0-56)	20 (0-60)#
Diagnostic peritoneal lavage	5 (3-8)	8.5 (4-31)	17 (9-27)	8.5 (3-31)*

KEY:

Values are medians (range); * no significant difference between Glasgow, Ranson and modified Ranson; # significantly different from modified Ranson and diagnostic peritoneal lavage ($p < 0.01$); * significantly different from Ranson and Bank and Wise ($p < 0.01$), and from Glasgow and modified Ranson ($p < 0.001$).

Discussion

A problem faced during any attempt to compare predictive systems is how to define the outcome of an attack (Table 33). Many of the endpoints quoted are vague or ill-defined, and, for example, the length of ICU or hospital stay may depend on many factors including the policy of the individual consultant, the availability of such beds and the demand for such beds from other patients.

Clinical assessment of the severity and likely outcome of an attack of acute pancreatitis early in the course of an attack is notoriously unreliable. The accuracy of clinical assessment in identifying attacks with a complicated outcome has been reported to improve with time and one study has suggested that 83% of such attacks were correctly predicted 48 hours after admission[226]. The data reported here do not support this. While clinical assessment appeared to provide the most accurate overall prediction of outcome, the sensitivity remains low and even at 48 hours is only 44%, thus clinical assessment performs poorly in its most important function, that of predicting complicated and potentially fatal attacks. Individual clinical parameters, although showing a significant difference between mild and complicated attacks, were each present in too few patients to be of overall value.

Of the multiple factor scoring systems the Glasgow scoring system was most accurate overall, although its sensitivity was lower than for either of the Ranson scoring systems. The Ranson scoring systems appeared to be better in identifying attacks with a complicated outcome but at the cost of a lower specificity. The

TABLE 33.

Comparison of the definitions for severe attacks of acute pancreatitis used in the description and analysis of different scoring systems.

Authors	Year	Mild attack	Severe attack
Ranson et al.	1974	No serious illness	Seriously ill Death
Ranson and Pasternack	1977	Recovered without major life-threatening complications	Requiring >7 days in ICU Death
Imrie et al.	1978	Not stated	Not stated
Osborne et al.	1981	Symptoms settled on conservative treatment	Symptoms and signs failed to settle
McMahon et al.	1980	Not stated	Hospital stay >14 days Severe complication: renal/respiratory failure pancreatic abscess Death
Mayer and McMahon	1985	Not stated	Hospital stay >14 days Pseudocyst or abscess Death
Corfield et al.	1985	Not stated	Death Complication: abscess pseudocyst, necrosis septicaemia, cholangitis, renal failure, diabetes, venous thromboembolism, myocardial infarction, GI haemorrhage, pulmonary complications and hypoxia and/or X-ray changes.

modification of the Ranson scoring system for gallstones improved the overall accuracy while reducing the sensitivity. Although applied retrospectively here, the delay from admission until gallstones are positively identified might diminish its value in clinical practice. The original Ranson scoring system appeared to be the method of choice for attacks associated with alcohol.

There was little to chose between the Glasgow and Ranson scoring systems as regards the delay to prediction. The Ranson system predicted deaths earlier although this difference did not reach statistical significance.

The Bank and Wise scoring system was the most extreme example of losing specificity as sensitivity was increased. It correctly identified all the patients with a complicated outcome but with a very low specificity, all except 14 patients having severe disease predicted. In this patient population at least, the Bank and Wise scoring system appeared worthless.

Diagnostic peritoneal lavage permitted the prediction of severity significantly earlier than the multiple factor scoring systems. Its ability to predict patients with a complicated outcome and its overall accuracy appeared to be poorer than either the Glasgow or Ranson scoring systems, although as it was applied in relatively few patients in the current study, a direct comparison cannot be made. It nevertheless appeared less successful in predicting attacks complicated by respiratory insufficiency, although correctly predicting 4 of the 5 patients developing local pancreatic complications, a fact perhaps recognised by its originators. In the

original analysis of the technique renal and respiratory failure were included amongst the complications[226] but have been excluded in the most recent report (Table 33)[234].

One valuable aspect of diagnostic peritoneal lavage was confirmed here by the early identification of 2 patients with abdominal pain and hyperamylasaemia due to causes other than acute pancreatitis. Both patients would have had their diagnosis and institution of the correct therapy delayed had diagnostic peritoneal lavage not been performed.

Multiple factor scoring systems are considered to be worth recording as an objective measure of the severity of an attack. They may be of particular value in comparing groups of patients within clinical trials and in some instances may provide useful warning of a complicated outcome. Prediction of outcome, particularly by the Ranson scoring systems, depends on the measurement of a large number of factors and the result may be delayed for 48 hours or more, thus limiting its value in clinical practice.

CHAPTER 8. A COMPARATIVE STUDY OF PREDICTION OF OUTCOME IN ACUTE PANCREATITIS BY APACHE II - A SEVERITY OF DISEASE CLASSIFICATION SYSTEM.

Introduction

The original APACHE illness grading system (acute physiology and chronic health evaluation) was developed on the premise that the severity of an acute disease could be measured by quantifying the degree of abnormality of multiple physiological variables - the acute physiology score[178]. The original APACHE system provided weightings (from 0 - normal to 4 - most abnormal) for 34 potential physiological and laboratory measurements, each being determined from the most abnormal recording made during that particular 24 hour period. The sum of all the measurements recorded represented the patient's total physiology score - the acute physiology score. The higher the score the sicker the patient[178]. To this was added an assessment of the patients pre-admission health status (A - fit to D - severely compromised health) to give the overall APACHE classification.

The original APACHE system was complex and difficult to use and a much simplified modification the APACHE II was reported in 1985[180]. APACHE II utilised only 12 routine physiological and laboratory measurements with an additional weighting for age and pre-admission health status. An increasing APACHE II score was found to correlate with the subsequent risk of hospital death in 5815 intensive care admissions[180]. Furthermore, it was suggested that APACHE II would provide prognostic stratification of patients and might assist

investigators in comparing new or differing forms of treatment[180].

To date the APACHE II system has been assessed exclusively in intensive care patients and reports of its application to the study of patients with acute pancreatitis have not been published.

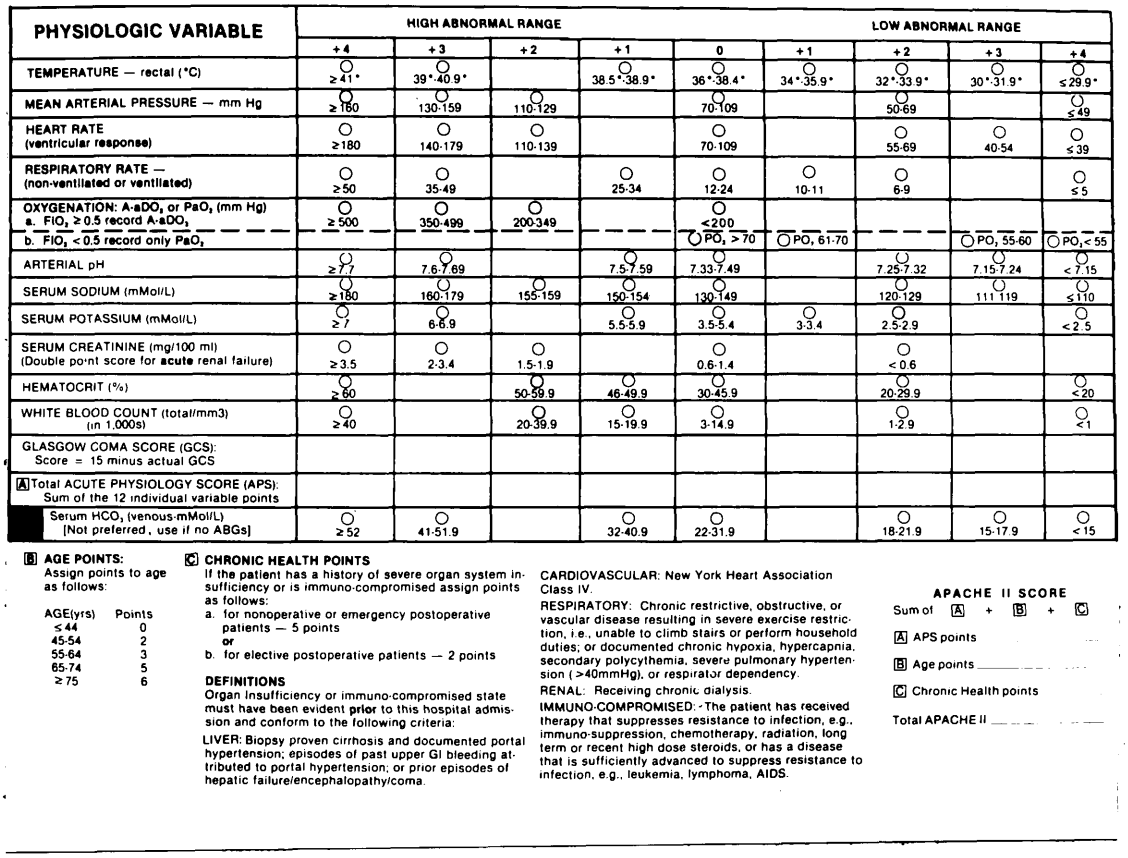
The aims of the current study were to evaluate prospectively the APACHE II severity of disease classification in patients with acute pancreatitis and compare it with the other illness-specific multiple factor scoring systems.

Methods

All 160 patients documented in the current trial had the appropriate laboratory and physiological data recorded prospectively each day to permit subsequent calculation of their daily APACHE II scores (Fig. 28). The only difference between the calculation here and the published table was in the use of SI. units for serum creatinine. The conversion used throughout between SI. and traditional units was 1μmol/l = 0.01mg/100mls.

The 7 laboratory variables and 5 physiological variables were added separately to provide sub-totals and then the overall APACHE II score was calculated by adding the weightings for age and chronic health state.

The aetiology and outcome of the attacks were as defined in chapter 4. Thus 160 patients were studied with 3 exclusions. A fatal outcome was recorded in 12 patients and a complicated outcome in another 26 patients who survived. An uncomplicated outcome was recorded in the remaining 119 patients.



PHYSIOLOGIC VARIABLE	HIGH ABNORMAL RANGE					LOW ABNORMAL RANGE			
	+4	+3	+2	+1	0	+1	+2	+3	+4
TEMPERATURE — rectal (°C)	≥41°	39°-40.9°		38.5°-38.9°	36°-38.4°	34°-35.9°	32°-33.9°	30°-31.9°	≤29.9°
MEAN ARTERIAL PRESSURE — mm Hg	≥160	130-159	110-129		70-109		50-69		≤49
HEART RATE (ventricular response)	≥180	140-179	110-139		70-109		55-69	40-54	≤39
RESPIRATORY RATE — (non-ventilated or ventilated)	≥50	35-49		25-34	12-24	10-11	6-9		≤5
OXYGENATION: A-aDO$_2$ or PaO$_2$ (mm Hg) a. FIO$_2$ ≥ 0.5 record A-aDO$_2$	≥500	350-499	200-349		<200				
b. FIO$_2$ < 0.5 record only PaO$_2$					PO$_2$ > 70	PO$_2$ 61-70		PO$_2$ 55-60	PO$_2$ < 55
ARTERIAL pH	≥7.7	7.6-7.69		7.5-7.59	7.33-7.49		7.25-7.32	7.15-7.24	< 7.15
SERUM SODIUM (mMol/L)	≥180	160-179	155-159	150-154	130-149		120-129	111-119	≤110
SERUM POTASSIUM (mMol/L)	≥7	6-6.9		5.5-5.9	3.5-5.4	3-3.4	2.5-2.9		< 2.5
SERUM CREATININE (mg/100 ml) (Double point score for **acute** renal failure)	≥3.5	2-3.4	1.5-1.9		0.6-1.4		< 0.6		
HEMATOCRIT (%)	≥60		50-59.9	46-49.9	30-45.9		20-29.9		< 20
WHITE BLOOD COUNT (total/mm3) (in 1,000s)	≥40		20-39.9	15-19.9	3-14.9		1-2.9		< 1
GLASGOW COMA SCORE (GCS): Score = 15 minus actual GCS									
[A] Total ACUTE PHYSIOLOGY SCORE (APS): Sum of the 12 individual variable points									
Serum HCO$_3$ (venous-mMol/L) [Not preferred, use if no ABGs]	≥52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	< 15

[B] AGE POINTS:
Assign points to age as follows:

AGE(yrs)	Points
≤44	0
45-54	2
55-64	3
65-74	5
≥75	6

[C] CHRONIC HEALTH POINTS
If the patient has a history of severe organ system insufficiency or is immuno-compromised assign points as follows:
a. for nonoperative or emergency postoperative patients — 5 points
or
b. for elective postoperative patients — 2 points

DEFINITIONS
Organ insufficiency or immuno-compromised state must have been evident **prior** to this hospital admission and conform to the following criteria:

LIVER: Biopsy proven cirrhosis and documented portal hypertension; episodes of past upper GI bleeding attributed to portal hypertension; or prior episodes of hepatic failure/encephalopathy/coma.

CARDIOVASCULAR: New York Heart Association Class IV.

RESPIRATORY: Chronic restrictive, obstructive, or vascular disease resulting in severe exercise restriction, i.e., unable to climb stairs or perform household duties; or documented chronic hypoxia, hypercapnia, secondary polycythemia, severe pulmonary hypertension (>40mmHg), or respirator dependency.

RENAL: Receiving chronic dialysis.

IMMUNO-COMPROMISED: The patient has received therapy that suppresses resistance to infection, e.g., immuno-suppression, chemotherapy, radiation, long term or recent high dose steroids, or has a disease that is sufficiently advanced to suppress resistance to infection, e.g., leukemia, lymphoma, AIDS.

APACHE II SCORE
Sum of [A] + [B] + [C]
[A] APS points
[B] Age points
[C] Chronic Health points
Total APACHE II

FIGURE 28.

The APACHE II severity of disease classification system (Knaus WA et al., Crit Care Med 1985;13:818-29). Reproduced by permission of the editor and publishers, Williams & Wilkins (1985).

In the comparison with the multiple factor scoring systems the original Ranson system[290] (Table 27) was used throughout as the diagnosis of gallstones was not usually available on admission.

Statistical analysis

Statistical analysis was conducted by the Mann-Whitney U test, Chi square test and Fisher's exact test. The diagnostic performance of the scoring systems have been analysed as described in chapter 6[119].

Results

Examining the component parts of the APACHE II score, the laboratory data score, the physiology data score, the combined laboratory/physiology score and the total score, while all provide useful separation between the groups of patients it was the total score which best separated the 3 groups throughout the 7 day period.

Admission APACHE II

The differences between the 3 groups of patients were significant on the day of admission to hospital, the "on admission" values often being the most abnormal values recorded. A scattergram showing the distribution of the day 1 values is shown in Figure 29. The "cut-off" value providing the best discrimination was calculated by taking the highest product of the sensitivity and specificity and was found for an APACHE II score of >5. This score provided a high sensitivity of 95% with a positive predictive value of 40%, 64% of

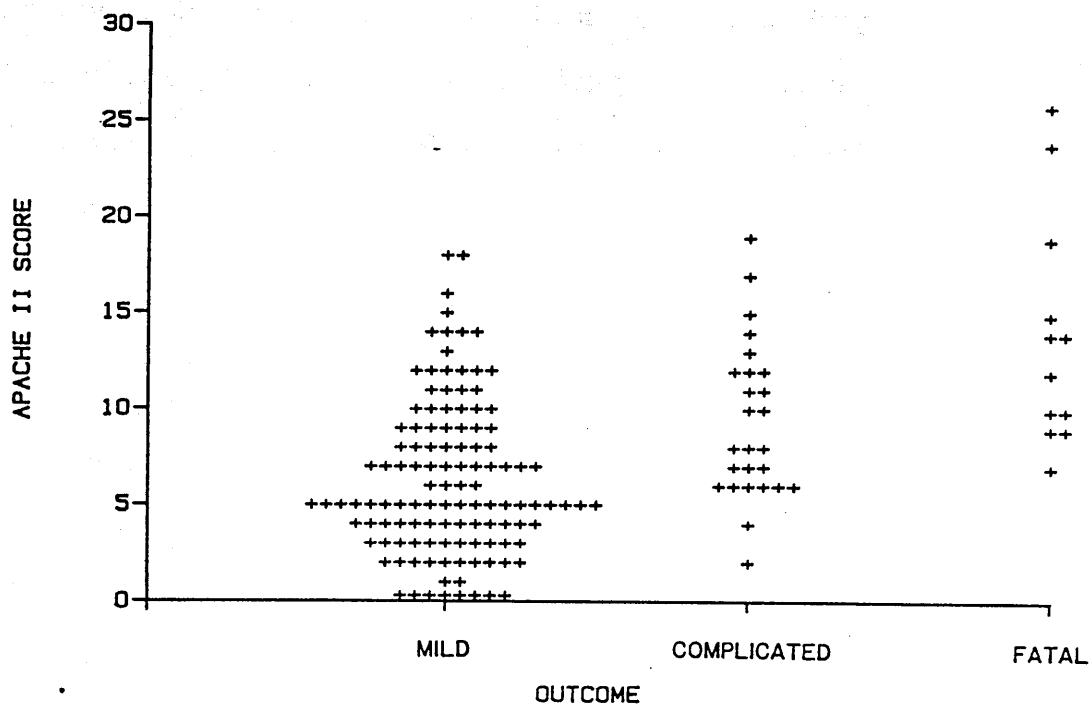


FIGURE 29.

Scattergram shows the distribution of the on admission APACHE II scores in relation to the outcome from acute pancreatitis.

attacks overall being graded correctly (Table 34). Raising the cut-off to >7 resulted in a slightly greater overall accuracy with some loss of sensitivity.

When the 14 individual components of the "on admission" APACHE II score were examined potassium, pO_2, heart rate, respiratory rate and age were all found to separate the uncomplicated attacks from complicated or fatal attacks. Sodium, the Glasgow coma scale and the chronic health evaluation were each found to be abnormal in very few patients (Table 35).

Peak APACHE II

A rather better discrimination between uncomplicated, complicated and fatal attacks was provided if the peak

APACHE II score (during the first 3 days) was used (Fig. 30). A score of >9 provided a sensitivity of 82% with 76% of attacks being predicted correctly, rising to 83% if the cut-off was raised to >12 (Table 34). Table 34 compares the admission and peak APACHE II scores with the other illness-specific prognostic scoring systems. Table 36 details the comparison of the scoring systems for attacks associated with the 2 major aetiologies, gallstones and alcohol respectively.

No deaths occurred in the patients with a peak APACHE II score of <10 and of these 95 patients, only 6 (6%) developed a complication (Fig. 31). As the peak APACHE II score increased so too did the incidence of death and complication.

TABLE 34.

Analysis of "admission" and "peak" APACHE II scores and comparison with multiple factor scoring systems and diagnostic peritoneal lavage in the prediction of outcome from acute pancreatitis.

	Sensitivity	Specificity	Predictive value +ve	Predictive value -ve	% Correct
On admission:					
APACHE II (>5)	95%	54%	40%	97%	64%
APACHE II (>7)	68%	67%	40%	87%	67.5%
Peak:					
APACHE II (>9)	82%	74%	50%	93%	76%
APACHE II (>12)	53%	92%	69%	86%	83%
Glasgow	71%	88%	66%	90.5%	84%
Ranson	87%	70.5%	48.5%	94%	74.5%
Diagnostic peritoneal lavage	67%	62.5%	77%	50%	65%

KEY:

Peak APACHE II score = highest score in day 1 - 3.

Sensitivity = % of all complicated attacks predicted correctly by test.
Specificity = % of all mild attacks predicted correctly by test.
PV +ve = % complicated attacks among all predicted severe by test.
PV -ve = % mild attacks among all predicted mild by test.
% Correct= % correctly classified.

TABLE 35.

Examination of the value of the 14 individual parameters comprising the admission APACHE II score in discriminating complicated from uncomplicated attacks.

Parameter	Complicated/ uncomplicated attack	Number of patients scoring	Weighting score distribution 1	2	3	4	5	6	p value
Sodium	complicated	2	2						NS
	uncomplicated	1	1						
Potasium	complicated	9	7	1		1			p <0.05
	uncomplicated	9	8			1			
Creatinine	complicated	9		5	1	2		1	NS
	uncomplicated	12		11	1				
Arterial pH	complicated	3		2		1			NS
	uncomplicated	7	4	2	1				
Arterial pO_2	complicated	17	7		3	7			p <0.02
	uncomplicated	27	17		6	4			
Haematocrit	complicated	7	4	3					NS
	uncomplicated	24	18	6					
White cell count	complicated	9	4	5					NS
	uncomplicated	23	15	8					
Temperature	complicated	17	16			1			NS
	uncomplicated	39	39						
Heart rate	complicated	25		19	6				p <0.02
	uncomplicated	50		43	7				
Mean blood pressure	complicated	26		19	5	2			NS
	uncomplicated	63		49	13	1			
Respiratory rate	complicated	15	12		3				p <0.001
	uncomplicated	11	10		1				
Glasgow coma scale	complicated	2		1	1				NS
	uncomplicated	3	2	1					
Age	complicated	31		5	10		11	5	p <0.01
	uncomplicated	64		11	20		20	13	
Chronic health evaluation	complicated	1					1		NS
	uncomplicated	2					2		

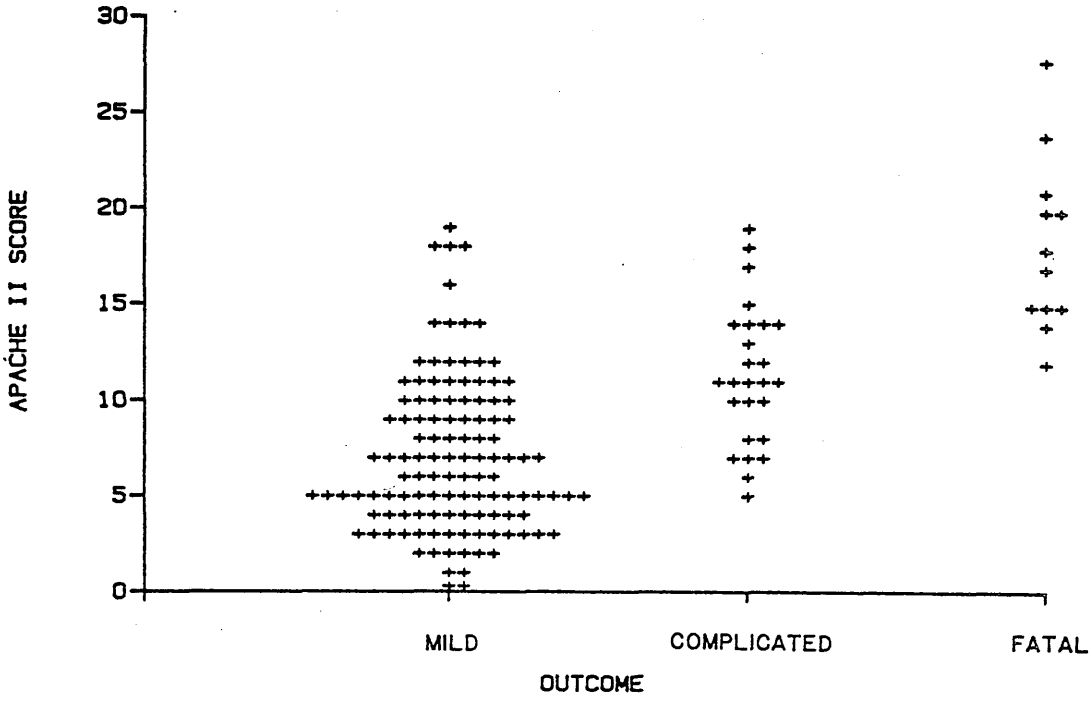


FIGURE 30.

Scattergram shows the distribution of the peak APACHE II scores (day 1 - 3) in relation to the outcome from acute pancreatitis.

TABLE 36.

Comparison of the peak APACHE II score with multiple factor scoring systems and diagnostic peritoneal lavage for gallstone and alcohol-associated attacks of acute pancreatitis.

	Sensitivity	Specificity	Predictive value +ve	Predictive value -ve	% Correct
Gallstone					
Peak APACHE II (>9)	93%	66%	45%	97%	72%
Glasgow	64%	78%	47%	88%	75%
Ranson	86%	52%	35%	92%	60%
Diagnostic peritoneal lavage	50%	100%	100%	60%	71.5%
Alcohol					
Peak APACHE II (>9)	57%	88%	44%	92%	83%
Glasgow	57%	98%	80%	93%	92%
Ranson	86%	98%	86%	98%	96%
Diagnostic peritoneal lavage	100%	25%	62.5%	100%	67%

KEY: as for table 34.

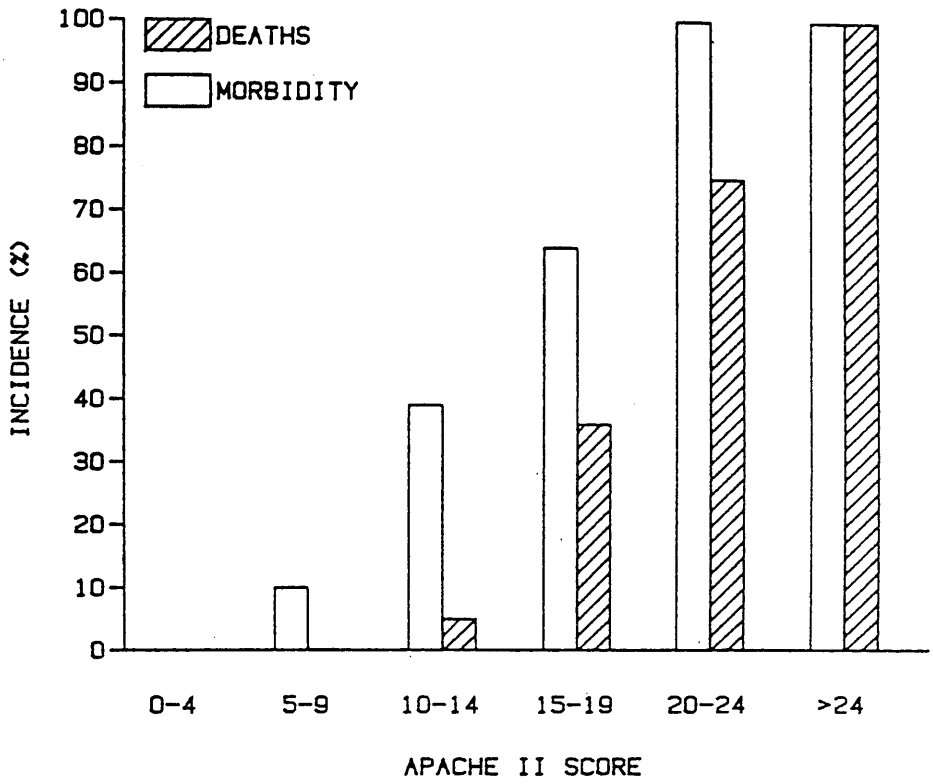


FIGURE 31.

Incidence of death and morbidity from acute pancreatitis in relation to the peak APACHE II score recorded.

Monitoring the course of illness

Figure 32 shows the daily mean APACHE II scores plotted for each of the 3 groups of patients: fatal attacks, complicated attacks and uncomplicated attacks. There were highly significant differences throughout the 7 days studied between those patients with a mild, uncomplicated attack and both those patients with a complicated attack and those with a fatal attack.

Patients with an uncomplicated attack of pancreatitis showed a steady fall each day in their mean laboratory, physiology and total APACHE II scores with resolution of the attack. Patients with a complicated outcome who survived showed a higher but similar overall pattern except for a transient elevation of the laboratory and total APACHE II scores on the third day. Their physiology score was also slower in falling.

A different pattern was seen amongst the 12 patients with a fatal outcome. They showed an early rise in both the laboratory and total scores, peaking on the third day before falling. This group also showed persistent elevation of their physiology score presumably reflecting the continuing disease activity. The worsening scores recorded in the 7 patients dying within the first week of their illness are shown in figure 33. Thus monitoring the APACHE II score during the first 7 days may provide an objective measure of the degree of illness and appears to reflect improvement or deterioration in the patients' clinical condition.

No differences emerged between the mean APACHE II scores over the first 7 days in patients with attacks complicated by respiratory

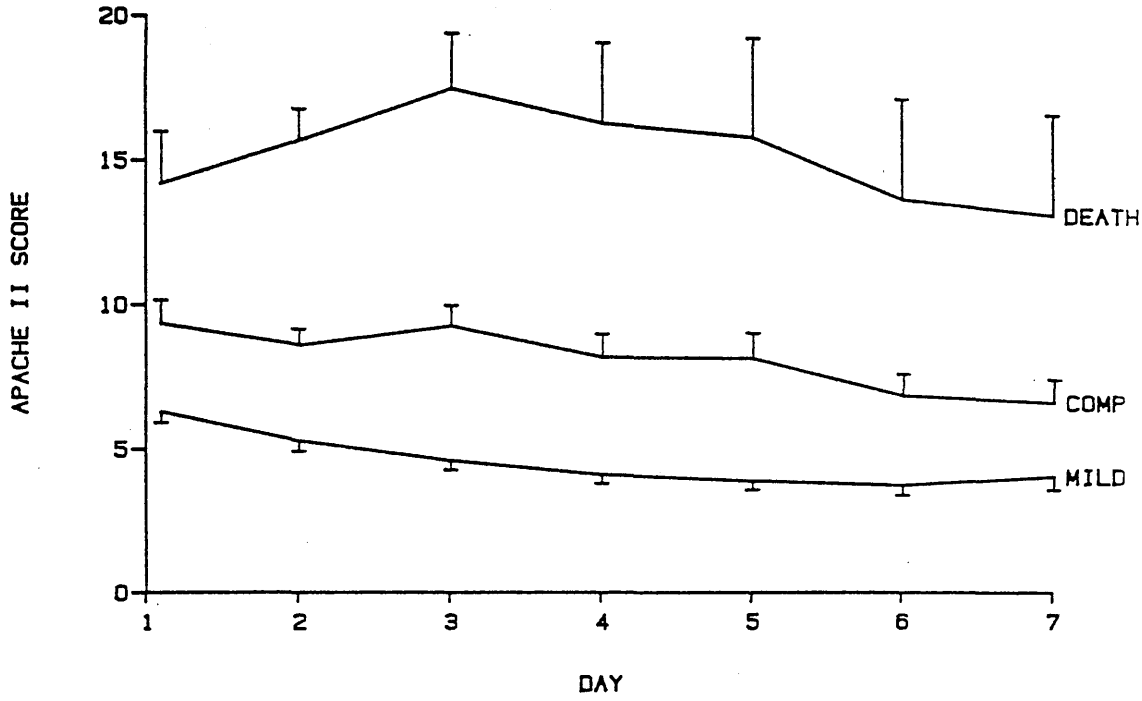


FIGURE 32.

Mean daily APACHE II scores by outcome in 119 patients with an uncomplicated course (mild), 26 patients with a complicated course (comp) and in 12 patients with a fatal outcome (death). The differences between fatal and uncomplicated and between complicated and uncomplicated were highly significant each day, $p < 0.001$.

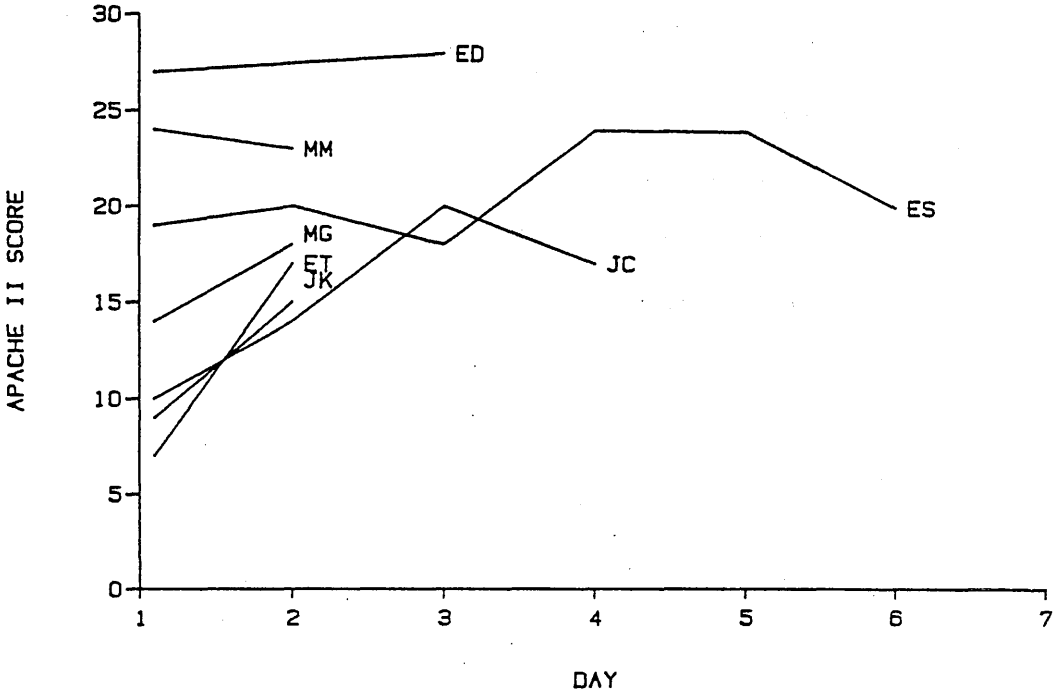


FIGURE 33.

Shows the rising pattern of the APACHE II scores in the 7 patients who died from acute pancreatitis during the first week of their illness.

insufficiency or by local pancreatic complications such as necrosis or pseudocyst.

Discussion

APACHE II has been shown to provide useful discrimination between mild, complicated and fatal attacks of acute pancreatitis within a few hours of admission to hospital. The laboratory tests required are simple and routine and are readily available "out of hours" which is not generally the case for the calcium or the enzyme assays required for the Ranson and Glasgow multiple factor scoring systems.

On admission neither the overall accuracy nor the predictive value of APACHE II were particularly high but appeared to compare favourably with diagnostic peritoneal lavage in this study. Diagnostic peritoneal lavage was not performed in all patients in the current study and thus is not strictly comparable but in previous studies has correctly predicted the outcome in between 82%[70] and 90% of cases[226]. Although providing an early prediction of outcome diagnostic peritoneal lavage remains an invasive test with a small risk of visceral injury and has not found widespread acceptance outwith clinical trials.

APACHE II was superior to clinical assessment on admission which, although shown to have a higher overall accuracy in the previous chapter, failed to identify 2/3rds of the attacks with a complicated or fatal outcome. Furthermore, APACHE II as it depends on the measurement of laboratory and physiological parameters is

objective and reproducible.

When the peak APACHE II scores were considered, its diagnostic accuracy improved to levels equivalent to the illness-specific multiple factor scoring systems, although this would then incur a similar delay. When gallstone and alcohol-associated attacks were examined separately it can be seen that the APACHE II score performed as well as the established multiple factor scoring systems for attacks associated with gallstones. The Ranson scoring system performed particularly well for attacks associated with alcohol abuse and APACHE II performed slightly less well in such cases.

Thus the APACHE II scoring system may prove to be a useful addition to the management and study of these patients. It may provide an indication of the severity and possible outcome of an attack soon after admission to hospital, permitting early, non-invasive selection of patients for study in clinical trial. The peak APACHE II score provided a more accurate prognostication of outcome similar to that of the standard multiple factor scoring systems and was able to grade patients by their percentage risk of major morbidity or death.

While the standard illness-specific scoring systems provide a single "one-off" prognostication, usually within the first 48 hours of the attack, but sometimes delayed 60 hours or more, the APACHE II score can be repeated daily to monitor the course of the attack. Patients dying within the first 4 days of admission showed a rising score reflecting their deteriorating organ function, whereas patients with an uncomplicated or complicated outcome demonstrated a falling

trend during the first 7 days of their illness with resolution of the systemic manifestations of their attack. The APACHE II score appeared to reflect continuing disease activity and may, therefore, be a useful means of monitoring the course of the illness and any response to therapy.

There were no differences in the mean APACHE II scores whether the attack was complicated by respiratory insufficiency or local pancreatic complications such as necrosis or pseudocyst. The design of the study did not allow for the continued monitoring of patients who went on to develop a late complication, to look for signs of secondary deterioration. This aspect might be examined in future studies.

Several components of the overall APACHE II score contributed to the total in only a few patients and other parameters failed to provide significant discrimination between the patient groups (Table 35). Other potential drawbacks of the APACHE II score when used to monitor the disease course included scoring patients who were clinically well during the latter half of their attack for a low normal heart rate or for an abnormal bicarbonate (used if pO_2 was not available). Perhaps the system might be improved by placing less weight on low normal values in an otherwise well patient. Nevertheless, it is an attractive concept to have a single scoring system applicable to all illnesses, although this will inevitably be associated with less accuracy than scoring systems designed for one particular condition.

PART 5.

INTRAPERITONEAL ANTIPROTEASE THERAPY

CHAPTER 9. STUDY OF FREE PROTEOLYTIC ACTIVITY IN PANCREATITIS EXUDATE AND THE EFFICACY OF INTRAPERITONEAL ANTIPROTEASE THERAPY IN EXPERIMENTAL ACUTE PANCREATITIS IN RATS

Introduction

Peritoneal proteolytic enzyme activity has previously been investigated in dogs with pancreatitis induced by the closed duodenal loop technique and by retrograde injection of bile into the pancreatic duct[262,263], overwhelming of the peritoneal antiprotease defences being associated with the onset of shock, and subsequent death of the animals.

Rats have in recent years become the most frequently used animal for studies of experimental acute pancreatitis but the exudates associated with the most commonly used taurocholate-induced pancreatitis model have not previously been examined for protease activity and adequacy of their antiprotease defences.

Formal peritoneal lavage has been shown to prolong the survival of rats with taurocholate-induced pancreatitis, addition of the antiprotease aprotinin to the lavage fluid in one series of experiments providing no additional benefit[197]. Therapy with intraperitoneal aprotinin alone was not associated with an improvement in survival, nor reduction in the degree of pancreatic damage in another study[34].

The aim of the current study was to investigate proteolytic enzyme activation and the adequacy of the peritoneal antiprotease defences in the exudates associated with taurocholate-induced

pancreatitis in the rat and secondly, to determine whether a novel treatment comprising removal of the exudate followed by instillation of an antiprotease solution, might be therapeutic.

Pilot studies

Preliminary studies were performed to investigate the severity and reproducibility of the bile salt model of acute pancreatitis originally described by Lankisch and co-workers[197] and later by others[7]. Both groups described a "free hand" retrograde injection, of 0.6ml and 0.2ml of sodium taurocholate solution respectively, into the bile-pancreatic duct of the rat. Using a "free hand" injection technique it is difficult to standardise both the rate of infusion and the pressures generated and thus the degree of pancreatic ductal rupture and pancreatic injury. This might be expected to produce variability in the severity of the pancreatic lesion induced and thus a lack of reproducibility which could be important, particularly if only small numbers of animals were to be studied. Pilot studies were therefore conducted, to determine the mortality rate and the pattern of the survival time associated with acute pancreatitis induced by a controlled retrograde infusion of sodium taurocholate.

A further modification of the technique was also studied entailing the slow retrograde infusion of a combined sodium taurocholate/enterokinase solution (Leese T, personal communication).

Methods

Male Wistar rats weighing 170-200g were used throughout. The

animals were fasted overnight prior to the induction of pancreatitis but were permitted free access to water. The body weight of the rats was determined just prior to use to permit calculation of the anaesthetic dose and volumes of subsequent intraperitoneal therapy. Anaesthesia was induced by intraperitoneal injection of pentobarbitone sodium (Sagatal, May and Baker, Dagenham, UK) 6mg/100g body weight.

Cannulation of the pancreatic duct

The abdomen was opened through a midline laparotomy and the duodenal loop with the head of pancreas was delivered out of the wound. A puncture was made in the antimesenteric border of the duodenum, just distal to the entry point of the common bile-pancreatic duct on the mesenteric border. Prior to introducing a cannula into the duodenum the cannula was primed with the appropriate bile salt solution. The tip of a size 23g x 22mm teflon cannula (Wallace Y-Can, H G Wallace Ltd., Essex, UK) was then passed into the duodenal lumen and manipulated into the bile-pancreatic duct via the ampulla. The cannula was secured in place in the duct by an encircling 3/0 silk ligature placed around the bile-pancreatic duct in the head of the gland, just proximal to its entry into the duodenal wall. A neurosurgical aneurysm clip was then placed across the common hepatic duct at the liver hilum, above the highest pancreatic ductal branch, to prevent retrograde injection of the bile salt solution into the liver bile duct radicles (Fig. 34).

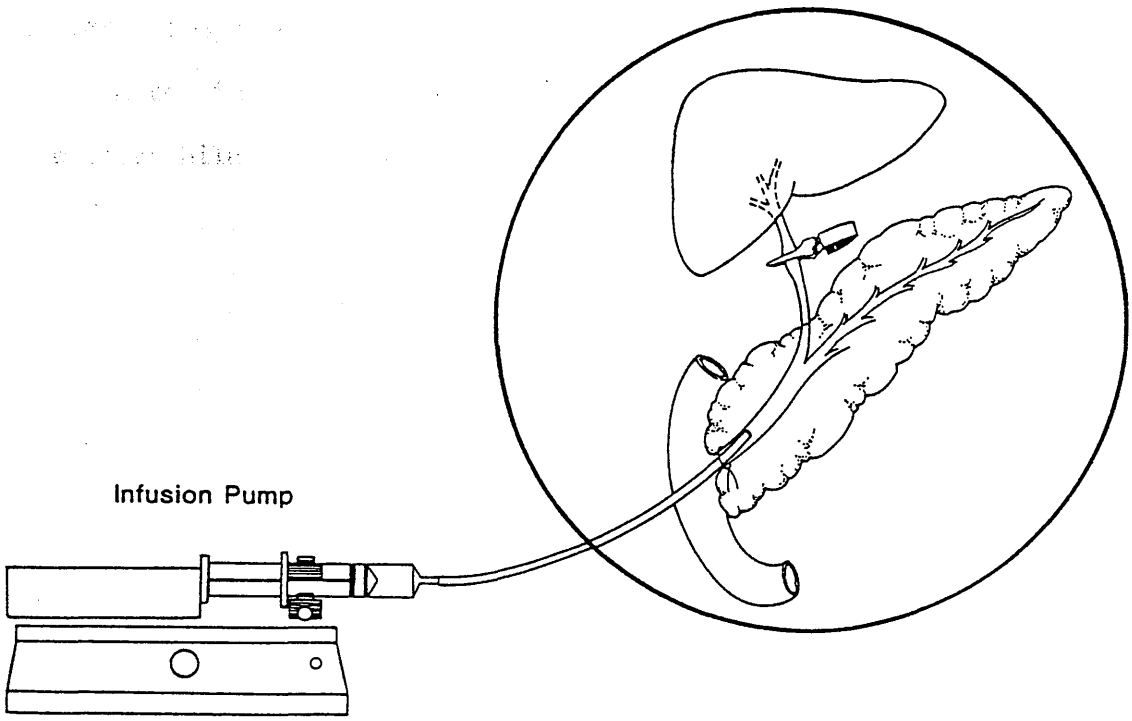


FIGURE 34.

Diagrammatic representation of the method of inducing experimental pancreatitis showing transduodenal placement of a cannula in the rat bile-pancreatic duct. A neurosurgical clip is applied across the proximal bile duct, at the liver hilum, prior to commencement of the bile salt infusion.

Induction of acute pancreatitis

The cannula in the bile-pancreatic duct was connected by standard extension tubing to a 5ml capacity glass syringe mounted in a syringe driver (Braun-Melsungen, West Germany). The infusion of the bile salt solution was timed and at the required volume the syringe driver was stopped. Calibration of the pump for a delivered volume of 200µl at an infusion rate of 100µl/minute resulted in a coefficient of variation of 0.9%.

After infusion of the bile salt solution the aneurysm clip at the liver hilum was removed. The ligature encircling the cannula in the distal bile-pancreatic duct was released and the cannula removed completely. Finally the puncture wound in the duodenal wall was repaired with a pursestring suture of 6/0 Ethilon. The appearance of the pancreas during the induction of pancreatitis is shown in figures 35-37.

Placement of peritoneal cannula

The pancreas was returned to the abdomen and a peritoneal dialysis cannula was placed in the peritoneal cavity. The dialysis cannula was fashioned from an intravenous infusion extension set comprising a three-way tap, a 10cm length of plastic tubing and a luer lock (Connecta, Viggo AB, Helsingborg, Sweden). This was selected because the plastic tubing was rigid and did not collapse on applying suction when aspirating fluid. The luer lock was excised and several side holes cut in the distal 5cm of the tubing thus fashioning a peritoneal dialysis cannula.

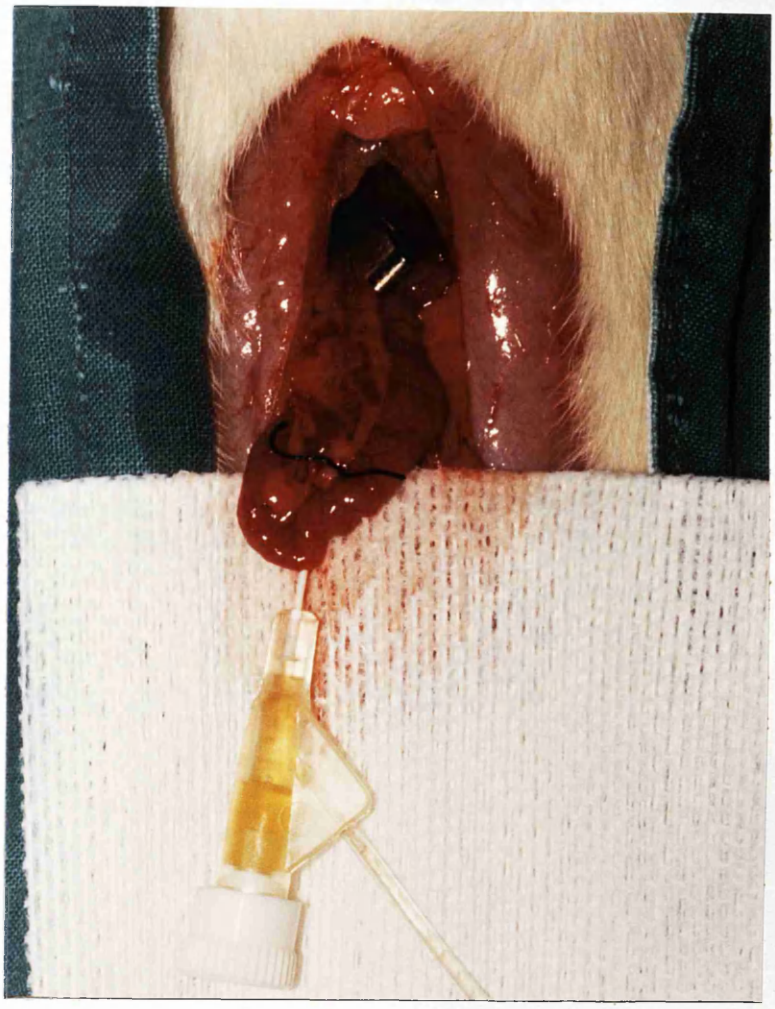


FIGURE 35.

Induction of pancreatitis. Cannula secured in bile-pancreatic duct with an encircling silk ligature. Appearance of pancreas soon after commencement of infusion showing oedema and haemorrhage around the main pancreatic duct.

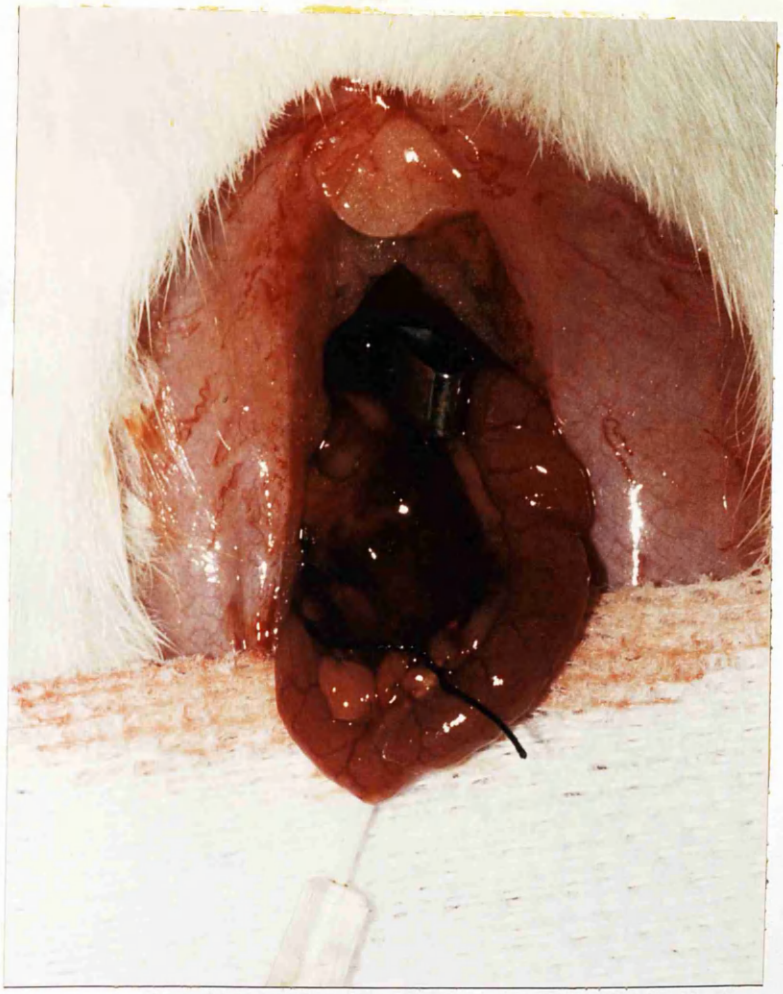


FIGURE 36.

Appearance of the pancreas at the end of the infusion, showing marked oedema, swelling and haemorrhage in the head of the gland.

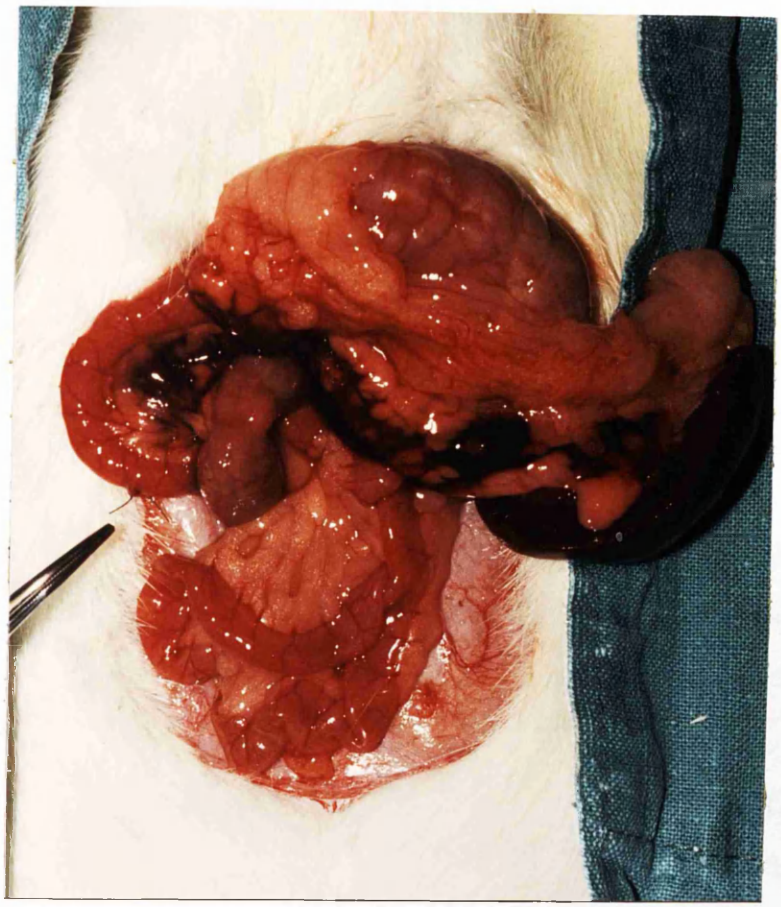


FIGURE 37.

Appearance of gland after removal of cannula showing swelling, oedema and haemorrhage of head, body and tail of gland extending to splenic hilum. Note pursestring suture of the puncture site in the duodenal wall.

The tip of a haemostat was passed from within the peritoneal cavity through the peritoneum, muscle and skin to emerge high on the animal's back, just to the right of the midline. Withdrawing the haemostat the cannula was pulled into the peritoneal cavity and the three-way tap was secured to the skin of the animal's back with a single silk suture. The open end of the cannula with side holes was placed adjacent to the pancreas, to lie in the most dependent portion of the peritoneal cavity with the animal upright. Finally the laparotomy wound was closed in layers.

Pilot studies

To determine the most suitable model for study a series of experiments were run utilising different concentrations of sodium taurocholate (BDH Chemicals Ltd., Poole, UK) in solution with 0.9% saline solution, prepared immediately before use. In one series of experiments porcine enterokinase (Sigma Chemical Co, St Louis, USA) was added to the test solution to a concentration of 0.2%.

The test infusions and the infusion rates studied are detailed as follows:-

sodium taurocholate 3%, 0.6ml infused at 200µl/minute

sodium taurocholate 5%, 0.2ml infused at 100µl/minute

sodium taurocholate 5%, 0.6ml infused at 200µl/minute

sodium taurocholate 5%, 2ml/kg infused at 50µl/minute

sodium taurocholate 3.5% with enterokinase 0.2%, 2ml/kg body weight infused at 50µl/minute.

These animals did not have a peritoneal cannula placed and

received no additional therapy postoperatively. The survival times were recorded and any animals remaining alive at 72 hours were sacrificed.

Experiment - intraperitoneal therapy

All rats had standard induction of pancreatitis with sodium taurocholate 5%, 0.6ml, freshly prepared and infused at 200µl/minute. Each animal had a peritoneal dialysis cannula placed prior to closure of the laparotomy wound. Only after successful completion of the procedure were the animals randomly allocated to one of the 4 treatment groups or to the control group, the randomisation ensuring 5 groups each of 10 rats.

Therapy (see below) was carried out on all rats exactly one hour after the induction of pancreatitis, the animals at this stage remained sedated or were only just beginning to recover from the earlier pentobarbitone injection.

In rats from the 4 treatment groups (randomised to receive intraperitoneal therapy) the peritoneal cannula was aspirated with a 5ml syringe and the volume and colour of the exudate was recorded (Figs. 38,39), the colour being compared against a standard colour chart[226]. In each case a 1ml aliquot of the fluid was centrifuged at 4°C and the supernatant stored at -20°C for subsequent analysis.

The peritoneal cavity was then washed out by a single peritoneal lavage with 0.9% saline solution (1.5ml/100g body weight). This was chosen to correspond to an approximate volume of 1000mls in a 70kg man, as suggested for diagnostic peritoneal lavage of patients

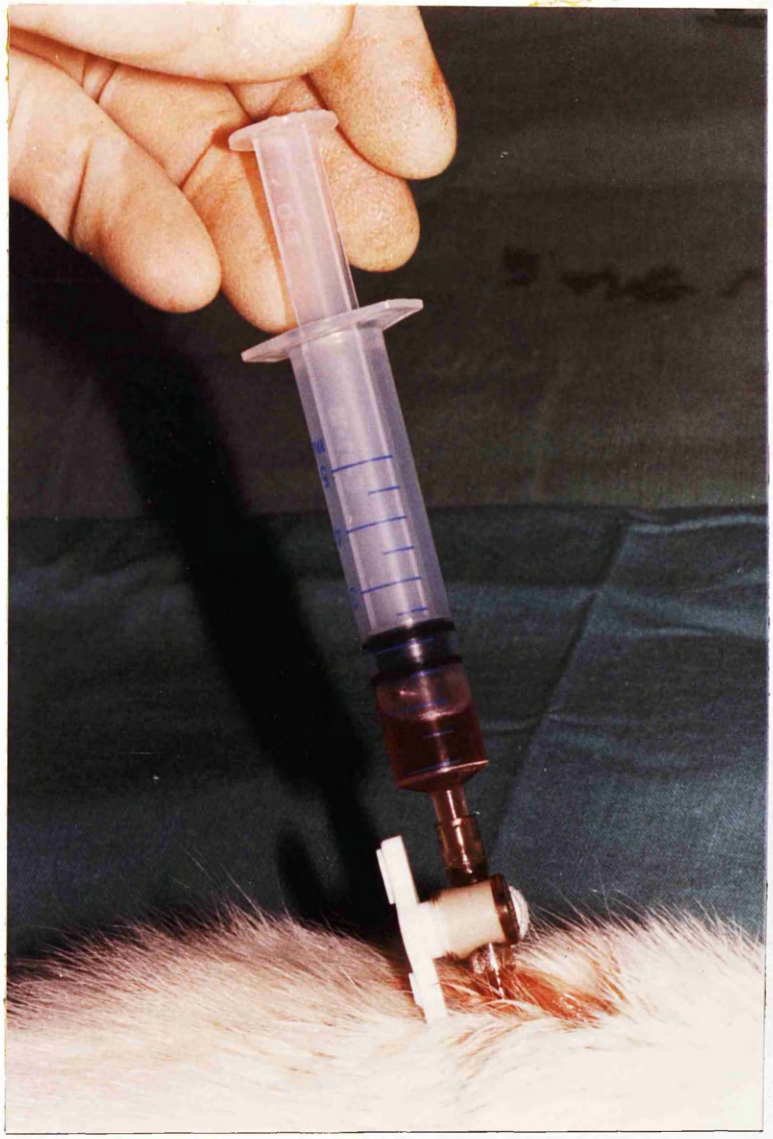


FIGURE 38.

Aspiration of free peritoneal exudate via the peritoneal dialysis cannula, one hour after the induction of experimental pancreatitis.

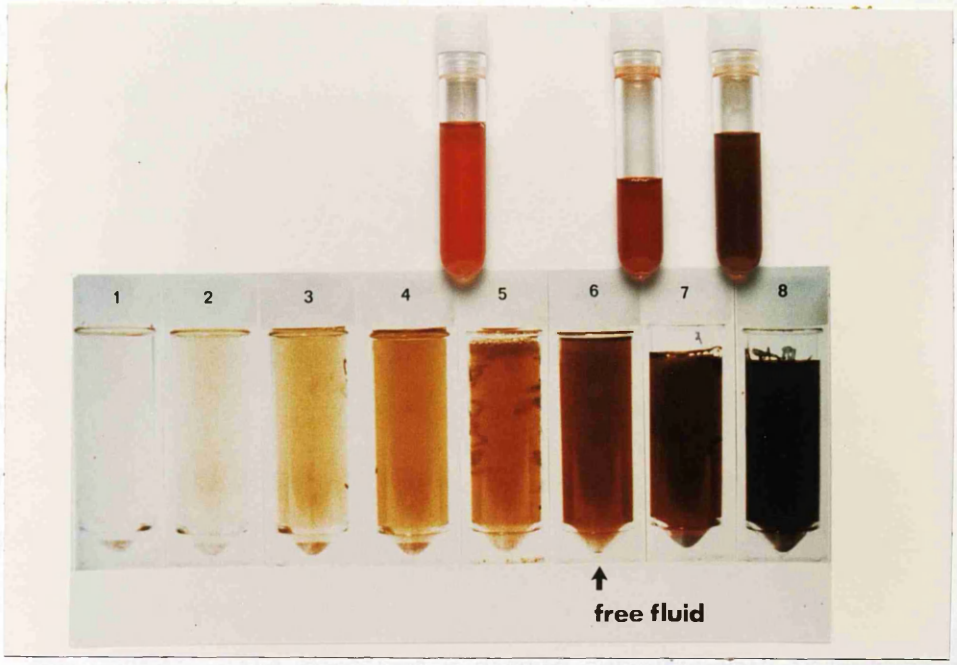


FIGURE 39.

Shows Leeds colour chart for identification of severity of acute pancreatitis with samples of peritoneal fluid from a rat with experimental pancreatitis, from left to right: peritoneal fluid after saline lavage, free exudate one hour after induction of pancreatitis and free exudate obtained at post mortem.

with acute pancreatitis[226]. After circulating the fluid by gently squeezing the animal's abdomen the fluid was then aspirated and the volume and colour recorded as before. An antiprotease or similar inactive solution was then instilled into the peritoneal cavity (1.5ml/100g body weight) and finally the peritoneal cannula was withdrawn leaving the fluid in situ (Fig. 40). The net fluid balance was calculated by subtracting the total volume of fluid aspirated from the total volume of fluid instilled and the balance recorded.

Active antiprotease or inactive solutions were studied as follows:- aprotinin (5000 KIU/ml in 0.9% saline solution) or 0.9% saline solution, human fresh frozen plasma (whole plasma containing clotting factors and the natural antiproteases) or human plasma protein solution (a colloid solution, predominantly of albumin at a concentration of 45g/l, devoid of active antiproteases and other active plasma factors).

The control rats received a single subcutaneous injection of 0.9% saline solution (1.5ml/100g body weight) exactly one hour after the induction of pancreatitis. The peritoneal cannula was then withdrawn without removing any free ascitic fluid from the peritoneal cavity (Fig. 41).

Animals from all groups were kept in cages of 4 with free access to water. Food pellets were reintroduced 24 hours postoperatively. The animals were examined every hour and the time of death recorded. Animals surviving to 72 hours were sacrificed. A post mortem was performed on all animals immediately after death. The volume and colour of any peritoneal exudate was recorded as described

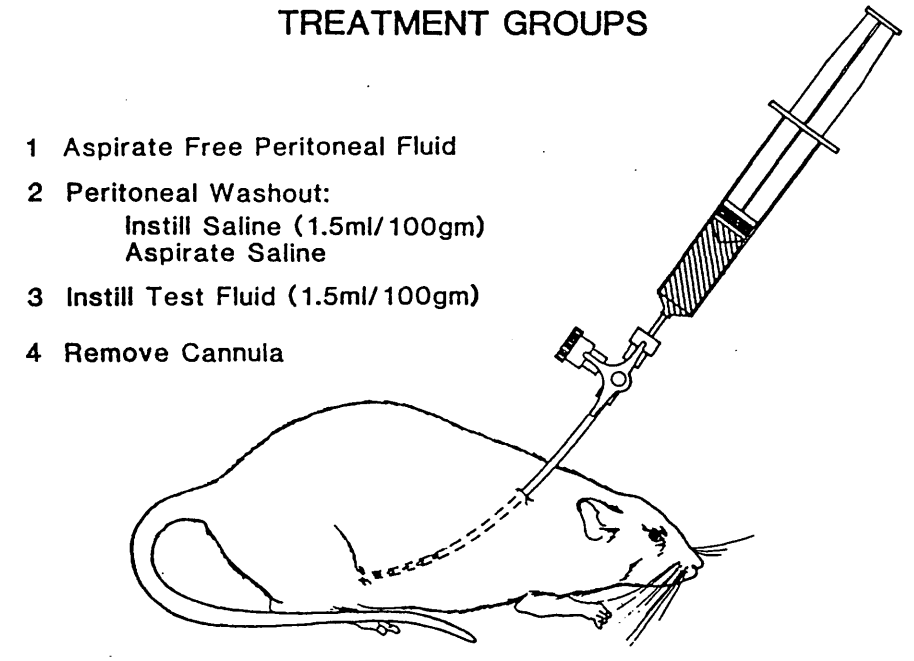


FIGURE 40.

Experimental protocol in rats randomised to receive intraperitoneal therapy at one hour.

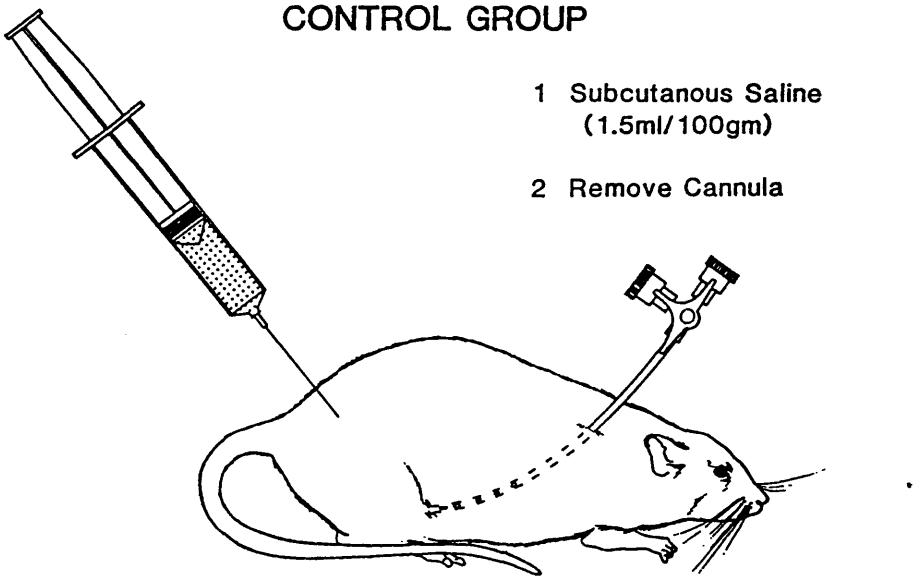


FIGURE 41.

Experimental protocol in rats randomised to the control group.

above (Fig. 39), and a 1ml aliquot of fluid was centrifuged at 4°C and the supernatant stored at -20°C for subsequent analysis.

The extent of the pancreatitis in the head and tail of the gland was graded macroscopically on a scale 1 to 4, corresponding to a normal appearance, mild inflammation/ thickening, severe inflammation/thickening or necrosis respectively (Fig. 42). The extent of fat necrosis in the peritoneal cavity was recorded as none, localised to the pancreas, regional or generalised. The pancreas was excised and the pancreatic weight recorded. The pancreas was then fixed in formalin, processed, sectioned and stained with haematoxylin and eosin for histological examination.

Biochemical analyses

The trypsin binding capacities of the 4 test solutions were determined in chapter 5. The peritoneal fluid samples obtained from the rats in the 4 treatment groups at one hour post-induction of pancreatitis and from all rats at autopsy were examined for evidence of free proteolytic activity as described in chapter 5. The peritoneal fluid specimens were thawed at room temperature and 20ul samples of each specimen were applied to a 0.2% plasminogen-free fibrin plate. Free proteolytic activity was judged to be present if a zone of lysis appeared on the plate following overnight incubation at 37°C.

Statistics

Statistical analysis of the survival times was conducted by the

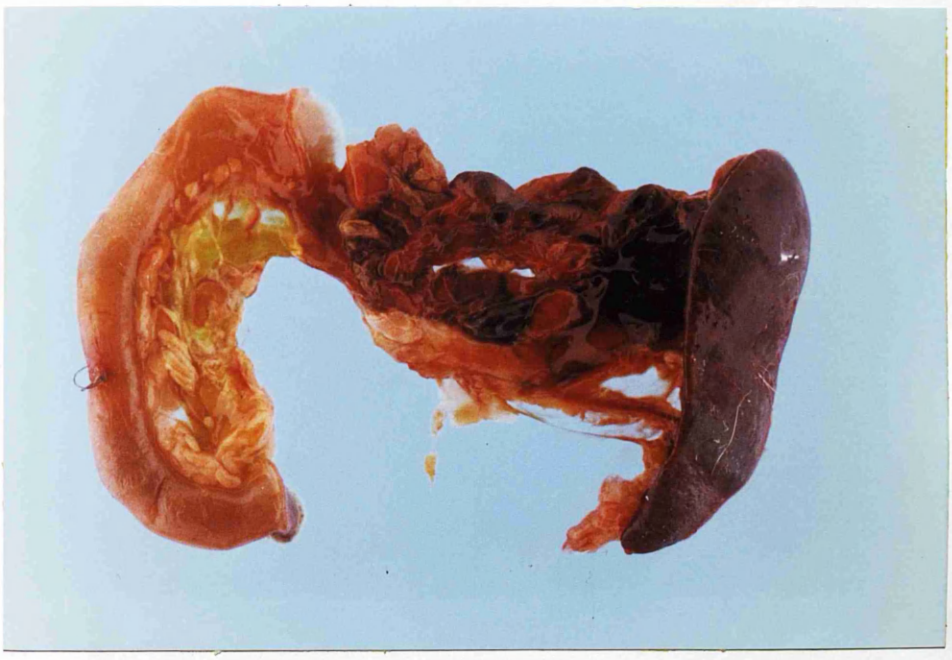


FIGURE 42.

Pancreas excised at post mortem showing extensive necrosis of gland (grade 4).

Log rank test. Simultaneous comparisons between all 5 groups were made with the Kruskal Wallis test and comparisons between 2 groups, by the Mann-Whitney U test.

Results

Pilot study

Table 37 shows the survival times associated with induction of pancreatitis by retrograde infusion of various concentrations of sodium taurocholate. Infusion of 5% sodium taurocholate, 0.6ml at 200µl/minute was the only method to produce a consistently high mortality. Animals surviving 48 hours remained well and were sacrificed at 72 hours.

Trypsin binding capacity

Trypsin binding capacities determined on the 4 test solutions demonstrated both 0.9% saline solution and human plasma protein solution to have no appreciable trypsin binding capacity. Human fresh frozen plasma was able to bind 80µg of trypsin/100µl, lysis appearing after the addition of 100µg of trypsin/100µl. Aprotinin solution was able to bind over 400µg of trypsin before lysis appeared after the addition of 450µg of trypsin/100ul and thus had the highest trypsin binding capacity of the 4 test solutions.

Experiment

The mean body weights of the rats from all 5 groups were comparable (Table 38). The mean volumes of exudate recovered from the

TABLE 37.

Pilot study investigating the mortality associated with different methods of inducing pancreatitis: varying the concentration, volume and rate of retrograde bile infusion.

Bile salt(concentration)	Volume	Rate	No.	Survival <24hrs	24-48hrs	>48hrs
Na taurocholate (3%)	0.6ml	200µl/min	2	-	-	2
Na taurocholate (5%)	0.2ml	100µl/min	8	1*	-	7
Na taurocholate (5%)	0.6ml	200µl/min	18	15	-	3
Na taurocholate (5%)	2ml/kg	50µl/min	2	-	-	2
Na taurocholate (3.5%)/ Enterokinase (0.2%)	2ml/kg	50µl/min	6	3	-	3

* leakage from repaired duodenal puncture wound at post mortem.

TABLE 38.

Comparability of peritoneal exudate characteristics, fluid balance and morphological parameters in the control group and in the 4 groups receiving intraperitoneal therapy.

Parameter	Control group	Intraperitoneal therapy groups				*p value
		Saline	Plasma protein solution	Fresh frozen plasma	Aprotinin	
Body weight (Gm)	186±7	187±7	184±6	186±8	185±7	NS.
Exudate Vol.(mls) at 1 hour	-	1.3±0.4	1.7±0.4	1.55±0.3	1.5±0.5	NS.
Colour	-	6.5	6.5	6.5	6.5	NS.
Net fluid balance post-therapy (mls)	2.8±0.1	1.8±0.7	1.2±0.5	1.4±0.5	1.7±0.6	p<0.01
Exudate Vol.(mls) at post mortem	6.7±2.7	5.3±2.2	6.9±3	3.25±2.5	6.55±2.5	NS.
Colour	6.8±1	6.15±0.9	6.3±0.75	6.2±0.9	6.25±1	NS.
Macroscopic score Head	3.9±0.3	3.8±0.4	4	3.7±0.6	3.9±0.3	NS.
Tail	3.35±0.4	3.3±0.3	3.35±0.55	3.25±0.75	3.3±0.6	NS
Pancreatic weight (mg)	119±38	117±32	110±18	131±32	124±21	NS.

Results expressed as means ± s.e.m.

* Kruskal Wallis test.

rats in the 4 treatment groups one hour post induction of pancreatitis ranged between 1.3 and 1.7mls and were not significantly different and the mean fluid colour scores were identical (Table 38). Following peritoneal lavage and instillation of the test solution, the mean net fluid gains amongst rats in the 4 treatment groups were similar. The control group had a significantly higher net fluid gain ($p < 0.01$), having had no peritoneal exudate removed (Table 38).

At post mortem the mean volumes of exudate were highest in the human plasma protein solution, control and aprotinin groups and was lowest in the group treated by human fresh frozen plasma although these differences were not significant (Table 38). The volume of exudate was lowest in the rats surviving to sacrifice at 72 hours. The exudate recovered at post mortem tended to be darker in the control rats but these differences were not significant.

Morphological aspects

Macroscopic assessment of the degree of pancreatitis at post mortem uniformly recorded necrosis in the head of the gland amongst the early deaths, those rats surviving to sacrifice at 72 hours showing severe pancreatic inflammation and thickening. The appearances of the body and tail of the glands was more variable, some showing necrosis mainly in the central portion of the gland around the main duct with more normal looking pancreas peripherally, other glands showed complete necrosis. No trend was apparent between the macroscopic appearances and the treatment administered. The mean scores are shown in Table 38 and no significant differences emerged

between the groups.

Fat necrosis was generalised in all animals and did not distinguish the groups or whether death occurred early or late.

The pancreatic weight was slightly greater in the 2 groups of rats receiving antiprotease solutions but these differences just failed to reach statistical significance (Table 38). Histology confirmed acute pancreatitis in all rats.

Survival time

The 4 groups of rats receiving intraperitoneal therapy had significant prolongation of their median survival times compared to controls (Fig. 43). When the intraperitoneal therapy groups were compared individually with the control group only the group treated with human fresh frozen plasma had a significantly longer median survival (Table 39). Therapy with human fresh frozen plasma significantly prolonged survival when compared with human plasma protein solution (Fig. 44). Therapy with aprotinin appeared no better than therapy with saline alone (Fig. 45) although when compared with the control group the improvement in survival of the aprotinin group just failed to reach statistical significance (Table 39).

Free proteolytic activity

Exudates aspirated one hour post-induction of pancreatitis demonstrated free proteolytic activity in between 4 and 5 of the rats in each treatment group (mean lysis area 359 $\pm$ 82mm^2, equivalent to 63μg free bovine trypsin/ml) (Table 40). The median survival time

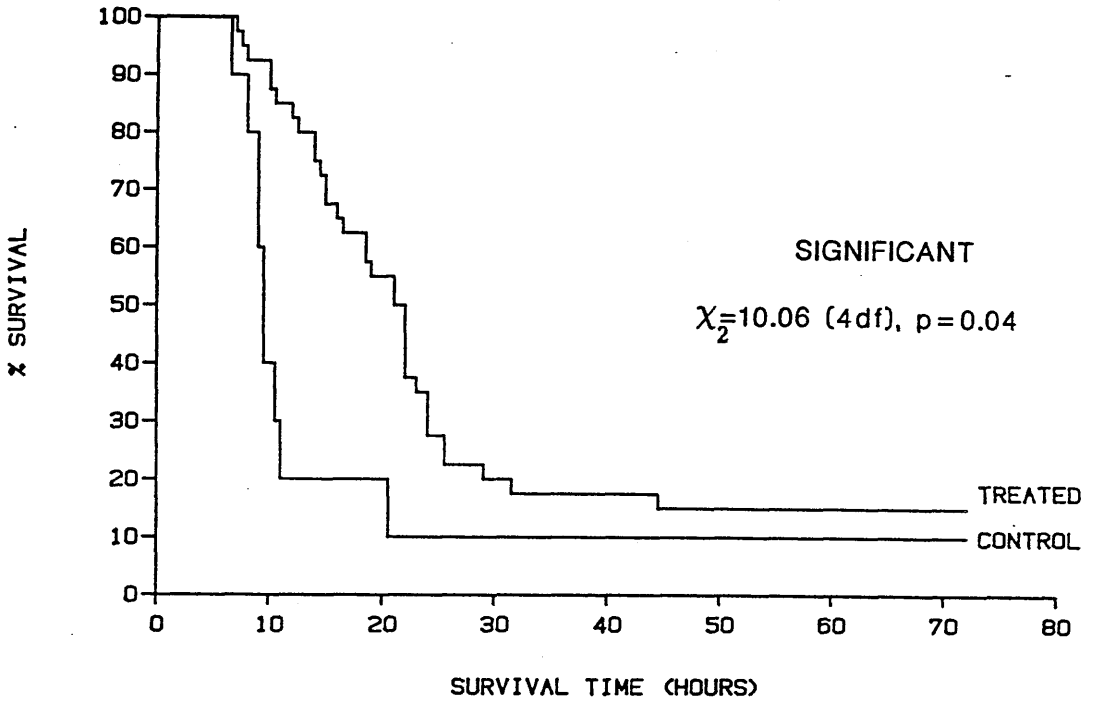


FIGURE 43.

Life table showing improved survival time amongst the 40 rats having intraperitoneal therapy (treated) compared to the 10 controls (control).

TABLE 39.

Comparison of survival times in the 4 treatment groups and in the control group following the induction of pancreatitis.

	No.	Median survival (hours)	Interquartile range (hours)	p value compared to control group
Control	10	9.5	8.8 - 13.4	-
Saline	10	20.3	7.9 - 51.4	0.236
Plasma protein solution	10	17.5	14.9 - 22.5	0.116
Fresh frozen plasma	10	24.8	18.8 - 72	0.01*
Aprotinin	10	19.8	14.0 - 23.3	0.065

* significant, log rank test.

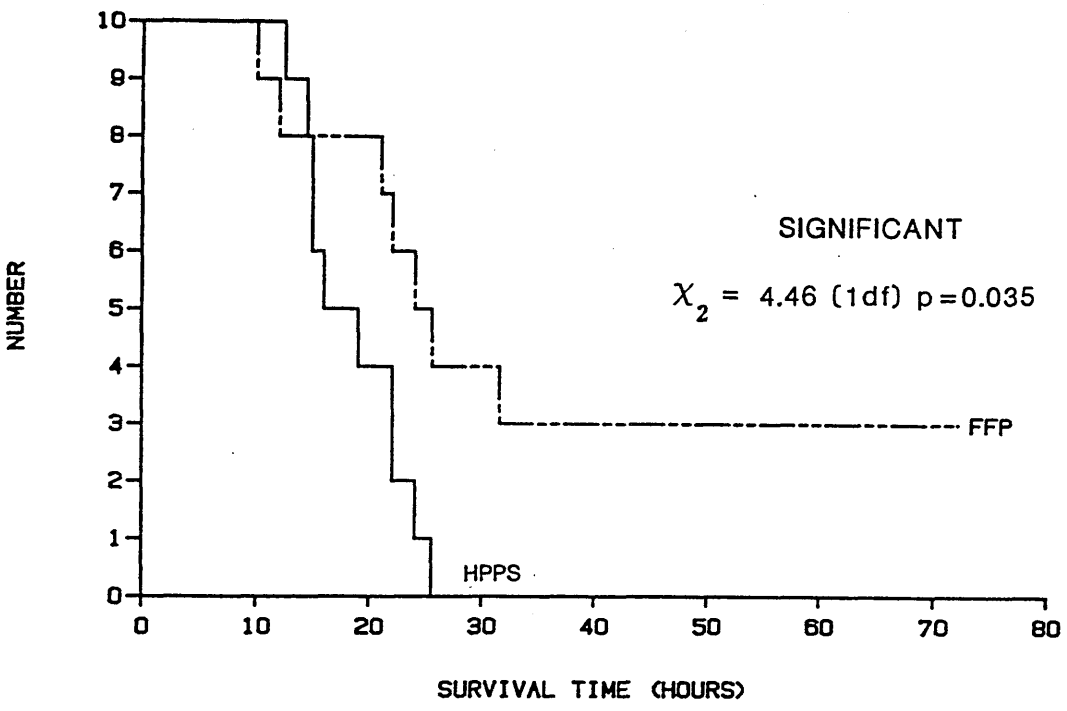


FIGURE 44.

Life table showing improvement of survival time in the 10 rats treated with intraperitoneal fresh frozen plasma (FFP) compared to the 10 treated with intraperitoneal human plasma protein solution (HPPS).

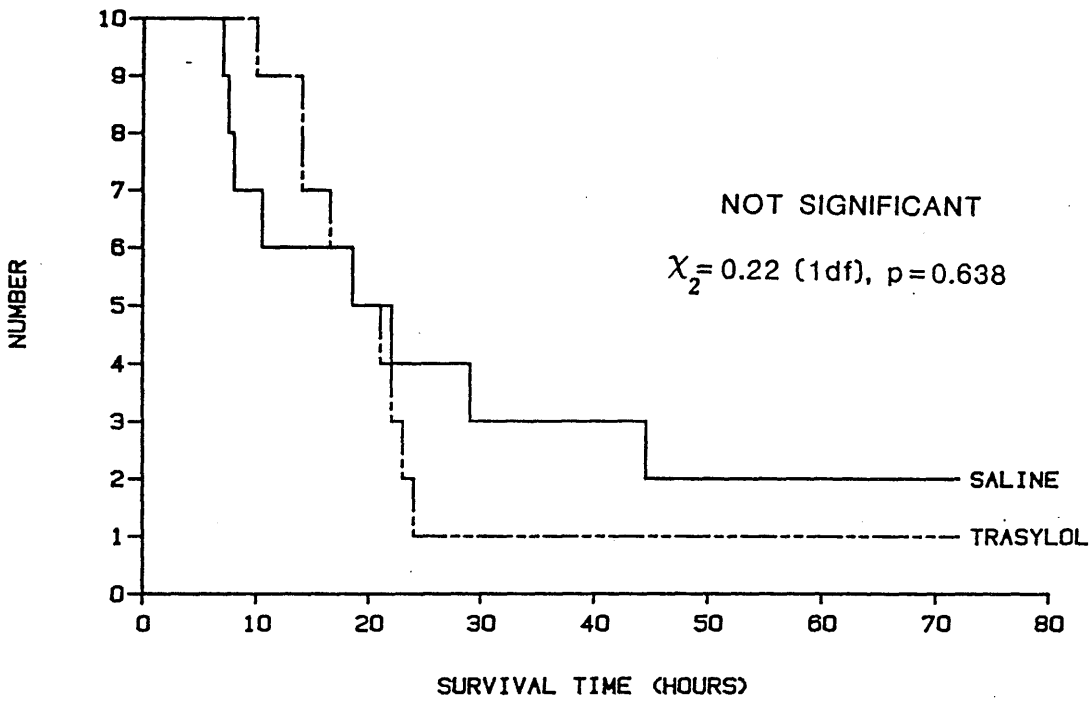


FIGURE 45.

Life table showing survival time of the 10 rats treated with intraperitoneal aprotinin (trasylol) compared to the 10 treated with intraperitoneal saline (saline).

TABLE 40.

Incidence and extent of free proteolytic activity in peritoneal exudates in the 5 groups of rats at one hour post-induction of pancreatitis and at post mortem.

Exudate tested	Control group	Intraperitoneal therapy groups			
		Saline	Plasma protein solution	Fresh frozen plasma	Aprotinin
One hour (no.)	-	5/10	5/10	4/10	4/10
Mean lysis area (mm^2)	-	378±166	384±160	176±76	659±205
Post mortem (no.)	6/10	2/10	1/10	1/10	1/10
Mean lysis area (mm^2)	273±145	44±8	trace	trace	trace

(No.) = number in each group of 10 showing activity.
Mean lysis area = area of zone of fibrinolysis (indicating free proteolytic activity).

amongst the rats with free proteolytic activity at one hour (20.5 hours) was not significantly different from the rats who had no free proteolytic activity demonstrated (median survival time 21.5 hours).

At post mortem the fluid from 6 of the 10 control rats showed free proteolytic activity (mean lysis area 273 $\pm$ 145mm^2, equivalent to 50µg free bovine trypsin/ml) (Table 40). Two of the rats in the saline treated group showed residual proteolytic activity at post mortem (equivalent to 10µg free bovine trypsin/ml), only one rat in each of the 3 other treatment groups showing a trace of activity.

Discussion

When the efficacy of a given therapy is judged on the basis of it prolonging the survival time, then the assessment of such a benefit is dependent only upon the proportion of untreated animals having a fatal outcome. This dictates that the experimental model should have a high mortality, close to 100% in the control group. Furthermore, the study of small numbers of animals within each treatment limb necessitates that the pancreatic injury be standard and reproducible. These goals appear to have been achieved here, the pilot study showing that the retrograde intraductal infusion of 5% sodium taurocholate at a constant rate by syringe pump produces a severe pancreatitis with a 83% mortality within 24 hours.

Such a mortality is unknown in the context of human acute pancreatitis, a mortality rate of 10% being more representative. Furthermore, only a proportion of the patients dying of acute

pancreatitis die within the first few days of their illness. Extensive necrosis is the usual result of taurocholate-induced pancreatitis whereas in man this degree of pancreatic damage may occur in fewer than 5% of attacks. However, in seeking to influence the course of the disease it does seem valid to examine therapy in the most severe cases, as it is these patients which present major problems in management, the majority of the remainder settling on simple supportive therapy.

Of the various methods of inducing acute pancreatitis experimentally, retrograde infusion of bile salt appears an appropriate choice having certain similarities with the theory of initiation of pancreatitis in man by reflux of bile. Although an invasive method, it reliably produces a rapidly evolving pancreatitis with similar macroscopic and histological features to man, including the production of fat necrosis and a haemorrhagic peritoneal exudate[7].

There is evidence of proteolytic enzyme activation in the peritoneal fluid with just under half the rats having exudate sampled at one hour demonstrating fibrinolysis representing free proteolytic activity and indicating overwhelming of the peritoneal antiprotease defences. Taurocholate-induced pancreatitis in rats thus appears similar to the pancreatitis induced in dogs by retrograde injection of bile where the highest enzyme levels in peritoneal exudate were found one hour after the induction of pancreatitis, associated with saturation of the antiprotease molecules and the presence of free proteolytic (caseinolytic) activity[263].

The free proteolytic activity demonstrated in the present study does not reflect activity due to the presence of protease-alpha$_2$macroglobulin complexes as these are unable to attack large substrate molecules such as fibrin. The presence of trypsin might be confirmed by demonstrating tryptic amidase (BAPNA splitting activity) but attempts to examine for this were unsuccessful due to the haemorrhagic coloration of the exudates, both at one hour and at the time of post mortem.

Although serial sampling of the exudate for analysis was not performed, free proteolytic activity presumably was present at one hour and persisted until death in the majority of the control rats but was diminished at post mortem in the rats which had received intraperitoneal therapy. The rats receiving intraperitoneal therapy had prolongation of their median survival time suggesting that removal of the peritoneal exudate was beneficial. Among these rats the median survival time was the same whether the peritoneal exudate removed at one hour possessed free proteolytic activity or not.

In the control rats not receiving intraperitoneal therapy the median survival time did not vary whether free proteolytic activity was present at post mortem or not, although free proteolytic activity was not found in the animal surviving longest at 72 hours. This suggests that factors other than the presence of free proteolytic activity may determine the timing of death from taurocholate-induced pancreatitis.

Both human fresh frozen plasma and particularly aprotinin were found to possess significant trypsin inhibitory activity, the binding

capacity of fresh frozen plasma being similar to that reported previously[54,207]. Human plasma protein solution and saline were confirmed to have no measurable trypsin binding capacity. Instillation of human fresh frozen plasma appeared to be the most effective of the intraperitoneal therapies tested when each of the treatment groups were compared in turn with the control group, the improvement associated with aprotinin therapy just failing to reach statistical significance. These data suggest that the instillation of a solution possessing antiprotease activity may be superior to one possessing no such activity.

Although human fresh frozen plasma was not significantly better than aprotinin, the trend towards a longer median survival in association with fresh frozen plasma may suggest that the nature of the antiprotease solution, rather than its overall capacity to bind trypsin, might be the more important factor.

Complexation of protease with aprotinin, as with $alpha_1$antiprotease leads to inhibition of the enzyme and such complexes possess no residual proteolytic activity. The mode of clearance of aprotinin-protease and $alpha_1$antiprotease-protease complexes from the peritoneal cavity is unknown but as in the bloodstream may involve transfer of the protease molecules to $alpha_2$macroglobulin, and hence to the peritoneal macrophages where the complex may be taken up and broken down. Thus it may be that the concentration of $alpha_2$macroglobulin, as appears to be the case in the bloodstream, may be the rate-limiting step in the clearance of proteases from the peritoneal cavity. Provision, therefore, of

exogenous alpha$_2$macroglobulin in fresh frozen plasma may boost the clearance of activated proteases from the peritoneal cavity although it is also recognised that the persistent proteolytic activity associated with such complexes may in itself be pathogenic.

The numbers of rats studied in the present study were too small to permit confident conclusions as to the superiority of fresh frozen plasma over aprotinin. Nevertheless the study has demonstrated that this form of intraperitoneal treatment is therapeutic and that the addition of antiproteases to the peritoneal cavity may confer additional benefit. An investigation of this approach to the treatment of acute pancreatitis in man would seem to be justified.

CHAPTER 10. EXAMINATION OF PANCREATIC ENZYMES AND ANTIPROTEASES IN PERITONEAL EXUDATES AND PSEUDOCYST FLUID

Introduction

Peritoneal effusions are thought to resemble slightly diluted plasma with respect to their protein composition[37]. The peritoneal exudate complicating acute pancreatitis comprises proteins, including the major antiproteases alpha$_2$macroglobulin and alpha$_1$antiprotease, active enzymes such as amylase and lipase, zymogens and activated proteolytic enzymes such as trypsin, chymotrypsin and elastase bound to antiproteases, and also includes prostaglandins, complement breakdown products and kinins[126,129,202,204]. The volume, colour and biochemical characteristics of the peritoneal exudate, including its albumin, total protein and aspartate aminotransferase content, have all been shown to reflect the severity of an attack of acute pancreatitis[227,277].

The peritoneal fluid associated with an attack of acute pancreatitis has been considered to arise from an exudation of enzyme-rich fluid from the pancreas. This fluid presumably excites a peritonitis leading to a migration of white blood cells and an out-pouring of fluid containing plasma proteins and antiproteases from the peritoneum, thus diluting and complexing any potentially dangerous proteolytic enzymes.

The toxic factor or factors in peritoneal fluid have not been fully characterised but may include pancreatic enzymes, complement breakdown products, kinins or other small vasoactive peptide

molecules or even prostaglandins. Some workers consider that saturation of the peritoneal antiprotease defences may be a central event mediating this toxicity, at least in experimental pancreatitis[261-3]. Alpha$_2$macroglobulin appears to be the key protective antiprotease in this situation, preferentially binding activated proteases. The onset of shock in these experimental models appears to occur following saturation of alpha$_2$macroglobulin, which is rapidly followed by activation of the trypsinogen and saturation of the remaining alpha$_1$antiprotease.

Activated proteolytic enzymes have been demonstrated in the peritoneal exudate in man in complex with alpha$_2$macroglobulin and alpha$_1$antiprotease and high degrees of complexation of alpha$_2$macroglobulin, up to 100%, have been demonstrated, suggesting this mechanism may also be important in man[17,263]. Free tryptic activity, which would indicate overwhelming of the peritoneal antiprotease defences, was not observed in 12 exudates studied by Geokas and colleagues[126]. One study has reported free proteolytic activity to be present in 6 of 13 exudates sampled at the time of surgery[376], but no clinical details were presented as to whether these patients were shocked or not.

The aim of the present study was to investigate further the extent of proteolytic enzyme activation and adequacy of the peritoneal antiprotease reserve in human acute pancreatitis. It is not clear whether antiproteases are normally present in peritoneal fluid and, if so, what the normal concentrations are. In health only a trace of fluid exists in the peritoneal cavity, insufficient for

sampling and analysis, and therefore a series of patients with a reactive effusion secondary to intestinal obstruction have been studied to permit comparison.

The release of active proteolytic enzymes in duodenal contents following perforation of a duodenal ulcer might in some ways mimic the enzyme release occurring in acute pancreatitis and samples of peritoneal exudates from a group of these patients have also been studied.

Finally cyst fluid from a series of patients with pseudocysts were also studied. Pseudocysts often communicate with the pancreatic duct and the fluid might represent pancreatic juice or possibly resemble the "pancreatic" component of pancreatitis exudate associated with an acute attack of pancreatitis.

Methods

Peritoneal exudate was obtained at diagnostic paracentesis from 21 patients with acute pancreatitis as described in chapter 4. Patients were admitted a median of 9 hours from the onset of their symptoms and peritoneal cannulation was performed at a median time of 8 hours after admission to hospital (range 4-31 hours). The 21 patients studied have been divided into two groups, 10 patients who died and 11 who survived their illness. The clinical details of the patients in the 2 groups are shown in table 41.

Free peritoneal fluid was obtained at the time of laparotomy in 7 patients with a perforated peptic ulcer (duodenal - 6, gastric - 1) and from 8 patients with various forms of mechanical intestinal

TABLE 41.

Comparison of age, sex, aetiology and complications in relation to the outcome from acute pancreatitis.

	Acute pancreatitis	
	Fatal attacks (n = 10)	Survivors (n = 11)
Sex	8 M : 2 F	9 M : 2 F
Age	55.4 ± 4.4	52.2 ± 4.8
Aetiology: gallstones	4 (40%)	5 (45%)
alcohol	2 (20%)	5 (45%)
other	-	1 (9%)
unknown	3 (30%)	-
gallstones/alcohol	1 (10%)	-
Mean prognostic factor score: Glasgow (8 factors)	4.5 ± 0.5	2.6 ± 0.43
Ranson (11 factors)	7.2 ± 0.8	3.7 ± 0.77
Complications:		
fulminant pancreatitis	3	-
necrosis	5	1
abscess	1	1
pseudocyst	-	2
respiratory insufficiency	1	3
none	-	4

obstruction. Samples of fluid were also collected from one patient with hyperamylasaemia due to extensive mesenteric infarction resulting from mesenteric venous thrombosis. Twelve patients with a pancreatic pseudocyst had their cyst fluid sampled, 10 at the time of cystogastrostomy and at percutaneous cyst drainage in two.

In all cases samples were centrifuged at 4°C and the supernatants separated and deep frozen, usually within one hour of sampling. Samples were stored at -20°C until assay.

Biochemical investigations

The following determinations were performed as described in chapter 5. Fluid total protein, albumin, $alpha_2$macroglobulin and $alpha_1$antiprotease concentrations and amylase, lipase and tryptic amidase activities were assayed. Free proteolytic activity and the trypsin binding capacity were determined using the plasminogen-free fibrin plate technique. Eleven of the patients with acute pancreatitis also had serum $alpha_2$macroglobulin and $alpha_1$antiprotease concentrations assayed in blood samples taken at the time of their peritoneal cannulation.

Statistical analysis

Results are expressed as means $\pm$ s.e.m. Differences between groups have been compared using the Mann-Whitney U test and by regression analysis.

Results

The results are detailed fully in Appendices 2 and 3.

Plasma proteins

The total protein and albumin concentrations were similar in all 3 exudates and were approximately 1/3rd lower than normal serum levels. Total protein concentrations tended to be lower amongst the pancreatitis patients with a fatal outcome compared to the survivors (38.4 $\pm$ 2g/l vs 44.4 $\pm$ 2.3g/l) although just failing to reach statistical significance. The mean total protein concentration was significantly lower in pseudocyst fluid (Fig. 46) as was the mean albumin concentration (Table 42).

Antiproteases

The $alpha_2$macroglobulin concentrations in the exudates were typically below the lower limit of normal for serum (1.1 - 3.9g/l). In the 10 patients dying of acute pancreatitis the mean $alpha_2$macroglobulin concentration was significantly lower than for the 11 survivors (0.51 $\pm$ 0.05g/l vs 0.7 $\pm$ 0.06g/l, $p < 0.05$). The mean peritoneal $alpha_2$macroglobulin concentrations were significantly higher in patients with perforated ulcer and lower in pseudocyst fluid (Fig. 47).

$Alpha_1$antiprotease concentrations in exudate were typically within or just below the lower limit of normal for serum (1.3 - 3.2g/l). The mean peritoneal $alpha_1$antiprotease concentrations were slightly higher in patients with intestinal obstruction and lower in

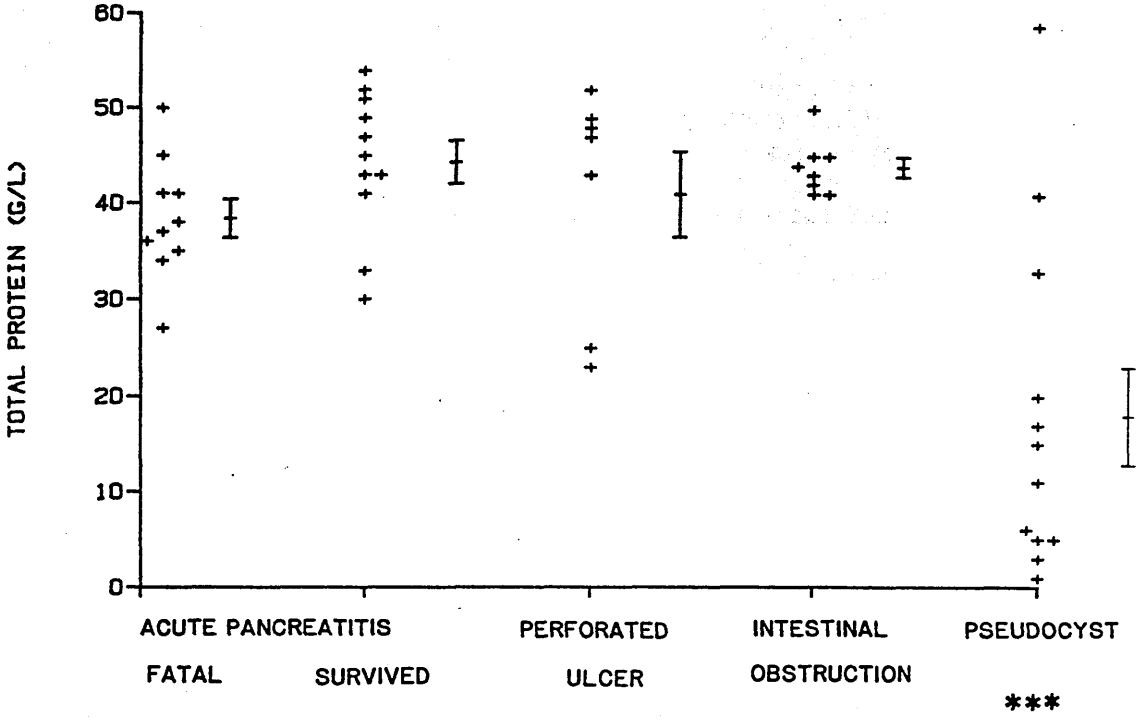


FIGURE 46.

Total protein concentrations in the 3 peritoneal exudate samples and in pseudocyst fluid. Mean concentration in pseudocyst fluid compared to pancreatitis exudate, *** p <0.001.

TABLE 42.

Comparison of pancreatic enzymes, total protein, antiproteases and trypsin binding capacity in exudates from patients with pancreatitis, perforated ulcer and intestinal obstruction and in pseudocyst fluid.

Parameter	Pancreatitis Dead (n = 10)	Pancreatitis Survived (n = 11)	Perforated peptic ulcer (n = 7)	Intestinal obstruction (n = 8)	Pseudocysts (n = 12)
Amylase (IU/l)	39021 ±11511	27556 ±6273	6056** ±2787	84*** ±24	153578 ±60505
Tryptic amidase (A/hr)*	0.141 ±0.073	0.267 ±0.063	0.133 ±0.061	0.005*** ±0.003	0.469 ±0.245
Total protein (g/l)	38.4 ±2	44.4 ±2.3	41 ±4.5	43.9 ±1	18*** ±5.1
Albumin (g/l)	23.1 ±1	29 ±2.2	24.9 ±2.9	25.4 ±0.96	9.4** ±2.9
$Alpha_2$-macroglobulin (g/l)	0.51 ±0.05	0.7 ±0.06	0.9* ±0.12	0.68 ±0.14	0.27** ±0.07
$Alpha_1$-antiprotease (g/l)	1.62 ±0.2	1.77 ±0.16	1.31 ±0.09	1.91 ±0.11	1.23 ±0.28
Trypsin binding capacity (µg trypsin/100µg)	62 ±11	72 ±8.5	35.7* ±10.2	115*** ±7.3	11.3*** ±4.6

* Units of absorbance change A_{405nm}/hr.

Means ± s.e.m, * = p <0.05, ** = p <0.01, *** = p <0.001.

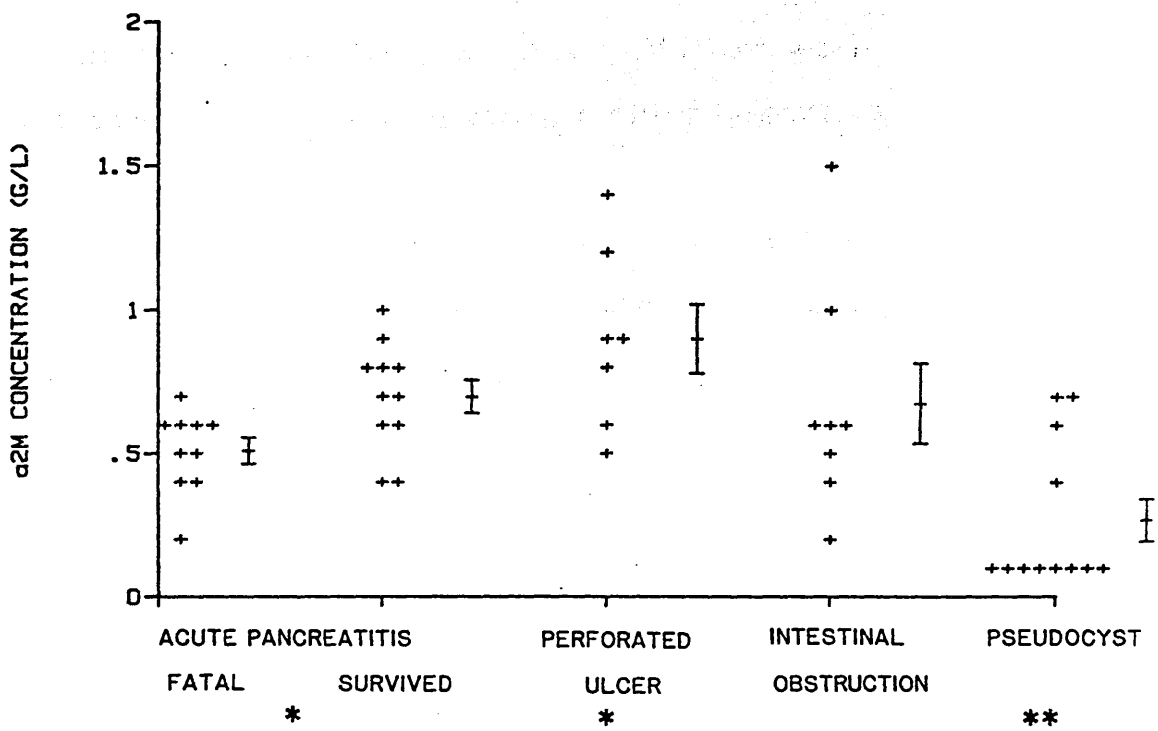


FIGURE 47.

Alpha$_2$macroglobulin concentrations in the 3 peritoneal exudate samples and in pseudocyst fluid. Mean concentration in fatal pancreatitis exudate compared to survivors, * ($p < 0.05$) and in perforated ulcer exudate and pseudocyst fluid compared to pancreatitis exudate, * and ** ($p < 0.01$) respectively.

patients with perforated ulcers and pseudocysts, which showed a wide range of concentrations of $alpha_1$antiprotease, but these differences were not significant (Fig. 48).

There were only minor differences in the concentrations of peritoneal antiproteases whether the attack was due to alcohol or gallstones (Table 43).

Serum levels of $alpha_2$macroglobulin were within the normal range in all but 2 of the 11 patients studied and were always significantly higher than the corresponding concentrations in exudate (1.68 ± 0.18g/l vs 0.64 ± 0.06 g/l, $p < 0.001$). Serum levels of $alpha_1$antiprotease were all within the normal range and again were always significantly higher than in exudate (2.68 ± 0.23g/l vs 1.62 ± 0.14g/l, $p < 0.01$).

Pancreatic enzymes

Patients with acute pancreatitis had very high fluid amylase activities exceeded only by pseudocyst fluid, although this difference was not significant (Fig. 49). Amylase activities tended to be higher in pancreatitis patients who subsequently died compared with the survivors, but the mean values were not significantly different. Amylase activities were elevated in all but one of the patients with a perforated ulcer, but in none of the patients with intestinal obstruction. In both conditions the mean amylase activities were significantly lower than in the exudates from patients with pancreatitis (Table 42).

Tryptic amidase activity, indicating trypsinogen activation,

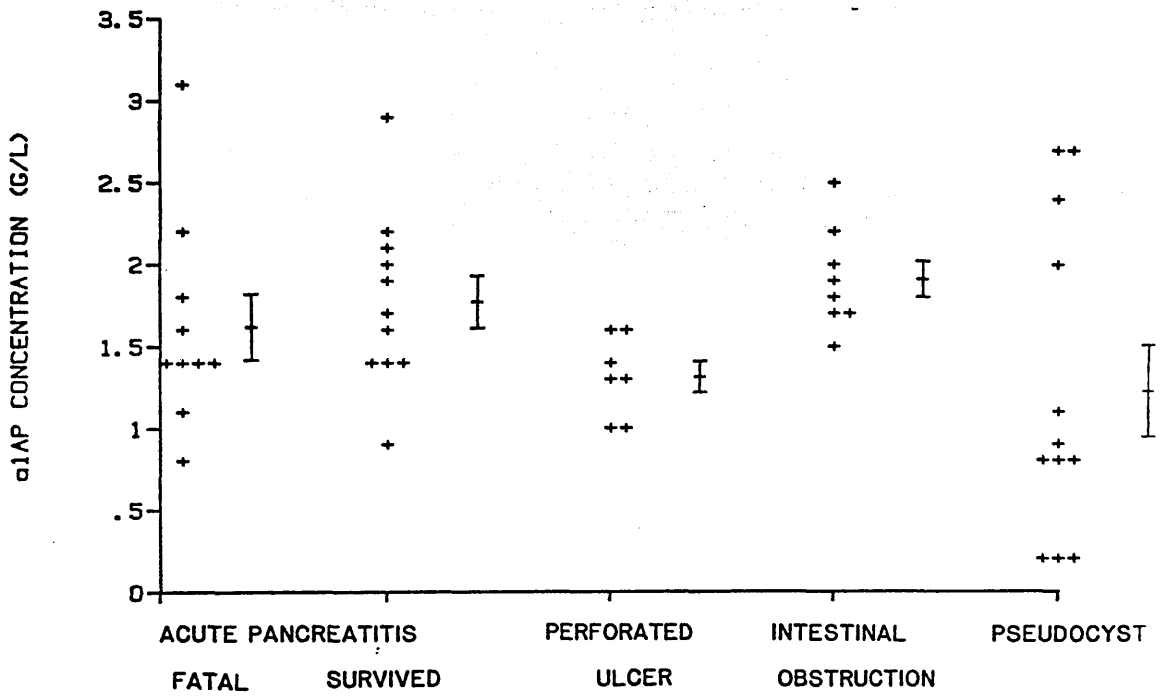


FIGURE 48.

Alpha$_1$antiprotease concentrations in the 3 peritoneal exudate samples and in pseudocyst fluid. No significant differences compared to mean concentration in pancreatitis exudate.

TABLE 43.

Comparison of pancreatic enzymes, protein, antiproteases and trypsin binding capacity of exudates from patients with pancreatitis associated with alcohol and gallstones.

	Acute pancreatitis		
Parameter	Alcohol (n = 7)	Gallstones (n = 9)	p value
Amylase (IU/l)	32984 ± 7297	20636 ± 4553	p = 0.29
Lipase (U/l)	34643 ± 3526	19156 ± 4807	p = 0.567
Tryptic amidase (A/hr)*	0.309 ± 0.046	0.199 ± 0.094	p = 0.37
Total protein (g/l)	44.4 ± 3.4	39.6 ± 2.4	p = 0.23
$Alpha_2$macroglobulin (g/l)	0.74 ± 0.06	0.57 ± 0.09	p = 0.055
$Alpha_1$antiprotease (g/l)	1.53 ± 0.16	1.79 ± 0.2	p = 0.37
Trypsin binding capacity (µg trypsin/100µl)	61.4 ± 12.5	75 ± 8.2	p = 0.43

* Units of absorbance change A_{405nm}/hour.

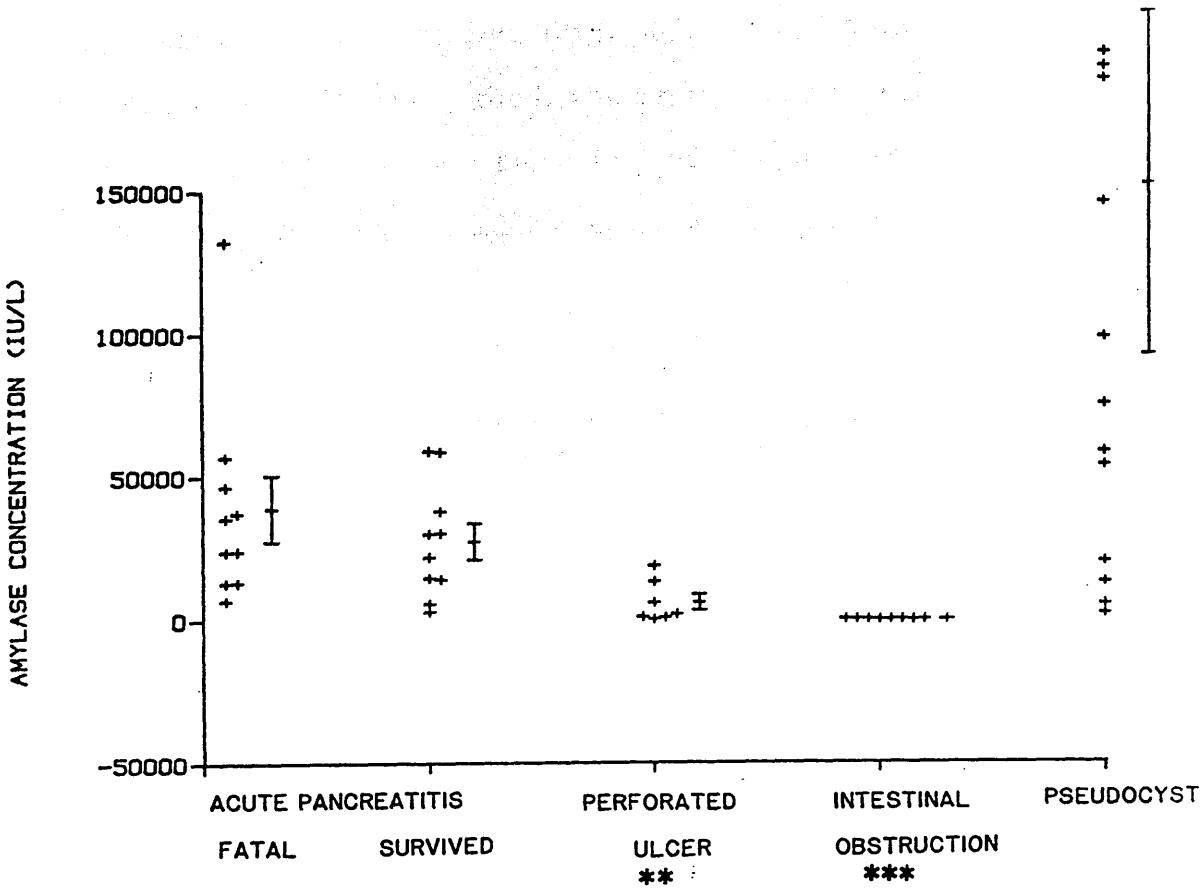


FIGURE 49.

Amylase concentrations in the 3 peritoneal exudate samples and in pseudocyst fluid. Mean concentration in perforated ulcer and intestinal obstruction exudates compared to pancreatitis exudate, ** ($p < 0.01$) and *** ($p < 0.001$) respectively.

was demonstrated in all the patients with acute pancreatitis in whom it could be measured, equivalent to free bovine trypsin concentrations of up to 18μg/ml (Fig. 50). Of the 10 patients with pancreatitis who ultimately died, the typical dark, haemorrhagic coloration of the peritoneal fluid in 6 of the patients interfered with the assay and tryptic amidase could not be determined in these patients.

None of the pancreatitis exudate samples demonstrated fibrinolytic activity that would suggest the presence of free proteolytic activity, and thus that the antiprotease defences had been overwhelmed.

Patients with pancreatitis secondary to alcohol abuse tended to have higher amylase, lipase and tryptic amidase activities in exudate than patients with a gallstone aetiology, although these differences failed to reach statistical significance (Table 43).

Four of the 6 patients with peritonitis due to perforation of a duodenal ulcer showed tryptic amidase activity. One other patient with perforation of a gastric ulcer showed a trace of amidase activity. Two of the 8 patients with intestinal obstruction showed a trace of tryptic amidase activity but in neither condition was fibrinolytic activity found to indicate free proteolytic activity.

Tryptic amidase activity could not be assayed on the single patient with extensive mesenteric infarction due to dark, haemorrhagic coloration of the peritoneal exudate. He was the only patient with peritoneal exudate demonstrating free proteolytic activity, equivalent to 270μg/ml of free bovine trypsin.

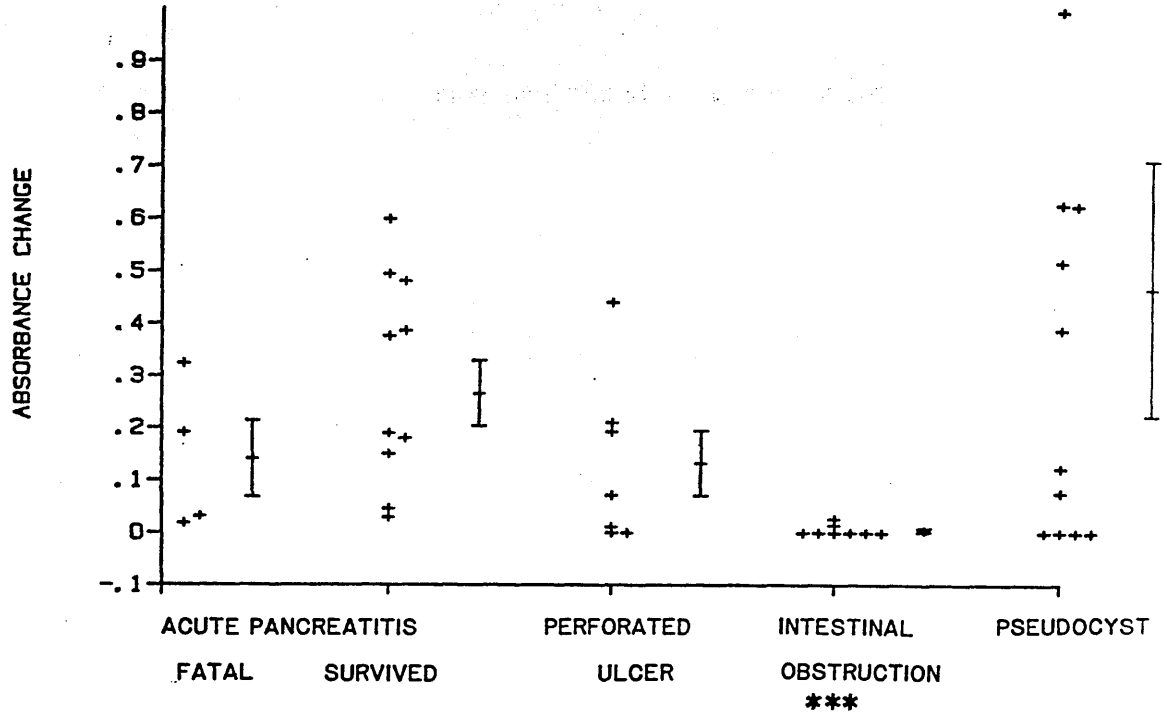


FIGURE 50.

Tryptic amidase activities in the 3 peritoneal exudate samples and in pseudocyst fluid. Mean activity in intestinal obstruction exudate compared to pancreatitis exudate, *** $p < 0.001$.

In pseudocyst fluids, 7 of the 11 patients in whom it could be measured showed tryptic amidase activity. Five of these patients demonstrated fibrinolytic activity and one other patient a trace of activity on the fibrin plate, indicating the presence of free proteolytic activity equivalent to free bovine trypsin activities ranging from 13.5μg/ml to 67μg/ml.

Trypsin binding capacity

No significant difference was found in the trypsin binding capacity amongst patients with acute pancreatitis who died compared to those who survived, although 3 patients dying early, including 2 who died within 24 hours of sampling, had very low binding capacities indicating almost complete saturation of their antiprotease reserve (Fig. 51). There were only minor differences between patients with an alcohol and gallstone aetiology (Table 43).

The trypsin binding capacity of the pancreatitis exudate was found to correlate significantly with the $alpha_1$ antiprotease concentration ($r = 0.764$, $p < 0.001$) but not with $alpha_2$ macroglobulin concentration (Fig. 52).

Exudates from patients with perforated peptic ulcers showed a significantly lower mean trypsin binding capacity and those with intestinal obstruction a significantly higher mean trypsin binding capacity than those with acute pancreatitis (Table 42).

Eleven of the 12 pseudocyst fluids studied showed a markedly decreased trypsin binding capacity indicating complete or almost complete overwhelming of their antiprotease defences.

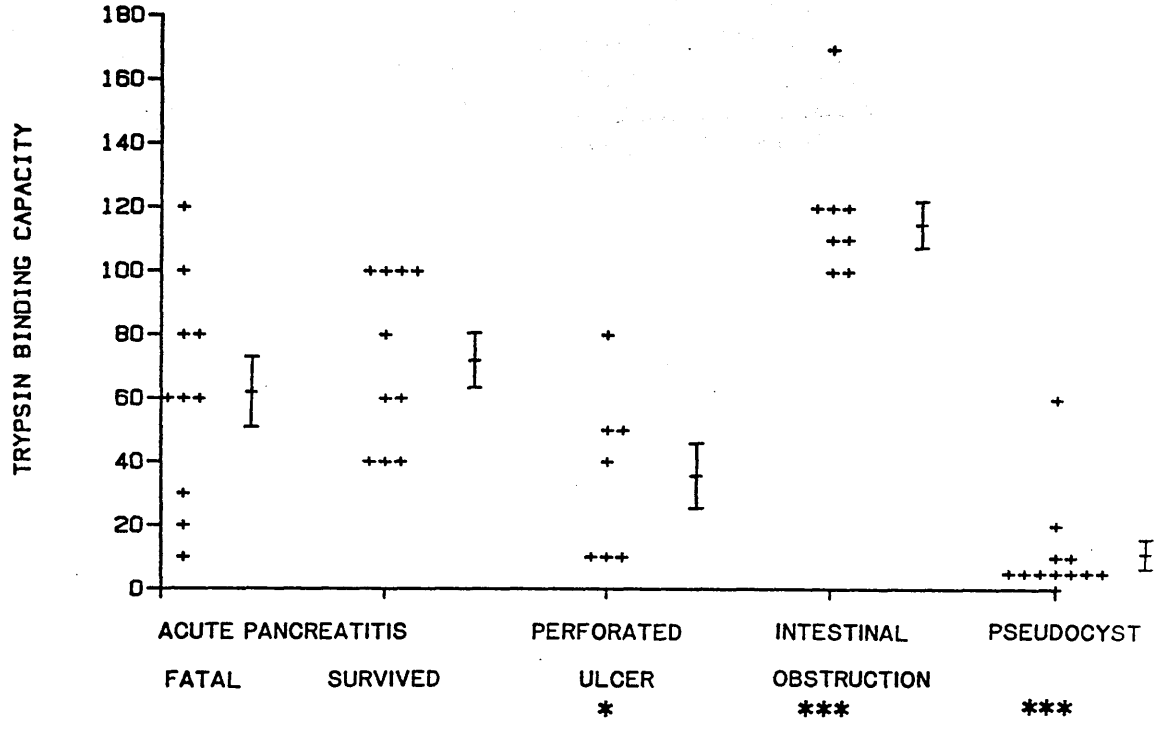
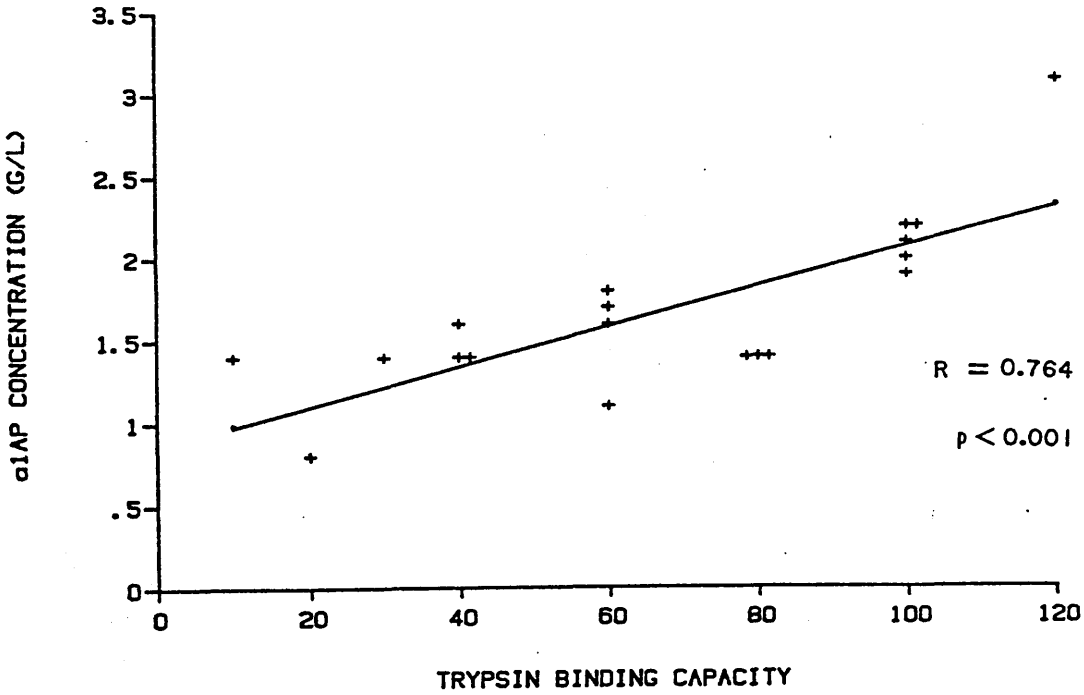


FIGURE 51.

Trypsin binding capacities in the 3 peritoneal exudate samples and in pseudocyst fluid. Mean binding capacity in perforated ulcer exudate, intestinal obstruction exudate and in pseudocyst fluid compared to pancreatitis exudate, * ($p < 0.05$), *** ($p < 0.001$) and *** respectively .



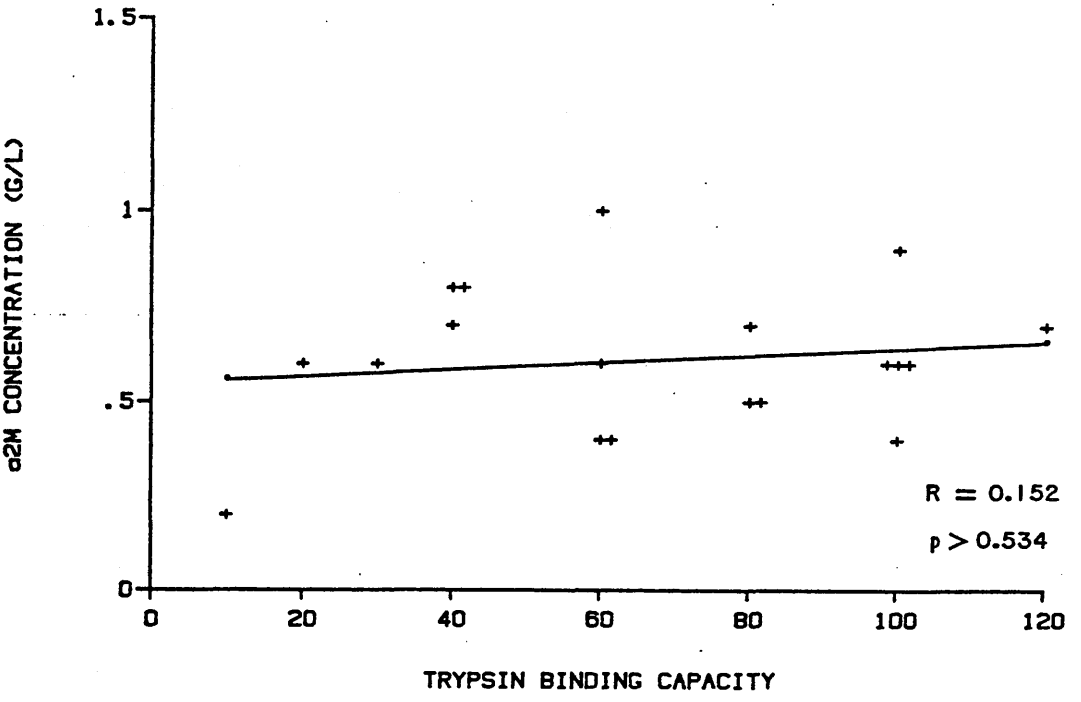


FIGURE 52.

(a) Correlation of trypsin binding capacity and $alpha_1$antiprotease concentration.

(b) Correlation of trypsin binding capacity and $alpha_2$macroglobulin concentration.

Discussion

Unfortunately the tryptic amidase activity could not be measured in 7 of the patients with acute pancreatitis, including 6 with an ultimately fatal outcome. There was measurable tryptic amidase activity in all the remaining patients indicating that trypsinogen activation had occurred, in agreement with the findings of others[37,126]. The presence of tryptic amidase activity in the absence of demonstrable free proteolytic activity has previously been confirmed to be due to the presence of trypsin-alpha$_2$macroglobulin complexes[126].

Similar levels of tryptic amidase activity were found in the patients with a perforated peptic ulcer, the trypsin in these cases arising from the escape of duodenal contents, where trypsinogen had been activated in the duodenum by enterokinase.

Two of the patients with intestinal obstruction also showed low grade tryptic amidase activity; one of these had a strangulated external hernia, although the bowel was viable. It is possible that trypsin may have migrated across the compromised bowel wall in this case although the explanation in the other case is unclear. Bieth and co-workers also noted low grade BAPNA splitting activity in ascitic fluids from conditions such as ovarian carcinoma, cardiac failure and cirrhosis, the cause of which was unexplained[37].

Peritoneal exudates were all shown to have a lower total protein concentration than serum, with reduced levels of albumin (mw. 68,000), alpha$_2$macroglobulin (mw. 725,000) and alpha$_1$antiprotease (mw. 55,000). Concentrations of alpha$_1$antiprotease in exudate were

around the lower limit of the normal serum values suggesting the concentrations of these plasma protein molecules in peritoneal exudate may be determined by their molecular size, with large molecules such as alpha$_2$macroglobulin less able to pass into the exudate.

In the 11 patients with acute pancreatitis for whom data were available, concentrations of the major antiproteases in exudate were always lower than the corresponding serum values. Previous studies on pancreatitis exudate have produced conflicting results; alpha$_1$antiprotease concentrations in exudate have been reported to be higher[376], lower[204] and markedly lower[126] than serum levels, although all found reduced alpha$_2$macroglobulin levels in exudate.

The assay of the trypsin binding capacity provides an overall assessment of the free antiprotease reserve available for binding. Alpha$_1$antiprotease is a much smaller molecule than alpha$_2$macroglobulin and on a molar basis is able to complex many more molecules of protease than alpha$_2$macroglobulin. Alpha$_1$antiprotease accounts for 90% of the measured trypsin binding capacity in serum[352], and that it accounts for the major part of the trypsin binding capacity of peritoneal exudate is confirmed here by the significant positive correlation between the two. Alpha$_2$macroglobulin accounts for most of the remaining trypsin binding capacity in serum but its relative contribution in exudate is not known. The additional contribution of the other antiproteases including inter-alpha-trypsin inhibitor, antichymotrypsin, and pancreatic secretory trypsin inhibitor must also be considered, but are thought to be relatively

unimportant in vivo.

The mean trypsin binding capacity of exudates from patients with intestinal obstruction was 115µg trypsin bound/100µl of sample and was thus similar to the trypsin binding capacity of normal human serum[6,54,207]. In comparison the trypsin binding capacities were significantly reduced in the exudates from patients with acute pancreatitis and perforated peptic ulcer. Apart from the higher $alpha_2$macroglobulin concentration in perforated ulcer exudates, the levels of $alpha_2$macroglobulin and $alpha_1$antiprotease were broadly similar amongst all 3 exudates. The reduced trypsin binding capacity reflects then a degree of saturation of the antiproteases in the exudates from patients with pancreatitis and perforated ulcer and is supported by the finding of tryptic amidase activity in these patients, indicating the presence of trypsin-$alpha_2$macroglobulin complexes.

No significant differences were found between fatal and recoverable attacks of pancreatitis in terms of their mean trypsin binding capacity although 3 patients did have markedly reduced levels. All 3 were shocked at the time of sampling and 2 died of fulminant pancreatitis within 24 hours. The low trypsin binding capacities in these patients indicates incipient saturation of the peritoneal antiprotease defences. As $alpha_2$macroglobulin binds trypsin more readily and has been shown to become complexed first[262], marked reduction of the total trypsin binding capacity suggests that actual or incipient saturation of $alpha_2$macroglobulin is likely, which in dogs precedes the development of shock and death of the

animal. Thus complete saturation of the antiprotease defences in pancreatitis exudate has not been confirmed to occur in this study but, from the data, it appears possible that it may occur in a few instances, were proteolytic enzyme release or zymogen activation to continue.

A low trypsin binding capacity was also demonstrated in 3 exudates from patients with a perforated ulcer. This situation is not, therefore, unique to acute pancreatitis and may cast some doubt on the overall importance of this phenomenon as an explanation for the systemic toxicity in acute pancreatitis. The low trypsin binding capacity in these cases may be explained on the basis of the sudden escape of proteolytic enzymes into the peritoneal cavity associated with the ulcer perforation. The resulting peritonitis and secondary influx of fluid and antiproteases will, over a period of time, dilute and complex the activated proteolytic enzymes, but in the early stages, the antiproteases are likely to show high degrees of saturation. These patients all had the typical features of peritonitis but did not manifest shock, as judged by their heart rate and blood pressure. Thus the trypsin binding capacity may frequently be low or overwhelmed soon after the actual perforation but, in this situation, its clinical relevance is uncertain.

Free proteolytic activity in peritoneal exudate was found in only one patient in the present study. He had extensive mesenteric infarction due to venous thrombosis and was shocked, anuric and in extremis when seen. He was resuscitated and taken to theatre where he underwent an extensive bowel resection but died soon afterwards.

Marked free proteolytic activity unchecked within the peritoneal cavity was confirmed in this one patient to be associated with profound systemic toxicity. Whether the peritoneal proteolytic activity was responsible for his shocked state, a contributing factor or an unrelated phenomenon is not known.

Data from the pseudocyst fluids are interesting to compare. In comparison with the peritoneal exudates pseudocyst fluids had a lower protein content but higher enzyme activities. Antiprotease levels were usually lower and in the majority there was complete or almost complete saturation of the antiproteases as indicated by the reduced or absent trypsin binding capacity. Two of the patients showing free proteolytic activity had no demonstrable tryptic amidase activity and one must postulate that another proteolytic enzyme from the pancreas was responsible for the fibrinolysis in each case.

Free proteolysis is then a common finding in pancreatic pseudocysts but confined within the fibrous wall of the cyst appears to pose no threat. There are occasional reports of shock and death in such patients in association with acute rupture of a pseudocyst[184] and it may be postulated that this could occur due to the sudden appearance of a large amount of free proteolytic activity within the peritoneal cavity. This scenario does not seem to hold for cases of pancreatic ascites. The leak from the cyst or from the pancreatic duct is often small and, although it can give rise to extremely high enzyme levels within the ascitic fluid, the chronic escape of enzymes rather than the sudden catastrophic leak described above does not appear to be associated with any acute, systemic effects.

None of the patients with pancreatitis showed free proteolytic activity in their peritoneal exudate and this is in agreement with the work of others employing a different assay for free trypsin[126]. The results, however, contrast with those of Wendt and co-workers, despite free proteolytic activity having been sought in the current study using the same plasminogen-free fibrin plate technique as they had employed[376]. In their study they stated that 6 out of 13 samples of exudate taken at the time of surgery demonstrated free proteolytic activity equivalent to 20.9 $\pm$ 16.8μg trypsin/ml[376].

It would be unusual to operate early in the course of an attack of acute pancreatitis and the exudates reported in their study may not be comparable to those reported here, or those in an earlier study where the exudates were also sampled by peritoneal cannulation soon after admission to hospital[126]. Wendt and co-workers provided no other details about their patients and, in particular, when the samples were collected in relation to the onset of the attack and whether the samples were truly of free peritoneal exudate, loculated peripancreatic collections or even of acute pseudocysts was not clear. The current study has shown that free proteolytic activity occurs in 50% of pseudocysts, a strikingly similar incidence to that reported in their study where 6 of 13 "exudates" showed free proteolytic activity[376].

The peritoneal antiprotease defences appeared to be adequate in the majority of patients with acute pancreatitis for the degree of proteolytic enzyme release occurring, there being no evidence, at the time of sampling, of free proteolytic activity. The antiprotease

defences appeared close to being overcome at the time of sampling in only 3 patients. The pancreatitis induced by retrograde infusion of bile to dogs is associated with extensive destruction of the gland and a massive early release of pancreatic enzymes systemically and intraperitoneally, quickly overwhelming the peritoneal antiprotease defences[263]. Such a severe, rapidly evolving lesion is presumably a rare occurrence in man, a slower release of enzymes providing time for a protective response by the accumulation of antiproteases within the peritoneal cavity.

No protease-antiprotease imbalance was demonstrated early in the attack when the exudate is thought to be most toxic. It should be noted, however, that sampling was delayed a median of 17 hours from the onset of symptoms in the current study compared with the one hour delay reported in experimental pancreatitis. Continued release of enzymes, or activation of zymogens, perhaps by the action of trypsin-alpha$_2$macroglobulin complexes[302], might lead to overwhelming of the antiprotease defences later during the course of the attack giving rise to shock. Equally, the continued presence of irritant fluid in the peritoneal cavity may lead to continued outpouring of fluid and antiproteases from the peritoneal capillaries which, presumably in most patients, keeps pace with the rate of proteolytic enzyme activation occurring.

CHAPTER 11. EFFECT OF INTRAPERITONEAL ANTIPROTEASE THERAPY WITH APROTININ ON PERITONEAL FLUID CHARACTERISTICS AND BIOCHEMISTRY

Introduction

Examination of peritoneal exudates from patients with acute pancreatitis have demonstrated high levels of the pancreatic enzymes with evidence of trypsin activation. The aim of the current study was to examine the effect of intraperitoneal antiprotease therapy on the peritoneal enzyme activities and protein concentrations in patients randomised to receive this treatment modality.

Methods

Peritoneal cannulation was performed as detailed in chapter 4. Aliquots of any free fluid obtained on aspiration were retained as were samples drained following lavage with a single litre of saline and samples drained 8 hours after the first instillation of aprotinin. Thus a maximum of 3 fluid samples were available for each patient.

The volume of the fluid drained on each occasion was recorded and the colour of the fluid was compared against a standard colour chart[226]. In all cases the samples were centrifuged at 4°C within one hour of sampling, the supernatants were separated and stored at -20°C until analysis. Total protein and albumin content, amylase, lipase and tryptic amidase activities were measured and the presence of free proteolytic activity was sought as detailed in chapter 5.

Of the 25 patients randomised to receive intraperitoneal therapy with aprotinin, one refused to consent to treatment and 2 others were found on peritoneal cannulation to have free fluid which was not characteristic of acute pancreatitis, both of whom subsequently underwent surgery. The remaining 22 patients had sequential study of their peritoneal fluid biochemistry.

The patients were studied in two groups based on the presence or absence of free peritoneal fluid at the initial cannulation. Ten patients had free fluid present in amounts ranging from 6 to 200 mls (median 45 mls). Of the remaining 12 patients, 4 had small quantities of free peritoneal fluid of less than 1ml on aspiration, insufficient for analysis, and have been studied with the patients who had no free fluid on aspiration. Table 44 details the characteristics of the patients in the 2 groups. Alcohol was the commonest aetiological factor amongst the patients with free peritoneal fluid present.

Significantly more fluid was recovered following saline lavage in the patients with free peritoneal fluid, but 8 hours after the instillation of aprotinin there was no significant difference in the mean volume of fluid returned from each group (Table 44).

Statistical analysis

Results have been expressed as means $\pm$ s.e.m. Statistical analysis was conducted by the Mann-Whitney U test and Wilcoxon rank sum test.

TABLE 44.

Comparison of age, sex, aetiology, delay to cannulation and volumes of fluid recovered during intraperitoneal therapy in the patients with and without free peritoneal fluid.

Parameter	Free fluid	No free fluid	p value
Sex	9M : 1F	7M : 5F	NS
Mean age (yrs)	51.8 ± 4.5	55.3 ± 4.5	NS
Aetiology: alcohol	5	1	
gallstones	3	5	
unknown	2	4	
other	-	2	
Median delay onset* to cannulation (hrs)	25 (12-47)	16.5 (8-61)	NS
Volume returned after saline lavage (mls)	740 ± 70	461 ± 84	p <0.05
Volume returned 8 hrs after instillation of aprotinin (mls)	480 ± 70	320 ± 64	NS

* median (range).

Results

Peritoneal fluid colour

Free peritoneal fluid ranged in colour from straw coloured (No. 3 on chart) to dark haemorrhagic (No. 8). In all but 2 patients in whom the colour was unchanged, the colour score of the fluid, according to the standard colour chart, decreased following lavage with a single litre of saline (4.5 ± 0.6 vs 5.9 ± 0.5, $p < 0.02$) (Fig. 53). Despite recovering most of the instilled saline after the single peritoneal lavage and the instillation of a further litre of saline with aprotinin, the returned fluid was darker 8 hours later in 15 of the 22 patients (Fig. 53), although this difference was not significant.

Protein content

Figure 54 shows the total protein concentration to have fallen after a single litre saline lavage, the concentration rising again in the returned fluid in both groups of patients 8 hours after the instillation of aprotinin. Albumin levels demonstrated a similar pattern (not shown).

Pancreatic enzymes

Figures 55 and 56 show similar patterns for the amylase and lipase activities respectively.

Of the 10 patients with free peritoneal fluid, tryptic amidase activity could be measured in 8 of them. In the other 2 patients the dark, haemorrhagic coloration of the fluid prevented its measurement.

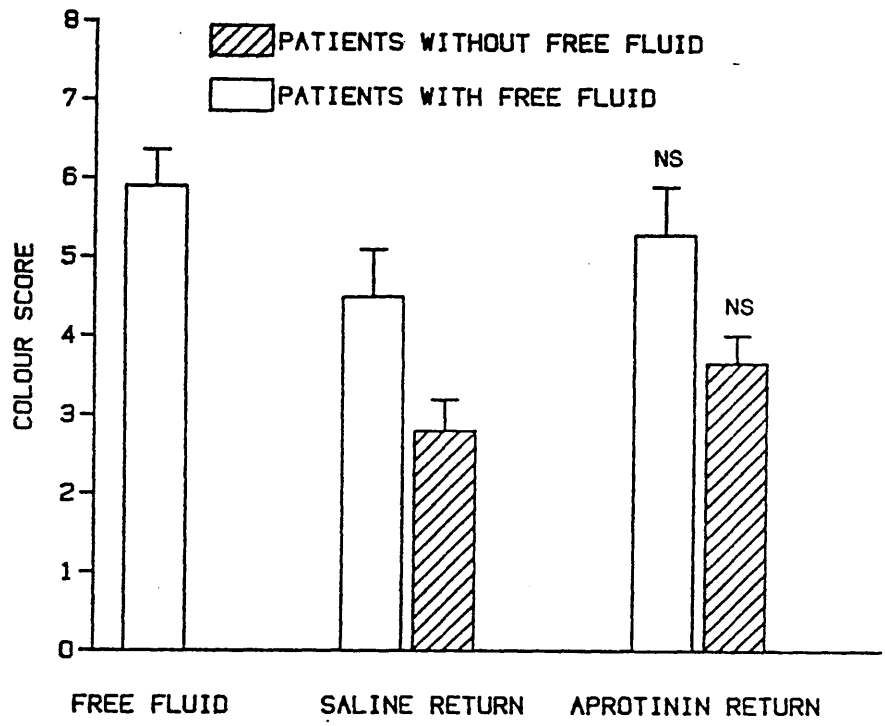


FIGURE 53.

Mean colour scores in free peritoneal fluid, following saline lavage and 8 hours after the instillation of aprotinin solution, in patients with and without free fluid at the initial cannulation. Differences between saline return and aprotinin return not significant (NS).

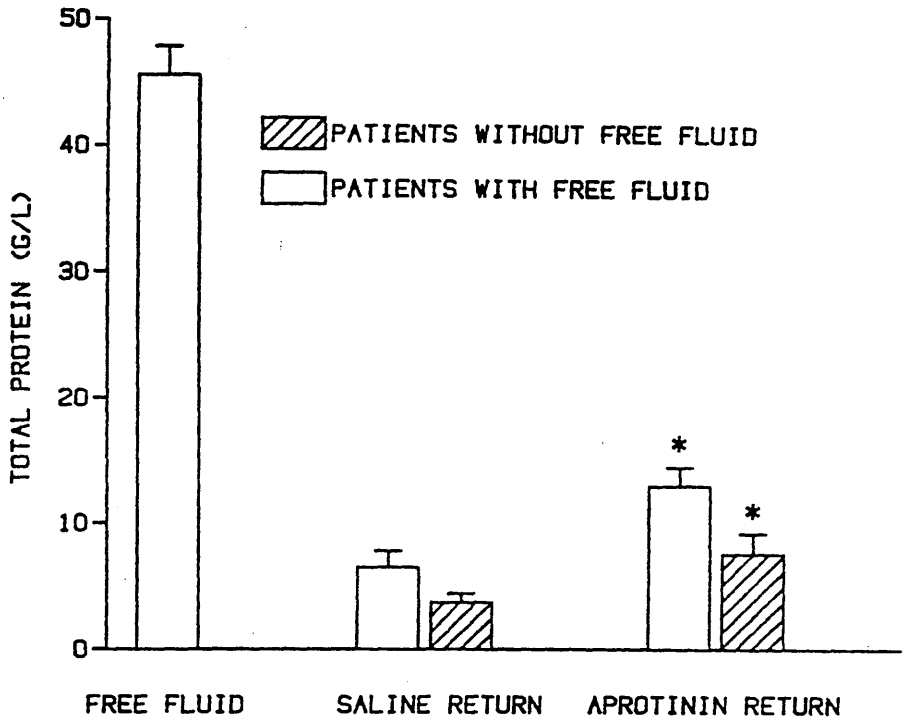


FIGURE 54.

Mean total protein concentrations in free peritoneal fluid, following saline lavage and 8 hours after the instillation of aprotinin solution in patients with and without free fluid at the initial cannulation. Differences between saline return and aprotinin return, * $p < 0.05$.

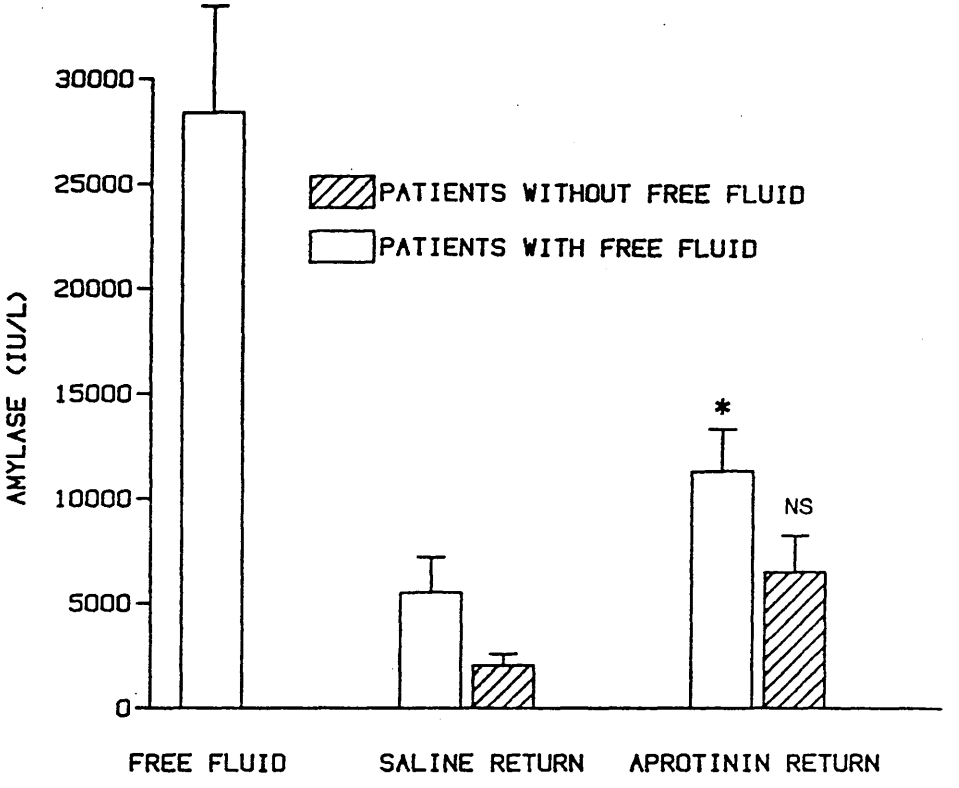


FIGURE 55.

Mean amylase concentrations in free peritoneal fluid, following saline lavage and 8 hours after the instillation of aprotinin solution in patients with and without free fluid at the initial cannulation. Differences between saline return and aprotinin return, * ($p < 0.05$) and NS (not significant) respectively.

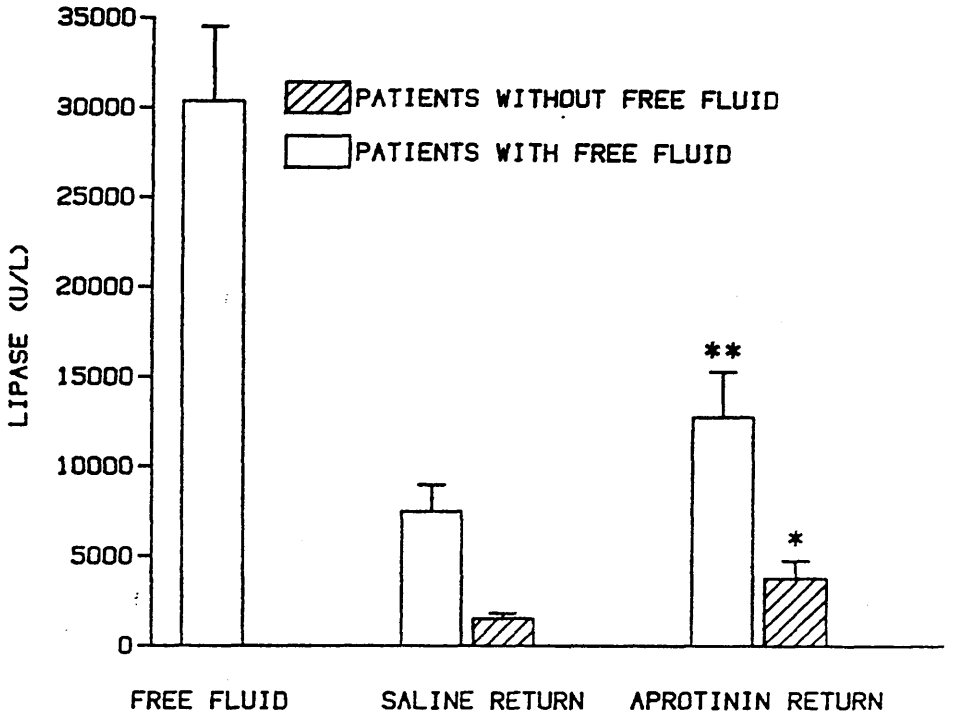


FIGURE 56.

Mean lipase concentrations in free peritoneal fluid, following saline lavage and 8 hours after the instillation of aprotinin solution in patients with and without free fluid at the initial cannulation. Differences between saline return and aprotinin return, ** ($p < 0.01$) and * ($p < 0.05$) respectively.

No patients were found to have free proteolytic activity on the fibrin plate assay.

Patients with no free peritoneal fluid had low levels of tryptic amidase activity detected following a single litre saline lavage. Unlike for amylase and lipase which both demonstrated an increased activity in the fluid returned 8 hours after the instillation of aprotinin, tryptic amidase activity fell significantly further in both groups (Fig. 57).

Free proteolytic activity was not found on the fibrin plate assay in the return fluids of any patient following saline lavage or 8 hours after instillation of aprotinin.

Discussion

The results of this study have confirmed high pancreatic enzyme activities in the peritoneal exudate associated with acute pancreatitis. The demonstration of tryptic amidase activity in the fluid confirms trypsin activation and the absence of free proteolytic activity indicates this is due to the presence of trypsin-alpha$_2$macroglobulin complexes.

The levels of protein and enzymes in peritoneal fluid were reduced approximately 10-fold following the aspiration of free fluid and a single litre saline lavage. Despite draining off a median of 670 mls from the litre of saline lavage fluid and diluting this further by instilling a another litre of saline with aprotinin, the concentrations of amylase, lipase, albumin and total protein had all risen significantly in the returned fluid 8 hours later, even in the

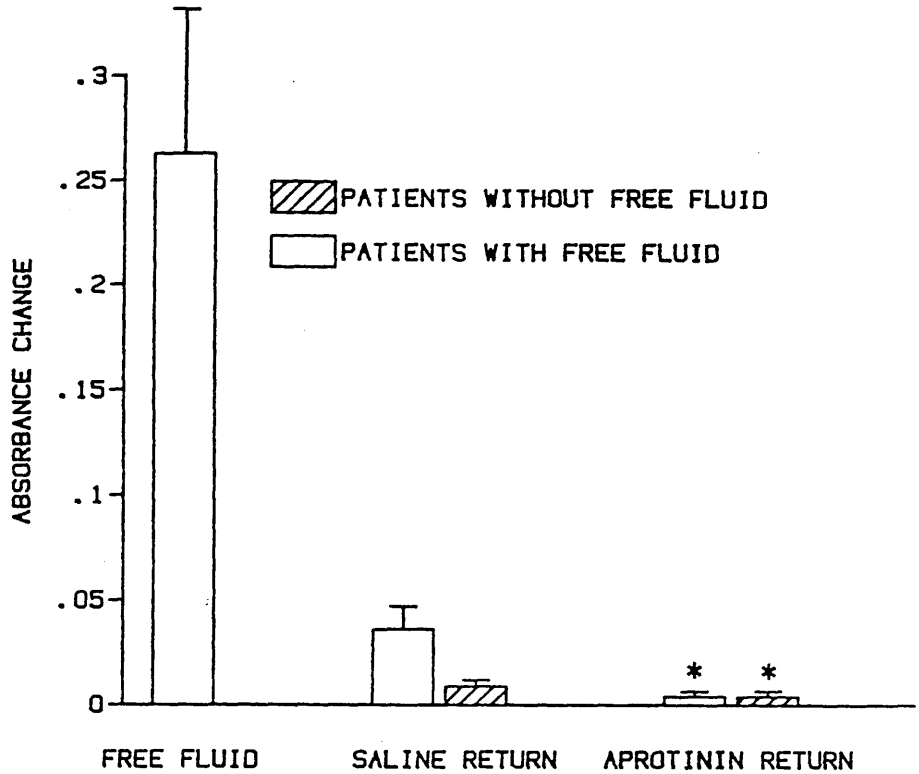


FIGURE 57.

Mean tryptic amidase activities in free peritoneal fluid, following saline lavage and 8 hours after the instillation of aprotinin solution in patients with and without free fluid at the initial cannulation. Differences between saline return and aprotinin return, * $p < 0.05$.

cases where there had been no free fluid at the initial peritoneal cannulation.

Three explanations can be suggested for this finding. Firstly, if blood enzyme levels were higher than levels in peritoneal fluid, enzymes might diffuse down the concentration gradient and thus equilibrate with the serum levels. Against this is the fact that peritoneal enzyme levels tend to be higher, only 3 patients having serum levels documented to be higher than the final peritoneal fluid levels 8 hours after the instillation of aprotinin. Although the dynamic changes in amylase and lipase activities were not monitored during the initial 8 hours of intraperitoneal therapy and serum levels could have been transiently higher than peritoneal fluid levels at some point in a greater number of the patients, the amount of enzyme required to cross the peritoneum to raise the concentration, of in excess of a litre of peritoneal fluid would be considerable and unlikely to have occurred in practice.

If selective reabsorbtion of water occurred from the peritoneal fluid, then the relative concentration of the enzymes would increase. A median of 670 mls of the single litre saline lavage was drained off, leaving at least 230mls of fluid in situ with a mean amylase concentration of 4216IU/l. Addition of one litre of saline with aprotinin to this would give an approximate amylase concentration of 970IU/l. As the mean amylase concentration in the return fluid after 8 hours was 9054IU/l, selective water reabsorbtion would need to account for a 10-fold increase in the enzyme concentration and on the basis of the volume of fluid drained 8 hours after the instillation

of aprotinin, this explanation seems unlikely.

The most plausible explanation would appear to be continued enzyme release from the pancreas into the peritoneal cavity during the initial 8 hour period during which the aprotinin solution was in situ.

This pattern of an increasing protein concentration and enzyme activity in peritoneal fluid, during the 8 hours of the initial aprotinin instillation, was not observed for the tryptic amidase activity. Trypsin release from the pancreas or activation of trypsinogen within the peritoneal exudate may have ceased spontaneously or as a result of the action of aprotinin. Alternatively aprotinin may be inhibiting the tryptic amidase activity of any trypsin-alpha$_2$macroglobulin complexes present in the exudate thus preventing its detection by the assay. Aprotinin, because of its relatively large molecular size, is considered to be a poor inhibitor of the trypsin-alpha$_2$macroglobulin complex due to steric hindrance[148,164], nevertheless, others consider its high concentration and massive molar excess may be sufficient to inhibit much of the residual tryptic amidase activity[121].

The results of this study support the continued release of enzymes from the pancreas, at least during the first 8 hours of intraperitoneal therapy. The instillation of aprotinin (5×10^6KIU) following peritoneal aspiration and a single litre saline lavage markedly reduces tryptic amidase activity in the peritoneal cavity during the 8 hour period of the instillation.

CHAPTER 12. INFLUENCE OF INTRAPERITONEAL ANTIPROTEASE THERAPY WITH APROTININ ON THE OUTCOME FROM ACUTE PANCREATITIS: INTERIM REPORT OF A RANDOMISED, CONTROLLED CLINICAL TRIAL

Introduction

The aims of this study were to compare and contrast the patients randomised to receive standard therapy with those randomised to receive additional intraperitoneal antiprotease therapy with aprotinin. The patients were analysed firstly, to determine whether the groups were similar with respect to their presentation, clinical characteristics and severity of illness, and secondly to determine if therapy resulted in any differences in outcome as judged by the incidence of death and major complication.

The effect of intraperitoneal therapy with aprotinin and any complications attributable to its use were also investigated based on clinical criteria and on the examination of various laboratory and physiological criteria, including the APACHE II illness scoring system.

Methods

Of the 160 patients with acute pancreatitis assessed and documented on admission to hospital, 51 (32%) satisfied the inclusion and exclusion criteria detailed in chapter 4 and were randomised, 26 to receive standard therapy alone (controls) and 25 to receive additional intraperitoneal therapy with aprotinin.

Exclusions

Two patients were found on peritoneal cannulation to have frank blood (No. 21) and foul smelling, dark, haemorrhagic fluid (No. 129) respectively on aspiration. Both were submitted to urgent laparotomy and were treated for a traumatic transection of the body of the pancreas and mesenteric infarction due to mesenteric venous thrombosis respectively and have been excluded. One patient (No. 131) randomised to receive intraperitoneal aprotinin declined to consent to peritoneal cannulation and has been excluded from further consideration. Twenty-two patients, therefore, underwent intraperitoneal therapy and were compared with the 26 control patients.

Determination of outcome

Death or the development of a major complication, as defined in chapter 4, were the primary endpoints by which the effectiveness of therapy was judged. In addition the patient's clinical condition, analgesic requirements, and the physiological and laboratory response to illness were recorded daily to look particularly at any short-term benefit of intraperitoneal therapy with aprotinin which might not be reflected in the eventual outcome.

Statistical analysis

Statistical analysis was conducted by Mann-Whitney U test, Fisher's exact test and by the Chi square test.

Comparability of groups

Table 45 compares various clinical, biochemical, aetiological and prognostic factors in the 2 groups of patients. A greater number of the patients in the control group were female, were slightly older and had gallstones found more frequently, although these differences were not significant. There was a greater incidence of hypoxia on admission in the treatment group (not significant), but overall the groups appeared to be well matched.

Results

Intraperitoneal therapy

Therapy with intraperitoneal aprotinin was associated with no adverse effects although in 5 patients there was a problem establishing cannula flow. In 2 of these patients the cannulas had to be repositioned after instillation of a litre of saline. In 2 other patients only a small amount of the first instillation of aprotinin was returned after 8 hours. In the final patient the cannula flow was slow and was attributed to a plug of fibrin, partially blocking the cannula tip and discovered on removal of the cannula after the second instillation of aprotinin.

A scanty growth of organisms was cultured from the peritoneal exudate in two patients and from the saline lavage return fluid in one instance. One of these patients subsequently developed a pancreatic abscess but unfortunately bacteriology samples were not sent when the abscess was drained 4 months later. No adverse effects were noted in the other 2 patients. Culture of the peritoneal cannula

tip revealed skin contaminants, usually S. albus, in 8 patients but in no instance were any adverse effects apparent.

Death and major complications

Four of the 22 patients (18%) who received intraperitoneal antiprotease therapy with aprotinin died compared to 5 of the 26 control patients (19%) (Table 46). Three (60%) of the control patients died within the first week of their illness, all within 4 days of admission. These early deaths were due to fulminant acute pancreatitis with shock, one in addition showing evidence of disseminated intravascular coagulation. The other two patients died later (on day 19 and day 38) as a result of pancreatic necrosis and sepsis.

Only one of the patients receiving intraperitoneal aprotinin died during the first week due to cardiac failure and hypothermia. The remaining deaths occurred in the second or subsequent week of the illness. One had fulminant acute pancreatitis due to pancreatic necrosis and died on day 9. The others died late (on day 50 and day 64) as a result of pancreatic necrosis and sepsis. Two of these 3 patients underwent surgical treatment prior to their death.

The median time to death in the control patients was 4 days compared to 29.5 days in the treatment group, although this difference was not significant. Of the remaining patients 9 (35%) of the controls developed a major complication compared with 10 (45%) of the patients receiving intraperitoneal aprotinin (Table 46).

TABLE 46.

Outcome: death, major complications and clinical outcome amongst patients receiving intraperitoneal aprotinin or standard therapy.

	Intraperitoneal aprotinin (n = 22)	Control (n = 26)	p value
Death	4 (18%)	5 (19%)	NS
Fulminant pancreatitis	1	3	
Pancreatic necrosis/sepsis	2	2	
Other - hypothermia	1	-	
Major complication	10 (45%)	9 (35%)	NS
Local complications:			
pseudocyst	4	2	
necrosis	-	2	
abscess	1	-	
Systemic complications:			
respiratory insuffic[y]	5	3	
septicaemia	-	2	
Uncomplicated	8 (36%)	12 (46%)	NS
"Slow settling"	5	6	
Settled quickly	3	6	

Clinical course

Five (23%) of the therapy group and 6 (23%) of the control group with an ultimately uncomplicated outcome were considered to be "slow settling" while 3 (14%) of the therapy group and 6 (23%) of the control group settled quickly following admission (Table 46). The median hospital stay was 11.5 days in the control group and 14 days in the group receiving intraperitoneal aprotinin, although this difference was not significant (Fig. 58). Table 47 compares various clinical features amongst the 2 groups of patients, no significant differences being found.

Clinical parameters

The initial clinical assessment performed soon after admission was repeated 24 and 48 hours later. The results of these assessments are shown in figure 59. The pancreatitis was graded as severe on admission in 7 of the patients receiving intraperitoneal aprotinin, falling to 4 at 48 hours. Only 4 of the control patients were graded severe on admission rising to 7 at 48 hours (excluding 2 other patients who had already died).

The degree of abdominal tenderness assessed unblinded each day was slightly more marked on admission in the intraperitoneal therapy group and was less than in controls on the 3rd and subsequent days, none of these differences reaching statistical significance (Fig. 60).

Eleven of the control patients became shocked (pulse >130/min, systolic BP <100mmHg) at some time during the first 3 days of their

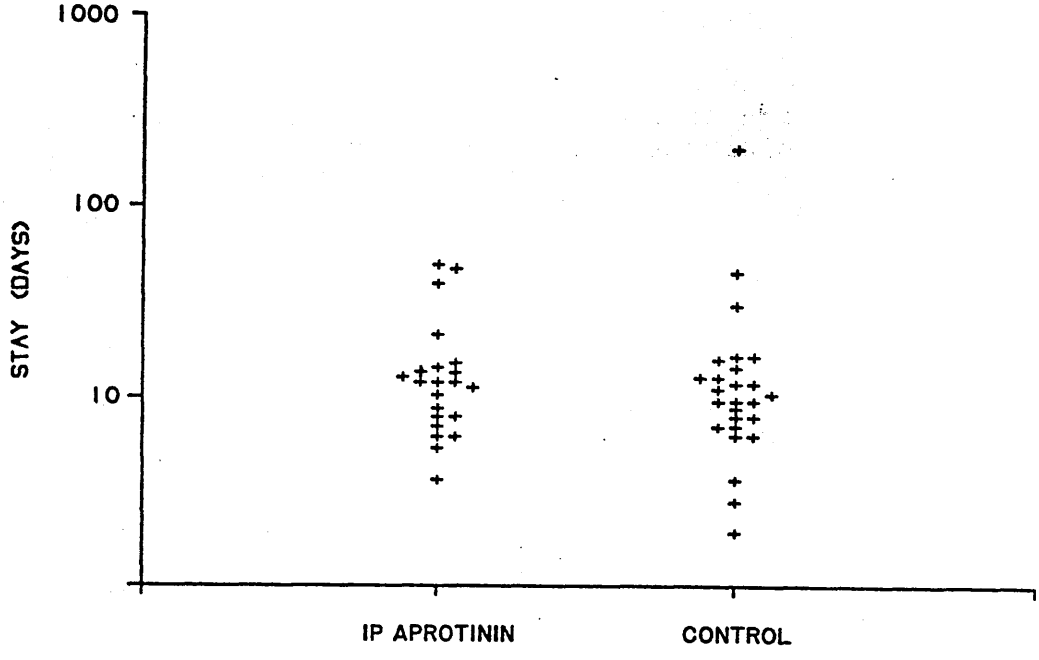


FIGURE 58.

Duration of hospital stay (log scale) in patients receiving intraperitoneal aprotinin or standard therapy (control).

TABLE 47.

Comparison of treatment amongst patients receiving intraperitoneal aprotinin or standard therapy.

Parameter	Intraperitoneal aprotinin (n = 22)	Control (n = 26)	p value
Nasogastric tube:			
number	22 (100%)	22 (85%)	NS
duration (days)*	3 (0-13)	3 (0-23)	NS
Ileus:			
number	14 (64%)	16 (62%)	NS
duration (days)*	1.5 (0-9)	1 (0-12)	NS
Intravenous fluids:			
duration (days)*	6.5 (3-43)	7 (1-57)	NS
Re-start oral fluids (day)*	4 (1-24)	4 (1-12)	NS
Recommence diet (day)*	6 (4-25)	8 (5-48)	NS
TPN employed: number	3 (14%)	4 (15%)	NS
Oxygen therapy:			
number	18 (82%)	21 (81%)	NS
duration (days)*	4.5 (0-42)	4 (0-20)	NS
ventilated	2 (9%)	6 (23%)	NS

* Median (range).

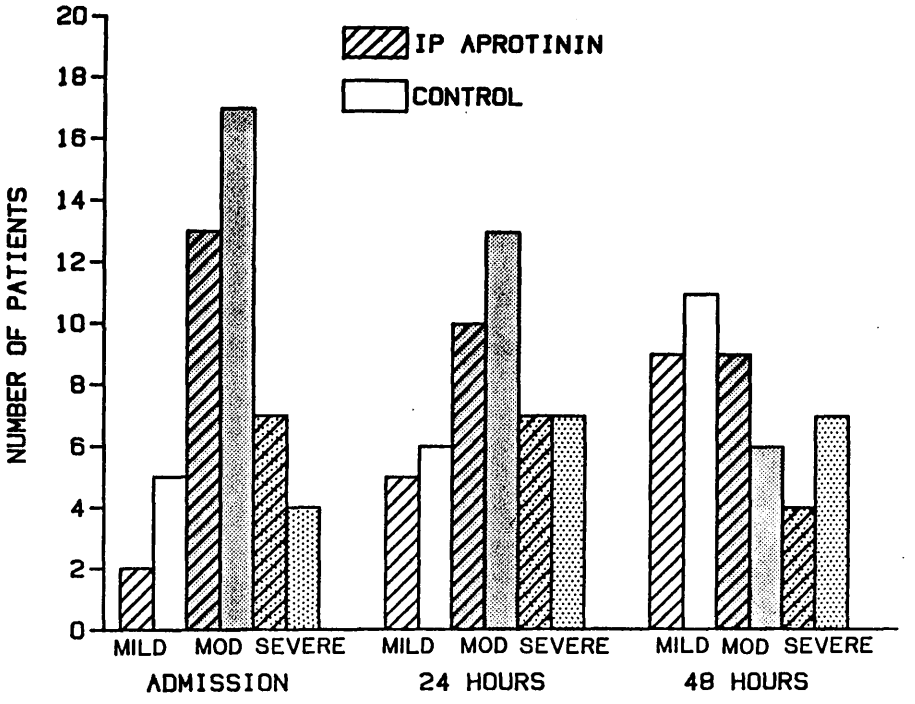


FIGURE 59.

Results of clinical examination at initial assessment, and 24 and 48 hours later, in patients receiving intraperitoneal aprotinin or standard therapy (control).

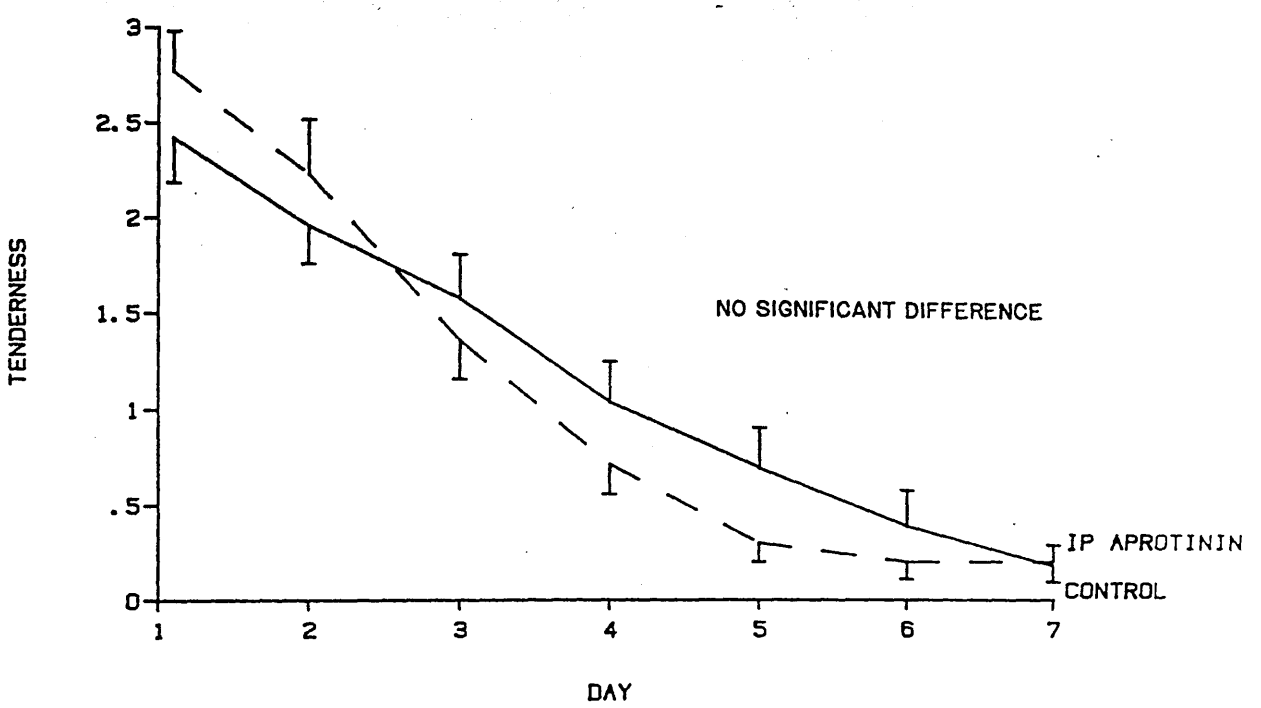


FIGURE 60.

Assessment of degree of abdominal tenderness in patients receiving intraperitoneal aprotinin or standard therapy (control).

illness compared with only 6 of the treated group although this difference was not significant. Aprotinin therapy appeared to have had little effect on the maximum or minimum mean blood pressures or heart rates (Fig. 61).

Patients receiving additional fluid as intraperitoneal aprotinin had a higher positive fluid balance, persisting throughout the first 7 days. This was most marked in the first 2 days and was partly compensated for by a lower positive balance during the 3rd and 4th days following admission (Fig. 62).

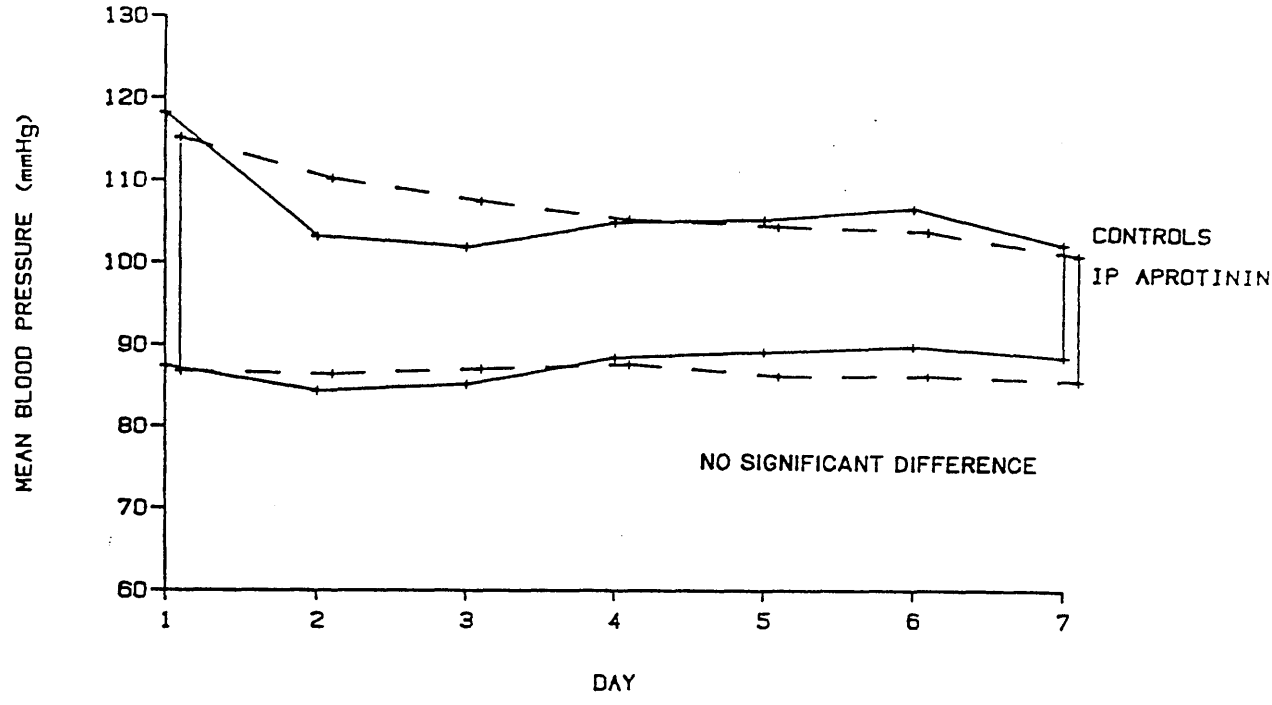
Subjective improvement in their pain following peritoneal washout and instillation of aprotinin was reported by 16 patients (73%). Four reported no improvement and 2 did not know. Any improvement was short-lived, their pain usually having returned within a few hours and their mean cumulative intramuscular analgesic requirement was identical to the control group (Fig. 63).

Laboratory studies: Renal function

The mean serum urea was slightly higher in the control patients from admission until day 4 (Table 48). The mean serum creatinine remained elevated at the upper limit of normal throughout the first week of the illness in the aprotinin treated group while falling steadily after the 2nd day in the control patients (Table 48). These differences did not reach statistical significance.

Pancreatic effects

The uncorrected serum calcium showed a similar falling pattern



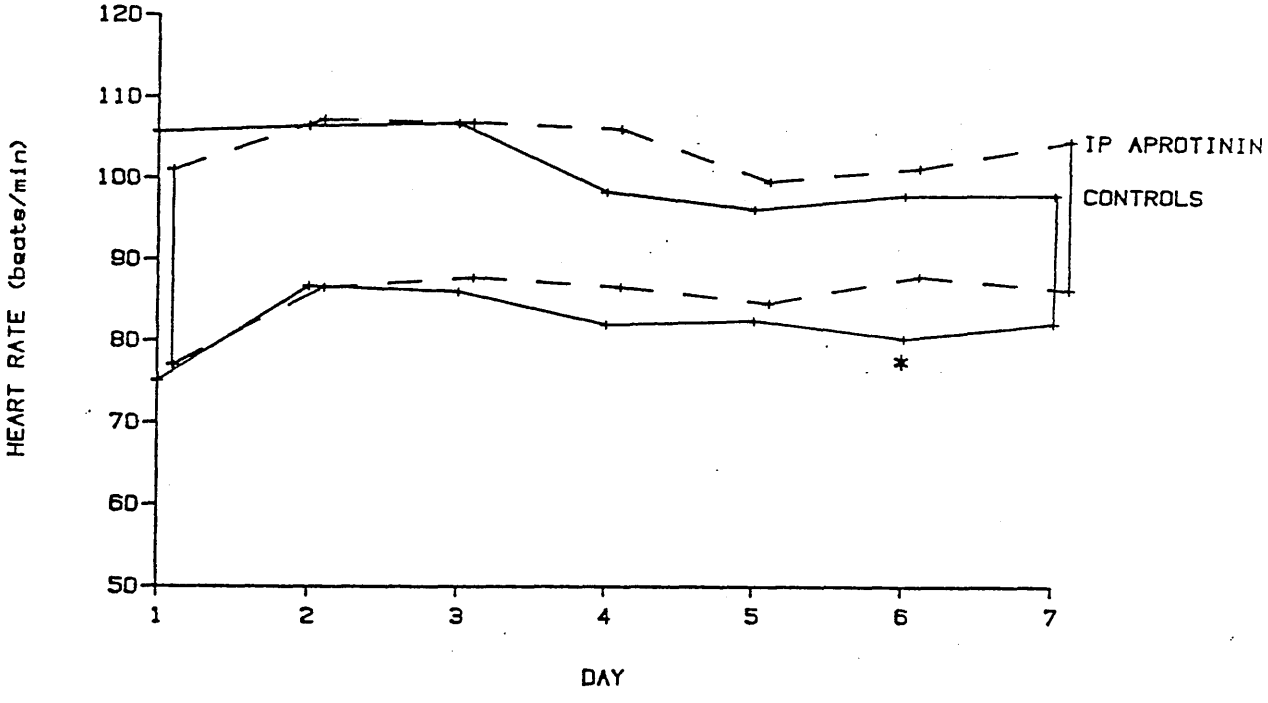


FIGURE 61.

(a) Maximum and minimum mean blood pressure in patients receiving intraperitoneal aprotinin or standard therapy (control).

(b) Maximum and minimum heart rate in patients receiving intraperitoneal aprotinin or standard therapy (control), * $p < 0.05$.

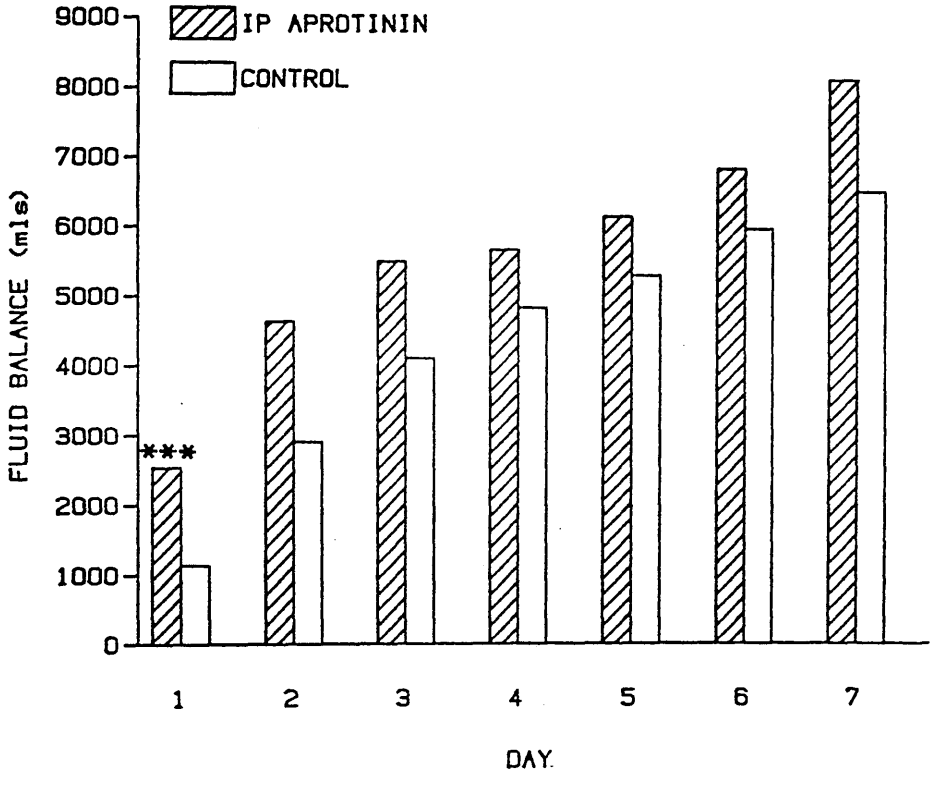


FIGURE 62.

Cummulative daily fluid balance in patients receiving intraperitoneal aprotinin or standard therapy (control) *** p <0.001.

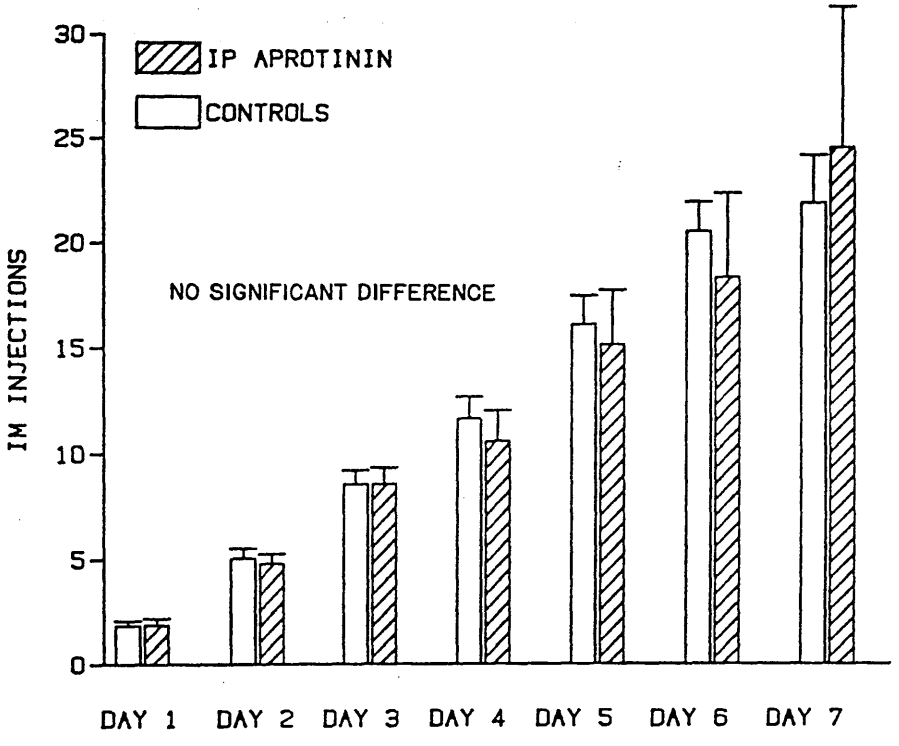


FIGURE 63.

Cummulative daily parenteral analgesic requirement in patients receiving intraperitoneal aprotinin or standard therapy (control).

TABLE 48.

Daily serum biochemical values in patients receiving intraperitoneal aprotinin therapy or standard therapy.

Parameter		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Urea	(T)	6.39 ±0.75	6.35 ±0.98	6.19 ±1.1	6.8 ±1.4	7.09 ±1.4	6.02 ±1.3	6.09 ±1.6
	(C)	7.74 ±1.2	8.3 ±1.1	7.32 ±1.1	6.1 ±1	5.94 ±1	5.26 ±0.8	6.74 ±1.3
Creatinine	(T)	112 ±9	110 ±13	111 ±12	111 ±15	105 ±16	100 ±15	103 ±20
	(C)	107 ±9	116 ±12	100 ±8	89 ±11	79 ±5	78 ±4	76 ±7
Calcium	(T)	2.22 ±0.04	2* ±0.04	1.95 ±0.06	2.01 ±0.07	2 ±0.05	2.07 ±0.08	2.1 ±0.04
	(C)	2.3 ±0.05	2.15 ±0.04	1.97 ±0.06	2.02 ±0.05	2.06 ±0.06	2.16 ±0.04	1.97 ±0.06
Glucose	(T)	8.7 ±0.7	8.2 ±0.8	7.2 ±0.5	7.1 ±0.7	6.4 ±0.5	7.7 ±1	9.9 ±1.8
	(C)	9.7 ±0.9	8.5 ±0.5	6.9 ±0.5	7.1 ±0.4	7.7 ±0.7	6.6 ±0.5	7.3 ±0.5
Amylase	(T)	5110 ±940	2099 ±293	1401 ±304	838 ±293	405 ±113	401 ±88	310 ±66
	(C)	5241 ±966	3268 ±625	1617 ±360	710 ±145	344 ±56	308 ±40	251 ±51
pO_2	(T)	65.8* ±3.4	87.3 ±7.2	74.7 ±6.3	77 ±4.4	88.4 ±9.5	93.3 ±9.8	84.8 ±8.2
	(C)	76.8 ±3.2	75 ±2.9	77.9 ±5.1	89 ±5	74.2 ±4.1	80.6 ±5.5	78.3 ±8.6
FiO_2/pO_2	(T)	2.6* ±0.2	2.92 ±0.18	4.1* ±0.45	3.36 ±0.43	3.12 ±0.39	2.67 ±0.32	2.98 ±0.56
	(C)	2.07 ±0.1	3.12 ±0.38	2.87 ±0.3	3.09 ±0.29	3.1 ±0.37	3.37 ±0.45	3.1 ±0.37

KEY:

Means ± s.e.m.
* $p < 0.05$, otherwise no significant differences found

(C) Standard therapy

(T) Intraperitoneal aprotinin

in both groups but with significantly lower levels in the treated group on day 2 (Table 48). Random blood glucose was elevated in both groups on admission showing an identical pattern thereafter (Table 48).

Serum amylase levels fell rapidly in both groups, approaching the upper limit of normal by day 5 (Fig. 64) (Table 48). The mean serum amylase had fallen further by day 2 in the treatment group but this difference failed to reach statistical significance.

Respiratory effects

The pO_2 was significantly lower on admission in the treatment group but thereafter showed a similar pattern in both groups (Table 48). As many patients received oxygen therapy the adequacy of oxygenation may be better expressed by the ratio of the inspired oxygen concentration (FiO_2) to the pO_2. The maximum ratio was calculated daily to reflect the poorest gas exchange and was found to be significantly higher in those patients receiving intraperitoneal aprotinin during days 1 and 3, although no consistent pattern emerged (Table 48).

Liver function

Aspartate and alanine aminotransferases were elevated initially in both groups of patients. Levels fell steadily from day 1 in the control patients whereas the aprotinin treated group showed a transient elevation on day 2 followed by a similar fall (Table 49). Alkaline phosphatase showed an identical falling pattern in both

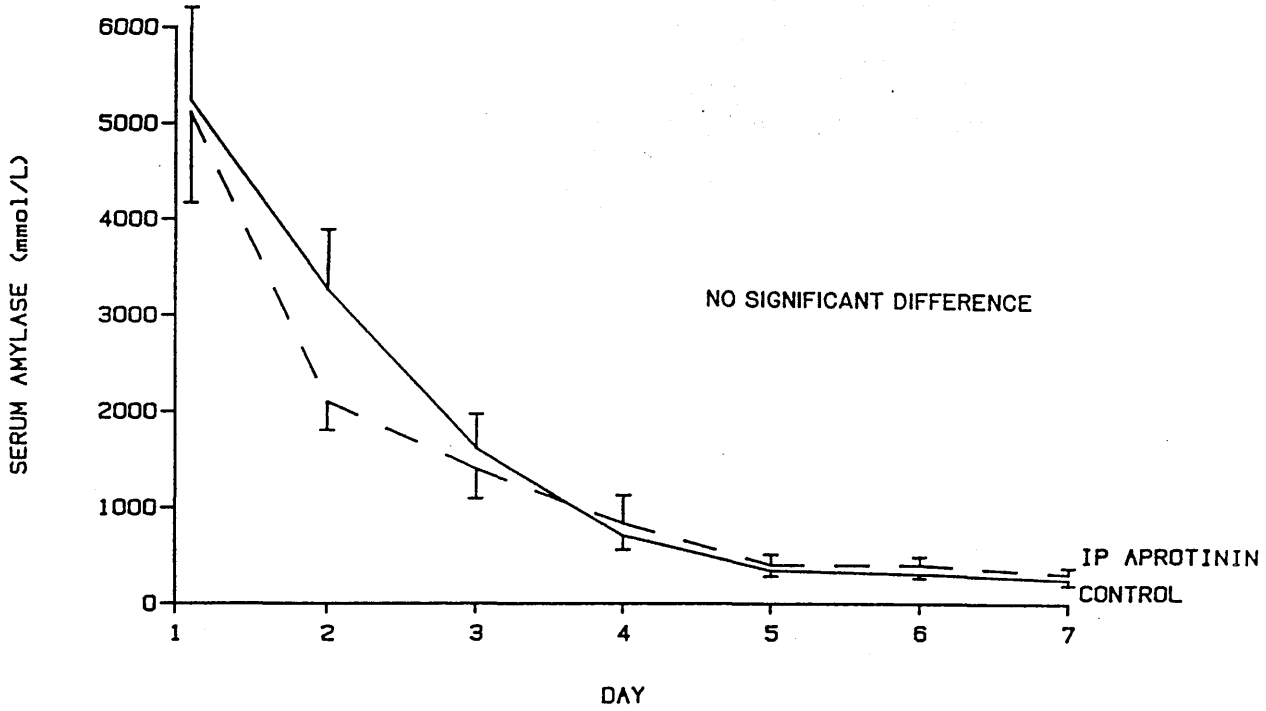


FIGURE 64.

Mean daily serum amylase in patients receiving intraperitoneal aprotinin or standard therapy (control).

TABLE 49.

Daily serum liver function tests in patients receiving intraperitoneal aprotinin therapy or standard therapy.

Parameter		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Aspartate amino-transferase	(T)	160 ±51	256 ±163	150 ±70	55 ±7	60 ±8	56 ±10	81* ±24
	(C)	190 ±40	112 ±23	66 ±9	61 ±12	45 ±5	46 ±7	37 ±5
Alanine amino-transferase	(T)	117 ±42	141 ±56	114 ±40	64 ±21	56 ±13	51 ±10	50 ±8
	(C)	170 ±47	118 ±25	68 ±13	57 ±10	55 ±8	43 ±6	39 ±5
Alkaline phosphatase	(T)	372 ±71	223 ±24	232 ±22	227 ±24	252 ±30	277 ±36	319 ±55
	(C)	384 ±56	284 ±32	241 ±24	235 ±24	264 ±24	272 ±29	320 ±35
Lactate dehydrogenase	(T)	567 ±72	799 ±207	764 ±117	736 ±102	812* ±68	764* ±106	903 ±223
	(C)	578 ±49	637 ±104	575 ±60	611 ±85	511 ±68	511 ±46	605 ±77
Bilirubin	(T)	23.9 ±3.8	26.6 ±4.6	34.5 ±7.7	31.5 ±9.7	36 ±12.8	32.7 ±14.3	39.5 ±20
	(C)	18.5 ±2.7	28.9 ±5	31.5 ±6.7	23 ±3.1	27.9 ±5	20.8 ±3.4	26 ±8.4

KEY:

Means ± s.e.m.
* $p < 0.05$, otherwise no significant differences found.

(C) Standard therapy

(T) Intraperitoneal aprotinin

groups (Table 49). Lactate dehydrogenase tended to rise through the course of the illness in the aprotinin treated group while remaining fairly constant at the upper limit of normal in the control group. These differences were statistically significant on days 5 and 6 (Table 49). Bilirubin showed a transient elevation in the control group whereas levels in the aprotinin treated group continued to rise to the end of the first week.

Plasma proteins

Serum albumin showed an identical falling pattern in both groups (Table 50). C-reactive protein showed marked elevation in both groups of patients, peaking around the 3rd day and no significant differences were found (Fig. 65)(Table 50). Levels of $alpha_2$macroglobulin fell in both groups of patients, reaching a trough around the 3rd and 4th days and rising slowly again thereafter (Fig. 66). $Alpha_1$antiprotease showed a similar rising pattern in both groups of patients, peaking between days 4 and 5 (Fig. 67)(Table 50).

Haematology

The mean haematocrit showed an identical falling pattern in both groups (Table 50). The white cell count showed high initial values, falling to within the normal range in both groups by day 6.

APACHE II illness scoring system

The overall APACHE II score was slightly higher in the aprotinin treated group of patients on admission and, with the

TABLE 50.

Daily values of plasma proteins, haematocrit and white cell count in patients receiving intraperitoneal aprotinin therapy or standard therapy.

Parameter		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Albumin	(T)	39.9 ±1.2	37.5 ±0.96	34.5 ±1	34.6 ±1.1	33.9 ±1	32.8 ±1.3	32.5 ±1.2
	(C)	40.5 ±1.3	38.4 ±1	34.7 ±0.9	33.7 ±1	34.5 ±1.5	34.5 ±1	30 ±1.6
C-reactive protein	(T)	56 ±15	156 ±21	231 ±19	232 ±22	186 ±18	153 ±17	158 ±20
	(C)	58 ±14	169 ±16	217 ±20	198 ±18	176 ±23	145 ±18	133 ±21
$Alpha_2$macro -globulin	(T)	1.72 ±0.12	1.39 ±0.12	1.28 ±0.11	1.26 ±0.11	1.4 ±0.12	1.39 ±0.13	1.38 ±0.14
	(C)	1.64 ±0.1	1.47 ±0.08	1.29 ±0.09	1.3 ±0.09	1.33 ±0.11	1.31 ±0.09	1.35 ±0.1
$Alpha_1$anti -protease	(T)	2.48 ±0.15	2.95 ±0.16	3.86 ±0.22	4.52 ±0.28	4.4 ±0.31	4.49 ±0.32	4.53 ±0.3
	(C)	2.38 ±0.14	3.1 ±0.13	3.77 ±0.16	4.17 ±0.19	4.33 ±0.21	4.17 ±0.24	4.12 ±0.25
Haematocrit	(T)	45.6 ±0.9	42.4 ±0.7	40 ±1.1	38.3 ±1.3	36 ±1.1	37.6 ±1.3	38.3 ±1.4
	(C)	44.5 ±1.5	43.1 ±1.3	39.3 ±1.2	36.4 ±1.1	36.3 ±1.5	36.7 ±0.9	35.4 ±1.7
White cell count	(T)	17.1 ±2.2	14.5 ±1	13.8 ±1.2	11.5 ±1.4	11.5 ±1.3	9.6 ±1.1	10.4 ±1.2
	(C)	16.6 ±1.5	16.1 ±1.6	15.4 ±1.7	12.3 ±1.1	10.7 ±1.1	10.1 ±1	12.6 ±1.6

KEY:

Means ± s.e.m.
No significant differences found

(C) Standard therapy

(T) Intraperitoneal aprotinin

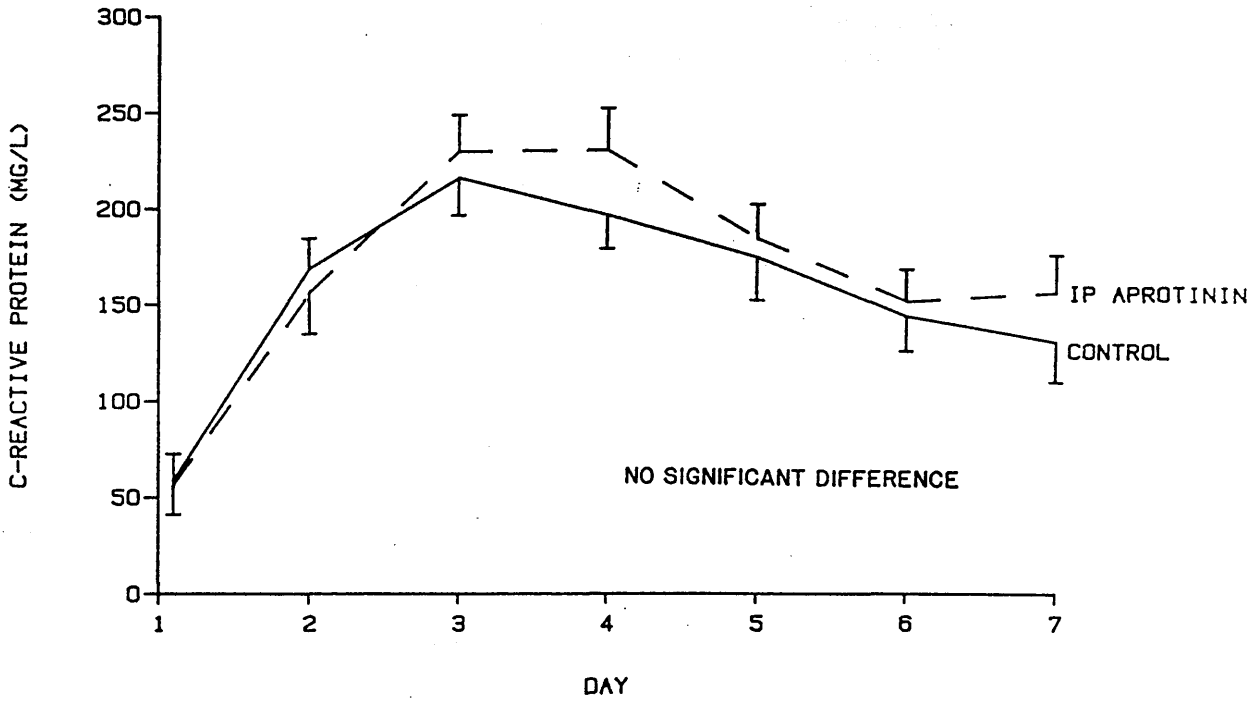


FIGURE 65.

Mean daily C-reactive protein concentration in patients receiving intraperitoneal aprotinin or standard therapy (control).

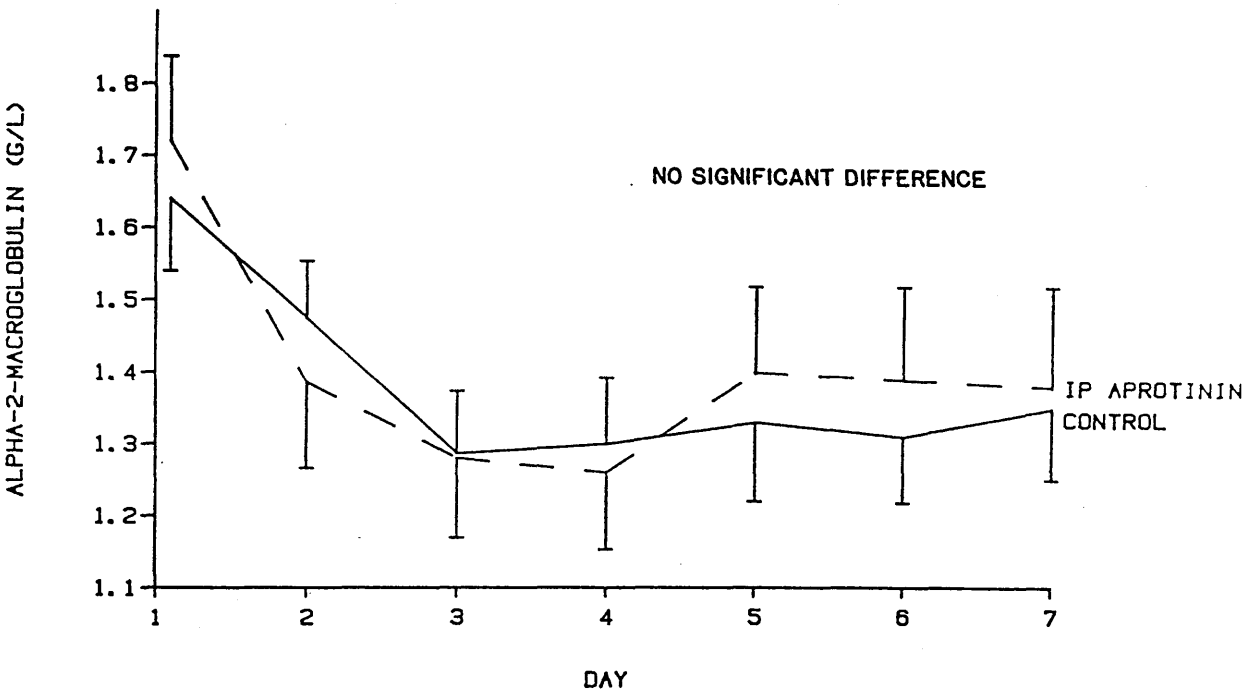


FIGURE 66.

Mean daily alpha$_2$macroglobulin concentration in patients receiving intraperitoneal aprotinin or standard therapy (control).

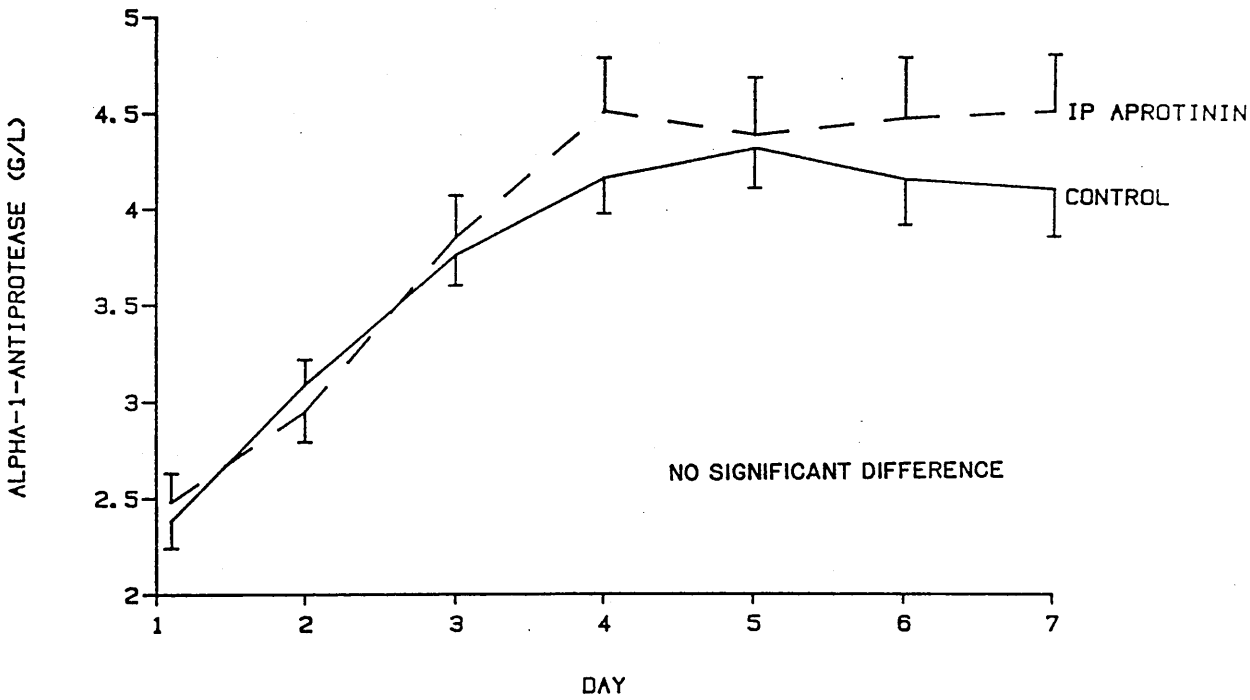


FIGURE 67.

Mean daily alpha$_1$antiprotease concentration in patients receiving intraperitoneal aprotinin or standard therapy (control).

exception of day 2, remained slightly higher throughout the first week, although these differences were not statistically significant (Fig. 68).

Discussion

The 2 exclusions and one refusal to participate in the study have resulted in a slight imbalance in the numbers between the 2 groups but nevertheless the groups appeared to be reasonably well matched. This finding of 2 patients with alternative diagnoses serves to emphasise the potential value of diagnostic peritoneal aspiration in patients with abdominal pain and hyperamylasaemia suspected of having severe acute pancreatitis, a finding noted previously by others[153,235].

Intraperitoneal antiprotease therapy with aprotinin, although having been shown to markedly reduce intraperitoneal tryptic amidase activity, had no appreciable effect on outcome as judged by the overall mortality rate and incidence of major complications in this interim report. There were, nevertheless, a number of features which might be interpreted as suggesting some therapeutic benefit.

Early death from fulminant pancreatitis occurred more often amongst the control patients and shock was observed more frequently in these patients. This may indicate an effect attributable to intraperitoneal therapy with aprotinin, although none of these differences reached statistical significance. This possible effect cannot be attributed directly to the drug and may merely reflect the greater positive fluid balance early in the course of the illness in

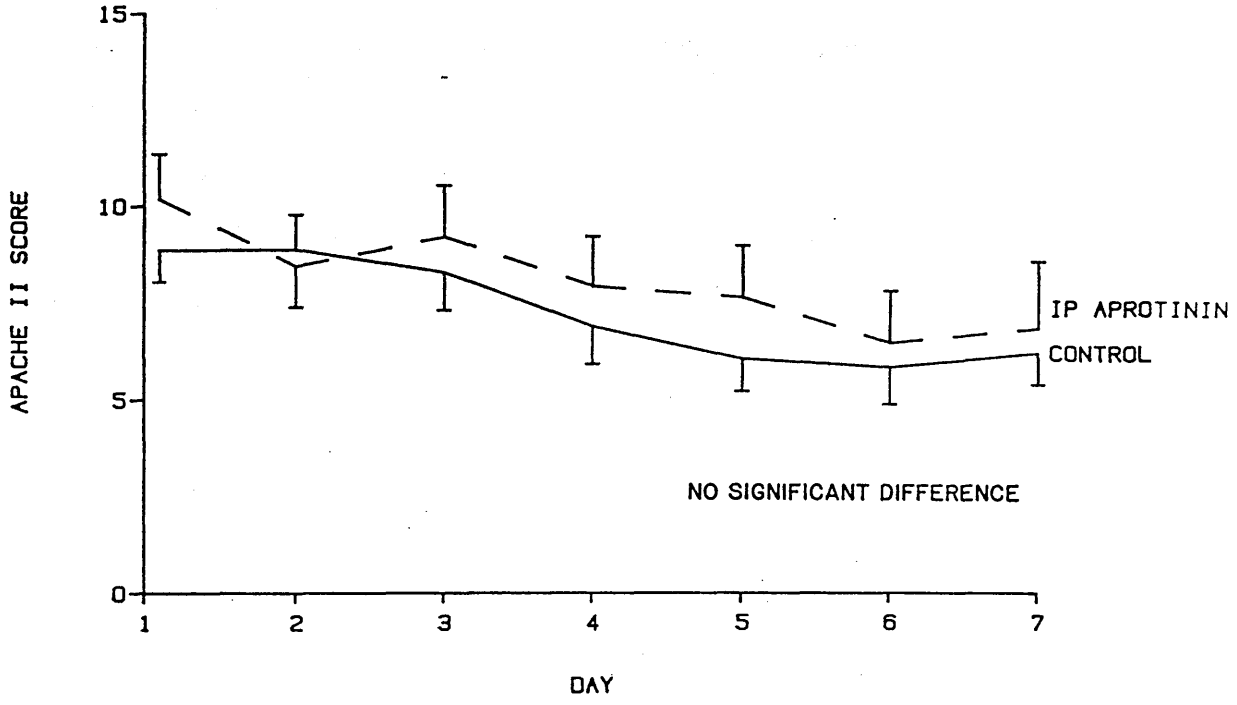


FIGURE 68.

Mean daily APACHE II score in patients receiving intraperitoneal aprotinin or standard therapy (control).

the patients undergoing intraperitoneal therapy. Ranson considered that the shock in acute pancreatitis often reflects a fluid deficit[287] and simply by providing more fluid (by the intraperitoneal route) over the first 48 hours might have diminished the incidence of shock.

A greater proportion of the patients receiving intraperitoneal therapy were assessed clinically as severe on admission (7 vs 4). By 24 hours 7 patients in each group were considered severe and by 48 hours this applied to only 4 of the treatment group whereas 7 of the control patients were still considered severe, excluding another 2 patients who had already died. This difference was not statistically significant and, although subjective and unblinded, suggested a trend towards a worsening clinical condition in a number of the control patients compared with the apparent improvement in the group treated with intraperitoneal aprotinin.

Against there being any important early differences between the groups was the failure to detect important variations in the APACHE II scores, or in other objective physiological parameters such as the mean heart rate and mean blood pressure, although it is recognised that significant changes in only a few patients may have been obscured by the lack of change in the majority.

Overall 14 (64%) of the intraperitoneal therapy group and 14 (54%) of the control group had a fatal or complicated clinical course suggesting that the treatment was associated with no major advantage in terms of these clinically important endpoints. Thus the initial results of this study are in agreement with the negative results of

the two recent studies investigating formal cycled peritoneal lavage[153,235]. Earlier studies in a predominantly[292] or exclusively alcoholic patient population[339] had suggested an early beneficial effect but too few such patients had been randomised in the current study to permit a separate analysis.

Relief of pain has been claimed to be a beneficial effect of peritoneal lavage in acute pancreatitis, particularly in the earlier anecdotal reports. There has been only one previous attempt to quantify the patient's degree of pain during treatment, based on a 12 hourly assessment by the attending nurse, grading the pain as absent - 0 to severe - 2. In this rather subjective assessment no difference emerged between the patients undergoing formal cycled peritoneal lavage and the control group[153]. The majority of patients undergoing a single peritoneal lavage and instillation of aprotinin in the current study claimed, when questioned, to have had relief of their pain, but this appeared to be short-lived and was not reflected in the more objective assessment of a diminution in their intramuscular analgesic requirement throughout the remainder of their illness.

Peritoneal washout appeared to produce a slightly greater fall in the mean serum amylase by day 2 in the patients undergoing intraperitoneal therapy, although this difference failed to reach statistical significance. Mayer and co-workers found that in dogs with experimental pancreatitis drainage of the peritoneal exudate did not lead to reduced serum enzyme levels[236], nor did peritoneal lavage in man[235] although, in another study in man, peritoneal lavage led to the serum amylase falling more rapidly[153]. The small effect on the

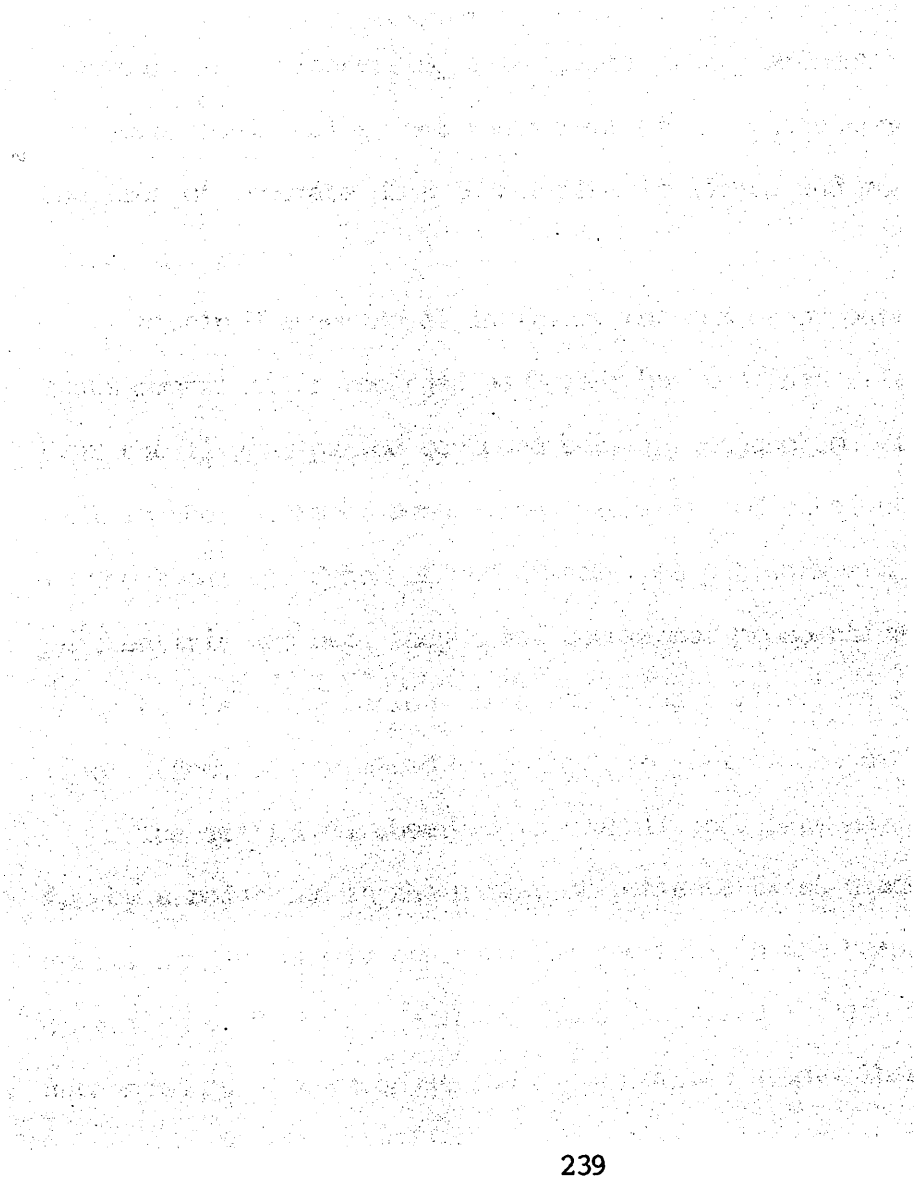
serum amylase levels postulated to have occurred in the current study does not appear to reflect greater haemodilution in the therapy group as the haematocrit and serum albumin levels were similar in both groups of patients throughout the first 7 days of their illness.

Minor differences between the groups emerged for the transaminases and lactate dehydrogenase. The highest lactate dehydrogenase levels were found in the illest patients in the treatment group towards the end of the first week by which time 3 of the corresponding patients in the control group had already died. Serum creatinine levels appeared to behave differently in the therapy group although the differences failed to reach statistical significance. The possible reasons for this remain unclear.

None of the specific protein markers were able to distinguish the two groups of patients. In particular serum levels of $alpha_2$macroglobulin did not appear to be influenced by intraperitoneal administration of an antiprotease solution nor was the acute phase response to illness, as reflected by the elevation in C-reactive protein and $alpha_1$antiprotease concentrations, diminished by therapy. Similarly, although APACHE II had earlier appeared to be a promising marker of the course of illness, no important differences were apparent in the analysis of patients receiving intraperitoneal therapy compared to those receiving standard therapy alone.

In conclusion, this interim report has demonstrated that intraperitoneal antiprotease therapy with aprotinin appears to have had no important effect on the incidence of death or major complications amongst the patients in the treatment group. There was

a suggestion of a trend towards earlier clinical improvement and a possible reduction in the incidence of shock and fulminant acute pancreatitis amongst the treated patients. These effects may be clinically important and will be examined further when a greater number of patients have been randomised.



CHAPTER 13. CONCLUSIONS

Incidence and mortality

The apparent incidence of acute pancreatitis, as reflected by the number of discharges recorded, has increased dramatically over the past 25 years. This is largely thought to reflect an increase in the diagnostic rate, particularly of mild cases who are less likely to die and thus accounting for the contemporaneous fall in the case mortality rate. It is likely that the true incidence of acute pancreatitis is increasing also, particularly amongst elderly females and young adult males, but the extent of this is unknown highlighting the lack of accurate data on the true incidence and mortality trends across the country.

Within Glasgow Royal Infirmary the number of patients with acute pancreatitis recorded each year has remained relatively stable over the 11 year period reviewed but, in conjunction with the 23% fall in the catchment population, probably indicates a true increase in the local incidence of the disease. As patients with acute pancreatitis had been sought and documented prospectively for 3 years prior to the study period, this was thought likely to rule out any major effect of increased investigative zeal on the diagnostic rate.

The small fall observed in overall mortality may be accounted for by a reduction in the number of patients first diagnosed at post mortem and is largely explained by the fall in the hospital's post mortem rate. Mortality amongst those diagnosed in life remained unchanged at 9% throughout the period of the study. This would appear

to confirm the contention that improvements in therapy over this period may have failed to influence outcome.

Closer examination of the data suggests that, at least for gallstone-associated attacks, improvements in therapy may have contributed to the 47% reduction in number of deaths. A reduction in the number of fatal recurrent attacks has contributed to this fall, perhaps reflecting the current policy of early cholecystectomy for gallstone-associated pancreatitis, performed during the initial admission. Surgical eradication of gallstones also appears to have been more successful during the latter half of the study and, finally, the incidence of gallstone-associated pancreatitis itself, may be decreasing amongst the local population.

The study has also confirmed the persistent early mortality associated with acute pancreatitis, almost half the patients dying within 7 days of admission. Surgery rarely, if ever, has a role in the treatment of this phase of the illness and if this early mortality is to be improved upon then we need to be able to identify these cases more accurately and more rapidly. Early ERCP and endoscopic sphincterotomy may well become the treatment of choice for gallstone-associated pancreatitis in the future[253] but in other forms of severe acute pancreatitis an effective, specific therapy is required.

Prediction of severity

Measurement of 5 different complement factors provided no useful discrimination between uncomplicated and complicated attacks.

As markers of severity the antiproteases provided useful discrimination, particularly during the second half of the first week of the illness. Alpha$_2$macrogloblin demonstrated a falling pattern in severe attacks, presumably indicating its consumption. Its serum levels are normally stable during illness and the magnitude of its fall may reflect the degree of protease complexation and uptake by the reticulo-endothelial system, and hence the degree of proteolytic enzyme activation.

The increase in alpha$_1$antiprotease, which accounts for the bulk of the serum antitrypsin activity, appears to more than adequately compensate for the reduction in alpha$_2$macroglobulin. The serum antitrypsin defences are boosted in acute pancreatitis, a more than 300% increase in the trypsin binding capacity having been reported during the course of the illness, although this increase appeared to be delayed in the severe cases[133]. Whether a reduced alpha$_2$macroglobulin concentration in serum is of importance in acute pancreatitis is not clear. Levels of alpha$_2$macroglobulin were restored by the infusion of fresh frozen plasma in one recent study but was not associated with evidence of any beneficial effect within the treated group[208]. Overwhelming of the serum antiprotease defences in acute pancreatitis has not been reported in man.

C-reactive protein provided a better prediction of outcome although its elevation is not specific to acute pancreatitis, rather it acts as an acute phase reactant, levels rising in response to any insult or injury. The non-specific nature of its elevation should mean that it truly reflects the severity of inflammation or illness

and is uninfluenced by other factors such as the age of the patient or the underlying aetiology of the attack which are drawbacks of the current scoring systems.

The peak levels of C-reactive protein provided the best discrimination but may not be attained until the 3rd or 4th day and thus C-reactive protein has no advantage over multiple factor scoring systems in terms of speed. C-reactive protein can be measured repeatedly thus permitting sequential monitoring of an attack. Persistently high levels were found in complicated attacks and in those developing a late complication. Furthermore, measurement of a single factor is much simpler than the currently used systems and more likely to be adopted into daily clinical practice.

Clinical assessment provided the highest overall accuracy although, even at 48 hours post-admission, failed to accurately identify patients with severe disease likely to have a complicated outcome. In this respect the present data is at variance with that reported by McMahon and colleagues and highlights the lack of objectivity of clinical assessment, particularly with respect to what constitutes a complicated attack[226]. The illest patients are often identifiable on clinical assessment as are those with an obviously mild attack, the problem being the identification of severe attacks amongst patients with clinical features lying between these two extremes.

Examination of several individual clinical parameters demonstrated that while they usually provided significant discrimination between uncomplicated and complicated attacks, they

were typically recorded in only a small proportion of patients developing a complicated attack.

Both the Ranson and Glasgow scoring systems performed well with little to choose between them, although the Ranson system appeared superior for alcohol-associated attacks. Diagnostic peritoneal lavage performed less well, although as pointed out earlier, not all patients were assessed by this technique. In the original description 72% of severe attacks had been correctly predicted (sensitivity) and 95% of mild attacks (specificity) with 90% of attacks overall correctly predicted[226]. In a subsequent 3 centre analysis of patients entered into the recent UK trial of therapeutic peritoneal lavage, 65% of complicated attacks were correctly predicted, 90% of mild attacks and overall 82% of attacks were correctly predicted[70].

Diagnostic peritoneal lavage is probably as accurate as the multiple factor scoring systems and may be better for alcohol-associated attacks[70]. Multiple factor scoring systems are not widely utilised outwith clinical studies and remain complex to apply. Diagnostic peritoneal lavage has the advantage of speed but may be less accurate for gallstones[70]. It is invasive and despite the advantage of occasionally detecting an erroneous diagnosis earlier in the course of an attack, many clinicians remain reluctant to apply it.

The APACHE II score has some advantages, not least the fact that it is objective, uses easily available data and provides reasonable accuracy soon after admission. The accuracy of the peak APACHE II score (within the first 3 days) was equivalent to the

multiple factor scoring systems but then has no clear advantage in terms of delay to prediction. APACHE II can be measured daily and used to follow the course of the illness which may prove to be its most valuable role, overcoming some of the drawbacks of the standard scoring systems. The APACHE II remains complicated to apply and from the analysis of the individual factors on admission, not all contribute to the overall score in this illness. The preliminary results nevertheless seem encouraging and worthy of further investigation.

All the laboratory based methods for prediction of severity examined here were indirect, often dependent upon secondary changes within other systems, and were typically slow in providing a prediction of outcome. A more direct assessment of the degree of proteolytic enzyme activation may more closely reflect the extent of the pancreatic injury. Phospholipase A_2 is produced by activation of its zymogen by trypsin. It has no natural inhibitors in the body and levels may more accurately reflect the degree of trypsin activation. In a recent study phospholipase A_2 levels were found to correlate with outcome, the highest levels being found in fatal attacks[284]. C-reactive protein was also assessed, appearing to provide similar discrimination between the groups, but a comparative analysis of these 2 factors was not provided[284].

Trypsin-alpha$_1$antiprotease complexes can be detected in serum and may reflect the amount of trypsin activation occurring[42,43]. The method is complicated, however, and remains purely a research technique.

A development which promises to be of great importance in this field of understanding is the recent description of an assay for the trypsinogen activation peptide released when trypsinogen is activated to trypsin[139]. This peptide can be detected in blood and in urine and directly reflects the amount of trypsinogen activation occurring. Increased levels have been detected on admission and early in an attack of pancreatitis in patients with a severe outcome[139]. Such an assay may allow us to monitor both the timing and extent of trypsinogen activation and may even provide guidance as to the need for antiprotease therapy.

Protease-antiprotease balance.

Free proteolytic activity, indicating overwhelming of the antiprotease defences, is frequently detected in exudates soon after the induction of experimental pancreatitis by the retrograde intraductal injection of bile[263]. Later in the course of pancreatitis induced by the closed duodenal loop method, saturation of the peritoneal antiproteases has been shown to be associated with the development of irreversible shock and death. The activation of zymogens and the complement and kinin systems with release of vasoactive peptides including kinins and histamine, which may cross the peritoneum into the circulation to exert a systemic effect, are thought to mediate this toxic effect.

The experimental study in rats confirmed the previous reports of frequent, early overwhelming of antiprotease defences associated with retrograde intraductal injection models of pancreatitis. The

improved survival amongst rats receiving intraperitoneal therapy appeared to confirm that the exudate was toxic and that its removal was beneficial. Survival was prolonged both when free proteolytic activity had been demonstrated in the exudate and when absent.

The addition of fresh frozen plasma after removal of the exudate proved the most effective therapy, possibly by the replenishment of active, natural antiproteases. Other active factors are present in fresh frozen plasma including clotting factors and fibronectin which conceivably could have played a role, although unlikely. Alpha$_2$macroglobulin is considered likely to be the key constituent of fresh frozen plasma and further studies might confirm this by examining the effectiveness of the various components of fresh frozen plasma which can be separated by fractionation techniques.

The trypsin binding capacities of peritoneal exudates were reduced in acute pancreatitis in man, indicating a degree of saturation of their antiproteases. The antiprotease defences seemed adequate in the majority of patients, appearing to be critically reduced in only 3 cases. Thus similar changes as reported in experimental pancreatitis, although less marked, were observed in human acute pancreatitis.

If overwhelming of the antiprotease defences is important to the toxicity of exudate in human pancreatitis, it would appear to be a rare phenomenon occurring in only a small proportion of patients. This contrasts with the results of a similar study where 6 of 13 exudates, sampled at the time of surgery, demonstrated free

proteolytic activity indicating overwhelming of the antiprotease defences[376].

No data is available on whether human exudate is toxic in the absence of overwhelming of its antiproteases. Alpha$_2$macroglobulin, due to its large size, appears less able than alpha$_1$antiprotease to enter the peritoneal exudate. Alpha$_2$macroglobulin appears, however, to be the key protective antiprotease and when saturated kinin release and complement activation can proceed, despite only limited saturation of alpha$_1$antiprotease[19,20]. The binding capacity or degree of saturation of alpha$_2$macroglobulin in exudate may then be more important than assessment of the total trypsin binding capacity and should be examined in future studies. Nothing is known of the dynamic changes in protease-antiprotease balance in exudates during human acute pancreatitis and serial investigation of these parameters would be of interest in any subsequent studies.

The results reported here suggest that intraperitoneal antiprotease therapy may be only rarely be indicated in human acute pancreatitis for overwhelming of the antiprotease defences. High degrees of alpha$_2$macroglobulin complexation in exudate appear to be more common[204] and antiprotease therapy with aprotinin might also be expected to benefit these patients, aprotinin having been shown in vivo and in vitro to be protective against trypsin mediated kinin release and complement activation[19,203,205]. Whether intraperitoneal antiprotease therapy might benefit the remaining patients is doubtful, given the failure of recent studies to show benefit from removing the exudate by formal peritoneal lavage[153,235], despite

previous reports of successful therapy[292,339].

The preliminary report of the clinical study comparing standard therapy alone or with additional intraperitoneal antiprotease therapy with aprotinin confirmed the protocol to be workable and the treatment regime acceptable to the patients and clinicians involved. Diagnostic peritoneal lavage was performed on the small group randomised to receive intraperitoneal antiprotease therapy without morbidity, and with the additional bonus of detecting two cases with alternative diagnoses requiring early surgery.

Clinical assessment proved an effective method for selecting an "at risk" group of patients with a high incidence of death and morbidity and thus suitable for study, early in the course of an attack.

Evidence was presented that the pancreas appears to continue to exude enzymes into the peritoneal cavity during the early phase of an attack. Intraperitoneal antiprotease therapy with aprotinin was shown to markedly reduce tryptic amidase activity in the peritoneal cavity during the first 8 hours of therapy.

In this preliminary report intraperitoneal antiprotease therapy with aprotinin produced no clear benefit in terms of the clinically important end points of death and incidence of major morbidity. There was a trend suggesting possible benefit with reduction in the incidence of shock and early death from fulminant acute pancreatitis, but too few patients had been studied at this point to permit formal statistical analysis and exclude a type 2 error.

Acute pancreatitis is an increasingly common condition the mortality of which has remained static at Glasgow Royal Infirmary over the period 1974 to 1984. No systemic therapy has yet been shown to significantly alter the outcome when investigated in a properly conducted prospective trial. The evidence presented in this thesis supports the theory of toxicity of peritoneal exudate in experimental pancreatitis in association with saturation of the peritoneal antiprotease defences. Similar, although less marked changes in the protease-antiprotease balance, have been demonstrated in the peritoneal exudates from patients with acute pancreatitis. It is suggested, but has not been proven, that overwhelming of these defences may occur occasionally in man.

A novel therapy comprising aspiration of the peritoneal exudate, a single litre saline lavage and instillation of the antiprotease aprotinin (5×10^6KIU on 2 occasions during the first 8 hours) has been investigated in a clinical study since February 1986. The interim results have shown no significant benefit in terms of the incidence of death or major complication although there appeared to be a trend towards some early clinical improvement with a reduction in the incidence of shock and early death in the treated patients which requires confirmation in a larger group of patients.

APPENDIX 1. CLINICAL STUDY: PATIENT DATA

No.	Sex	Age	Study group	Aet	Home/ death (day)	Prognostic factor score		Out-come	Complications
						Glas	Ranson		
001	F	69	C	GB	15	5	7	Mild	Slow settling
002	F	80	NR	UK	14*	6	4	Mild	No *social reasons
003	F	63	T	GB	>14	5	5	Mild	Slow settling
004	F	60	C	GB	12	2	2	Comp	Resp insufficiency/ BWS
005	M	65	C	UK	3	3	4	Died	Fulminant
006	M	39	NR	UK+	4	0	1	Mild	No
007	F	39	NR	GB+	5	2	1	Mild	No
008	M	36	C	ALC+	58	3	4	Comp	Necrosis
009	M	62	T	GB	50	3	4	Died	Necrosis
010	M	35	NR	GB	7	2	2	Mild	No
011	F	66	C	OTH	284	5	7	Comp	Necrosis
012	M	34	NR	ALC+	4	0	0	Mild	No
013	M	32	T	ALC	14	2	1	Comp	Pseudocyst
014	M	30	Ex	ALC+	9	0	0	Mild	Slow settling
015	M	31	NR	ALC+	8	1	1	Mild	No
016	M	89	NR	GB	12	2	5	Mild	No
017	M	51	T	OTH	14	4	6	Comp	Resp insufficiency
018	M	35	NR	ALC	10	2	2	Mild	No
019	M	56	Ex	UK	12	4	5	Mild	Lap diagnosis
020	M	32	NR	ALC	8	0	1	Mild	DTs
021	M	31	T	OTH	12	0	1	Mild	No
022	M	50	T	ALC	6	0	0	Mild	No
023	F	66	NR	GB+	8	1	3	Mild	No
024	M	25	C	ALC	9	1	1	Mild	Slow settling/ Collection
025	M	23	C	ALC+	7	0	0	Mild	No
026	M	48	NR	GB	4	1	1	Mild	No
027	F	56	T	GB	15	3	6	Comp	Resp insufficiency
028	F	39	NR	UK	6	1	0	Mild	No
029	F	52	T	GB	14	6	6	Comp	Resp insufficiency
030	M	67	NR	UK+	7	2	2	Mild	No
031	F	23	NR	GB	6	0	0	Mild	No
032	M	36	NR	ALC+	8	0	0	Mild	No
033	M	44	NR	ALC	5	0	0	Mild	No
034	F	74	C	GB	11	1	4	Mild	No
035	M	33	NR	ALC	5	2	1	Mild	No
036	M	39	NR	ALC	19	1	0	Mild	Collection
037	M	37	NR	OTH+	11	1	1	Mild	No
038	F	53	Ex	ALC	38	2	3	Comp	Resp insufficiency/ DTs
039	M	70	T	UK	13	2	2	Mild	Slow settling
040	M	64	C	UK	7	1	2	Mild	No
041	F	82	NR	GB	12	0	1	Mild	No
042	M	30	NR	OTH	4	0	0	Mild	No

043	F	86	Ex	OTH	3	5	7	Died	Fulminant/ARF
044	F	69	C	GB	2	4	7	Died	Fulminant/DIC
045	M	55	T	ALC	9	6	9	Died	Fulminant/ Necrosis/BWS/DIs
046	F	82	NR	GB	17*	2	4	Mild	No *social reasons
047	M	25	NR	ALC+	6	0	0	Mild	No
048	F	36	NR	GB/A+	13	2	2	Mild	DIs
049	F	26	NR	GB	16*	1	1	Mild	No *cholecyst
050	M	37	NR	OTH+	7	2	2	Mild	No
051	M	83	NR	UK	21*	1	2	Mild	No *ERCP
052	M	46	NR	GB/A	6	0	0	Mild	No
053	M	65	T	GB	9	2	3	Comp	Resp insufficiency
054	M	31	C	ALC	8	0	1	Mild	No
055	M	73	C	OTH	19	3	5	Died	Necrosis
056	M	40	C	ALC+	8	1	2	Mild	No
057	F	80	Ex	OTH	10	3	5	Died	ARF/Septicaemia
058	M	59	NR	GB	6	3	2	Mild	No
059	M	78	NR	GB	67	2	4	Comp	LVF/Septicaemia
060	M	75	T	GB+	60	3	7	Comp	Resp insufficiency/ Recurrent attack
061	F	47	NR	GB/A	9	1	3	Mild	No
062	F	36	T	UK	18	2	4	Comp	Pseudocyst
063	F	36	NR	GB	16*	1	2	Mild	No *cholecyst
064	M	29	NR	ALC	6	0	0	Mild	No
065	F	82	NR	UK+	8	0	1	Mild	No
066	M	54	NR	GB/A	15	5	2	Comp	Resp insufficiency
067	M	33	T	ALC	7	1	1	Mild	No
068	F	52	NR	ALC	4	0	0	Mild	No
069	M	29	NR	ALC	8	0	0	Mild	No
070	F	46	Ex	ALC+	8	2	3	Mild	No
071	F	60	NR	UK	11	2	4	Mild	No
072	F	72	NR	GB	35	2	3	Comp	Abscess
073	M	23	NR	ALC+	5	0	0	Mild	No
074	M	56	NR	ALC	8	1	1	Mild	No
075	M	55	NR	ALC+	6	0	0	Mild	No
076	M	57	T	UK	64	5	9	Died	Necrosis/BWS
077	M	77	NR	UK	10	2	4	Mild	No
078	F	49	NR	ALC	10	0	0	Mild	No
079	M	45	NR	ALC	7	0	1	Mild	No
080	M	34	NR	GB	8	0	0	Mild	No
081	F	29	NR	GB	11*	1	2	Mild	No *cholecyst
082	F	78	NR	UK	10	1	2	Mild	No
083	F	46	C	UK	14	1	5	Mild	Slow settling/ Confusion
084	F	69	C	GB	20	4	5	Comp	Resp insufficiency
085	F	65	NR	GB	16*	0	1	Mild	*Cholecyst/ Ketoacidosis
086	M	55	C	GB	17	1	2	Comp	Septicaemia
087	F	78	C	GB	9	0	2	Mild	No
088	F	83	T	OTH	4	6	8	Died	Hypothermia
089	F	39	NR	GB	7	2	3	Mild	No
090	M	25	NR	GB	5	2	0	Mild	No

091	F	59	C	UK	14	3	4	Mild	Slow settling/ Confusion
092	F	64	NR	GB+	7	0	1	Mild	No
093	M	63	T	UK	17	4	5	Comp	Resp insufficiency/ Late abscess
094	M	85	Ex	UK	5	5	5	Died	?Aneurysm
095	M	73	C	UK	11	4	5	Comp	Septicaemia
096	F	72	NR	UK	8	0	2	Mild	No
097	M	37	NR	ALC	9	2	2	Mild	No
098	M	33	NR	ALC+	7	0	1	Mild	No
099	F	71	NR	GB	12	1	3	Mild	Slow settling
100	M	24	NR	GB+	6	1	1	Mild	No
101	F	77	NR	GB	13	1	5	Mild	No
102	M	29	NR	ALC+	6	0	0	Mild	No
103	M	65	T	GB	10	3	3	Mild	Slow settling
104	M	69	NR	GB	12	1	3	Mild	Slow settling
105	M	63	NR	GB	8	3	4	Mild	No
106	M	56	C	GB	38	6	7	Died	Necrosis/BWS
107	M	79	NR	UK	18	2	5	Comp	Resp insufficiency
108	F	59	NR	UK	14	1	3	Mild	Recurrent attack
109	M	31	T	ALC+	12	2	3	Comp	Pseudocyst
110	F	64	T	GB	7	4	4	Mild	No
111	F	40	NR	GB	9	3	3	Mild	No
112	M	29	Ex	OTH	29	3	5	Comp	Resp insufficiency/ BWS
113	M	72	NR	GB	21*	2	2	Mild	No *social reasons
114	F	29	NR	GB+	21*	1	2	Mild	No *cholecyst
115	M	24	NR	ALC+	8	1	0	Mild	No
116	M	40	NR	UK+	6	0	0	Mild	No
117	F	31	NR	GB	7	1	1	Mild	No
118	M	37	T	ALC	26	2	2	Mild	Slow settling/DTs
119	M	81	NR	GB	15*	1	3	Mild	No *social reasons
120	M	74	NR	GB	10	0	3	Mild	No
121	F	56	NR	GB	5	1	2	Mild	No
122	M	56	NR	UK	9	1	3	Mild	No
123	M	40	NR	UK+	4	0	3	Mild	No
124	F	69	NR	GB	11	3	4	Mild	No
125	F	74	C	GB	10	4	3	Comp	Resp insufficiency
126	M	41	T	UK	8	0	1	Mild	Slow settling
127	M	50	NR	ALC	1	0	0	Mild	Self discharge
128	M	56	NR	ALC+	6	0	1	Mild	No
129	M	49	T	ALC	-	-	-	Died	Bowel infarction
130	M	66	Ex	ALC+	25*	3	2	Mild	No *social reasons
131	M	48	T	ALC	23	5	3	Comp	Pseudocyst
132	M	61	NR	ALC	12	1	1	Mild	No
133	M	40	T	UK	16	2	1	Comp	Pseudocyst
134	F	44	NR	GB	19*	0	0	Mild	No *cholecyst
135	M	21	NR	ALC	8	1	0	Mild	No
136	M	72	Ex	UK+	10	2	4	Mild	Slow settling
137	M	33	C	ALC	11	2	2	Mild	Slow settling
138	F	39	NR	UK	9	1	2	Mild	No

139	F	69	C	GB	20	3	6	Comp	Pseudocyst
140	F	44	NR	ALC	5	0	1	Mild	No
141	M	58	NR	OTH	5	1	1	Mild	No
142	M	58	C	UK	15	4	5	Comp	Pseudocyst
143	F	69	NR	GB	8	1	3	Mild	No
144	F	68	C	GB	13	4	3	Mild	Slow settling
145	F	71	NR	GB	28*	5	7	Mild	No *social reasons
146	M	66	NR	GB	7	2	3	Mild	No
147	M	31	NR	ALC+	5	1	0	Mild	No
148	M	62	NR	ALC+	9	2	2	Mild	No
149	F	80	NR	GB	15*	0	2	Mild	No *social reasons
150	F	50	NR	ALC+	5	0	0	Mild	No
151	M	29	Ex	ALC	2	3	6	Died	Fulminant/Necrosis/ ARF
152	F	83	NR	GB	24*	1	2	Mild	No *social reasons
153	F	15	Ex	UK	10	2	4	Mild	No
154	M	25	NR	GB	7	0	0	Mild	No/Recurrent attack
155	M	56	NR	GB/A	9	2	5	Mild	No
156	M	66	C	GB/A	4	4	6	Died	Fulminant/Necrosis/ DTs
157	M	46	NR	ALC	7	0	1	Mild	No
158	F	20	NR	GB	13*	0	1	Mild	No *cholecyst
159	M	56	NR	ALC	15*	1	1	Mild	No *social reasons
160	M	53	NR	OTH	9	2	2	Mild	No

KEY:

Study group: NR = not randomised (mild), Ex = exclusion,
T = intraperitoneal aprotinin therapy, C = standard therapy.

Aetiology (Aet): ALC = alcohol, GB = gallstones, OTH = other,
UK = unknown, GB/A = gallstones & alcohol, + = recurrent attack.

Prognostic factor scores: Glas = Glasgow scoring system,
Ranson = Ranson scoring system. (scores $\geqslant 3$ = severe attack predicted).

Complications: ARF = acute renal failure, BWS = body wall staining,
collection = small peripancreatic fluid collection on ultrasound,
DIC= disseminated intravascular coagulation, DTs = delirium tremens,
fulminant = fulminant acute pancreatitis, lap diagnosis = laparotomy
diagnosis, LVF = left ventricular failure, necrosis = pancreatic
necrosis, recurrent attack = recurrent attack during same admission,
resp insufficiency = acute respiratory insufficiency.

*cholecyst/ERCP/social reasons = discharge delayed by elective early
cholecystectomy/ERCP/"social reasons".

APPENDIX 2. RESULTS OF BIOCHEMICAL STUDIES OF PANCREATITIS EXUDATES

Patient	TBC	a_2M	a_1AP	TP	Alb	Amylase	Lipase	Tryptic amidase
Fatal pancreatitis:								
Early <7days								
JK	20	0.6	0.8	27	19	6890	16690	NA
MM	10	0.2	1.4	37	25	56900	35500	0.018
TG	80	0.5	1.4	36	23	13330	15900	NA
SK	30	0.6	1.4	50		24000	38390	0.324
Late >7days								
BE	100	0.6	2.2	41	27	13030	8930	0.031
JS	120	0.7	3.1	38	21	132400	38940	NA
AP	80	0.5	1.4	34	25	24140	20780	NA
MR	60	0.6	1.1	35	22	35600	30430	0.191
TR	60	0.4	1.8	41		37400	40860	NA
WC	60	0.4	1.6	45		46560	35810	NA
Complicated pancreatitis:								
MW	40	0.8	1.4	41	28	58700	37219	0.482
JF	40	0.8	1.6	47	32	38000		0.496
DMcT	100	0.9	1.9	43		30000	30960	0.190
JR	60	0.8	0.9	52		14700	38560	0.600
JC	100	0.6	2.0	30		14380	15370	0.029
WM	100	0.4	2.2	43		5660	4510	NA
JMcG	60	1.0	1.7	54		22000	46750	0.377
PM		0.4	2.9	33	21		10470	0.046
Uncomplicated pancreatitis:								
MW	40	0.7	1.4	49	33		31248	0.180
JG	80	0.7	1.4	45		30300	33610	0.150
GS	100	0.6	2.1	51	31	59000	38870	0.387

KEY:

No free proteolytic activity found on fibrin plate assay.

TBC = trypsin binding capacity (µg trypsin inhibited/100µl), a_2M = alpha$_2$macroglobulin (g/l), a_1AP = alpha$_1$antiprotease (g/l), TP = total protein (g/l), Alb = albumin (g/l), Tryptic amidase = BAPNA splitting activity (change in absorbance units A_{405nm}/hour), FPA = free proteolytic activity (mean lysis area on fibrin plate assay mm^2.), NA = not assayable (due to haemorrhagic coloration of fluid sample).

APPENDIX 3. RESULTS OF BIOCHEMICAL STUDIES OF OTHER EXUDATES AND PSEUDOCYST FLUID

Patient	TBC	a_2M	a_1AP	TP	Alb	Amylase	Tryptic amidase	FPA
Perforated ulcers:								
MMcK	10	1.2	1.0	23	15	6000	0.072	0
WW	10	0.6	1.0	25	13	13410	0.442	0
AB	80	0.9	1.6	47	30	213	0	0
WK	50	1.4	1.3	49	30	1935	0	0
DC	40	0.8	1.6	52	32	741	0.211	0
WMcG	50	0.9	1.3	43	27	19020	0.193	0
JL	10	0.5	1.4	48	27	1077	0.011	0
Intestinal obstruction:								
EB	100	0.2	1.5	43	24	30	0.027	0
JG	110	0.6	1.8	50	31	45	0	0
FB	120	0.6	1.9	41	22	126	0	0
GD	120	0.6	2.0	45	26	51	0	0
AH	100	0.5	1.7	45	26	204	0.015	0
JF	120	1.0	1.7	44	26	159	0	0
JD	160	0.4	2.5	42	23	33	0	0
JR	110	1.5	2.2	41	25	27	0	0
Mesenteric infarction:								
JC	0	1.0	1.6	50	14	352000	NA	1590
Pseudocysts:								
PC	20	0.4	1.2	20	11	460000	0.077	0
AF	0	<0.2	0.9	11	5	99300	0.520	254
ER	<10	<0.2	0.8	5	4	54000	0.390	64
GP	10	<0.2	0.8	15	8	13100	0.628	0
JR	<10	<0.2	0.2	5	3	147000	0	0
EB	<10	<0.2	2.0	17	12	1994	0.124	380
MMcN	<10	<0.2	<0.2	3	1	231360	0	64
TB	<10	0.6	2.7	33	9	58810	2.792	Trace
BC	10	0.7	2.4	41	25	5370	0.630	0
MM	60	0.7	2.7	59	33	20450	NA	0
AL	<10	<0.2	0.8	6	1	676000	0	0
TI	<10	<0.2	<0.2	1	<1	75540	0	113

KEY: as for appendix 2.

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