FRESH FROZEN PLASMA THERAPY IN ACUTE PANCREATITIS

BY

TREVOR LEESE

MA(Cantab), MB,BS(London), FRCS

A Thesis Submitted for the Degree of Doctor of Medicine

from

The Department of Surgery, The University of Leicester

June 1987

UMI Number: U010406

UMI

Dissertation Publishing

ProQuest

The material which this dissertation is based

is my own independent work except where

acknowledged.

<div style="text-align: right">

Trevor Leese

May 1987

</div>

To Brigitte and the boys

"That sweetbread gazing up at me

Is not what it purports to be...

Since it is neither sweet nor bread,

I think I'll take a bun instead."

Ode to Sweetbread by Ogden Nash, 1953

CONTENTS

SUMMARY

Despite improvements in general supportative measures, the mortality from acute pancreatitis in the United Kingdom still approaches 10%. Although many specific therapies have been proposed, none has consistently been shown to improve outcome in controlled clinical trials.

Intravenous fresh frozen plasma has been advocated as a therapy for acute pancreatitis. It may supplement declining natural antiprotease capacity, particularly $alpha_2$ macroglobulin which appears to have a central role in the elimination of disseminated pancreatic enzymes in man.

Initially, the effect of fresh frozen plasma therapy on survival in an experimental model of acute pancreatitis was studied. A rat model of acute pancreatitis was established including the facility for continuous intravenous infusion of fluid. Intravenous therapy including fresh frozen plasma significantly improved survival at 72 hours in the model when compared with crystalloid ($p < 0.001$) and colloid ($p < 0.01$) controls.

Subsequently a controlled clinical trial was performed involving five hospitals over a twenty three month period. Two hundred and two patients admitted with acute pancreatitis were randomised to receive either low volume fresh frozen plasma therapy (two units daily for three days) or colloid control. The major serum antiproteases were measured during the course of the disease.

There were no significant differences between the two groups of patients in terms of clinical outcome. Serum $alpha_2$ macroglobulin levels were reduced in both groups on day one and

continued to fall significantly from day one to day three in the colloid control group (p<0.005) whilst remaining substantially unaltered in patients receiving fresh frozen plasma (p = 0.6527).

The ability of relatively low volumes of fresh frozen plasma to supplement declining alpha$_2$ macroglobulin levels in the early stages of acute pancreatitis has therapeutic implications. The administration of larger volumes of fresh frozen plasma or alpha$_2$ macroglobulin concentrates may improve clinical outcome.

CHAPTER ONE

ACUTE PANCREATITIS

1.1 Classification of Pancreatitis

At the crucial international meeting of pancreatic specialists in Marseilles in 1963 (Sarles H, 1965), pancreatitis was separated into four main categories:

"1. Acute pancreatitis defined as a single attack of pancreatitis resulting in a variable outcome

2. Relapsing acute pancreatitis defined as a condition in which recurring attacks produced clinical and biological restitution of the gland

3. Chronic relapsing pancreatitis defined as a condition in which acute exacerbations occurred against a background of chronic disease

4. Chronic pancreatitis defined as a condition in which anatomical and/or functional damage occurred to the gland"

This classification had a major influence on the study of pancreatitis in subsequent years, but a number of difficulties have persisted with the system. The Marseilles categories of relapsing acute and chronic relapsing pancreatitis are difficult to define with precision, and a single attack of acute pancreatitis may leave functional and/or morphological alterations, yet a patient with silent underlying disease may present with an acute attack. At the 1983 international conference in Cambridge, England, the following new definitions were proposed:

"1. Acute Pancreatitis is an acute condition typically presenting with abdominal pain, and usually associated with raised pancreatic enzymes in the blood or urine due to inflammatory disease of the pancreas

2. Chronic Pancreatitis is a continuing inflammatory disease

of the pancreas characterised by irreversible morphological

loss of function"

It was emphasised that acute pancreatitis may recur, and that

patients with chronic pancreatitis may have acute exacerbations

but the condition may be completely painless (Sarner and Cotton,

1984).

The Marseilles classification was revised in 1984 also

omitting the terms relapsing acute pancreatitis and chronic

relapsing pancreatitis (Sarles, 1985). They suggested the

following definitions:

"Acute Pancreatitis

Clinically acute pancreatitis is characterised by acute

abdominal pain accompanied by increased pancreatic enzymes in

blood and/or urine. Although it usually runs a benign course,

severe attacks may lead to shock with renal and pulmonary

insufficiency which may prove fatal. Acute pancreatitis may be a

single episode or recur.

Morphologically there is a gradation of lesions in acute

pancreatitis. In the mild form peripancreatic fat necrosis and

interstitial oedema can be recognised but, as a rule, pancreatic

necrosis is absent. The mild form may develop into the severe

form with extensive peri- and intrapancreatic fat necrosis,

parenchymal necrosis and haemorrhage. The lesions may be either

localised or diffuse. Occasionally, there may be little

correlation between the severity of the clinical features and the

morphological findings.

Both exocrine and endocrine functions of the pancreas are

impaired to a variable extent for a variable duration.

If the primary cause and complications such as pseudocysts are eliminated in acute pancreatitis, clinical, morphological and functional restitution normally occurs. In some cases scarring and pseudocysts persist. Only rarely does acute pancreatitis lead to chronic pancreatitis.

Chronic Pancreatitis

Clinically chronic pancreatitis is characterised by recurrent or persisting abdominal pain, although chronic pancreatitis may present without pain. Evidence of pancreatic insufficiency, e.g. steatorrhoea or diabetes, may be present.

Morphologically, chronic pancreatitis is characterised by an irregular sclerosis with destruction and permanent loss of exocrine parenchyma which may be either focal, segmental or diffuse. These changes may be associated with varying degrees of dilatation of segments of the duct system. Thus, dilatation of the duct of Wirsung and of its small ducts may occur together or independently. No obvious cause for the duct dilatation may be found, but most often it is associated with strictures of the ducts or intraductal protein plugs and calculi (calcification). All types of inflammatory cells may be present in varying degrees as well as oedema and focal necrosis. Cysts and pseudocysts, with or without infection, which may or may not communicate with ducts, are not uncommon. Compared with the degree of acinar destruction, the islets of Langerhans are relatively well preserved. Based on the predominating structural features, the following descriptive terms can be used: chronic pancreatitis with focal necrosis; chronic pancreatitis with segmental or diffuse fibrosis; and chronic pancreatitis with or without calculi.

A distinctive form of chronic pancreatitis is obstructive chronic pancreatitis. It is characterised by dilatation of the ductal system proximal to the occlusion of one of the major ducts (e.g. by tumour or scars), diffuse atrophy of the acinar parenchyma, and uniform diffuse fibrosis. Calculi are uncommon.

In chronic pancreatitis (with the exception of obstructive chronic pancreatitis), the irreversible morphological changes in the pancreas may lead to a progressive or permanent loss of exocrine and endocrine pancreatic function. In obstructive chronic pancreatitis both structural and functional changes tend to improve when the obstruction is removed."

1.2 Acute Pancreatitis - Historical

Senn in 1886 said that "acute ideopathic pancreatitis is an exceedingly rare affection and found only in a few well-authenticated cases from the literature". The earliest report was by Mayo in 1836 which, like all the earliest cases, was a post mortem finding. The patient was a pregnant 21 year old who was noted to have a pancreas that was "throughout of a deep and dull red colour". Classen(1842) described cases collected from the literature considered to be acute inflammation of the pancreas though Fitz (1889) doubted the diagnosis in some of these earliest cases. He summarised the state of knowledge at that time and attempted to define the relationship between pancreatic haemorrhage and pancreatitis in terms of both the gross and microscopic appearance. He described the characteristic clinical features and pathologically recognised three types of pancreatitis: suppurative, haemorrhagic and gangrenous.

Contributions by Klebs (1870), Rokitansky (1861) and Friedreich (1878) were prominent in the early literature. Koerte's

monograph (1898) reported several cases where, at laparotomy for abdominal pain, oedema of the pancreas was the only abnormality found. Opie (1910a) discussed and cited more cases of acute interstitial inflammation and in 1919 Mercade described an apparently definite incidence of acute oedematous pancreatitis. Zoepffel (1922) added detailed accounts of four further cases. It was only after Elman's thoroughly researched and well argued contribution in 1933 that the oedematous form of pancreatitis was fully recognised.

Only relatively recently has the concept of oedematous pancreatitis progressing occasionally to haemorrhagic pancreatitis, possibly leading to necrosis and suppuration, been widely accepted (Howard, 1960). This was despite the case report by Quick (1932) of a patient who at laparotomy had an oedematous gland and was later found to have acute necrotising pancreatitis at post mortem examination.

1.3 Aetiology

Gallstones were noted to be present in the early post mortem reports of acute pancreatitis. Koerte (1898) mentions that diseases of the bile passages, especially cholelithiasis, are frequently associated with pancreatic lesions. Opie's attention was directed towards the relationship between acute pancreatitis and gallstones by a case of suppurative pancreatitis with a stone impacted at the ampulla of Vater at post mortem and multiple similar stones in the gallbladder (Opie, 1901a). Since Opie's further contributions (1901b, 1903,1904,1909) there has been an undisputed association between gallstones and acute pancreatitis, though the pathogenic mechanism he proposed remains controversial

to this day.

In the United Kingdom the incidence of gallstones in collected series of acute pancreatitis varies between 34 and 57% whereas in most North American centres a much lower incidence is reported because of the higher proportion of alcohol associated disease (Table 1).

The importance of alcohol in precipitating attacks of acute pancreatitis was also appreciated by many early writers. Fitz (1889) noted that a small fraction of his patients were addicted to the abuse of alcohol. Halsted (1901) suggested that excessive alcohol use was a factor associated with pancreatitis and Lando (1906) and Poggenpohl (1909) also recognised the relationship. Egdahl (1907) found that 16% of 105 patients with acute pancreatitis were alcoholic, Rich and Duff (1936) 29% and Myers and Keefer (1934) 21%. Table 1 shows the incidence of alcoholism in patients presenting with acute pancreatitis in the UK and USA since 1970. Alcoholic pancreatitis is common in USA and France and though there has been a recent increase in Scotland (Blamey et al 1984) there has been a relatively small rise in incidence in England.

Trauma can lead to acute pancreatitis. This may be a blunt or penetrating injury. In this country blunt injury is the more common - particularly the fracturing of the pancreas across the vertebral bodies seen following road traffic accidents as a steering wheel or seat-belt injury. Post-operative trauma, however, is a more common cause (Trapnell, 1966). McCutcheon and Race reviewed post operative acute pancreatitis in 1962 and found it occurred particularly after gastric surgery, especially Billroth II (polya) partial gastrectomy. It can follow any

Table 1

Incidence of associated biliary tract disease and alcoholism in series of acute pancreatitis since 1970

	No. of cases	% of biliary disease	% alcoholic
UK			
Gillespie 1973	93	34	22
Bourke 1975	202	49	6
Imrie and Whyte 1975	78	51	25
Trapnell and Duncan 1975	590	54	5
Read et al. 1976	47	57	17
MRC Multicentre Trial 1977	257	55	9
Imrie et al. 1978a	161	52	31
De Bolia and Obeid 1984	897	57	21
Blamey et al. 1984	405	44	33
Total	2730	53	18
USA			
Jordan and Spjut 1972	51	16	44
Olsen 1974	100	11	78
Howes et al. 1975	95	5	91
Ranson et al. 1976	300	17	69
Kelly 1976	519	46	31
Jacobs et al. 1977	125	45	32
Total	1190	31	50

manipulation of the ampulla of Vater, but is also seen after surgery remote from the pancreas. Post-operative pancreatitis has a tragically high mortality (25-40%) particularly when it follows choledochal and pancreatic duct manipulation and instrumentation. Acute pancreatitis is seen following endoscopic sphincterotomy in about 5% of cases (Leese et al. 1985) but is usually only mild.

Infection as an aetiological factor intermittently receives attention. Elevated mycoplasma antibodies have been found following otherwise unexplained acute pancreatitis (Schmid and Blaich, 1976; McMahon and Freeman, 1979). Coxsackie and mumps virus infections (Imrie et al. 1977; Gladisch et al. 1976) typhoid (Borisova et al. 1977) infectious mononucleosis (Hedstrom and Belfrage, 1976) as well as Lambliasis and other helminth parasites (Koszarska et al. 1977; Das 1977) have all been proposed as causes of pancreatitis.

Hyperlipidaemia is commonly associated with acute pancreatitis and may predispose to its development. In series where alcoholism is common, hyperlipidaemia is found in as many as 37.5% of patients with acute pancreatitis (Cameron et al. 1974) and the reported incidence of hyperlipidaemia in acute pancreatitis reflects the percentage of alcoholic pancreatitis in the series (Table 2). Alcohol abuse causes hyperlipidaemia (Stanbury et al. 1976) and most cases of hyperlipidaemia associated with acute pancreatitis are not primary in origin but secondary to alcohol abuse. Whether the episodes of pancreatitis are caused directly by alcohol or by the lipid abnormality is unclear and it may be that the link is only the common aetiology. Those who continue to ingest alcohol continue to have raised serum lipids, but those who abstain show improvement of their

lipid abnormalities (Dickson et al. 1984). Some patients
exhibiting Fredrickson type I,IV or V hyperlipidaemia can develop
recurrent attacks of central and upper abdominal pain, often
associated with raised serum amylase (Stanbury et al. 1976). A
proportion of patients who sustain an attack of acute
pancreatitis whilst taking oestrogens also have Fredickson type
IV or V hyperlipidaemia. Serum amylase estimations in those
patients are often only moderately elevated or even normal,
because hyperlipidaemia interferes with the amylase assay
(Cameron et al. 1973). Serial dilutions of serum samples may
reveal a diagnostically high amylase, though this may not be
reliable and measurement of urinary amylase is probably more
accurate (Dickson et al. 1984).

Table 2

The association between alcoholism and hyperlipidaemia in
patients with acute pancreatitis

	No. of patients	% alcohol aetiology	% hyperlipidaemia
Cameron et al. 1974	48	80	37.5
Greenberger et al. 1966	25	48	12.0
Buch et al. 1980	117	75	32.5
Dickson et al. 1984	311	34	4.5

The first reported case of an association between hyperparathyroidism and acute pancreatitis was by Erdheim in 1903 when he described a patient with a necrotic pancreas and a parathyroid adenoma. By 1962 a review of the literature collected 62 patients in whom the conditions were associated (Mixter et al. 1962). Hyperparathyroidism is often quoted as an aetiological factor in acute pancreatitis (Pyrah et al. 1966) but the incidence of acute pancreatitis in patients with hyperparathyroidism is probably well under one percent and certainly much lower than the 5-6% reported in some series (Bess et al. 1980; Shearer and Imrie, 1986). Rarely acute necrotizing pancreatitis may supervene within 12 hours of parathyroid surgery. Acute pancreatitis has been associated with hypercalcaemia due to causes other than hyperparathyroidism.

Most cases of acute pancreatitis following renal transplantation occur some six months or more after surgery. The incidence is two to seven percent and the mortality is high, reported at 20-70 percent. There are several predisposing factors in these patients including immunosuppressive therapy, viral infection, secondary and tertiary hyperparathyroidism, hyperlipidaemia and vasculitis.

Drug induced acute pancreatitis was recently the subject of comprehensive critical reviews (Mallory and Kern, 1980; Lendrum, 1981). These concluded that the following drugs can cause pancreatitis : Azathioprine, thiazides, sulphonamides, frusemide, oestrogens and tetracycline. There was less convincing but suggestive evidence for L-asparagine, chlorthalidine, corticosteroids, ethacrynic acid, phenformin and procainamide. It was also concluded that there was insufficient evidence to

implicate any other drug, although many have been proposed.

Although pregnancy has been suggested as a cause of pancreatitis in the past (Trapnell and Duncan, 1975) it has been shown that nearly all cases are due to gallstones (McKay et al. 1980). Hyperlipidaemia and high oestrogen levels may account for others.

Many other factors are well documented in their association with acute pancreatitis including severe hypotension (Banks, 1979), Caroli's Disease (Sahel et al. 1976) and anatomical anomalies such as pancreas divisum (Cotton, 1980) and juxtampullary bile-filled duodenal duplication cysts (Jones et al. 1982a). A list of conditions associated with acute pancreatitis is given in Table 3. Investigation of patients who have sustained an attack of acute pancreatitis must be thorough in order to deal with the underlying disorder and prevent further attacks.The proportion of patients ascribed to the ideopathic category is artificially high in many series (up to 40%) because of inadequate follow-up investigations. No patient should be placed in the unknown group until they have had at least ERCP, an investigation which can reveal gallstones missed by ultrasonography and oral cholecystography, and which can reveal anatomical lesions such as pancreas divisum and stenosis of the minor papilla.

In children, trauma is the most common cause of acute pancreatitis. The injury is often relatively mild - falling on a toy or poking a bicycle handlebar in the epigastrium. Often initial symptoms are mild resulting in a delay in diagnosis. The pancreas has usually been vertically transected over the vertebral column and the injury is often isolated unless caused by a road traffic accident. Rarer causes of childhood acute

Table 3

Reported Aetiologies of Acute Pancreatitis

1. Gallstones
2. Alcohol Abuse   }   Account for 90-98% in
3. Idiopathic   most series

4. Trauma   - Surgical   - Pancreaticobiliary
                                   Upper abdominal
                                   Endoscopic sphincterotomy
                - Non-iatrogenic - Blunt abdominal injury
                                   Penetrating injury
                - Investigative - ERCP
                                   Aortography

5. Periampullary
   Obstruction   - Ampullary tumour
                  Pancreas divisum
                  Annular pancreas
                  Ampullary stenosis
                  Choledochal cyst
                  Duodenal diverticulum
                  Duodenal duplication
                  Malrotation of gut

6. Infections   - Mumps
                  Coxsackie B virus
                  Echovirus
                  Congenital rubella
                  Hepatitis A virus
                  Epstein-Barr virus
                  Ascaris lumbricoides
                  Clonorchis sinensis
                  Mycoplasma pneumoniae
                  Leptospirosis
                  Typhoid
                  Malaria
                  Lambliasis

7. Metabolic
   Disorders   - Hyperlipidaemia
                  Hypercalcaemia
                  Protein-calorie malnutrition
                  Diabetic ketoacidosis
                  Reye's Syndrome

8. Ischaemia   - Circulatory shock
                  Cardiac surgery
                  Vasculitis
                  Hypothermia
                  Malignant hypertension
                  Emboli
                  Dissecting aortic aneurysm

9. Drugs   - Definite association - Azathioprine
                                            Thiazides
                                            Sulphonamides
                                            Frusemide
                                            Oestrogens
                                            Tetracycline

Possible association - Corticosteroids
                                            L-asparagine
                                            Chlorthalidine
                                            Phenformin
                                            Ethacrynic acid
                                            Procainamide

10. Neoplasms   - Primary pancreatitis
                    Secondary pancreatic
                    Lymphoma

11. Hereditary
    Diseases   - Hereditary pancreatitis
                    Cystic fibrosis
                    Alpha$_1$ antiprotease deficiency

12. Miscellaneous Causes - Post-renal transplantation
                              Afferent loop obstruction
                              Transurethral resection of prostate
                              Large meal following fasting
                              Pregnancy
                              Thrombotic thrombocytopenic purpura
                              Acute intermittent porphyria
                              Tropical pancreatitis
                              Henoch-Schonlein purpura
                              Systemic lupus erythematosus
                              Scorpion bite
                              Emotional stress
                              Occupational exposure to hydrocarbons

pancreatitis include mumps, steroids, hyperlipidaemia and
familial pancreatitis (Ravitch, 1975).

1.4 Pathogenesis

Chiari (1896) was the first to suggest that the fundamental
pathogenetic principle of acute pancreatitis was autodigestion of
the organ, and this has been widely accepted ever since. Trypsin has
been assumed to occupy a dominant role in this process (Elliot,
1957; Haverback et al. 1969). The activation of trypsinogen and the
subsequent initiation of the cascade of activation of the other
dangerous zymogens, pro-carboxypeptidases, proelastase and
phospholipase A_2, can be brought about by trypsin, by enzymes of the
blood clotting or fibrinolytic systems and by certain lysosomal
enzymes notably cethepsin B (Greenbaum et al. 1959; Geokas and
Rinderknecht, 1974; Neurath and Walsh, 1976). Once released within
the gland in active form they rapidly destroy the surrounding
supporting structure, plasma membranes of acinar cells
and subcellular components, and, by releasing further digestive
enzymes, add to the developing fire (Hermon-Taylor, 1977).

Under normal circumstances enterokinase initiates activation of
trypsinogen into trypsin and pancreatitis can be induced by
intraductal infusion of commercially available preparations of
enterokinase (Mann 1980; Mann et al. 1977; Mann and Mann, 1979).
Retrograde pancreatic injections of trypsin (Beck et al. 1964;
Schmidt and Creutzfeldt, 1969) and Chymotrypsin (Anderson et al.
1969) can also induce pancreatitis experimentally.

The role of elastase and other pancreatic enzymes has been
studied by Geokas (1968; 1972) and Geokas and Rinderknecht (1974).
Phospholipase A_2 may be important in the pathogenesis of acute

pancreatitis because its reaction products, which include lysolecithin and lysocephalin, are potent cytotoxic substances (Schmidt and Creutzfeldt, 1969). Lecithin and cephalin are major constituents of the lipid membrane of acinar and other cell walls. Anderson et al. (1969) reported producing experimental acute pancreatitis with lipase though Creutzfeldt and Schmidt (1970) subsequently questioned the purity of their enzyme preparation.

Undoubtedly many pancreatic enzymes contribute to the local and metastatic damage seen in acute pancreatitis but other mediators of shock have been proposed (Colman and Wong, 1979) including prostaglandins (Glazer and Bennett, 1976), histamines (Schult et al. 1979) and components of the complement system.

Pancreatic enzymes are capable of producing kinins from substrate kininogens (Orlov and Belyakov, 1978). Activation of the kallikrein-kinin system may result in the increased capillary permeability and pain which are features of acute pancreatitis (Hiltunen et al. 1985). The kallikrein-kinin system is thought to participate in the pathogenesis of acute pancreatitis (Ofstad, 1970). Two major classes of kininogens have been identified in plasma and are known as low molecular weight and high molecular weight kininogen. The proteolytic enzyme kallikrein, which exists in plasma as an inactive precursor (prekallikrein), has the property of splitting off biologically active polypeptide fragments preferentially from the high molecular weight kininogen, while glandular kallikrein, present for example in the pancreas, preferentially cleaves low molecular weight kininogen. The fragments are known generically as kinins and include the nonapeptide bradykinin. Normally bradykinin and other kinins are rapidly inactivated by peptidases commonly called kininases.

Intravenous administration of bovine trypsin to the dog causes a depletion of kininogen in plasma, the release of bradykinin into the circulation, and a fall in blood pressure (Corrado et al. 1966). The trypsin induced bradykinin release may be explained by a direct attack on the kininogen molecule, via activation of prekallikrein, or indirect activation of prekallikrein via factor XII.

Bradykinin causes vasodilatation, increased vascular permeability, invasion of leucocytes and pain. Local kinin release appears to predominate over systemic kinin activity in acute pancreatitis (Amundsen, 1967; Propieraitis and Thompson, 1979). Kininogen concentration in plasma is lowered in experimental haemorrhagic pancreatitis and in the peritoneal exudate an intense kinin formation is accompanied by a high rate of kininogen consumption (Ofstad, 1970).

The serum complement system may be recruited in acute pancreatitis (Goldstein et al. 1978; Seelig et al. 1977; Smith et al. 1977). Components of the complement system are potential substrates for some active proteases and may be a source of biologically active peptides capable of provoking pathological changes in acute pancreatitis. The activated complement system may produce the initial membrane lesions seen in acute pancreatitis (Goldstein et al. 1978; Seelig et al. 1975; Smith et al. 1977). Trypsin can convert C_3 to C_{3a} and C_5 to C_{5a}. C_{3a} and C_{5a} are biologically active fragments which are leucotactic, provoke histamine release from mast cells, contract smooth muscle and cause increased capillary permeability. Serum C_3 levels fall in acute pancreatitis while serum C_{3a} levels rise (Balldin et al. 1981).

The coagulation and fibrinolytic systems are activated during acute pancreatitis which can induce disseminated intravascular coagulation (Simon and Giacobino, 1970).

Hypotension is one of the most characteristic features of severe acute pancreatitis. Sequestration of fluid resulting in hypovolaemia and release of hypotensive factors into the circulation are key factors in the pathogenesis of shock. Many of these hypotensive agents may be formed within the pancreatic broth prior to absorption into the bloodstream, but the presence of detectable protease activity in plasma suggests that some may be liberated within the circulation.

Despite the acceptance of autodigestion, there has been much debate as to the mechanisms triggering the inappropriate activation of pro-enzymes leading to acute pancreatitis. The literature on these possible mechanisms is vast and evidence often conflicting. A review of the most popular postulated mechanisms follows.

a) Bile Reflux

Opie described the finding at autopsy of a stone impacted in the ampulla of Vater in association with acute pancreatitis (Opie, 1901a). Subsequently he reported other cases and proposed the common-channel theory (Opie, 1901b; 1904). He postulated that the presence of a common channel for bile and pancreatic juice allowed reflux of bile into the pancreatic duct system if the ampulla of Vater should be obstructed by a gallstone. The common channel theory was later modified suggesting that the presence of a common channel allowed pancreatic juice to pass into the common bile duct where it was activated by bile and the bile-enzyme mixture then refluxed back into the pancreatic ducts initiating acute

pancreatitis (Elliot et al. 1957).

To support his theory Opie studied the anatomical arrangement
in 100 autopsies and found a common channel in 89 (Opie, 1903) and
many subsequent studies, autopsy, perfusion and vinyl cast, reported
a high incidence of the common channel required to support Opie's
theory (Table 4).

Table 4

<div align="center">The Incidence of Common Channel</div>

Author	Cases	Present
Opie (1903)	100	89
Mann and Giordano (1923)	299	65
Cameron and Nobel (1924)	100	74
Dardinsky (1935)	100	47
Chodkowski (1936)	322	80
Mehen (1938)	449	61
Reinhoff and Pickrell (1945)	250	69
Howard and Jones (1947)	150	73
Millbourn (1950)	200	85
Berman et al. (1960)	130	89
Mester et al. (1963)	100	90

Cameron and Noble (1924) and Howard and Jones (1947)
demonstrated reflux in 66% and 54% of cases respectively where
they placed a stone in the ampulla at autopsy and then injected
dye into the common bile duct. However, until recently, stones in
the common bile duct were found in only small numbers of patients
with acute pancreatitis. Thus in 1,278 cases collected by

Schmieden and Sebening (1927) a stone in the common channel was found in a mere 4.4%. Dragstedt et al. (1934) suggested the stone may have passed into the duodenum or been dislodged back into the common bile duct at operation or autopsy. Good evidence that this does occur was provided by Acosta and Ledesma in 1974 when they found that 34 of 36 patients with gallstone associated pancreatitis passed a stone per rectum within 10 days and Kelly (1976) reported a similar finding. Recent publications suggest that if patients with gallstone associated pancreatitis are subjected to early laparotomy within 72 hours of presentation, then 63-72% will have stones within the common bile duct (Acosta et al. 1980; Kelly, 1980). Early endoscopic retrograde cholangiopancreatography in gallstone acute pancreatitis confirms a high incidence of common bile duct stones (50%, Neoptolemos et al. 1986a). In contrast, the proportion of patients found to have choledocholithiasis after 72 hours is smaller, varying from 3% to 33% and remaining relatively constant from the end of the first week of the attack until several months later (Kelly, 1980; Dixon and Hillam, 1970; Acosta et al. 1980; Stone et al. 1981; Ranson, 1979; Paloyan et al. 1975). Current evidence indicates, therefore, that a major factor in initiating an attack of gallstone pancreatitis is obstruction of the confluence of the lower common bile duct and pancreatic duct by a stone or oedema of the ampulla of Vater secondary to stone migration.

Causes of obstruction creating a common channel other than stones have been postulated and include spasm of the sphincter of Oddi (Archibald, 1919; Ivy, 1947; Doubilet and Mulholland, 1948) and acute oedema of the duodenal mucosa (Balo and Ballon, 1929; Dreiling et al, 1952). Oedema and spasm have been suggested as the mechanism

whereby alcohol precipitates attacks of acute pancreatitis (The, 1978).

If a common channel does allow bile reflux then trypsinogen may be activated by constituents of bile, the most strongly implicated of which are dihydroxy bile salts, endotoxins, oxygen derived free radicles (Sanfey et al. 1985; Parks et al. 1983) and lipid peroxides produced by aberrant hepatic mixed function oxidase activity.

Against the common channel theory is that the actual incidence of common channel is probably only 50% and direct bile reflux is probably impossible in the remainder of individuals. Acute pancreatitis remains uncommon despite the frequency of common channel and has a limited age incidence despite the channel being present throughout life. Pressure in the pancreatic ductal system is higher than that in the common bile duct and there is no good evidence for reversal of flow if biliary pressure is artificially increased (Anderson et al. 1960; Elmslie et al. 1966). If a gallstone initiates reflux then its size would need to be critical to impact whilst leaving room for reflux. Biliary reflux is often seen at T-tube and per-operative cholangiography and appears harmless (Hicken and McAllister, 1952) and, indeed, bile can be diverted through the pancreatic ductal system without causing acute pancreatitis providing physiological pressures are not exceeded (Thal et al. 1957; White and Magee, 1960).

b) Obstruction, Hypersecretion

Many authors considered acute pancreatitis to result from obstruction of the pancreatic duct in the presence of an actively secreting gland (Lium and Maddock, 1948; Nardi, 1963). The evidence

against this is that organic obstruction of the pancreatic duct is
not present in the majority of cases of acute pancreatitis (Longmire
and Wallner 1956; McCutcheon, 1962; Shader and Paxon, 1966). Also
experimental obstruction of the main pancreatic duct in animals
produces atrophy of acini without pancreatitis (Wang et al. 1951;
Radakovich et al. 1952; Isaksson et al. 1983) and even with
stimulated secretion produces only pancreatic oedema and not
necrotising pancreatitis (Creutzfeldt and Schmidt, 1970). Neoplasms
of the pancreas obstructing the main duct are rarely associated
with acute pancreatitis (Brunschwig, 1949).

c) Duodenal Reflux

Williams and Busch (1907) thought that the passage of a
gallstone might dilate the ampulla allowing reflux of intestinal
contents into the pancreatic duct system initiating pancreatitis.
Archibald (1919) reported experiments in which he found it
impossible to force free fluid from the duodenum into the common
bile duct or pancreatic ducts despite a sustained high duodenal
pressure, but in 1935 Perman described four cases of acute
pancreatitis occurring post-operatively where there was obstruction
to an afferent loop and he postulated that duodenal contents were
forced into the pancreatic duct.In 1958 Wallensten reported twelve
deaths from post operative acute pancreatitis in 1,769 Polya
gastrectomies and none in 605 Billroth I gastrectomies. McCutcheon
revived the theory in 1962 and provided support from the result of
experiment in dogs by creating Pfeffer loops. He proved duodenal
reflux occurred and that the resulting pancreatitis was prevented by
ligating the pancreatic duct. It is conceivable that a small
pressure change in the duodenum allows reflux into the pancreatic

duct system if the papilla has been damaged or made incompetent by sphincterotomy, the long arm of a T-tube or the passage of a gallstone (McCutcheon and Race, 1962) and mucosal oedema in alcoholics may permit reflux (McCutcheon, 1968). Acute pancreatitis is seen only occasionally after surgical or endoscopic destruction of the sphincter of Oddi.

d) Others

Direct or vascular injury seems to be responsible for traumatic pancreatitis. Viral disease may initiate pancreatitis through direct cell injury or by some pancreotropic effect (Creutzfeldt and Schmidt, 1970). Acute fatty infiltration of the acinar cells may account for the pancreatitis associated with hyperlipidaemia (Braunsteiner et al. 1967). The pancreatitis associated with hyperparathyroidism and hypercalcaemia may be due to the calcium-dependent convertion of trypsinogen to trypsin (Goebell and Hotz, 1976), however, few patients with hypercalcaemia not associated with hyperparathyroidism develop pancreatitis (Banks, 1979) and in most reports other predisposing factors have been implicated (Leeson and Fourman, 1966; Meltzer et al. 1962). Two patients have been described who developed acute pancreatitis following excessive intravenous calcium replacement resulting in hypercalcaemia (Manson, 1974; Hochgelerent and David, 1974). Some cases of pancreatitis may follow a vasculitis or hypersensitivity reaction. The role of bacteria is not clear. Coliforms have been found in acute pancreatitis and pancreatic abscesses, but infection is usually thought to be a secondary event. Some authors, however, suspect a more important primary role for bacteria in the pathogenesis of acute pancreatitis (Keynes, 1981). Direct pancreatic duct infusion

with bacterial suspensions can produce acute pancreatitis, and introduction of antibiotics into Pfeffer loops can reduce the incidence of pancreatitis (Williams and Byrne, 1968). Some cases of 'idiopathic' pancreatitis may be due to exposure to occupational chemicals (Braganza et al. 1986) or to emotional stress leading to a low flow state mediated through neurohumoral mechanisms with ischaemia of the pancreatic microcirculation (Kaplan, 1986).

1.5 Clinical

a) History

The cardinal symptom of acute pancreatitis is abdominal pain. Notoriously variable in its character, the pain typically commences in the epigastrium, is of fairly sudden onset, increases gradually in severity, radiates through to the back and is associated with nausea and vomiting. So severe can its presentation be that the essential feature in diagnosis is its consideration in all patients with acute abdominal pain. There is a tendency for the patient to bend forwards in an attempt to relieve the pain, but in the presence of generalised peritonitis a flat motionless position is adopted.

On rare occasions acute pancreatitis occurs without abdominal pain (Toffler and Spiro 1962; Dooner and Aliaga 1965; Read et al. 1976).Under these circumstances there is usually severe pancreatic necrosis and haemorrhage associated with shock and coma.

There may be a history of recent excessive intake of food and/or alcohol ("gourmand pancreatitis") or of previous biliary tract disease (biliary colic, cholecystitis, intermittent obstructive jaundice, etc.)

b) Examination

The usual physical signs are epigastric tenderness and
guarding. In severe cases the abdomen may be rigid with absent
bowel sounds. Other signs which may be present include
tachycardia, hypotension, jaundice (Schmidt and Creutzfeldt,
1973) fever and tachypnoea (Jacobs et al. 1977). Many additional
signs have been described whose occurrence is infrequent and
inconsistent and assists little in diagnosis (Durr, 1979).

Certain cutaneous manifestations, although rare, are of
diagnostic interest. Grey-Turner's sign is a bluish
discolouration of the flanks (Grey-Turner, 1919) and Cullen's
sign is a similar discolouration of the periumbilical region
(Cullen, 1918). Both represent ecchymosis due to entrance of
blood-stained fluid into the fascial planes and both are seen in
haemorrhagic pancreatitis. The incidence of these signs in
various series of acute pancreatitis ranges from 0-3% and their
presence is commonly associated with a poor prognosis, though a
recent study showed a mortality of only 37% when the signs were
present (Dickson and Imrie, 1984). They are not pathognomonic of
acute pancreatitis and Cullen actually described his sign in a
patient with ruptured extrauterine pregnancy.

Extra-abdominal fat necrosis may involve the skin to produce
erythematous subcutaneous indurations, or the synovia to produce
swollen joints.

The circulatory shock of acute pancreatitis can be related
to decreased peripheral resistance, impaired myocardial function
and increased capillary permeability resulting in massive fluid
sequestration in the intestinal lumen, retroperitoneal tissues
and peritoneal cavity. This shock situation may be refractory to

all forms of intravenous fluid replacement and cardiovascular support.

c) Special Investigations

Radiographic changes on chest x-ray are only visible in 10-20% of cases on admission, although 40-70% of all patients with acute pancreatitis have asymptomatic hypoxaemia. This is probably due to increased right-to-left shunting and is possibly caused by pulmonary intravascular thrombi due to subclinical disseminated intravascular coagulation (Ranson et al. 1977; Murphy et al. 1980) and loss of surfactant leading to atelectasis. In severe pancreatitis disruption of the alveolar-capillary membrane produces frank pulmonary oedema and possibly pulmonary fibrin deposition (Berry et al. 1981) with severe respiratory insufficiency and occasionally a picture of adult respiratory distress syndrome. Although these processes can be reversed, once intubation and ventilatory support become necessary only 10-20% of patients survive.

Serious renal impairment may occur in the absence of circulatory collapse. This is accompanied by a selective increase in renal vascular resistance, a reduction in renal blood flow, decreased oxygen uptake and impairment of renal tubular protein reabsorption (Werner et al. 1974). Reduced glomerular filtration may occur because of fibrin deposits in the glomeruli and deposits of fibrinogen and IgC in the glomerular basement membrane. Fibrin thrombi may be formed as a result of activation of the coagulation system by thrombin. Simple correction of hypovolaemia may not, therefore, be sufficient to prevent renal failure, though it should be the initial consideration.

Hyperglycaemia occurs in 15-25% of patients with acute

pancreatitis, but is usually mild and transient. The effect is largely attributable to marked elevation of glucagon (up to 10-15 times above normal) which more than compensates for the modest rise in insulin (usually 2-3 times above normal). Significant hyperglycaemia is rarely seen even in the presence of massive pancreatic necrosis, but insulin treatment is often required if parenteral feeding is instituted.

Low serum levels of calcium usually indicate a severe attack of pancreatitis, but are not usually sufficiently reduced to cause tetany. Hypocalcaemia is largely secondary to reduced albumin in the vascular compartment but may also be influenced by enzymic damage of circulating parathormone and sequestration of calcium in soap deposits associated with fat necrosis. Hyperlipidaemia, hypoalbuminaemia, elevated white blood count and deranged liver function tests may also be present in varying combinations.

Plain x-ray film of the abdomen is essential to exclude pneumoperitoneum, small bowel obstruction and aortic aneurysm. Three or more air-fluid levels are strongly indicative of small bowel pathology rather than acute pancreatitis and laparotomy should be considered (Moossa, 1984). The film may show localised ileus (sentinel loop or colon cut off), pancreatic calcification or gallstones.

Computed tomography is the imaging modality of choice for evaluating the pancreas in acute pancreatitis. It is far superior to ultrasonography in detecting acute pancreatitis and defining its extent because the associated paralytic ileus hinders thorough ultrasound examination of the gland (Moossa, 1982; Silverstein et al. 1981) which is fully visualised in only about

20% of patients with pancreatitis. However ultrasonography is 90%
accurate in the diagnosis of gallstones as a cause of
pancreatitis (Moossa, 1984). The most imporant role of computed
tomography is to diagnose and define the complications of
pancreatitis, particularly necrosis pseudocysts and abscesses,
and to follow the course of these lesions with serial
examinations.

Radionucleotide scans (99m-Tc labelled HIDA, PIPIDA and
DICIDA) were thought to accurately differentiate between acute
pancreatitis and acute cholecystitis but in fact they do not
achieve this objective because non-filling of the gallbladder in
acute pancreatitis is common and is due to concomitant
cholecystitis or 'gallbladder ileus' (Neoptolemos et al. 1984a).

d) Pancreatic Enzyme Markers

A marked elevation of total serum amylase (>1000 IU/L)
provides the single most useful diagnostic marker of acute
pancreatitis. A normal value(0-300 IU/L) is so rare that the
diagnosis should be questioned if the amylase level is normal.
However, in the patient who presents a few days after the onset
of the disease, the peak amylase may be missed. Amylase levels
may also be distorted if serum is lipaemic in which case a random
urinary amylase concentration may be helpful.

Normally the main sources of serum amylase are the salivary
glands (S-amylase) and the pancreas (P-amylase) in about equal
amounts. There are several isoenzymes of each type and the
pancreas may also secrete a small amount of S-amylase. Minor
quantities of amylase are found in other tissues including
fallopian tube, ovary, prostate, lungs and liver.

Macroamylasaemia occurs in up to 0.4 per cent of

individuals. This biochemical abormality is due to complexing of P- or S-amylase to a large molecule such as IgA or IgM resulting in a macromolecular complex usually around 160,000 daltons in size. This may be associated with a low renal clearance of amylase.

The causes of mild to moderate elevations of serum amylase(300-1000 IU/L) are diverse (Burnett and Ness, 1955; Elliott and Williams, 1961; Adams et al. 1968; Salt and Schenker, 1976; Wilson and Imrie, 1986) and are listed in Table 5. Differential diagnosis most commonly includes biliary colic, cholecystitis, perforated peptic ulcer and myocardial infarction (Durr, 1979). If there is any doubt as to the diagnosis in patients with hyperamylasaemia after initial resuscitation, then laparotomy should be undertaken. A more specific marker of acute pancreatitis would be desirable and several alternatives have been examined. Separation of total serum amylase into pancreatic isoenzymes by means of electrophoresis, isoelectric focusing or chromatography is theoretically attractive (Warshaw, 1977; O'Donnell et al. 1977; Kolars et al. 1984) and confers a small increase in sensitivity but serum levels do not remain elevated any longer than total amylase. Differential inhibition of P-amylase by a specific wheat germ inhibitor has recently been shown to be potentially valuable as an alternative to actual determination of isoenzymes (Delcourt, 1982).

About 24% of serum amylase is removed by the kidneys and the normal clearance ratio is 2-3 ml per minute compared with creatinine clearance of at least 100 ml per minute. Levitt et al. (1969) reported that the renal clearance of amylase was raised during acute pancreatitis, from which the amylase-creatinine

Table 5   Causes of Raised Serum Amylase

Pancreatic Disease	Acute pancreatitis Chronic pancreatitis Trauma Pancreatic carcinoma Periampullary tumours and pseudo-tumours
Hepato-Biliary Disorders	Acute cholecystitis Hepatitis Cirrhosis Choledocholithiasis ERCP
Gastrointestinal Disorders	Peptic ulcer Acute intestinal obstruction Mesenteric infarction Intestinal perforation Crohns disease Afferent loop syndrome Peritonitis Acute appendicitis
Gynaecological Disorders	Salpingitis Torted ovarian cyst or tumour Ruptured ectopic pregnancy Endometriosis
Salivary Gland Disease	Mumps Parotitis Sialadenitis Trauma Tumours Maxillo-facial surgery
Renal Disease	Acute renal failure Chronic renal failure
Macroamylasaemia	
Neoplasms	Bronchogenic tumours Ovarian cystadenocarcinoma Colonic adenocarcinoma
Miscellaneous causes	Burn injury Ruptured aortic aneurysm Dissecting aortic aneurysm Cerebral trauma Post-operative (especially intra- abdominal and cardiothoracic) Prostatic disease Diabetic ketoacidosis Intravenous steroids Intravenous secretin Morphine-prostigmine test

clearance ratio emerged. This ratio has been proposed as a more accurate method of diagnosing acute pancreatitis than serum amylase alone (Murray and Mackay, 1977) with the advantages that it remains elevated for several days after serum amylase has returned to normal. It is elevated even if serum is lipaemic and a normal ratio excludes acute pancreatitis (Levitt et al. 1977; Leckie et al. 1980). Initial enthusiasm has been tempered by reports that the diagnostic accuracy of the ratio is poor when compared with patients with other acute abdominal conditions rather than normal controls (Farrer and Calkins, 1976; McMahon et al. 1982). The test has failed to gain wide acceptance and is probably of no value in clinical practice (McMahon et al. 1982; Moossa, 1984).

Measurement of serum lipase should probably be more widely adopted as a diagnostic aid for acute pancreatitis (Kolars et al. 1984) particularly as methods of rapid determination have become available (Lifton et al. 1974; Grenner et al. 1982). Advantages of serum lipase are that it is more specific than amylase as the pancreas is the only major source (Kasper and Sommer, 1976) and levels tend to rise earlier and are sustained longer than serum amylase (Delcourt, 1982; Kolars et al. 1984). Serum lipase is not elevated in salivary gland disease and macroamylasaemia, but may be increased in peptic ulcer perforation, intestinal infarction, intestinal perforation, acute cholecystitis and choledocholithiasis. There is no lipase in urine (Rick, 1972).

Serum trypsin, elastase and phospholipase A_2 have also been measured and may occasionally be helpful in the atypical non-urgent situation. These methods are not always available and do not add notably to accuracy in diagnosing typical cases of acute

pancreatitis (Delcourt, 1982; Elias et al. 1977; Berry et al. 1982).

1.6 Incidence and Mortality

Regional surveys in Great Britain and the United States have been performed in defined population areas in order to evaluate the true incidence of acute pancreatitis. Trapnell and Duncan (1975) found an incidence of 53.8 cases of acute pancreatitis per million of the population per year over a seven year period in the Bristol area. Bourke's (1975) figures for Nottingham were similar - 47.5 cases per million of the population per year over the period 1969-1974. The American figures for Rochester, Minnesota (O'Sullivan et al. 1972) were double at 110 cases per million per year. The incidence of acute pancreatitis is probably rising in North America and Europe possibly because of the ageing gallstone population and the increasing incidence of alcoholism, particularly in urban areas.

Acute pancreatitis can occur at all ages and has even been reported in the unborn foetus (Seifert, 1956). However it is rare in children (Goebell and Hotz, 1976). The male to female ratio differs widely in collected series, but overall is approximately 1:1 (Kasper and Sommer, 1976). Generally speaking males predominate where alcohol associated pancreatitis is common, and females where gallstones are the more frequent aetiological factor(Goebell and Hotz, 1976). Gallstone pancreatitis is as least twice as common in females with a peak incidence of presentation at 50-70 years of age. Alcoholic pancreatitis is over four times commoner in men with peak incidence of presentation at 30-40 years.

At the beginning of the century mortality rates from acute pancreatitis exceeded 50% (Korte, 1911; Guleke 1924; de Klimko, 1936). There followed an apparent decrease with the recognition of milder cases after Elman et al. (1929) popularised serum amylase estimation and drew attention to the milder forms of the disease (Elman, 1933). Up until this time surgery had been advocated in the acute attack (Moynihan, 1925) and it was only after publication of the high mortality rate in these reviews that this policy was questioned.

Coincident upon the swing to non-operative management as advocated by Rowland (1934), Mikkelson (1934) and Katsch (1939), the mortality fell to rates averaging 22%. It must be remembered, however, that this was also the time when the milder forms of the disease were being recognised for the first time.

The ensuing three decades saw little change in mortality rates in this country of 26% (Pollock, 1959) 23% (Efron, 1966) 23% (Trapnell, 1966) 17% (Trapnell and Duncan, 1975).

Improved methods of diagnosis and early recognition of severe cases resulting in vigorous resuscitative measures, intensive care nursing and early specific treatment measures resulted in the lower mortality rates in more recent series (Table 6) and the mortality rate in this country at present is probably 5-10%.

Table 6   Published Mortality Rates 1976-1984 for Acute
                                        Pancreatitis

Author	No.of Cases	% Mortality
Ranson et al. (1976)	300	7.3
Goebell et al. (1977)	93	5.4
Jacobs et al. (1977)	519	12.9
MRC Group (1977)	103	11.6
Paul et al. (1977)	257	11.0
Imrie et al. (1978a)	161	8.7
Durr (1978)	69	13.0
Blamey et al. (1984)	897	11.0
De Bolla and Obeid (1984)	405	10.6
Total	2,804	10.6

1.7 Early Assessment of Severity

Acute pancreatitis is a disease with a wide spectrum of severity. Eighty per cent of patients have a mild attack and rapidly recover with conservative treatment, usually with a hospital stay of only three to ten days. The remaining twenty per cent of patients have a severe attack with a protracted clinical course associated with local and systemic complications. About half of these patients will die.

Williamson (1984) suggested three reasons for separating patients into good and bad prognosis on admission:

1. The more severe group need more vigorous resuscitation and monitoring including intensive care facilities, urethral catheter and central venous pressure monitoring. This would be

costly if applied to all patients with acute pancreatitis.

2. Aggressive treatment measures such as peritoneal lavage, early surgery or endoscopic sphincterotomy might be justified in patients with a poor outlook but not in the majority of patients with a mild attack which will quickly abort.

3. A general acceptance of criteria of severity would facilitate international comparisons for prospective studies.

Unfortunately in no other abdominal condition is the outcome so unpredictable at the outset. Differentiation between mild and fulminating pancreatitis at presentation on clinical grounds alone is quite unreliable. Although patients with severe pancreatitis may arrive in hospital shocked and comatose, they are more likely to appear clinically well on initial assessment, only to deteriorate in the ensuing hours or days. Initial clinical evaluation is successful in identifying only 34-39% of patients with severe pancreatitis (McMahon et al. 1980b; Corfield et al. 1985). This figure does improve to 73% at 24 hours and 83% at 48 hours (McMahon et al. 1980b; Corfield et al. 1985). There is therefore a need for supplementary indices of severity, but these are of limited value unless they provide an answer within 48 hours of admission.

a) Single Prognostic Factors

Clinical signs such as cyanosis, shock, fever, Grey-Turner's sign, tetany and an abdominal mass all suggest severe disease, but are often absent in patients who develop severe complications.

The presence of Methaemalbumin in the blood has been shown to be associated with the haemorrhagic form of acute pancreatitis (Mazumber, 1961; Northram, 1963; Winstone, 1965). Methaemalbumin

indicates intravascular haemolysis, methaem (haematin) being a
breakdown product of haemoglobin which combines with albumin. Two
studies have shown that its presence in blood or ascitic fluid
carries a 54-66% chance of death (Geokas et al. 1974; Lankisch et
al. 1978) but other reports suggest that it is of little or no
value (Ranson et al. 1974; McMahon et al. 1980b; Berry et al.
1982). The main drawback lies in the delay of 72 hours before
methaemalbumin appears in the circulation.

Plasma concentrations of fibrinogen reflect the coagulopathy
of acute pancreatitis and are raised on admission and correlate
with other indicators of severity, notably hypoxaemia (Murray and
Mackay, 1977; Ranson et al. 1977; Berry et al. 1981). Adult
respiratory distress syndrome may result from deposition of
fibrin in the lungs.

Hypoxaemia is a common feature of acute pancreatitis. An
arterial pO_2 of 8 kPa or below is usually taken to indicate a
severe attack, though the actual fall in oxygen saturation will
reflect the patient's age and pre-existing respiratory function.
In one series half the patients who developed serious
complications or died had arterial hypoxia (<8kPa) on admission
(Cooper et al. 1982).

Early hypocalcaemia has long been recognised as a grave
prognostic feature in acute pancreatitis (Trapnell, 1966; Ranson
et al. 1976). Some calcium combines with fatty acids liberated by
pancreatic enzymes to form soaps, but more important are the
increased concentrations of glucagon and calcitonin and decreased
concentrations of magnesium and parathormone associated with
acute pancreatitis (Condon et al. 1975; McMahon et al. 1978;
Croton et al. 1981). Subsequent hypocalcaemia reflects the fall

in serum albumin (Allam and Imrie, 1977). A corrected serum
calcium <2.0 mmol/L was associated with a severe outcome in 98%
of cases but was only present in 2.99% of severe cases in one
study (Cooper et al. 1982).

Many other single factors have been proposed as early
indicators of severity in acute pancreatitis including lactate
dehydrogenase, age, leucocyte count, blood glucose, liver
function tests, urea, creatinine, C-reactive protein, serum
ribonuclease, declining concentrations of complement proteins
(Foulis et al. 1982) and continuing fluid sequestration during
the first three days (Overy et al. 1980) though few on their own
show the specificity and sensitivity required for clinical
usefulness. Computed tomography scan obtained within 24-48 hours
of admission may be able to distinguish oedematous and necrotic
pancreatitis (Dammann et al. 1980; Hill et al. 1982).

b) Multi-Factor Analysis

Multi-factor analysis was first proposed by Ranson et al.
(1974). They studied 43 clinical, biochemical and haematological
factors determined within 48 hours of admission in 100 patients
with acute pancreatitis. Eleven factors had prognostic
significance and the presence of three or more of the criteria
shown in Table 7 correlated well with severe outcome. This
scoring system has been confirmed as an important method of
predicting outcome (Ranson et al. 1976; Jacobs et al. 1977;
Pickford et al. 1977; Satiani and Store, 1979; Ranson, 1982) but
was derived from a study group comprising mainly alcoholic
pancreatitis and correlates less well with the outcome in
patients with gallstone associated pancreatitis. A similar study
confined to patients with cholelithiasis-associated pancreatitis

dropped one of the eleven factors (hypoxaemia) and adjusted eight of the remaining ten factors (Ranson, 1979).

Imrie and colleagues (1978a) have also reliably modified Ranson's original criteria for use in a patient population which is more elderly and contains a large proportion who have non-alcoholic causes for acute pancreatitis as encountered in Britain. They describe nine criteria (Table 7) and in their original report all patients who died had been correctly predicted as severe on the basis of three or more of the criteria present within 48 hours of admission.

Imrie's multi-factor analysis has also gained wide acceptance in Britain though modifications have been proposed including omission of AST/ALT (Blamey et al. 1984) reducing the criteria to eight or omission of age in the subgroup of patients with gallstone associated pancreatitis (Osborne et al. 1981). 70-80% of patients can be correctly predicted as severe or mild using multi-factor analysis.

Multivariate analysis of risk factors has been used by Ranson and Pasternack (1977) for accurate prediction of severity in acute pancreatitis using as many as nine variables. Blamey et al. (1984) sought to minimise the number of variables by using only those factors with independent significance (lactate dehydrogenase and calcium). This may prove an alternative method for identifying high risk patients, but must await prospective verification.

One of the problems encountered with early prediction of severity in acute pancreatitis is that of obtaining an objective and widely applicable definition of clinically severe outcome. Death is an obviously severe outcome and the only gold standard

Table 7   Multi-Factor Analysis for Predicting Severity
                 of an Attack of Acute Pancreatitis

*Ransons Criteria   (Ranson et al. 1974)

At Admission: 1. Age > 55 years
                                   2. Leucocyte count $>16 \times 10^9$/L
                                   3. Blood glucose >10 mmol/L
                                      (no history of diabetes)
                                   4. Serum LDH >700 IU/L
                                   5. Serum AST >250 Sigma Frankel
                                                 Units %

During first 48 hours: 6. Haematocrit fall >10%
                                   7. BUN rise >5 mg%
                                   8. Arterial pO_2 < 8kPa
                                   9. Base deficit >4 mEq/L
                                  10. Serum calcium <8.0 mg/dl
                                  11. Estimated fluid sequestration >6L

*Imries Criteria   (Imrie et al. 1978a)

During first 48 hours: 1. Age >55 years
                                   2. Leucocyte count $>15 \times 10^9$/L
                                   3. Blood glucose >10mmol/L
                                      (no history of diabetes)
                                   4. Arterial pO_2 < 8kaPa
                                      (no O_2 for 15 min)
                                   5. Serum albumin <32 g/L
                                   6. Serum LDH >600 IU/L
                                   7. Serum calcium< 2.0 mmol/L
                                   8. Serum AST or ALT >100 IU/L
                                   9. Blood urea >16 mmol/L

* In both methods a severe attack is predicted if three or more
  factors are recorded.

for comparing clinical results is the mortality. Criteria such as
the period of admission, the need for early operation (Blamey et
al. 1984) admission to ITU or length of stay in ITU (Ranson et
al. 1976) or delay before resuming normal oral intake are
influenced by availability of facilities and the management
policies of individual clinicians. Criteria such as respiratory
or renal failure depend in part for their definition on factors
also used in predicting outcome. Other disadvantages of multi-
factor analysis are that data collection is often incomplete or
is not completed for 48 hours or longer following admission by
which time clinical assessment is probably as good in predicting
outcome (McMahon et al. 1980b; Corfield et al. 1985).

c) Diagnostic Peritoneal Lavage

Many of the clinical signs of acute pancreatitis are due to
release of a vaso-active enzyme-rich broth which enters the
circulation by absorption from the peripancreatic tissue and from
accumulated ascitic fluid through the peritoneum. It was
therefore postulated that diagnostic peritoneal aspiration might
detect evidence of severe disease before its clinical appearance.
Attempts to use the concentration of amylase in peritoneal fluid
obtained by needle or cannula aspiration to diagnose acute
pancreatitis had been reported. These suffered from the same
drawback as the traditional four quadrant tap for the diagnosis
of abdominal trauma in that fluid was only obtained in a
proportion of cases. Thus a lavage technique was proposed,
performed as soon as possible after diagnosis of acute
pancreatitis (Pickford et al. 1977).

After emptying the stomach and bladder, a peritoneal
dialysis catheter was inserted through the linea alba 2-5 cm

below the umbilicus using local anaesthetic. Any free ascitic
fluid was aspirated and its volume and colour noted. One litre of
warmed normal saline was then instilled into the abdominal cavity
and the patient turned from side to side to facilitate mixing.
The fluid was then allowed to run out into the same bag and its
colour noted (Pickford et al. 1977).

The ascitic fluid can be analysed and significant
differences in albumin, ALT, total protein, amylase, urea,
calcium, potassium, bilirubin, alkaline phosphatase and white
blood count are seen when comparing severe and mild cases. In the
original study best markers for predicting severe disease were
albumin (>3 g/L), ALT (>10 IU/L) and total protein (>7.5 g/L).
Comparative serum and peritoneal amylase and lipase levels may
be reliable indicators in the early prognosis of acute
pancreatitis (Robert et al. 1986). However, these measurements
offer no better prediction than Ranson's or Imrie's multi-factor
analysis and are equally time-consuming. Of greater value is the
immediate prediction of severity based on the volume and colour
of the ascitic fluid.

In the original study performed on twenty seven patients
with acute pancreatitis (Pickford et al. 1977) the presence of
at least 10 ml of free peritoneal fluid and a straw-coloured or
darker return fluid after lavage were taken to predict severe
disease and successfully predicted severe disease in five
patients who had been clinically assessed as mild. In a later
paper from the same centre, 96 attempted diagnostic lavages were
reported and the criteria for predicting severe disease were
amended (McMahon et al. 1980a) to:

    1. At least 10 ml of peritoneal fluid (or 20ml, Mayer

et al. 1985).

and/or   2. The presence of brown coloured free fluid ("prune-
            juice").

and/or   3. Return lavage fluid of straw colour or darker
            ("unmilked tea" or "average bitter beer").

Lavage was achieved in 89 of the 96 patients (5 failures due
to incorrect location of the cannula and 2 due to suspected
viscus perforation). There was one complication - a patient who
had not had the stomach emptied prior to the procedure sustained
a minor stomach perforation and underwent laparotomy. 76% of
severe attacks were correctly predicted by lavage performed a
mean of 7 hours after admission (range 1-30 hours) compared with
38% predicted by clinical criteria on admission.

The technique has the advantages that it appears to relieve
pain in about one third of patients and that it allows
therapeutic dialysis to be started immediately through the
cannula should the volume or appearance of the fluid suggest
severe disease. The technique also occasionally identifies
unsuspected abdominal pathology such as biliary peritonitis,
intestinal perforation or infarction either because the ascitic
fluid clearly contains bile or fibres or because large numbers of
intestinal organisms are seen if the lavage fluid is sent for
microscopy (Bradley et al. 1981).

In a comparative study (McMahon et al. 1980b) clinical
assessment at 48 hours was comparable to multiple prognostic
criteria in predicting severe attacks (83% vs 82%). The presence
of methaemalbumin in serum or hypocalcaemia were of no additional
value. Diagnostic peritoneal lavage was the most accurate guide
to severity and predicted all patients who developed shock or

died. This study also provided a colour coding chart for assessing the ascitic fluid and lavage return fluid.

The value of peritoneal lavage in early prediction of severity in acute pancreatitis has been confirmed by other centres. Cooper et al. (1982) incorporated diagnostic lavage in a multi-factor analysis with hypotension (systolic BP < 100mmHg) hypocalcaemia (corrected $Ca^{++} < 2.0$ mmol/L) and hypoxaemia ($PaO_2 < 8kPa$). The presence of one or more of these factors was associated with a 43% mortality while the absence of all four was associated with a 3% mortality. Measurement of PaO_2 and diagnostic peritoneal lavage were the most sensitive and it was proposed that they could be used without the other two factors.

Diagnostic peritoneal lavage undoubtedly offers a good method for early prediction of severity in acute pancreatitis. It can usually give an answer within a few hours of admission and thus has an advantage over multi-factor analysis which often takes 48 hours to complete. It probably should not be used in patients who have had previous abdominal surgery. It is an invasive technique and cases of visceral perforation have occurred. It can only be justified if important differences in treatment depend upon the findings.

1.8 Treatment

a) General

The only therapeutic manoeuvre universally accepted as having provided benefit in the reduction of mortality in acute pancreatitis is that of intravenous fluid replacement. A better understanding of the pathophysiological changes that occur in

pancreatitis led Keith and Watman (1954) to the finding that up to 30% of circulatory volume may be lost. The obvious need for replacement of these losses meant that few clinical trials were performed although Elliot (1957) demonstrated that whenever there was a suspicion of a deficit in colloid or whole blood, their replacement significantly reduced mortality. In these patients monitoring central venous pressure, systemic arterial pressure and hourly urine output is essential. A Swann-Ganz catheter may with benefit be inserted and cardiac output measured. In mild acute pancreatitis the intravenous infusion of crystalloid solutions is sufficient.

The arterial blood gasses should be estimated at least 12 hourly for the first three days. Although radiographic changes on chest x-ray are visible in only 20% of patients on admission, 40-70% of all patients with acute pancreatitis will have asymptomatic hypoxaemia. Humidified oxygen should be given in such patients.

Adequate analgesia is essential as the pain is often severe and contributes to the shocked state of the patient. Recommended analgesics include pethidine and buprenorphine hydrochloride, but morphine and its close derivatives should be avoided because they may cause spasm of the sphincter of Oddi.

The role of routine administration of antibiotics is controversial. There is no evidence that they influence the outcome of acute pancreatitis although published trials included too few patients to disprove their efficiency (Craig et al. 1975; Howes et al. 1975; Finch et al. 1976). It may be reasonable to use antibiotics in gallstone pancreatitis and/or fulminant pancreatitis but not in mild to moderate alcoholic pancreatitis.

Blood calcium and glucose levels should be followed during the course of acute pancreatitis. Calcium supplements may be given prophylactically or can be used if symptoms or signs of hypocalcaemia develop. Hyperglycaemia is not infrequent and up to 10% of patients will require insulin therapy, though the diabetic state is usually of short duration.

Severe acute pancreatitis is frequently associated with multisystem failure requiring intensive supportive care in order to sustain cardiovascular, respiratory and renal function. It is essential that patients are carefully monitored and potential organ failure anticipated, particularly within the first 48-72 hours of an attack.

b) Non-Invasive Therapeutic Approaches

A vast literature has accrued relating to the conservative management of acute pancreatitis and this may conveniently be divided into the following non-operative therapeutic approaches:

(i) Resting the pancreas

The main principle in many treatment methods for acute pancreatitis is, according to Ettien and Webster (1980) "to put the pancreas to rest". Various methods of suppressing pancreatic secretion have been advised but their value has not been established. Experimental evidence suggests the pancreas is already fully "shut down" during the acute phase of the disease. This is presumably why no treatment designed to suppress pancreatic secretion has yet been shown to be useful.

Since the early reports of Katsch (1939) and others the beneficial effects of restriction of oral intake of food and fluids has been so widely accepted on the grounds of clinical observation that it has never been proven under strictly

controlled conditions (Durr, 1979). Liberal oral consumption of clear fluids has been shown to have no effect on the outcome of patients with acute pancreatitis in a recent study (Lange and Pedersen, 1983). Similarly naso-gastric decompression, which prevents emptying of gastric acid into the duodenum and thereby reduces the secretin-mediated pancreatic secretion of fluid and bicarbonate, had become a generally accepted cornerstone in the management of acute pancreatitis. However, the only controlled trials have shown no benefit (Levant et al. 1974; Switz et al. 1975; Naeije et al. 1978; Field et al. 1979; Fuller et al. 1981; Loiudice et al. 1984). The additional discomfort of a naso-gastric tube cannot be justified routinely and may increase the risk of aspiration pneumonia. A tube is clearly indicated, however, when duodenal obstruction or paralytic ileus develop and may help in relieving vomiting. It should be used selectively rather than mandatorily.

A natural progression in attempts to reduce acid stimulation was the use of simple antacids (Goebell, 1971; White, 1974). It was inevitable that H_2 receptor blockers would be advocated for similar reasons (Banks, 1979). Trials of cimetidine in acute pancreatitis have shown no benefit compared with controls receiving placebo (Meshkinpour et al. 1979; Broe et al. 1982; Loiudice et al. 1984) or with controls with naso-gastric suction (Goff et al. 1982).

(ii) Anticholinergics

The use of anticholinergics was advocated to "dry up" the pancreas (Elmslie, 1967). Cogbill and Song (1970) reported a reduction in the complication rate when anticholinergics were administered ($p < 0.01$). The predominant clinical experience,

however, is that neither probanthine nor atropine influence the morbidity or mortality (Trapnell, 1966; Ranson et al. 1976). Both can cause significant side effects and should therefore not be used.

(iii) Glucagon

The hormone glucagon which is secreted by the alpha-cells of the Islets of Langerhans has been shown to decrease the volume and enzyme concentration of pancreatic exocrine secretion in animals (Walker and Necheles, 1956; Dyck et al. 1969) and man (Zajtchuk et al. 1967; Dyck et al. 1970; Knight et al. 1971). Administration of the hormone in animals results in a marked degranulation of the pancreas thought to be due to inhibition of enzyme synthesis (Jarrett and Lacy, 1962). Raised plasma glucagon levels occur in pancreatitis in dogs (Paloyan et al. 1966) and man (Lawrence, 1966). A later rapid fall in plasma levels coincident with the onset of severe shock and preceding death was observed by Knight et al. (1972) in experimental pancreatitis in dogs. Extrapolating from these observations they suggested that infusion of exogenous glucagon might further suppress pancreatic activity and reduce mortality. Uncontrolled clinical use suggested that both mortality and morbidity were reduced in patients with acute pancreatitis treated with glucagon (Condon et al. 1973). Experimentally Waterworth and Bevan, (1976) and Manabe and Steer (1979) were able to demonstrate a protective effect of glucagon in experimental pancreatitis. Condon et al. (1974) and Lankisch et al. (1974) failed to demonstrate prolonged survival in experimental controlled trials.

When used clinically in the MRC Multicentre controlled trial (1980) glucagon was not found to diminish the risk of death nor

to provide rapid relief of pain which had been claimed by others (Condon et al. 1973; Waterworth and Bevan, 1976).

(iv) Somatostatin

A reduction of exocrine pancreatic secretion has been demonstrated with the use of somatostatin (Creutzfeldt et al. 1975; Domschke et al. 1977; Kayasseh et al. 1977; Folsch et al. 1978). Somatostatin and a long-acting somatostatin analogue have been shown to have a beneficial effect on survival rate and histological changes in experimental acute pancreatitis (Baxter et al. 1985a). Somatostatin may not mediate its beneficial effect in established pancreatitis by inhibiting pancreatic secretion (which may be minimal) but rather by its cytoprotective (Szabo and Usadel, 1982) and reticulo-endothelial stimulating (Baxter et al. 1985b; Baxter et al. 1986) actions.

Lankisch et al. (1977a) showed that linear somatostatin had no effect on mortality rate or the amount of pancreatic necrosis, oedema and leucocyte infiltration in sodium taurocholate-induced experimental pancreatitis in the rat, though the increase in amylase and lipase in serum was reduced. A co-operative European prospective clinical study of somatostatin (Usadel et al. 1982) has shown it to have no effect on the mortality rate or parameters indicating the severity of the disease (Usadel et al. 1985).

Infused peptide hormones, including somatostatin, may be hydrolysed rapidly by the presence of circulating proteolytic complexes in acute pancreatitis (Hermon-Taylor et al. 1981) in which case it would not be surprising that the results of their use are not encouraging.

(v) Others

A vast array of other drugs has been proposed in the treatment of acute pancreatitis including corticosteroids, tranquillizers, isoprenaline, heparin, prostoglandin E_2, vasopressin, carbonic anhydrase inhibitors, calcitonin, 5-fluorouracil, propylthiouracil, dextran and scavengers of free oxygen radicles. Experimental animal work and uncontrolled clinical studies have been produced to support many substances, but none has been proven beneficial in properly conducted controlled clinical trials. Trasylol will be considered in chapter three.

c) Prevention of Dissemination of Activated Pancreatic Enzymes

In acute pancreatitis there is an outpouring into the retroperitoneum and surrounding tissues of a fluid rich in activated enzymes, histamine releasing factors and low molecular weight vasoactive peptides. These substances reach the blood in part by absorption from the peritoneal cavity and partly by lymphatic drainage via the thoracic duct.

Bradykinin and kallidin are formed from kininogens, which are alpha-2-globulins present in plasma and lymphatics, by the action of kallikrein and trypsin. Kallikreinogen, which is present in pancreatic tissue as well as plasma, is converted to active kallikrein by plasmin, trypsin and kallikrein itself. These and probably other unidentified factors are responsible for the systemic effects of acute pancreatitis which include impaired cardiac function, reduced vascular tone, impaired capillary permeability, complement activation and initiation of disseminated intravascular coagulation. Major respiratory insufficiency can be initiated by high concentrations of free

fatty acids and phospholipase A_2 which damages the pulmonary surfactant layer. Vasoactive substances may produce intrarenal vasoconstriction and reduced glomerular filtration.

To limit this severe 'metastatic' damage invasive measures including peritoneal lavage, pancreatic resection and thoracic duct drainage have been proposed in fulminating pancreatitis to reduce the amount of toxic pancreatic broth reaching the bloodstream in the hope of reducing mortality and morbidity.

(i) Therapeutic Peritoneal Lavage

Following anecdotal reports of success using peritoneal lavage in patients with severe acute pancreatitis (Wall, 1965; Gjessing, 1967) and evidence of clear benefit in studies with experimental animals (Lankisch et al. 1979; Rogers and Carey, 1966; Rasmussen, 1967; Rosato et al. 1972) a series of papers put the view more firmly that it was an important and possibly life-saving advance in the management of the most severe forms of the disease (Ranson et al. 1976; Ranson and Spencer, 1978; Balldin and Ohlsson, 1979; Stone and Fabian, 1980; Neher and Schuster, 1982; Fagniez et al. 1982; Kauste et al. 1983). The rationale for using peritoneal lavage therapy was that the peritoneal cavity forms an important reservoir of pancreatic enzymes and that lavage in the early phase of the disease precludes the systemic absorption of potentially toxic compounds. The reports which encouraged this belief commented on the rapid relief of pain, rapid improvement in clinical status, reduced early mortality and reduction in respiratory problems.

Peritoneal lavage may be performed following the operative placement of several sump drains around the pancreas, particularly in the lesser sac. At one time this was often

combined with biliary decompression (irrespective of the aetiology of the attack) and the insertion of a gastrostomy tube for prolonged gastric aspiration, but the value of this "triple-tube drainage" was never established. Nearly all the patients treated were suffering from alcoholic pancreatitis and either the studies were not properly controlled or the numbers included were small. Subsequently it was shown that lavage using percutaneously placed catheters resulted in a better outcome than when formal laparotomy was performed.

Against this background a pilot study in the UK was conducted in Bristol and Scarborough (Cooper et al. 1982). Lavage emerged as potentially beneficial, but sufficient doubt lingered to fuel the demand for a controlled study. A study appeared from Sweden (Ihse et al. 1982) which showed no advantage in the lavage group but the number of patients included was small. A larger study was embarked upon in three cities (Leeds, Bristol and Glasgow) including a total of 24 hospitals (Mayer et al. 1985). Of 413 patients with acute pancreatitis, 91 met the inclusion criteria for severe acute pancreatitis (on multiple prognostic criteria or diagnostic peritoneal lavage). Half were randomised to receive peritoneal lavage.This was performed through a peritoneal dialysis catheter inserted through the abdominal wall in the midline as for diagnostic lavage. Hourly two-litre cycles of warmed balanced isotonic peritoneal dialysis solution (Dialaflex 61,Boots Ltd., Nottingham, UK) containing added potassium and heparin were continued for 72 hours, or longer if the lavage return fluid remained dark. Mortality in the lavage group was 27% and major complications were seen in 38%. Mortality in the control group was 28% with major complications

in 35%.

Thus this large multicentred British trial showed no beneficial effect from peritoneal lavage. In particular there was no reduction in early mortality, no rapid relief of pain, no rapid improvement in clinical status and no advantage in the alcoholic group of patients as had been suggested by others (Stone and Fabian, 1980). It is possible that the delay between the onset of pancreatitis and lavage may account for discrepancies between experimental results and the results of this clinical trial. The addition of potentially therapeutic substances such as antiproteases to lavage fluid has been proposed (Imrie, 1985) but in an animal study the addition of leupeptin, antipain and elastinal to lavage fluid has not shown shown any benefit over simple lavage alone (Terry et al. 1986).

(ii) Pancreatic resection for acute necrotising pancreatitis

As for peritoneal lavage, good results have been claimed in terms of achieving immediate clinical improvement for pancreatic resection in acute necrotising pancreatitis (Kivilaakso et al. 1981) and the only prospective randomised trial comparing pancreatic resection and peritoneal lavage favoured resection for fulminating alcoholic pancreatitis (Kivilaakso et al. 1984). Indications for early laparotomy include peritonitis, an unclear diagnosis with suspected visceral perforation and rapid deterioration of a patient with suggested acute pancreatitis despite intensive medical treatment (Nordback and Auvinen, 1985). Approximately 10% of cases of acute pancreatitis would fulfill these criteria. Resection has been reported to the level of the portal vein, middle of the head of the pancreas or near total leaving only a 1-2 cm sickle-shaped pancreatic remnant. The

biliary tract and pancreatic bed are then drained (Autio et al. 1979; Nordback et al. 1985a). It is important to differentiate between such a radical and controversial surgical approach and conventional surgery performed at a later stage for drainage of pancreatic abscesses and pseudocysts or sequestrectomy for pancreatic slough.

Enthusiasm for pancreatic resection has decreased and many now condemn it (Herman et al. 1974; Ranson, 1981 and 1983; Eggink et al. 1984; Smadja and Bismuth, 1986). Even some pioneers in this field have since criticised ablative surgery (Hollender et al. 1983; Nordback and Auvinen, 1985) but surgery may have a role in extensive acute pancreatic necrosis (Kivilaakso et al. 1984; Hollender et al. 1983; Nordback et al. 1986; Watts, 1963). Unfortunately it remains very difficult to decide which patients might benefit from such aggressive surgery. Enhanced CT scanning (Ranson et al. 1985; Block et al. 1986), radiolabelled leucocyte scanning (Fawcett et al. 1979; Anderson et al. 1983) and ERCP (Gebhardt et al. 1983) have been reported as being helpful.

Remarkably good results have recently been reported from Ulm for necrosectomy and postoperative local lavage in patients with necrotizing pancreatitis, with hospital mortality of only 8.3% (Berger et al. 1986). Indications for laparotomy included persistent organ failure or sepsis or proof of extended pancreatic or extra pancreatic necrosis by contrast-enhanced CT scan. However, mortality following pancreatic resection for acute fulminating pancreatitis is generally reported at 13-64% with a mean of just under 40% (Boutelier and Edelman, 1971; Hollender et al. 1971; Norton and Eiseman, 1974; Edelman and Boutelier, 1974; Francillon et al. 1974; Kummerle et al. 1975; Dritsas,

1976; Autio et al. 1979; Frey, 1979; Alexandre and Guerrieri, 1981; Gebhardt and Gall, 1981; Kivilaakso et al. 1981; Neher and Schuster, 1982; Smadja and Bismuth, 1986; Nordback et al. 1986, Wilson et al. 1986). It must be remembered that these patients are usually at very high risk when selected for surgery. There is also a very high incidence of later complications in the alcoholic group of patients having this treatment including polyneuropathy, dyspeptic symptoms and diabetes, though few problems seem to occur from exocrine dysfunction (Nordback and Auvinen, 1985).

(iii) Thoracic duct drainage

The principle route of transfer of enzymes from the pancreas to the plasma in acute pancreatitis is probably via the lymphatics and veins that drain the gland (Mayer and McMahon, 1985a) which may explain the failure of therapeutic peritoneal lavage. Brzek and Bartos (1969) suggest that the thoracic duct lymph plays an important role in the transport of pancreatic enzymes to the bloodstream and proposed that thoracic duct drainage would reduce metastatic enzyme damage. There are several techniques described for thoracic duct drainage (Cevese et al. 1975). Sim et al. (1966) reported favourable effects of thoracic duct fistulas in dogs with acute pancreatitis and Brzek and Bartos (1969) showed clinical improvement in most of ten patients in whom external drainage of the thoracic duct was instituted in an uncontrolled study. Komarov et al. (1983) instituted thoracic duct drainage in patients with severe pancreatitis with lymphabsorption and reinfusion of cleansed lymph. They suggested some clinical benefit. No properly conducted prospective randomised trials have been performed except for a small trial comparing thoracic duct

drainage to peritoneal lavage which showed no benefit for thoracic duct drainage and encountered complications from the technique itself (Stone et al. 1983).

1.9 Gallstone Associated Pancreatitis

Gallstones are the comonest cause of acute pancreatitis in the UK (MRC Working Party, 1977; Blamey et al. 1984; DeBolla and Obein, 1984; Corfield et al. 1985; Goodman et al, 1985) and parts of the USA (Dixon and Hillam, 1970; Kelly, 1980). Mortality from acute pancreatitis due to gallstones is 8-10% during a first attack. The mortality is higher than in patients with alcoholic pancreatitis, possibly reflecting the older age of patients with gallstones.

Cholecystectomy should be performed in patients who have suffered an attack of biliary pancreatitis because the risk of further biliary symptoms, especially recurrent acute pancreatitis, is 25-53% (Howard and Jordan, 1960; Elfstrom, 1978; Kelly, 1980) and this risk is reduced by more than 90% following cholecystectomy (Howard and Ehrlich, 1962; Dixon and Hillam, 1970; Kelly, 1974; Osborne et al. 1981; Kelly and Swaney, 1982). The timing of biliary tract surgery remains the subject of much debate.

Interest in immediate biliary tract surgery (within 72 hours of admission) has been revived by recent evidence linking choledocholithiasis with the development of acute pancreatitis. Gallstones are passed in the stool of 86-92% of patients with biliary pancreatitis within ten days of the acute attack (Acosta and Ledesma, 1974; Acosta, 1975; Kelly, 1980) compared to 12% of patients with a recent diagnosis of gallstones without a history

of pancreatitis and no patients with alcoholic pancreatitis.
Stones are to be found in the common bile duct of 50-72% of
patients within 72 hours of the onset of biliary pancreatitis
(Acosta et al. 1978; Kelly, 1980; Neoptolemos et al. 1986a) and
this figure falls to 3-33% by one week and remains fairly
constant for several months thereafter (Dixon and Hillam, 1970;
Paloyan et al. 1975; Ranson, 1979; Acosta et al. 1980; Kelly,
1980; Stone et al. 1981). Immediate biliary surgery is advocated
by some authors in the hope that removal of impacted common bile
duct calculi early in the attack will reduce the severity of
acute pancreatitis and thereby reduce mortality (Acosta et al.
1978 and 1980; Stone et al. 1981; Mercer et al. 1984). Mortality
as low as 2-3% has been reported for immediate biliary
surgery(Acosta et al. 1978; Stone et al. 1981).

The argument against immediate biliary surgery is that it
results in many patients being subjected to common bile duct
exploration for retrieval of stones most of which would have
passed spontaneously within a few days if surgery were delayed.
It is difficult to select a group of patients pre-operatively
with common bile duct calculi who would be improved by immediate
removal of an impacted comon bile duct calculus. It is also the
experience of most authors that immediate biliary surgery in
acute pancreatitis is associated with an unacceptably high
mortality of 15-20% (Paloyan et al. 1975; Ranson, 1979; Ong et
al. 1979; Kelly, 1980; Osborne et al. 1981; Tondelli et al. 1982)
compared with conventional treatment, though a properly
controlled study is required.

Cholecystectomy performed once the attack has subsided but
prior to hospital discharge (early surgery) with per operative

cholangiogram and common bile duct exploration where indicated, has been shown to have a negligible mortality (Ranson, 1979; Kelly, 1980) and is to be preferred to a policy of cholecystectomy at a subsequent elective admission (delayed surgery) because of the high risk of recurrent biliary pancreatitis or other biliary symptoms. There are still advocates of delayed biliary surgery however (De Bolla and Obein, 1984) and the operative mortality is certainly low (Kelly, 1974; Ranson, 1979; Ong et al. 1979; Osborne et al. 1981). This policy gained considerable favour because it allows the inflammatory process to subside and permits radiological confirmation of gallstones.

In order to pursue a policy of immediate or early biliary surgery in gallstone pancreatitis identification of patients with gallstones early in the attack is necessary. Oral cholecystography and intravenous cholangiography are of little value for several weeks following the onset of acute pancreatitis due to paralytic ileus and derangement of liver function. Although ultrasonography can detect gallstones in 90-95% of patients in the outpatient situation (McIntosh and Penney, 1980; Krook et al. 1980; Cooperbery and Burhenne, 1980) the gallbladder is visualised in only 80% of patients in the first 72 hours following admission with acute pancreatitis (Mckay et al. 1982) and only 58-67% of patients with biliary pancreatitis are detected by ultrasound in the first 72 hours following admission (McKay et al. 1982; Neoptolemos et al. 1984a). This figure can be increased to 78-93% if ultrasound is repeated following the patients clinical improvement.

For diagnosis of gallstones within 72 hours of admission the results of ultrasonography can be combined with clinico-

biochemical prediction of gallstones (McMahon and Pickford, 1979;
Blamey et al. 1983; Van Gossum et al. 1984; Neoptolemos et al.
1984a; Mayer and McMahon, 1985b; Goodman et al. 1985; Nordback et
al. 1985b; Neoptolemos et al. 1986b) though the accuracy of these
systems has been doubted (Dammann et al. 1980) and some patients
will still be missed. The age and sex of the patient as well as
bilirubin, alkaline phosphatase, Gamma GT, ALT, AST and amylase
levels have all been shown useful in predicting biliary
pancreatitis. Clinico-biochemical scoring systems have been
proposed to predict common bile duct calculi. Neoptolemos et al.
(1984a) used four criteria (bilirubin >40 umol/L, Gamma GT >250
IU/L, Alkaline phosphatase >225 IU/L and age >70 years), of which
bilirubin was the most useful.

Radionuclide biliary scanning was shown by Fonseca et al.
(1979) to be of potential value in determining gallbladder
pathology in acute pancreatitis, but there has been a wide
variation in reported accuracy with this technique (Frank et al.
1980; Glazer et al. 1981; Neoptolemos et al. 1981; Stone et al.
1981; Serafini et al. 1982). In one study the gallbladder was not
visualised in only 47% of patients with biliary pancreatitis and
in as many as 31% of patients with non-biliary pancreatitis
(possibly due to oedema around the cystic duct due to retrograde
lymphangitis) suggesting little place for this investigation in
the diagnosis of biliary pancreatitis (Neoptolemos et al. 1981).

The continuing difficulty of urgently identifying patients
with gallstones, particularly those with common bile duct stones,
has prompted invasive investigations of the biliary tree within
72 hours of admission. Percutaneous transhepatic cholangiography
has been advocated (Coppa et al. 1981) as it was suspected that

ERCP might worsen the pancreatitis or lead to septic complications and in particular to abscess formation. A number of centres have now established that ERCP is a relatively safe procedure in acute pancreatitis provided it is performed by an experienced endoscopist (Safrany and Cotton, 1981; Neoptolemos et al. 1986a and b). Cholangiography can be successfully achieved in over 90% of patients and complications are rare. Because of technical difficulties and potential complications of ERCP simple methods of pre-selecting patients who are most likely to have stones in the common bile duct such as ultrasound and clinico-biochemical criteria remain valuable.

ERCP can be safely combined with endoscopic sphincterotomy and common bile duct stone extraction with dramatic clinical improvement in some cases (Safrany et al. 1980; Safrany and Cotton, 1981; Delmotte et al. 1982; Rosseland and Solhaug, 1984; Von Reimann and Lux, 1984; Neoptolemos et al. 1986a) offering a therapeutic alternative in patients with severe gallstone associated pancreatitis which may well prove superior to urgent surgery as it has for acute gallstone cholangitis (Leese et al. 1986). In most patients who have undergone urgent endoscopic sphincterotomy, cholecystectomy should be performed during the convalescent period, though this can sometimes be avoided in the elderly and frail (Neoptolemos et al. 1984b). In one large study of urgent ERCP in acute pancreatitis, 70 patients with suspected gallstone pancreatitis were randomised equally to receive either ERCP within 72 hours of admission (combined with endoscopic sphincterotomy and stone extraction where common bile duct stones were present) or conventional treatment. ERCP was achieved in 89% of cases and common bile duct stones were demonstrated and

removed after endoscopic sphincterotomy in 50% of the gallstone group. There were no serious complications from ERCP or endoscopic sphincterotomy. The only two deaths occurred in the control group, both with gallstone pancreatitis. One patient had a stone impacted at the ampulla on post mortem examination and therefore might have benefited from endoscopic sphincterotomy (Neoptolemos et al. 1986a).

No clear benefit for urgent ERCP and endoscopic sphincterotomy has yet been demonstrated, but it is clearly a safe procedure and in particular there seems to be no increased incidence of infective complications of pancreatitis as predicted by some authors (Ranson, 1979; Coppa et al. 1981) or of bleeding complications as experienced in earlier studies from Germany (Roesch and Demling, 1982). In fact the overall complications following endoscopic sphincterotomy in acute pancreatitis are no higher than might be anticipated in other cases (Leese et al. 1985) and acute pancreatitis should no longer be regarded as a relative contraindication to endoscopic sphincterotomy as it has been in the past (Geenen et al. 1981).

An aetiological factor will not have been discerned in up to 40% of patients by the time recovery has occurred from an attack of acute pancreatitis. At this stage the decision needs to be taken as to whether these patients should be further investigated as outpatients or remain as inpatients. Clinico-biochemical predictors of gallstones can be used to select patients for further detailed investigations, particularly ERCP (Goodman et al. 1985). Microlithiasis of the gallbladder can be very difficult to detect and may cause recurrent pancreatitis as the small size of calculi favours migration (Houssin et al. 1983). An aggressive

attitude towards the diagnosis of gallstones can reduce the idiopathic group to under 10%.

1.10 Pancreatic Pseudocyst

Pancreatic pseudocysts are defined as fibrous-lined collections of walled-off pancreatic fluid and/or necrotic debris in the lesser sac, pancreas or retroperitoneum formed by the action of extravasated, activated enzymes. They arise in up to 10% of patients following an attack of acute pancreatitis, particularly if due to alcohol (Warshaw, 1974; Bradley et al. 1976; Boggs et al. 1982) or trauma (Van Heerden and ReMine, 1975; Thomford and Jesseph, 1969).

Pain is the usual presenting feature and anorexia, nausea and vomiting are less common complaints. An upper abdominal mass is palpable in 50-60% of patients (Boggs et al. 1982) and jaundice (Gonzales et al. 1965) paralytic ileus or ascites are found in approximately 10% of patients. In children a common presentation is rapid weight loss following an apparently trivial injury (Cooney and Grosfeld, 1975).

The serum amylase is often raised and remains elevated during the process of pseudocyst formation in 52% of patients (Shatney and Lillihei, 1979). Ultrasonography is the best diagnostic investigation as it is effective, safe and relatively cheap with a diagnostic accuracy greater than 90% (Bradley and Clements, 1974; Boggs et al. 1982). It is invaluable in determining the size, site and number of pseudocysts and has superceded the use of plain or contrast radiology. Computed tomography occasionally picks up pseudocysts not visualised on ultrasonography, but is not the investigation of first choice.

The majority of pseudocysts develop between two and four

weeks after an attack of acute pancreatitis and are multiple in
2.5-14.3% of cases (Frey, 1978). Initially a policy of
observation should be adopted as 40% of pseudocysts undergo
spontaneous resolution within the first six weeks, but rarely
afterwards (Bradley et al. 1976 and 1979; Sankaran and Walt,
1975; Boggs et al. 1982). The incidence of complications
associated with pseudocysts rises progressively after this time
from 20% to 60% beyond three months (Warshaw, 1974). Smaller
pseudocysts (less than 5cm diameter) will usually resolve whereas
those greater than 7.5cm usually require drainage.
Ultrasonography is invaluable for following the progress of
pseudocysts.

By six weeks a pseudocyst has matured and the cyst wall is
sufficiently strong to hold anastomatic sutures permitting
internal drainage (Shatney and Lillihei, 1981; Van Heerden and Re
Mine, 1975). The type of internal drainage is largely dictated by
the site of the pseudocyst. Cystogastrostomy is associated with
an 8-10% recurrence rate (Becker et al. 1968; Joyce et al. 1979)
and a post operative complication rate of 30% (Shatney and
Lillihei, 1979; Van Heerden and ReMine, 1975) with
gastrointestinal haemorrhage being particularly common.
Cystoduodenostomy has a reported cyst recurrence rate of 0-20%
(Rosenburg et al. 1969; Joyce et al. 1979) with a low mortality
and morbidity. Cystojejunostomy using a Roux-en-Y loop is the
procedure of choice when the cyst is not closely adherent to the
upper gastrointestinal tract and has a cyst recurrence rate
reported at 10% and a 30% post-operative complication rate (Boggs
et al. 1982). Pseudocysts in the body and tail can be removed by
distal pancreatectomy and splenectomy with or without drainage of

the pancreatic stump into a limb of jejunum (Joyce et al. 1979).
Pancreaticoduodenectomy for a cyst of the head carries a prohibitive
morbidity. Internal drainage into the stomach has been achieved
endoscopically (Cotton, 1983) and by percutaneous transgastric
cystogastrostomy using a drainage catheter.

Intervention before six weeks is indicated if the patient
develops a rapidly enlarging pseudocyst, intra-abdominal sepsis,
intraperitoneal rupture of the cyst or haemorrhage. If the cyst
wall looks thin on ultrasonography, then drainage should be
external, even though this is associated with a complication rate
as high as 72-86% (Boggs et al. 1982; Eeftinck et al. 1982)
including fistulae (often persisting for several months) sepsis
and cyst recurrence in 25-40% of patients (Shatney and Lillihei,
1979; Warshaw, 1974). External drainage has more recently been
achieved by needle aspiration or catheter drainage under
ultrasound control (MacErlean et al. 1980; Cooperman, 1980).

Infection within a pseudocyst is a rare complication
associated with clinical deterioration of the patient who
develops pain, tachycardia, pyrexia and rigors (Frey et al.
1979). A gas-fluid level or pockets of gas may develop within the
pseudocyst. ERCP may provoke infection of a communicating
pseudocyst and if a cavity is demonstrated at ERCP it should not
be filled with contrast and parenteral antibiotics should be
administered. An infected pseudocyst should be treated by prompt
external drainage.

Perforation of a pseudocyst may lead to generalised
peritonitis requiring emergency laparotomy and irrigation of the
peritoneal cavity with drainage of the pseudocyst. The mortality
is high. A pseudocyst may spontaneously rupture into the

intestines leading to sudden disappearance of a pseudocyst and rapid clinical improvement.

Occasionally a mediastinal pseudocyst can occur. Banks et al. (1984) reviewed 30 cases in the world literature and added one. Symptoms of dyspnoea, coughing and chest pain are added to epigastric pain and weight loss. Pleural effusion with a high amylase content is often present with a large mediastinal shadow on chest x-ray. Usually the mediastinal pseudocysts communicates with an abdominal pseudocyst so that effective drainage of both components can be achieved by draining the abdominal part alone.

Pancreatic ascites occasionally follows acute pancreatitis and is usually due to continued leakage of pancreatic fluid following spontaneous decompression of a pseudocyst into the peritoneal cavity (Weaver et al. 1982). The ascites has an elevated amylase level and protein content greater than 2.5 grams per cent (Cameron et al. 1967). Initial treatment is medical with drainage of ascites and intravenous hyperalimentation, but if resolution is not complete by three weeks surgery should be undertaken preceded by endoscopic retrograde pancreatography under antibiotic cover. This usually defines the leak enabling treatment to be planned. Lesions of the body or tail are usually treated by resection, lesions of the head by internal drainage (Weaver et al. 1982).

1.11 Pancreatic Abscess

A pancreatic abscess may be defined as a collection of pus and devitalised tissue both within the pancreas and extending out from it. The incidence is 2-9% of all cases of acute pancreatitis (Altemeier and Alexander, 1963; Warshaw, 1972; Miller et al. 1974; Ranson and Spencer, 1977; Donohue et al. 1980; Becker et al. 1984)

and as high as 25% in fulminating pancreatitis (Kune and King, 1973;
Warshaw, 1980). Pancreatic abscesses are present in 16-65% of
patients dying from acute pancreatitis (Warshaw, 1972).

A pancreatic abscess may be primary if it develops de novo
and secondary when infection occurs in a pre-existing pseudocyst
(Donohue et al. 1980). A primary pancreatic abscess usually
arises 2-4 weeks after the onset of acute pancreatitis and should
be suspected if the progress of the patient is slow. Some
patients develop abscesses at a much later stage (Miller et al.
1974; Shi et al. 1984). The clinical features are tachycardia,
pyrexia, abdominal pain and distension and an epigastric mass is
present in 50% (Warshaw, 1972). Usually there is a leucocytosis
$15-20x10^9$/L, but serum amylase is elevated in only 30% of patients
and blood cultures are usually negative. Plain abdominal radiology
reveals extraluminal gas in some patients ('soap bubble sign').
Indium-111-labelled leucocyte scanning may detect abscess formation
in acute pancreatitis (Fawcett et al. 1979) but is generally
disappointing.

Computerised tomography and ultrasonography have facilitated
accurate diagnosis enabling differentiation of pancreatic
swellings without cyst or abscess formation (pancreatic phlegmon)
(Jeffrey et al. 1982; McKay et al. 1982; Ranson et al. 1985).
Serial ultrasound scanning has demonstrated progression of
pancreatic swellings to cyst or abscess formation in some
patients (McKay et al. 1982) but computerised tomography is more
accurate than ultrasound for abscess detection (McClave et al.
1986). Delays in diagnosis of have therefore been reduced and the
process can be facilitated by CT-guided needle aspiration (Hill et
al. 1984; Crass et al. 1985).

The abscess may be sterile, but when organisms are grown the infection is usually polymicrobial (Aranha et al. 1982; Kaushik et al. 1984; Kune, 1982). The most common organisms grown are E.Coli, enterococcus and klebsiella though pseudomonas, bacteroides, proteus, staphylococcus and streptococcus may be present.

The pancreatic abscess may rupture into the peritoneal cavity, stomach or duodenum, common bile duct, splenic flexure of the colon, bladder or bronchus. Obstruction of the duodenum, common bile duct or colon may occur (Shi et al. 1984; Kaushik et al. 1984). Vascular complications are not uncommon, particularly after surgical or spontaneous decompression of an abscess and include erosion of local vessels causing haemorrhage (Shi et al. 1984) and thrombosis of the hepatic, portal or systemic veins. The abscesses are often multilocular and are usually situated in the retroperitoneal space, commonly retropancreatic, rather than in the lesser sac (Shi et al. 1984). The abscesses are invasive in nature (Warshaw, 1972) probably due to a combination of enzyme digestion and bacterial infection. They often extend into the root of the transverse mesocolon, root of the small bowel mesentery, paracolic gutters and subdiaphragmatic spaces.

Treatment of pancreatic abscesses involves an early laparotomy and extensive debridement of all necrotic tissue with wide sump drainage of all peripancreatic tissues via multiple drains. The inframesocolic approach or from lateral to the flexures of the colon is best in view of the predominantly retroperitoneal nature of the pathology (Hubbard et al. 1972; Camer et al. 1975; Shi et al. 1984). Duodenal obstruction may necessitate gastro-jejunostomy. Colonic necrosis may require resection with colostomies. 'Marsupilisation' of the abscess (suturing its wall to the abdominal

wall) has been used (Bolooki et al. 1968; Davidson and Bradley, 1981) but usually the wall is ill defined and cannot be sutured. An open packing technique to facilitate drainage enables removal of further areas of necrotic tissue and pus at change of dressings (Bradley and Fulenwider, 1984). Percutaneous drainage of pancreatic abscesses after CT localisation has been achieved (Karlson et al. 1982) but their multilocular nature and the large necrotic tissue fragments present within them would seem to contraindicate this approach.

Further surgical drainage is required in about 30% of patients after an apparently adequate initial procedure (Warshaw, 1974; Becker et al. 1984). Continued sepsis is a major factor contributing to the high mortality rate. Without drainage pancreatic abscess is uniformly fatal. With drainage the reported mortality ranges from 14-75% (Warshaw, 1974; Owens and Hamit, 1977; Becker et al. 1984; Shi et al. 1984; Bradley and Fulenwider, 1984). The main complications are continued sepsis, haemorrhage, pancreatic fistula and organ failure. A good outcome depends upon early diagnosis and aggressive treatment and even then the clinical course is usually complicated and protracted.

1.12 Vascular Complications

Patients with acute pancreatitis may develop gastric or duodenal erosions resulting in haematemesis or melaena which usually stops with conservative treatment. Massive bleeding occurs in 1.7-2.5% of patients with pancreatitis (Trapnell, 1971; Marks et al. 1967) and may be associated with necrotising pancreatitis, pancreatic abscess or a pseudocyst. Bleeding may be gastrointestinal, retroperitoneal or intraperitoneal and is a

frequently lethal complication with reported mortality of 50-70%
(Jordan and Spjut, 1972; Stroud et al. 1981).

Erosion of vessels related to the pancreatic inflammatory
process can lead to true aneurysms or pseudoaneurysms when a
vessel bleeds into a pseudocyst. Venous erosion is a less
frequent cause of massive haemorrhage. The most commonly involved
major artery is the splenic (Gadacz et al. 1978; Stroud et al.
1981) followed by the gastroduodenal, left gastric and right
colic arteries. Erosion of a pseudocyst into an adjacent viscus
can also produce profuse bleeding, particularly into the stomach,
duodenum or colon but also the oesophagus or common bile duct.
Spontaneous pre-operative bleeding from pseudocysts is seen in
approximately 7.5% with 60% mortality (Boggs et al. 1972), but
bleeding can also occur after drainge of a pseudocyst. Frey
(1978) recommended selective angiography of the superior
mesenteric artery and coeliac axis as part of the pre-operative
evaluation of pseudocysts to diagnose pseudoaneurysms. 'Hemosuccus
Pancreaticus' is the term used to describe bleeding from the
pancreatic duct into the duodenum. This may be seen
endoscopically and occurs when major haemorrhage communicates
with the pancreatic ductal system (Bivens et al. 1978).

Emergency selective mesenteric angiography and arterial
embolisation have been applied to some cases of massive
haemorrhage with limited success (Frey et al. 1982; Steckman et al.
1984) but the mainstay of treatment remains surgical with
ligation of bleeding vessels away from the inflammatory process
and appropriate surgical treatment of the pancreatic pseudocyst,
necrosis or abscess.

Thrombosis of major veins is uncommon but when it occurs it

usually involves the splenic vein. This can lead to left-sided

portal hypertension and bleeding from gastro-oesophageal varices.

CHAPTER TWO

PROTEASE INHIBITORS WITH SPECIFIC REFERENCE TO THEIR
ROLE IN ACUTE PANCREATITIS

2.1 Human Plasma Protease Inhibitors

a) Introduction

The plasma protease inhibitors contribute by weight the third largest group of functional proteins in human plasma after albumin and immunoglobulins. They represent nearly 10% of the total protein in plasma. A list of the principal protease inhibitors in human plasma is given in Table 8. $Alpha_1$ antiprotease and $alpha_2$ macroglobulin account for more than 90% of the protease inhibitory capacity of plasma. Concentrations of $alpha_1$ antiprotease and $alpha_2$ macroglobulin are 1.1-2.4 g/L and 1.45-3.7 g/L respectively (Leicester Royal Infirmary normal ranges). However the molar concentration of $alpha_1$ antiprotease is 5-10 times that of $alpha_2$ macroglobulin.

Table 8

Principal Protease Inhibitors in Human Plasma

Name	Concentration g/L	Mol Wt. (daltons)	No. of polypeptide Chains
$Alpha_1$ antiprotease	1.75 ± 0.35	52,000	1
$Alpha_2$ macroglobulin	2.6 ± 0.6	720,000	4
$Alpha_1$ antichymotrypsin	0.49 ± 0.07	69,000	1
Inter-alpha-trypsin inhibitor	0.5	160,000	1
Antithrombin III	0.24 ± 0.02	65,000	1
$Alpha_2$ antiplasmin	0.07 ± 0.01	70,000	1
$C\bar{1}$-inhibitor	0.24 ± 0.03	70,000	1

The antiprotease mechanism not only manifests itself in diseases such as acute pancreatitis, but in everyday control of a variety of events associated with connective tissue turnover, coagulation, fibrinolysis, complement activation, and inflammatory reactions. It is extremely efficient in preventing the existence of active proteases in the circulation, a state which would be incompatible with life.

Some of the plasma inhibitors are congenitally deficient in certain individuals. When this occurs the development of a specific disease state may help determine a physiological function of the inhibitor, as in the case of $alpha_1$ antiprotease deficiency causing familial emphysema. The difficulty in determining the role of protease inhibitors could be easily clarified if the rate of complex formation between inhibitor and suspected enzyme were available together with the distribution of the enzyme in question among all the inhibitors in plasma. It is difficult without this information to determine which reactions are physiologically significant. In vivo half-times for inhibition have been calculated and indicate that some of the proposed functions of plasma inhibitors are too slow to be of physiological significance. For most inhibition reactions a half-time of 100 msecs is the maximum that should be allowed for effective control of a particular enzyme (Travis and Salvesen, 1983). Kinetic data now becoming available suggest that it is probable that several plasma inhibitors specifically control the activity of individual proteases, and that many of the currently used names must be revised as their functions become more clear.

There are at least nine separate well-characterised proteins in human plasma that share the capacity to inhibit the activity

of various proteases. Several inhibitors appear to share
features, in particular their mechanism of action, but they are
not sufficiently homologous to be considered as inhibitor
families and only inter-alpha-trypsin inhibitor seems to be
related to any of the families of proteases suggested by
Laskowski and Kato (1980).

b) Mechanism of Action

Most protein protease inhibitors act competitively by
allowing their target enzyme to bind directly to a substrate-like
region contained within the amino acid sequence of the inhibitor
(Laskowski and Kato, 1980). This reaction between enzyme and
inhibitor is essentially second order and the resultant complex
contains one molecule of each reactant with the exception of
those inhibitors that contain more than one inhibitory domain
(the "multiheaded inhibitors"). To be an efficient inhibitor the
protein must combine rapidly with its target proteinase to form
a stable complex. Apparent second order association rates are
generally faster than $10^5 M^{-1} sec^{-1}$ and dissassociation rates are
usually less than $10^5 M^{-1} sec^{-1}$ giving an equilibrium constant of
10^{10} or greater.

Inhibition occurs as a consequence of binding of the active-
site substrate-binding region of a protease to the corresponding
substrate-like region (reactive site) on the surface of the
inhibitor. At or near the centre of the reactive site is an amino
acid residue specifically recognised by the primary substrate
binding site of the target protease and termed the P_1 residue
(Schechter and Berger, 1967). Adjacent to P_1 in the direction of
the carboxy terminus is the residue referred to as P_1'. It is the
peptide bond joining these two residues, known as the reactive

site peptide bond, that is hydrolysed during complex formation between inhibitor and its target enzyme. Other interactions, including hydrogen bonds, Van der Waal's forces and possibly salt bridges, in the close contact area between the reactive site and the corresponding substrate-binding region of the protease all contribute to the characteristic stability of the protease/antiprotease complex.

c) Alpha$_1$ antiprotease

Alpha$_1$ antiprotease has the highest molar concentration of all inhibitors found in plasma, 5-10 times that of alpha$_2$ macroglobulin. Its concentration in normal plasma is approximately 1.1 - 2.4 g/L (Leicester Royal Infirmary reference range). It was originally named alpha$_1$ antitrypsin (Schultze et al. 1962) because of its ability to inactivate pancreatic trypsin, but in vitro it actually inhibits a wide variety of serine proteases including trypsin, chymotrypsin and elastase. In vivo it is most effective at inhibiting elastase. Alpha$_1$ proteinase inhibitor (Pannell et al. 1974) or alpha$_1$ antiprotease are thus better names.

Interest in alpha$_1$ antiprotease was aroused because individuals with circulating levels of this inhibitor less than 15% of normal are susceptible to the development of emphysema at an early age. It therefore has an important role in controlling proteolysis of the connective tissue of the lung.

Human alpha$_1$ antiprotease is a glycoprotein of approximately 52,000 daltons with 12% carbohydrate. The inhibitor exists as a single polypeptide chain with no internal disulfide bonds and only a single cysteinyl residue normally intermolecularly disulfide-linked to either cystein or glutathione. The primary

structure of the inhibitor has been elucidated through both classical protein chemistry techniques (Carrell et al. 1982) and cloning and sequencing of cDNA from baboon and human $alpha_1$ antiprotease (Kurachi et al. 1981). The results indicate that the mature protein consists of 394 amino acids with an amino terminal glutamic acid and a carboxy terminal lysine.

Human $alpha_1$ antiprotease occurs in at least 20 different forms in the general population. A classification system based on the electrophoretic mobility of the inhibitor isoforms in individual samples has been developed (Pierce et al. 1976). This method of phenotyping known as the Pi system refers to the commonest genotype of $alpha_1$ antiprotease as Pi_m with the phenotype being Pi_{mm}. However, even in such homozygous individuals there are at least five isoinhibitor forms occurring as two major and three minor forms. Differences in the carbohydrate side chains provide much of the explanation for the microheterogenicity of $alpha_1$ antiprotease in a homozygous individual.

Data clearly indicate at least one fundamental difference in the primary structure of the various inhibitors secreted by individual $alpha_1$ antiprotease genotypes. Such studies were initiated to explain the low concentration of $alpha_1$ antiprotease in the plasma of individuals first described as developing lung disease. This group secretes a form of $alpha_1$ antiprotease whose electrophoretic pattern, referred to as Pi_{zz}, indicates that the isoinhibitor forms have isoelectric points further away and higher up than that of Pi_{mm}. The Z inhibitor is fully active, but is present in serum at only 15-20% of the normal concentration (Jeppson et al. 1978) with plasma concentration of less than

0.15 g/L. The problem in Pi_{zz} individuals is one of secretion
rather than synthesis as inclusion bodies consisting primarily of
an insoluble polymerized form of $alpha_1$ antiprotease are
localised within the rough endoplasmic reticulum of their
hepatocytes (Eriksson and Larsson, 1975). A single amino acid
replacement of glutamic acid in $alpha_1$ Pi_{mm} for lysine in $alpha_1$
Pi_{zz} at position 342 in the amino acid sequence produces
conformational changes reducing the rate of processing with
constant retention of $alpha_1$ Pi_{zz} in the liver. A similar
explanation probably applies to other varients as well, including
the Pi_s genotype for which a glutamic acid to valine substitution
has been reported at residue 264 (Hercz and Harpaz, 1980).

The control of elastolytic activity secreted primarily by
neutrophils appears to be the primary function of this protein.
An individual with normal serum levels of $alpha_1$ antiprotease has
sufficient active inhibitor to control proteolysis in the lung.
However, upon inhalation of foreign particulate matter, cigarette
smoke for example, a protease-antiprotease imbalance can be
brought about. This occurs because the foreign matter contains
oxidants which directly reduce $alpha_1$ antiprotease levels in
lungs or serum. Alternatively activation and recruitment of
phagocytic cells may lead to increased oxidant levels causing
$alpha_1$ antiprotease inactivation. In any disease in which the
primary defect is abnormal connective tissue turnover, the major
cause may be inactivation of $alpha_1$ antiprotease, presumably by
phagocytic cells attracted to the tissue in question. For
example, in immune complex diseases neutrophils and/or
macrophages may migrate to the disease site and increase oxidant
levels locally thus inactivating $alpha_1$ antiprotease. The result

would be the same as in the emphysema condition with a change in equilibrium between alpha$_1$ antiprotease and the proteases released by the phagocytic cells.

Human alpha$_1$ antiprotease has been shown to inactivate virtually all mammalian serine proteases tested to date including pancreatic and neutrophil elastase, pancreatic trypsin and chymotrypsin, neutrophil cathepsin G, thrombin, plasmin, acrosin, tissue kallikrein, factor Xa, factor XIa, skin and synovial collagenases and urokinase. However, inactivation of an enzyme is not, in itself, a sufficient criterion for a physiological role, as outlined earlier. The primary function of alpha$_1$ antiprotease is to control the activity of neutrophil elastase since the constant for the association of inhibitor and enzyme is more than tenfold higher than that of other proteases tested to date.

The binding of alpha$_1$ antiprotease with serine proteases occurs through strong interactions presumably caused by proteolytic attack on the inhibitor, the latter acting initially as a substrate for the enzyme. The reactive site serine residue of the protease appears to be involved in some type of covalent bonding with the inhibitor to stablise the inhibitor-enzyme complex. There are also substantial ionic and hydrophobic components involved in the formation of the complex. The inhibition is almost instantaneous and is stoichiometrically 1:1 on a molar basis for all the various proteases (Bundy and Mehl, 1959).

During complex dissociation, some active enzyme and a modified form of the inhibitor are released. The molecular weight of the modified inhibitor is reduced to less than 50,000 daltons with cleavage of a small peptide of 8,000 daltons from the N-

terminal end (Johnson and Travis, 1976).

Crossed immunoelectrophoresis (Ohlsson and Tegner, 1973) can be used for tracing complexes between alpha$_1$ antiprotease and proteases in biological fluids. The mobility of complexes on agar gel electrophoresis is different from that of free alpha$_1$ antiprotease. The more cationic the enzyme, the more cationic the complexes. The in vitro half-life of trypsin alpha$_1$ antiprotease complexes is 4-14 days (Moroi and Yamasaki, 1974). However, in vivo alpha$_1$ antiprotease may only be a temporary inhibitor of trypsin (Oda et al. 1977). Trypsin in complex with alpha$_1$ antiprotease administered intravenously has a circulating half-life of 45 minutes in the dog (Ohlsson et al. 1971) and 3.5 hours in man (Ohlsson and Laurell, 1976). The elimination of protease-alpha$_1$ antiprotease complexes is not adequately expressed as a simple exponential decay. Evidence for the dissociation of trypsin-alpha$_1$ antiprotease complexes is implied by the appearance of trypsin-alpha$_2$ macroglobulin complexes in the blood of both dog and man. It appears that trypsin is tranferred to alpha$_2$ macroglobulin and the trypsin-alpha$_2$ macroglobulin complexes are rapidly eliminated from the circulation. The released alpha$_1$ antiprotease molecules are not able to bind protease again (Balldin et al. 1978a; Johnson and Travis, 1976; Oda et al. 1977). This key role of alpha$_2$ macroglobulin in the elimination of trypsin may not be true for other proteases such as chymotrypsin (Balldin, 1980).

Alpha$_1$ antiprotease may therefore serve in part as a carrier protein for proteases particularly in the extracellular space where the concentration of alpha$_2$ macroglobulin is low (Ganrot, 1972; Ohlsson et al. 1971; Ohlsson and Laurell, 1976; Beatty et

al. 1982).

d) Alpha$_2$ Macroglobulin

Human alpha$_2$ macroglobulin is a glycoprotein with molecular weight 720,000 daltons. It contains 8-11% carbohydrate (Dunn and Spiro, 1967) and is present in plasma at concentrations of 1.45 - 3.7 g/L (Leicester Royal Infirmary reference range). It is relatively easy to isolate because of its large molecular weight and the purified protein is composed of a tetramer of identical sub-units of approximately 185,000 daltons linked in pairs by disulfide bonds. Two pairs associate by non-covalent bonds to form the native tetrameric molecule (Jones et al. 1972). Each sub-unit contains approximately 1450 amino acids with an amino terminal serine and carboxyterminal alanine.

Alpha$_2$ macroglobulin binds trypsin, chymotrypsin, elastase, collagenase, papain, cationic aspartate, amino transferase, subtilin A, plasmin, thrombin and kallikrein (Barrett and Starkey, 1973). Complexes of alpha$_2$ macroglobulin with most endoproteases are characterised by sub-unit molecular weights of 80,000-100,000 daltons (Harpel, 1973).

The reaction between alpha$_2$ macroglobulin and protease is unique in at least one respect, the retention of proteolytic activity of bound protease against low-molecular weight substrates (Haverback et al. 1962; Rinderknecht and Geokas, 1973; Hermon-Taylor et al. 1981). This contrasts with the almost total inhibition of bound protease against high-molecular weight substrates. The activity of alpha$_2$ macroglobulin-protease complexes against small-molecular-weight substrates can be inhibited by small protein protease inhibitors such as pancreatic trypsin inhibitor, but not by larger ones such as soya bean

trypsin inhibitor or trasylol (Eddeland and Ohlsson, 1978a).
Clearly in the complexes the active site of the protease is quite
open, but the access of substrate and inhibitors to this site is
sterically hindered.

Alpha$_2$ macroglobulin thus exhibits non-active site
directed inhibition of virtually all endoproteases. Although we
now have a clear impression of the unique mechanism by which
alpha$_2$ macroglobulin functions, little is known of its
physiological role, principally because it reacts with such a
vast array of proteases having different specificity, catalytic
mechanisms and tissue sources.

The characteristics of the reaction of protease with
alpha$_2$ macroglobulin is so markedly different from all other
known inhibitors that some scientists question whether it should
be put in this class at all (Laskowski and Kato, 1980). The 'Trap
Hypothesis' was proposed by Barrett and Starkey (1973) to explain
the mechanism by which alpha$_2$ macroglobulin inactivates protease.
The hypothesis suggests the following reaction between protease
and alpha$_2$ macroglobulin:

$$\alpha 2M + P \longrightarrow \alpha 2M\text{-}P \longrightarrow \alpha 2M^*\text{-}P \longrightarrow \alpha 2M^*\text{-}(P)$$
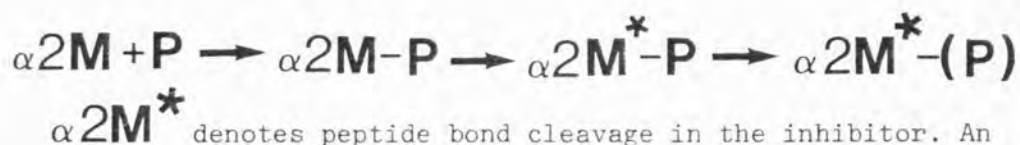
$\alpha 2M^*$ denotes peptide bond cleavage in the inhibitor. An
active endoprotease is pictured as recognising a particular
sequence in the alpha$_2$ macroglobulin sub-unit to form a loose
complex (step 1).Proteases with vastly different specificities
recognise a 'bait region' in the middle of the alpha$_2$
macroglobulin which is a relatively short stretch of residues
containing a sequence of amino acids reflecting the substrate
specificity of all endoproteases. The target bond is then

hydrolysed (step 2) causing a rapid conformational change which physically entraps the enzyme molecule within the bulk of the alpha$_2$M* molecule (step 3). Entrapment of the protease results in a sterically induced blockage of some of its properties symbolised here as (P).

Thus the proposed conformational alteration is the key to the action of alpha$_2$ macroglobulin causing inhibition by hindering the access of high molecular weight proteins to the entrapped protease. The change can be demonstrated by an increased rate of migration of the complexes on polyacrylic gels (Barrett et al. 1979) and by sedimentation experiments, but the most convincing evidence comes from electron micrographs revealing a more compact shape for alpha$_2$ macroglobulin protease complexes relative to native alpha$_2$ macroglobulin (Barrett et al.1974).

Only molecules of very high molecular weight can act by this mechanism. The cooperation of the sub-units and the involvement of liberated sulphadyl groups and metal ions seems to be important in producing the conformational changes in alpha$_2$ macroglobulin (Van Leuven, 1984). A three dimensional model has been proposed recently compatible with the trap hypothesis (Feldman et al. 1985) and a schematic mechanism of the interaction of alpha$_2$ macroglobulin with protease is given in figure 1.

Complexes formed between alpha$_2$ macroglobulin and proteases are so stable that no leakage of protease, as measured by transfer to other inhibitors, has ever been detected even after prolonged incubation with alpha$_1$ antiprotease, soya bean trypsin inhibitor or C$\bar{1}$-inhibitor. Once bound a protease cannot

Figure 1

Schematic representation of the "trapping reaction" of
alpha$_2$ macroglobulin with protease

-S-S-   Disulphide bridges linking polypeptide chains.
----   Non covalent interactions holding pairs of
         polypeptide chains together to form a tetramer

Thiol ester subsequently   (⊖)
hydrolysed

Bait region
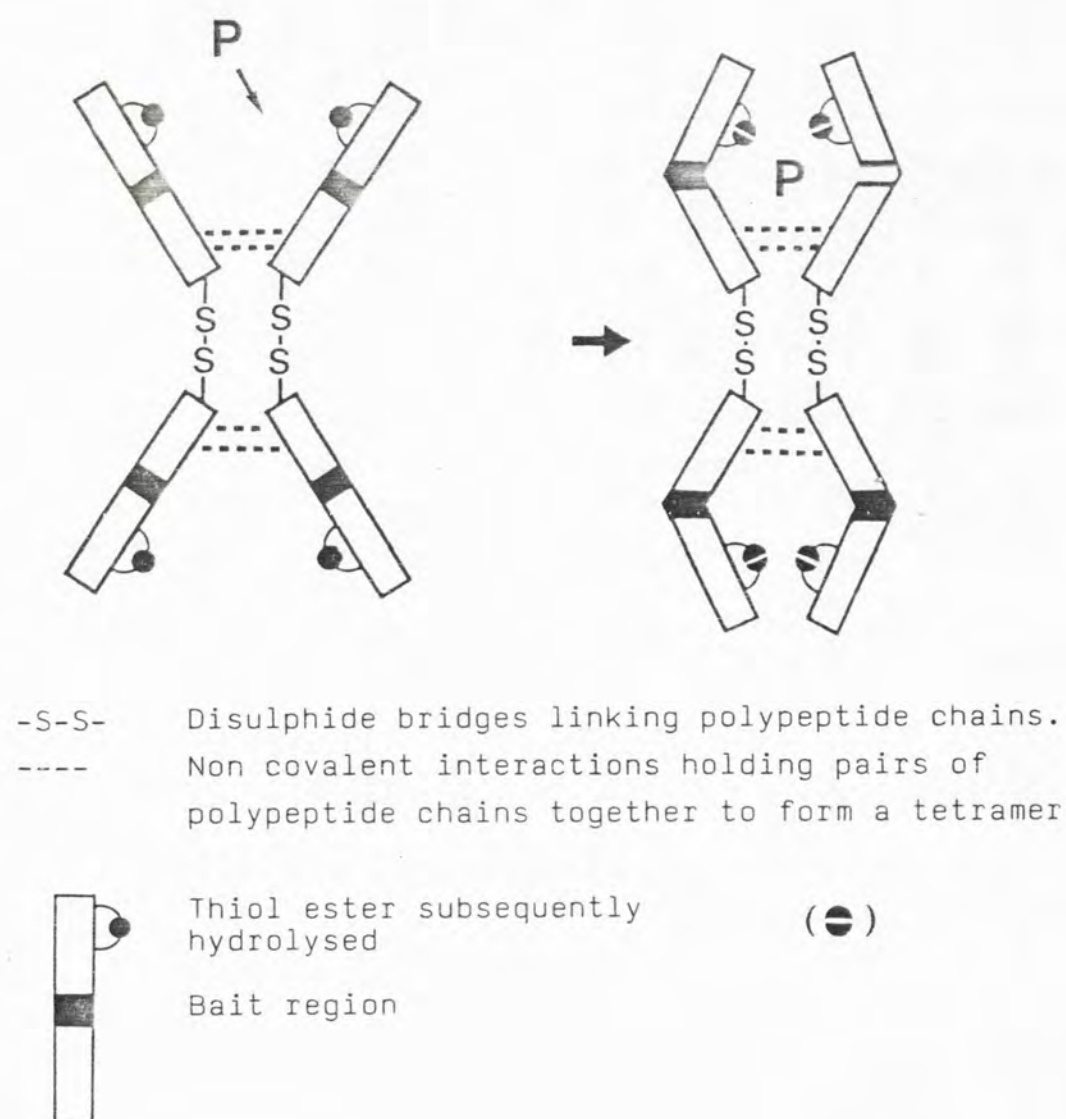
be displaced from its complex by another protease.

Given that binding of protease to alpha$_2$ macroglobulin is essentially irreversible, the only meaningful kinetic parameters, at least from a physiological point of view, is the rate of binding. Conventional estimates of such rates are complicated by the fact that alpha$_2$ macroglobulin-protease complexes retain variable amounts of proteolytic activity. Recently, however, several workers determined the apparent K_{ass} of various proteases with alpha$_2$ macroglobulin yielding values of 1.5×10^7 $M^{-1}sec^{-1}$ for bovine trypsin at $25°C$, 4.0×10^7 M^{-1} sec^{-1} for neutrophil elastase, 3.1×10^6 M^{-1} sec^{-1} for cathepsin G, 2.0×10^6 M^{-1} sec^{-1} for bovine chymotrypsin, 4.8×10^4 M^{-1} sec^{-1} for plasma kallikrein, 5.0×10^2 M^{-1} sec^{-1} for thrombin and 6.7×10^7 M^{-1} sec^{-1} for factor Xa. The different rates of inhibition of proteases by alpha$_2$ macroglobulin probably directly reflect the rates of hydrolysis of the target peptide bonds in the bait region, as once the trap is sprung the conformational changes in the molecule probably proceed at a standard rate determined solely by the structure of the inhibitor molecule.

One or two molecules of protease are trapped by one molecule of alpha$_2$ macroglobulin (Ganrot, 1966; Ganrot, 1967a; Harpel, 1973). The actual binding ratio varies with experimental conditions. The most simple explanation is that alpha$_2$ macroglobulin can bind two molecules of protease if the rate of conformational change is much slower than the initial binding step whereas only one molecule is bound if the first step is much slower than the conformational change, i.e. two molecules of protease can be trapped, but only one is required to spring the trap.

The trap theory of protease inhibition by alpha$_2$ macroglobulin is well supported but the validity of the hypothesis has been challenged recently following the discovery that proteases can become covalently attached to alpha$_2$ macroglobulin. This covalent reaction occurs at a site other than the active site of the protease and the extent of covalent linking is dependent on the protease and varies considerably (Wu et al. 1981). These covalent bonds may help stabilise complexes or act as a trigger promoting the conformational change in the molecule.

Alpha$_2$ macroglobulin also binds some proteases quite separately from its reaction with active protease. This 'adherence' reactivity is unrelated to trapping and covalent linking reactions and is probably mediated by different parts of the molecule. Binding occurs largely through ionic and hydrophobic interactions and several basic proteins including carboxypeptidase A are bound to the acidic alpha$_2$ macroglobulin molecule. This binding is reversible and protease can be displaced by other inhibitors. Adherence to alpha$_2$ macroglobulin does not prevent trapping of proteases and is not associated with any conformational change. There is no evidence that the adherence reaction serves any physiological role.

Upon addition of small quantities of enzyme to a mixture of alpha$_2$ macroglobulin and other plasma inhibitors, complexes with both inhibitors are formed in a ratio that depends upon the relative values of the second order rate constants for the associations. However, the complexes with a typical inhibitor dissociate, albeit slowly, while the alpha$_2$ macroglobulin complex is 'irreversible'. The result in an experiment is that all the

proteases are ultimately transferred to alpha$_2$ macroglobulin
unless there is enough enzyme to saturate alpha$_2$ macroglobulin.
In vivo this effect is greatly exaggerated as alpha$_2$
macroglobulin-protease complexes are cleared very rapidly with a
plasma half-life of approximately ten minutes in man (Balldin et
al. 1978a). Clearance of the complexes is by receptor-mediated
endocytosis in the reticulo-endothelial system. Thus essentially
all injected proteases are cleared by the alpha$_2$ macroglobulin
pathway.

In evolutionary terms it would appear that alpha$_2$
macroglobulin is an ancient protein as homology with species as
primitive as hagfish and lamprey has been demonstrated; these
incidentally are the species possessing the earliest forms of
immunoglobulins. Thus, whatever its physiological function, it is
of early and presumably fundamental derivation. Its importance is
indicated by the fact that no total deficiency of alpha$_2$
macroglobulin is reported despite large scale searches (Laurell
and Jeppson, 1975).

The role of alpha$_2$ macroglobulin in the control of
coagulation (Abildgaard, 1979) and fibrinolysis (Mullertz, 1979)
have been examined. The inhibitor does not inhibit either factor
XIIa or XIa but it does complex with factor Xa and thrombin,
albeit at slow rates. Comparison with antithrombin III indicates
that it is a far less effective inhibitor during normal
coagulation. Therefore if alpha$_2$ macroglobulin aids in
controlling the coagulation cascade, it is probably through
inhibition of plasma kallikrein - a role it shares with C1
inhibitor. Alpha$_2$ macroglobulin can only act in a minor role in
controlling normal fibrinolysis, the main regulator being alpha$_2$

antiplasmin. It is only when the local or systemic concentration of an inhibitor becomes lowered significantly that alpha$_2$ macroglobulin exerts significant control over the coagulation and fibrinolytic systems, for example during pathological episodes such as DIC during which the majority of antithrombin III is exhausted and during thrombolytic therapy, when alpha$_2$ antiplasmin levels drop precipitously.

The rapid clearance of alpha$_2$ macroglobulin-protease complexes by reticuloendothelial cells, particularly the Kupffer cells of the liver (Ohlsson, 1971; Ohlsson and Laurell, 1976) suggests that it controls proteolytic activity in the circulation by removing enzymes and that inhibition is largely incidental. Alpha$_2$ macroglobulin-trypsin complexes have a plasma half-life of seven minutes in the dog (Ohlsson 1971) and ten minutes in man (Ohlsson and Laurell, 1976). This compares with a half-life in excess of 100 hours for free alpha$_2$ macroglobulin in man (Blatrix et al. 1973). The suggestion that protease inhibitors in plasma function to complex protease and 'transfer' these to alpha$_2$ macroglobulin for rapid clearance (Ohlsson and Laurell, 1976) is now in doubt because of the finding that the clearance of alpha$_2$ macroglobulin complexes in the mouse is separate from the clearance of other inhibitor complexes (Fuchs et al. 1982). It is not therefore completely clear whether this 'piggyback' mechanism is physiologically significant.

Clearance of alpha$_2$ macroglobulin complexes from the circulation is almost certainly accomplished by their binding to cellular receptors, as shown by their internalisation via receptor-mediated endocytosis by macrophages in vitro (Ohlsson, 1971; Imber and Pizzo, 1981). They are recognised by cellular

receptors and internalised very rapidly (Topping and Seilman,
1979). The complex uptake is independent of the protease involved
so it is the altered conformation of the molecule which appears
to be recognised by the receptor. Alpha$_2$ macroglobulin bound to
the cell surface receptor is located in coated pits and
internalisation is via the formation of coated vesicles
(Willingham et al. 1979). There is rapid intracellular
degradation of ingested proteins (Van Leuven et al. 1979;
Goldstein et al. 1979). The receptors appear to be recycled onto
the cell surface in association with membrane recycling (Van
Leuven, 1984).

By virtue of its broad specificity, alpha$_2$ macroglobulin may
contribute as a defence against invading pathogens and parasites
by inactivating proteases elaborated by these organisms, for
which no specific inhibitor is available, should they reach the
bloodstream. It may also function as an important backup
inhibitor when levels of more specific inhibitors are lowered.
Alpha$_2$ macroglobulin should perhaps be considered as part of the
immune defence system.

e) Alpha$_1$ Antichymotrypsin

Alpha$_1$ antichymotrypsin is a glycoprotein with inhibitory
activity towards chymotrypsin. It is a major acute phase protein
whose concentration increases rapidly and dramatically after
surgery, burns and in acute pancreatitis (Aronsen et al. 1972;
Lasson and Ohlsson, 1984; McMahon et al. 1984; Goodman et al.
1986). With the exception of C-reactive protein whose
concentration in normal serum is usually undetectable, alpha$_1$
antichymotrypsin shows the most immediate response as an acute
phase protein, doubling in concentration within eight hours of

insult. Thus this inhibitor may play an important role in controlling specific systems associated with inflammatory episodes. In acute pancreatitis maximum levels are reached on day five and the rise in levels in severe attacks may lag behind the rise seen in mild attacks (McMahon et al. 1984), a difference not seen with C-reactive protein.

Studies on the structure and function of $alpha_1$ antichymotrypsin, unlike those of $alpha_1$ antiprotease and $alpha_2$ macroglobulin, are quite limited. This is partly due to the failure to demonstrate any clear function for $alpha_1$ antichymotrypsin and because of its relatively low concentrations in plasma (0.25 - 0.75 g/L).

$Alpha_1$ antichymotrypsin stoichiometrically inhibits chymotrypsin to give a 1:1 complex. The molecular weight of $alpha_1$ antichymotrypsin is 69,000 daltons. It contains 26% carbohydrate and has some structural homology with $alpha_1$ antiprotease and antithrombin III, suggesting a common evolutionary origin.

Human $alpha_1$ antichymotrypsin is a specific inhibitor of chymotrypsin-like proteases forming stable complexes with bovine and human chymotrypsin, human neutrophil cathepsin and dog and human mast cell 'chymases'. No inhibition of human trypsin or neutrophil elastase has been found. The role of this inhibitor is not proven, though it may involve the control of connective tissue breakdown and cell-cell interactions. A more likely role is the regulation of angiotensin II production where both cathepsin G and mast cell chymase can act as effective angiotensin converting enzymes and both are inhibited by $alpha_1$ antichymotrypsin. Since angiotensin I to angiotensin II

conversion is an inflammatory reaction, the effect of increased synthesis of alpha$_1$ antichymotrypsin would be to produce sufficient inhibitor to control smooth muscle contraction and aldosterone production. Alpha$_1$ antichymotrypsin may also have a role in controlling the chymotrypsin-like enzymes found in chromatin and it may even be a protective agent secreted by tumour cells to protect against lysis by natural killer lymphocytes (Travis and Salvesen, 1983).

f) Inter-Alpha-Trypsin-Inhibitor

Inter-alpha-trypsin-inhibitor is a serine protease inhibitor first described and isolated from plasma by Steinbuch and Loeb in 1961. Its function was not established until 1965 when it was found to have inhibitory activity against trypsin.

Inter-alpha-trypsin-inhibitor has a molecular weight of 160,000 daltons and is a single chain glycoprotein containing 8.4% carbohydrate and nearly 1.0 gram atoms of zinc. It rapidly inhibits human and bovine trypsins and chymotrypsins by forming 1:1 complexes, although those with the human enzymes are not stable. The mechanism of complex formation is not known and as yet no physiological role for inter-alpha-trypsin inhibitor has been elucidated. The inhibitor does combine rapidly with pancreatic proteases but it is doubtful whether it plays a role in controlling them, even though structural studies indicate its relationship to the pancreatic trypsin inhibitor (Kunitz) family of protease inhibitors. Based on its rapid turnover under malignant or inflammatory conditions, it is more likely to be involved in controlling enzymes released from tissues other than the pancreas (Travis and Salvesen, 1983). It accounts for about 3% of total protease inhibitory capacity of serum (Ganrot, 1967b)

and its serum levels fall during acute pancreatitis (Lasson and Ohlsson, 1984).

g) Antithrombin III

Human antithrombin III is a glycoprotein with normal serum concentration of approximately 0.2 g/L. It plays a major role in controlling serine proteases in the coagulation cascade scheme. In particular it inactivates thrombin, an effect enhanced by the presence of heparin (Abildgaard, 1968). The purified protein is a single-chain molecule of 65,000 daltons containing 15% carbohydrate and six disulphide bridges. The amino acid sequence shows extensive homology with $alpha_1$ antiprotease and ovalbumin.

In addition to thrombin, human antithrombin III inactivates factors IXa, Xa and XIa, plasmin, plasma kallikrein, trypsin and chymotrypsin. Rate studies have not been performed on all these proteases to determine the physiological importance, but it is likely that only the reaction with thrombin is significant.

Heparin appears to act as an allosteric activator of antithrombin III effecting a conformational change to accelerate the inhibition of thrombin. In the presence of heparin this inhibitor can rapidly shut down the coagulation process by inactivation of thrombin.

h) Alpha$_2$ Antiplasmin

In 1974 Mullertz postulated the existence of a plasma inhibitor of plasmin separate from $alpha_2$ macroglobulin and $alpha_1$ antiprotease on the basis of the rapid inactivation of this enzyme by an unknown component in plasma. Alpha$_2$ antiplasmin (Antiplasmin/primary plasmin inhibitor/alpha$_2$ plasmin inhibitor) has since been isolated and is a single-chain glycoprotein of 70,000 daltons containing 11% carbohydrate and asparagine

and leucine as the amino and carboxy terminal residues respectively (Wiman and Collen, 1979). The normal plasma concentration is 0.07 g/L.

Alpha$_2$ antiplasmin is an extremely efficient, fast-reacting inhibitor of plasmin. It also inactivates trypsin, plasma kallikrein, chymotrypsin, thrombin, Factor Xa and Factor XIa, but the inhibition of all these enzymes, with the exception of trypsin, is very slow and it is unlikely that alpha$_2$ antiplasmin plays any role in controlling these activities. However, bovine trypsin reacts reasonably rapidly with alpha$_2$ antiplasmin but this inhibitory activity is masked by the higher concentration of other inhibitors in plasma including alpha$_2$ macroglobulin and alpha$_1$ antiprotease. This inhibitor may play some role in controlling tryptic activity in tissues other than blood (Travis and Salvesen, 1983).

Alpha$_2$ antiplasmin plays a central role in the molecular mechanism of fibrinolysis. Control of clot dissolution in vivo requires a continuous replacement at the fibrin surface of plasminogen because of inactivation of plasmin by alpha$_2$ antiplasmin at this site. The plasminogen would then be activated by fibrin bound plasminogen activator molecules.

The half-life of circulating alpha$_2$ antiplasmin-plasmin complexes is about 0.52 days compared with that of 2.6 days for unreacted alpha$_2$ antiplasmin. There is no data to indicate any 'transfer' of alpha$_2$ antiplasmin-complexed plasmin to alpha$_2$ macroglobulin. The inhibitor is temporarily exhausted during thrombolytic therapy because of the formation of complexes with plasmin and patients with alpha$_2$ antiplasmic deficiency are subject to episodes of bleeding. It is unlikely that the

inhibitor has other physiological functions except perhaps the control of one or more forms of plasminogen activator. Alpha$_2$ antiplasmin levels fall in acute pancreatitis in dogs (Izquierdo et al. 1984) suggesting greater activity of plasmin-mediated fibrinolysis.

i)C$\bar{1}$- Inhibitor

C$\bar{1}$- inhibitor, as its name implies, was identified originally as an inhibitor of the activated form of the first component of complement (Levy and Lepow, 1957) and hence it is also referred to as C$\bar{1}$ esterase inhibitor and C$\bar{1}$ inactivator.

Human C$\bar{1}$ - inhibitor is a single chain glycoprotein of 70,000 daltons containing approximately 35% carbohydrate. The normal concentration in plasma is 0.24 g/L. Both C$\bar{1}$r and C$\bar{1}$s are inhibited by C$\bar{1}$- inhibitor. In addition plasma kallikrein, factors XIa and XIIa and plasmin are inactivated by this inhibitor. Trypsin, porcine pancreatic elastase and bovine chymotrypsin are not inhibited.

C$\bar{1}$ r and C$\bar{1}$ s are subcomponents of C$\bar{1}$ and possess only limited proteolytic activity C1r is activated to C$\bar{1}$r by a process believed to involve a change in conformation promoted by C1 - antibody-antigen complexes. This in turn leads to activation of C$\bar{1}$s by limited proteolysis. C$\bar{1}$s activates C4 and C2 by a similar proteolytic cleavage. Thus both enzymes play a critical role in complement activation. C$\bar{1}$-inhibitor is the only known plasma inhibitor of C$\bar{1}$r and C$\bar{1}$s and thus has a pivotal role in controlling activation of the classical pathway of complement. Isolated C$\bar{1}$r and C$\bar{1}$s react to form equimolar complexes with C$\bar{1}$-inhibitor.

Plasma kallikrein is also inactivated by C$\bar{1}$- inhibitor but

although the rate of inhibition is greater than that seen with alpha$_2$ macroglobulin, the concentration of alpha$_2$ macroglobulin in normal plasma is higher. The enzyme may be controlled equally by C$\bar{1}$-inhibitor and alpha$_2$ macroglobulin in plasma (Schapira et al. 1981). This has tremendous potential importance since plasma kallikrein is thought to have two main functions; generation of the pharmacologically active peptide bradykinin from high-molecular-weight kininogen and amplification of the early phase of the intrinsic pathway of blood coagulation through a feedback system to activate factor XIIa. People with C$\bar{1}$-inhibitor deficiency develop hereditary angioneunotic oedema suggesting the main function of the inhibitor is controlling complement activation rather than intrinsic pathway activation or bradykinin generation.

j) Beta$_1$ Anticollagenase

The inhibitory activity in serum towards mammalian collagenases appears to reside in three proteins, alpha$_2$ macroglobulin, beta$_1$ anticollagenase and a third as yet uncharacterised cationic protein (Borth et al. 1981). Human serum beta$_1$ anticollagenase is a single chain glycoprotein of 30,000-33,000 daltons containing only traces of carbohydrate. It specifically inactivates collagenases from skin, rheumatoid synovial fluid, gastric mucosa and granulocytes. Complexes are taken up rapidly by phagocytic cells. Beta$_1$ anticollagenase accounts for only about 5% of anti-collagenase activity in serum. Alpha$_2$ macroglobulin is responsible for most of the remainder. Beta$_1$ anticollagenase with a low molecular weight may have a more important role within the tissues, particularly in synovium.

k) Alpha Cysteine Protease Inhibitor

Plasma contains 0.5 g/L of alpha-cysteine proteinase inhibitor which has at least two forms with molecular weight of 57,000 - 175,000 daltons. The high molecular weight forms are probably multimes. Alpha cysteine protease inhibitor is highly specific for cysteine proteases such as cathepsin H and cathepsin L. The presence of a plasma inhibitor specific for cysteine proteases seems redundant when one considers that there are no known members of the circulating proteases that use a cysteine catalytic mechanism. All the well characterised human cysteine proteases are thought to function within cells. It is probably the release of these intracellular proteases at cell death or other related events that requires the presence of an extracellular antiprotease to prevent harmful protein degradation. Low molecular forms of alpha cysteine protease inhibitor are probably important (like $alpha_1$ antiprotease) in diffusing from blood into tissue spaces to deal with spilt enzymes.

Should cysteine proteases ever find their way into blood they would be inhibited not only by alpha cysteine protease inhibitor but also by $alpha_2$ macroglobulin which also reacts with cathepsins B and H. $Alpha_2$ macroglobulin is probably the more important within the circulation and alpha cysteine protease inhibitor levels are high only to maintain protective levels against cysteine proteases in the intracellular tissue spaces.

1) Other Inhibitors

Numerous reports appear indicating new inhibitors, but most are probably leaking tissue inhibitors or impure examples of already characterised inhibitors. Many intracellular protease inhibitors occur and the importance of mammalian cellular

proteases in the normal functioning and pathological states of cells, with the extensive but controlled amount of proteolysis that occurs have only become appreciated recently. Our knowledge of cellular proteases and their inhibitors is in a state of infancy but is growing rapidly.

Several low molecular weight serine protease inhibitors are found in tissues and secretions but not in plasma (Laskowski and Kato, 1980) including the pancreatic secretory trypsin inhibitor which will be discussed later. Serine protease inhibitors homologous to the Kazal inhibitor have been isolated from the seminal plasma and vesicles in different species. Some proteases may act on B and T cells involved in the regulation of immune function.

Heparin cofactor II is a single chain glycoprotein of 65,000-70,000 daltons which forms a covalent 1:1 molar complex with thrombin. Its action is augmented by heparin. No physiological role has yet been found but it may be involved in the control of thrombin activities.

An inhibitor of protein C is found in serum. It is a glycoprotein with a molecular weight of 96,000 daltons.

m) Summary

In summary, the data now available about plasma antiproteases strongly suggests the following specific inhibitor-target enzyme pairings:

1. Alpha$_1$ antiprotease with neutrophil elastase

2. Antithrombin III with thrombin

3. Alpha$_1$ antichymotrypsin with cathepsin G and mast cell chymase

4. Alpha$_2$ antiplasmin with plasmin

5. C$\bar{1}$-inhibitor with C$\bar{1}$r and C$\bar{1}$s

Human alpha$_2$ macroglobulin, by virtue of its ability to inactivate proteases from all classes, may act as a rapid and efficient clearing agent for these enzymes when they appear free in the circulation. Currently, clear functional roles for inter-alpha-trypsin inhibitor, beta$_1$ anticollagenase and alpha cysteine protease inhibitor are not known, despite the narrow specificity of these inhibitors.

2.2 Rat Plasma Protease Inhibitors

There are interesting differences between rat and human plasma protease inhibitors. The trypsin inhibitory capacity of rat serum is three times greater than that of human serum due to the presence of additional inhibitors (Takahara et al. 1983).

Much of the inhibitory capacity resides with the alpha$_1$ antiproteases. In rat, as in man, multiple forms of alpha$_1$ antiprotease have been identified, but in the rat they appear to be the product of at least two different genes while in man the multiple forms are immunologically identical. The nomenclature is confusing, but the main rat alpha$_1$ antiprotease which accounts for about 68% of the trypsin inhibitory capacity of this fraction (Kuehn et al. 1984) has a molecular weight of 55,000 daltons. It apears to be related to the alpha$_1$ antiprotease of man and is an acute phase protein with plasma levels increasing 35-50% above baseline within twelve hours of turpentine injection. It preferentially inhibits elastase but also inhibits trypsin and chymotrypsin (Frazer et al. 1985). A second alpha$_1$ antiprotease is present accounting for about 14% of the trypsin inhibitory capacity of this fraction with a molecular weight of 64-65,000 daltons (Kuehn et al. 1984). It is antigenically distinct from

the first and plasma levels do not increase during the acute
phase response (though levels of its coding mRNA in liver
increase 300% in 6-9 hours according to Frazer et al. 1985). It
preferentially inhibits trypsin but will also inhibit
chymotrypsin. There may be a third $alpha_1$ antiprotease named
contrapsin by Takahara and Sinohara (1982) accounting for the
remaining 18% of the trypsin inhibitory capacity of this fraction
(Kuehn et al. 1984). It has a molecular weight of 55-65,000 and
preferentially inhibits trypsin.

Two alpha macroglobulins are present in rat plasma each with
a large molecular weight and four sub-units capable of
undergoing conformational changes similar to those occurring in
$alpha_2$ macroglobulin in man. They both inhibit all classes of
endoprotease. The first is $alpha_1$ macroglobulin (Ganrot, 1973)
present in concentrations of 3.88 ± 0.48 g/l. It is not an acute
phase protein and plasma levels fall during acute pancreatitis as
do $alpha_2$ macroglobulin levels in man. The second is $alpha_2$ acute
phase macroglobulin which has a molecular weight of 770,000
daltons and contains 10% carbohydrate. It has an amino acid
composition similar to that of $alpha_1$ macroglobulin (Gauthier and
Mouray, 1976) and is an acute phase protein with levels
increasing 10-100 fold during acute inflammation (Koj et al.
1982).

Two isoforms of murinoglobulin, not found in man, are
present in rat plasma at relatively high concentration ($14.1 \pm$
1.49 g/L) accounting for one third of plasma globulin. They are
monomeric glycoproteins of molecular weight 210,000 daltons with
12% carbohydrate (Saito and Sinohara, 1985). They may have
evolved from an $alpha_2$ macroglobulin homologue in an early

ancestor of the rodent line.

Alpha$_1$ inhibitor$_3$ is found in rat plasma (Esnard et al.
1985) with immunological and antiprotease properties very similar
to those of human inter-alpha-trypsin-inhibitor and the two
proteins are assumed to be homologous. Rat plasma also contains
an antithrombin III, an alpha$_2$ antiplasmin and an alpha cysteine
protease inhibitor similar to those found in man (Esnard and
Gauthier, 1983).

Low molecular weight thial protease inhibitors are found in
rat plasma at low concentration (Iwata et al. 1982) possibly
originating from the tissues.

2.3 Plasma Protease Inhibitors in Acute Pancreatitis

Using immunochemical methods Ohlsson and Eddeland (1975)
found free active proteases (trypsin, chymotrypsin, elastase)
appeared promptly in pancreatic exudate collected from the
surface of the gland in bile-induced pancreatitis in dogs
following complete saturation of alpha$_1$ antiprotease and alpha$_2$
macroglobulin in the exudate. Proteases are soon detectable in
the peritoneal exudate, but initially only in the form of
complexes with alpha$_1$ antiprotease and alpha$_2$ macroglobulin. The
protein content of the peritoneal exudate is approximately one
third of that of plasma and similar reductions are found in the
concentrations of the main protease inhibitors (Wendt et al.
1984). The inhibitor capacity in the peritoneal exudate may be
inadequate to deal with large amounts of protease. Alpha$_2$
macroglobulin consumption is pronounced in the peritoneal fluid
(Lasson and Ohlsson, 1984) and shock developed in a dog model of
acute pancreatitis coincident with saturation of the inhibitors

in the peritoneal exudates (Ohlsson et al. 1971; Ohlsson and
Tegner, 1973; Ohlsson and Eddeland, 1975).

Clearance of complexes from the peritoneal fluid is much
slower than clearance of complexes from plasma, and the complexes
may become more abundant than the free inhibitors in the peritoneal
exudate (Balldin and Ohlsson, 1979). In the peritoneal exudate
5-25% (mean 10%) of alpha$_1$ antiprotease and 45-100% (mean 65%) of
alpha$_2$ macroglobulin were found in complexed form in dogs with
acute pancreatitis (Balldin et al. 1981). Eighty six per cent
saturation of peritoneal alpha$_2$ macroglobulin was reported by
Wendt et al. (1984) who also reported the presence of free
trypsin which was inhibited by adding alpha$_2$ macroglobulin. High
degrees of saturation of alpha$_2$ macroglobulin in the peritoneal
exudate of patients with acute pancreatitis has been shown to be
an ominous sign (Balldin and Ohlsson, 1979; Balldin et al. 1981).

Acute pancreatitis is characterised by the rapid appearance
of pancreatic enzymes in plasma. Increased concentrations of
amylase and lipase are present as active enzymes and are thus
readily detected by bioassay. The elaborate system of protease
inhibitors probably prevents the existence of free proteolytic
enzymes such as trypsin and elastase in plasma, but marked
increases in immunoassayable enzymes are found (Elias et al.
1977; Borgstrom and Ohlsson, 1978; O'Connor et al. 1981)
indicating the presence of circulating inhibitor-enzyme
complexes.

Inhibitor-enzyme complexes enter the circulation both by
direct absorption of exudate into the bloodstream from the
inflamed retroperitoneal tissues and peritoneum as well as via
the lymphatics up the thoracic duct. Whether complex formation

occurs principally in ascitic fluid and oedema fluid adjacent to the inflamed pancreas or whether it occurs to a significant extent in the plasma is still unclear. According to Geokas et al. (1978) substantial amounts of alpha$_2$ macroglobulin-protease complexes entering the circulation from the peritoneal cavity may be responsible for some of the complications of acute pancreatitis such as coagulopathies, acute renal failure and shock. Pancreatectomy, therapeutic peritoneal lavage and thoracic duct drainage have been proposed as methods of limiting the volume of toxic pancreatic broth entering the circulation (see Chapter 1.8).

Normally alpha$_2$ macroglobulin-protease complexes are rapidly removed from the circulation by the reticulo-endothelial system, particularly the Kupffer cells of the liver as discussed previously. In acute pancreatitis the clearance of complexes may be retarded (Hermon-Taylor and Heywood, 1986) and, although the presence of alpha$_2$ macroglobulin-protease complexes in plasma can be hard to detect (Balldin et al. 1981), the plasma of patients with acute pancreatitis displays enhanced esterolytic activity (Worthington and Cuschieri, 1976; Sumi et al. 1978) and degrades parathormone (Brodrick et al. 1981). This indirect evidence is consistent with the presence of alpha$_2$ macroglobulin-protease complexes in plasma and is reinforced by the observation that immunodetectable trypsin was released from the alpha$_2$ macroglobulin of the serum of patients with acute pancreatitis after acid incubation (Brodrick et al. 1979).

If small amounts of complexed alpha$_2$ macroglobulin are present, this may contribute to the multi-organ derangement as the complexes retain activity against low-molecular-weight

substrates (Haverback et al. 1962; Hermon-Taylor et al. 1981; Rinderknecht and Geokas, 1973).

Alpha$_1$ antiprotease is considered an acute phase reactant (Aronsen et al. 1972) and its plasma levels rise significantly in the early stages of acute pancreatitis (Goodman et al. 1986; Lasson and Ohlsson, 1984) reaching a peak at about five days (McMahon et al. 1984). The rapid rise in alpha$_1$ antiprotease seen in mild pancreatitis may be delayed in severe disease (Goodman et al. 1986).

Crossed immunoelectrophoresis (Ohlsson and Tegner, 1973) can be used for tracing complexes between alpha$_1$ antiprotease and protease in biological fluids. The more cationic the protease, the more cationic the complex. Further analysis using specific antibodies reveals the presence of trypsin, chymotrypsin and elastase complexed with alpha$_1$ antiprotease in the peritoneal exudate and blood of patients with acute pancreatitis (Balldin and Ohlsson, 1979; Balldin et al. 1981).

Alpha$_1$ antiprotease may function as a carrier protein by rapidly forming complexes with proteases and subsequently transferring them to alpha$_2$ macroglobulin (Beatty et al. 1982; Ohlsson et al. 1971; Ohlsson and Laurell, 1976). The evidence for this has already been discussed. The high-molecular-weight alpha$_2$ macroglobulin is located mainly intravascularly (Laurell and Jepson, 1975) whereas the low-molecular-weight alpha$_1$ antiprotease can more easily act extravascularly. In severe attacks of pancreatitis, low alpha$_2$ macroglobulin levels and the presence of alpha$_1$ antiprotease-trypsin complexes in blood coincides with delay of the acute phase for alpha$_1$ antiprotease (in which high levels are reached on the 6th day in severe

attacks instead of the 3rd day in other acute phase reactions)
(Aronsen et al. 1972). This is evidence for the protective role
of alpha$_1$ antiprotease in acute pancreatitis. The increased
synthesis of this acute phase protein during the disease
counteracts the alpha$_2$ macroglobulin deficiency.

Plasma alpha$_2$ macroglobulin levels are low on admission and
there is a gradual decrease in levels during the first three days
(Balldin et al. 1981; Goodman et al. 1986; Lasson and Ohlsson,
1984; McMahon et al. 1984) reaching values below 40% of normal in
plasma and peritoneal fluid in severe attacks (Lasson and
Ohlsson, 1984). With increasing severity of pancreatitis, the
more marked and the more sustained is the fall in alpha$_2$
macroglobulin levels (McMahon et al. 1984). Mean plasma levels of
1.35 and 1.36 g/L were found in severe attacks (Balldin et al.
1981; McMahon et al. 1984; Mero et al. 1982). The remaining
alpha$_2$ macroglobulin may have reduced trypsin binding capacity
indicating some circulating alpha$_2$ macroglobulin-protease
complexes.

Part of the decrease in plasma alpha$_2$ macroglobulin levels
can probably be accounted for by correction of the
haemoconcentration seen on admission and possibly by altered
distribution of plasma proteins between intra- and extra-vascular
compartments in severe disease. In these cases there is also a
rapid onset of malnutrition which may depress protein synthesis
in the hepatocyte which might be expected to result in decreased
alpha$_2$ macroglobulin levels. There is no evidence, however, of
gross deficiency in protein synthesis as judged by plasma levels
of acute phase proteins and immunoglobulins. Low levels of
albumin certainly occur (Imrie et al. 1978b) but may be due to

losses from plasma due to increased capillary pore size rather than depressed synthesis. Plasma levels of alpha$_2$ macroglobulin are not depressed during septicaemia (McMahon et al. 1984). The low levels of alpha$_2$ macroglobulin in severe pancreatitis are primarily due to consumptive depletion of plasma alpha$_2$ macroglobulin as alpha$_2$ macroglobulin-protease complexes are removed from the circulation by the reticulo-endothelial system. The continuing decline of plasma alpha$_2$ macroglobulin levels observed in acute pancreatitis suggests that active protease release may be an important factor in pathogenesis for several days after admission.

Plasma alpha$_2$ macroglobulin levels may normalise after one week followed by some overshoot in the next week (Balldin et al. 1978a), though recovery of plasma levels takes a variable length of time from 48 hours to more than 15 days (Nilehn and Ganrot, 1967; Spotti and Holzknecht, 1970).

The measurement of alpha$_2$ macroglobulin-protease complexes in biological fluids is difficult. It can be achieved by immunological methods using antisera against proteases and antiproteases including electroimmunoassay and radioimmunoassay (Eddeland and Ohlsson, 1978b; Harpel, 1973; Laurell, 1972; Ohlsson and Skude, 1976). The inhibitory capacity of alpha$_2$ macroglobulin can be quantitatively studied by titration of patients blood or peritoneal fluid with bovine trypsin. The trypsin binding to alpha$_2$ macroglobulin can be measured using a suitable substrate assuming one molecule of alpha$_2$ macroglobulin binds two molecules of trypsin. Alternatively plasma trypsin can be measured by radioimmunoassay in terms of its immunological concentration and not its enzymic activity (Elias et al. 1977). This can be

compared with total alpha$_2$ macroglobulin concentration to obtain
the percentage of functionally active alpha$_2$ macroglobulin in the
patients serum (Ganrot, 1967a; Sottup-Jensen et al. 1981;
Donnelly et al. 1984). Alpha$_2$ macroglobulin complexes can be
removed from the fluid phase by an immunocapture step using an
antibody directed against alpha$_2$ macroglobulin and bound to an
enzyme. Complexes immobilised on the antibody-containing surface
can be quantified using a suitable substrate.

As far as levels of other plasma inhibitors in acute
pancreatitis are concerned, alpha$_1$ antichymotrypsin is a major
acute phase protein and its plasma concentration rises rapidly in
acute pancreatitis (Aronsen et al. 1972; Goodman et al. 1986;
Lasson and Ohlsson, 1984; McMahon et al. 1984). As with alpha$_1$
antiprotease, levels reach a peak around day five and in severe
attacks levels may lag behind those seen in mild attacks (McMahon
et al. 1984). Plasma concentrations of inter-alpha-trypsin
inhibitor fall during acute pancreatitis (Lasson and Ohlsson,
1984). Alpha$_2$ antiplasmin concentrations fall in acute
pancreatitis in dogs (Izquierdo et al. 1984).

Alpha$_2$ macroglobulin, alpha$_1$ antiprotease and alpha$_1$
antichymotrypsin are quantitatively the most important plasma
protease inhibitors in man. Of this trio, alpha$_2$ macroglobulin
may occupy a key role. On a simple functional basis, the theory
that acute pancreatitis leads to the consumption of the
protective role of the plasma protease inhibitors is an
attractive one. However, this assumes a static model and does not
take into account increased production of certain inhibitors,
particularly alpha$_1$ antiprotease and alpha$_1$ antichymotrypsin,
which accompanies an inflammatory reaction, the so-called 'acute

phase response'. This profound and rapid increase in plasma concentration of $alpha_1$ antiprotease and $alpha_1$ antichymotrypsin may appear slightly delayed in patients with severe acute pancreatitis due to increased consumption or a severely compromised immune system unable to mount an inflammatory response.

The total protease inhibitory capacity of plasma may actually increase during the first few days following the onset of acute pancreatitis (Adham et al. 1972) but this measurement is of limited clinical application. More important is the relative consumption of specific protease inhibitors whose function cannot be served by the other antiproteases. The concentrations of $alpha_2$ macroglobulin, antithrombin III, inter-alpha-trypsin-inhibitor and probably $alpha_2$ antiplasmin fall. The drop in $alpha_2$ macroglobulin levels represents its position as the final link in the antiprotease chain and shows that in acute pancreatitis the complexing and elimination of $alpha_2$ macroglobulin exceeds the production of $alpha_2$ macroglobulin. The magnitude of the fall in $alpha_2$ macroglobulin levels correlates well with the severity of acute pancreatitis (Balldin et al. 1981; Lasson and Ohlsson, 1984; McMahon et al. 1984; Mero et al. 1982) and the fall in $alpha_2$ macroglobulin may be proportional to the total output of proteases.

Low concentrations of $alpha_2$ macroglobulin in plasma and, possibly more importantly, the reflected low concentration and high saturation of $alpha_2$ macroglobulin in peritoneal exudate, may be important in the pathogenesis of both the early shock-like illness and later complications. There is a prompt release of bradykinin and activation of the complement system when $alpha_2$

macroglobulin becomes saturated in the peritoneal exudate and when plasma concentrations fall below 30% (Balldin et al. 1980; 1981; Lasson and Ohlsson, 1982; Lasson et al. 1983). Also protease complexes with alpha$_2$ macroglobulin are present in the plasma of patients with acute pancreatitis (Lasson and Ohlsson, 1986; Donnelly et al.1983; Abham et al. 1972). These complexes can have profound biochemical (Hermon-Taylor et al. 1981) and immunological effects (Donnelly et al. 1983) and their presence can result in up to 20% reduction in the functional capacity per unit weight of alpha$_2$ macroglobulin (Donnelly et al. 1984).

2.4 Other Protease Inhibitors

a) Introduction

Much work has gone into attempts to inactivate pancreatic proteolytic enzymes to advance the conservative management of acute pancreatitis. This would be expected since inappropriate activation of zymogen remains the generally held pathogenetic concept.

Protein inhibitors of proteases are ubiquitous, they are present in multiple forms in numerous tissues of animals, plants and microorganisms (Vogel et al. 1966). Their physiological function is prevention of unwanted proteolysis. The lack of identification of the true target enzymes for the majority of inhibitors is one of the major stumbling blocks to the understanding of inhibitor evolution and specificity.

The number of known and partially characterised inhibitors of serine proteases is enormous and their nomenclature confusing. They have been classed into families on the basis of extensive homology or topographical relationships between disulfide bridges

and the location of the reactive site (Laskowski and Kalo, 1980).
Although these attempts at grouping are not necessarily
valid, they will be used for purposes of convenience (Table 9).

TABLE 9

Families of Protease Inhibitors

(Laskowski and Kato, 1980)

I   Bovine pancreatic trypsin inhibitor (Kunitz) family

II   Pancreatic secretory trypsin inhibitors (Kazal) family

III   Streptomyces subtilisin inhibitor family

IV   Soyabean trypsin inhibitor (Kunitz) family

V   Soyabean protease inhibitor (Bowman-Birk) family

VI   Potato I inhibitor family

VII   Potato II inhibitor family

VIII Ascaris trypsin inhibitor family

IX   Other families

b) The pancreatic trypsin inhibitor (Kunitz) family

In 1936 a protease inhibitor was isolated by Kunitz and
Northrop from bovine pancreas. This basic kallikrein-trypsin
inhibitor was independently discovered in bovine lymph nodes by
Kraut, Frey and Werle (1930) and called kallikrein inactivator.
According to Fritz et al. (1979), using an indirect
immunofluorescence technique, the Kunitz inhibitor can be
localised to tissue mast cells of bovine lung, pancreas, liver and
parotid gland. The molecular weight was calculated to be 6513

daltons (Anderer and Hornle, 1965) with an isoelectric point about pH 10-10.5 and 58 amino acids. It is acid stable and resistant to heat and proteolytic degradation. It inhibits not only trypsin and kallikrein, but also chymotrypsin and plasmin (Vogel et al. 1966), though species differences exist and human chymotrypsin is not inactivated by the inhibitor (Coan and Travis, 1971).The Kunitz inhibitor depresses the activity of the fibrinolytic enzyme system by inhibition of both plasminogen activation and of active plasmin (Maki and Beller, 1966).

Studies on the metabolism of this inhibitor in rats showed a half-life of 70 minutes after intravenous injection (Werle, 1969)compared with 140 minutes in man (Beller et al. 1966). Elimination occurs mainly through the kidneys (Kaller, 1964) and much of the inhibitor is inactivated in the kidneys prior to excretion in urine. Only 10% is found in a biologically active form in urine in man (Kunitz and Northrop, 1936).

Trasylol (Aprotinin) is the commercially available preparation of this inhibitor purified from bovine lung and parotid gland. The activity of Trasylol is expressed in kallikrein-inactivator units (KIU). One KIU is usually equivalent to 0.14 ug of Trasylol (Trautschold et al. 1966). The inhibitor has also been synthesised in the laboratory (Yajima et al. 1974).

Functionally this inhibitor is probably involved in the regulation of mast cell protease activity. The chymotrypsin-trypsin- or kallikrein-like substrate specificity of the mast cell proteases favours such an assumption (Fritz et al. 1979).

Because of the ability of Trasylol to inhibit the actions of trypsin and kallikrein in man, it was proposed for the treatment of acute pancreatitis and introduced in 1950 by Frey et al. A

number of enthusiastic reports were published describing its value in experimentally induced pancreatitis in animals (Grozinger et al. 1964; McHardy et al. 1963; Nemir et al. 1963; Smith et al. 1963) and in human disease mainly in the German literature (Bedacht, 1958; Forell, 1963; Maurer, 1961; McHardy et al. 1963; Weiss et al. 1960). In initial controlled trials, however, no benefit was demonstrated (Baden et al. 1969; Skyring et al. 1965; Trapnell et al. 1967) leading Goebell in a review in 1971 to conclude that there was no unquestionable evidence of a beneficial effect from Trasylol. These early controlled trials were open to criticism as the series were small and the dosage of Trasylol administered was later considered inadequate. Trapnell revived the discussion in 1974 by publishing results of a multicentre controlled trial in 105 patients using 200,000 units of Trasylol as an initial dose followed by 200,000 units intravenously six-hourly for 5 days. He included patients only if it was their first attack of acute pancreatitis with gallstone or ideopathic aetiology. He showed a mortality of 7.5% in the Trasylol-treated patients compared with 25% in the control group. Particular benefit was demonstrated for Trasylol administration to elderly patients.

This trial was criticised on several counts, particularly the high mortality in the control group, the exclusion of alcoholic patients and the proportion of patients with mild disease in the Trasylol-treated group (Dreiling et al. 1976). Three further randomised clinical trials have been published, all of which failed to demonstrate any advantage to using Trasylol (MRC Multicentre Trial, 1980; Gauthier et al. 1978; Imrie et al. 1978a).

A possible explanation for Trasylol's apparent failure in acute pancreatitis may be that it is too large a molecule to complete inhibition of alpha$_2$ macroglobulin-protease complexes (Hermon-Taylor et al. 1981) which continue to be active against biologically important low-molecular-weight substrates. There are still advocates of Trasylol therapy in acute pancreatitis (Takasugi and Toki, 1980; Trapnell, 1981) and it has been used in therapeutic peritoneal lavage fluid with some reported success. Trasylol may be able to protect against trypsin-induced bradykinin release once alpha$_2$ macroglobulin becomes saturated (Balldin et al. 1980) and trypsin binds to Trasylol in preference to alpha$_1$ antiprotease once alpha$_2$ macroglobulin is saturated.

Though the pancreatic trypsin inhibitor is found only in bovids and capids, cows and pigs have a homologous inhibitor, the colostrum trypsin inhibitor. At least two Kunitz-type domains are present in the inter-alpha-trypsin inhibitor of mammalian serum. Kunitz-type inhibitors have also been found in snake venom, red sea turtle eggs, garden snails mucus and sea anemone. The gene coding for Kunitz-type inhibitors is very old and very widely distributed.

c) The Pancreatic secretory trypsin inhibitor (Kazal) family

This family is named after bovine pancreatic secretory trypsin inhibitor (Kazal et al. 1948) which is found in all vertebrates examined. The inhibitor is stored in zymogen granules and secreted with the zymogens into pancreatic juice. It is recovered in pancreatic juice in an amount corresponding to 1-3% of the total pancreatic protein (Pubols et al. 1974). It is an acid-stable one-chained polypeptide with a molecular weight of 6,500 daltons whose biological function is thought to be the

prevention of premature activation of trypsinogens within the pancreatic gland. Consistent with this function is the clear specificity for inhibition of trypsin, failure to interact with trypsinogen and failure to inhibit enterokinase or other pancreatic serine proteases of the same species (the inhibitor would otherwise be diverted from its essential target, trypsin).

In healthy blood donors the plasma level of pancreatic secretory trypsin inhibitor is about 8 ug/l quantitated by a radioimmunoassay. The inhibitor, as other low molecular weight proteins, is excreted in urine and found in concentrations of about 15 ug/l (Eddeland and Ohlsson, 1978b). Levels do increase during acute pancreatitis probably due to a leakage phenomenon (Lasson and Ohlsson, 1984) and plasma levels increase most if there is renal insufficiency during acute pancreatitis as the inhibitor is normally excreted in urine (Eddeland and Ohlsson, 1978b). Pancreatic secretory trypsin inhibitor is not found complexed with trypsin in plasma or peritoneal exudates probably as the complexes are cleaved in the presence of plasma protease inhibitors (Ogawa et al. 1981). The role of pancreatic secretory trypsin inhibitor is thus probably restricted to within the pancreatic gland.

Large numbers of low-molecular-weight inhibitors have been isolated from sperm, seminal plasma and seminal vesicles of various mammals, most of which are referred to as acrosin inhibitors, since acrosin is a trypsin-like enzyme located in sperm acrosomes. These appear to be homologous to Kazal pancreatic secretory inhibitors. The inhibitors found in dog submandibular glands, turtle egg whites and leeches are also Kazal-type as are the ovoinhibitors found in avian egg whites and

chicken plasma (Laskowski and Kato, 1980).

d) The Streptomyces subtilisin inhibitor family

Various species of streptomyces excrete protease inhibitors into the medium, some of which have been extensively studied. Umezawa initiated the screening of enzyme inhibitors found in microbial cultures. He suggested that microorganisms which produce strong proteases might also produce inhibitors to protect their cells from the harmful effects of the enzyme (Umezawa and Aoyagi, 1977). Protease inhibitors had been found previously in cultures of clostridium botulinium, aspergillus, penicillium, rhizopus, E.Coli and other gram negative bacteria. The major breakthrough came from Tokyo when Leupeptin was recovered from culture filtrates of Streptomycetes freshly isolated from soil.

Leupeptin is a group of low-molecular-weight oligopeptides comprising propionyl or acetyl L-leucyl-L-leucyl-L-argininal and their analogues in which leucine is replaced by isoleucine or valine (Kondo et al. 1969; Kawamura et al. 1969). It is found in culture filtrates of many species of Streptomyces (Aoyagi et al. 1969a,1969b). Leupeptin inhibits plasmin, trypsin, kallikrein, thrombokinase, papain and cathepsin B but not chymotrypsin or elastase (Aoyagi et al. 1969b; Umezawa, 1976).

The same laboratories isolated Antipain from culture filtrates of Streptomyces michrogoensis and yokosukaemis (Suda et al. 1972) which inhibits papain, trypsin, thrombokinase, cathepsin A and B and plasmin. It also weakly inhibits kallikreins. Chymostatin was isolated from filtrates of Streptomyces hygroscopicus and lavendulae. Chymostatin inhibits chymotrypsin, cathepsin A and B and papain (Ikezawa et al. 1971). Elastinal was isolated from a culture filtrate of Streptomyces

griseorubor. This is a specific inhibitor of elastase and does not inhibit other proteases (Umezawa and Aoyagi, 1977). Pepstatin, Phosphoramidon and Bestatin are among other protease inhibitors which have been isolated and their structure and properties have been comprehensively reviewed (Umezawa and Aoyagi, 1977).

Some of these oligopeptide protease inhibitors have been studied in acute pancreatitis. They are small enough to complete inhibition of proteases bound to alpha$_2$ macroglobulin (Hermon-Taylor et al. 1981) where Trasylol failed due to steric constraints. Leupeptin has a molecular weight of only 474 daltons,whereas Trasylol has a molecular weight of approximately 6500 daltons and contains three intra-chain disulphide bridges. Leupeptin and antipain in vitro at low concentration inhibit the effect of alpha$_2$ macroglobulin-trypsin complexes on parathormone and hydrolysis of porcine cholecystokinin whereas Trasylol was ineffective even in massive molar excess (Hermon-Taylor et al. 1981).

Continuous intravenous infusion of a partially purified preparation of leupeptin was shown to significantly prolong survival in rats with acute haemorrhagic pancreatitis whereas Trasylol was without effect (Jones et al. 1982b). In mice Leupeptin, Pepstatin, Chymostatin and Antipain decreased the severity of acute pancreatitis whereas Trasylol and chloroquine did not (Lombardi and Rao, 1982).

A combination of sterically favourable water soluble low-molecular-weight inhibitors may provide effective specific chemotherapy for acute pancreatitis (Hermon-Taylor and Heywood, 1986). Oligopeptide aldehyde protease inhibitors have been

suggested as a useful adjuvant to therapeutic peritoneal lavage fluid (Imrie, 1985) though no distinct benefit has been shown in an animal model in which leupeptin or antipain plus elastinal were added to lavage fluid (Terry et al. 1986).

e) Other inhibitor families

The first plant inhibitor to be well characterised was soyabean trypsin inhibitor (Kunitz). Its isolation and crystallisation and that of its complex with trypsin by M.Kunitz is one of the classic achievements of the inhibitor industry (Kunitz, 1947). Similar inhibitors have been isolated from winged bean, rice and barley (Laskowski and Kato, 1980).

In 1953 Hoffman et al. reported that soyabean trypsin inhibitor was ineffective in the treatment of acute haemorrhagic pancreatitis in the dog, though some beneficial effects have been reported by other workers (Cannon and Turner, 1961; Ribacoff et al. 1961).

The Bowerman-Birk inhibitors are readily isolated from seeds of all leguminous plants and a large number have been described (Richardson, 1977). All the sequenced ones consist of two tandem homology regions on the same polypeptide chain each with a reactive site.

Ascaris lumbricoides are round parasitic worms living in the digestive system of man and pig. They produce a family of inhibitors for all the digestive enzymes of the host (Peanasky et al. 1974).

Potatoes and related plants are extremely rich sources of protease inhibitors grouped into several families.

Oda and co-workers (1971) reported a beneficial effect of chlorophyll-a in patients with chronic relapsing pancreatitis. It

was suggested that chlorophyll-a converts to chlorophyllin in vivo which inhibits trypsin. Orda et al. (1974) showed a reduced mortality in an animal model of acute pancreatitis using chlorophyll-a and later showed that intraperitoneal chlorophyll-a has a similar effect (Wiznitzer et al. 1976). Durr (1979) reviewing the literature was of the opinion that treatment with chlorophyll-a deserved further evaluation in man.

Epsilon-amino caproic acid (EACA) and the still more potent omega amino acids, para-amino benzoic acid (PAMBA) and 4-aminomethylcyclohexanecarboxylic acid (AMAC) effectively inhibit activation of plasminogen and, in high concentration, also plasmin and trypsin (Markwardt et al. 1966). Reduced mortality in animal studies was reported by Gabryelewicz et al. (1969) but no beneficial effect in acute pancreatitis was demonstrated by either Lankisch et al. (1977b) or Diwok et al. (1971). In a clinical trial a reduced mortality was reported using EACA (Kontinnen, 1971) but results did not reach significance. Meinel and Wolff (1975) used PAMBA in a clinical trial and concluded with the ambiguous statement that PAMBA was as effective as Trasylol.

FOY (ethyl-4-(6-guanidinotexanoyloxy)benzoate methane sulfonatel)and FOY-305 (camostate) are guanidine acid esters which are strongly basic molecules with a high affinity for serine proteases. They represent a new class of synthetic inhibitors of various proteases, as well as phospholipase A_2, originally synthesised as clinically applicable trypsin inhibitors (Muramatsu and Fujii, 1972). They may also enter pancreatic acinar cells and decrease pancreatic secretion of enzymes (Adler et al. 1984). Oral FOY-305 is metabolised to FOY-

255 which is detectable in plasma with a half-life of about 80 minutes (Saitoh, 1982). It inhibits the activity of pancreatic enzymes in the circulation (Takasugi and Toki, 1980).

Animal work reported improved survival in acute pancreatitis following administration of FOY (Lankisch et al. 1983). A small multicentre double-blind trial from Hannover (Freise et al.1986) reported that FOY therapy reduced the number of complications of acute pancreatitis and the hospitalisation, but did not reduce mortality. Serum amylase levels showed a significant decline in the FOY-treated group when compared with controls. They concluded that further clinical studies are necessary.

FOY has been in clinical use in Japan since 1977. A multicentre trial involving 38 hospitals and 150 attacks of acute pancreatitis showed that FOY significantly increased the incidence of favourable clinical outcomes compared with a control group receiving Trasylol therapy. A double blind trial involving four hospitals showed a significant increase in favourable clinical outcomes in patients receiving oral FOY-305 compared with controls receiving inactive placebo. (Tanaka et al. 1979). The number of patients involved in this study was small and further clinical trials are required to evaluate FOY.

CHAPTER THREE

THE SCOPE OF THE PRESENT STUDY

The review of the literature makes it clear that our
knowledge of the aetiology and pathogenesis of acute pancreatitis
is, at present, incomplete and that treatment is
unsatisfactory.The available evidence from clinical and
experimental studies suggests that a broad spectrum of direct or
indirect insults trigger a final common critical event which is
the inappropriate activation of digestive zymogen within the
gland leading to autodigestion. A toxic broth of activated
pancreatic enzymes is released into the retroperitoneum and
peritoneal cavity. These enzymes reach the bloodstream by direct
absorption and via the lymphatics.

Circulating digestive enzymes produce systemic
pathophysiological disturbances throughout the body which can
lead to respiratory, myocardial and renal failure, toxic
confusional states and disseminated intravascular coagulation.
Raised plasma levels of active proteolytic enzymes have been
demonstrated in acute pancreatitis. When free trypsin,
chymotrypsin and elastase are released into the circulation they
immediately complex with the plasma protease inhibitors,
particularly $alpha_1$ antiprotease and $alpha_2$ macroglobulin. The
$alpha_1$ antiprotease-enzyme complex is catalytically inert, but
the $alpha_2$ macroglobulin-enzyme complex retains a proportion of
esterolytic and proteolytic activity against substrates of
varying molecular size. Whether complex formation occurs
principally in ascitic fluid and oedema fluid adjacent to the
inflamed pancreas or whether it occurs to a significant extent in
plasma is unclear.

Normally these complexes are rapidly eliminated from the
circulation by the reticulo-endothelial system and circulating

tryptic activity is insignificant. In acute pancreatitis the formation of complexes is greatly accelerated and the capacity to remove them may be inhibited or overwhelmed. Tryptic activity is detectable in plasma and circulating $alpha_2$ macroglobulin-enzyme complexes are present. Plasma concentrations of $alpha_1$ antiprotease increase as part of the acute phase response but plasma concentrations of $alpha_2$ macroglobulin fall as consumption exceeds production, lowest levels correlating with a severe clinical outcome. Although there is still an apparent excess of antiprotease capacity in plasma, low concentrations of plasma $alpha_2$ macroglobulin and high degrees of saturation of ascitic $alpha_2$ macroglobulin correlate with the inappropriate activation of the complement, fibrinolytic and kallikrein-kinin systems which may be important in the pathogenesis of both the early shock-like illness and later complications.

This thesis was stimulated by an uncontrolled study from Dundee (Cuschieri et al. 1983) reporting a low mortality from acute pancreatitis in patients given fresh frozen plasma as part of their intravenous fluid therapy. 239 consecutive patients admitted with 336 attacks of acute pancreatitis were given a five-day regimen of fresh frozen plasma. Each received 2-3 units (500-725ml) intravenously during the first 12 hours and subsequently 1-2 units (225-500ml) daily for the next 4 days. A substantial abatement of pain and abdominal tenderness was reported in all patients within 24 hours of fresh frozen plasma therapy with an improvement in the general condition and cardiovascular and respiratory state of 65 of 69 patients with severe disease. A beneficial effect on serum amylase and blood glucose levels was also suggested - though it must be emphasised

that there was no control group. No complications were encountered from the fresh frozen plasma therapy, and in particular no hepatitis. The hospital mortality in this study was 3.7 percent with overall mortality rate per attack of 2.7 percent. This is substantially lower than the 8-10 percent which we might now expect from acute pancreatitis in the UK.

When looking for a mechanism to explain the apparent beneficial effect of fresh frozen plasma therapy in acute pancreatitis, its colloid value must first be considered. There is a massive fluid shift from the intravascular space in acute pancreatitis and the degree of fluid sequestration correlates with mortality (Sauven et al. 1986). Repletion of the volume deficit improves the clinical state and survival rates, but there is controversy concerning the choice of resuscitative fluid. In the past the purported antitryptic factor concentrated in albumin preparations (Landsteiner, 1900) was thought to ameliorate the course of acute pancreatitis (Kenwell and Wels, 1953) but Elliot et al. (1955) showed that the benefit of albumin was not in the inhibition of trypsin but rather by support of plasma volume. Both colloid and crystalloid resuscitative solutions have been shown to be effective in acute pancreatitis (Martin et al. 1984). The question arises as to whether or not colloid osmotic pressure must be maintained for adequate tissue perfusion. Virgilio et al. (1979) showed that the key factor in resuscitation of haemodynamically deteriorating patients was maintenance of plasma volume measured by pulmonary capillary wedge pressure. Decrease in serum oncotic pressure associated with crystalloid infusion was not detrimental to the patient and may, in fact, have allowed the Starling equation to work advantageously by increasing tissue

pressure. An increase in tissue pressure essentially decreases fluid filtration from the vascular bed, and the use of a balanced salt solution allows replenishment of the interstitial fluid compartment with appropriate ions.

A possible detrimental effect of colloid administration is the marked increase in plasma volume with little increase in the interstitial fluid volume, with consequent failure to replace actual fluid loss. Colloid administration is associated with a dramatic elevation of pulmonary capillary wedge pressure, left ventricular stroke volume and cardiac output with a decline in haematocrit (Martin et al. 1984). This may lead to heart failure or pulmonary oedema. Albumin resuscitation does not help to mobilise extravascular pulmonary water, rather it leads to a worsening of adult respiratory distress syndrome (Weaver et al. 1978).

Thus the colloid content of fresh frozen plasma is not necessarily beneficial in acute pancreatitis, and in any case in the Dundee study it represented only 20-30% of the colloid administered to patients with severe disease.

Fresh frozen plasma contains several protein systems which might be beneficial in acute pancreatitis including clotting factors, fibronectin (Mosesson and Amrani, 1980; Saba and Jaffe, 1980) and C-reactive protein, but the replenishment of the plasma antiprotease system is the most attractive theory (Cuschieri et al. 1983). Alpha$_1$ antiprotease and alpha$_2$ macroglobulin provide the bulk of antiprotease activity in plasma and both retain their specific activity after storage at -20°C. If fresh frozen plasma therapy does improve outcome by replenishing the plasma antiprotease system, then it is probably the alpha$_2$ macroglobulin

which it provides that is important, as the acute phase response significantly increases the patients plasma levels of $alpha_1$ antiprotease during acute pancreatitis while $alpha_2$ macroglobulin levels fall. $Alpha_2$ macroglobulin appears to have a central role in the elimination of antiproteases.

The present study was designed to further evaluate the use of fresh frozen plasma therapy in acute pancreatitis. The Dundee study suggested the need for a prospective clinical trial of fresh frozen plasma in acute pancreatitis using a colloid control group. If all patients with acute pancreatitis are to be included in such a trial, in order to demonstrate a significant reduction in severe outcome, say from 20% to 10%, with a type I error set at 0.05 and a type II error set at 0.1, over 250 patients would be required in each limb. To demonstrate a significant reduction in mortality, say from 10% to 5%, over 550 patients would be required in each limb (Pocock, 1983). To achieve such numbers in a reasonable timescale a multicentre study is required.

Before embarking on such a major clinical undertaking more evidence was sought for a beneficial effect of fresh frozen plasma therapy in an experimental model of acute pancreatitis. The development of an animal model of acute pancreatitis is described in Chapter 4 and the development of a system for continuous intravenous infusion of fluid and continuous body temperature monitoring in the unrestrained rat is described in Chapter 5. Chapter 6 describes studies performed to ensure that animals received adequate analgesia during the experiments. The collection of fresh frozen plasma from a rat colony, its

effect on survival times and the morbid anatomy and metabolic
changes compared with control animals in experimental acute
pancreatitis is reported in Chapter 7. The subsequent multicentre
prospective controlled clinical trial of fresh frozen plasma therapy
in acute pancreatitis is reported in Chapter 8. This trial includes
the monitoring of the major serum antiproteases during acute
pancreatitis to assess the effect of fresh frozen plasma therapy
on their concentrations.

CHAPTER FOUR

AN EXPERIMENTAL MODEL OF ACUTE PANCREATITIS

4.1 Introduction

Preliminary studies of rat pancreatic ductal pressures and capacity were made to confirm the requirements for a reproducible model of acute pancreatitis. With this information modifications were made to the method of inducing acute pancreatitis described by Lankisch et al. (1974).

In all experiments inbred male albino AS rats weighing 250-300 g (8-10 weeks old) were used. The colony was kept according to Home Office regulations in the animal unit of the Clinical Sciences Building, Leicester University.

4.2 Materials and Methods

a) Anaesthesia

Animals were allowed free access to food (Rat and Mouse Economy Pellets, Labsure, Lavender Mill Manea, Cambridgeshire, UK) and water prior to anaesthesia which was induced with a mixture of 5% halothane and oxygen at 2 litre/min delivered from a Boyle's machine via a conical head mask. Once the animals became unconscious the halothane was reduced to 1.5%. Anaesthesia was completed and maintained using an intraperitoneal injection of 1ml/kg of a mixture of one part small animal Immobilon (a neuroleptoanalgesic comprising 0.074 mg/ml etorphine and 18 mg/ml methotrimeprazine, C-Vet Ltd., Bury St.Edmunds, UK) to three parts Hypnovel (5mg/ml Midazolam, Roche Products Ltd., Welwyn Garden City, UK). The injection was given through the anterior abdominal wall in the midline using a 23G Microlance needle (Becton Dickinson and Company Ltd, Dunlaoghaire, Co.Dublin,Eire) (Figure 2). Once this injection had been administered the animal was removed from the conical head mask and placed on its side in

its cage to await the surgical procedures.

b) Common pancreatico-biliary ductal cannulation

The anterior abdominal wall was shaved and the skin cleaned with a solution of 0.5% cetrimide and 0.05% chlorhexidine. The animal was then placed on a cork board over an electric warming pad (Pet Warmer, Johnson and Calverley, Elland, West Yorkshire, UK) to prevent excessive heat loss during surgery. The anterior abdominal wall was opened in the midline for 2cm below the xiphisternum and the incision held open with a pair of eyelid retractors. The duodenal loop was delivered, wiping off any congenital adhesions to the colon.

Initially multiple cannulations were performed in non-recovery animals before the common duct could be reliably cannulated transduodenally. Several catheter systems were tried and the eventual choice was a 24G Quick-Cath (Travenol Laboratories Ltd., Castlebar, County Mayo, Ireland). This system has a 16mm over-the-needle teflon catheter with a plug and luer fitting (Figure 3).

The duodenal loop was held in such a way that the lower end of the common pancreatico-biliary duct was clearly displayed as it passed obliquely through the duodenal wall (the rat has no ampulla). The loop was stabilised using a 2/0 silk sling passed through an avascular window distal to the common duct with the proximal duodenum held gently between finger and thumb of the non-dominant hand. The cannula was introduced from the antimesenteric border into the lumen of the duodenum at which point the central trochar was removed. The teflon cannula tip was then advanced into the olique opening of the common duct on the mesenteric border of the duodenum using a gentle rotatory action

Figure 2   Anaesthesia induced via a conical head mask
             and completed with an intraperitoneal injection.
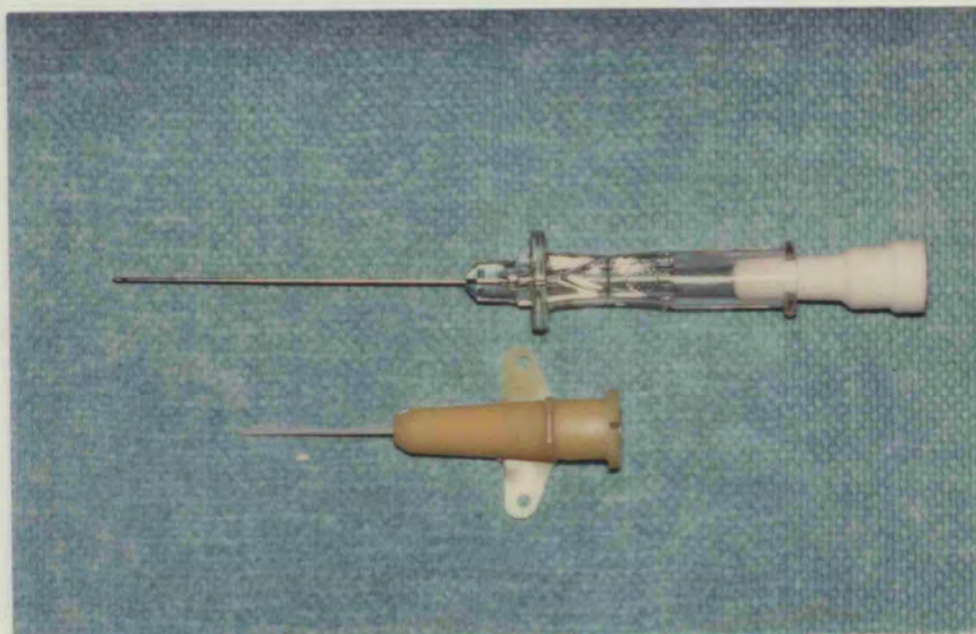
Figure 3   Cannula system separated to show central
             trochar and over-the-needle teflon catheter.

and advanced for 5-8mm up the transparent duct (Figure 4). The cannula was held in place with a 5/0 silk tie placed around the lower end of the common duct which also occluded the common duct temporarily thus creating a closed pancreatico-biliary system.

c) Induction of pancreatitis

With the teflon cannula located in the common pancreatico-biliary duct, a small microvascular clamp (C3V, John Weiss and Son,London, UK) was placed on the bile duct just below the liver hilum. This prevented passage of the retrograde infusate into the liver and created a closed pancreatic ductal system.

2ml/kg (0.5-0.6ml) of a freshly prepared mixture of 0.2% enterokinase (Sigma Chemical Company Ltd., Poole, Dorset, UK) and 3.5% sodium taurocholate (BDH Chemicals Ltd., Poole, Dorset, UK) in 150 mmol/L NaCl was infused into the pancreatic duct system at a steady rate of 3ml/hr from a syringe pump. The cannula and giving set were carefully filled with the mixture before starting the infusion to avoid dead spaces. Infusion times ranged from 10 minutes for a 250 g animal to 12 minutes for a 300 g animal.

At the termination of the infusion the microvascular clamp was removed from the bile duct at the liver hilum, the retaining tie around the distal common duct and cannula was divided, and the cannula was removed. This restored biliary and pancreatic drainage. The puncture hole in the antimesenteric border of the duodenum was closed with a single 5/0 silk suture and the duodenal loop was returned to the abdomen. The abdominal wall was closed with a continuous layer of 5/0 silk to peritoneum and muscle and a second layer of continuous 5/0 silk to skin. The wound was sealed with a spray dressing (Op-Site, Smith and Nephew Ltd., Welwyn Garden City, Herts, UK) and the animal placed on its

Figure 4

Transduodenal cannulation
of the transparent common
pancreatico-biliary duct.
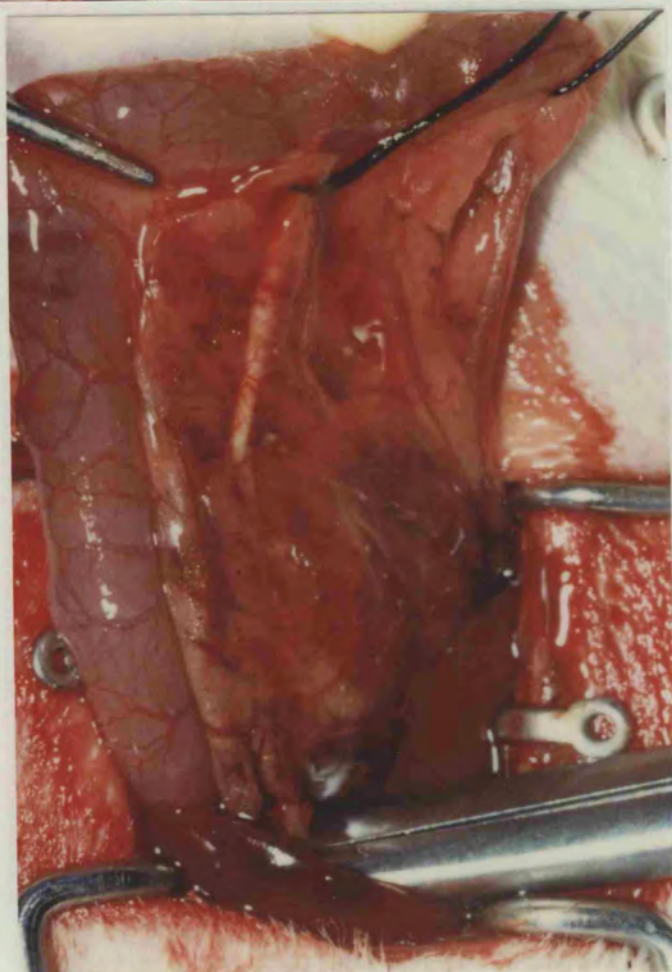
Figure 5

Post-infusion appearance
with visible oedema and
haemorrhage of pancreatic
tissue within the duodenal
mesentery.

side on sawdust in the bottom of its cage whilst awaiting

recovery from the anaesthetic. Electric warming pads were

positioned under the cage to prevent excessive heat loss during

recovery and these were turned off once the animal was mobile.

The recovered animals were allowed free access to food and water.

4.3 Preliminary Studies

a) Radiographic contrast studies

To ensure that the volume of fluid infused retrogradely into

the pancreatic ductal system was adequate to fill the pancreatic

tree and not produce major ductal disruptions, 3ml/hr of

radiographic contrast material (Urografin 370, Schering AG,

Berlin, W.Germany) was infused from a syringe pump into the

closed pancreatic ductal systems of three 250g animals and x-rays

taken at 0,5,10 and 15 minutes to assess the pancreatograms.

b) Pressure Studies

To ensure that 2ml/kg of the enterokinase/bile salt mixture

infused at 3ml/hr did not produce excessive pressures likely to

cause major duct disruption and inconsistent pancreatic perfusion,

the infusion systems in three animals were connected via T-

junctions to pressure transducers (EM 751A, Elcomatic Ltd.,

Neilston, Glasgow, UK) and thence via an amplifier (8805C,

Hewlett Packard Ltd., Winneish, Wokingham, Berks, UK) to a Gould

Brush 260 Multichannel Recorder (Gould Electronics Ltd.,

Hainault, Ilford, Essex). This provided continuous recordings of

the pressure within the closed systems during the infusions.

4.4 Characterization of the Model

a) Mortality

Acute pancreatitis was induced in thirty animals in groups

of six and the disease allowed to run its course. Animals were carefully observed during the experiment and the time of any deaths was recorded.Three survivors at 72 hours and three survivors at one week were sacrificed and all animals underwent post mortem examination.

b) Haematology and biochemistry

In a second group of animals acute pancreatitis was induced and three animals were sacrificed at each of the times shown in Table 10. Three survivors of the mortality experiment were sacrificed at 72 hours and the remaining three at one week.

TABLE 10

Haematological, Biochemical, Macroscopic and Microscopic Studies on the Rat Model of Acute Pancreatitis

Time from start of retrograde infusion	No. of animals sacrificed for study
0 min (controls)	3
5 min	3
10 min	3
30 min	3
1 hr	3
3 hr	3
6 hr	3
12 hr	3
24 hr	3
48 hr	3
72 hr (Survivors from 1st experiment)	3
1 week " " "	3

Because of the high mortality in the model some animals died before their alloted time for sacrifice and replacements were required. Animals for sacrifice were anaesthetised with 5% halothane and oxygen at 2 litre/min delivered from a Boyle's machine via a conical head mask. They then underwent cardiac puncture and exanguination using a 19G needle mounted on a 10ml syringe yielding 5-12 ml of whole blood per animal. Three drops of whole blood were placed in a B-D Microtainer containing EDTA anticoagulant and inert mixing beads (Becton Dickinson Vacutainer Systems, Rutherford, New Jersey, USA) for haematological measurements whilst the remainder was placed in a plain tube and the serum separated for biochemical measurements.

Haemoglobin, white blood cell count and packed cell volume were measured using a Coulter Counter S-Plus IV (Coulter Electronics Ltd., Luton, Beds, UK) which had previously been checked against manual methods to ensure accuracy was achieved with rat blood samples.

Amylase was measured using a standard Phadebas technique (Pharmacia Diagnostics AB, Uppsala, Sweden) which uses cibacron brilliant blue linked to starch. The free dye is liberated by amylase and is measured and expressed at 37°C. An alkaline pH is used to stop the reaction and stabilise the end colour which is measured spectrophotometrically.

Albumin, alkaline phosphatase, bilirubin and calcium were measured using Technicon standard methods on a SMAC Mark I analyser using spectrophotometry of end colours (Technicon Instrument Company, Basingstoke, Hants, UK). The measurement of albumin is a dye binding technique involving bromocresol green at pH 4.5 in the presence of a wetting agent and succinate buffer.

Alkaline phosphatase is measured by incubating serum with paranitrophenyl phosphate at pH 9.8 with an aminomethyl propanol buffer and measuring the release of paranitrophenol (yellow). The enzyme measured and expressed at 37°C. Bilirubin measured using a diazo method. Diazotised sulphanalic acid reacts with bilirubin in acid medium to produce a red colour. Caffeine benzoate is used as an uncoupler to release bilirubin from its binding proteins and get the full reaction. The reaction is then stopped with alkali which produces a stable blue colour. Total calcium is measured using cresolphthalein complexone in an alkaline buffer. Serum is diluted in an acid buffer to liberate calcium from protein carriers and it is then dialysed with the cresolphthalein complexone to produce a purple colour.

c) Macroscopic features

Following cardiac puncture and exanguination of the animals cervical fracture was performed followed by an immediate limited post mortem examination. The abdomen and thorax were opened. The colour of any ascites was noted and a small amount drawn off with a syringe for amylase estimation. The volume of ascites was estimated by absorbing it from the abdominal cavity into cotton wool balls and subtracting the known dry weight of the cotton wool balls from their wet weight assuming 1ml of fluid weighs 1g. The presence and distribution of fat necrosis and the macroscopic appearance of the pancreas and other major thoracic and abdominal viscera was noted.

d) Microscopic features

Total pancreatectomy was performed together with the spleen and duodenal loop. The lungs, heart, liver and kidneys were also removed and placed immediately in 10% formalin for 24 hours. The

organs were then processed to paraffin wax and multiple 4u

sections taken which were stained with haematoxylin and eosin.

The histology was reviewed 'blind' by one pathologist who

was not aware of the duration of pancreatitis in each specimen.

He assessed all the organs, but concentrated on the pancreas in

which he developed a scoring system of 0-3 for each of the four

major microscopic features of acute pancreatitis (necrosis,

inflammatory infiltration, haemorrhage and oedema).

4.5. Results

As far as the anaesthetic is concerned, there were initially

occasional problems with the airway which were overcome by

pulling forward the animals tongue. Apnoea was induced if the

halothane was kept at 5% for too long before reducing it to 1.5%.

With practice this anaesthetic was easy to administer and

reliable. There was sometimes a brief period of recovery from

halothane before the intraperitoneal injection took effect, but

the mixture of small animal Immobilon and Hyponoval enabled the

surgical procedures to be started within five minutes of its

administration and provided 1-3 hours of operating time with a

mean of $1\frac{1}{2}$ hrs. With practice all the surgical procedures,

including intravenous access, were completed within 45 minutes

and animals were then placed on their sides in their cages to

recover. Failure of animals to recover from anaesthetic was

unusual and when it occurred the animal was withdrawn from the

subsequent experiments before randomisation.

Cannulation of the common pancreatico-biliary duct required

preliminary practice in non-recovery animals. Initially there was

a tendency for the cannula to perforate the mesenteric border of

the duodenum or the duct itself. This was more common with some
of the stiffer cannulae which were tried before adopting to the
Quick-Cath. With practice the cannulation became a quick and
reliable procedure and during the main experiments there were
very few technical failures.

During infusion of the sodium taurocholate/enterokinase
mixture into the pancreatic ductal system, the gland became
noticably swollen with macroscopic evidence of haemorrhagic
pancreatitis even before the duodenal loop was returned to the
abdominal cavity (Figure 5). Radiographic contrast studies
revealed that a good pancreatogram was achieved by infusion of
2ml/kg of Urografin 370 at 3ml/hr (Figure 6) suggesting a good
distribution of this volume of fluid throughout the gland with no
obvious major duct disruption. Pressure studies within the
closed system revealed a gradual rise during infusion of the
sodium taurocholate/enterokinase mixture from a resting pressure
of 5-12 cm of water to a maximum pressure of 35-50cm of water by
the end of the infusion.

The survival of thirty animals in whom acute pancreatitis
was initially induced is shown in Figure 7. The mortality at 72
hours was 80% with most of the deaths occurring within the first
48 hours. Three of the six survivors were sacrificed at 72 hours
and the remaining three at one week for haematological,
biochemical, macroscopic and microscopic studies.

The haematological and biochemical results obtained from
blood acquired by cardiac puncture are given in appendix 1.
Although numbers are small, haemoglobin and haematocrit levels
appear to increase initially, but after 12 hours concentrations
start to fall reaching their lowest values around 72 hours with

Figure 6

a) Control x-ray of rat with transduodenal cannula (arrowed) in common pancreaticobiliary duct.

b) Pancreatogram (arrowed) produced by infusion at 3ml/hour of 0.5ml of contrast into the closed pancreatic ductal system of a 250g rat.

Figure 7

Survival in a Preliminary Group of Animals

Following Induction of Acute Pancreatitis

No. of animals = 30
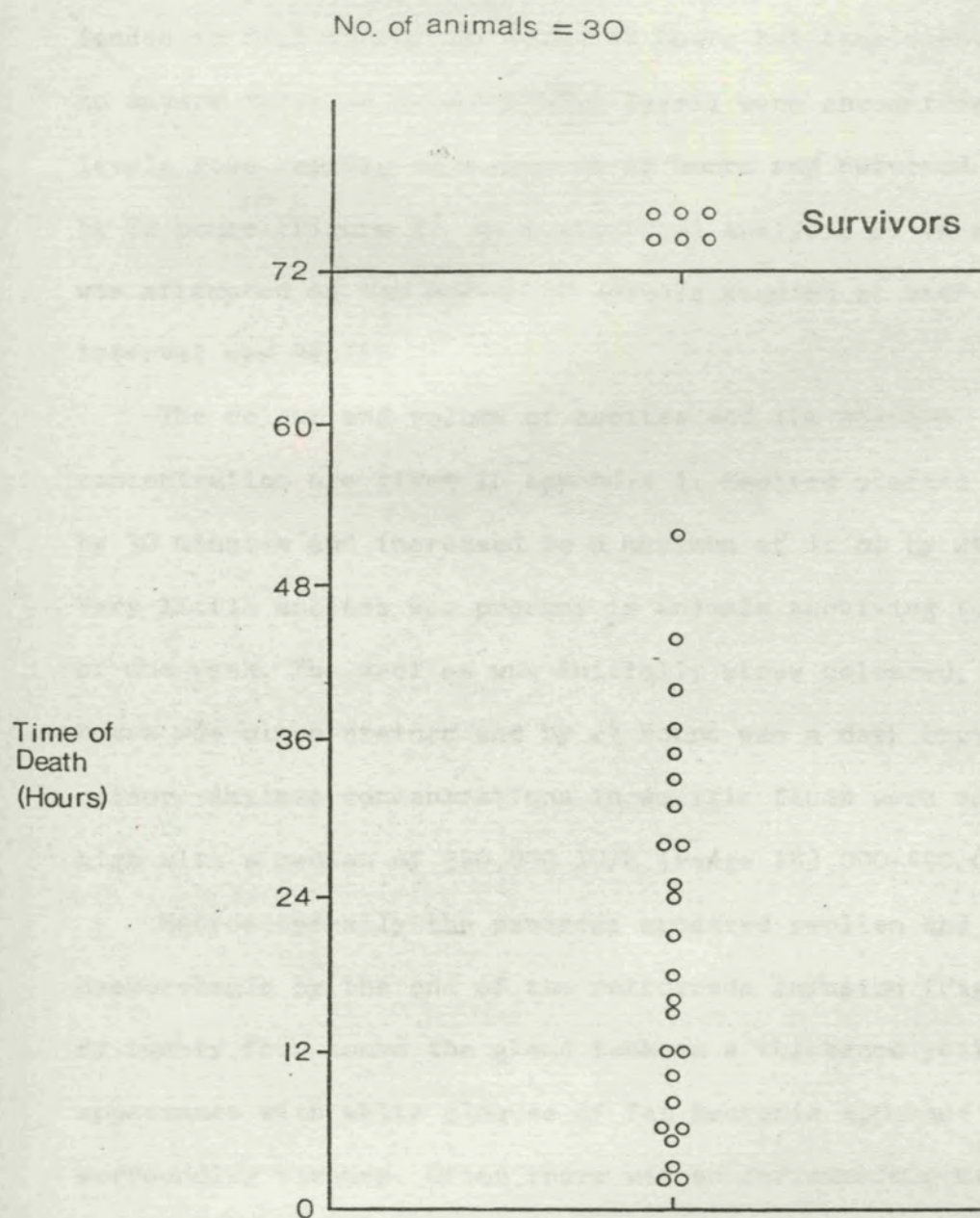
Survival at 72 hours = 20%

some recovery by one week (Figure 8). The white blood count

increased steadily to 48 hours and then dramatically to 72 hours

returning towards normal by one week (Figure 8). Some derangement

of liver function occurred, but was inconsistent. Albumin levels

tended to fall during the first 48 hours but then recovered and

no severe falls in serum calcium levels were encountered. Amylase

levels rose rapidly to a peak at 12 hours and returned to normal

by 72 hours (Figure 9). No statistical analysis of these figures

was attempted as the number of animals studied at each time

interval was small.

The colour and volume of ascites and its amylase

concentration are given in appendix 1. Ascites started to appear

by 30 minutes and increased to a maximum of 12 ml by 24 hours.

Very little ascites was present in animals surviving to 72 hours

or one week. The ascites was initially straw coloured, but by six

hours was blood-stained and by 24 hours was a dark brown/red

colour. Amylase concentrations in ascitic fluid were consistently

high with a median of 320,000 IU/L (range 183,000-490,000 IU/L).

Macroscopically the pancreas appeared swollen and

haemorrhagic by the end of the retrograde infusion (Figure 5).

By twenty four hours the gland took on a thickened yellow/grey

appearance with white plaques of fat necrosis apparent in the

surrounding tissues. Often there was an inflammatory mass

('phlegmon') present by 48 hours with omentum and small bowel

adherent to the inflamed gland. Occasionally a loculus of fluid

('pseudocyst') was present within the mass. Macroscopic abscess

formation was not encountered but necrotic areas were visible

within the pancreas and mesenteries by 48 hours. There were no

obvious macroscopic changes in the liver, kidneys, lungs or heart

147

Figure 8

Haematology Results in the Experimental Model of Acute Pancreatitis
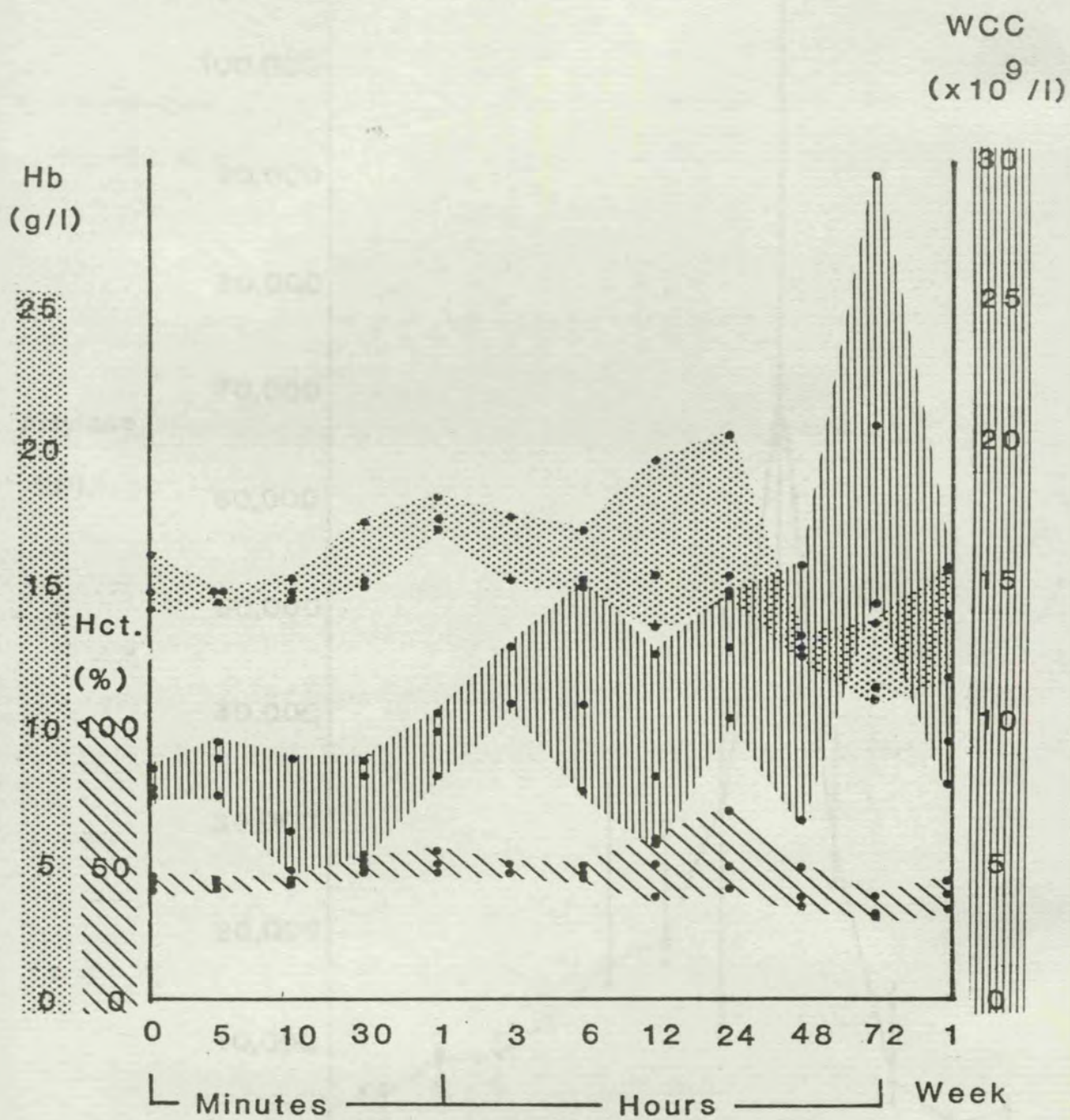
Time from start of retrograde infusion

Figure 9

Serum Amylase Concentrations in the Rat Model

of Acute Pancreatitis

Amylase

IU/L

110,000
100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000

0 5 10 30   1   3   6   12   24   48 72   1

minutes   hours   week

Time
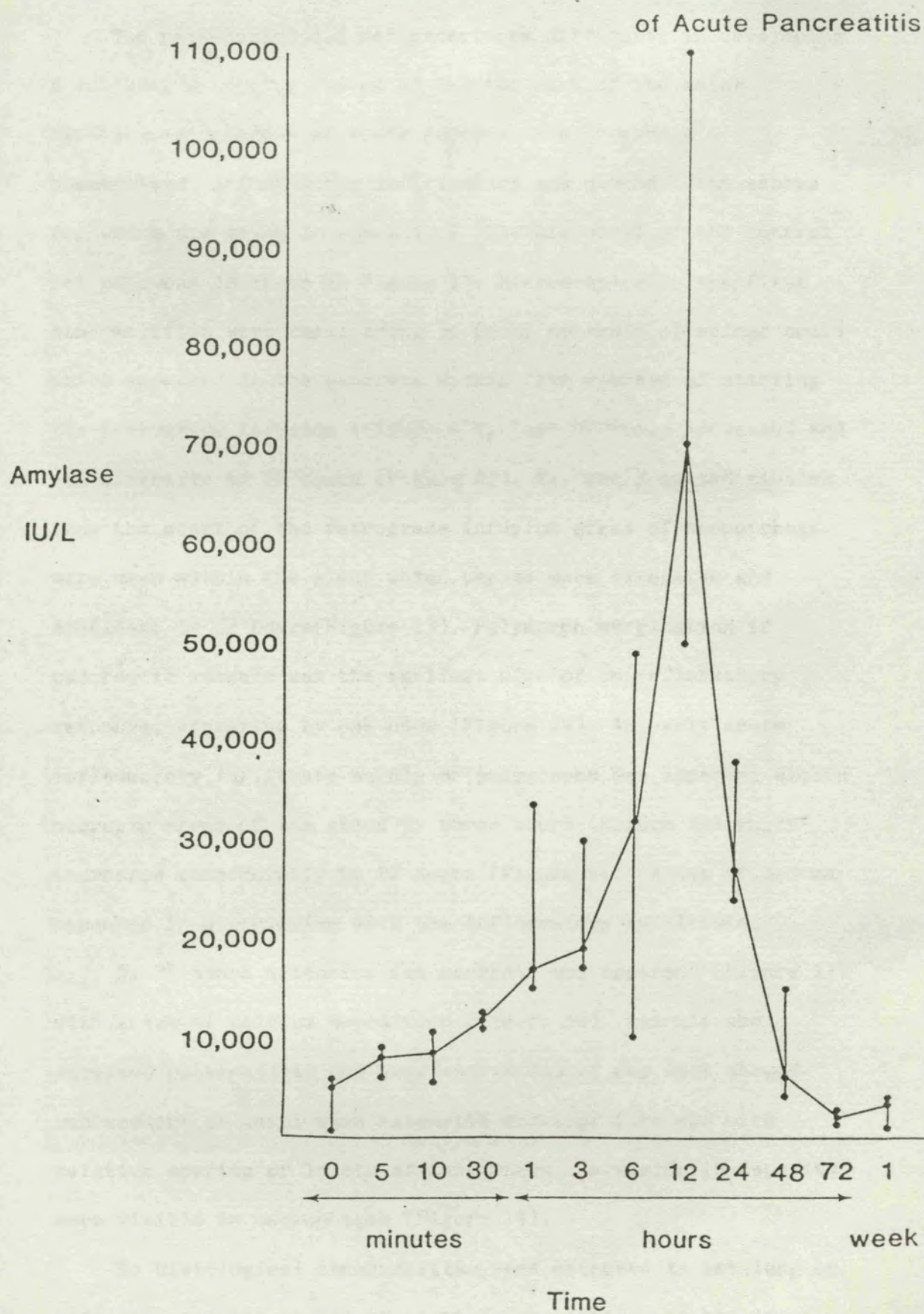
though small pleural effusions (< 1ml) were occasionally

encountered.

The pathologist did not experience difficulty in developing

a subjective scoring system of 0-3 for each of the major

histological changes of acute pancreatitis (necrosis,

haemorrhage, inflammatory infiltration and oedema), the scores

for which are given in appendix 1. The histology of the control

rat pancreas is shown in Figure 10. Microscopically the first

abnormalities were small areas of focal necrosis of acinar cells

which appeared in the pancreas within five minutes of starting

the retrograde infusion (Figure 11). This necrosis increased and

was extensive by 12 hours (Figure 12). As early as ten minutes

from the start of the retrograde infusion areas of haemorrhage

were seen within the gland which became more extensive and

confluent by 12 hours(Figure 13). Polymorph margination in

pancreatic vessels was the earliest sign of an inflammatory

response, appearing by one hour (Figure 14). An early acute

inflammatory infiltrate mainly of polymorphs was apparent within

necrotic areas of the gland by three hours (Figure 15) which

increased considerably by 12 hours (Figure 16). Areas of oedema

appeared in association with the inflammatory infiltrate.

By 24 hours extensive fat necrosis was apparent (Figure 17)

with areas of calcium deposition (Figure 18). Animals who

survived pancreatitis and were sacrificed at one week showed

replacement of acini with extensive areas of fibrosis with

relative sparing of Islets of Langerhans. Haemosiderin deposits

were visible in macrophages (Figure 19).

No histological abnormalities were detected in rat lung or

kidney during the course of acute pancreatitis, but the liver
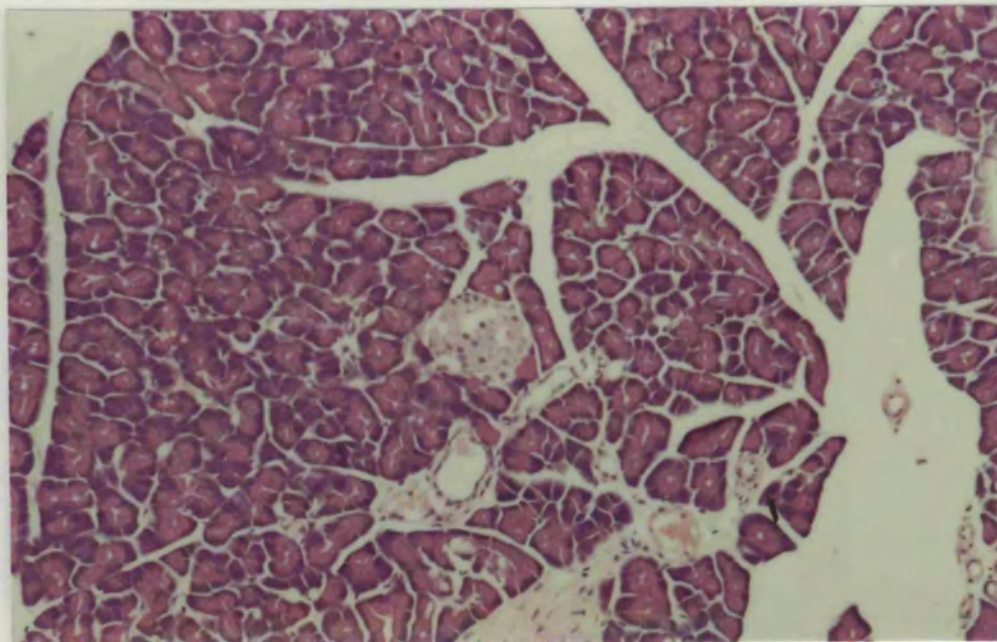
Figure 10

Histology of the rat pancreas in a control animal
(H and E, original magnification x 40).
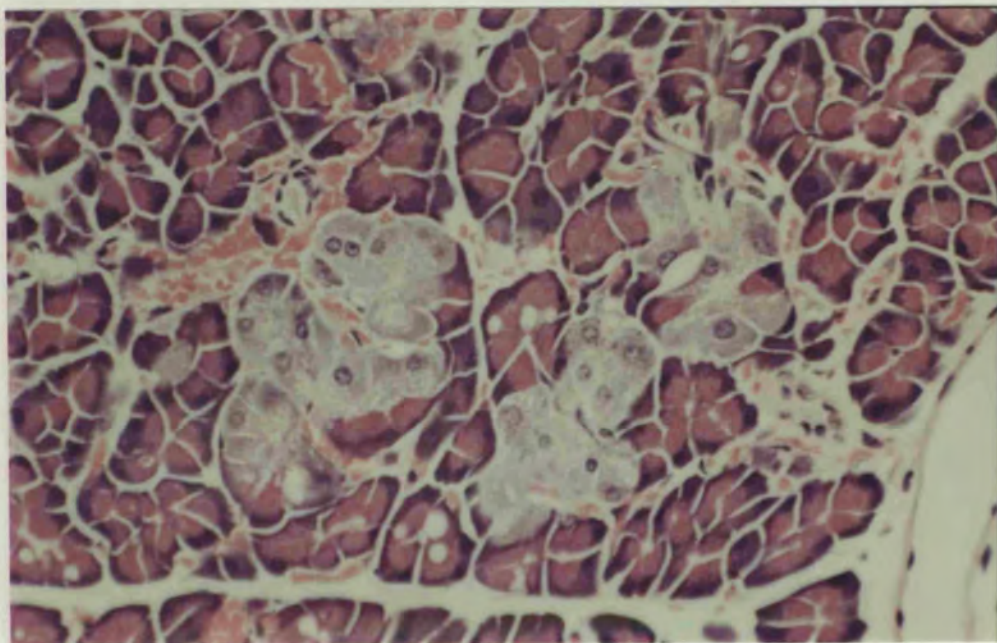
Figure 11

Focal necrosis of acinar cells within five minutes
of retrograde infusion into pancreatic ductal system
(H and E, original magnification x 100).
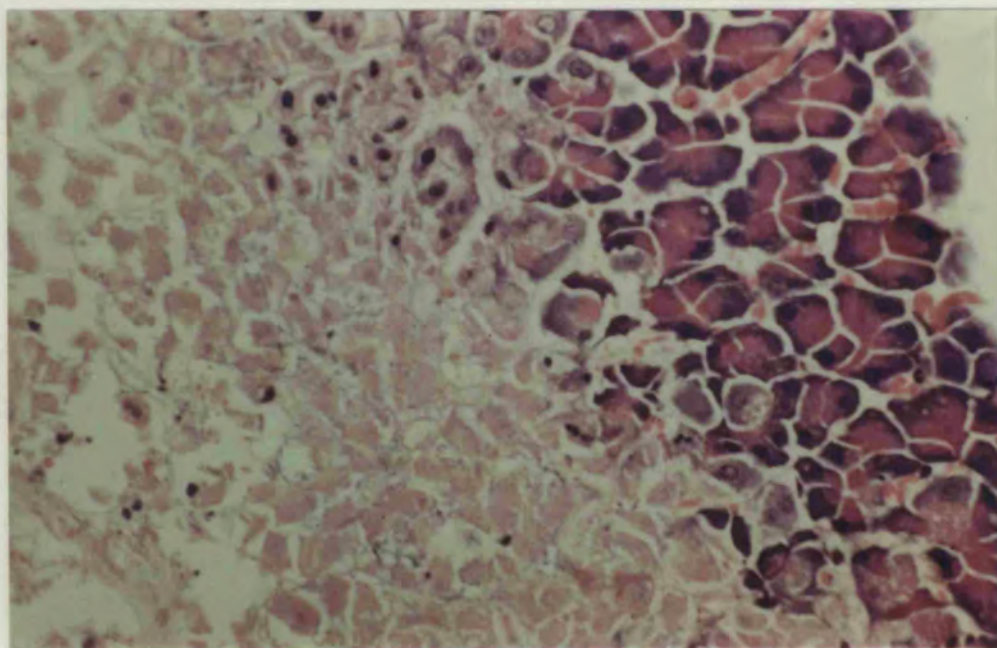
Figure 12

Extensive areas of necrosis within the pancreas at
twelve hours.
(H and E, original magnification x 100).
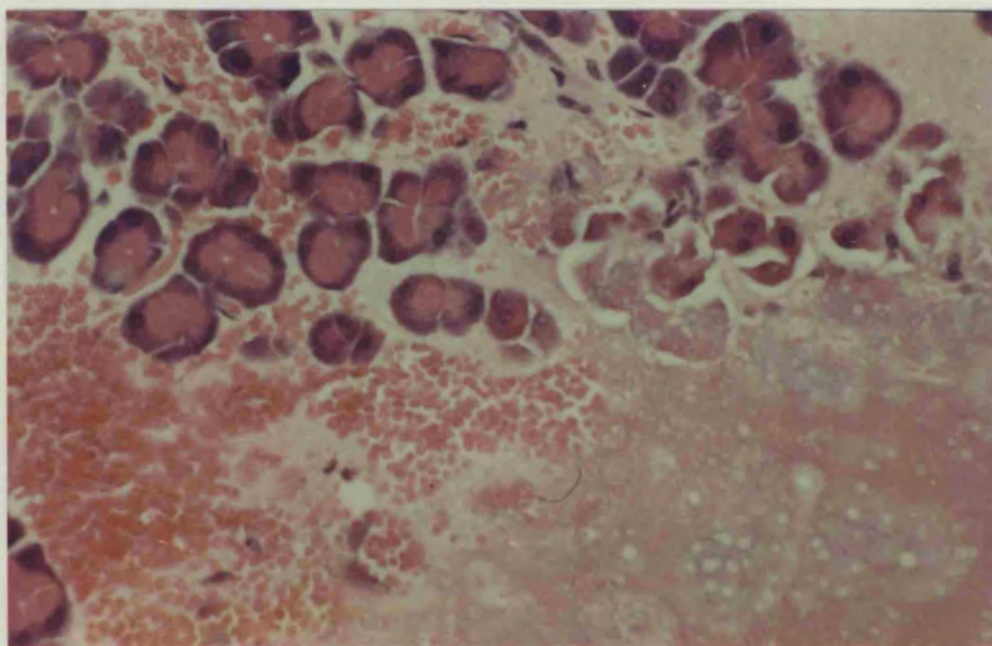
Figure 13

Haemorrhage into necrotic area of the gland
(H and E, original magnification x 100).
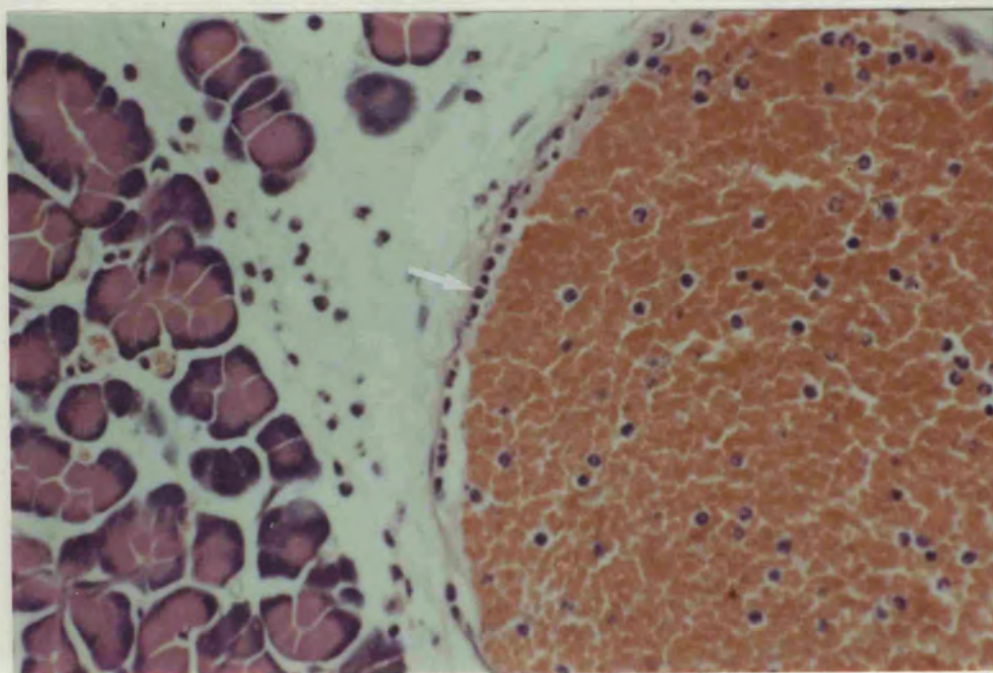
Figure 14

Polymorph margination (arrowed) in a pancreatic
blood vessel at one hour
(H and E, original magnification x 100)
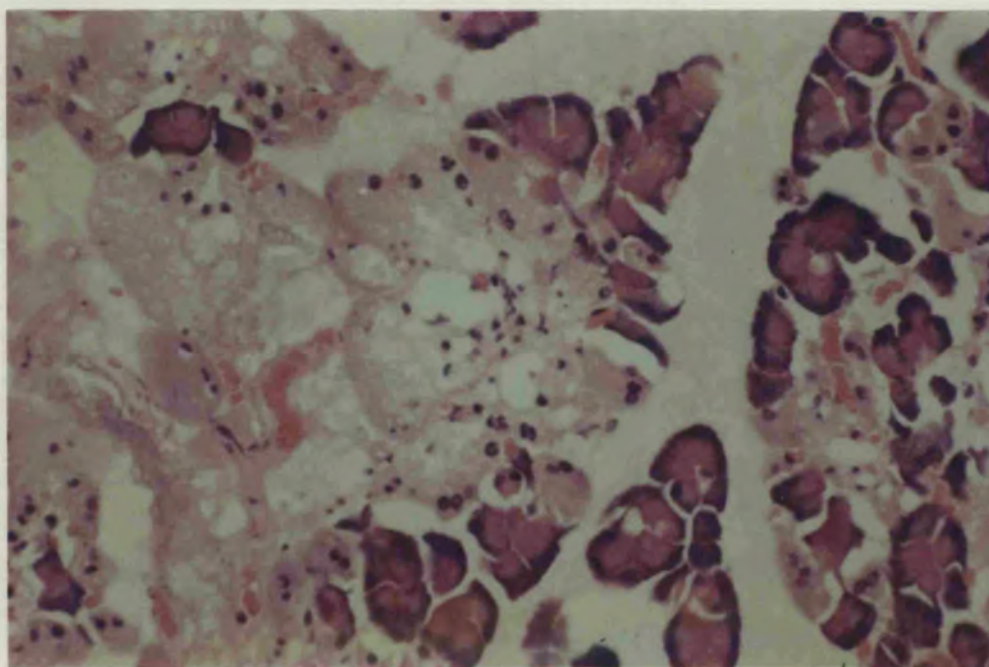
Figure 15

An early acute inflammatory infiltrate in necrotic
areas of the pancreas at three hours
(H and E, original magnification x 100)
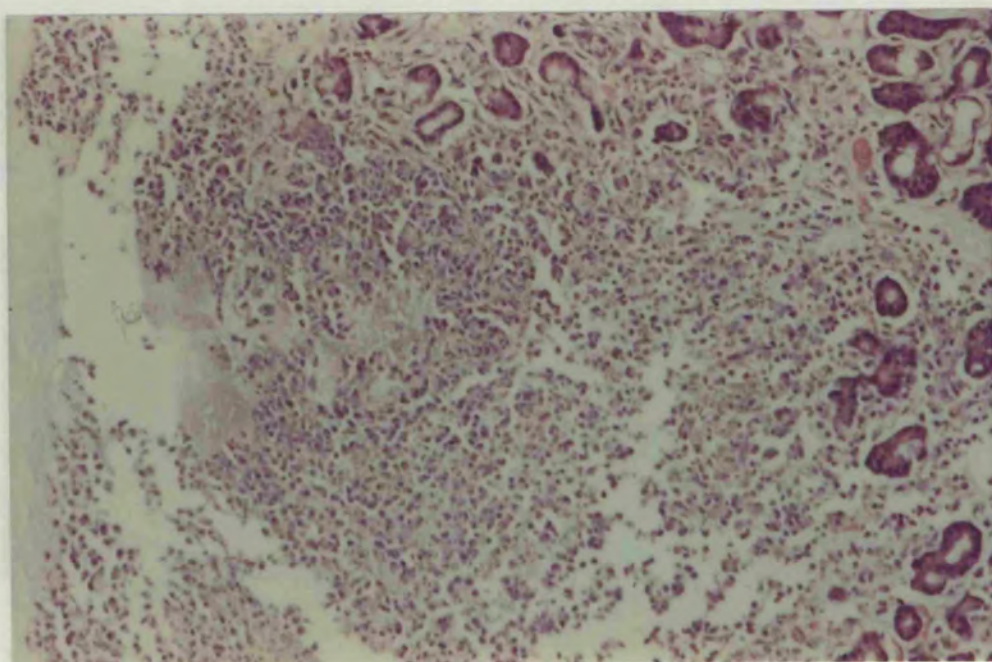
Figure 16

Dense acute inflammatory infiltrate within the gland
at twenty four hours
(H and E, original magnification x 40)
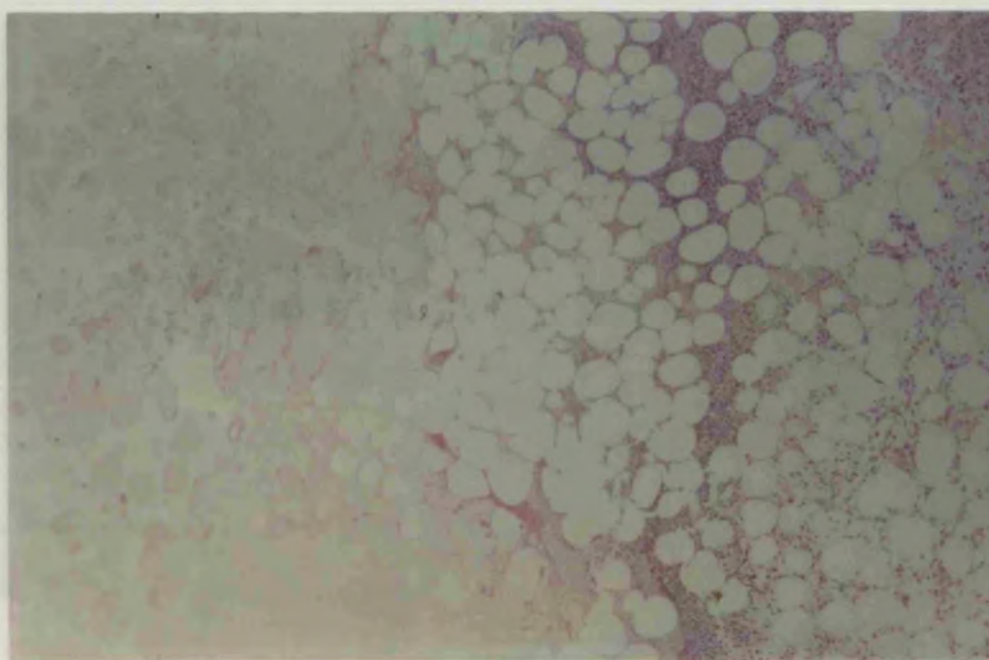
Figure 17

Extensive fat necrosis at twenty four hours
(H and E, original magnification x 40)
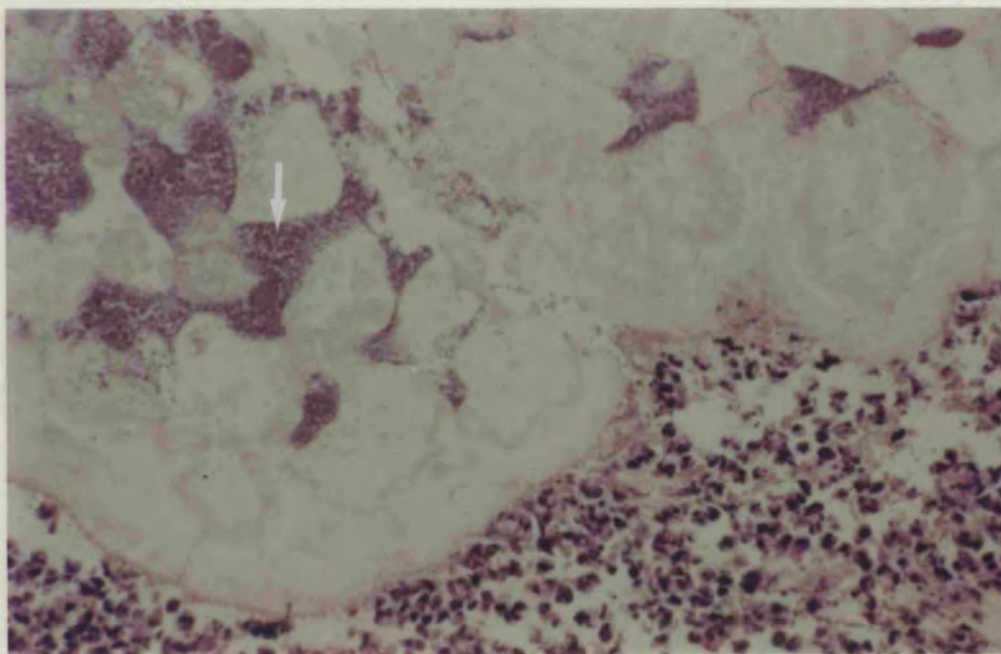
Figure 18

Calcium deposition (arrowed) in an area of fat
necrosis at twenty four hours
(H and E, original magnification x 100)
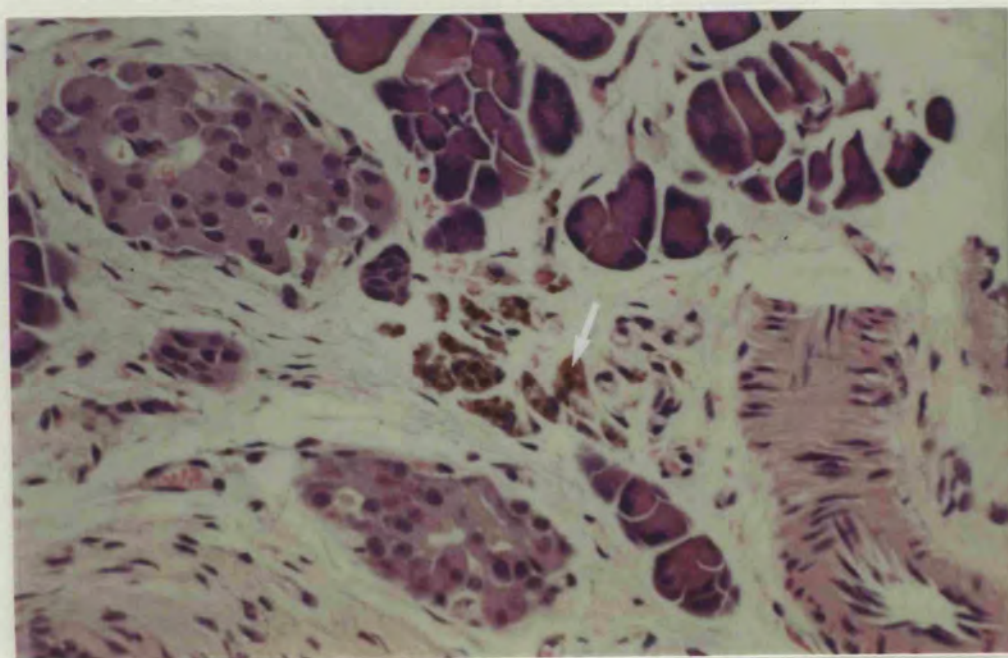
Figure 19

Extensive fibrosis within the pancreas at one week with
relative sparing of Islets of Langerhans. Haemosiderin
deposits are visible within macrophages(arrowed)
(H and E, original magnification x100)
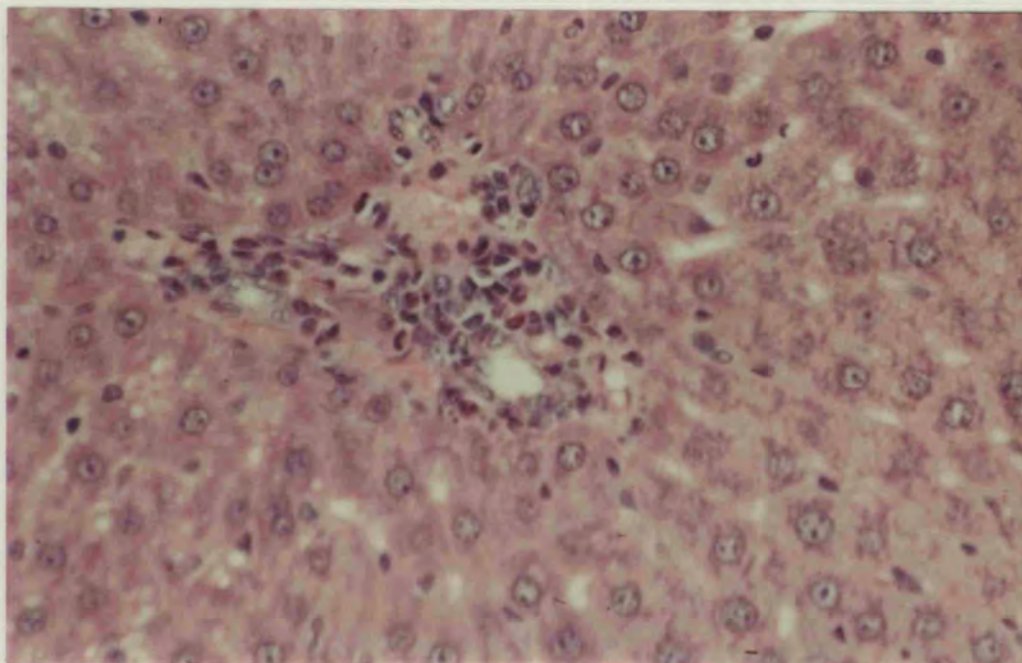
Figure 20

Liver showing inflammation in a portal tract
(H and E, original magnification x 100)
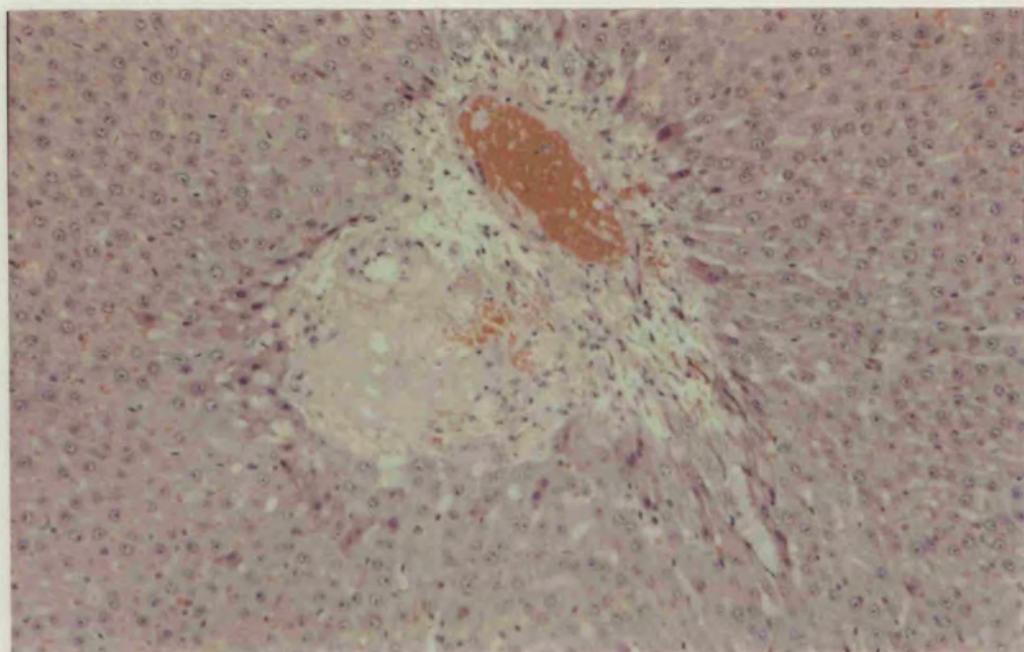
Figure 21

Liver showing small infarct adjacent to a portal tract
(H and E, original magnification x 40)

showed some inflammation in the portal tracts (Figure 20) and occasional adjacent small infarcts (Figure 21) suggesting some retrograde passage of infusate into the biliary tree on removal of the hilar clamp. These changes probably account at least in part for the derangement of liver function seen in some animals (appendix 1).

4.6 Discussion

Bernard in 1856 was the first to document the development of fatal extensive pancreatic necrosis following the instillation of olive oil into the pancreatic ducts. Subsequently numerous animal models of acute pancreatitis have been developed to investigate the pathogenic mechanisms and evaluate methods of treatment (Elliot, 1971). A wide variety of animals have been employed including mice, rats, guinea pigs, cats, dogs, pigs and monkeys.

A commonly used method of inducing pancreatitis has been the retrograde injection of various noxious agents into the pancreatic ductal system. These have included olive oil (Hess, 1909), bile (Hallenbeck et al. 1953), bile salt (Hansson, 1967; Aho et al. 1980; Lankisch et al. 1974), pancreatic enzymes (Wanke, 1970), enterokinase (Hammond and Mann, 1977) and Pfeffer loop fluid (Williams and Byrne, 1967). Ductal cannulation is a prerequisite in these methods.

Creation of a closed duodenal loop in dogs was the basis of Pfeffer's well-known model (Pfeffer et al. 1957). This produced a rapidly fatal pancreatitis probably resulting from reflux of duodenal contents, including enterokinase and activated pancreatic enzymes, into the pancreatic ducts (McCutcheon and Race, 1962). Nevalainen and Seppa (1975) produced a similar model

with a simple closed duodenal loop around the entry of the pancreatic duct. They overcame gastric outlet obstruction by using a transduodenal intraluminal tube. Chetty et al. (1980) modified this model by including injection of infected human bile under pressure into the duodenal loop producing a more severe pancreatitis. These models avoid the difficult ductal cannulation in small animals, but their validity has been questioned as they may produce duodenal necrosis and death from sepsis rather than acute pancreatitis (Dickson et al. 1986).

Obstruction of the pancreatic duct alone may cause oedema of the pancreas but does not produce acute pancreatitis (Elliot, 1971; Gamklou and Edlund, 1966; Isaksson et al. 1983) though secondary stimulation may do so (Lium and Maddock, 1948; Gamklou and Edlund, 1966).

Dietary manipulations causing acute pancreatitis in rats were first reported by Farber and Popper(1950) and Goldberg et al. (1950) using an ethionine supplemented diet. When ethionine supplementation was combined with choline deficiency a consistently lethal pancreatitis was produced (Lombardi et al. 1975). The primary biochemical defect induced by these dietary measures has not been fully established, but it involves interference with RNA metabolism and protein synthesis.

Acute pancreatitis has also been induced in animals using intraparenchymal injections of bile salt and trypsin (Waterman et al. 1969; Bawnik et al. 1974), intravascular clotting and ischaemia (Hardway and McKay, 1958; McPhedran and Lee, 1963), endotoxins (Shohl et al. 1960; Cotlar et al. 1960), bacteria (Williams and Byrne, 1968) immune mechanisms (Thal, 1955; Janigan et al. 1975), scorpion venom (Bartholomew et al. 1976) and

subcutaneous high dose caerulein injections (Wood et al. 1982).

The rat was chosen for this animal model of acute
pancreatitis as it is a convenient and economical laboratory
animal. The anticipated numbers required precluded the use of
larger more expensive animals and the requirements for continuous
intravenous infusion of fluids would have been more difficult in
smaller animals which also tend to tolerate surgical procedures
under general anaesthetic less well than the rat. The Department
of Surgery already maintained an inbred AS colony in the Clinical
Sciences Building at Leicester University. An inbred colony was
required to facilitate collection and administration of fresh
frozen plasma in subsequent experiments (Chapter 7).

The anaesthesia used had already been tried extensively on
rats in the animal unit. The agents certainly proved easy to use
and reliable providing adequate operating time.

Ductal cannulation with retrograde infusion into the
pancreatic ductal system was chosen as the method for inducing
pancreatitis as this is a widely used technique capable of
producing consistent results. I gained some experience of this
technique during a study performed at St.Georges Hospital in
London (Jones et al. 1982b). Sodium taurocholate and enterokinase
were chosen as the agents for retrograde infusion as they have
been successfully used previously to induce acute pancreatitis in
the rat and they reflect the bile and duodenal reflux mechanisms
proposed for the initiation of acute pancreatitis in man.

'Low pressure' injections below 40cm of water are suggested
by Elliot (1971) when inducing pancreatitis by retrograde
infusions in order to prevent duct disruptions and ensure a more
even and reproducible distribution of noxious agents within the

gland. Slow hand injections produce a widely fluctuating pressure
with peaks well over 100cm of water, and the use of a constant
pressure system is unsatisfactory as the time for infusion of a
given volume of infusate is very variable (P.Jones, personal
communication). The method chosen was therefore the slow
controlled infusion of a constant volume into the closed
pancreatic duct system using a syringe pump. This seemed to
produce a consistently severe pancreatitis. The contrast and
pressure studies performed on the model suggested an even
distribution of agents within the gland without major duct
disruptions and with maximum pressures during infusion in the
range 35-50 cm of water.

The induction of pancreatitis was confirmed by the typical
macroscopic and microscopic features of the disease as well as
the marked increase in serum amylase levels and the high amylase
concentrations in ascitic fluid. The development of a simple
scoring system for the main microscopic features of acute
pancreatitis enabled subsequent comparison of the severity of
local disease in different treatment groups (Chapter 7).

A high mortality in an animal model is required to
facilitate the demonstration of significant differences between
potential treatment groups. The mortality in this model was 80%
at 72 hours with most deaths occurring within 48 hours. Survival
to 72 hours was taken as the end point for subsequent experiments
in order to minimise animal suffering. Animals alive at 72 hours
in these initial experiments seemed clinically to have recovered
and were eating normally. The three animals allowed to progress
beyond 72 hours were all alive at one week.

CHAPTER FIVE

A SYSTEM FOR CONTINUOUS INTRAVENOUS INFUSION AND BODY

TEMPERATURE MONITORING IN THE UNRESTRAINED RAT

5.1 <u>Introduction</u>

The continuous intravenous infusion of fluids to the
experimental animals for a period of seventy two hours was a
prerequisite for this study. A number of methods of continuous
intravenous infusion in rats have been developed. These include
close restraint which is ethically undesirable and leads to acute
gastric erosions (Bonfils et al. 1959) and sedation of the
animal (Saarni and Viikari, 1976) which is unsuitable for long-
term infusions. For this study it was decided that the
intravenous infusions should be administered to the unrestrained
rat and that the cannula should be protected in a coiled spring
leading outside the cage. Tail veins commonly provide a site for
infusion in mice and small rats, but in rats heavier than 200g,
as used in this study, horny tail skin renders this extremely
difficult to achieve without surgical exposure of the lateral
tail vein prior to cannulation. The right external jugular vein
was selected for cannulation in this study with the cannula tip
located in the cranial vena cava. This provided a good-sized vein
unlikely to thrombose during the seventy two hour course of the
experiment and remote from the abdominal procedure. A
modification of a system originally described by Lemmel and Good
(1971) was used to protect the cannula.

It seemed likely that the comparison between treatment
regimens in the rat model would be made on the basis of duration
of survival following induction of acute pancreatitis.
Modifications were therefore made to a system developed at
St.Georges Hospital Medical School for the automatic estimation
of time of death based upon the continuous measurement of body
temperature. The time of death can be accurately calculated from

the exponential fall in body temperature occurring thereafter.
The wires from a platinum resistance thermometer implanted into
the unrestrained animal could conveniently be brought away from
the animal to a recorder in the same coiled spring used to
protect the intravenous cannula.

5.2 Materials and Methods

a) Cages

Six purpose built cages were constructed to accommodate one
rat each. Cages with an open top were required to allow
uninhibited movement of the protective spring system passing
between the animals and the intravenous infusion systems and
continuous body temperature recording systems mounted on a shelf
above the cages. The sides of the cage therefore needed to be
high enough to prevent the animals escaping. Transparent material
was used in constructing the cages to enable animals to be
closely observed during experiments. Additional requirements were
that the cages should have an access point for standard water
drinking bottles, a container for food and be easy to clean
between experiments.

Box cages were constructed from 3mm perspex sheeting (Figure
22) (Smiths Brothers Asbestos,Leicester, UK) and consisted of
four sides 12" square and a base 12⅛ " square. The eight joins
were reinforced with ½" x ½" x " aluminium angle held to the
perspex sheets with 6BA raised head countersunk screws.
Construction was such that one edge butts on the next side and
the bottom overlaps all four sides (Figure 23).

A window was cut into one side of the box 9/16" wide, 1"
deep and 3" above the base of the box so that the standard water

Figure 22

The specially constructed perspex cages
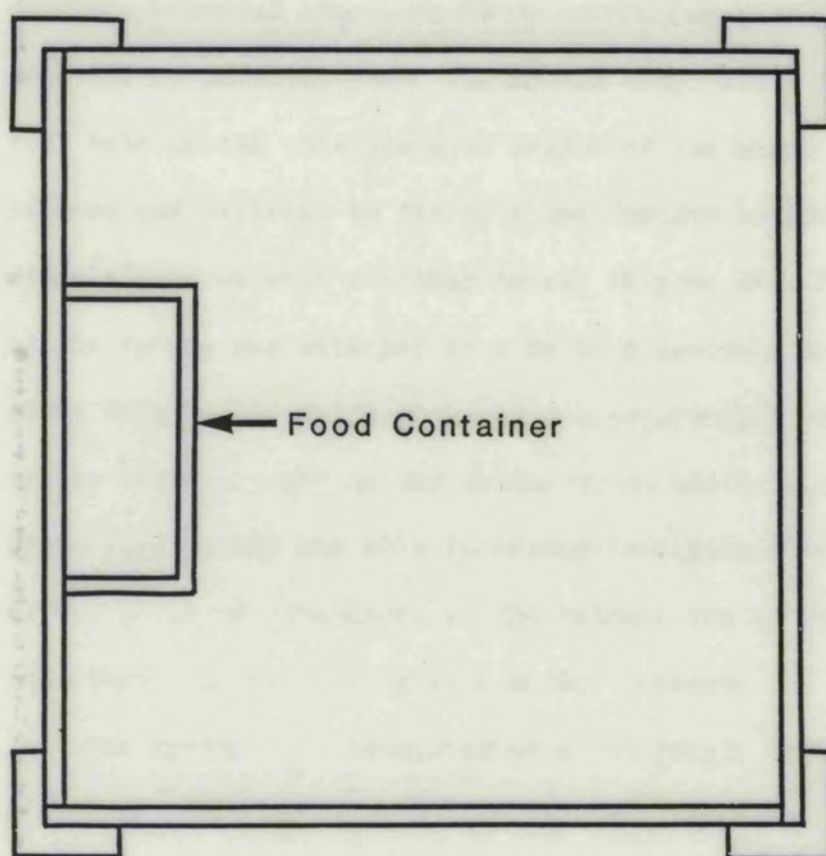
← Food Container

Figure 23

Diagram of construction of perspex cages showing
edges of sides butting onto the next reinforced
with aluminium angle.

bottle spout entered the cage when the bottle was hung on that side. A food container was constructed using a lip of perspex 1" high, 3" wide and 2" deep glued to the base and one side.

All joints were glued after assembly with Tensol 70 (Smiths Brothers Asbestos,Leicester,UK) to ensure a watertight box to make cleaning easier. During experiments sawdust was placed on the cage floor and standard food (Rat and Mouse Economy Pellets, Labsure, Lavender Mill Manea,Cambridge, UK) was placed in the food container. Between experiments cages were first cleaned by hand and then put through an automated cage washer.

b) Protective Spring Systems

Modifications were made to a system already in use in the Department of Medicine at Leicester University for the protection of intra arterial lines providing continuous recording of blood pressure in ambulant rats. The system comprised a flexible spring coil held to the interscapular region of the animal with a harness pad stitched to the skin and further supported around the animals' thorax with a ribbon jacket (Figure 24). The other end of the spring was attached to a balance assembly held in a retort stand (Figure 25) which included a counterweight to keep the spring coil straight as the animal moved about in the cage. The protective spring was able to rotate freely in a radial direction at its point of attachment to the balance arm to prevent twisting and kinking of the spring with animal movement.

The spring coil consisted of a 15" length of open coiled stainless steel spring 0.25" outside diameter made of 0.02" diameter wire with 25 turns per inch (Coil Springs, Bevans Holdings, South Wigston,Leicester,UK). This was strong enough to prevent animals biting into it and the lumen was large enough to
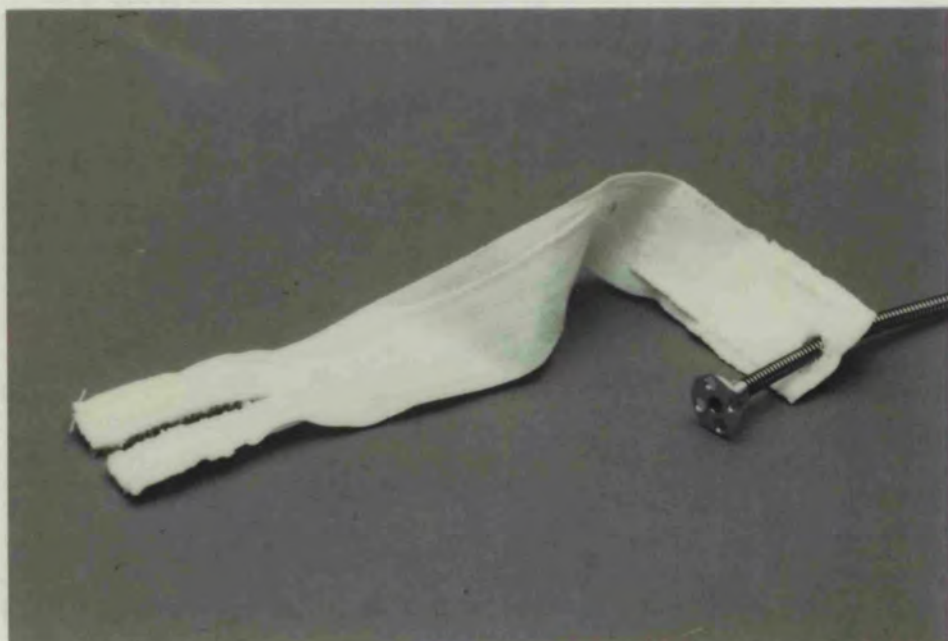
Figure 24

The lower end of the flexible steel spring showing
harness pad and ribbon jacket for attachment to the
animals thorax

Figure 25

The balance assembly for the protective spring
system held in a retort stand

enable both the intravenous cannula and the platinum resistance
thermometer to be threaded down with ease.

One end was glued with 'araldite' to a harness pad made out of
duralumin (Figures 24 and 26) with four holes for sutures
attaching it to the animals' skin around the point of entry of
the cannula and thermometer wires. A one inch wide ribbon jacket
with velcroe pads for fastening around the animals' thorax was
also attached to the lower end of the spring (Figure 24).

The upper end of the spring passed through a delrin spring
guide (Figure 26) in which it could rotate freely in a radial
fashion. This guide was attached to the end of the pivot arm by a
press fit. Above the guide the upper end of the spring passed
into a delrin disc $\frac{3}{4}$" circumference and $\frac{1}{4}$" in height held to the
spring by a 6BA screw (Figure 26). Into the lumen of the spring
at the upper end a spring brush of dural was inserted to prevent
damage to the cannula and wires at their point of emergence from
the spring system (Figure 26).

The balance assembly consisted of a balance arm 24" in
length of $\frac{1}{4}$" circumference duarlumin bar. A fulcrum made of
duralumin was glued to the centre of the balance arm with
araldite (Figure 27). A pivot was constructed of stainless steel
one end of which could be fixed into a retort stand and the other
end attached to the fulcrum by a 1/16" split pin (Figure 27). An
adjustable duralumin balance weight was fitted onto the back end
of the balance arm retained by a 2BA nylon screw (Figure 27).

c) Continuous Intravenous Infusion

One end of a 90cm length of fine polyethylene tubing
(internal diameter 0.28mm, external diameter 0.61mm, Portex
800/100/100/100, Fisons Medical Equipment, Loughborough,

Figure 26 The Upper and Lower Ends of the Protective Spring System
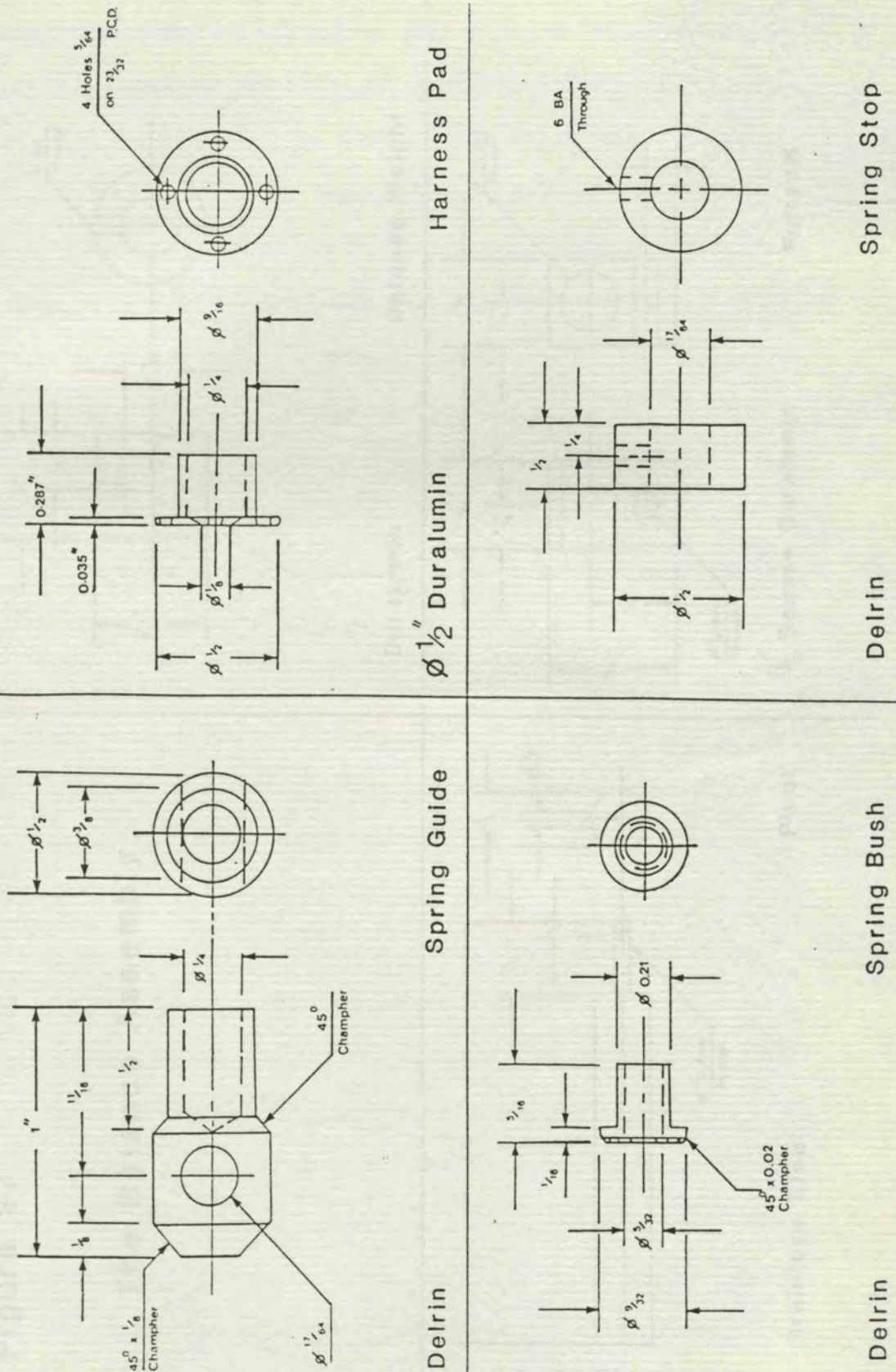
Figure 27

The Balance Assembly

2 BA Through

∅ 1/64"

1/2"

1"

∅ 1"

Balance Weight

∅ 1/16 Hole Through

1/8

1/8

3/16

Fulcrum

Duralumin

3/16

1/8

1/8

9/16

∅ 1/8 Hole Through

3/8" Square Duralumin

∅ 1/8

∅ 1/2

1/16

3/8

∅ 1/4 Hole Through

3 3/8
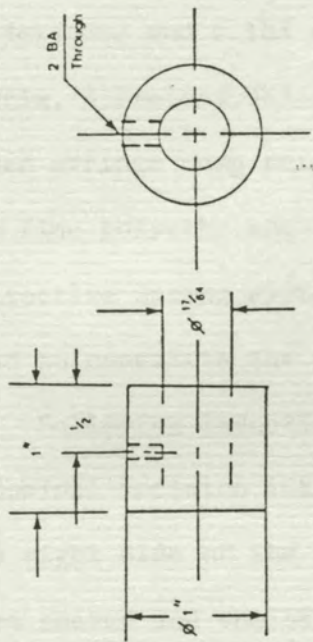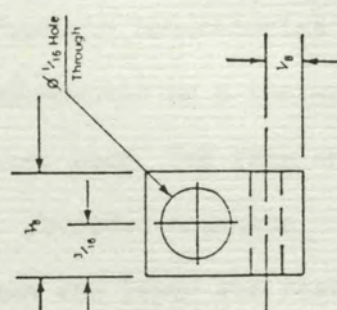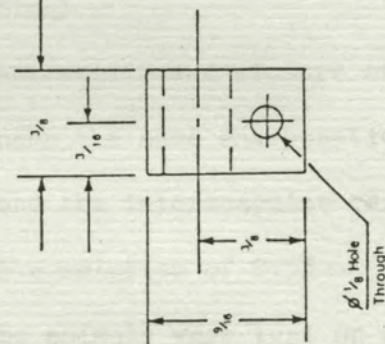
Pivot
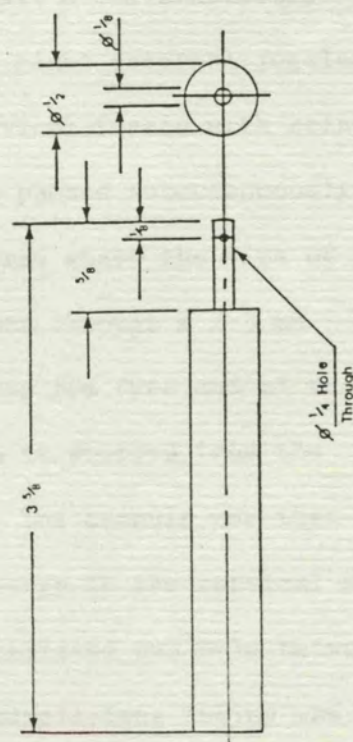
Stainless Steel

Leicestershire,UK) was threaded onto a size 27 hypodermic needle (12 x 0.4mm, Monoject,Sherwood Medical, County Oak, Crawley, W.Sussex,UK) connected via a luer lock to a 60ml sterile syringe. The whole system was carefully filled with a sterile solution of 4% dextrose and 0.18% saline (Galen Research Laboratories Ltd., Antrim, N.Ireland,UK). The syringe was mounted on a variable speed syringe pump set to run at 1ml per hour. The free end of the fine polyethylene tubing was then threaded down the protective spring system to emerge from the lower end ready to be used to cannulate the experimental animal.

Following induction of acute pancreatitis and closure of the abdominal incision (Chapter 4) and under the same anaesthetic, the right side of the animals' neck and the interscapular region were shaved and the skin cleaned with a solution of 0.5% cetrimide and 0.05% chlorhexidine. The animals were kept on a cork board over an electric warming mat. A one centimetre incision was made vertically over the right external jugular vein which was exposed by dividing the cervical fascia with scissors. A pair of mosquito artery forceps was passed subcutaneously from the neck wound to the interscapular area where the tips of the jaws were delivered to the skin surface through a 2-3 mm incision. The jaws were opened to grasp the free end of the 90cm length of fine polyethylene tubing as it emerged from the protective spring system (Figure 28). The cannula was then drawn through the subcutaneous tunnel to emerge in the cervical wound.

The external jugular vein was mobilised and held between two 5/0 silk slings. One centimetre of polyethylene tubing was excised to provide a clean obliquely-cut end and to remove the segment crushed by the artery forceps. The cannula tip was

introduced into the vein through a small incision made with the tips of a pair of iris scissors. The cannula was advanced until the tip lay within the cranial vena cava and the caudal silk sling was tied around the vein and cannula. Two further 5/0 silk ties were secured around the cannula holding it to the cervical fascia, being careful to avoid compression of the lumen. The cervical wound was closed with a single layer of continuous 5/0 silk and the wound sealed with spray dressing (Op-site, Smith and Nephew Ltd., Welwyn Garden City, Herts, UK).

The animals thus received a continuous infusion of fluids at 1ml per hour from a syringe pump mounted on a shelf above the cage. The infusion was carried to the animal in the fine polyethylene tubing protected by the spring system. The cannula tip, having been introduced via the right external jugular vein, was located in the cranial vena cava.

d) Continuous Body Temperature Monitoring

Body temperature was recorded on a 6 channel chart recorder with its own amplifier (Din-Size 880, Sekonic, Rostol Ltd., Ashvale, Hants, UK). This was slightly modified in that the six terminals behind the machine, which were difficult to connect wires to, were brought out to Jack sockets facing anteriorly on the right hand side of the machine for ease of use (Figure 29). Safety standards were improved by putting on a new mains lead terminated properly to improve earthing, and by sealing the back of the machine. The machine was calibrated to measure a temperature range of 0-50°C with a chart speed set at one centimetre per hour. Six animal temperatures could be recorded simultaneously, each in a different colour ink for ease of identification.
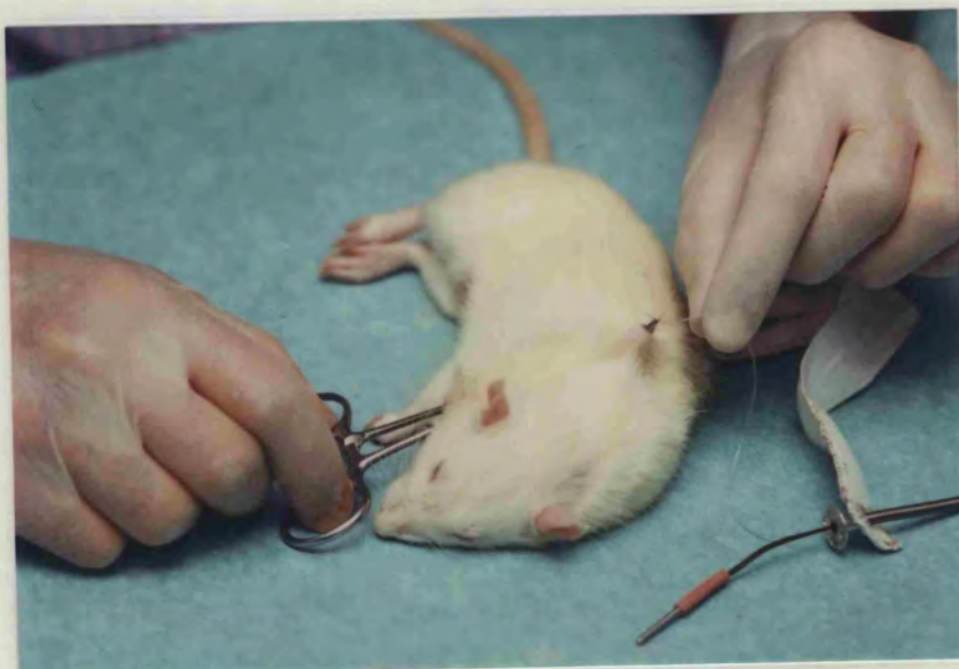
Figure 28

Grasping the free end of the polyethylene tubing as it
emerges from the protective spring system to draw it
subcutaneously to emerge in the cervical wound.
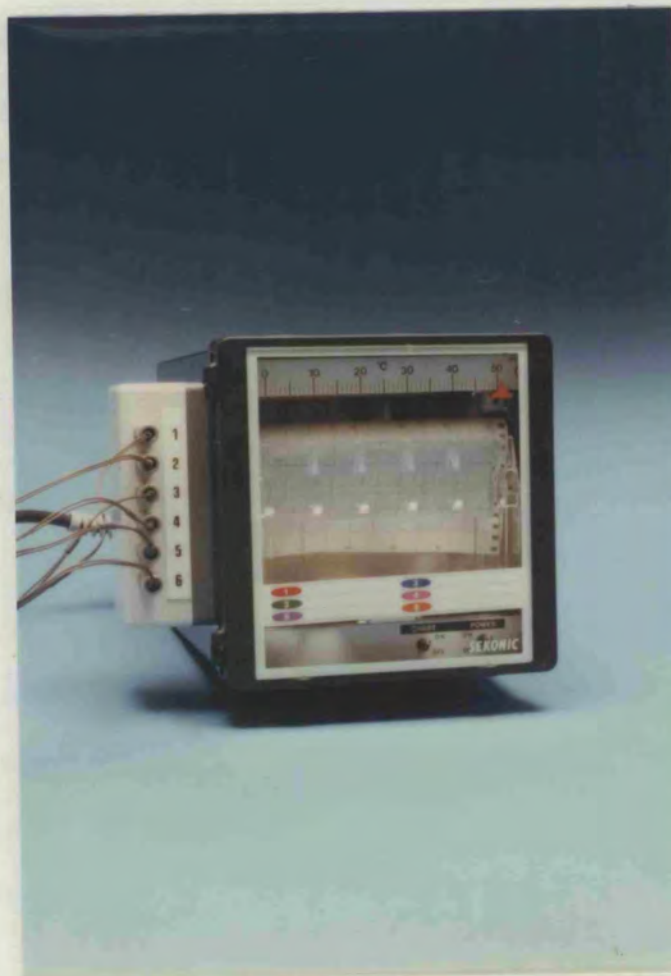
Figure 29

The six-channel
temperature recorder
with the added Jack
sockets.

2mm x 30mm platinum resistance thermometers with integral wire leads (Rostol Ltd., Ashvale, Hants, UK) were used to measure the animals' temperature. These were modified by applying an acrylic cement seal at the base of the thermometer to reduce the incidence of wire fracture caused by repeated angulations brought about by movement of the animal. The distal ends of the wires were fitted with 3.5mm Jack plugs to enable easy connection to the multichannel recorder. The thermometers were threaded down the protective spring system together with the intravenous cannula before the surgical procedure was undertaken on each animal.

The platinum resistance thermometer was implanted subcutaneously into the rats after the venous cannulation was completed and the cervical wound closed. The animal was turned over to reveal the intravenous cannula emerging from the interscapular incision to pass into the protective spring. The thermometer was inserted through the same interscapular incision to lie in the subcutaneous tissues over the animals' thoracic spine (Figure 30).

Following implantation of the thermometer, the metal harness pad on the end of the flexible spring system was sutured over the interscapular incision with four 5/0 silk sutures so that the intravenous cannula and thermometer wires emerging from the animal passed directly into the protective spring (Figure 31). The tape jacket was fastened around the animals' thorax using the velcroe pads to further support the system (Figure 32). The rat was then placed on its side on sawdust in the bottom of its cage to recover over a warming mat. On regaining mobility the animal was able to move in an unrestrained fashion within its cage with
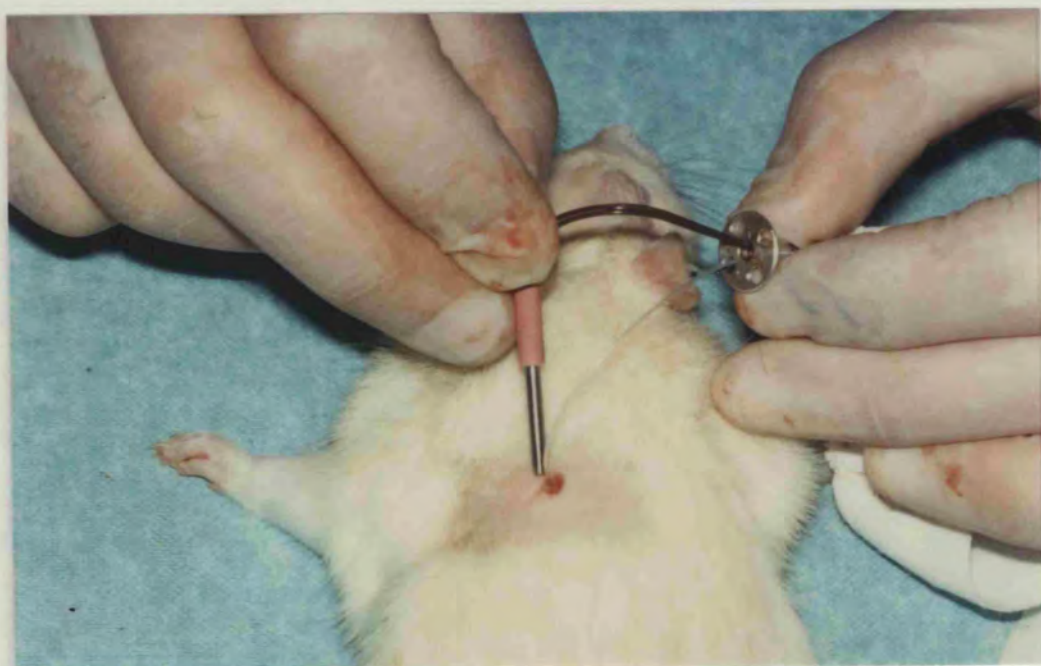
Figure 30

The platinum resistance thermometer with integral
wire leads being implanted subcutaneously through
the interscapular incision

Figure 31

Suturing the metal harness pad to the animals
skin over the site of emergence of the
polyethylene tubing and thermometer wires
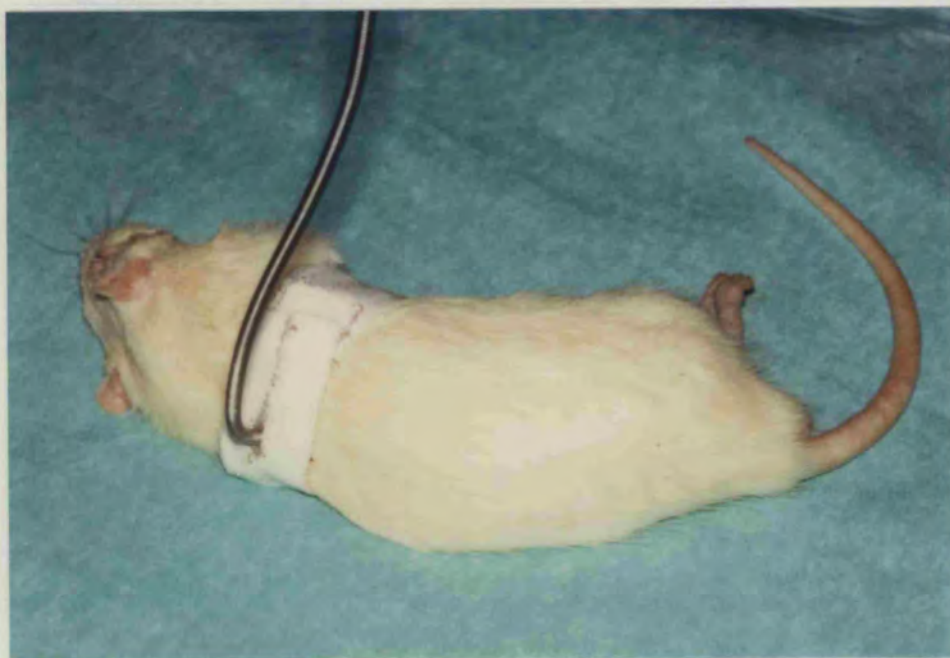
Figure 32

The supporting ribbon jacket fastened around
the animals thorax with velcroe pads
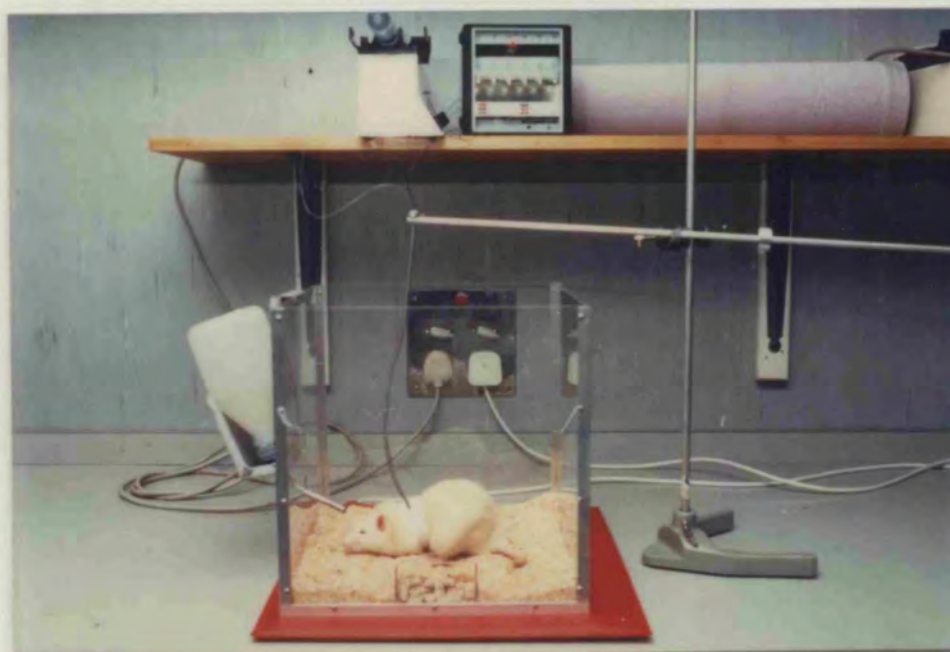
Figure 33

A recovered animal allowed unrestrained movement
within its cage with free access to food and water.
The infusion pump and temperature recorder are
mounted on the shelf above the cage

free access to food and water (Figure 33) whilst receiving a continuous intravenous infusion of fluids from a syringe pump mounted on a shelf above the cage, and whilst having its body temperature continually recorded on the chart recorder also mounted above the cage. The multichannel recorder enabled six animals to be set up simultaneously. A time mark was made on the chart paper at the completion of the surgical procedures as each rat was returned to its cage.

5.3 Results

The cages and protective spring systems gave excellent service. The rats were clearly observed through the transparent cages during the experiments and the cages were readily cleaned between experiments using the automated cage washer. The only minor criticisms of the cages were that they were rather brittle and tended to crack if dropped during cleaning, and that two rats who were recovering from pancreatitis managed to jump out through the open tops of their cages. These problems could be overcome by making the cages from a less brittle plastic and adding six inches to the height.

The protective springs rotated freely in an axial direction with movement of the rat in its cage and were held straight by the counterweight on the suspensory arm. This prevented undue kinking of the spring, and even when this did occur the twists were easily undone without impairing the intravenous infusion.

More than 300 rats received continuous intravenous infusions of 24 ml fluid daily for 72 hours during the course of this study. The cannulation procedure took less than ten minutes with practice. The only surgical problem encountered was the

occasional introduction of air embolus during cannulation of the external jugular vein. This was manifest as a bubbling sound from the rats' thorax followed by cardiac arrest. It occurred during early experience with the technique when the cannula was allowed to produce too large an opening in the vein wall due to an excessive length of free cannula in the cervical wound during introduction. This problem was eliminated by keeping the cannula length in the cervical wound to a minimum and keeping tension on the caudal sling around the vein until it was tied in place around the cannula.

Failures of the infusion system were rare. Due to predominant clockwise or anticlockwise movement of the recovered animals in their cages the length of cannula between the syringe pump and spring system did tend to twist and product loops. This problem was rarely a cause of infusion failure as a reasonable length of redundant cannula was left between the top of the spring and the infusion pump to accommodate any twisting. Twists were periodically taken out of the system by removing the 60ml syringe from the syringe pump and straightening the cannula before replacing the syringe in the pump. The two animals who recovered enough from their acute pancreatitis to be able to jump out of the open cage tops both caused infusion failures by biting through the cannulae above the spring system. Blockage of the cannula lumen was unusual, and when it did occur it was usually overcome by flushing the line with 0.2ml of dextrose saline from a hand-held 1ml syringe.

The warming mat underneath the rat cages prevented excess heat loss during the anaesthetic period. The mat was switched off once the animals were able to stand. The continuous recording of

body temperature in the rat enabled accurate extrapolation of time of death. Animals cooled to room temperature within 5-6 hours of death. Small changes in body temperature occurred whilst the animals were alive post-operatively, and some rats showed a gradual fall in body temperature of $2-4°C$ up to the point of death. However, so sharp was the fall in body temperature from the time of death that the moment of death could be calculated to within 15 minutes using the time markers on the recording.Typical examples are shown in Figure 34. The moment of death is clearly shown in each animal and the use of a colour code facilitated identification of the animals.

Some problems were encountered with the integral wire leads attached to the platinun resistance thermometers. Firstly they tended to fracture at the point of exit from the thermometer due to angulations produced by animal movements. This was improved by surrounding the wires with acrylic cement at their point of emergence. Secondly, like the intravenous cannulae, the wires tended to form twists and loops above the protective spring system in response to the animals' predominant clockwise or anticlockwise movement within the cage. This was overcome by periodically disconnecting the jack plugs from their sockets to undo any twists. The wires did tend to develop fatigue fractures with time, however. In later experiments, when it became obvious that survival at 72 hours was to be the criterion for comparison of intravenous treatment regimens, the importance of accurate . timing of any deaths was diminished. The tempeature recording system was therefore abandoned and times of deaths occurring during periods when the experiment was not directly supervised were estimated as the midpoint from the time at which the animal

Figure 34

Tracing of Continuous Body Temperature
Records in Six Rats
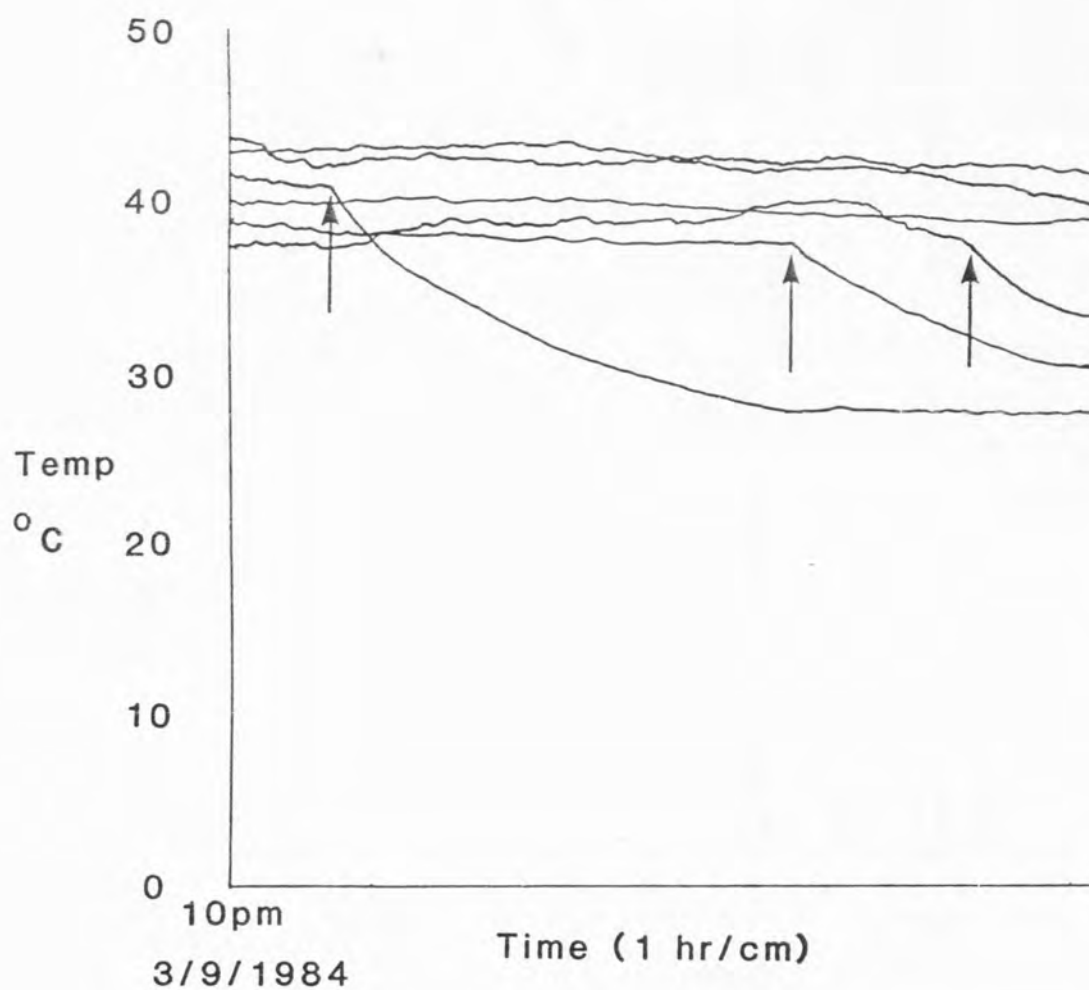
During this record three animals died and
the moment of death can be clearly identified
from the sharp change in temperature trace
occuring at the time of death (arrows)

had last been checked.

5.4 Discussion

Intravenous infusion of fluids is often required in experimental animals. This entails either severe restraint, sedation of the animal or the application of a protective device to prevent the animals biting through the intravenous cannula. Close restraint is ethically undesirable and in rats caused acute gastric erosions (Bonfils et al. 1959). Sedation (Bezman-Tarcher, 1969; Saarni and Viikari, 1976) is unsuitable for long-term infusions. Several systems for the protection of cannulae to mobile animals have been developed. The cannula can be protected electrically (Edmonds and Thompson, 1970) or mechanically by coiled springs leading outside the cage (Dalton et al. 1969; Lemmel and Good, 1971; Jones and Hynd, 1981; Shaw, 1982). In this study a protective spring system was used.

Tail veins commonly provide a site of infusion in mice and small rats (Saarni and Viikari, 1976), but in rats heavier than 200g the thick horny tail skin renders this extremely difficult to achieve without surgical exposure of the tail vein prior to cannulation. For long-term infusions the cannula tip needs to be sited in a major vein to reduce the risk of thrombophlebitis leading to infusion failure. Cannula tips have been sited in the femoral vein and caudal vena cava (Bezman-Tarcher, 1969; Jones and Hynd, 1981; Shaw, 1982) since emergence of the cannula near the tail facilitates the application of a protective device. The jugular vein has been used by Dalton et al (1969) and Evans (1970) and the external jugular vein was chosen in this study as the route of entry of the cannula tip which was subsequently

advanced into the cranial vena cava. This site was remote from the potentially contaminated abdominal procedure and provided a large enough vein for continuous infusions lasting 72 hours. The use of a jacket to hold the infusion system to the animals thorax was originally described in mice by Lemmel and Good (1971). They brought the cannula out from the cervical wound within the jacket rather than tunneling it subcutaneously to reach the interscapular area.

Rotational forces producing twisting and kinking of the cannula, brought about by turning of the recovered animals within their cages, could have been overcome by using swivel devices (Eve and Robinson, 1963), but these are expensive and can be unreliable. The problem was overcome in this study by leaving a long redundant loop of cannula between the top of the spring and the infusion pump to absorb the rotational forces, and then periodically removing the syringe from the syringe pump to undo any twists. This required very close supervision of experiments. More recently (Kamada and Collier, 1986) a simple system for avoiding twists has been developed involving formation of a 12mm diameter spiral from the polyethylene cannula and suspension of the syringe pump from a string above the cage allowing the whole system to turn in response to rotational forces. An alternative is to fix the upper end of the spring so that the spring itself limits the rotational movement of the animal in its cage. This is ethically less desirable and can led to frequent knotting of the spring causing stress to the animal.

Intravenous infusion rates of 1ml per hour have been shown to be tolerated well by rats of this size with acute pancreatitis (Jones et al. 1982b). Blockage of the cannula was

uncommon at this infusion speed and when it did occur it was usually overcome by flushing the line with 0.2ml of dextrose saline from a hand-held 1ml syringe. Heparin was not included with infused fluids as has been suggested in other studies (Saarni and Viikari, 1976; Jones and Hynd, 1981). The rat is very sensitive to heparin and other workers have confirmed that intravenous infusions can be administered without its use (Kamada and Collier, 1986). The cannula was not used to withdraw blood samples as the rat blood volume is small and it was felt that repeated blood sampling would add an additional physiological upset to the experimental model. Instead animals were sacrificed at intervals for haematological and biochemical studies, conveniently combined with post-mortem macroscopic and microscopic studies of body organs.

The entire intravenous infusion system was simple and inexpensive to construct. The animals appeared unstressed by the apparatus. The flexible springs rarely became twisted and kinked as they were able to rotate freely in a radial direction. When twists and kinks did occur they were easily undone without failure of the infusion. The operative technique is straight-forward and within the capability of any research worker. The system enabled reliable continuous intravenous infusion in large numbers of unrestrained rats. The open-topped cages were ideal for viewing the animals and avoiding spring snagging. The only modifications which I would suggest would be to make the cages six inches taller as two animals managed to jump out of the twelve inch cages and to use less brittle plastic.

The methods for determining the length of survival in studies of experimental acute pancreatitis are often not

precisely defined. Some workers have provided constant
supervision while others have relied on survival at a set period
of time such as 24 hours. Janku et al. (1962) devised a complex
and expensive apparatus indicating death as the moment the
delicately suspended animal cages ceased to move producing an
acoustic signal from a central control unit. This was
susceptible to vibrations and still required continuous
supervision. The system used in this study to provide a fully
automated method for determination of the time of death was
developed at St. Georges Hospital Medical School in London.
Lutsky et al. (1966) described a method for estimating the time
of death in dogs by serial rectal temperature measurements to
determine the time elapsed since death by backwards
extrapolation. The St.Georges workers realised that the
continuous monitoring of body temperature in the experimental
system would permit accurate estimation of the time of death.

In this study I have confirmed that their system, which I
have modified only slightly, produces a reliable method of
automated quantitation of survival in rats. The main problem
encountered with the system was the tendency of the integral
solid wire leads supplied with the platinum resistance
thermometers to fracture. This could have been ovecome by
changing the wires for a less brittle multibraided type and
incorporating a swivel device or forming a spiral of the wires as
they emerged from the spring as performed by Kamada and Collier
(1986) for the intravenous cannula. In this study the need did
not arise as it soon became obvious that survival to 72 hours was
to be the measurable end-point for comparison of treatment
regimes and the exact measurement of the times of deaths

occurring before this was not necessary. The continuous recording of body temperature was therefore abandoned and the times of any unsupervised deaths were estimated more crudely as the mid-point of the unsupervised period.

CHAPTER SIX

BUPRENORPHINE ANALGESIA IN THE RAT MODEL

OF ACUTE PANCREATITIS

6.1 Introduction

    There is an increasing awareness of the need to provide
adequate analgesia for experimental animals, particularly when
potentially painful pathology such as acute pancreatitis is
inflicted. There are few objective standards for the assessment of
pain in animals. Most of the criteria used are subjective, and
are often based on a general clinical impression of the animals
appearance and behaviour. The apparent lack of a clearly
identifiable response to pain in animals has led to the
suggestion that they do not experience pain in the same manner as
do human beings. Whilst this may be so, the argument must not be
extended to suggest that attempts to provide pain relief in
laboratory animals are therefore unnecessary. Until further
progress is made in assessing the nature of pain in animals, it
should be assumed that if a procedure is likely to cause pain in
man, it will produce a similar degree of pain in animals and the
relief of pain must be considered essential.

    Acute pancreatitis is undoubtedly a painful pathology in man
and therefore it was decided that all rats in which acute
pancreatitis was induced should receive analgesia throughout the
course of the experiment. A centrally acting agent was chosen as
they are generally the most potent analgesics. Buprenorphine
('Temgesic', Reckitt and Colman, Kingston-upon-Hull, Humberside,
UK) was preferred to morphine or pethidine because:

a) it may be 50-100 times more potent in some of its analgesic
   properties when compared with morphine and pethidine (Cowan et
   al. 1977; Shannon, 1983)

b) it is longer acting than morphine or pethidine with a half
   life of at least six hours in animals and possibly ten to

twelve hours (Flecknell, 1984) allowing eight or twelve hourly bolus injections rather than four hourly as is required with morphine and pethidine.

c) it has marked narcotic antagonist activity and may reverse the narcosis and respiratory depression produced by neurolept-analgesic combinations such as Immobilon used as an anaesthetic agent in these experiments. The prospect of reversing narcosis and respiratory depression yet maintaining (and prolonging) analgesia by the use of buprenorphine is extremely attractive (Flecknell, 1984).

d) Tolerance to morphine is produced rapidly in rats and dosages must be continuously increased to produce equivalent analgesic effects (Cowan et al. 1977). Tolerance has been produced with repeated injection of buprenorphine, but its onset is much slower and its manifestation would not be anticipated during the 72 hour course of the planned experiments.

e) Buprenorphine does cause some respiratory depression in rats with increased arterial pCO_2 and reduced pO_2, but only with large doses beyond those required for analgesia, and the effect is much less than with morphine (Cowan et al. 1977).

Initially, on the manufacturers' advice, rats in whom active pancreatitis had been induced received 0.03 mg/kg of buprenorphine as an intramuscular injection at the end of the surgical procedures followed by a continuous intravenous infusion infusion of 0.03 mg/kg/8hr. The buprenorphine was added to the intravenous infusion fluid contained in syringes for mounting on the syringe pump. This route of administration was chosen to provide more stable analgesic levels and because continuous intravenous infusion was already incorporated in the study.

On this dosage analgesia appeared inadequate as assessed by senior animal house staff experienced in the assessment of laboratory animals. The rats showed behavioural changes such as immobility, failure to groom, abnormal posture ('tucked up' appearance), and decreased food and water intake. There were also changes in appearance such as loss of eye colouration and fur discolouration, but most obvious was the pain response of squeaking and attempted biting or withdrawal elicited on abdominal palpation.

It was therefore apparent that higher doses of buprenorphine were required. Broad plateau, biphasic or inverted U-shaped dose-response curves have been obtained with buprenorphine for some of its actions. A simple experiment was therefore devised to ensure that higher doses of buprenorphine did provide increasing analgesic effect without encoutering significant antagonist effects or other side effects, and also to ensure that tolerance was not encountered within the 72 hour expected duration of the main animal experiments.

6.2 Materials and Methods

Buprenorphine was generously donated by the manufacturers as pure solid buprenorphine hydrochloride which was made up to a solution containing 1mg/ml by dissolving it in 0.9% NaCl. This was kept frozen at $-20°C$ under which conditions the manufacturers report it remains quite stable (personal communication). Small aliquots were thawed as required for each experiment and diluted with normal saline to a solution containing 0.1 mg/ml from which volumes for intramuscular and intravenous administration were drawn using a graduated 1ml

syringe.

Under general anaesthetic male AS rats 250-300g underwent
right internal jugular vein cannulation with the polyethylene
cannula and protective spring systems as previously described in
Sections 4.2a and 5.2 a-c. Recovered animals were allowed
unrestrained movement in their cages with free access to food and
water whilst receiving a continuous intravenous infusion from a
syringe pump mounted above the cage.

Thirty animals were used in this study in groups of six.
Animals received 4% dextrose 0.18% saline infusions at a rate of
1ml per hour. Two hours after recovery from anaesthetic they were
randomised on the opening of a sealed envelope to one of six
dosages of buprenorphine (Table 11) ranging from none to 0.5
mg/kg/8hr, which were added to the dextrose saline intravenous
infusions. Animals also received a bolus intramuscular loading
dose equivalent to sixteen hours intravenous dosage as suggested
by Flecknell (1984).

TABLE 11

Buprenorphine Experiments

No. of Rats	Intramuscular loading dose of buprenorphine	Continuous intravenous dose of buprenorphine
5	Nil	Nil
5	0.2 mg/kg	0.1 mg/kg/8hr
5	0.4 mg/kg	0.2 mg/kg/8hr
5	0.6 mg/kg	0.3 mg/kg/8hr
5	0.8 mg/kg	0.4 mg/kg/8hr
5	1.0 mg/kg	0.5 mg/kg/8hr

Animals were assessed 'blind' by a senior animal house technician experienced in rat behaviour. He did not know which dosage of analgesia the rats were receiving. He saw the animals six-hourly over a 72 hour period and scored them on their reaction to toe and tail pinch. Toe pinch was performed by picking the animal up and pinching a digit of a hind leg between finger and thumb. Tail pinch was performed by pinching the tip of the tail between finger and thumb. The response was scored for each of these tests according to the following scale:

3 = Normal brisk withdrawal response often associated with
    squeaking and attempted biting

2 = reduced response

1 = minimal response

0 = no response

The two scores were recorded every six hours together with any comment about the animals general appearance and behaviour. Animals were sacrificed after 72 hours.

Data were analysed using a Minitab Computer package to perform the Wilcoxon matched pairs signed ranks test.

6.3 Results

There were no apparent ill effects on the animals even at the highest dosage of buprenorphine. Their appearance remained unchanged and there were no obvious behavioural changes. In particular the eating habits did not obviously differ between the groups. The scores of toe and tail pinch are given in Appendix 2.

In animals No.1,6,7,11 and 13 intravenous access was lost during the course of the experiment through mechanical failures

as described in Chapter 5. This fairly high incidence of infusion failure (16.7%) reflects the fact that these experiments were performed very soon after the intravenous infusion system had been developed and represents teething problems which were not encountered in subsequent experiments. Animals in whom the infusion system failed were sacrificed and only toe and tail pinch scores obtained before infusion failure were used in the analysis of results.

All scores of toe and tail pinch were analysed against the dosage of buprenorphine administered. Figure 35 shows the mean scores of combined toe and tail pinch plotted for each animal together with the mean score for all five animals at each buprenorphine dosage. All dosages of buprenorphine significantly lowered the mean score for toe and tail pinch when compared with no analgesia at all with $p < 0.0001$. The mean score for animals receiving only 0.1mg/kg/8hr was significantly higher than the score for animals receiving 0.4 mg/kg/8hr or 0.5 mg/kg/8hr with $p < 0.05$.

In order to exclude the development of analgesic tolerance during the 72 hour course of the experiment, the scores for toe and tail pinch at 6,12,18 and 24 hours (day 1) were compared with the scores at 54,60,66 and 72 hours (day 3) (Table 12). There was no detectable increase in scores for toe and tail pinch from day 1 to day 3 at any dosage of buprenorphine suggesting that tolerance had not developed over this timescale.

Figure 35

Combined Toe and Tail Pinch Scores
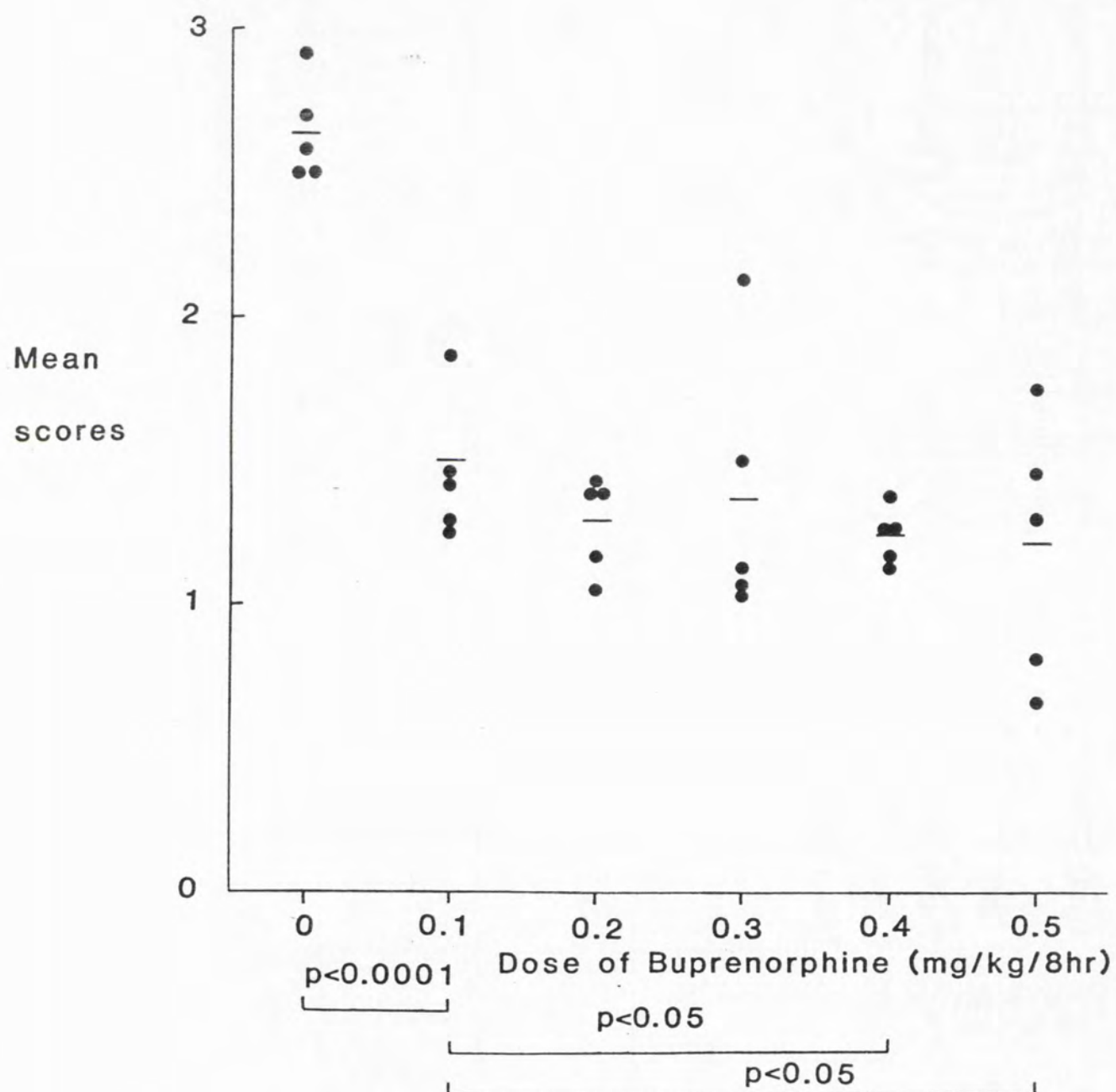
TABLE 12

A Comparison of Combined Toe and Tail-Pinch Scores on Days One and Three

Dose of Buprenorphine	Scores	Day 1	Day 3	'P' Value
Nil	median	3	3	0.6101
	range	(0-3)	(0-3)	
0.1 mg/kg/8hr	median	2	2	0.6362
	range	(0-3)	(0-3)	
0.2 mg/kg/8hr	median	1.5	1	0.7637
	range	(0-3)	(0-3)	
0.3 mg/kg/8hr	median	1	1	0.3640
	range	(0-3)	(0-3)	
0.4 mg/kg/8hr	median	1	1	0.6180
	range	(0-3)	(0-3)	
0.5 mg/kg/8hr	median	1	1	0.8339
	range	(0-3)	(0-3)	
TOTAL	median	1	1	0.4488
	range	(0-3)	(0-3)	

6.4 Discussion

The provision of adequate analgesia to rats with acute pancreatitis was of fundamental importance to this project. It is a major problem to determine whether an animal is in pain. Observations of the animals behaviour may be helpful and pain may be present if the animals become relatively inactive or completely imobile, show an abnormal gait or posture ('tucked up' appearance), become abnormally aggressive or restless, stop eating and drinking or show abnormal vocalisation such as squeaking. Experienced animal laboratory staff were not satisfied with the analgesic effect of 0.03mg/kg/8hr buprenorphine because the animals became immobile and anorexic with a failure to groom and a 'tucked up' appearance. Animals also lost eye colouration, developed fur discolouration and showed an obvious pain response of squeaking, withdrawal and attempted biting on abdominal palpation. Buprenorphine is increasingly used for relief of pain during acute pancreatitis in man where it has the additional potential advantage of not causing spasm of the bile duct sphincter (Staritz et al. 1986). It may be regarded as the most generally useful agent at present which can be used to provide effective, long lasting analgesia in a wide range of species (Flecknell, 1984).

Buprenorphine [N-cyclopropylmethyl-7 -(1-5-hydroxy,1,2,2,trimethylpropyl)-6,14 endoethano-6,7,8,14,-tetrahydronororipavine] is a C-bridged oripavine derived from thebaine. It is a partial agonist of the morphine type (Shannon, 1983) and a potent antinociceptive agent. It is excreted mainly in bile in the rat. Lower doses of buprenorphine suppress morphine abstinence whereas higher doses precipitate a mild

abstinence syndrome in morphine-dependent dogs (Martin et al. 1976). In addition ceiling effects occur with single doses of buprenorphine and the slopes of the dose-response curves for suppression and precipitation of morphine abstinence are relatively flat. These pharmacologic properties are characteristic of a partial agonist.

On the other hand biphasic or inverted U-shaped dose-response curves have been obtained with buprenorphine in rats for certain behavioural measures including induction of catalepsy, tail flick, vocalisation threshold tests for analgesia and EEG and measures of behavioural arousal (Cowan et al. 1977; Kareti et al. 1980; Dum et al. 1981; Dum and Herz, 1981). The mechanisms for these biphasic actions of buprenorphine remain uncertain. Biphasic dose response curves are not readily accounted for within the context of current receptor theory for partial agonists (Dum and Herz, 1981). To account for the biphasic dose response curves it has been postulated that buprenorphine may act at one receptor to produce its agonist action, and at a sterochemically related but distinct second receptor at which the effect produced at the first receptor is antagonised (Rance, 1979).

These analgesia studies were performed to determine whether larger dosages of this partial agonist could be administered with an increased analgesic effect and without running into serious side effects. It also enabled an assessment of the development of tolerance.

Animal analgesic testing is performed using thermal methods (hot plate test, tail-flick test) chemical methods, (mouse writhing test) or electrical methods (electric grid shock, tail

shock, tooth pulp shock) which can be combined with avoidance behaviour such as the pressing of a lever to reduce the severity of the shock in order to get a measure of pain levels (Flecknell, 1984). Here mechanical methods of toe and tail pinch were used as they are well accepted procedures which are quick, easy and cheap to perform and do not require the animals to be subjected to prolonged periods of pain.

The problem of extrapolating from the types of painful stimulus used in analgesic tests to the type of pain likely to be encountered by the rats during acute pancreatitis is common to all the available test methods. Nevertheless some indications can be gained of the dosage of analgesics that are likely to be effective. The acute pancreatitis model was not used for analgesia testing as it did not seem justifiable to expose animals to a potentially continuous pain until adequate analgesia was provided.

This experiment demonstrated that the analgesic effect of buprenorphine increases over the range 0-0.5 mg/kg/8hr. All doses of buprenorphine tested produced a significant reduction in toe and tail pinch score when compared with no analgesia and 0.4 and 0.5 mg/kg/8hr produced a significant reduction in score when compared with 0.1 mg/kg/8hr. It appears that within this dosage range the antagonist effects of buprenorphine are not enough to reduce the analgesic effect.

The rats seemed to tolerate buprenorphine even at the highest dose tested with no obvious side effects or behavioural changes when compared with animals receiving no buprenorphine. There was also no evidence for the devleopment of tolerance within the 72 hour time course of the experiments - the toe and

tail pinch scores during the first 24 hours were not significantly different from those obtained during the last 24 hours. This is an obvious advantage of burprenorphine when compared with morphine for which tolerance develops rapidly (Cowan et al. 1977).

As this work was being completed a paper was published on the relief of pain in laboratory animals (Flecknell, 1984) which reported clinical experience with analgesics in laboratory animals and suggested a dose of 0.1-0.5 mg/kg buprenorphine subcutaneously 8 or 12 hourly to provide analgesia in the rat. This was in broad agreement with my findings and therefore it was decided that the following policy should be adopted for analgesia in this model of acute pancreatitis:

a) Animals should receive a loading dose of 0.6 mg/kg buprenorphine at the end of the surgical procedures.

b) All intravenous fluids administered to the animals should contain buprenorphine at a concentration providing 0.3 mg/kg/8hr (ten times the dose originally suggested by the manufacturers).

c) Animals should be assessed regularly throughout the course of the experiment and any animal judged to be suffering pain should have the dose of analgesia increased to 0.5 mg/kg/8hr with a suitable additional loading dose given intramuscularly.

d) Any animals still thought to be suffering unduly should be sacrificed.

This policy was followed during the course of the study and animals remained acceptably comfortable as assessed by experienced animal technicians and a veterinary surgeon. Of over 300 animals in whom acute pancreatitis was induced and who were

allowed to recover from anaesthetic, in only 5% was it necessary to increase the dosage of buprenorphine from 0.3 mg/kg/8hr to 0.5 mg/kg/8hr. Only two of these animals were sacrificed before their experiment was completed because of continued unacceptable behavioural abnormalities suggesting persistent pain.

Subsequently a report has appeared of the successful use of much higher doses of buprenorphine in rats with experimental acute pancreatitis (Wereszczynska-Siemiatkowska et al. 1986). 15 mg/kg/8hr was administered via a jugular catheter to male Wistar rats with bile salt induced acute pancreatitis. This represents a 400-fold increase of the dosage used in this study and is greatly in excess of doses recommended for use in laboratory animals (Flecknell, 1984). Such large doses of buprenorphine are unnecessary and may be counter-productive if side effects such as respiratory depression occur. The same study reported no effect on pancreatic enzyme elevation in serum or ascitic fluid and no alteration of the histological parameters of acute pancreatitis in animals receiving buprenorphine when compared with colloid controls. The authors concluded that buprenorphine could be recommended for use in experimental acute pancreatitis when studying therapeutic regimens without interfering with them.

CHAPTER SEVEN

CONTROLLED TRIALS OF FRESH FROZEN PLASMA THERAPY

IN EXPERIMENTAL ACUTE PANCREATITIS

7.1 Introduction

Having established an animal model of severe acute pancreatitis incorporating continuous intravenous infusion and automated estimation of time of death, the next phase of the study was a comparison of the effect of fresh frozen plasma therapy on survival of the animal model compared with crystalloid and colloid control groups.

A method for the collection of fresh frozen plasma from male rats in the same inbred AS colony was developed as a preliminary to these experiments.

7.2 Materials and Methods

a) Collection of fresh frozen plasma

Pooled whole blood was collected from male rats 250-350g from the same inbred AS colony used for the acute pancreatitis model. The blood was collected into a standard double blood pack (Fenwal double blood pack, Travenol Laboratories Ltd., Thetford, Norfolk, UK) designed for the collection of 450 ml of human blood. The pack consisted of one bag containing 63 ml of citrate phosphate dextrose adenine formula 1 anticoagulant solution, with a second empty bag connected via a length of tubing enabling the plasma to be transferred within the closed system upon the breaking of the communicating seal.

Each animal was anaesthetised with a mixture of 5% halothane and oxygen at 2 litres per minute delivered from a Boyle's machine via a conical head mask. Once the animal became unconscious the halothane was reduced to 1.5% and the left lateral thoracic wall was cleaned with a solution of 0.5% cetrimide and 0.05% chlorhexidine. The animal was then exsanguinated by direct

cardiac puncture using a 19G needle mounted on a 10ml sterile syringe. This yielded 5-12 ml (median 10ml) of whole blood per animal. A fresh sterile needle and syringe was used for each animal. Once no more blood could be obtained from the cardiac needle, the syringe was disconnected and the animal sacrificed by cervical fracture.

Rat whole blood clotting time is very short (less than 2 minutes) and, to prevent blood clotting before reaching the blood bag, the syringes had to contain an anticoagulant. Initially this was provided by preliminary filling of the syringe dead space with heparin solution ('Hep-Flush', Sodium Heparin 100 units/ml, Edwin Burgess Ltd. Princes Risborough, Bucks, UK). This procedure had to be abandoned when rats receiving infusions of fresh frozen plasma collected in this way were found to be dying of haemorrhagic complications. Coagulation studies revealed gross prolongation of whole blood clotting time and thrombin time, suggesting over-heparinisation. (See Results, 7.3).Anticoagulant was then provided for the syringes by withdrawing some of the acid citrate anticoagulant from the blood bag, paying careful attention to sterile technique, and placing 0.5 ml in each syringe.

A total of 45-50 animals had to be bled in this way in order to fill a blood bag. Whole blood was transferred from the syringes to the blood bag through a sampling site coupler (Fenwall Laboratories, Deerfield, Illinois, USA) using a fresh sterile needle. The bleeding was performed in one session (2-3 hours work) following which the full blood bag was immediately spun in a refrigerated centrifuge at 5000 rpm for twenty minutes. Plasma was separated from the packed cells using a simple plasma

extractor press (4R4414 Fenwall Laboratories, Deerfield, Illinois, USA) and breaking the communicating seal to allow the plasma to flow over into the second bag. A yield of 250-300ml of plasma was obtained from each bag of pooled whole blood.

Buprenorphine was added to the bag so that the plasma contained 0.01 mg/ml of buprenorphine. This would then provide approximately 0.3 mg/8hr buprenorphine as analgesia during subsequent infusions to animals with pancreatitis assuming a mean animal weight of 275g (see Chapter 6). Once again careful sterile technique was employed when adding buprenorphine to the plasma.

After fitting a fresh sampling site coupler, plasma was withdrawn from the bag using a sterile needle so that 8ml aliquots were placed into 60 ml syringes. These syringes were sealed with a blind hub and stored at -20°C.

Thus within 3-4 hours of starting the cardiac punctures to obtain whole blood, the pooled plasma was frozen in 8 ml aliquots inside 60 ml syringes ready for mounting direct onto syringe pumps once thawed.

b) The controlled trials

Acute pancreatitis was induced in male AS rats 250-300g as described in Chapter 4 and a continuous intravenous infusion system was used as described in Chapter 5. The system for automated estimation of time of death (Chapter 5) was used initially, but later abandoned.

The animal model of acute pancreatitis was set up using six animals at a time. Following the surgical procedures they were placed in their cages to recover from anaesthetic, receiving an intravenous infusion of 1ml per hour of a sterile solution of 4% dextrose and 0.18% saline ('Intraven', Galen Research

Laboratories Ltd., Antrim,N.Ireland). Two hours after the completion of the surgical procedure the animals were inspected. If they had recovered from anaesthetic they were randomised on the opening of a sealed envelope to one of three intravenous fluid regimes. Within each batch of six animals, two were randomised to each treatment group. Animals who had not survived the anaesthetic and surgical procedures were therefore not included in the trials.

Each experiment then ran for 72 hours during which time the animals were monitored carefully and the syringes on the pumps changed according to the intravenous rota. All infused fluids were placed in 60ml sterile syringes for mounting onto the syringe pumps and care was taken to ensure good sterile technique during filling. Each infusion was prepared just before it was required and all fluids contained buprenorphine as described in Chapter 6. The syringes containing fresh frozen plasma were removed as required from their storage at -20°C and thawed at room temperature before mounting direct onto the syringe pumps.

In the first experiment an intravenous regimen in which one third of fluid (8ml/24 hours) was administered as fresh frozen plasma was compared with intravenous regimens in which the fresh frozen plasma was replaced by crystalloid control (0.9% saline, 'Intraven', Galen Research Laboratories Ltd., Antrim, N.Ireland) or colloid control ('Haemaccel', Hoechst UK Ltd., Hounslow, Middlesex,UK) (Table 13). In each case fluid was administered at 1ml per hour and fluid rotas were repeated each 24 hours to the completion of the experiment. Thirty animals were included in each of the three groups.

In the second experiment the same high volume fresh frozen

plasma group and colloid control group were used again, but the
third group of animals received a lower volume of fresh frozen
plasma (2ml/24 hours), the remaining colloid (6ml/24 hours) being
administerd as 'Haemaccel' (Table 13).

During experiments the animals were monitored carefully to
ensure that their fluid rotas were accurately adhered to. This
entailed the changing of syringes in the syringe pump when
indicated and the undoing of any twists and kinks developing in
the infusion lines or occasionally the flushing of infusion lines
as described in Chapter 5. At all times great care was taken over
sterile technique when handling the infusion systems.

The times of any deaths were recorded. The timing of any
unsupervised deaths was initially estimated from the exponential
loss of body temperature shown on the multichannel recorder, but
this system was later abandoned. Subsequent deaths were estimated
as the midpoint from the time the animal had last been checked.
All animals surviving to 72 hours were sacrificed by cervical
fracture after inducing anaesthesia with 5% halothane and oxygen
delivered at 2 litres per minute from a Boyle's machine via a
conical head mask. Animals underwent a postmortem examination of
the abdominal cavity noting in particular the presence of
ascites, fat necrosis and the appearance of the pancreas and
surrounding organs.

Survival at 72 hours was compared between the treatment
groups using the Chi-squared test (Minitab Computer Package).

c) Comparison of treatment groups

In order to see whether the different infusion regimens
affected the severity of local and systemic pathological changes
of pancreatitis, further animals with acute pancreatitis were

TABLE 13

<u>Intravenous Fluid Therapy</u>

<u>Experiment 1</u>

Group		Intravenous fluids per 24 Hours*	No.Animals
A	Crystalloid controls	8ml 0.9% sodium chloride	30
		16ml 4% dextrose 0.18% saline	
B	Colloid controls	8ml 'Haemaccel'	30
		16ml 4% dextrose 0.18% saline	
C	Fresh frozen plasma	8ml Fresh frozen plasma	30
		16ml 4% dextrose 0.18% saline	

<u>Experiment 2</u>

Group		Intravenous fluids per 24 hours*	No.Animals
D	Colloid controls	8ml 'Haemaccel'	30
		16ml 4% dextrose 0.18% saline	
E	Low volume fresh frozen plasma	2ml Fresh frozen plasma	30
		6ml 'Haemaccel'	
		16ml 4% dextrose 0.18% saline	
F	High volume fresh frozen plasma	8ml Fresh frozen plasma	30
		16ml 4% dextrose 0.18% saline	

* = repeated each 24 hours to 72 hours

randomised to receive either high volume fresh frozen plasma
therapy or colloid control (rotas B and C of experiment 1, Table
13). Two animals from each group were sacrificed at each of the
following times; 1hr, 2hr, 4hr, 8hr, 16hr, 32hr, 64hr. Where
animals died before their allocated time of sacrifice,
replacements were set up.

Haematological (haemoglobin, white blood cell count and
packed cell volume) and biochemical (amylase, bilirubin, alkaline
phosphatase, calcium and albumin) measurements were made on whole
blood obtained by cardiac puncture (as described in 4.4b).
Macroscopic and microscopic examination was performed in the
abdominal cavity and thorax (as described in 4.4c and 4.4d).
Again histology was assessed 'blind' by one pathologist who was
not aware of the duration of pancreatitis in each specimen nor
the treatment group. He assessed all the organs (lungs, kidneys,
liver and pancreas) but concentrated on the pancreas in which he
used the subjective scoring system of 0-3 previously developed
for each of the four main microscopic features of acute
pancreatitis (necrosis, inflammatory infiltration, haemorrhage
and oedema).

7.3 Results

a) Collection of fresh frozen plasma

The collection of pooled fresh frozen plasma was relatively
easy to perform, although large numbers of animals had to be bled
(approximately 350) to provide enough plasma for the experiments.
The initial heparinisation of syringes prior to cardiac puncture
was a mistake as nine of ten animals without pancreatitis given
intravenous infusions of fresh frozen plasma collected in this

way died within 48 hours. The likely causes of this mortality
with the fresh frozen plasma were bacterial contamination or
excessive anticoagulation. The inbred nature of the colony made
it unlikely that immune reactions were responsible.

Bacterial contamination of fresh frozen plasma might have
occurred as a result of the need to bleed so many individual rats
to fill one blood bag. The risk of bacterial contamination had
been kept to a minimum by:

a) Cleaning the animals skin with 0.5% cetrimide and 0.05%
chlorhexidine prior to percutaneous cardiac puncture.

b) Using a fresh sterile syringe and needle for each cardiac
puncture and using a second sterile needle to transfer the whole
blood to the blood bag.

c) Keeping to a minimum the time between bleeding the animals and
freezing the plasma.

Significant bacterial contamination of fresh frozen plasma
samples was excluded by allowing four 8ml aliquots to thaw and
then dividing each equally into aerobic and anaerobic blood
culture bottles incubated for three days at 37°C. Subcultures
were taken each 24 hours. One subculture grew a staphylococcal
contaminant in low numbers, but the others were sterile.
Bacterial contamination did not, therefore, appear to be a major
problem as far as the collection of fresh frozen plasma was
concerned.

An overdose of anticoagulant in animals receiving the
initial batch of fresh frozen plasma was suggested by the
presence of obvious haemorrhagic complications in the nine
animals which died. Six had blood around the nose and mouth and
when subjected to a limited post mortem examination, four had

large amounts of blood within the intestine and two had

haemoperitoneum. Whole blood clotting times, thrombin times and

reptilase times were therefore measured on plasma from three

control animals who had received only 4% dextrose and 0.8% saline

infusion and three animals who had received 8ml of intravenous

fresh frozen plasma over 8hrs. Thrombin times were performed by

mixing 100ul of saline with 100ul of rat plasma and then adding

100ul of bovine thrombin and timing the appearance of fibrin

clot. The reptilase time was performed by adding 100ul of

reptilase (Malayan pit-viper venom) to 200ul of rat plasma and

timing the appearance of fibrin clot. The results (Table 14)

showed that the whole blood clotting time and thrombin time

(which are influenced by heparin) were greatly prolonged in the

animals which had received fresh frozen plasma compared with the

controls, while the reptilase times (which are not influenced by

heparin) were not. In addition the thrombin time and reptilase

TABLE 14

Clotting Studies

Rat No.		Whole blood clotting time (mins)	Thrombin time (secs)	Reptilase time (secs)
1		1	38	38
2	Controls	1¼	38.5	38
3		2½	36	36
4		15	>300	37
5	Post fresh frozen plasma	>180	>300	38
6		6	200	36

time were measured on two recently thawed samples of heparinised fresh frozen plasma diluted 50/50 with human plasma. Once again the thrombin times were greatly prolonged (180 seconds) while the reptilase times were normal (17.0 and 17.5 seconds).

These results suggested that gross over heparinisation of animals had occurred following infusion of the fresh frozen plasma. The use of heparin as anticoagulant in the syringes used for cardiac puncture was therefore abandoned and instead some of the acid citrate anticoagulant was withdrawn from the blood bag and 0.5ml added to each syringe. Intravenous infusions of fresh frozen plasma collected in this way resulted in no deaths in ten animals in a preliminary trial lasting 72 hours, and thrombin times measured on plasma collected from animals at the end of the study were normal.

b) Results of the controlled trials

Survival of animals in the first experiment is shown in Figure 36. Thirty percent of animals receiving crystalloid control (Group A) survived to 72 hours compared with 43% receiving colloid control (Group B) and 73% receiving fresh frozen plasma (Group C). Using the Chi-squared test, the difference in survival between the crystalloid and colloid control groups did not reach significance, but the improved survival in the fresh frozen plasma group reached significance at the 0.1% level when compared with the crystalloid controls and the 5% level when compared with the colloid controls.

Survival of animals in the second experiment is shown in Figure 37. Fifty percent of animals receiving colloid control (Group D) survived to 72 hours compared with 56.7% receiving low volume fresh frozen plasma(Group E) and 66.7% receiving high

volume fresh frozen plasma(Group F). In this experiment no significant differences are present between the three treatment groups in terms of 72 hour survival, though a trend towards improved survival in the fresh frozen plasma treated groups is seen.

If the results of experiments one and two are combined, however, (Figure 38), 46.7% of the sixty animals who received colloid control (Groups B and D) survived to 72 hours compared with 70% of the sixty animals receiving the higher volume (8ml/24hr) of fresh frozen plasma (Groups C and F). Using the Chi-squared test, this difference reaches significance at the 1% level.

c) Comparison of the treatment groups

No significant differences were apparent in terms of the macroscopic appearance of the abdominal contents at post mortem examination between the treatment groups. The ascitic fluid, fat necrosis and macroscopic appearance of the pancreas were similar to those noted in earlier studies on the model (Chapter 4).

There were no obvious differences between the high volume fresh frozen plasma therapy group and colloid control groups in terms of their haematology, biochemistry, macroscopic or histological features of acute pancreatitis (Appendix 3). The changes were similar to those noted in the preliminary studies (Chapter 4) and both treatment groups appeared to behave in a similar fashion as far as the local disease and measured changes inblood parameter changes were concerned. In particular no difference between the two groups was apparent in terms of serum amylase nor of histological scoring of the four main parameters of acute pancreatitis, though the number of animals involved in

Figure 36

Results of Experiment One Comparing High Volume Fresh Frozen Plasma to Crystalloid and Colloid Controls in the Treatment of Experimental Acute Pancreatitis.

No. of animals in each group = 30

| Survival at 72 hours | 30 % | 43 % | 73 % |

Chi-squared test

N.S.

P<0.001

P<0.05
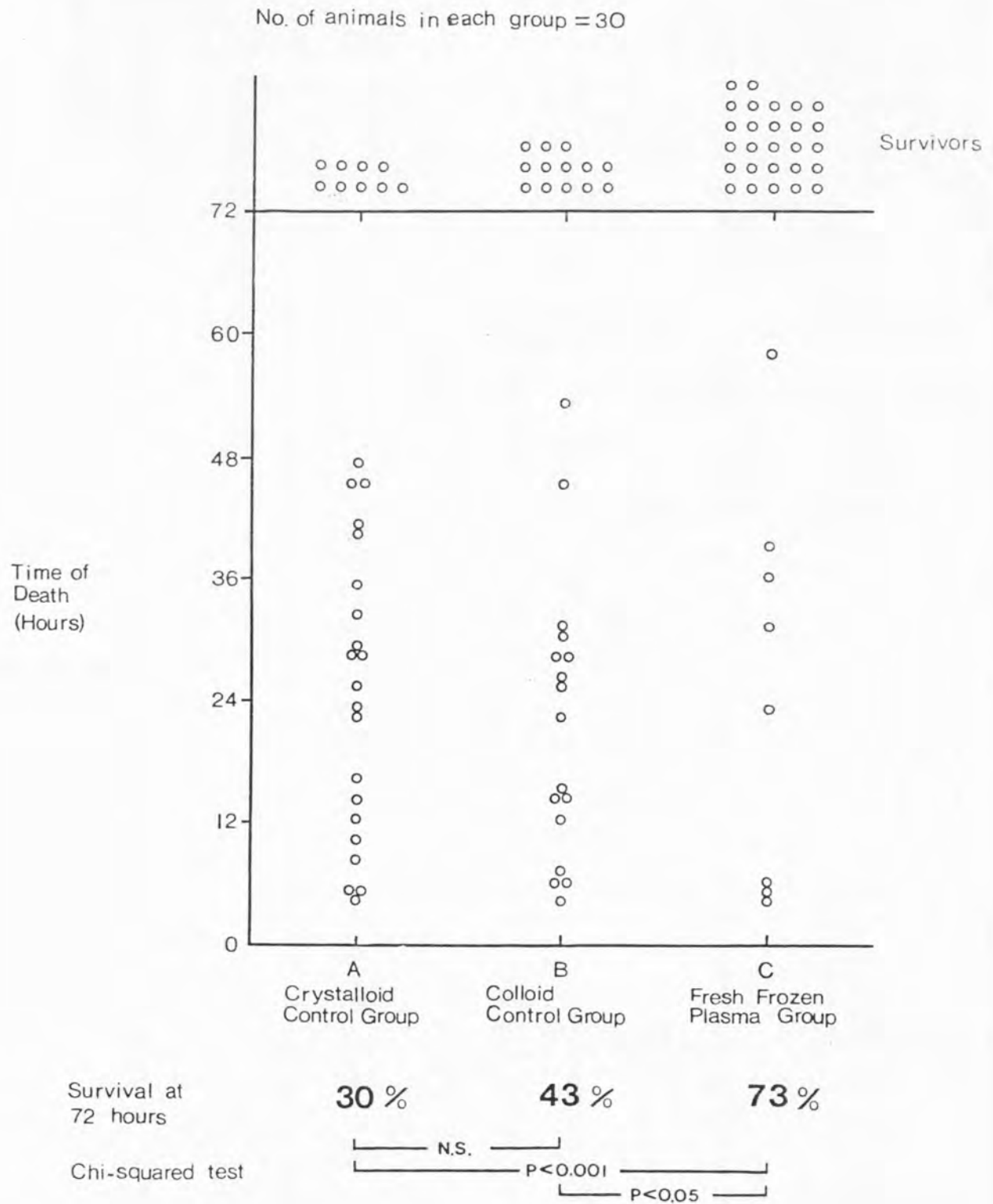
Figure 37

Results of Experiment Two Comparing High Volume Fresh
Frozen Plasma to Low Volume Fresh Frozen Plasma and
Colloid Control in the Treatment of Experimental Acute
Pancreatitis. No. of animals in each group =30

Time of Death (Hours)

	D Colloid Control Group	E Low Volume Fresh Frozen Plasma Group	F High Volume Fresh Frozen Plasma Group
Survival at 72 hours	50 %	56.7 %	66.7 %

Chi-squared test

N.S.

N.S.

N.S.
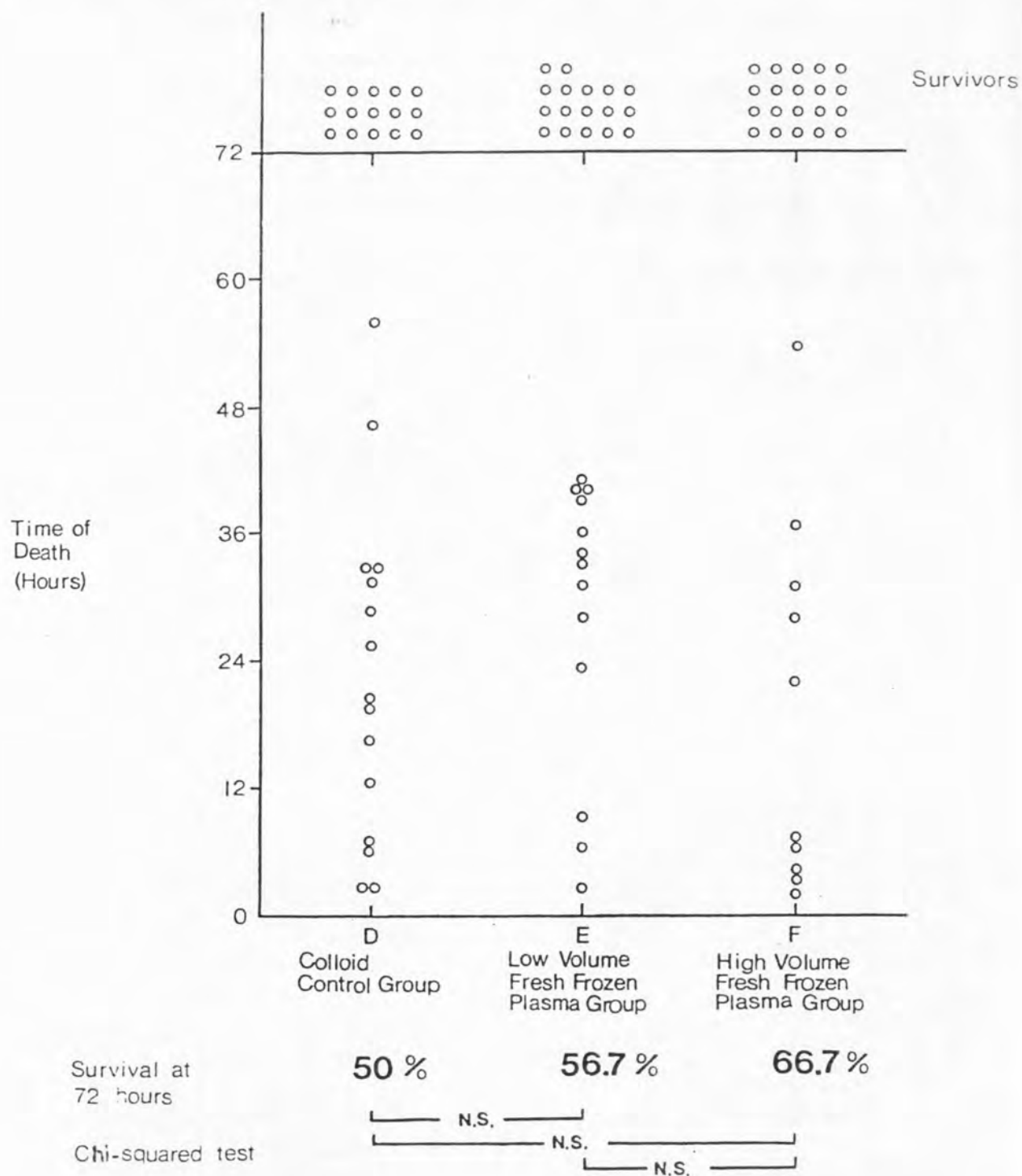
Figure 38

Combined Results of Experiments One and Two Comparing
High Volume Fresh Frozen Plasma to Colloid Control in the
Treatment of Experimental Acute Pancreatitis.

No. of animals in each group = 60

Survivors

Time of
Death
(Hours)

B and D
Colloid
Control Groups

C and F
High Volume
Fresh Frozen
Plasma Groups

Survival at
72 hours

46.7%   70%

Chi-squared test   �——— P<0.01 ———⌡
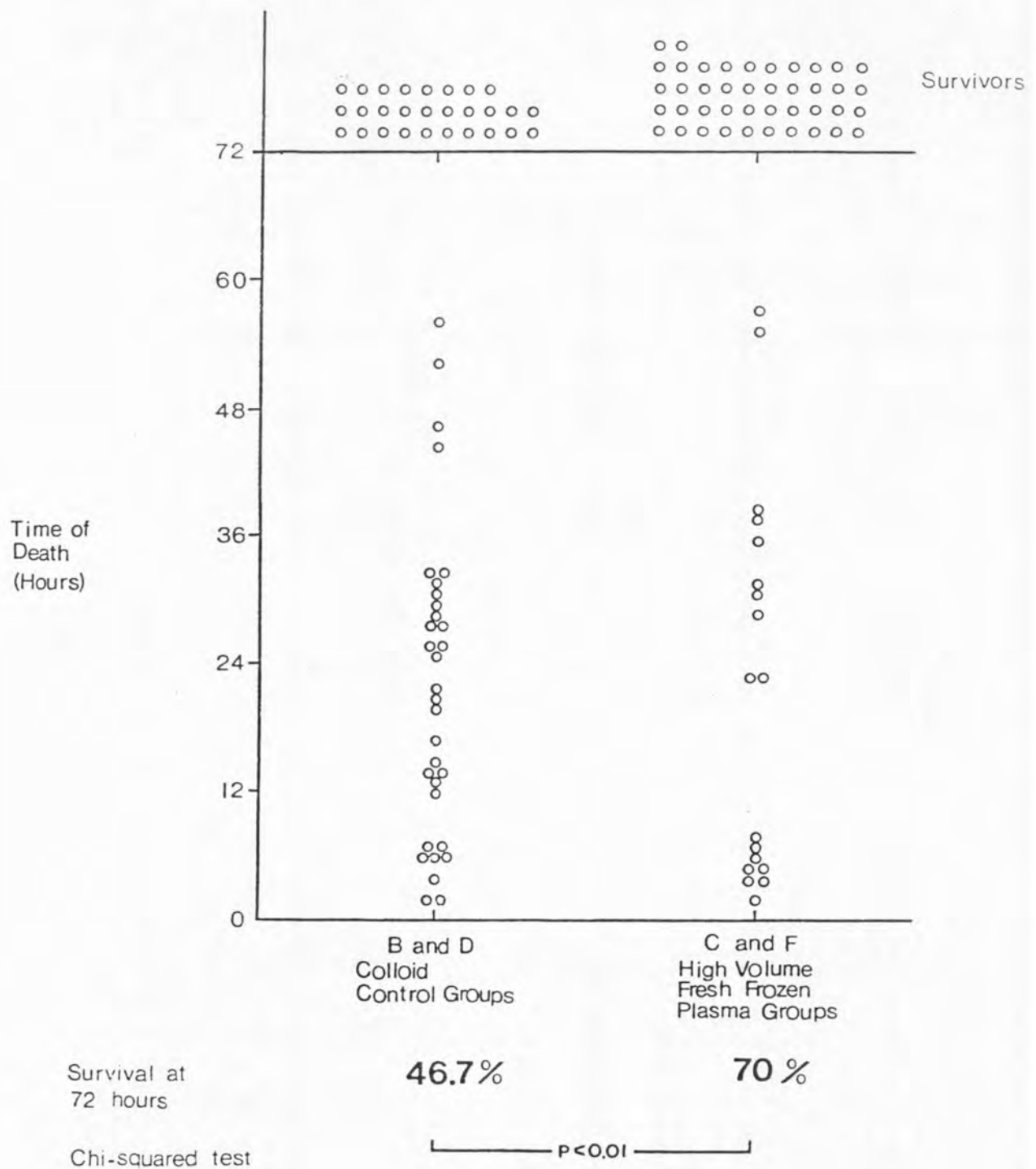
these studies was small, making any statistical comparison
difficult.

7.4 Discussion

Large volumes of rat fresh frozen plasma were required for
this study and I was unable to find any previous description of
the collection of fresh frozen plasma in the rat. The method
developed for this study appears to work well. Large numbers of
animals had to be sacrificed to provide enough fresh frozen
plasma and the rat was a reasonably economical animal to use in
this respect. The use of an inbred colony permitted the pooling
of whole blood and the avoidance of immunological problems during
fresh frozen plasma therapy. Only male rats were used as they
grow to a larger size with greater blood volumes, and cyclical
changes in female hormonal levels were avoided.

The introduction of infection during bleeding of multiple
animals appears to have been avoided by careful attention to
sterile technique. This included the cleansing of the animals
skin, the use of fresh sterile needles and syringes for each
cardiac puncture, and of fresh needles for each transfer of fluid
into or out of the blood bag. Infective complications were also
avoided by keeping to a minimum the time between collection of
the whole blood and freezing of the aliquots of fresh frozen
plasma, and also the time between thawing of the fresh frozen
plasma samples and mounting onto syringe pumps for infusion. Thus
any bacteria which might be present within the fresh frozen
plasma samples were given little chance to multiply. The results
of bacteriology performed on the fresh frozen plasma samples and
the beneficial effect of fresh frozen plasma therapy on survival
of the animal model of acute pancreatitis both suggest that

bacterial contamination of the fresh frozen plasma was not a significant problem.

The short whole blood clotting time in rats necessitated the use of anticoagulant in the syringes used for collecting whole blood. The initial dead-space heparinisation of syringes was a mistake as the infusion of fresh frozen plasma collected in this manner produced a coagulopathy in the animals resulting in deaths from spontaneous haemorrhage. The coagulation studies confirmed overheparinisation as the cause of the problem. The prolongation of thrombin time in animals who had received fresh frozen plasma could have been due to subnormal fibringen levels, excessive fibrin degradation products or the presence of excessive amounts of heparin in the rats plasma. The normal reptilase times, however, confirmed an excess of heparin in the rat plasma as reptilase acts direct on fibrinogen to produce fibrin clot and is immune to heparin, but would have been influenced by subnormal fibrinogen levels or excessive fibrin degradation products in the rat plasma. The possibility that the bovine thrombin test simply did not work on rat plasma was excluded by the results obtained on the control animals and the results of the thrombin test on the 50/50 mixture of human plasma samples.

It therefore appeared that the rats could not tolerate the use of heparin as anticoagulant and instead acid citrate from the blood bag was used. Each blood bag contained 63ml of acid citrate (each 100ml of which contains sodium citrate Ph.Evr.2.63g, anhydrous dextrose BP 2.90g, Citric acid monohydrate Ph.Evr.327mg, sodium acid phosphate BP 251mg and Adenine 27.5mg). 25ml was withdrawn from the bag using a sterile syringe and 0.5ml aliquots added to each syringe to be used for cardiac

puncture. This anticoagulant did not appear to harm animals receiving fresh frozen plasma therapy as none of the preliminary ten animals receiving the infusions over a seventy two hour period died, and all had normal thrombin times at 72 hours.

The freezing of fresh frozen plasma in 8 ml aliquots ready for mounting direct onto syringe pumps enabled the avoidance of repeated thawing and freezing of the whole fresh frozen plasma volume. This might have had deleterious effects on some of the constituent proteins as well as encouraging bacterial proliferation.

As far as the controlled trials are concerned, in the first experiment intravenous infusions incorporating large volumes of fresh frozen plasma were compared with crystalloid and colloid control groups. One third of the total volume of intravenous fluid was given as fresh frozen plasma with the remainder made up of crystalloid (dextrose saline). In the control groups the fresh frozen plasma was replaced by normal saline (crystalloid) or 'Haemaccel' (colloid). The crystalloid control group was used to provide a baseline mortality for the model with intravenous infusion and to see if the inclusion of colloid influenced outcome. A colloid control group was included to try and separate the potential colloid value of fresh frozen plasma therapy from the postulated specific value of some of its constituent proteins.

The choice of a colloid for the colloid control group was not easy. Ideally purified rat albumin solution should have been used as the closest colloid match for fresh frozen plasma devoid of antiproteases and other potentially useful specific proteins. Enquiries into the feasibility of harvesting purified rat albumin

solution suggested that it would be an extremely expensive process to set up, also involving the sacrifice of many animals in order to obtain enough plasma for processing. Therefore an alternative colloid was sought with a similar molecular size to albumin. 'Haemaccel' is a well-known plasma substitute used widely in clinical practice particularly in the treatment of hypovolaemic states. It contains 35g/litre of a degraded and modified beef gelatine (polygeline) of average molecular weight 35,000 (a similar concentration and molecular weight to plasma albumin). It also contains electrolytes at a similar concentration to plasma (sodium 145 mmol/L, potassium 5.1 mmol/litre, calcium 6.26 mmol/L, chloride 145 mmol/L and traces of phosphate and sulphate). Isotonic equilibrium is made up by the polypeptides and its pH is 7.3 ± 0.3. Its oncotic pressure at $37^{\circ}C$ is 3.432-3.824 KPa (350-390 mmH_2O). 'Haemaccel' does not interfere with blood grouping and cross matching or the coagulation system in man. It is also non-immunogenic and does not induce antibody formation. In clinical practice it is often preferred to 'Dextrans' which can interfere with blood group cross match and biochemical measurements. 'Haemaccel' is only slowly metabolised and is reported to have a plasma half life of 4-8 hours. It can be safely administered to rats (personal communication from manufacturers) and was therefore chosen as colloid control for these experiments.

Animals were randomised to their treatment group on the opening of a sealed envelope two hours after the completion of the surgical procedures. This delay was introduced partly to enable anaesthetic deaths to be excluded (though in practice these were infrequent) but also to allow pancreatitis to become

established before commencing therapy. In clinical practice patients usual present some hours after the onset of pancreatitis and immediate therapy is not realistic in animal models. A delay of two hours was judged appropriate on the timescale of the rat pancreatitis as preliminary studies in the animal model (Chapter 4) had demonstrated that the disease process was well established by this time. In each group of six animals, two were randomised to each of the three treatment groups. This method of randomisation was adopted to cancel out any variation in the composition of the bile/enterokinase mixture between batches. Fresh batches of the mixture were prepared for each group of animals and it therefore seemed wise to ensure that the same number of animals in each group were randomised to each treatment limb.

In these experiments, as in the preliminary studies on the model reported in Chapter 4, nearly all the observed deaths occurred within 48 hours and very few deaths occurred after 48 hours. This supported the choice of a 72 hour end point to the experiments to minimise animal suffering.

In the first experiment, survival at 72 hours in the crystalloid control group (30%) did not statistically differ from survival in the colloid control group (43%), though the numbers in each group (30) were relatively small. Survival at 72 hours without any intravenous fluid therapy in this model was 20% (Chapter 4). Both colloid and crystalloid resuscitative solutions have been shown to be effective in acute pancreatitis (Martin et al. 1984) and colloid is not necessarily advantageous over crystalloid as has already been discussed (Chapter 3). This study demonstrates no obvious advantage for 'Haemaccel' versus

crystalloid. However, the high volume fresh frozen plasma therapy group showed a significantly improved survival at 72 hours (73%) when compared with the crystalloid control group (p<0.001) and colloid control group (p<0.05).

In the second experiment an attempt was made to determine whether smaller volumes of fresh frozen plasma could also be shown to improve survival. The same colloid control group ('Haemaccel') and high volume fresh frozen plasma group were used as in the first experiment. The high volume fresh frozen plasma group received one third of their intravenous fluid therapy as fresh frozen plasma which in human terms represents approximately eight units of fresh frozen plasma (1800ml) per twenty four hours. The Dundee study (Cuschieri et al. 1983) suggested that a beneficial effect can be achieved in patients with much lower volumes (2-3 units per 24 hours) (Chapter 3). A low volume fresh frozen plasma group was therefore included in this second experiment receiving only 2ml/24 hrs of fresh frozen plasma with the remaining colloid volume provided by 'Haemaccel'.

Survival in the first and second experiments in the colloid control group (43% and 50%) and in the high volume fresh frozen plasma group (73% and 66.7%) were encouragingly similar suggesting good reproducibility of results, but in this second experiment the difference in survival between the two groups (50% vs 66.7%) did not reach significance. However, the comparison of 72 hour survival between high volume fresh frozen plasma and colloid control groups if both experiments are combined (46.7% vs 70%) was significant with p<0.01. The low volume fresh frozen plasma group showed a survival of 56.7% at 72 hours which did not differ significantly from the high volume fresh frozen plasma or

colloid control groups - though a trend towards improved survival in the two groups receiving fresh frozen plasma was present.

In summary these experiments have demonstrated that intravenous infusions incorporating fresh frozen plasma can significantly improve survival in a rat model of acute pancreatitis when compared with crystalloid and colloid controls. This beneficial effect of fresh frozen plasma was clearly demonstrated for large volumes (one third of total intravenous fluid therapy). Smaller volumes of fresh frozen plasma were associated with increased survival when compared with colloid controls, though the difference was not significant.

This study does not clarify the mechanism of the improved survival in the fresh frozen plasma group except to suggest that it is not merely the colloid content of fresh frozen plasma that is beneficial. The basic haematological, biochemical and histological features which were compared between the fresh frozen plasma and colloid treatment groups showed no obvious differences in terms of local or systemic manifestations of the disease. The effect of fresh frozen plasma on serum antiprotease levels was not measured because this was technically impracticable in the rat.

Extrapolation from animal studies to human disease processes is always difficult, but this study, taken in conjunction with the uncontrolled clinical study from Dundee (Cuschieri et al. 1983) in which fresh frozen plasma therapy was associated with a low mortality in acute pancreatitis (Chapter 3), seemed to justify a controlled clinical trial of fresh frozen plasma therapy in this disease. This trial, which included the monitoring of the major serum antiproteases during therapy, is

described in the next chapter.

CHAPTER EIGHT

A MULTICENTRE PROSPECTIVE CLINICAL TRIAL OF

FRESH FROZEN PLASMA THERAPY IN ACUTE PANCREATITIS

8.1 Introduction

Having demonstrated that fresh frozen plasma therapy had a beneficial effect on survival in an experimental model of acute pancreatitis, a clinical trial of fresh frozen plasma therapy was planned.

The trial was designed to reflect the original uncontrolled clinical study from Dundee (Cushieri et al. 1983). Similar low volumes of fresh frozen plasma were administered and all patients with acute pancreatitis were entered into the study, rather than selecting out patients with evidence of more severe disease. A colloid control group of patients was included to try and separate the potential colloid value of fresh frozen plasma from the postulated specific value of some of its constituent proteins. The major serum antiproteases were measured during the disease in both groups of patients to assess the effect of fresh frozen plasma therapy on their concentrations.

Five hospitals were involved in order to speed the recruitment of patients into the trial.

8.2 Patients and Methods

This study involved the Leicester General Hospital, the Leicester Royal Infirmary, Glenfield General Hospital, Peterborough District Hospital and the George Eliot Hospital, Nuneaton. During the period 1st March 1985 to 31st January 1987, patients referred to these surgical units with a clinical diagnosis of acute pancreatitis and serum amylase >1000 IU/I were entered into the trial. One hundred and thirty patients admitted to the Leicester Royal Infirmary during the study period, who were entered into a separate trial of endoscopic

sphincterotomy in acute biliary pancreatitis (Neoptolemos et al. 1986a) were excluded.

After informed consent the patients were randomised on the opening of a sealed envelope to receive either two units of fresh frozen plasma daily for three days as part of their intravenous fluid therapy or a similar volume (400 mls daily) of human albumin solution as colloid control. Each hospital used a separate set of randomisation envelopes. The remaining intravenous fluid therapy was left entirely to the medical staff looking after the individual patient. An accurate record was kept of all intravenous fluid administered during the first three days and any adverse reactions to fresh frozen plasma or human albumin solution were noted.

The nine Imrie criteria (Chapter 1,7b,Table 7) for predicting severity in acute pancreatitis were recorded as soon as possible after diagnosis and in every case within 48 hours of admission. The presence of three or more of these adverse criteria predicts a severe attack of pancreatitis.

Serum lactate dehydrogenase activity, which was not available as a routine test from the chemical pathology department during this study, was measured using the Merckotest R kit (EM Diagnostics, Poole, Dorset, England) in accordance with the recommendations of the Deutsche Gesellschaft fur Klinische Chemie, pyruvate being the substrate, at $37°C$ on a Cobas Bio centrifugal analyser. The between-run coefficient of variation was 1.6% at 411 IU/L and 1.8% at 622 IU/L. Comparison of results obtained on external quality control material demonstrated a negative bias of 8.5% with respect to national mean values by this method, the percentage bias being constant over the range

216-854 IU/L. The regression equation was $y=0.915x + 0.02$, $r=1.000$, $n=7$, where y is the observed activity in IU/L and x is the national mean(expected) activity.

The principle serum antiproteases were measured on days one, three and seven. Serum specimens were stored at $-20°C$ until analysis. Serum $alpha_1$ antiprotease concentration was measured using goat antiserum and Calibrator-1 (Atlantic Antibodies Ltd., Winnersh, Berkshire, UK) on a Cobas Bio centrifugal analyser on turbimetric mode. The method recommended by Atlantic Antibodies was used with the exception that the antiserum was diluted in 0.9% saline instead of 0.1 M phosphate buffered saline. The $alpha_1$ antiprotease levels thus obtained were multiplied by a correction factor of 0.8 so as to bring the results into agreement with those obtainable using the Sheffield Protein Reference Unit calibrant SPS-01. The between-run coefficient of variation was 8.3% at 1.2 g/l.

Serum $alpha_2$ macroglobulin was also measured using goat antisera and Calibrator 1 following the protocol recommended by Atlantic Antibodies for the Cobas Bio analyser. The between-run coefficient of variation was 2.9% at 1.8 g/l.

Reference ranges of 1.1-2.4 g/l for $alpha_1$ antiprotease and 1.45-3.7 g/l for $alpha_2$ macroglobulin were established using data from a sample of healthy adults selected randomly from the general population in and around Leicester.

The patients history, treatment and progress were carefully recorded on trial proformas including follow-up of survivors after discharge from hospital. A severe outcome from acute pancreatitis was defined as death, a major complication or a hospital stay exceeding twenty days, having excluded social

problems or elective surgery as reasons for prolonging the admission.

The two tailed Mann-Whitney Ranking Test was used to analyse
continuous parameters (Minitab Computer Package) and the Wilcoxon matched
pairs signed ranks test for analysis between days. The Chi-squared test
was used to analyse clinical variables between the two groups with Yates
correction where indicated.

8.2 Results

Two hundred and three patients were referred for entry into
the trial. One patient refused randomisation after informed
consent. This patient subsequently recovered from a mild attack
of biliary pancreatitis. The remaining two hundred and two
patients were randomised. One hundred were randomised to receive
fresh frozen plasma but one patient was subsequently withdrawn
from this group. This was a seventy-one year old female admitted
moribund with generalised abdominal tenderness, maximal in the
left iliac fossa. She was entered into the trial because of a
serum amylase of 1350 IU/L. She failed to respond to
resuscitation and died twenty hours after admission. Post mortem
examination revealed perforated carcinoma of the sigmoid colon
with generalised peritonitis, but no evidence of pancreatitis.

One hundred and two patients were randomised to receive
colloid control, but three patients were subsequently withdrawn
from this group. Two patients with mild attacks of alcoholic
pancreatitis were randomised in error as their serum amylase was
less than 1000 IU/1. The third patient was a sixty year old
female admitted in shock unable to give a history. She had upper
abdominal tenderness on examination and was entered into the
trial because of a serum amylase of 1498 IU/1. Efforts at

resuscitation failed and she died within twenty-four hours of
admission. Post mortem examination revealed that death was caused
by multiple pulmonary emboli from an extensive deep vein
thrombosis. There was no evidence of pancreatitis.

This left one hundred and ninety-eight patients in the study
equally randomised to fresh frozen plasma and colloid control
groups. The individual data from these patients is given in
appendix 4. The sex distribution was not statistically different
with 41 males and 58 females in the fresh frozen plasma group and
54 males and 45 females in the colloid control group. The two
groups were well matched in terms of the aetiology of
pancreatitis (Table 15). Gallstones accounted for 56.6%, alcohol
20.7%, other aetiologies 8.1% and 14.6% remain idiopathic,
although some of the latter group of patients are still under
investigation. In 78 patients in the fresh frozen plasma group
and 83 patients in the colloid control group there had been no
previous attack of pancreatitis. The remaining patients (18.6%)
had experienced a median of 2 (range 1-6) previous attacks.

TABLE 15 - The aetiology of acute pancreatitis

	Fresh Frozen Plasma	Colloid Control	Total
Gallstones	60	52	56.6%
Alcohol	19	22	20.7%
Other	6	10	8.1%
Idiopathic	14	15	14.6%
	———	———	
Total	99	99	
	———	———	

Thirty-four of the fresh frozen plasma group and 38 of the
colloid control group were predicted severe on the Imrie
criteria. The distribution of prognostic scores did not differ
significantly between the two groups, nor did any of the

TABLE 16 - Distribution of prognostic scores and individual prognostic markers between the treatment groups

		Fresh Frozen Plasma	Colloid Control	
Predicted Severe		34/99	38/99	NS*
Prognostic Score	Median (range)	2 (0-7)	2 (0-7)	NS +
Age (years)	Median (range)	59 (17-89)	58 (13-91)	NS
WCC (x 10^9/1)		14.3 (5.2-25.0)	14.3 (3.3-38.3)	NS
Urea (mmol/1)		5.5 (1.5-31.6)	5.8 (2.0-61.0)	NS
Glucose (mmol/1)		7.5 (4.7-66.4)	7.55 (3.6-25.7)	NS
Albumin (g/1)		37 (25-46)	37 (16-48)	NS
Calcium (mmol/1)		2.23 (1.4-2.67)	2.24 (1.15-3.09)	NS
PaO_2 (KPa)		10.1 (6.1-17.2)	10.6 (5.9-17.8)	NS
ALT (IU/1)		54 (4-1111)	59 (5-846)	NS
LDH (IU/1)		306 (71-1800)	351 (97-3500)	NS

*Chi Squared Test

+Two-tailed Mann Whitney Ranking Test

individual risk factors (Table 16). This suggests that the two groups were well matched in terms of predicted severity of pancreatitis. There was also no difference between the two groups when looking at other haematological and biochemical parameters recorded within forty-eight hours of admission (Table 17).

No statistical difference was demonstrable between the two groups when looking at the level of monitoring which they received or other potential treatments offered for acute pancreatitis (Table 18). The only difference between the two groups in terms of treatment was the intravenous fluid therapy. The colloid administered for the purposes of this trial was the only colloid given during the first three days in 80.7% of patients. Sixteen patients in the fresh frozen plasma group and 22 patients in the colloid control group received additional colloid during this period. Twenty-six of these patients (68.4%) had predicted severe disease on the Imrie criteria and 17 (44.7%) had severe outcomes. In this group of patients the colloid specified by the trial accounted for a median of 50% (range 10-75%) of colloid administered during the first three days. Three patients developed mild pyrexia and skin rashes whilst receiving fresh frozen plasma. None of these reactions were severe enough to necessitate withdrawing fresh frozen plasma therapy. No other adverse reactions were reported to either fresh frozen plasma or human albumin solution.

There was no statistical difference between the two groups in terms of clinical outcome (Table 19). There were fourteen severe outcomes in the fresh frozen plasma group with eight deaths and twenty severe outcomes in the colloid control group with nine deaths. Overall mortality was 8.6% in a total of 17.2%

TABLE 17 - Distribution of other Haematological and Biochemical
            Results between the Treatment Groups

	Fresh Frozen Plasma	Colloid Control	
Serum Amylase Median (IU/1) (range)	3,400 (1022-74,000)	2,805 (1001-19,890)	NS*
Haemaglobin (g/1)	14.3 (11.4-18.3)	14.35 (9.4-18.8)	NS
Haematocrit (%)	42.9 (26.9-56.3)	42.7 (28.4-54.6)	NS
Creatinine (umol/1)	88 (32-407)	89.5 (39-619)	NS
Total protein (g/1)	65 (51-88)	65 (44-89)	NS
Bilirubin (umol/1)	18 (3-127)	17 (4-194)	NS
Alkaline Phosphatase(IU/1)	109 (8-893)	118 (10-1200)	NS

*Two-tailed Mann Whitney Ranking Test

TABLE 18 - Comparison of Monitoring and other Potential Treatments
            Offered for Acute Pancreatitis

	Fresh Frozen Plasma	Colloid Control	
Monitoring			
Central venous pressure line	14	17	NS*
Urinary catheter	49	49	NS
Transfer to intensive care unit	5	12	NS
Other Potential Treatments			
Nasogastric tube	56	57	NS
Oxygen therapy	36	37	NS
Antibiotics	68	61	NS
Early surgery	10	9	NS
ERCP(+ endoscopic sphincterotomy)	6(3)	7(3)	NS
Calcium infusion	9	3	NS
Insulin	5	3	NS
Parenteral feeding	7	8	NS

*Chi Squared Test $\pm$ Yates Correction

TABLE 19 - Clinical Outcome

		Fresh Frozen Plasma	Colloid Control	
Severe outcome		14	20	NS*
Death		8	9	NS*
Hospital Stay	Median	8	9	NS+
(Days)	(Range)	(3-56)	(3-68)	

* Chi Squared Test
+ Two-tailed Mann Whitney Ranking Test

TABLE 20 - Major Complications

	Fresh frozen Plasma	Colloid Control
Respiratory complications/ failure	8	10
Renal failure	3	4
Cardiac failure	5	4
Multisystem failure	1	6
Pancreatic phlegmon	2	3
Pancreatic pseudocyst	4	6
Pancreatic abscess	2	4
Pancreatic necrosis	0	3
Gastrointestinal haemorrhage	1	4
Gastric outlet obstruction	0	3
Myocardial infarction	1	0
Cerebrovascular accident	0	1
Disseminated intravascular coagulation	1	0
Deep venous thrombosis	1	0
Patients with severe complications	14	20
Deaths	8	9

severe outcomes which were evenly distributed amongst the five
hospitals involved in the study. The major complications are
listed in Table 20. To eliminate incidental findings at
ultrasound and CT scanning, pancreatic mass lesions were only
included as major complications if they resulted in hospital stay
in excess of twenty days or if pseudocysts and abscesses required
percutaneous or surgical drainage. Hospital stay did not differ
significantly between the two groups. Patients were followed up
for a median of 3.5 months (range 0-22) after discharge from
hospital.

Of the 72 patients predicted severe on Imrie criteria, 29
had a severe outcome (40.3%) while five of 126 predicted mild had
a severe outcome (4.0%). This produces a sensitivity of 85% and a
specificity of 74% for the prognostic scoring system. When
assessing the individual markers (Table 21), the differences in
distribution between mild and severe outcome groups all reached
significance except for alanine transaminase.

Serum $alpha_1$ antiprotease levels (Figure 39) in both groups
were elevated on day one when compared with our reference
population and a significant rise occurred from day one to day three
($p < 0.0001$). Levels remained elevated at day 7. Fresh frozen plasma
appears to have no effect on the magnitude of this rise.

When serum $alpha_2$ macroglobulin levels (Figure 40) are
considered, on all three days both groups showed significantly
reduced levels compared to our reference population. In the colloid
control group $alpha_2$ macroglobulin levels fell significantly from
day one to day three ($p < 0.005$) whilst remaining substantially
unaltered in the fresh frozen plasma group ($p = 0.6527$). The
difference between the two groups reached significance on day three

(p<0.01). There were no statistically significant changes between
the levels on day three and day seven in either group.

TABLE 21 - Assessment of the Imrie Criteria Comparing Severe to
Mild Outcomes for all Patients

		Severe outcomes	Mild outcomes	
Age (years)	Median (range)	71 (22-87)	56 (13-91)	p<0.001*
WCC $(x\ 10^9/1)$		18.65 (5.6-38.3)	13.55 (3.3-28.6)	p<0.0001
Urea (mmol/l)		9.9 (1.5-61.0)	5.2 (1.9-31.6)	p<0.0001
Glucose (mmol/l)		9.3 (4.1-66.4)	7 (3.6-35.4)	p<0.002
Albumin (g/l)		35 (16-46)	37 (28-48)	p<0.05
Calcium (mmol/l)		2.1 (1.4-3.09)	2.24 (1.15-2.67)	p<0.002
PaO_2 (KPa)		7.65 (5.9-11.6)	10.6 (6.5-17.8)	p<0.0001
ALT (IU/l)		63 (12-798)	54 (4-1111)	NS
LDH (IU/l)		457 (132-3500)	306 (71-1697)	p<0.001

*Two-tailed Mann Whitney Ranking test

When the serum antiprotease results are analysed in terms of
the severity of clinical outcome, similar changes in $alpha_1$
antiprotease levels occurred in mild and severe groups. No
statistical differences are present on any day between the mild and
severe outcomes within the fresh frozen plasma or colloid control
groups.

Figure 39

Serum Alpha-1-Antiprotease Concentrations in the Two
Treatment Groups
The reference range is given in continuous lines.
All results are shown as medians with interquartile ranges.

Days after admission to hospital
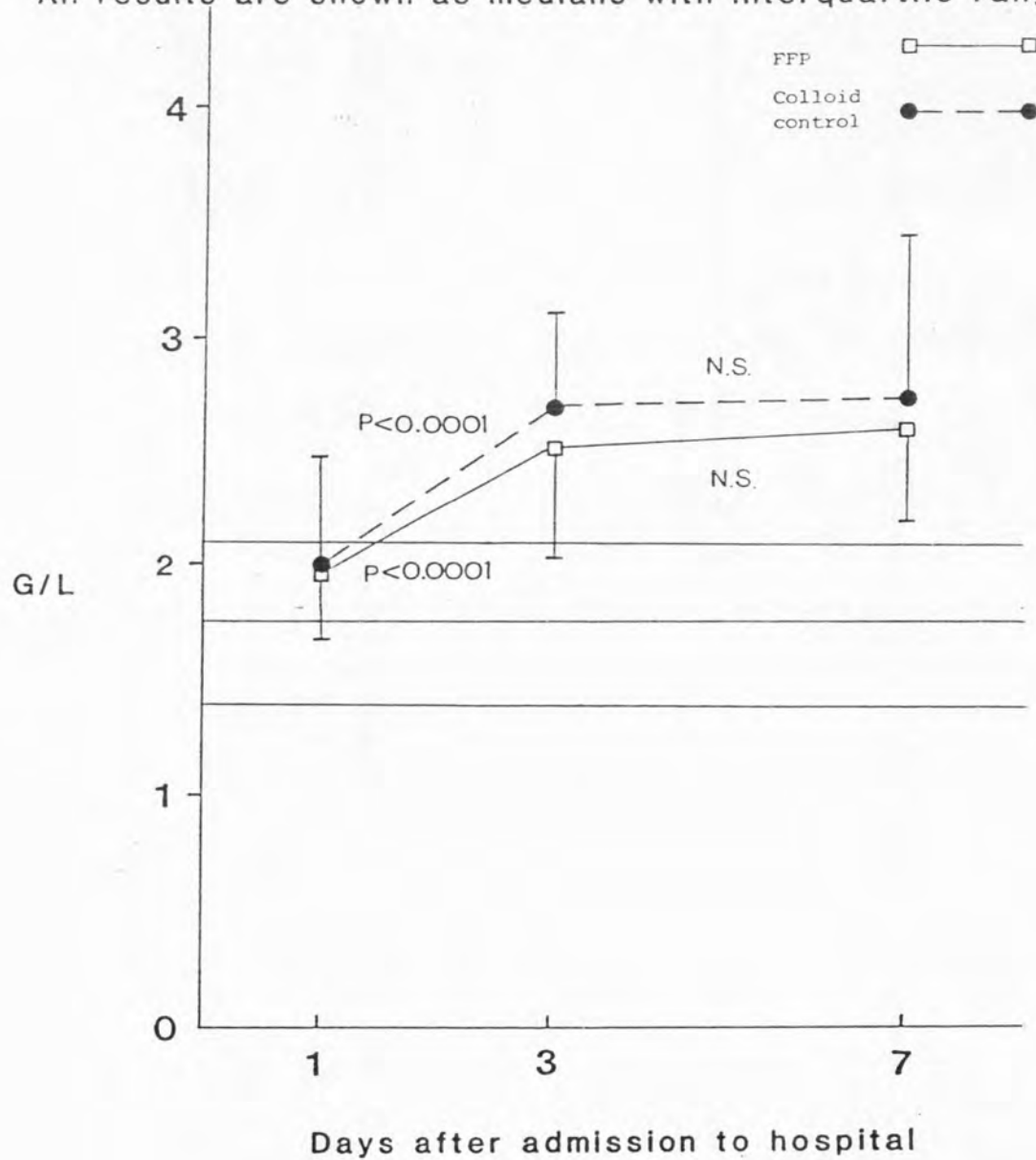
Figure 40

Serum Alpha-2-Macroglobulin Concentrations in the Two Treatment Groups.

The reference range is given as continuous lines.

All results are shown as medians with interquartile ranges.
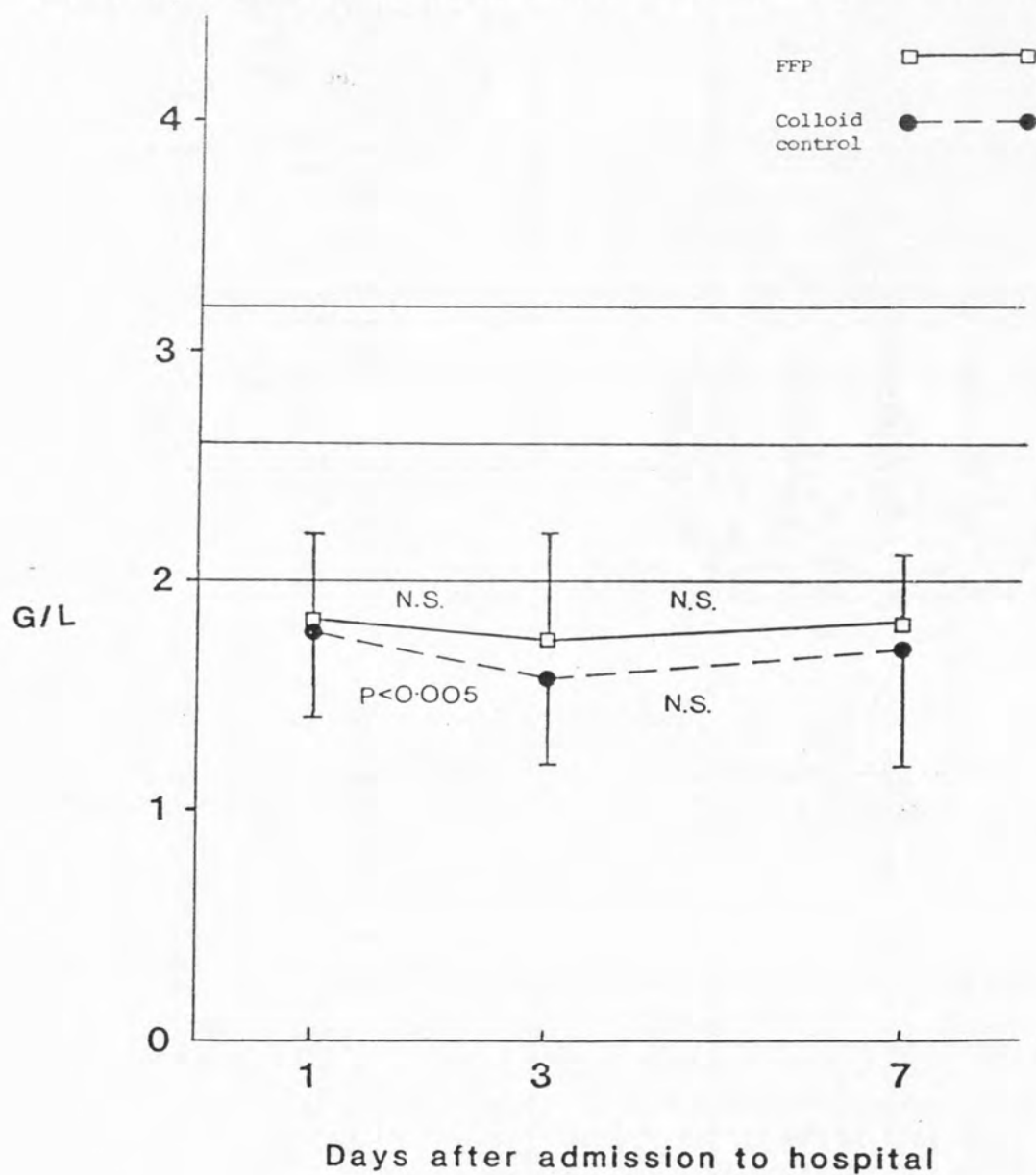
Figure 41

Serum Alpha-2-Macroglobulin Concentrations Comparing
Mild and Severe Clinical Outcomes.
The reference range is given as continuous lines.
All results are shown as medians with interquartile ranges.
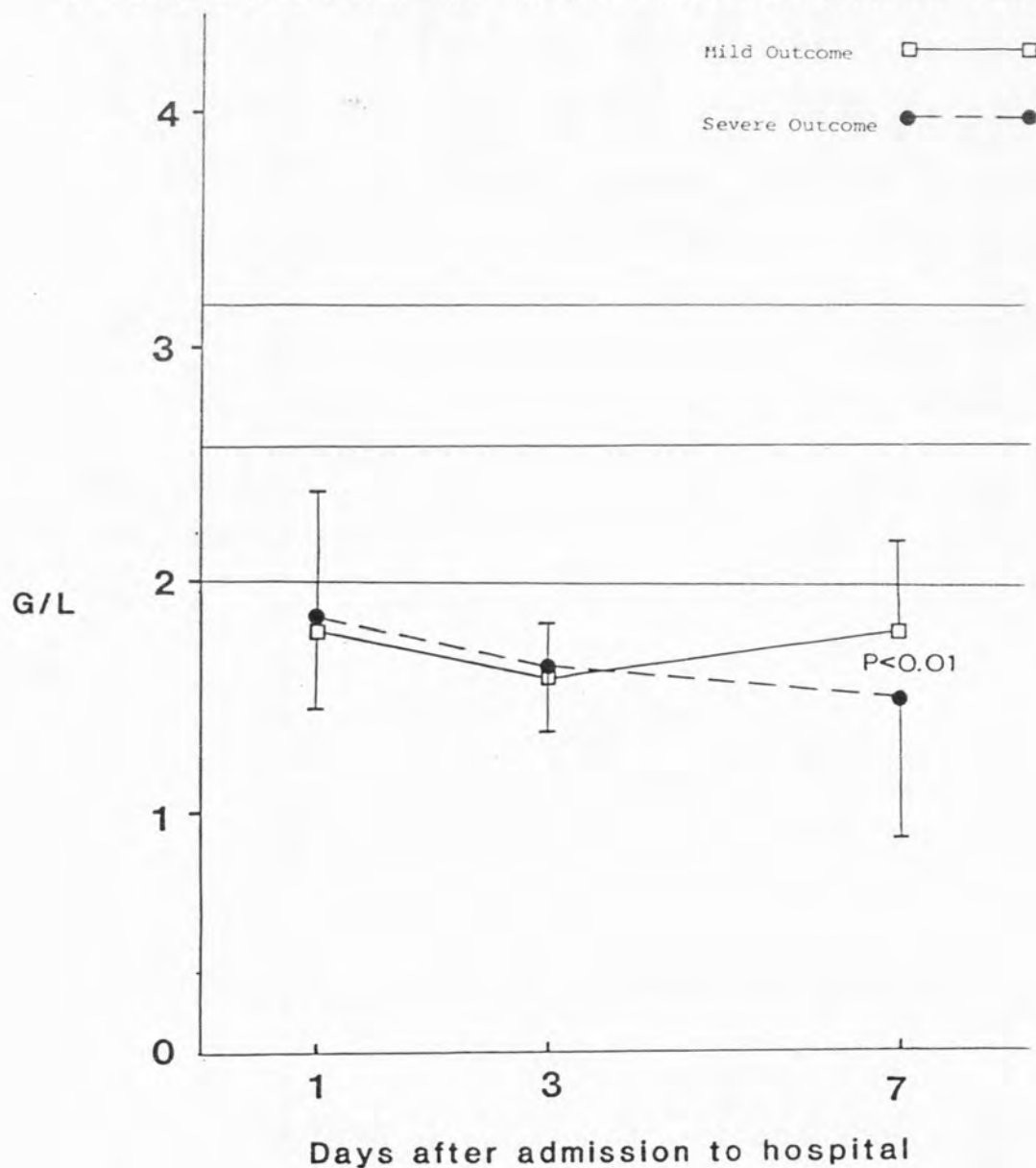
If the alpha$_2$ macroglobulin levels are analysed in the same way the only difference between the fresh frozen plasma and colloid control groups is that on day seven levels in the colloid control group were significantly lower in patients with a severe outcome than in patients with a mild outcome (p<0.05). This difference does not reach significance in the fresh frozen plasma group (p = 0.1576). When both groups are combined (Figure 41) the alpha$_2$ macroglobulin levels were significantly lower on day seven in patients with a severe outcome than in patients with a mild outcome (p<0.01).

8.4 Final Discussion

Acute pancreatitis is characterised by the release of pancreatic enzymes into the retroperitoneum, peritoneal cavity and circulation resulting in disseminated proteolytic damage affecting in particular the lungs, kidneys and myocardium. This metastatic injury accounts for much of the mortality and morbidity associated with the disease. In the United Kingdom, where biliary pancreatitis predominates over alcoholic pancreatitis, approximately 80% of patients can be expected to experience a mild illness and recover fully within three weeks. The remaining 20% of patients will suffer a severe illness of which, despite recent advances in general supportive measures, approximately half will die. Many specific therapies have been proposed for acute pancreatitis (chapter 1) but none has consistently been shown to improve clinical outcome in controlled clinical trials.

Normal human plasma contains powerful antiproteases and there is a reserve of inhibitory activity which has to be

exhausted before systemic pathological effects accrue from the release of activated enzymes into the circulation. Human serum albumin was advocated by Kenwell and Wels in 1953 as therapy for acute pancreatitis when it was suggested that the albumin preparation contained antiprotease activity. It was subsequently shown that the albumin had no capacity to inhibit trypsin (Elliot et al. 1955). More recently interest in fresh frozen plasma therapy for acute pancreatitis has been stimulated by the publication of an uncontrolled study from Dundee (Cuschieri et al. 1983) reporting a low mortality in a consecutive series of 239 patients experiencing 336 separate attacks of acute pancreatitis. The overall mortality was 3.7% per patient population and 2.7% per total number of separate attacks. In an experimental study performed in response to this report (chapter 7) intravenous therapy incorporating fresh frozen plasma was shown to significantly improve survival at 72 hours in a rat model of acute pancreatitis when compared with colloid control. These two studies appeared to justify a controlled clinical trial of fresh frozen plasma therapy in acute pancreatitis.

When looking for a mechanism to explain the apparent beneficial effect of fresh frozen plasma therapy the potential colloid value has first to be considered. The arguments for and against colloid therapy were discussed in chapter 3 and colloid therapy is not necessarily advantageous over crystalloid. In our preliminary experimental study (chapter 7) simple colloid was not shown to significantly improve survival in the animal model when compared with crystalloid control. However, because of some uncertainty about the value of colloid therapy in acute pancreatitis, it was decided to include a colloid control group

in the present study. Human albumin solution was useful in this respect as its protein content is almost pure albumin. It is devoid of other potentially valuable proteins present in fresh frozen plasma and in particular contains no active antiproteases.

Several proteins contained in fresh frozen plasma might be of specific benefit in the therapy of acute pancreatitis. These include clotting factors, fibronectin and C-reactive protein; but the replenishment of the plasma antiprotease system is the most promising explanation for the apparent beneficial effect of fresh frozen plasma therapy (Cuschieri et al. 1983). The plasma antiproteases constitute by weight the third largest group of functional proteins in human plasma, after albumin and immunoglobulins, accounting for nearly 10% of total plasma protein (chapter 2). They remain active after storage at -20°C in fresh frozen plasma. In man the principle antiproteases are $alpha_1$ antiprotease and $alpha_2$ macroglobulin which account for more than 90% of antiprotease capacity. The normal plasma concentrations of $alpha_1$ antiprotease and $alpha_2$ macroglobulin are 1.1-2.4 g/l and 1.45-3.7 g/l respectively, however the molar concentration of $alpha_1$ antiprotease is five times that of $alpha_2$ macroglobulin.

The primary role of $alpha_1$ antiprotease is the control of elastolytic activity secreted by neutrophils, but during acute pancreatitis it binds a wide range of proteases. It exhibits an acute phase response with a significant increase in plasma levels during the early stages of acute pancreatitis. The response is displaced slightly, reaching a peak on the fifth or sixth day rather than the third day as seen with other acute phase proteins (Aronsen et al. 1972; McMahon et al. 1984; Goodman et al. 1986).

In vivo alpha$_1$ antiprotease may only be a temporary inhibitor of trypsin (Oda et al. 1977) and when complexes of alpha$_1$ antiprotease and trypsin are administered intravenously in dog (Ohlsson et al. 1971) and man (Ohlsson and Laurell, 1976) the complexes dissociate with the appearance of alpha$_2$ macroglobulin-trypsin complexes in blood. Alpha$_1$ antiprotease may therefore serve in part as a carrier protein for proteases, particularly in the extracellular space where concentrations of alpha$_2$ macroglobulin are low (Ohlsson et al. 1971; Ohlsson and Laurell, 1976; Ganrot, 1972).

Alpha$_2$ macroglobulin may be the final link in the antiprotease chain and its importance is suggested by the fact that complete deficiency of alpha$_2$ macroglobulin has not been described in man. It is a very large glycoprotein (m.w. 725,000 daltons) which forms complexes with a wide variety of proteases. The mechanism of complex formation is unique and involves a rapid conformational change to physically entrap the protease within the bulk of the alpha$_2$ macroglobulin (Barrett and Starkey, 1973). This leaves the active site of the protease still open within the complex to act on smaller molecular weight substrates (Hermon-Taylor et al. 1981), but the access of larger substrates and inhibitors to the site is sterically hindered. The complexes are very stable and are subsequently rapidly eliminated from the circulation and destroyed by the reticulo-endothelial system. The half life of complexes in the circulation is ten minutes in man (Ohlsson and Laurell, 1976).

During acute pancreatitis plasma levels of alpha$_2$ macroglobulin fall as complexing and elimination of alpha$_2$ macroglobulin exceeds its production de novo. Lowest plasma levels are encountered on day

three, but the more severe the pancreatitis, the more marked and
sustained the fall in alpha$_2$ macroglobulin levels (McMahon et al.
1984; Goodman et al. 1986; Balldin et al. 1981; Mero et al.
1982). Low concentrations of alpha$_2$ macroglobulin in plasma and,
possibly more importantly, the reflected low concentrations and
high saturation of alpha$_2$ macroglobulin in peritoneal exudate,
may lead to the inappropriate activation by proteases of
secondary agents such as the complement and kinin system. These
secondary agents may be important mediators of the systemic
effects of acute pancreatitis (Balldin et al. 1981).

In the present study fresh frozen plasma was administered
during the first three days of the illness, the period of time
during which plasma alpha$_2$ macroglobulin levels are falling most
rapidly. Six units were administered compared with the Dundee
study (Cuschieri et al. 1983) in which six to eleven units (1500-
2500 mls) were infused over a longer period of five days. In that
study the fresh frozen plasma accounted for 20-30% of colloid
administered to patients with severe disease. Despite medical
teams being free to administer additional colloid in the present
study, the majority of patients (80.7%) did not receive any
colloid during the first three days beyond that specified by the
trial. In the remaining patients, who generally had more severe
disease, the trial colloid accounted for 10-75% (median 50%) of
total colloid administered.

Using these relatively small volumes of colloid, no patient
had to be withdrawn from the trial because of contraindications
to the fluid therapy such as congestive cardiac failure or acute
renal failure. Three possible adverse reactions to fresh frozen
plasma were encountered in the form of mild pyrexia and skin

rashes. No severe reactions or cases of transmitted infection were recorded. The donors for fresh frozen plasma were screened for antibodies to hepatitis B surface antigen throughout the trial and for antibodies to Human Immunodeficiency Virus from October 1985.

The fresh frozen plasma and colloid control groups were well matched in terms of predicted severity of pancreatitis. Using the nine Imrie criteria (Imrie, 1978a) 36% of patients were predicted severe which correlates with previous studies (Neoptolemos et al. 1986a; Blamey et al. 1984). The Imrie multi-factor analysis has gained wide acceptance in Britain, though modifications have been proposed including reducing the criteria to eight by the omission of the transaminase (Blamey et al. 1984) or age (Osborne et al. 1981). In this study, using the original nine criteria, the sensitivity of the prediction was 85% and the specificity 74%. Omitting the transaminase these figures become 79% and 80% respectively and omitting age they become 74% and 85%. The predictive values of a score of three or more adverse criteria are 40.3% using all nine factors, 45.0% omitting transaminase and 51.0% omitting age. On a simple statistical analysis (table 21) alanine transaminase appears to be the least useful of the nine criteria in predicting severity of outcome in this study. It may be more useful in the early diagnosis of biliary pancreatitis (McMahon and Pickford, 1979).

The definition of a severe outcome in acute pancreatitis is difficult. Death is an obviously severe outcome but other criteria which have been used, such as the period of hospital admission, the need for surgery or admission to ITU, the length of stay on ITU and the delay before resuming oral intake, are all

influenced by the availability of facilities and management policy of individual clinicians. This problem is compounded when several hospitals are involved in the study. In this trial death, the development of a major complication or hospital stay in excess of twenty days have been taken as indicating a severe outcome. The use of a shorter period of hospital stay seemed unrealistic in the predominantly elderly gallstone population of patients encountered in the Midlands. Hospital stays prolonged beyond twenty days purely because of elective surgery or social problems were excluded. Pancreatic mass lesions, demonstrated on ultrasound and CT investigations, were only included as major complications if they resulted in hospital stay in excess of twenty days or if they required surgical or percutaneous drainage.

When including all patients with acute pancreatitis in a controlled trial of a new therapy, over 250 patients are required in each limb to demonstrate a significant reduction in severe outcomes from 20% to 10% with a type one error set at 0.05 and a type two error set at 0.1, and over 550 in each limb to demonstrate a significant reduction in mortality from 10% to 5% (Pocock, 1983). In practice these numbers are difficult to achieve, even with a multicentre study. This problem can be reduced by pre-selecting a group of patients with more severe disease using methods such as multi-factor analysis (Imrie et al. 1978a; Ranson et al. 1974) or diagnostic peritoneal lavage (Pickford et al. 1977). All patients with acute pancreatitis were entered into this study to reflect the original Dundee work (Cuschieri et al. 1983). The decision to terminate the trial was taken after the analysis of two hundred patients revealed no

significant difference in clinical outcome between the fresh frozen plasma and colloid control groups. The overall incidence of major complications (17.2%) and the mortality (8.6%) in this study reflect the present outcome of acute pancreatitis in this country. It seems likely that, even if low volume fresh frozen plasma therapy does improve clinical outcome, the effect is not great enough to justify its routine administration to all patients with acute pancreatitis.

Alpha$_1$ antiprotease levels showed a significant increase from day one to day three in both fresh frozen plasma and colloid control groups. Levels remained elevated at day seven. This confirms the acute phase response reported by other workers (Aronsen et al. 1972; McMahon et al. 1984; Goodman et al. 1986). Fresh frozen plasma appears to have no effect on the magnitude of this rise. A delay in the rise of serum alpha$_1$ antiprotease levels in severe pancreatitis which has been reported by other workers (Goodman et al. 1986), was not encountered in this study. No significant differences were present in alpha$_1$ antiprotease levels between mild and severe outcomes.

The effect of relatively low volumes of fresh frozen plasma on serum alpha$_2$ macroglobulin levels was interesting. Alpha$_2$ macroglobulin levels were reduced on days 1, 3 and 7 in both the fresh frozen plasma and colloid control groups when compared with the reference population. In the colloid control group alpha$_2$ macroglobulin levels continued to fall from day one to day three (p$<$0.005) whilst levels were maintained in the fresh frozen plasma group (p = 0.6527). The difference between the two groups on day three was significant with p$<$0.01. This conflicts with a recent study (Goodman et al. 1986) in which patients with

predicted severe pancreatitis were randomised to receive three
units of fresh frozen plasma daily or conventional treatment.
Fresh frozen plasma showed no ability to supplement declining
$alpha_2$ macroglobulin levels. The numbers of patients involved,
however, was small with only three patients receiving fresh
frozen plasma and two in the control group. Four of these
patients died.

The results of this controlled clinical trial demonstrate
that low volume fresh frozen plasma therapy can supplement
declining $alpha_2$ macroglobulin levels during acute pancreatitis.

CHAPTER NINE

CONCLUSIONS AND PROSPECTS FOR RESEARCH

This study has demonstrated that:

a) High volume fresh frozen plasma therapy (one third of total intravenous fluid) significantly improves survival in an animal model of acute pancreatitis when compared with crystalloid and colloid control groups. It does so without reducing the local damage to the pancreas.

b) Low volume fresh frozen plasma therapy does not improve clinical outcome when compared with colloid control and it cannot be recommended for acute pancreatitis.

c) Fresh frozen plasma can be used to supplement declining serum alpha$_2$ macroglobulin levels seen during the early stages of this disease.

It is reasonable to speculate that the administration of larger volumes of fresh frozen plasma in man may result in the normalisation of serum alpha$_2$ macroglobulin levels. Whether or not larger volumes of fresh frozen plasma would improve clinical outcome in acute pancreatitis is unclear, but the animal study suggests that it might.

A clinical trial of high volume fresh frozen plasma therapy in acute pancreatitis will start on 1st June 1987. This will be a multicentre trial co-ordinated in Leicester. Within six hours of the diagnosis of acute pancreatitis (serum amylase > 1000 IU/L) the eight modified Glasgow criteria (Blamey et al. 1984) will be measured enabling the attack to be predicted as mild or severe. Only patients with predicted severe disease will be entered into the trial. This group of patients has the potential for 50-60%

severe clinical outcomes and therefore smaller numbers will be
required to show clinical benefit from a new therapy than would
be needed if all patients with acute pancreatitis were included.

After informed consent, patients will be randomised on the
opening of a sealed envelope to receive either eight units of
fresh frozen plasma intravenously or a similar volume (1800 ml)
of human albumin solution per 24 hours for 72 hours. Any patient
unable to tolerate the specified volume of colloid (for example
patients with congestive cardiac failure or acute renal failure)
will be withdrawn from the trial. The remaining intravenous fluid
therapy will be decided by the medical team caring for the
patient.

The major plasma antiproteases (alpha$_1$ antiprotease, alpha$_2$
macroglobulin, alpha$_1$ antichymotrypsin and antithrombin III) and
serum C-reactive protein and fibronectin will be measured on days
one, three and seven. This will demonstrate the effect of fresh
frozen plasma therapy on the concentration of these potentially
useful specific proteins. D-dimer will also be measured as an
indicator of disseminated intravascular coagulation.

If high volume fresh frozen plasma therapy is shown to
improve clinical outcome in prognostically severe acute
pancreatitis, then further studies using specific plasma
fractions, in particular alpha$_2$ macroglobulin concentrates, will
be performed.

Appendix 1

Results of Preliminary Studies on the Experimental Model of Acute Pancreatitis

Rat No.	Time from Start of Infusion	Ascites: Vol. (mls)	Colour	Amylase content (iu/l)	Blood results: Hb (g/l)	WCC (x10^9/l)	Hct. (%)	Amylase (iu/l)	Bilirubin (umol/l)	Alk Phos (iu/l)	Calcium (mmol/l)	Alb (g/l)	Histology scores: Necrosis	Inflammation	Haemorrhage	Oedema
1	0	0	-	-	15.9	8.2	43.7	40	3	248	2.61	33	0	0	0	0
2	0	0	-	-	14.5	7.3	41.4	5010	5	165	2.35	29	0	0	0	0
3	0	0	-	-	13.9	7.5	39.5	6060	4	162	2.46	29	0	0	0	0
4	5	0	-	-	14.6	9.2	41.0	9000	9	138	2.45	29	1	0	0	0
5	5	0	-	-	14.6	7.3	40.7	8160	6	126	2.43	29	2	0	0	0
6	5	0	-	-	14.2	8.6	39.5	6020	15	165	2.40	29	1	0	0	0
7	10	0	-	-	15.0	6.0	42.0	5760	4	154	2.56	29	3	0	2	1
8	10	0	-	-	14.3	4.5	41.3	10,800	3	123	2.47	29	2	0	0	0
9	10	0	-	-	14.6	8.6	42.6	8600	9	146	2.43	28	1	0	1	0
10	30	2	straw	320,000	14.8	8.5	45.0	12,120	4	-	2.46	28	2	0	0	0
11	30	1	straw	-	15.0	5.1	48.0	10,920	5	169	2.49	28	2	0	2	0
12	30min	0.5	red	-	17.1	8.0	46.0	12,020	6	140	2.43	28	3	0	1	1
13	1	0.5	red	-	18.0	10.3	53.0	33,800	5	175	2.45	28	2	1	1	0
14	1	2	straw	425,000	17.2	8.0	46.0	17,400	6	42	2.56	28	2	1	1	0
15	1	3	red	263,000	16.8	9.6	48.0	15,200	8	169	2.40	28	2	0	1	1
16	3	1	straw	-	17.3	10.6	48.6	30,000	23	187	2.23	28	1	0	1	1
17	3	6	red	183,000	clotted			17,000	24	156	2.20	26	2	1	1	1
18	3	4	straw	289,000	15.0	12.6	46.0	19,000	10	165	2.40	26	2	1	2	1
19	6	4	red	427,000	14.8	7.5	44.0	10,000	7	126	2.07	23	2	1	1	1
20	6	3	red	277,000	16.8	14.9	46.0	49,000	27	211	2.33	27	2	1	1	1
21	6	4	red	436,000	15.0	10.6	48.0	22,000	10	175	2.23	26	3	2	1	0
22	12	6	red	210,000	13.4	8.0	37.0	50,000	152	621	2.16	23	3	3	2	3
23	12	4	red	410,000	19.4	5.6	58.0	110,000	insufficient				3	3	2	3
24	12	8	brown	490,000	15.2	12.4	49.0	70,000	24	211	2.23	26	3	2	3	2
25	24	6	red	460,000	20.2	10.1	58.0	38,000	27	146	2.40	26	2	2	1	2
26	24	10	brown	186,000	14.5	14.5	40.0	24,000	32	175	2.20	25	3	3	2	3
27	24	12	brown	193,000	15.2	12.6	48.0	27,000	24	187	2.49	25	2	2	2	3
28	48	6	red	436,000	13.0	12.3	37.0	15,000	38	595	2.51	25	3	3	2	3
29	48	10	brown	343,000	12.3	6.5	34.5	4300	89	771	2.42	25	3	3	3	3
30	48	8	brown	243,000	12.6	15.6	48.0	6400	23	187	2.23	28	3	2	3	2
31	72	0	-	-	11.2	14.2	31.0	2152	4	203	2.44	28	3	3	1	2
32	72	2	brown	-	10.8	20.6	30.0	2760	196	511	2.57	28	1	2	0	2
33	72hrs	0	-	-	13.5	29.5	37.5	1772	107	602	2.56	28	3	3	1	?
34	1	0	-	-	15.5	7.9	43.1	3908	3	301	2.69	35	1	0	0	0
35	1	0	-	-	11.6	15.5	33.2	996	67	606	2.46	28	1	1	0	2
36	1week	0	-	-	13.9	9.3	38.6	3456	5	96	2.49	31	2	2	1	2

Appendix 2

Results of Analgesia Studies.

Rat No.	Dosage of Buprenorphine (mg/kg/8 hours)	Score for Tail Pinch Time (hours)												Score for Toe Pinch Time (hours)											
		6	12	18	24	30	36	42	48	54	60	66	72	6	12	18	24	30	36	42	48	54	60	66	72
3	0	3	3	3	0	3	1	3	3	3	3	3	3	3	3	2	3	3	0	3	2	3	3	1	3
8	0	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	3
18	0	0	3	3	3	0	3	3	3	3	3	3	3	3	3	3	0	3	3	3	3	0	3	3	3
19	0	3	3	3	3	3	3	3	0	3	3	3	3	3	3	3	2	3	3	3	3	0	3	3	3
26	0	3	3	3	3	3	1	3	3	0	3	3	3	3	3	3	2	3	2	3	3	3	0	3	3
2	0.1	0	3	3	2	2	2	1	2	1	2	2	2	1	0	1	1	2	1	1	1	2	1	1	1
7	0.1	2	0											1	2										
16	0.1	2	0	1	3	0	2	0	3	2	1	3	0	0	1	2	0	1	1	1	2	2	2	0	2
24	0.1	2	2	3	2	1	2	2	2	3	2	0	2	3	2	1	2	3	1	2	0	3	2	1	2
27	0.1	1	2	2	2	1	3	2	0	2	2	1	2	1	0	1	2	0	1	2	2	1	1	1	2
1	0.2	1	0	3										1	0	2									
12	0.2	3	2	2	1	2	3	1	2	2	0	3	2	0	2	1	1	1	1	2	1	1	1	2	1
17	0.2	2	0	1	2	0	3	2	0	0	2	1	0	3	0	1	2	2	3	1	2	2	3	1	0
20	0.2	2	3	0	2	3	2	0	0	2	3	3	1	1	0	2	2	0	1	1	2	0	0	2	1
28	0.2	2	0	1	2	0	1	2	0	0	1	1	2	0	2	1	2	0	1	2	0	1	1	2	1
5	0.3	3	2	3	0	3	3	2	3	3	0	3	3	3	1	0	3	2	1	2	2	2	2	2	3
11	0.3	1												2											
15	0.3	0	2	0	3	0	3	0	0					1	0	2	0	0	3	2	1				
23	0.3	0	1	1	1	1	0	2	1	2	1	0	1	0	1	1	2	1	2	2	2	2	1	1	1
29	0.3	1	0	2	0	1	1	2	0	1	0	1	1	0	1	1	2	1	2	2	2	2	1	1	0
6	0.4	2	2	0	1	2	0	2	2					1	1	0	1	1	1	1	1				
9	0.4	0	2	1	1	2	1	2	2	2	1	1	1	1	3	1	1	1	1	1	1	1	1	1	1
14	0.4	3	1	1	0	1	1	1	1	0	0	2	2	2	0	1	0	1	2	2	0	2	2	3	2
21	0.4	2	1	3	0	1	1	2	2	1	1	2	1	0	1	3	3	1	1	2	2	1	0	1	1
25	0.4	1	0	3	0	1	1	2	2	1	1	2	1	0	1	3	0	1	1	2	2	1	0	1	1
4	0.5	0	1	2	0	1	2	1	0	0	1	0	1	0	0	1	2	1	1	0	0	1	0	1	0
10	0.5	1	3	1	2	2	2	2	2	3	3	2		1	2	2	1	0	1	1	1	1	2	2	3
13	0.5	1	0	0	0	1	0	1	2					2	0	0	1	2	2	1	0				
22	0.5	1	0	3	2	1	1	2	2	0	0	2	1	3	2	0	3	1	2	2	3	1	1	1	1
30	0.5	1	3	0	2	1	1	0	2	0	0	2	1	1	1	0	2	3	3	1	0	2	3	1	1

Appendix 3

Haematological, Biochemical, Macroscopic and Microscopic Comparison of the High Volume Fresh Frozen Plasma and Colloid Treatment Groups in the Experimental Model of Acute Pancreatitis.

Rat No.	Infusion	Time From Start of Infusion (hours)	Ascites Vol. (mls)	Colour	Hb (g/l)	WCC (x10⁹/l)	Hct. (%)	Amylase (iu/l)	Bilirubin (umol/l)	Alk Phos (iu/l)	Calcium (mmol/l)	Alb (g/l)	Necrosis	Inflammation	Haemorrhage	Oedema
1	Control	0	0	-	14.0	7.6	41.2	8800	5	173	2.61	29	0	0	0	0
2	Control	0	0	-	15.1	8.0	42.5	7900	5	182	2.69	30	0	0	0	0
3	FFP	1	0.5	straw	15.4	9.1	47.6	18600	5	233	2.58	29	1	0	0	0
4	Colloid	1	1	straw	15.8	9.6	42.8	30000	5	377	2.72	27	2	1	0	0
5	FFP	1	1.5	straw	14.9	8.6	43.6	20500	9	236	2.43	29	2	1	1	1
6	Colloid	1	1	red	16.2	10.0	41.9	18200	6	169	2.40	28	2	1	1	1
7	FFP	2	1	straw	14.9	12.1	49.0	26200	5	190	2.65	26	2	1	1	1
8	Colloid	2	3	red	15.4	10.6	47.6	22200	5	356	2.65	25	2	0	1	1
9	FFP	2	3	red	15.5	9.2	42.1	29300	15	49	2.46	28	1	1	2	1
10	Colloid	2	4	red	15.3	9.7	44.0	28700	5	146	2.43	28	2	1	0	0
11	FFP	4	4	red	14.8	13.5	46.0	35600	8	154	2.56	26	2	1	2	0
12	Colloid	4	2	red	15.4	12.6	47.3	30100	5	175	2.40	24	3	2	0	1
13	FFP	4	3	red	15.9	10.2	44.8	21100	5	185	2.46	26	2	2	1	1
14	Colloid	4	3	red	15.4	10.0	47.2	27000	10	382	2.44	22	2	1	2	1
15	FFP	8	4	brown	17.2	9.5	50.1	29500	29	1213	2.61	30	2	2	2	1
16	Colloid	8	5	red	14.6	12.6	48.6	24500	33	979	2.44	14	3	1	1	2
17	FFP	8	6	red	14.9	13.0	44.2	45000	5	187	2.23	26	2	2	2	0
18	Colloid	8	4	red	16.2	10.7	45.1	3700	11	476	2.16	25	2	2	2	2
19	FFP	16	8	red	14.7	12.4	42.4	29800	5	665	2.23	26	1	3	2	1
20	Colloid	16	4	brown	15.6	10.7	49.6	23000	5	165	2.35	19	2	2	2	3
21	FFP	16	5	brown	15.6	13.6	51.3	80300	23	955	2.40	28	3	3	3	2
22	Colloid	16	9	red	14.9	14.2	47.6	32000	27	876	2.22	28	3	2	3	2
23	FFP	32	6	brown	13.5	14.9	44.2	32100	32	90	2.36	30	2	3	2	1
24	Colloid	32	10	brown	14.6	10.1	47.6	32200	141	486	2.54	25	3	2	1	2
25	FFP	32	4	brown	14.9	11.6	40.2	15000	89	654	2.42	29	3	3	3	3
26	Colloid	32	6	brown	14.1	13.8	41.6	20100	38	435	2.23	28	3	3	3	2
27	FFP	64	2	brown	14.2	17.6	40.1	16300	5	862	2.61	26	3	3	2	3
28	Colloid	64	1	brown	13.5	12.5	38.6	18100	5	249	2.50	23	3	2	3	2
29	FFP	64	0	-	11.6	10.7	39.0	9600	107	854	2.56	28	3	3	1	3
30	Colloid	64	0	-	12.2	19.8	38.7	8500	67	301	2.46	29	2	3	1	3

Appendix 4

Details of Patients Included in the Clinical Trial

a) Patients randomised to receive fresh frozen plasma

Trial No.	Sex	Aet.	Stay (days)	Risk Score	Age (yrs)	WCC (x10^9/1)	Glucose (mmol/1)	Urea (mmol/1)	PaO2 (KPa)	Calcium (mmol/1)	LDH (iu/1)	Alb (g/1)	ALT (iu/1)	Alpha2 Macroglobulin (g/1) Day1	3	7	Alpha1 Antiprotease (g/1) Day1	3	7	Outcome
3	F	G	23	4	76	17.4	11.6	6.3	8.7	2.0	635	38	30	3.0	2.3	2.0	1.8	2.9	4.4	S(+)
5	F	G	8	4	86	12.5	10.5	6.0	7.2	2.2	-	40	168	2.5	2.6	2.7	2.1	2.4	2.0	M
6	F	G	56	4	82	21.3	6.3	19.7	8.5	1.63	217	32	17	1.9	1.8	1.7	3.0	2.9	3.5	S
8	M	G	14	4	63	12.8	15.1	8.6	7.0	2.15	-	37	104	1.6	1.7	1.6	1.8	4.0	4.2	M
10	M	I	27	4	76	22.9	7.9	9.8	7.12	2.06	757	39	12	2.2	1.1	-	2.7	3.0	1.9	S
11	F	G	10	2	72	18.8	7.9	3.9	8.9	2.24	-	42	34	-	1.3	1.3	-	4.7	4.7	M
13	M	G	20	1	51	8.8	6.1	5.7	10.3	2.17	429	42	969	1.7	1.6	1.9	1.9	2.7	3.2	M
14	F	G	21	2	68	18.3	9.4	5.7	9.4	2.34	296	37	19	2.0	2.5	2.5	2.2	2.3	2.2	M
15	M	G	9	2	32	15.1	8.3	6.4	10.6	2.39	387	44	673	1.6	2.0	1.2	1.5	1.3	3.4	M
16	F	G	9	0	53	13.6	9.4	4.7	10.5	2.15	-	43	84	1.9	2.1	-	1.8	2.6	-	M
18	M	G	16	4	67	14.5	11.5	6.4	6.9	2.55	282	38	687	1.3	1.4	2.1	2.0	2.5	2.5	M
20	F	G	6	3	64	15.2	7.0	5.0	8.7	2.0	339	42	276	1.1	1.4	1.6	1.6	2.7	3.0	M
21	M	I	17	1	70	12.7	9.6	8.0	8.9	2.31	132	35	83	2.7	-	-	1.9	-	-	S(+)
25	M	I	7	3	59	15.3	10.2	4.8	9.4	2.08	269	40	11	1.9	1.7	1.9	1.8	2.7	3.1	M
28	M	A	10	1	69	12.0	9.6	2.6	9.1	2.3	258	32	6	-	1.5	1.6	-	2.9	3.3	M
29	F	G	16	3	69	14.1	9.8	15.0	11.0	1.91	561	28	22	1.5	1.4	1.5	3.3	4.1	6.4	M
30	F	G	15	1	48	18.1	7.1	4.5	14.4	2.39	487	35	86	3.1	2.9	2.0	1.7	4.4	5.8	M
33	F	G	7	2	65	10.8	7.4	5.1	8.5	2.2	361	39	291	1.4	1.6	1.9	2.1	3.4	2.9	M
34	M	G	4	1	72	9.3	6.6	6.6	-	2.28	388	38	95	2.7	2.9	-	1.4	1.6	-	M
37	F	G	18	4	38	15.1	7.1	4.2	12.2	1.98	1623	43	1111	2.0	1.8	1.8	1.6	2.0	2.4	M
36	F	G	7	2	30	22.0	5.7	4.5	13.5	2.3	267	38	324	-	2.1	2.1	1.1	2.3	2.4	M
43	M	A	7	0	33	14.3	5.1	3.8	12.5	2.28	481	42	38	1.7	2.0	2.6	2.5	2.2	3.0	M
45	M	A	15	1	45	17.8	7.7	2.6	13.5	2.23	192	36	23	-	1.2	1.3	-	2.1	2.3	M
46	M	A	26	4	32	19.6	6.1	1.5	6.8	1.4	517	25	51	3.6	2.8	2.5	2.0	2.0	0.9	S
50	F	G	7	3	82	17.4	9.5	6.3	10.3	2.2	351	30	83	2.4	2.8	2.8	2.6	2.7	2.6	M
51	M	A	5	0	32	6.9	6.2	6.6	12.8	2.24	74	42	42	1.4	1.7	-	1.1	3.1	-	M
54	F	O	5	6	22	19.9	8.6	28.4	7.6	1.95	1800	30	60	1.9	1.8	-	2.3	3.6	-	S(+)
55	M	A	10	2	31	15.8	7.3	2.9	7.6	2.12	515	34	50	1.3	1.7	1.7	2.3	4.2	4.2	M
56	F	I	5	1	25	15.6	8.6	2.6	14.1	2.15	71	36	16	1.6	1.5	1.9	2.0	2.5	3.2	M
63	F	G	11	1	87	10.9	7.7	8.3	8.76	2.09	255	46	54	2.1	2.2	-	1.8	2.0	-	M
64	F	I	9	2	69	15.6	6.3	5.3	10.15	2.36	289	37	32	2.7	3.1	6.7	2.3	2.5	5.7	M
67	F	O	7	3	56	15.3	10.3	4.0	9.92	2.5	223	43	33	1.5	1.8	-	2.9	4.4	-	M
68	M	A	7	1	27	16.3	8.0	4.5	11.9	2.47	204	37	27	1.4	1.4	1.6	2.0	3.4	2.9	M
70	M	A	9	1	36	9.7	6.8	4.9	7.7	2.32	291	43	31	1.6	1.2	1.2	1.8	2.9	2.3	M
72	M	G	17	2	78	21.0	9.5	7.4	11.67	2.37	348	41	84	2.5	2.1	2.1	2.6	2.5	2.9	M
73	F	I	11	0	40	9.6	5.6	1.9	17.2	2.3	227	44	13	1.5	2.2	1.9	1.9	2.3	2.2	M
74	M	I	20	5	67	16.2	5.5	6.0	7.6	1.7	471	38	380	1.1	1.1	1.1	3.3	3.1	2.9	S
75	F	G	5	1	62	10.0	8.5	5.6	11.9	2.34	225	38	18	1.4	1.5	1.5	1.8	1.8	2.0	M
93	F	I	6	2	19	9.1	6.3	4.8	12.5	1.99	347	28	4	1.8	1.4	1.5	2.5	2.1	2.3	M
95	M	G	19	1	51	12.9	9.6	8.8	6.1	2.3	354	42	79	1.5	1.5	1.7	1.8	2.9	3.2	S
97	F	G	10	1	75	12.5	6.1	4.3	13.3	2.22	408	39	25	2.1	2.2	2.2	2.1	2.0	1.9	M
106	M	A	4	0	34	10.9	4.7	8.4	10.7	2.67	242	46	8	2.9	3.4	2.6	2.2	2.5	3.2	M
107	F	O	1	4	85	5.6	66.4	16.3	6.3	2.5	-	35	-	-	-	-	-	-	-	S(+)
109	F	G	12	2	35	16.5	6.8	3.1	-	2.23	222	40	272	1.3	1.4	1.5	2.1	2.1	2.5	M
58	F	G	23	1	71	13.1	6.1	7.7	10.11	2.4	135	35	41	-	2.4	2.3	-	2.2	2.0	M
114	F	G	30	2	74	7.8	13.7	5.5	10.3	2.13	364	41	66	1.8	1.6	1.7	1.9	2.6	2.9	M
124	M	G	8	3	32	16.5	8.3	5.1	9.3	2.29	460	31	305	1.3	1.2	1.4	1.7	2.7	3.2	M

Appendix 4 (continued)

Details of Patients Included in the Clinical Trial

a) Patients randomised to receive fresh frozen plasma

Trial No.	Sex	Aet.	Stay (days)	Risk Score	Age (yrs)	WCC (x10⁹/l)	Glucose (mmol/l)	Urea (mmol/l)	PaO₂ (KPa)	Calcium (mmol/l)	LDH (iu/l)	Alb (g/l)	ALT (iu/l)	Alpha₂ Macroglobulin (g/l) Day1	3	7	Alpha₁ Antiprotease (g/l) Day1	3	7	Outcome
100	M	I	5	0	21	13.1	6.3	6.0	13.4	2.29	278	42	17	1.8	2.1	1.9	1.5	1.8	1.6	M
86	F	G	3	1	37	12.9	5.6	3.8	11.8	2.32	138	40	176	1.7	2.0	2.2	1.8	1.9	1.8	M
130	F	G	10	2	68	9.4	5.8	4.6	10.4	2.19	244	37	646	2.5	2.7	2.6	1.9	2.2	2.0	M
151	M	G	7	2	78	11.7	6.5	5.3	7.8	2.47	306	37	11	2.9	2.3	2.8	3.0	3.1	2.9	M
133	M	A	12	0	32	14.8	7.5	5.9	9.4	2.27	323	33	10	1.3	1.4	1.1	2.4	5.4	1.5	M
129	F	G	24	4	65	22.5	5.8	14.1	9.9	1.64	494	36	798	0.7	0.9	0.8	1.1	1.9	2.8	S(+)
128	F	G	13	2	74	13.8	5.8	7.5	10.4	2.1	263	33	172	2.3	1.9	2.2	2.0	2.3	3.0	M
132	F	G	13	1	52	12.5	6.6	5.1	9.8	2.15	-	36	116	1.9	1.6	1.9	2.5	2.7	3.1	M
78	F	G	5	3	52	16.7	11.5	4.4	11.4	2.17	169	42	627	1.9	2.1	-	2.3	2.2	-	M
111	M	A	9	1	37	12.3	11.4	5.2	14.0	2.19	287	37	21	1.6	2.3	1.7	1.8	3.2	2.5	M
116	F	C	6	2	64	16.8	8.6	5.1	9.4	2.03	423	44	69	2.8	2.4	2.7	1.4	1.8	1.9	M
118	F	G	18	2	89	14.3	5.4	7.8	10.8	2.3	256	39	138	2.4	2.8	3.0	2.2	2.4	2.3	M
89	M	A	5	4	54	18.5	7.1	24.0	8.9	1.86	501	30	34	-	2.6	-	-	3.3	-	M
80	F	A	10	0	35	6.4	7.5	3.6	9.8	2.35	155	35	66	1.2	1.3	1.5	1.2	2.2	2.3	M
83	F	I	7	1	49	12.1	8.1	4.0	7.7	2.38	201	35	54	1.6	-	-	-	2.7	-	M
87	M	G	15	4	87	16.4	8.9	8.5	7.7	2.11	304	34	443	1.9	1.9	1.8	2.0	2.3	2.6	S
110	F	G	10	3	61	11.1	6.5	3.3	8.7	2.41	413	34	238	1.9	1.5	2.5	2.5	2.0	2.3	M
119	F	G	13	3	74	13.4	13.1	10.4	9.4	1.7	365	35	58	4.4	2.5	2.0	3.2	2.7	3.4	M
138	F	I	6	0	17	8.5	5.6	2.4	13.5	2.12	261	40	26	3.6	4.1	3.5	3.5	2.9	1.8	M
139	M	A	9	1	46	8.3	6.6	2.3	9.6	2.27	387	41	106	1.7	1.6	1.8	1.8	2.0	2.6	M
143	M	O	5	1	59	13.1	5.7	5.4	8.9	2.46	244	44	8	1.7	1.6	1.7	1.9	1.8	2.2	M
144	F	I	8	5	48	17.3	35.4	5.1	11.7	1.63	1154	29	14	1.2	1.2	1.3	1.6	4.0	4.2	M
145	M	O	5	1	59	10.2	5.6	5.4	9.9	2.29	232	39	12	1.7	1.9	-	2.2	1.8	-	M
151	M	C	7	2	78	11.7	6.5	5.3	7.8	2.47	306	37	11	2.9	2.3	2.8	3.0	3.1	2.9	M
149	M	G	14	4	78	18.6	14.4	6.9	-	2.05	263	37	106	2.2	1.8	1.5	1.9	3.3	2.9	M
163	F	I	10	3	80	14.4	12.6	12.0	10.1	1.67	286	35	15	1.4	1.2	1.1	2.0	4.3	4.8	M
196	F	G	12	1	48	7.8	5.6	2.8	10.8	2.41	356	42	896	1.6	1.4	1.1	2.1	2.2	3.4	M
172	M	G	19	5	72	17.8	9.1	11.3	7.1	2.54	233	30	109	1.7	1.7	1.7	2.1	2.2	2.4	S(+)
174	F	C	10	2	79	19.3	7.5	4.8	8.3	2.2	270	33	42	1.8	1.6	2.0	2.0	2.1	2.2	M
177	M	A	8	0	32	5.2	9.8	4.6	10.8	2.45	497	40	26	1.8	2.0	1.9	2.0	2.1	1.9	M
179	M	A	9	3	65	21.4	9.4	7.3	7.32	2.27	295	37	18	1.5	1.3	1.0	1.5	3.0	3.1	M
180	F	G	8	7	68	13.9	18.8	21.3	6.9	1.7	795	29	20	1.4	1.1	1.3	3.3	3.2	3.4	M
181	F	C	18	2	76	18.8	8.3	9.0	-	2.48	326	-	-	1.1	1.3	1.2	1.9	3.0	2.7	M
183	F	C	5	1	70	13.7	9.1	8.6	11.2	2.33	411	35	64	1.3	1.8	1.8	1.2	1.9	2.1	M
164	F	C	8	1	34	19.5	6.1	3.8	11.8	2.05	237	39	44	1.3	1.4	1.8	1.6	2.4	2.5	M
166	F	C	6	1	70	6.9	6.7	8.9	11.0	2.43	347	40	18	2.2	1.8	1.8	1.8	1.5	1.8	M
157	M	A	8	2	31	21.3	11.0	3.9	10.2	2.23	361	37	20	1.3	1.3	1.5	1.6	3.3	3.3	M
154	M	C	7	1	77	11.6	5.7	7.8	11.4	2.18	243	35	57	1.5	1.8	2.0	2.1	2.3	2.1	M
159	M	C	6	2	64	10.2	6.7	4.8	10.3	2.3	397	37	348	1.5	1.6	1.9	2.0	2.0	2.1	M
194	F	I	18	2	58	10.0	8.6	9.4	16.6	2.05	233	28	6	1.0	1.1	1.1	3.4	2.5	2.5	M
192	F	C	9	7	83	17.0	11.0	6.0	7.5	1.71	897	29	21	2.3	2.0	-	2.8	3.5	-	S(+)
160	F	C	4	1	53	14.8	5.3	4.8	10.1	2.18	453	36	293	1.9	2.2	-	1.7	1.9	-	M
188	M	A	4	0	20	11.7	6.6	5.1	10.4	2.38	229	-	-	2.1	2.2	-	1.3	2.0	-	M
198	F	C	9	4	83	15.1	7.3	31.6	10.9	1.99	434	33	31	3.1	2.2	2.3	2.5	1.9	2.1	M
199	F	C	8	2	37	15.3	6.3	2.5	11.4	2.25	312	37	201	1.8	1.7	1.8	3.1	2.7	2.5	M
168	F	C	8	2	66	14.0	7.5	6.7	8.6	1.92	360	-	-	1.8	1.4	1.5	1.7	2.8	2.5	M
169	F	O	33	3	33	22.2	11.9	10.7	7.9	2.16	329	42	66	1.5	0.4	0.9	1.6	1.4	3.1	S(+
187	F	C	22	3	76	25.0	16.1	10.4	12.6	2.49	305	34	24	1.4	1.5	1.6	1.5	2.0	2.0	M
197	F	G	5	1	55	9.3	6.3	3.4	11.5	2.26	434	36	456	1.9	1.6	1.9	1.6	1.7	2.2	M

Appendix 4 (continued)

Details of Patients Included in the Clinical Trial

b) Patients randomised to receive colloid control

Trial No.	Sex	Aet.	Stay (days)	Risk Score	Age (yrs)	WCC (x10^9/1)	Glucose (mmol/1)	Urea (mmol/1)	PaO2 (KPa)	Calcium (mmol/1)	LDH (iu/1)	Alb (g/1)	ALT (iu/1)	Alpha2 Macroglobulin (g/1) Day1	3	7	Alpha1 Antiprotease (g/1) Day1	3	7	Outcome
1	F	G	16	7	85	21.3	4.8	16.8	14.1	1.84	842	28	131	2.2	1.7	1.9	2.5	2.2	2.6	M
2	F	G	11	2	69	7.8	6.2	3.9	8.4	2.26	592	44	438	2.5	-	1.9	1.7	-	2.3	M
4	F	G	8	3	82	9.1	7.8	11.7	8.0	2.1	980	35	290	3.3	1.7	1.5	1.6	2.1	2.8	S(+)
7	F	I	8	3	72	14.2	12.0	7.5	6.5	2.24	-	36	60	1.6	1.1	1.3	1.6	2.6	4.8	M
9	M	O	8	0	25	10.2	5.5	4.7	9.99	2.24	-	33	86	-	-	-	3.7	3.7	-	M
12	M	A	13	2	30	6.9	9.7	5.6	10.9	1.97	-	40	121	1.8	1.8	1.6	3.4	3.2	2.3	M
17	M	A	10	1	23	18.2	7.9	5.5	12.9	2.26	449	42	8	1.8	2.1	2.3	4.0	3.2	2.5	M
19	M	G	5	1	56	14.3	6.4	5.5	8.9	2.13	542	40	17	1.9	1.7	-	1.9	3.1	-	M
22	M	I	9	1	83	10.3	6.6	11.4	9.5	2.14	343	33	10	2.1	2.0	2.1	2.9	3.6	2.2	M
23	F	G	7	2	28	9.2	5.2	2.9	13.0	1.97	224	31	64	2.5	2.1	2.6	2.4	2.4	2.7	M
24	F	I	10	0	40	11.7	6.2	7.6	11.1	2.1	200	36	10	1.8	1.8	-	1.8	2.8	-	M
26	M	G	6	4	78	9.9	5.1	5.8	7.6	2.21	707	32	149	1.8	1.5	1.5	2.9	2.9	2.7	M
27	M	A	6	0	46	14.9	8.0	4.6	13.3	2.16	197	42	8	1.4	1.3	1.4	1.9	3.2	2.9	M
31	M	A	8	0	38	13.1	8.3	4.9	11.5	2.48	187	38	32	1.9	1.4	2.3	1.9	3.5	4.7	M
33	F	G	22	6	58	22.0	7.7	7.6	7.4	1.77	707	39	496	2.4	2.1	1.5	2.1	6.3	6.3	S
32	F	G	12	3	65	15.1	11.7	7.0	8.9	2.55	144	40	22	1.1	0.7	1.0	1.2	2.2	2.8	M
38	M	I	22	4	69	18.3	16.9	10.0	8.7	1.92	364	37	25	-	1.9	2.2	-	4.8	7.8	S
39	M	G	8	5	91	17.5	12.3	8.7	9.6	2.3	746	35	174	5.0	2.2	3.9	2.5	1.5	2.1	M
42	M	O	7	1	73	10.6	6.5	12.6	10.6	2.36	518	41	31	2.2	1.5	-	2.3	2.8	-	M
44	M	A	11	1	34	12.1	6.4	6.1	10.8	2.08	422	35	131	1.5	1.0	1.2	4.0	4.6	2.8	M
47	F	G	26	4	78	16.2	8.6	10.1	11.0	2.29	680	33	148	2.8	1.6	2.0	1.8	2.4	2.1	M
48	M	A	3	0	41	6.4	4.8	2.5	13.7	2.4	307	40	63	3.0	2.8	2.6	1.8	2.0	1.7	M
49	M	G	10	5	66	28.6	10.2	9.1	7.7	2.09	300	37	734	1.1	1.1	1.0	2.1	3.0	3.2	M
52	M	A	11	2	42	17.1	10.1	2.9	10.1	2.65	259	45	66	1.5	1.0	1.3	2.0	3.3	3.3	M
53	M	O	12	1	16	21.2	8.3	5.6	11.7	2.29	394	40	18	1.2	1.3	2.5	1.8	3.6	7.0	M
61	M	A	9	0	36	10.9	6.7	4.4	8.8	2.37	291	43	46	1.6	1.2	1.5	1.8	2.7	2.2	M
62	M	A	17	2	39	18.2	6.6	2.8	12.1	2.4	306	36	124	2.5	2.2	2.4	2.9	3.2	3.4	M
65	M	O	5	2	58	12.1	5.6	4.9	7.7	2.28	542	44	8	3.5	-	-	2.4	3.1	-	M
66	F	G	10	3	75	12.2	7.5	6.5	7.5	2.28	285	37	267	-	3.9	5.5	-	3.4	6.3	M
69	F	I	1	5	78	5.8	7.7	17.0	10.0	3.09	2500	31	101	1.4	-	-	2.1	-	-	S(+)
71	M	A	9	0	36	11.4	6.8	4.6	10.6	2.3	291	41	24	1.6	1.2	1.5	1.8	2.9	2.2	M
91	F	G	7	1	33	11.1	10.0	3.3	14.0	2.15	306	39	280	-	1.2	2.5	-	2.1	3.6	M
92	M	G	12	3	80	15.4	5.1	3.7	13.0	2.27	542	36	114	1.5	1.1	1.2	1.6	2.0	2.3	M
94	M	G	8	1	73	10.6	6.6	4.6	-	2.23	483	42	72	1.4	1.4	-	1.7	1.6	-	M
96	M	G	17	3	58	16.3	8.8	4.9	11.4	2.19	389	41	292	2.5	2.2	1.8	2.6	5.5	3.6	M
98	M	G	8	2	81	12.4	8.2	10.9	6.1	2.3	428	45	20	3.2	2.7	-	1.7	3.0	-	M
101	F	G	23	2	73	18.6	7.3	5.2	9.5	2.36	235	35	5	3.0	2.6	2.7	1.8	2.4	2.6	M
102	M	G	7	2	76	15.9	5.8	9.3	8.8	2.14	190	35	14	1.8	1.7	2.2	2.3	3.1	2.9	M
103	F	G	10	1	71	14.3	6.0	5.9	11.6	2.15	117	40	16	2.0	1.3	1.5	2.5	3.1	3.5	M
104	F	G	7	0	23	12.4	6.5	4.0	12.5	2.21	126	40	43	1.1	1.1	1.2	2.8	2.1	2.1	M
105	F	G	9	2	74	10.2	6.9	6.4	9.9	2.05	542	40	287	2.9	2.5	2.5	1.7	2.1	2.1	M
40	F	A	11	0	32	11.3	9.6	5.1	11.4	2.06	542	36	54	2.4	1.8	2.4	2.4	3.1	6.0	M
57	F	G	5	0	34	12.4	6.5	4.0	12.5	2.1	291	40	43	3.5	2.2	2.4	2.4	3.1	3.4	M
121	F	G	29	5	69	16.5	13.7	11.2	7.4	1.9	349	32	82	1.2	0.9	0.9	1.9	2.8	3.2	S
122	F	I	7	6	49	28.1	6.1	28.2	6.5	2.14	1396	19	117	1.9	1.6	1.7	2.1	2.0	2.0	S(+)
123	M	A	7	1	31	15.1	4.1	4.3	15.7	2.48	258	45	7	1.8	1.6	2.7	2.5	2.1	3.2	M
112	F	A	6	0	41	12.2	8.7	2.0	10.2	2.06	323	35	-	1.0	0.9	1.0	2.3	3.2	4.1	M
120	F	I	5	4	81	19.0	4.1	29.8	6.0	-	531	-	-	1.7	1.8	-	2.9	3.1	-	S(+)
60	F	G	10	3	85	20.3	11.2	5.1	11.1	2.15	411	32	31	3.2	2.5	2.4	2.8	2.9	2.8	M
126	M	G	6	1	32	20.7	9.7	3.6	15.2	2.24	410	37	59	1.3	1.0	1.3	1.7	2.1	2.5	M

Appendix 4 (continued)

Details of Patients Included in the Clinical Trial

b)Patients randomised to receive colloid control

Trial No.	Sex	Aet.	Stay (days)	Risk Score	Age (yrs)	WCC$_9$ (x10^9/l)	Glucose (mmol/l)	Urea (mmol/l)	PaO$_2$ (KPa)	Calcium (mmol/l)	LDH (iu/l)	Alb (g/l)	ALT (iu/l)	Alpha$_2$Macroglobulin (g/l) Day1	3	7	Alpha$_1$Antiprotease (g/l) Day1	3	7	Outcome
81	F	G	4	1	15	15.9	5.3	4.0	11.8	2.4	164	45	85	2.0	1.9	1.9	0.9	0.9	0.9	M
85	M	I	35	3	52	21.3	9.8	5.8	7.1	1.65	138	44	19	1.1	0.9	0.9	1.4	2.8	2.8	S(+)
134	F	G	6	1	49	8.9	4.6	6.2	-	2.19	236	34	145	2.2	2.5	3.5	1.8	2.4	3.4	M
125	F	I	20	1	71	12.4	6.5	8.5	9.3	2.04	354	36	8	2.1	1.7	1.7	1.5	3.1	3.3	M
131	F	G	68	1	56	10.6	9.2	7.7	11.6	2.24	363	40	55	2.5	2.6	2.6	2.0	4.6	4.2	S
127	M	G	8	5	83	12.6	8.6	5.7	7.9	1.95	157	31	232	1.6	1.7	2.0	2.1	2.4	1.9	M
115	M	O	8	1	13	8.0	5.7	4.0	6.9	2.34	439	42	17	2.9	2.6	3.1	1.9	2.4	2.1	M
84	M	G	7	4	79	25.5	7.4	7.6	8.2	2.15	97	31	274	1.8	2.2	2.2	1.0	2.2	2.1	M
88	F	G	9	2	73	8.3	6.7	5.1	10.8	2.55	225	33	108	1.4	1.3	1.3	1.9	2.1	2.1	M
99	M	I	20	2	77	19.8	9.4	7.0	11.2	2.24	457	41	21	-	1.4	1.3	-	2.6	2.5	S
109	F	G	9	3	76	18.0	10.8	11.3	11.3	2.15	419	34	61	2.0	1.4	-	3.4	1.7	-	M
79	F	G	30	3	85	21.8	9.1	7.4	10.9	2.25	200	39	222	1.6	1.6	2.0	2.3	2.1	2.7	S
90	M	G	14	1	48	20.9	6.9	3.1	15.9	2.23	175	34	54	1.4	2.3	1.4	2.0	3.4	2.0	M
117	F	I	31	2	69	3.3	9.5	5.0	10.8	2.37	1697	-	-	2.1	1.3	1.6	2.7	3.1	3.4	M
135	F	I	18	3	72	26.1	9.9	8.0	7.7	2.25	555	46	19	1.0	1.0	-	2.8	3.2	-	S
136	M	G	13	3	69	10.8	10.7	8.1	-	2.24	238	33	122	1.7	1.6	1.6	2.9	3.1	3.5	M
137	F	G	10	1	38	12.5	3.6	6.9	9.8	1.85	446	32	77	1.7	0.9	0.9	1.7	2.9	2.1	M
140	M	O	3	1	59	13.5	4.8	5.2	9.2	2.4	189	38	12	1.7	1.7	1.7	-	2.1	1.9	M
141	M	G	46	1	48	15.1	7.6	4.6	9.2	2.24	160	34	17	0.8	0.9	0.9	1.6	1.7	2.1	S
142	M	G	31	5	86	9.9	10.1	10.6	7.0	2.23	744	42	232	2.9	1.7	2.7	1.7	2.6	1.6	S(+)
146	M	I	5	0	17	9.2	6.3	4.7	13.5	2.35	242	-	-	2.4	2.2	2.9	2.1	2.3	2.9	M
147	M	A	11	4	54	16.4	6.5	14.4	11.0	2.1	1701	29	642	2.0	1.8	1.8	2.7	2.5	2.9	S(+)
148	F	A	6	3	28	21.2	11.9	3.0	14.5	1.95	226	36	32	1.4	1.5	1.9	1.2	2.3	2.4	M
150	F	G	18	4	36	27.2	13.1	6.1	9.6	1.15	-	34	171	-	1.0	1.2	-	3.6	4.6	M
152	F	G	8	4	66	27.1	11.3	6.8	13.4	2.25	126	31	34	1.9	1.6	1.9	2.6	2.4	2.1	M
161	M	A	5	0	52	14.3	6.4	3.1	12.1	2.12	354	40	21	1.4	1.1	-	1.6	2.3	-	M
162	F	G	9	2	30	15.1	6.6	3.5	10.8	2.25	364	35	130	1.5	1.6	1.7	2.5	3.4	2.8	M
175	F	O	8	1	49	22.1	-	4.8	11.5	2.38	282	36	23	1.5	1.0	1.0	1.4	1.9	2.4	M
176	M	O	5	1	59	13.8	6.1	3.3	-	2.3	203	-	-	1.6	1.6	-	2.0	2.0	-	M
178	M	A	10	0	43	8.4	7.8	7.3	14.5	2.28	301	48	33	2.1	1.8	2.2	1.7	1.7	1.9	M
173	F	G	61	6	63	32.0	25.7	61.0	9.4	1.94	312	16	17	1.0	-	0.9	3.5	-	2.3	S
182	F	G	10	2	48	13.2	6.3	4.1	11.4	2.42	725	46	864	1.2	1.1	0.9	1.3	1.8	4.5	M
184	F	I	5	4	81	21.2	14.2	5.2	6.8	2.47	367	36	90	2.2	1.8	1.4	1.8	3.4	3.9	S(+)
185	M	I	8	0	47	9.5	6.8	5.9	17.8	2.37	285	43	14	1.6	1.5	1.7	1.5	1.8	1.6	M
186	F	O	6	1	40	12.9	6.7	4.0	10.9	2.39	341	42	115	1.4	1.1	1.3	1.4	1.8	1.7	M
165	M	A	16	1	40	15.0	6.1	4.9	11.9	2.23	-	37	25	1.2	1.1	1.2	1.3	2.8	2.8	M
156	M	A	17	0	55	13.7	6.3	4.6	8.3	2.1	351	-	30	-	-	1.1	-	-	3.4	M
155	M	G	11	2	78	12.1	12.4	7.8	10.8	2.0	144	32	10	1.4	1.5	-	2.1	2.2	-	M
153	F	G	9	5	71	16.9	8.5	6.8	6.7	2.27	610	37	413	2.2	-	1.9	1.8	-	2.3	M
158	F	G	23	5	82	38.3	14.9	10.3	5.9	1.66	408	34	43	2.5	1.5	1.0	2.1	3.2	2.3	S(+)
167	M	G	10	3	62	17.2	6.5	6.9	9.5	2.4	276	36	104	1.5	1.2	1.4	2.0	2.4	2.6	M
171	M	O	4	1	59	12.9	4.8	7.0	-	2.36	248	44	7	1.9	1.6	-	1.8	1.6	-	M
59	M	A	21	3	49	15.9	9.3	8.2	6.9	1.79	568	32	14	1.1	0.8	0.8	1.9	3.6	3.0	S
190	M	G	6	2	70	11.6	8.0	5.5	11.4	2.11	-	-	144	1.8	1.5	1.9	2.9	2.5	2.9	M
191	F	G	54	3	84	19.3	10.1	11.6	9.2	2.27	412	37	18	1.8	1.2	1.2	1.6	2.7	2.9	S
193	M	G	11	4	83	16.9	4.3	6.3	7.7	1.98	275	33	85	1.2	1.2	1.1	2.3	2.5	1.9	M
189	M	A	15	1	49	17.3	8.2	2.7	9.6	2.33	352	37	25	1.3	1.2	1.2	2.5	2.9	3.1	M
170	M	G	15	2	73	12.8	8.6	5.6	8.6	2.25	467	38	267	2.5	2.1	-	3.0	2.7	-	M
195	M	A	5	1	37	11.3	8.7	8.3	11.5	2.1	856	-	-	2.2	1.8	-	1.3	2.9	-	M

BIBLIOGRAPHY

Abildgaard U. Inhibition of the thrombin-fibrinogen reaction by heparin and purified cofactor. Scand J Haemat 1968,5:440-53.

Abildgaard U. ed. Collen D, Wirman B, Verstraete M. Physiological Inhibitions of Blood Coagulation and Fibrinolysis. Amsterdam,Elsevier 1979,pp239-41.

Acosta JM. Diagnosis of gallstone pancreatitis. Lancet 1975,1: 919.

Acosta JM, Ledesma CL. Gallstone migration as a cause of acute pancreatitis. N Engl J Med 1974,290:484-7.

Acosta JM, Pellegrini CA, Skinner DB. Etiology and pathogenesis of acute biliary pancreatitis. Surgery 1980, 88:118-25.

Acosta JM, Rossi R, Galli OM, Pellegrini CA, Skinner DB. Early surgery for acute gallstone pancreatitis: evaluation of a systematic approach. Surgery 1978,83:367-70.

Adams JT, Libertino JA, Schwartz SI. Significance of elevated serum amylase. Surgery 1968, 63:877-84.

Adham NF, Dyce B, Haverback BJ. Trypsin-binding $alpha_2$ macroglobulin in patients with acute pancreatitis. Gastroenterology 1972,61:365-72.

Adler G, Rauschu, Weidenbach F, Arnold R, Kern HF. General and selective inhibition of pancreatic enzyme discharge using a proteinase inhibitor (Foy-305) Klin Wochenschr 1984;62:406-11.

Aho HJ, Koskensalo SM-L, Nevalainen TJ. Experimental pancreatitis in the rat. Sodium taurocholate-induced acute haemorrhagic pancreatitis. Scand J Gastroent 1980,15:411-16.

Alexandre J-H, Guerrieri MT. Role of total pancreatectomy in the treatment of necrotising pancreatitis. World J Surg 1981,5:369-77.

Altemeier WA, Alexander JW. Pancreatic abscess. A study of 32 cases. Arch Surg 1963,87:80-9.

Amundsen E. Proteinase inhibitors in acute pancreatitis. Acta Chir Scand 1967, Suppl 378:111-13.

Anderer FA, Hornle S. Strukturuntersuchungen am kallikrein-inaktivator aus rinderlunge. I. Molekulargewicht, endgruppenanalyse und aminosaure - zusammensetzung. Z Naturforsch 1965(B) 20:457-62.

Anderer JR, Spence RAJ, Laird JD, Ferguson WR, Kennedy TL. Initial experience with Indium-III autologous leucocyte imaging in patients with acute pancreatitis. Br Med J 1983,287:637-8.

Anderson MC, Mehn WH, Method HL. An evaluation of the common

channel as a factor in pancreatic or biliary disease. Ann Surg 1960, 151:379-90.

Anderson MC, Needleman SB, Gramatica L, Toranto JR, Briggs DR. Further inquiry into the pathogenesis of acute pancreatitis. Arch Surg 1969,99:185-91.

Aoyagi T, Miyata S, Nanbo M, et al. Biological activities of leupeptins. J Antibiot (Tokyo) 1969b, 22:558-68.

Aoyagi T, Takeuchi T, Matsuzaki A, et al. Leupeptins, new protease inhibitors from actinomycetes. J Antibiot (Tokyo) 1969a,22:283-6.

Aranha GV, Prinz RA, Greenlee HB. Pancreatic abscess - an unresolved surgical problem. Am J Surg 1982, 144:534-8.

Archibald E. The experimental production of pancreatitis in animals as the result of common duct sphincter spasm. Surg Gynecol Obstet 1919,28:529-45.

Aronsen K-F, Ekelund G, Kindmark C-O, Laurell C-B. Sequential changes of plasma proteins after surgical trauma. Scand J Clin Lab Invest 1972, 29:Suppl 124:127-36.

Autio V, Juusela E, Lauslahti K, Markkula H, Pessi T. Resection of the pancreas for acute haemorrhagic and necrotising pancreatitis. World J Surg 1979,3:631-9.

Baden H, Jordal K, Lund F, Zachariae F. Prophylactic and curative action of Trasylol in pancreatitis. A double blind trial. Scand J Gastroenterol 1969,4:291-5.

Balldin G. On protease-antiprotease imbalance with special reference to the protective role of protease inhibitors in acute pancreatitis. Universitas Gothorum Carolina Lundensis Malmo General Hospital 1980.

Balldin G, Eddeland A, Ohlsson K. Studies on the role of the plasma protease inhibitors on C_3-activation in vitro and in acute pancreatitis. Scan J Gastroenterol 1981,16:603-9.

Balldin G, Gustafsson E-L, Ohlsson K. Influence of plasma protease inhibitors and Trasylol on trypsin-induced bradykinin-release in vitro and in vivo. Eur Surg Res 1980,12:260-9.

Balldin G, Laurell C-B, Ohlsson K. Increased catabolism of alpha macroglobulins after intravenous infusion of trypsin-alpha$_1$ antitrypsin complexes in dogs. Hoppe-Seylers Z Physiol Chem 1978a,359:699-708.

Balldin G, Ohlsson K. Demonstration of pancreatic protease-antiprotease complexes in the peritoneal fluid of patients with acute pancreatitis. Surgery 1979, 85:451-6.

Balo J, Ballon HC. Effects of retention of pancreatic secretion. Surg Gynecol Obstet 1929,67:1-13.

Banks PA. Pancreatitis. In: Topics in Gastroenterology. New York, Plenum Publishing Corporation 1979,pp 12-52.

Banks PA, McLellan PA, Gerzof SG, Splaine EF, Lintz RM, Brown ND. Mediastinal pancreatic pseudocyst. Dig Dis Sci 1984,29:664-8.

Bartholomew C, McGeeney KF, Murphy JJ, Fitzgerald O, Sankaran H. Experimental studies on the aetiology of acute scorpion pancreatitis. Br J Surg 1976,63:807-10.

Barrett AJ, Brown MA, Sayers CA. The electrophoretically 'slow' and 'fast' forms of the alpha$_2$ macroglobulin molecule. Biochem J 1979, 181:401-18.

Barrett AJ, Starkey PM. The interaction of alpha$_2$-macroglobulin with proteinases. Biochem J 1973, 133:709-24.

Barrett AJ, Starkey PM, Munn EA. Ed. Fritz H, Tscherche H, Greene LJ, Truscheit E. Proc. 5th Bayer Symp. Proteinase inhibitors. Berlin/Heidelberg/New York, Springer, 1974 pp 72-7.

Bawnik JB, Orda R, Wiznitzer T. Acute necrotizing pancreatitis. An experimental model. Dig Dis Sci 1974, 19:1143-7.

Baxter JN, Jenkins SA, Day DW, et al. Effects of somatostatin and a long-acting somatostatin analogue on the prevention and treatment of experimentally induced acute pancreatitis in the rat. Br J Surg 1985a, 72: 382-5.

Baxter JN, Jenkins SA, Day DW, et al. Effects of somatostatin analogue (SMS 201-995) on hepatic and splenic reticuloendothelial function in the rat. Br J Surg 1985b, 72: 1005-8.

Baxter JN, Jenkins SA, Mackie CR. Somatostatin in pancreatitis, Authors reply to letter. Br J Surg 1986, 73:242.

Beatty K, Travis J, Bieth J. The effect of alpha$_2$ macroglobulin on the interaction of alpha$_1$ proteinase inhibitor with porcine trypsin. Biochim Biophys Acta 1982, 704:221-6.

Beck IT, Kahn DS, Solymer J, Mckenna D, Zylberszac B. The role of pancreatic enzymes in the pathogenesis of acute pancreatitis. Gastroenterology 1964, 46:531-42.

Becker JA, Pemberton JH, DiMagno EP, Ilstrup DM, McIlrath DC, Dozois RR. Prognostic factors in pancreastic abscess. Surgery 1984, 96: 455-60.

Becker WF, Pratt HS, Ganji H. Pseudocysts of the pancreas. Surg Gynecol Obstet 1968, 127:744-7.

Bedacht R. Treatment of pancreatitis with a kallikrein inactivator. Argneim Forsch 1958, 12:371-6.

Beger HG, Buchler M, Bittner R, Block S, Roscher R. Necrosectomy and postoperative local lavage in patients with necrotizing pancreatitis - results of a prospective clinical trial. Digestion 1986,35:8.

Beller FK, Epstein MD, Kaller H. Distribution, half life and placental transfer of the protease inhibitor Trasylol. Thromb Diath Haemorrh 1966, 16: 302-10.

Bernard C. Memoire sur le pancreas et sur le role du sac pancreatique. CR Acad Sci 1856, Vol 1: Paris, JB Balliere.

Berry AR, Taylor TV, Davies GC. Pulmonary function and fibrinogen metabolism in acute pancreatitis. Br J Surg 1981, 68:870-3.

Berry AR, Taylor TV, Davies GC. Diagnostic tests and prognostic indicators in acute pancreatitis. J R Coll Surg Edinb 1982, 27:345-52.

Bess MA, Edis AJ, Van Heerden JA. Hyperparathyroidism and pancreatitis : Chance or a causal association. J Am Med Assoc 1980, 243:246-7.

Bezman-Tarcher A. Method for continuous intravenous infusion of large amounts of oleic acid into rats. J Lipid Res 1969, 10:197-206.

Bivens BA, Sacatelo CR, Chung VP, Brady P. Hemosuccus pancreaticus (Hemoductal pancreatitis) gastrointestinal haemorrhage due to rupture of a splenic artery aneurysm into the pancreatic duct. Arch Surg 1978, 113:751-3.

Blamey SL, Imrie CW, O'Neill J, Gilmour WH, Carter DC. Prognostic factors in acute pancreatitis. Gut 1984, 25: 1340-6.

Blamey SL, Osborne DH, Gilmour WH, O'Neill J, Carter DC, Imrie CW. The early identification of patients with gallstone pancreatitis using clinical and biochemical factors only. Ann Surg 1983, 198:574-8.

Blatrix C, Amouch P, Drouet J, Steinbuch M. Study on the plasmatic elimination of the alpha 2-macroglobulin/proteinase complexes. Pathol Biol 1973,21:11-14.

Block S, Maier W, Bittner R, Buchler M, Malfertheiner P, Beger HG. Identification of pancreas necrosis in severe acute pancreatitis : imaging procedures versus clinical staging. Gut 1986, 27: 1035-42.

Boggs BR, Potts JR III, Postier RG. Five year experience with pancreatic pseudocysts. Am J Surg 1982, 144:685-8.

Bolooki M, Jaffe B, Gliedman ML. Pancreatic abscesses and lesser omental sac collections. Surg Gynecol Obstet 1968, 126:1301-8.

Bonfils S, Richir CL, Potet F, Liefooghe G, Lambling A. "Ulcere" experimental de contrainte du rat blanc. II - Anatomo-pathologie des lesions gastriques et de differantes lesions viscerales. Evolution des ulceration apres cessation de la contrainte. Rev Fr d'Etudes Clin Biol 1959,4:888-94.

Borgstrom A, Ohlsson K. Immunoreactive trypsin is serum and peritoneal fluid in acute pancreatitis. Hoppe-Seylers Z Physiol

Chem 1978,359:677-81.

Borisova MA, Krylova AM, Rybka IN, Prudnikov BA, Solomaschchenko BS. Pancreatitis as a rare complication of typhoid. Vrach Delo 1977,9:140-2.

Borth W, Menzel EJ, Salzer M, Steffen C. Human serum inhibitors of collagenase as revealed by preparative isoelectric focusing. Clin Chim Acta 1981, 117:219-25.

Bourke JB. Variations in annual incidence of primary pancreatitis in Nottingham 1969-1974. Lancet 1975,2:967-9.

Boutelier PH, Edelman G. Tactique chirurgicale dans les pancreatites aigues necrosantes. Plaidoyer en faveour des sequesterectomies. Ann Chir 1971,26:249-51.

Bradley EL 3rd, Clements JL Jr. Implications of diagnostic ultrasound in the surgical management of pancreatic pseudocysts. Am J Surg 1974, 127:163-73.

Bradley EL 3rd, Clements JL Jr, Gonzalez AC. The natural history of pancreatic pseudocysts: A unified concept of management. Am J Surg 1979,137:135-40.

Bradley EL 3rd, Fulenwider JT. Open treatment of pancreatic abscess. Surg Gynecol Obstet 1984,159:509-13.

Bradley EL 3rd, Gonzalez AC, Clements JL Jr. Acute pancreatic pseudocysts: incidence and implications. Ann Surg 1976,184:734-7.

Bradley JA, Bradley P, McMahon MJ. Diagnostic peritoneal lavage in acute pancreatitis - the value of microscopy of the lavage fluid. Br J Surg 1981,68:245-6.

Braganza JM, Jolley JE, Lee WR. Occupational chemicals and pancreatitis : A link ? Int J Pancreatology 1986,1:9-19.

Braunsteiner H, Herbst M, Sailer S, Sandhofer F. Essentielle hyperlipaemie und akute pankreatitis. Schweiz Med Wochenschr 1967,97:698-702.

Brodrick JW, Geokas MC, Largman C, Fassett M, Johnson JH. Molecular forms of immuno-reactive pancreatic cationic trypsin in pancreatitis patient sera. Am J Physiol 1979,237:474-80.

Brodrick JW, Largman C, Ray SB, Geokas MC. Probolysis of parathyroid hormone in vitro by sera from acute pancreatitis patients. Proc Soc Exp Biol Med 1981, 167:588-96.

Broe RJ, Zinner MJ, Cameron JL. Cimetidine and acute pancreatitis. Surg Gynecol Obstet 1982,154:13-16.

Brunschwig A. Pancreatic physiology in the light of recent surgical experiences. Surg Gynecol Obstet 1949,88:266-7.

Brzek V, Bartos V. Therapeutic effect of the prolonged thoracic duct lymph fistula in patients with acute pancreatitis.

Digestion 1969,2:43-50.

Buch A, Buch J, Carlsen A, Schmidt A. Hyperlipidaemia and pancreatitis. World J Surg 1980,4:307-14.

Bundy HF, Mehl JW. Trypsin inhibitors of human serum. J Biol Chem 1959, 234:1124-8.

Burnett W, Ness TD. Serum amylase and acute abdominal disease. Br Med J 1955,24:770-2.

Butkiewicz T. Die gallige bauchfellentzundung ohne perforation der gallenwege. Arch Klin Chir 1936,185:55-140.

Camer SJ, Tan EG, Warren KW et al. Pancreatic abscess. A critical analysis of 113 cases. Am J Surg 1975,129:426-31.

Cameron AL, Noble JF. Reflux of bile up the duct of Wirsung caused by an impacted biliary calculus. J Am Med Assoc 1924,82:1410-4.

Cameron JL, Anderson RP, Zuidema GD. Pancreatic ascites. Surg Gynecol Obstet 1967, 125:328-32.

Cameron JL, Capuzzi DM, Zinderman GD, Margolis S. Acute pancreatitis with hyperlipemia. Ann Surg 1973,177:483-99.

Cameron JL, Capuzzi DM, Zuideme GD, Margolis S. Acute pancreatitis with hyperlipemia. Evidence for a persistent defect in lipid metabolism. Am J Med 1974,56:482-7.

Cannon RH, Turner MD. Retardation of the progress of experimental pancreatitis with trypsin inhibitors. Surg Forum 1961,12:367-9.

Carrell RW, Jeppsson JO, Laurell CB et al.Structure and variations of human $alpha_1$ antitrypsin.Nature 1982,298:329-34.

Cevese PG, Vecchioni R, Cordiano C, et al. Surgical techniques for operations on the thoracic duct. Surg Gynecol Obstet 1975,140:957-65.

Chetty U, Gilmour HM, Taylor TV. Experimental acute pancreatitis in the rat - a new model. Gut 1980, 21:115-17.

Chiari H. Uber die selbstverdaunung des menschlichen pankreas. Z Heilk 1896,17:69-96.

Chodkowski 1936 (Quoted by Butkiewicz).

Classen. Die Krankh d. Bauchspeicheider 1842. Cited by Fitz 1889.

Coan MH, Travis J. Interactions of human pancreatic proteinases with naturally occurring proteinase inhibitors. In: Proceedings of the International Research Conference on Proteinase Inhibitors. Berlin/New York. Walter de Gruyter 1971,pp 294-8.

Cogbill CL, Song KT. Acute pancreatitis. Arch Surg 1970,100:673-6.

Condon JR, Knight M, Day JL. Glucagon therapy in acute pancreatitis. Br J Surg 1973,60:509-11.

Condon RE, Woods JH, Poulin TL, Wagner WG, Pissiotis CA. Experimental pancreatitis treated with glucagon or lactated ringer solution. Arch Surg 1974,109:154-8.

Cooney DR, Grosfeld JL. Operative management of pancreatic pseudocysts in infants and children : a review of 75 cases. Ann Surg 1975,182:590-6.

Cooper MJ, Williamson RCN, Pollock AV. The role of peritoneal lavage in the prediction and treatment of severe acute pancreatitis. Ann R Coll Surg Engl 1982,64:422-7.

Cooperbery P, Burhenne HG. Real-time ultrasonography. Diagnostic technique of choice in calculous gallbladder disease. N Engl J Med 1980,302:1272-9.

Cooperman AM. Are pancreatic pseudocysts overtreated ? Surg Gynecol Obstet 1980,151:96.

Coppa GF, Le Fleur R, Ranson JHC. The role of chiba-needle cholangiography in the diagnosis of possible acute pancreatitis with cholelithiasis. Ann Surg 1981,193:393-8.

Corfield AP, Cooper MJ, Williamson RCN. Acute pancreatitis: A lethal disease of increasing incidence. Gut 1985,26:724-9.

Corrado AP, Reis ML, Carvalho IF, Diniz CR. Bradykininogen and bradykinin in the cardiovascular shock produced by proteolytic enzymes. Biochem Pharmacol 1966,15:959-70.

Cotlar AM, Hudson TL, Kaplan MH, Cohn I Jr. Experimental haemorrhagic pancreatitis produced by staphylococcal toxin. Surgery 1960,47:587-93.

Cotton PB. Congenital anomaly of pancreas divisum as cause of obstructive pain and pancreatitis. Gut 1980,21:105-14.

Cotton PB. Pancreatic orifice sphincterotomy; experience from 15 centres. Gut 1983,24:A967.

Cowan A, Doxey JC, Harry EJR. The animal pharmacology of buprenorphine, an oripavine analgesic agent. Br J Pharmacol 1977,60:547-54.

Craig RM, Doral E, Myles L. The use of ampicillin in acute pancreatitis. Ann Intern Med 1975,83:831-2.

Crass RA, Meyer AA, Jeffrey RB, et al. Pancreatic abscess: Impact of computerized tomography on early diagnosis and surgery. Am J Surg 1985,150:127-30.

Creutzfeldt W, Lankisch PG, Folsch UR. Hemmung der sekretin - und cholecystokinin - pancreozymin - indozierten seft - und enzymsekretion der pankreas und der gallenblasen kontraktion beim menschen durch somatostatin. Dtsch Med Wochenschr

1975,100:1135-8.

Creutzfeldt W, Schmidt H. Aetiology and pathogenesis of pancreatitis (current concepts). Scand J Gastroenterol 1970,5,Suppl 6:47-62.

Cullen TS. A new sign in ruptured extrauterine pregnancy. Am J Obstet Gynecol 1918,78:457-60.

Cuschieri A, Wood RAB, Cumming JRG, Meehan SE, Mackie CR. Treatment of acute pancreatitis with fresh frozen plasma. Br J Surg 1983,70:710-12.

Dalton RG, Touraine JL, Wilson TR. A simple technique for continuous intravenous infusion in rats. J Lab Clin Med 1969,74:813-15.

Dammann HG, Grabbe E, Runge M. Computed tomography and acute pancreatitis. Lancet 1980,2:860.

Dammann HG, Dopner M, von Wichert P, Harders H, Hornborstel H. Gallstones and acute pancreatitis. Lancet 1980,1:308.

Dardinsky VJ. The anatomy of the major duodenal papilla of man, with special reference to its musculature. J Anat 1935,69:469-78.

Das S. Pancreatitis in children associated with round worms. Indian J Pediatr 1977,14:81-3.

Davidson ED, Bradley EL 3rd. Marsupialization in the treatment of pancreatic abscess. Surgery 1981,89:252-6.

De Bolla AR, Obeid ML. Mortality in acute pancreatitis. Ann Roy Coll Surg Eng 1984,66:184-6.

de Klimko D. Surgical treatment of acute pancreatitis. Surg Gynecol Obstet 1936,63:89-95.

Delcourt A. Controversies in the biological diagnosis of acute pancreatitis.ed. Hollender LF. Controversies in Acute Pancreatitis. New York, Springer-Verlag 1982 pp 38-44.

Delmotte JS, Pommelet P, Houcke P et al. Initial duodenoscopic sphincterotomy in patients with acute cholangitis or pancreatitis complicating biliary stones. Gastroenterology 1982,82:1042.

Dickson AP, Imrie CW. The incidence and prognosis of body wall ecchymosis in acute pancreatitis. Surg Gynecol Obstet 1984,159:343-7.

Dickson AP, O'Neill J, Imrie CW. Hyperlipaemia,alcohol abuse and acute pancreatitis. Br J Surg 1984,71:685-8.

Dickson AP, Foulis AK, Imrie CW. Histology and bacteriology of closed duodenal loop models of experimental acute pancreatitis in the rat. Digestion 1986,34:15-21.

Diwok K, Leithauser W, Nowotny P. Zur wirkung von contrykal PAMBA

und heparin auf den verlauf einer experimentallen pankreatitis bei ratten. Dtsch Z Verdau Stoffwechselkr 1971,31:113-21.

Dixon JA, Hillam JD. Surgical treatment of biliary tract disease associated with acute pancreatitis. Am J Surg 1970,120:371-5.

Domschke S, Domschke W, Rosch W et al. Inhibition by somatostatin of secretin-stimulated pancreatic secretion in man: a study with pure pancreatic juice. Scand J Gastroenterol 1977,12:59-63.

Donnelly PK, Shenton BK, Alomran AM, Francis DM, Proud G, Taylor RM. The role of protease in immunoregulation. Br J Surg 1983,70:614-22.

Donnelly PK, Shenton BK, Proud G. et al. Plasma protease inhibitors: a therapeutic potential ? Ann Med Interne 1984,135:13.

Donohue PE, Nymus LM, Baker RI. Pancreatic abscess after alcoholic pancreatitis. Arch Surg 1980,115:905-9.

Dooner HP, Aliaga C. Painless acute necrotic pancreatitis. Arch Intern Med 1965,116:828-31.

Doubilet H, Mulholland JH. Recurrent acute pancreatitis : observations on etiology and surgical treatment. Am Surg 1948,128:609-38.

Dragstedt LR, Haymond HE, Ellis JC. Pathogenesis of acute pancreatitis (acute pancreatic necrosis). Arch Surg 1934,28:232-91.

Dreiling DA, Leichtling JJ, Greenstein AJ. Trasylol revisited: the value of proteolytic inhibitors in the therapy of pancreatitis. Mt Sinai J Med 1976,43:409-14.

Dreiling DA, Richman A, Fradkin NF. The role of alcohol in the aetiology of pancreatitis : study of effect of intravenous ethyl alcohol on the external secretion of the pancreas. Gastroenterology 1952,20:636-46.

Dritsas KG. Near total pancreatectomy in the treatment of acute haemorrhagic pancreatitis. Am Surg 1976,42:44-7.

Dunn JT, Spiro RG. The alpha$_2$ macroglobulin of human plasma. II Studies on the carbohydrate units. J Biol Chem 1967,242:5556-63.

Dum J, Herz A. In vivo receptor binding of the opiate partial agonist buprenorphine, correlated with its agonistic and antagonistic actions. Br J Pharmacol 1981,74:627-33.

Dum J, Blasig J, Herz A. Buprenorphine: Demonstration of physical dependence liability. Eur J Pharmacol 1981,70:293-300.

Durr HK. Alkoholschadigung des pankreas. Internist (Berlin) 1978,19:123-30.

Durr HK. Acute Pancreatitis. ed.Howat HT, Sarles H. The Exocrine Pancreas. London, Saunders 1979,pp352-401.

Dyck WP, Rudick J, Hoexter B, Janowitz HD. Influence of glucagon on pancreatic exocrine secretion. Gastroenterology 1969,56:531-7.

Dyck WP, Texter EC Jr, Lasater JM, Hightower NC Jr. Influence of glucagon on pancreatic exocrine secretion in man. Gastroenterology 1970,58:532-9.

Eddeland A, Ohlsson K. The elimination in dogs of trypsin-alpha-macroglobulin complexes inactivated by the Kazal or the Kunitz inhibitor. Hoppe-Seyler's Z Physiol Chem 1978a,359:379-84.

Eddeland A, Ohlsson K. A radioimmunoassay for measurement of human pancreatic secretory trypsin inhibitor in different body fluids. Hoppe-Seyler's Z Physiol Chem 1978b,359:671-5.

Edelman G, Boutelier P. Le traitement des pancreatites aigues necrosantes par l'ablation chirurgicale precoce de portions necrosees. Chirurgie 1974,100:155-9.

Edmonds CJ, Thompson BD. A method allowing intravenous infusion of unrestrained rats for several weeks. J Physiol (Lond) 1970,207:41-2P.

Eeftinck Schattenkerk M, De Vries JE, Bruining HA, Eggink WF, Obertop H. Surgical treatment of pancreatic pseudocysts. Br J Surg 1982,69:593-4.

Efron G. The natural history of pancreatitis. Br J Surg 1966,53:702-6.

Egdahl A. A review of 105 reported cases of acute pancreatitis with a special reference for aetiology : with report of two cases. Bull John Hopkins Hospital 1907,18:130-6.

Eggink WF, Schattenkerk ME, Obertop H, van der Ven WJ, Bruining HA. The role of early surgery in the treatment of acute haemorrhagic pancreatitis (AHNP). Neth J Surg 1984,36:6-9.

Elfstrom J. The timing of cholecystectomy in patients with gallstone pancreatitis. Acta Chir Scand 1978,144:487-90.

Elias E, Redshaw M, Wood T. Diagnostic importance of changes in circulating concentrations of immunoreactive trypsin. Lancet 1977,2:66-8.

Elliott DW. Treatment of acute pancreatitis with albumin and whole blood. Arch Surg 1957,75:573-80.

Elliot DW. Appraisal of the usefulness of various experimental models for the study of acute pancreatitis. ed. Beck IT, Sinclair TJ. The Exocrine Pancreas. London, Churchill 1971,pp86-104.

Elliot DW, Williams RD. A re-evaluation of serum amylase determinations. Arch Surg 1961,83:130-7.

Elliot DW, Williams RD, Zollinger RM. Alterations in pancreatic resistance to bile in the pathogenesis of acute pancreatitis. Ann Surg 1957,146:669-81.

Elliot DW, Zollinger RM, Moore R, Ellison EH. The use of serum albumin in the management of acute pancreatitis. Gastroenterology 1955,28:563-87.

Elman R. Acute interstitial pancreatitis. Surg Gynecol Obstet 1933,57:291-309.

Elman R, Arneson N, Graham EA. Value of blood amylase estimations in the diagnosis of pancreatic disease; clinical study. Arch Surg 1929,19:943-67.

Elmslie RG. Aspects of the management of acute pancreatitis. Med J Aust 1967,54:211-14.

Elmslie RG, White TT, Magee DF. The significance of reflux of trypsin and bile in the pathogenesis of human pancreatitis. Br J Surg 1966,53:809-16.

Erdheim J. Zur normalen und pathologischen histologie der glandule thyroidea, parathyroidea und hypophysis. Beit Path Anat 1903,33:158.

Eriksson S, Larsson C. Purification and partial characterization of pas-positive inclusion bodies from the liver in $alpha_1$-antitrypsin deficiency. N Engl J Med 1975,292:176-80.

Esnard F, Gauthier F. Rat $alpha_1$-cysteine proteinase inhibitor. J Biol Chem 1983,258:12443-7.

Esnard F, Gutman N, El Moujahed A, Gauthier F. Rat plasma $alpha_1$ inhibitor : a member of the alpha macroglobulin family. FEBS lett 1985,182:125-9.

Ettien JT, Webster PD. The management of acute pancreatitis. Adv Intern Med 1980,25:169-98.

Evans JS. A technique for prolonged infusion of unanesthetized and relatively unrestrained rats. J Appl Physiol 1970,29:275-7.

Eve C, Robinson SR. Apparatus for continuous long-term intravenous infusion in small animals. J Lab Clin Med 1963,62:169-74.

Faber E, Popper H. Production of acute pancreatitis with ethionine and its prevention by methionine. Proc Soc Exp Biol Med 1950,74:838-40.

Fagniez PL, Bonnet F, Hannoun S, Thomsen C, Julien M, Germain A. Traitment des pancreatites aigues necrosantes par dialyse peritoneale : une etude prospective. Chirurgie 1982,108:719-23.

Farrer WH, Calkins WG. A study of amylase creatinine clearance ratio in acute pancreatitis. Gastroenterology 1976,70:883.

Fawcett HD, Lin MS, Goodwin DA. Indium-III labelled leucocyte imaging in acute pancreatitis with suspected complicating abscess. Dig Dis Sci 1979,24:872-5.

Feldman SR, Gonias SL, Pizzo SV. Model of alpha$_2$-macroglobulin structure and function. Proc Natl Acad Sci 1985,82:5700-4.

Field BE, Hepner GW, Snabit MM et al. Nasogastric suction in alcoholic pancreatitis. Dig Dis Sci 1979,24:339-44.

Finch WT, Sawyers JL, Schenker S. A prospective study to determine the efficiency of antibiotics in acute pancreatitis. Ann Surg 1976,183:667-71.

Fitz RH. Acute pancreatitis. A consideration of pancreatic haemorrhage, haemorrhagic, suppurative and gangrenous pancreatitis and of disseminated fat necrosis. The Middleton Goldsmith Lecture for 1887. Bost Med Surg J 1889,120:181-7,205-7,229-35.

Flecknell PA. The relief of pain in laboratory animals. Lab Anim 1984,18:147-60.

Folsch VR, Lankisch PG, Creutzfeldt W. Effect of somatostatin on basal and stimulated pancreatic secretion in the rat. Digestion 1978,17:194-203.

Fonseca C, Greenberg D, Rosenthall L, Arzoumanian A, Lisbona R. 99m-Tc-IDA imaging in the differential diagnosis of acute cholecystitis and acute pancreatitis. Radiology 1979,130:525-7.

Forell MM. Therapy with kallikrein and protease inhibitors. Ann NY Acad Sci 1963,104:368-75.

Foulis AK, Murray WR, Galloway D et al. Endotoxaemia and complement activation in acute pancreatitis in man. Gut 1982,23:656-61.

Francillon J, Vignal J, Grandjean JP et al. Acute necrotising pancreatitis with a report of 14 cases. Chir Gastroenterol 1974,8:53-5.

Frank MS, Weissmann HS, Chun KJ, Sugarman LA, Brandt LJ, Freeman LM. Visualisation of the biliary tract with 99m-Tc-HIDA in acute pancreatitis. Gastroenterology 1980,78:1167.

Frazer JM, Nathoo SA, Katz J, Genetta TL, Finlay TH. Plasma protein and liver mRNA levels of two closely related murine alpha$_1$-protease inhibitors during the acute phase reaction. Arch Biochem Biophys 1985,239:112-19.

Freise J. Therapie der akuten pankreatitis mit dem synthetischen proteasen und phospholipase A$_2$ inhibitor gabexat mesilat. Fortschr Med 1983,101:1432-6.

Freise J, Melzer P, Schmidt FW, Horbach L. Gabexate mesilate in the treatment of acute pancreatitis. Results of a Hannover multicentre double-blind study with 50 patients. Z Gastroenterol

1986,24:200-11.

Frey CF. Pancreatic pseudocyst - operative strategy. Ann Surg 1978,188:652-62.

Frey CF. Haemorrhagic pancreatitis. Am J Surg 1979,137:616-23.

Frey CF, Lindenauer SM, Miller ThA. Pancreatic abscess. Surg Gynecol Obstet 1979,149:722-6.

Frey CF, Stanley JC, Eckhauser F. Haemorrhage. ed. Bradley EL 3rd. Complications of Pancreatitis. Philadelphia, WB Saunders 1982, pp 96-123.

Frey EK, Kraut H, Werle E. Kallikrein (Glumorin) Stuttgart Ferd Enke 1950.

Friedreich N. ed. von Ziemssen H. Cyclopedia of the Practice of Medicine. New York, Williams Wool and Co. 1878, pp 549-630.

Fritz H, Kruck J, Russe I, Liebich HG. Immunoflourescence studies indicate that the basic trypsin-kallikrein-inhibitor of bovine organs (Trasylol) originates from mast cells. Hoppe-Seyler's Z Physiol Chem 1979,360:437-44.

Fuchs HE, Shifman MA, Pizzo SV. In vivo catabolism of $alpha_1$ proteinase inhibitor-trypsin, antithrombin III - thrombin and $alpha_2$ macroglobulin-methylamine. Biochim Biophys Acta 1982,716:151-7.

Fuller RK, Loveland JP, Frankel MH. An evaluation of the efficiency of nasogastric suction treatment in alcoholic pancreatitis. Am J Gastroenterol 1981,75:349-53.

Gabryelewicz A, Niewiarowski S, Poropowicz J, Chlebowski J. Heparin and protease inhibitors in the prevention of experimental acute pancreatic necrosis in dogs. Digestion 1969,2:7-16.

Gadacz TR, Trunkey D, Kieffler RF. Visceral vessel erosion associated with pancreatitis - Case reports and a review of the literature. Arch Surg 1978,113:1438-40.

Gamklou R, Edlund Y. Ductal factors in the pathogenesis of acute pancreatitis in the rat. Scand J Gastroenterol 1966,1:94-100.

Ganrot K. $Alpha_2$ acute phase globulin in rat serum. Purification, determination and interaction with trypsin. Biochim Biophys Acta 1973,295:245-51.

Ganrot PO. The combining ratio between trypsin and serum $alpha_2$ macroglobulin. Acta Chem Scand 1966,20:2299-300.

Ganrot PO. Interaction of plasma and trypsin with $alpha_2$ macroglobulin. Acta Chem Scand 1967a,21:602-8.

Ganrot PO. Studies on serum protease inhibitors with special reference to $alpha_2$ macroglobulin. Acta Univ Lund 1967b,Section II,No.2.

Ganrot PO. Variations of the concentration of some plasma proteins in normal adults, in pregnant women and in newborns. Scand J Clin Lab Invest 1972,29.Suppl 124:83-8.

Gauthier A, Gillet R, Di Constanzo J, Camelot G, Sarles H. Kontollierte multicentre studies fur behandlung der akunten pankreatitis mit hochdosiertein aprotinin und mit glukagon. ed.Barthedheimer H,Classen M, Ossenberg F-W. Die Behandlung der Kranken Bauchspeicheldruse,Stuttgart,Thieme Verlag 1978.

Gauthier F, Mouray H. Rat alpha$_2$ acute-phase macroglobulin. Isolation and physicochemical properties. Biochem J 1976,159:661-5.

Gebhardt C, Gall FP. Importance of peritoneal irrigation after surgical treatment of hemorrhagic necrotizing pancreatitis. World J Surg 1981,5:379-85.

Gebhardt C, Riemann JF, Lux G. The importance of ERCP for the surgical tactic in haemorrhagic necrotizing pancreatitis (preliminary report). Endoscopy 1983,15:55-8.

Geenen JE, Vennes JA, Silvis SE. Resume of a seminar on endoscopic retrograde sphincterotomy (ERS). Gastrointest Endosc 1981,27:31-8.

Geokas MC. The role of elastase in acute pancreatitis. III The destructive capacity of elastase on pancreatic tissue in vivo and in vitro. Arch Path 1968,86:135-41.

Geokas MC. Acute pancreatitis. Calif Med 1972,117:25-39.

Geokas MC, Rinderknecht H. Free proteolytic enzymes in pancreatic juice of patients with acute pancreatitis. Am J Dig Dis 1974,19:591-8.

Geokas MC, Rinderknecht H, Brodrick JW, Largman C. Studies on the ascitic fluid of acute pancreatitis in man. Dig Dis Sci 1978,23:182-8

Geokas MC, Rinderknecht H, Walberg CB, Weissman R. Methaemalbumin in the diagnosis of acute haemorrhagic pancreatitis. Ann Intern Med 1974,81:483-6.

Gillespie WJ. Observations on acute pancreatitis. A retrospective clinical study. Br J Surg 1973,60:63-5.

Gjessing J. Peritoneal dialysis in severe acute haemorrhagic pancreatitis. Acta Chir Scand 1967,133:645-7.

Gladisch R, Hofmann W, Waldherr R. Myocarditis and insulitis following Coxsackie virus infection. Z Kardiol 1976,65:837-49.

Glazer G, Bennett A. Prostaglandin release in canine acute haemorrhagic pancreatitis. Gut 1976,17:22.

Glazer G, Murphy F, Clayden GS, Lawrence RG, Craig O. Radionuclide biliary scanning in acute pancreatitis. Br J Surg 1981,68:766-70.

Goebell H. Was ist gesichert in der therapie der akuntun und chronischen pankreatitis ? Internist (Berlin) 1971,12:498-504.

Goebell H, Ammann R, Akovbiantz A et al. Calcitonin in der behandlung der akuten pankreatitis. Eine-doppel-blindstudie. 32 Tagung der Deutschen gesellschaft fur Verdauugs - und staffwechselkrankheiten,Gottingen 1977.

Goebell H, Hotz J. Die atiologie der akuten pankreatitis. ed. Forell MM, Handbuch der Inneren Midizin. Band 3, Teil 6: Pankreas. S. Auflage. Berlin, Heidelberg, New York, Springer Verlag 1976.

Goff J, Feinberg L, Brugge W. A randomised trial comparing cimetidine to nasogastric suction in acute pancreatitis. Dig Dis Sci 1982,27:1085-8.

Goldberg RC, Chaikoff IL, Dodge AH. Destruction of pancreatic acinar tissue by DL-ethionine. Proc Soc Exp Biol Med 1950,74:869-72.

Goldstein IM, Cala D, Radin A, Kaplan HB, Horn J, Ranson J. Evidence of complement catabolism in acute pancreatitis. Am J Med Sci 1978,275:257-64.

Goldstein JL, Anderson RG, Brown MS. Coated pits, coated vesicles and receptor-mediated endocytosis. Nature 1979,279:679-85.

Gonzalez LL, Jaffe MS, Wiot JF et al. Pancreatic pseudocyst : a cause of obstructive jaundice. Ann Surg 1965,161:569-76.

Goodman AJ, Bird NC, Johnson AG. Antiprotease capacity in acute pancreatitis. Br J Surg 1986,73:796-8.

Goodman AJ, Neoptolemos JP, Carr-Locke DL, Finlay DBL, Fossard DP. Detection of gallstones after acute pancreatitis.1985,26:125-32.

Greenbaum LM, Hirschkowitz A, Schoichet I. The activation of trypsinogen by cathepsin B. J Biol Chem 1959,234:2885-90.

Greenberger NJ, Hatch FT, Drummery GD, Esselbacher KJ. Pancreatitis and hyperlipaemia. Medicine 1966,45:161-74.

Grenner G, Deutsch G, Schmidtberger R, Dati F. Hochempfindlicher enzymimmunoassay zur bestimmung von human-pankreas-lipase. J Clin Chem Clin Biochem 1982,20:515-19.

Grey-Turner G. Local discolouration of the abdominal wall as a sign of acute pancreatitis. Br J Surg 1919,7:394-5.

Grozinger EH, Hollis AV, Artz CD. Experimental studies on prevention of fatal pancreatitis. J Am Med Assoc 1964,187:652-4.

Guleke N. ed. Gross O, Guleke N. Die Erkankungen des Pankreas. Berlin, Julius Springer 1924.

Hallenbeck GA, Jordan GL Jr, Kelly AH. The effect of experimentally produced pancreatitis on canine external

pancreatic secretion. Surg Gynecol Obstet 1953,96:714-23.

Halsted WS. Retrojection of bile into the pancreas, a cause of haemorrhagic pancreatitis. Bull John Hopkins Hosp 1901,12:179-82.

Hammond JB, Mann NS. Pancreatitis following the intraductal injection of partially purified enterokinase in dogs. Am J Dig Dis 1977,22:182-8.

Hansson K. Experimental and clinical studies in aetiologic role of bile reflux in acute pancreatitis. Acta Chir Scand 1967 Suppl 375:102.

Hardway RM, McKay DG. Production of acute haemorrhagic pancreatitis in dogs by means of an episode of intravascular clotting in the pancreas. Surgery 1958,45:557-61.

Harpel PC. Studies on human plasma $alpha_2$-macroglobulin-enzyme interactions. J Exp Med 1973,138:508-21.

Haverback BJ, Dyce B, Bundy HF, Edmondson HA. Trypsin, trypsinogen and trypsin inhibitors in human pancreatic juice. Mechanisms for pancreatitis associated with hyperparathyroidism. Am J Med 1969,29:421-33.

Haverback BJ, Dyce B, Bundy HF, Wirtschafter SK, Edmondson HA. Protein binding of pancreatic proteolytic enzymes. J Clin Invest 1962,41:972-80.

Hedstrom SA, Belfrage I. Acute pancreatitis in two cases of infectious mononucleosis. Scand J Infect Dis 1976,8:124-6.

Hercz A, Harpaz N. Characterization of the oligosaccharides of liver Z variant $alpha_1$-antitrypsin. Can J Biochem 1980,58:644-8.

Herman RE, Al-Jurf AS, Hoerr SO. Pancreatitis. Surgical management. Arch Surg 1974,109:298-303.

Hermon-Taylor J. An aetiological and therapeutic review of acute pancreatitis. Br J Hosp Med 1977,18:546-52.

Hermon-Taylor J, Heywood GC. A rational approach to the specific chemotherapy of pancreatitis. Scand J Gastroenterol 1986,20 Suppl 117:39-46.

Hermon-Taylor J, Magee AI, Grant DAW, Jones PA, Marshall CE, Dunham J. Cleavage of peptide hormones by $alpha_2$-macroglobulin-trypsin complex and its relation to the pathogenesis and chemotherapy of acute pancreatitis. Clin Chim Acta 1981,109:203-9.

Hess O. Pankreasnekrose und chronische pankreatitis. Med Chir 1909,19:637-7.

Hicken NF, McAllister AJ. Is reflux of bile into the pancreatic ducts a normal or abnormal physiologic process ? Am J Surg 1952,83:781-6.

Hill MC, Barkin J, Isikoff MB, Silverstein W, Kalser M. Acute pancreatitis: Clinical vs CT findings. AJR 1982,139:263-9.

Hill MC, Dach JL, Barken J, Isikoff MB, Morse B. The role of percutaneous aspiration in the diagnosis of pancreatic abscess. AJR 1984,141:1035-8.

Hiltunen J, Kaukinen S, Pessi T, Ylitalo P. The kallikrein-kinin system in operated and non-operated patients with acute pancreatitis. Acta Chir Scand 1985,151:371-5.

Hochgelerent EL, David DS. Acute pancreatitis secondary to calcium infusion in a dialysis patient. Arch Surg 1974,108:218-19.

Hoffman HL, Jacobs J, Freedlander SO. Use of crystalline soybean trypsin inhibitor in acute haemorrhagic pancreatitis in dogs. Arch Surg 1953,66:617-23.

Hollender LF, Gillet M, Kohler JJ. Akute Pankreatitis Pankreatektomie. Arch Klin Chir 1971,328:314-27.

Hollender LF, Meyer C, Kauffman JP, Keller P, Sequin J, Pagliano G. Traitment chirurgical des pancreatites aigues-hemorragiques. Etude analytique et deductions prospectives de 58 observations. J Chir (Paris) 1983,120:595-601.

Houssin D, Castaign D, Lemoine J, Bismuth H. Microlithiasis of the gallbladder. Surg Gynecol Obstet 1983,157:20-4.

Howard JM. ed. Howard JM, Jordan GL. Surgical Diseases of the Pancreas. London, Pitman Medical Publishing Co.Ltd. 1960,pp123-53.

Howard JM, Ehrlich EW. Gallstone pancreatitis : A clinical entity. Surgery 1962,51:177-84.

Howard JM, Jones RJ.Anatomy of the pancreatic ducts. Etiology of acute pancreatitis. Am J Med Sci 1947,214:617-22.

Howard JM, Jordan GL. Surgical Diseases of the Pancreas. Philadelphia. JB Lippincott Co. 1960,p94.

Howes R, Zuidema GD, Cameron JL. Evaluation of prophylactic antibiotics in acute pancreatitis. J Surg Res 1975,18:197-200.

Hubbard TB, Eilber FR, Oldroyd JJ. The retroperitoneal extension of necrotising pancreatitis. Surg Gynecol Obstet 1972,134:927-30.

Ihse I, Evander A, Gustafson I. A controlled randomised study on the value of peritoneal lavage in acute pancreatitis. (an interim report). ed. Hollender LF. Controversies in Acute Pancreatitis. Berlin. Springer-Verlag 1982,pp200-2.

Ikezawa H, Aoyagi T, Takeuchi T, Umezawa H. Effect of protease inhibitors of actinomycetes on lysosomal peptide-hydrolases from swine liver. J Antibiot 1971,24:488-90.

Imber MJ, Pizzo SV. Clearance and binding of two electrophoretic 'fast' forms of human alpha$_2$-macroglobulin. J Biol Chem

1981,256:8134-9.

Imrie CW. Peritoneal lavage in severe acute pancreatitis. Br J Surg 1985,72:677.

Imrie CW, Allam BF, Ferguson JC, Blumgart LH. Hypoalbuminaemia: The common cause of hypocalcaemia in acute pancreatitis. Ann Roy Coll Surg Eng 1978b,59:135.

Imrie CW, Benjamin IS, Ferguson JC et al. A single centre, double blind trial of trasylol therapy in primary acute pancreatitis. Br J Surg 1978a,65:337-41.

Imrie CW, Ferguson JC, Sommerville RG. Coxsackie and mumps virus infection in a prospective study of acute pancreatitis. Gut 1977,18:53-6.

Imrie CW, Whyte AS. A prospective study of acute pancreatitis. Br J Surg 1975,62:490-4.

Isaksson G, Lundquist I, Ihse I. Effects on the exocrine and endocrine pancreas of duct occlusion with two different tissue glues in the rat. Eur Surg Res 1983,15:136-44.

Ivy AC. Motor dysfunction of the biliary tract. Analytical and critical consideration. AJR 1947,57:1-11.

Iwata D, Hirado M, Niinobe M, Fujii S. Existence of two kinds of thiol protease inhibitors in rat serum. Biochem Biophys Res Commun 1982,104:1525-31.

Izquierdo R, Sandberg L, Nora MO et al. Comparative study of protease inhibitors on coagulation abnormalities in canine pancreatitis. J Surg Res 1984,36:606-13.

Jacobs ML, Duggett WM, Civetta JM et al. Acute pancreatitis : analysis of factors influencing survival. Ann Surg 1977,185:43-51.

Janigan DT, Nevalainen TJ, MacAulay MA, Vethamany VG. Foreign serum-induced pancreatitis in mice. I. A new model of acute pancreatitis. Lab Invest 1975,33:591-607.

Janku J, Koldovsky O, Malec Z. Apparatus for indicating times of death of small laboratory animals kept in tens of cages. Physiol Bohemoslov 1962,11:249-56.

Jarett L, Lacy PE. Effect of glucagon on the acinar portion of the pancreas. Endocrinology 1962,70:867-73.

Jeffrey RB, Federle MP, Cello JP, Crass RA. Early computed tomographic scanning in acute severe pancreatitis. Surg Gynecol Obstet 1982,154:170-4.

Jeppson JO, Laurell CB, Fagerhol LM. Properties of isolated human alpha$_1$-antitrypsin of Pi types M,S and Z. Eur J Biochem 1978,83:143-53.

Johnson DA, Travis J. Human alpha-1-proteinase inhibitor mechanism of action: evidence for activation by limited proteolysis. Biochem Biophys Res Commun 1976,72:33-9.

Jones JM, Creeth JM, Kekwick RA. Thiol reduction of human alpha$_2$-macroglobulin. The subunit structure. Biochem J 1972,127:187-97.

Jones PA, Hermon-Taylor J, Grant DAW. Antiproteinase chemotherapy of acute experimental pancreatitis using low molecular weight oligopeptide aldehyde leupeptin. Gut 1982b,23:939-43.

Jones PA, Hynd JW. Continuous long-term intravenous infusion in the unrestrained rat - a novel technique. Lab Anim 1981,15:29-33.

Jones PA, Rayter Z, Knight MJ, Rosswick RPR. Juxtampullary bile-filled duodenal duplication cyst : another surgically correctable cause of acute pancreatitis. J R Soc Med 1982a,75:662-4.

Jordan GL, Spjut HJ. Haemorrhagic pancreatitis. Arch Surg 1972,104:489-93.

Joyce LD, Toledo-Pereyra LG, Humphrey EW. Pancreatic pseudocyst: a clinical review. Am Surg 1979,45:453-61.

Kaller H. Tierexperimentelle unter suchungen uber die wirkungsdauer und elimination von trasylon. ed. Heinkel K, Schon H. Pathogenese, Diagnostik, Klinik und Therapie der Erkrankungen des Exocrine Pankreas. Stuttgart. Schattauer 1964,p.307.

Kamada N, Collier D.St.J. A simple and economic technique for long-term continuous intravenous infusion in the unrestrained rat. Lab Anim 1986,20:118-20.

Kaplan MH. Pathogenesis of pancreatitis : a unified concept. Int J Pancreatology 1986,1:5-8.

Kareti S, Moreton JE, Khazan N. Effects of buprenorphine, a new narcotic agonist-antagonist analgesic on the EEG, power spectrum and behaviour of the rat. Neuropharmacology 1980,19:195-201.

Karlson KB, Martin EC, Frankuchen EL, Mathern RF, Schultz RW, Casarella WJ. Percutaneous drainage of pancreatic pseudocysts and abscesses. Radiology 1982,142:619-24.

Kasal LA, Spicer DS, Brahinsky RA. Isolation of a crystalline trypsin inhibitor. J Am Chem Soc 1948,70:3034-40.

Kasper H, Sommer H. Klinik der akuten pankreatitis. ed. Forell MM. Handbuch der Inneren Medizin. Band 3. Teil 6: Pankreas. Berlin/Heidelberg/New York. S.Auflage, Springer 1976.

Katsch G. Aktive internistische therapie der akuten pankreatitis. Z Klin Med 1939,135:554-61.

Kaushik SP, Vohra R, Verma GR, Kaushik S, Sabharwal A. Pancreatic abscess : a review of seventeen cases. Br J Surg 1984,71:141-3.

Kauste A, Hockerstedt K, Ahonen J, Tervaskari H. Peritoneal

lavage as primary treatment in acute fulminating pancreatitis.
Surg Gynecol Obstet 1983,156:458-63.

Kawamura K, Kondo S, Maeda K, Umezawa H. Structures and syntheses
of leupeptins Pr-LL and Ac-LL. Chem Pharm Bull (Tokyo)
1969,17:1902-9.

Kayasseh L, Stalder GA, Ghr K, Rittman WW, Girard J. Der einfluss
von somatostatin auf die mittels sekretin oder tetmahlzeit
stimulierte exokrine pankreas-sekretion beim hund. Schweiz Med
Wochenschr 1977,107:541-2.

Keith LM, Watman RN. Blood volume deficits in acute pancreatitis.
Surg Forum 1954,5:380-4.

Kelly TR. Gallstone pancreatitis. Arch Surg 1974,109:294-7.

Kelly TR. Gallstone pancreatitis : Pathophysiology. Surgery
1976,80:488-92.

Kelly TR. Gallstone pancreatitis : The timing of surgery. Surgery
1980,88:345-50.

Kelly TR, Swaney PE. Gallstone pancreatitis : The second time
around. Surgery 1982,92:571-5.

Kenwell HN, Wels BW. Acute haemorrhagic pancreatitis. report of
11 consecutive cases treated with human serum albumin. Surg
Gynecol Obstet 1953,96:169-70.

Keynes WM. Experimental acute pancreatitis. ed. Keynes WM, Keith
RG. The Pancreas. London, William Heinemann 1981.

Kivilaakso E, Lempinen M, Makelainen A, Nikki P, Scroder T.
Pancreatic resection versus peritoneal lavage for acute
fulminating pancreatitis. A randomised prospective study. Ann
Surg 1984,199:426-31.

Kivilaakso E, Fraki O, Nikki P, Lempinen M. Resection of the
pancreas for acute fulminating pancreatitis. Surg Gynecol Obstet
1981,152:493-8.

Klebs E. Handb d Path Anatomie 1870,12:553. Cited by Fitz 1889.

Knight MJ, Condon JR, Day JL. Possible role of glucagon in the
pathogenesis of acute pancreatitis. Lancet 1972,1:1097-9.

Knight MJ, Condon JR, Smith R. Possible use of Glucagon in the
treatment of pancreatitis. Br Med J 1971,2:440-2.

Koerte W. Die chirugischen krankheiten und die verletzungen des
pankreas. Stuttgart, F.Enke 1898,p.175.

Koj A, Dubin A, Kosperczyk H, Bereta J, Gordon AH. Biochem J
1982,206:545-53.

Kolars JC, Ellis CJ, Levitt MD. Comparison of serum amylase,
pancreatic isoamylase and lipase in patients with

hyperamylasemia. Dig Dis Sci 1984,29:289-93.

Komarov BD, Shimanko II, Milovanov YS, Musselius SG, Shirikova MN. Thoracic duct drainage and lymph reinfusion in patients with destructive pancreatitis and diffuse peritonitis. Khirurgiia (Mosk) 1983,4:52-8.

Kondo S, Kawamura K, Iwanaga J, et al. Isolation and characterization of leupeptins produced by actinomycetes. Chem Pharm Bull (Tokyo) 1969,17:1896-901.

Kontinnen YP. Epsilon-aminocaproic acid in treatment of acute pancreatitis. Scand J Gastroenterol 1971,6:715-18.

Korte. Die chirugische behandlung de akuten pankreatitis. Arch F Klin Chir 1911,96:557-615.

Koszarska J, Gruszchzynska M, Pilarska K. Lambliasis as a cause of acute pancreatitis. Wiad Lek 1977,30:875-7.

Kraut H, Frey EK, Werle E. Uber die inaktivierung des kallikreins. Hoppe-Seyler's Z Physiol Chem 1930,192:1-21.

Krook PM, Allen FH, Bush WH Jr, Malmer G, MacLean MD. Comparison of real-time cholecystosonography and oral cholecystography. Radiology 1980,135:145-8.

Kuehn L, Rutschmann M, Dahlmann B, Reinauer H. Proteinase inhibitors in rat serum: purification and characterization of three functionally distinct trypsin inhibitors. Biochem J 1984,218:953-9.

Kummerle F, Neher M, Schonborn H, Mangold G. Vortzeitige operation bei akuten hemorrhagisch-nekrotisierender pankreatitis. Dtsch Med Wochenschr 1975,100:2241-2.

Kune GA. Abscesses of the pancreas. Aust NZ J Surg 1982,38:125-8.

Kune GA, King R. The late complications of acute pancreatitis. Pancreatic swelling, cyst and abscess. Med J Aust 1973,1:1241-6.

Kunitz M. Isolation of crystalline protein compound of trypsin and of soybean trypsin-inhibitor. J Gen Physiol 1947,30:311-20.

Kunitz M, Northrop JH. Isolation from beef pancreas of crystalline trypsinogen, trypsin and trypsin inhibitor, and inhibitor-trypsin compound. J Gen Physiol 1936,19:991-1007.

Kurachi K, Chandra T, Degen SJ et al. Cloning and sequence of cDNA coding for alpha$_1$-antitrypsin. Proc Natl Acad Sci USA 1981,78:6826-30.

Lando DH. Uber veranderunger des pankreas bei leberzirrhose. Z Heilk 1906,27:60.

Landsteiner K. Zur kenntniss der antifermentativen, lytischen und agglutinirenden wirkungen des blutserums und der lymphe. Zbl Bakt 1900,27:357.

Lange P, Pedersen T. Initial treatment of acute pancreatitis. Surg Gynecol Obstet 1983,157:332-4.

Lankisch PG, Goke B, Stockmann F, Otto J, Winckler K, Creutzfeldt W. Protective wirkung von gabexant-mesilat (Foy) bei akuter pankreatitis ? Z Gastroenterol 1983,21:453.

Lankisch PG, Koop H, Otto J, Oberdieck U. Evaluation of methaemalbumin in acute pancreatitis. Scand J Gastroenterol 1978,13:975-8.

Lankisch PG, Koop H, Winckler K, Folsh UR, Creutzfeldt W. Somatostatin therapy in acute experimental pancreatitis. Gut 1977a,18:713-16.

Lankisch PG, Koop H, Winckler K, Kostering H. Therapie der akuten experimentellen pankreatitis mit dem antifibrinolytikum PAMBA. Z Gastroenterol 1977b,15:722-5.

Lankisch PG, Koop H, Winckler K, Schmidt H. Continuous peritoneal dialysis as treatment of acute experimental pancreatitis in the rat. I. Effect of length and rate of survival. Dig Dis Sci 1979,24:111-16.

Lankisch PG, Winckler K, Bokermann M, Schmidt H, Creutzfeldt W. The influence of glucagon on acute experimental pancreatitis in the rat. Scand J Gastroenterol 1974,9:725-9.

Laskowski M, Kato I. Protein inhibitors of proteinases. Ann Rev Biochem 1980,49:493-626.

Lasson A, Dittman B, Ohlsson K. Influence of plasma proteinase inhibitors and aprotinin on trypsin-induced bradykinin release in vitro in man. Hoppe-Seyler's Z Physiol Chem 1983,364:1315-22.

Lasson A, Ohlsson K. An in vitro study of the influence of plasma protease inhibitors and aprotinin on trypsin-induced C3 cleavage in human serum. Biochim Biophys Acta 1982,709:227-33.

Lasson A, Ohlsson K. Protease inhibitors in acute human pancreatitis. Correlation between biochemical changes and clinical course. Scand J Gastroenterol 1984,19:779-86.

Lasson A, Ohlsson K. Consumptive coagulopathy, fibrinolysis and protease - antiprotease interactions during acute human pancreatitis. Thromb Res 1986,41:167-83.

Laurell CB. Electroimmuno assay. Scand J Clin Lab Invest 1972,29:Suppl 124:21-37.

Laurell CB, Jeppson JO. ed. Putnam FW. Plasma Proteins. Structure, Function and Genetic Control. New York, Academic Press,1975,pp.229-64.

Lawrence AM. Radioimmunoassayable glucagon levels in man. Effects of starvation, hypoglycaemia and glucose administration. Proc Natl Acad Sci USA 1966,55:316-20.

Leckie PA, Ferreira P, Debas HT. Assessment of amylase-creatinine clearance ratio in postoperative patients. Ann Surg 1980,192:195-8.

Leese T, Neoptolemos JP, Baker AR, Carr-Locke DL. Management of acute cholangitis and the impact of endoscopic sphincterotomy. Br J Surg 1986,73:988-92.

Leese T, Neoptolemos JP, Carr-Locke DL. Successes, failures, early complications and their management following endoscopic sphincterotomy: results in 394 consecutive patients from a single centre. Br J Surg 1985,72:215-19.

Leeson PM, Fourman P. Acute pancreatitis from vitamin-D poisoning in patients with parathyroid deficiency. Lancet 1966,1:1185-6.

Lemmel EM, Good RA. Continuous long-term intravenous infusion in unrestrained mice-method. J Lab Clin Med 1971,52:1011-14.

Lendrum R. Drugs and the pancreas. Adverse Drug Reaction Bulletin 1981,90:328-31.

Levant JA, Secrist DM, Resin H, Sturdevant RAL, Guth PH. Nasogastric suction in the treatment of alcoholic pancreatitis. A controlled study. JAMA 1974,229:51-2.

Levitt MD, Johnson SG, Ellis CJ, Engel RR. Influence of amylase assay technique on renal clearance of amylase - creatinine ratio. Gastroenterology 1977,72:1260-3.

Levitt MD, Rapoport M, Cooperband SR. The renal clearance of amylase in renal insufficiency, acute pancreatitis and macroamylasemia. Ann Intern Med 1969,71:919-25.

Levy L, Lepow IH. Assay and properties of serum inhibitor of C1-esterase. Proc Soc Exp Biol Med 1957,101:608-11.

Lifton LJ, Slickers KA, Pragay DA, Katz LA. Pancreatitis and lipase : a re-evaluation with five minute turbidimetric lipase determination. JAMA 1974,229:47-50.

Lium R, Maddock S. Etiology of acute pancreatitis. An experimental study. Surgery 1948,24:593-604.

Loiudice TA, Lang J, Mehta H, Banta L. Treatment of acute alcoholic pancreatitis: the roles of cimetidine and nasogastric suction. Am J Gastroenterol 1984,79:553-8.

Lombardi B, Estes LW, Longnecker DS. Acute hemorrhagic pancreatitis (massive necrosis) with fat necrosis induced in mice by DL-ethionine fed with a choline-deficient diet. Am J Path 1975,79:465-80.

Lombardi B, Rao KN. Acute haemorrhagic pancreatic necrosis in mice : effects of proteinase inhibitors on its induction. Digestion 1982,23:57-64.

Longmire WP Jr, Wallner MA. Pancreatitis occurring in heterotopic

pancreatic tissue. Surgery 1956,40:412-18.

Lutsky I, Evans WE, Kayser K. Estimation of time of death in dogs. Am J Surg 1966,111:846-50.

McClave SA, McAllister EW, Karl RC, Nord HJ. Pancreatic abscess : 10 year experience at the university of South Florida. Am J Gastroenterol 1986,81:180-4.

McCutcheon AD. Aetiological factors in pancreatitis. Lancet 1962,1:710-12.

McCutcheon AD. A fresh approach to the pathogenesis of pancreatitis. Gut 1968,9:296-310.

McCutcheon AD, Race D. Experimental pancreatitis : a possible aetiology of post-operative pancreatitis. Ann Surg 1962,155:523-31.

MacErlean DP, Bryan PJ, Murphy JJ. Pancreatic pseudocysts : management by ultrasonically guided aspiration. Gastrointest Radiol 1980,5:255-7.

McHardy G, Craighead CC, Balart L, Cadic H, LaGrange C. Pancreatitis - Intrapancreatic proteolytic trypsin activity. Evaluation of a trypsin inhibitor. JAMA 1963,183:527-9.

McIntosh DM, Penney HF. Grey-Scale ultrasonography as a screening procedure in the detection of gallbladder disease. Radiology 1980,136:725-7.

McKay AJ, Imrie CW, O'Neill J, Duncan JG. Is an early ultrasound scan of value in acute pancreatitis ? Br J Surg 1982,69:369-72.

McKay AJ, O'Neill J, Imrie CW. Pancreatitis, pregnancy and gall stones. Br J Obstet Gynaecol 1980,87:47-50.

McMahon MJ, Bowen M, Mayer AD, Cooper EH. Relation of alpha$_2$ macroglobulin and other antiproteases to the clinical features of acute pancreatitis. Am J Surg 1984,147:167-70.

McMahon MJ, Freeman R. Elevated antibodies to mycoplasma pneumoniae and acute pancreatitis - causal relationship or coincidence. Ann R Coll Surg Engl 1979,61:232.

McMahon MJ, Pickford IR. Biochemical prediction of gallstones early in an attack of acute pancreatitis. Lancet 1979,2:541-3.

McMahon MJ, Pickford IR, Playforth MJ. Early prediction of severity of acute pancreatitis using peritoneal lavage. Acta Chir Scand 1980a,146:171-5.

McMahon MJ, Playforth MJ, Pickford IR. A comparative study of methods of predicting severity of attacks of acute pancreatitis. Br J Surg 1980b,67:22-5.

McMahon MJ, Playforth MJ, Rashid SA, Cooper EH. The amylase to creatinine clearance ratio - a non specific response to acute

illness ? Br J Surg 1982,69:29-32.

McPhedran NT, Lee SB. Effect of intraduct or intravascular
norepinephrine infusion on the development of acute haemorrhagic
pancreatitis. Surgery 1963,53:490-4.

Maki M, Beller FK. Comparative studies of fibrinolytic
inhibitors in vitro. Thromb Diath Haemorrh 1966,16:668-86.

Mallory A, Kern F. Drug-induced pancreatitis : a critical review.
Gastroenterology 1980,78:813-20.

Manabe T,Steer M. Experimental acute pancreatitis in mice.
Protective effects of glucagon. Gastroenterology 1979,76:529-34.

Mann FC, Giordano AS. The bile factor in pancreatitis. Arch Surg
1923,6:1-30.

Mann NS. Effect of intraductal enterokinase, S-azacytidine and
cyclohexamide on pancreatic protein content. Am J Proctol
Gastroenterol Colon Rectal Surgery 1980,31:15,16 and 21.

Mann NS, Fadian RS, Narenderan K. Prevention of enterokinase
induced acute pancreatitis with Trasylol and soyabean trypsin
inhibitor. Gastroenterology 1977,72:1097.

Mann SK, Mann NS. Effect of chlorophyll-a, fluorouracil and
pituitrin on experimental acute pancreatitis. Arch Pathol Lab Med
1979,103:79-81.

Manson RR. Acute pancreatitis secondary to iatrogenic
hypercalcaemia. Arch Surg 1974,108:213-15.

Marks IN, Bank S, Louw JH et al. Peptic ulceration and
gastrointestinal bleeding in pancreatitis. Gut 1967,8:253-9.

Markwardt F, Kloecking HP, Landmann H. Vergleichende
untersuchungen uber antifibrinolytika. Thromb Diath
Haemorrh 1966,15:561-9.

Martin DT, Steinberg SM, Kopolovic R, Carey LC, Cloutier CT.
Crystalloid versus colloid resuscitation in experimental
hemorrhagic pancreatitis. Surg Gynecol Obstet 1984,159:445-9.

Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE. The
effect of morphine - and nalorphine-like drugs in the non-
dependent and morphine-dependent chronic spinal dog. J Pharmacol
Exp Ther 1976,197:517-32.

Maurer G. A new treatment for acute pancreatic disease :
experience with trypsin inactivator. Med Press 1961,246:287-93.

Mayo HWJ. Outlines of Human Pathology, London PH Renshaw 1836.

Mayer AD, McMahon MJ. Enzyme transfer from pancreas to plasma in
acute pancreatitis: the contribution of ascitic fluid and
lymphatic drainage of the pancreas. Gut 1985a,26:876-81.

Mayer AD, McMahon MJ. Biochemical identification of patients with gallstone associated acute pancreatitis on the day of admission to hospital. Ann Surg 1985b,201:68-75.

Mayer AD, McMahon MJ, Corfield AP et al. Controlled clinical trial of peritoneal lavage for the treatment of severe acute pancreatitis. N Engl J Med 1985,312:399-404.

Mazumber PM. A case of acute pancreatitis with methaemalbuminemia Br Med J 1961,2:1617.

Mehen H. Die bedentung der mundungsverhaltnisse von gallen - und pankreasgang fur die entstehung der gallensteine. Arch Klin Chir 1938,192:559-71.

Meltzer LE, Palmon FP, Paik YK, Custer RH. Acute pancreatitis secondary to hypercalcaemia of multiple myeloma. Ann Intern Med 1962,53:1008-12.

Meinel H, Wolff E. Die behandlung der akuten pankreatitis mit dem antifibrinolytikum PAMBA. Das Deutsche Gesundheitswesen 1975,30:127-30.

Mercade S. Pancreatite aigue. Bull Mem Soc Chir (Paris) 1919,14:326-7.

Mercer LC, Saltzstein EC, Peacock JB, Dougherty SH. Early surgery for biliary pancreatitis. Am J Surg 1984,148:749-51.

Mero M, Schroder T, Tenhunen R, Lempinen M. Serum phospholipase A_2, immunoreactive trypsin and trypsin inhibitors during acute pancreatitis. Scand J Gastroenterol 1982,17:413-16.

Meshkinpour H, Molinari MD, Gardner L, Berk JE, Hoehler FK. Cimetidine in the treatment of acute alcoholic pancreatitis. A randomized double-blind study. Gastroenterology 1979,77:687-90.

Mester Z, Csanaky G, Mocsay L, Epstein O. Anatomy of duodenal papilla and genesis of pancreatic reflux. Arch Surg 1963,87:775-87.

Mikkelson O. Pancreatitis acuta : schwere falle besonders hinsichtlich ihrer konservativen behandlung. Acta Chir Scand 1934,75:373-415.

Millbourn E. On the excretory ducts to the pancreas in man, with special reference to the common bile duct and to the duodenum. Acta Anat 1950,9:1-34.

Miller TA, Lindenauer M, Frey CF et al. Pancreatic abscess. Arch Surg 1974,108:545-51.

Mixter CG, Keynes WM, Cope O. Further experience with pancreatitis as a diagnostic clue to hyperparathyoidism. New Engl J Med 1962,266:265-72.

Moossa AR. The impact of computed tomography and ultrasonography on surgical practice. Bull Am Coll Surg 1982,67:10-14.

Moossa AR. Diagnostic tests and procedures in acute pancreatitis. New Engl J Med 1984,311:639-43.

Moroi M, Yamasaki M. Mechanism of interaction of bovine trypsin with human alpha$_1$-antitrypsin. Biochim Biophys Acta 1974,359:130-41.

Mosesson MW, Amrani DL. The structure and biologic activities of plasma fibronectin. Blood 1980,56:145-58.

Moynihan Sir B. Acute pancreatitis. Ann Surg 1925,81:132-42.

MRC Multicentre Trial. Morbidity of acute pancreatitis : The effect of aprotinin and glucagon. Gut 1980,21:334-9.

MRC Working Party. MRC working party on the treatment of acute pancreatitis : death from acute pancreatitis. Lancet 1977,2:632-5

Mullertz S. Different molecular forms of plasminogen and plasmin produced by urokinase in human plasma and their relation to protease inhibitors and lysis of fibrinogen and fibrin. Biochem J 1974,143:273-83.

Mullertz S. ed. Collen D, Wirman B, Verstraete M. Physiological Inhibitors of Blood Coagulation and Fibrinolysis. Amsterdam,Elsevier,1979,pp239-41.

Muramatsu M, Fujii S. Inhibitory effect of W-guanidina acid esters on trypsin, plasmin, kallikrein and thrombin. Biochem Biophys Acta 1972,268:221-4.

Murphy D, Pack AI, Imrie CW. The mechanism of arterial hypoxia occurring in acute pancreatitis. Q J Med 1980,49:151-63.

Murray WR, Mackay C. The amylase-creatinine clearance ratio in acute pancreatitis. Br J Surg 1977,64:189-91.

Myers WK, Keefer CS. Acute pancreatic necrosis in acute and chronic alcoholism. N Engl J Med 1934,210:1376-80.

Naeije R, Salingret E, Clumeck N, De Troyer A, Devis G. Is nasogastric suction necessary in acute pancreatitis ? Br Med J 1978,2:659-60.

Nardi GL. Current concepts in therapy : pancreatitis. New Engl J Med 1963,268:1065-7.

Nash O. You Can't Get There from Here. Boston, Little, Brown and Co. 1953,p157.

Neher M, Schuster HP. Peritoneal lavage in severe pancreatitis. Hepatogastroenterology 1982,29:263-4.

Nemir P, Hoferichter J, Drabkin JL. The protective effect of proteinase inhibitor in acute necrotising pancreatitis : an experimental study. Ann Surg 1963,158:655-65.

Neoptolemos JP, Carr-Locke DL, Fraser I, Fossard DP. The

management of common bile duct calculi by endoscopic sphincterotomy in patients with gallbladder in situ. Br J Surg 1984b,71:69-71.

Neoptolemos JP, Fossard DP, Berry J. The value of IDA scanning in the diagnosis of acute gallbladder and pancreatic disease. Br J Surg 1981,68:808-9.

Neoptolemos JP, Hall AW, Finlay DF, Berry JM, Carr-Locke DL, Fossard DP. The urgent diagnosis of gallstones in acute pancreatitis : a prospective study of three methods. Br J Surg 1984a,71:230-3.

Neoptolemos JP, London N, Bailey I et al. The role of clinical and biochemical criteria and endoscopic retrograde cholangio-pancreatography in the urgent diagnosis of common bile duct stones in acute pancreatitis. Surgery 1986b,100:732-42.

Neoptolemos JP, London N, Slater ND, Carr-Locke DL, Fossard DP, Moossa AR. A prospective study of ERCP and endoscopic sphincterotomy in the diagnosis and treatment of gallstone acute pancreatitis. Arch Surg 1986a,121:697-702.

Neurath H, Walsh KA. Role of proteolytic enzymes in biological regulation (a review). Proc Natl Acad Sci USA 1976,73:3825-32.

Nevalainen TJ, Seppa A. Acute pancreatitis caused by closed duodenal loop in the rat. Scand J Gastroenterol 1975,10:521-7.

Nilehn JE, Ganrot PO. Plasmin, plasmin inhibitors and degradation products of fibrinogen in human serum during and after intravenous infusion of streptokinase. Scand J Clin Lab Invest 1967,20:113-21.

Nordback I, Auvinen O. Long-term results after pancreas resection for acute necrotising pancreatitis. Br J Surg 1985,72:687-9.

Nordback I, Auvinen O, Autio V. Clinicobiochemical factors in predicting gallstones in necrotizing pancreatitis. Surg Gynecol Obstet 1985b,161:139-41.

Nordback I, Auvinen O, Pessi T, Autio V. Determining necrosis in necrotising pancreatitis. Br J Surg 1985a,72:225-7.

Nordback I, Auvinen O, Pessi T, Autio V. Complications after pancreas resection for necrotizing pancreatitis. Acta Chir Scand 1986,152:49-54.

Northram BE, Row DS, Winstone NE. Methaemalbumin in the differential diagnosis of acute haemorrhagic and oedematous pancreatitis. Lancet 1963,1:348-52.

Norton L, Eiseman B. Near total pancreatectomy for haemorrhagic pancreatitis. Am J Surg 1974,127:191-5.

O'Connor CM, O'Donnell MD, McGeeney KF. Problems associated with the radioimmunoassay of serum trypsin. Clin Chim Acta

1981,114:29-35.

Oda K, Laskowski M Sr. Kress LF, Kowalski D. Human plasma alpha$_1$-proteinase inhibitor : temporary inhibition and multiple molecular forms of the complex with porcine trypsin. Biochem Biophys Res Commun 1977,76:1062-70.

Oda T, Yokono O, Yoshida A, Miyake K, Iino S. Successful treatment of pancreatitis with chlorophyll-a and inhibitory effects of its derivatives on trypsin and other protease activities in vitro. Gastroenterol Jpn 1971,6:49-54.

O'Donnell MD, Fitzgerald O, McGeeney KF. Differential serum amylase determination by use of an inhibitor, and design of a routine procedure. Clin Chem 1977,23:560-6.

Ofstad E. Formation and destruction of plasma kinins during experimental acute haemorrhagic pancreatitis in dogs. Scand J Gastroenterol 1970,5:Suppl 1:44.

Ogawa M, Takatsuka Y, Kitahara T, Matsuura K, Kosaki G. Radioimmunoassay of human pancreatic amylase. Methods Enzymol 1981,74:290-8.

Ohlsson K. Elimination of ^{125}I-trypsin-alpha-macroglobulin complexes from blood by reticuloendothelial cells in dog. Acta Physiol Scand 1971,81:269-72.

Ohlsson K, Eddeland A. Release of proteolytic enzymes in bile-induced pancreatitis in dogs. Gastroenterology 1975,69:668-75.

Ohlsson K, Ganrot P-O, Laurell C-B. In vivo interaction between trypsin and some plasma proteins in relation to tolerance to intravenous infusion of trypsin in dog. Acta Chir Scand 1971,137:113-21.

Ohlsson K, Laurell C-B. The disappearance of enzyme-inhibitor complexes from the circulation of man. Clin Sci Mol Med 1976,51:87-92.

Ohlsson K, Skude G. Demonstration and semiquantitative determination of complexes between various proteases and human alpha$_2$-macroglobulin. Clin Chim Acta 1976,66:1-7.

Ohlsson K, Tegner H. Experimental pancreatitis in dog. Demonstration of trypsin in ascitic fluid, lymph and plasma. Scand J Gastroenterol 1973,8:129-33.

Olsen H. Pancreatitis. A prospective clinical evaluation of 100 cases and review of the literature. Am J Dig Dis 1974,19:1077-90.

Ong GB, Lann KH, Lim TK, Wong J. Acute pancreatitis in Hong Kong. Br J Surg 1979,66:398-403.

Opie EL. The aetiology of acute haemorrhagic pancreatitis. Bull Johns Hopkins Hosp 1901a,121:182-8.

Opie EL. The relation of cholelithiasis to disease of the

pancreas and to fat necrosis. Am J Med Sci 1901b,121:27-43.

Opie EL. Disease of the Pancreas, its Cause and Nature. Philadelphia/London. JB Lippincott Co. 1903.

Opie EL. The relation of cholelithiasis to diseases of the pancreas. J Am Med Assoc 1904,43:1102-5.

Opie EL, Meakins JC. Data concerning the aetiology and pathology of haemorrhagic necrosis of the pancreas. (acute haemorrhagic pancreatitis). J Exp Med 1909,11:561-78.

Orda R, Wiznitzer T, Bawnik JB, Bubis JJ. Effect of chlorophyll-a in experimental acute pancreatitis. Isr J Med Sci 1974,10:630-6.

Orlov V, Belyakov N. Blood kallikrein kinin system in acute pancreatitis. Am J Gastroenterol 1978,70:645-8.

Osborne DH, Imrie CW, Carter DC. Biliary surgery in the same admission for gallstone - associated acute pancreatitis. Br J Surg 1981,68:758-61.

O'Sullivan JN, Nobrega FT, Morlock CG, Brown AL, Bartholomew LG. Acute and chronic pancreatitis in Rochester, Minnesota 1940-1969. Gastroenterology 1972,62:373-9.

Overy RD, Evans M, Pollock AV. Fluid sequestration : an accurate index of severity in acute pancreatitis. Br J Surg 1980,67:817.

Owens BJ, Hamit HF. Pancreatic abscess and pseudocyst. Arch Surg 1977,112:42-5.

Paloyan D, Paloyan E, Worobeck R, Ernst K, Deininger E, Harper PV. Serum glucagon levels in experimental acute pancreatitis in the dog. Surg Forum 1966,17:348-9.

Paloyan D, Simonowitz D, Skinner DB. The timing of biliary tract operations in patients with pancreatitis associated with gallstones. Surg Gynecol Obstet 1975,141:737-9.

Pannell R, Johnson D, Travis J. Isolation and properties of human plasma alpha$_1$-antiproteinase inhibitor. Biochemistry 1974,13:5439-45.

Parks DA, Bulkley GB, Granyer DN. Role of oxygen-derived free radicles in digestive tract disease. Surgery 1983,94:415-22.

Paul P, Ohnhaus EE, Hesch RD et al. Behandlung der akuten pancreatitis mit salm-calcitonin : ergebnisse einer prospektiven multicenter doppelblindstudie 32. Tagung der Deutschen gesellenschaft fur Verdauungs und Stoffwechselkrankheiten. Gottingen 1977.

Peanasky RJ, Abu-Erreish GM, Gaush CR et al. ed. Fritz H, Tschesche H, Greene LJ, Truscheit E. Proteinase Inhibitors. Berlin, Springer 1974,pp649-66.

Perman E. Surgical treatment of gastric and duodenal ulcer. Acta

Chir Scand 1935,77:Suppl 38:1-333.

Pfeffer RB, Stasior O, Hinton JW. The clinical picture of the sequential development of acute haemorrhagic pancreatitis in the dog. Surg Forum 1957,8:248-51.

Pickford IR, Blackett RL, McMahon MJ. Early assessment of severity of acute pancreatitis using peritoneal lavage. Br Med J 1977,2:1377-9.

Pierce JA, Jeppson JO, Laurell CB. Alpha$_1$ antitrypsin phenotypes determined by isoelectric focusing of the cysteine-antitrypsin mixed disulfide in serum. Anal Biochem 1976,74:227-41.

Pocock SJ. Clinical Trials : A Practical Approach. Chichester. John Wiley and Sons 1983,pp123-59.

Poggenpohl SM. Zur frage der veranderungen des pankreas bei leberzirrhose. Virchows Arch Pathol Anat 1909,196:466-501.

Pollock AJ. Acute pancreatitis. Analysis of 100 patients. Br Med J 1959,1:6-14.

Propieraitis AS, Thompson AG. The site of bradykinin release in acute experimental pancreatitis. Arch Surg 1979,98:73-6.

Pubols MH, Bartelt DC, Greene LJ. Trypsin inhibitor from human pancreas and pancreatic juice. J Biol Chem 1974,249:2235-42.

Pyrah LN, Hodgkinson A, Anderson CK. Primary hyperparathyroidism. Br J Surg 1966,53:245-316.

Quick B. Acute pancreatitis. Aust NZ J Surg 1932,2:115-32.

Radakovich M, Pearse HE, Strain WH. Study of the aetiology of acute pancreatitis. Surg Gynecol Obstet 1952,94:749-54.

Rance MJ. Animal and molecular pharmacology of mixed agonist-antagonist analgesic drugs. Br J Clin Pharmacol 1979,7:281-6s.

Ranson JHC. The timing of biliary tract surgery on acute pancreatitis. Ann Surg 1979,189:654-62.

Ranson JHC. Conservative surgical management of acute pancreatitis. World J Surg 1981,5:351-9.

Ranson JHC. Etiological and prognostic factors in human acute pancreatitis : A review. Am J Gastroenterol 1982,77:633-8.

Ranson JHC. ed. Brooks JR. Surgery of the Pancreas. Philadelphia,WB Saunders Co.1983.

Ranson JHC, Bathazar E, Caccavale R, Cooper M. Computed tomography and the prediction of pancreatic abscess in acute pancreatitis. Ann Surg 1985,201:656-63.

Ranson JHC, Lackner H, Berman IR et al. The relationship of coagulation factors to clinical complications of acute

pancreatitis. Surgery 1977,81:502-11.

Ranson JHC, Pasternack BS. Statistical methods for quantifying the severity of clinical acute pancreatitis. J Surg Res 1977,22:79-91.

Ranson JHC, Rifkind KM, Roses DF, Fink SD, Eng K, Spencer FC. Prognostic signs and the role of operative management in acute pancreatitis. Surg Gynecol Obstet 1974,139:69-81.

Ranson JHC, Rifkind KM, Turner JW. Prognostic signs and non-operative peritoneal lavage in acute pancreatitis. Surg Gynecol Obstet 1976,143:209-19.

Ranson JHC, Spencer FC. Prevention, diagnosis and treatment of pancreatic abscess. Surgery 1977,82:99-105.

Ranson JHC, Spencer FC. The role of peritoneal lavage in severe acute pancreatitis. Ann Surg 1978,187:565-75.

Rasmussen BL. Hypothermic peritoneal dialysis in the treatment of experimental haemorrhagic pancreatitis. Am J Surg 1967,114:716-21

Ravitch MM. The pancreas in infants and children. Surg Clin North Am 1975,55:377-85.

Read G, Braganza JM, Howat HT. Pancreatitis - a retrospective study. Gut 1976,17:945-52.

Reinhoff WR, Pickrell KL. Pancreatitis. An anatomic study of the pancreatic and biliary systems. Arch Surg 1945,51:205-19.

Ribacoff S, Hurwitz A, Khafif R, Siegman F. Study of the effect of trypsin inhibitor in acute haemorrhagic pancreatitis in dogs. Surg Forum 1961,12:370-2.

Rich AR, Duff GL. Experimental and pathological studies on pathogenesis of acute pancreatitis. Bull Johns Hopkins Hosp 1936,58:212-59.

Richardson M. THe proteinase inhibitors of plants and micro-organisms. Phytochemistry 1977,16:159-69.

Rick W. Chemical methods in the diagnosis of pancreatic disease. Clin Gastroenterol 1972,1:3-25.

Rinderknecht H, Geokas MC. On the physiological role of alpha$_2$ macroglobulin. Biochim Biophys Acta 1973,295:233-44.

Robert JH, Meyer P, Rohner A. Can serum and peritoneal amylase and lipase help in the early prognosis of acute pancreatitis. Ann Surg 1986,203:163-8.

Rodgers RE, Carey LC. Peritoneal lavage in experimental pancreatitis in dogs. Am J Surg 1966,111:792-4.

Roesch W, Demling L. Endoscopic management of pancreatitis. Surg Clin North Am 1982,62:845-52.

Rokitansky C Von. Lehrb d path Anat 3k Anfl 1861,iii:309,242. Cited by Fitz 1889.

Rosato EF, Chu WH, Mullen JL, Rosato FE. Peritoneal lavage treatment of experimental pancreatitis. J Surg Res 1972,12:138-40

Rosenberg IK, Kahn JA, Walt AJ. Surgical experience with pancreatic pseudocysts. Am J Surg 1969,117:11-17.

Rosseland AR, Solhaug JH. Early or delayed endoscopic papillotomy (EPT) in gallstone pancreatitis. Ann Surg 1984,199:165-7.

Rowland VC. Pancreatitis, with special reference to its non-surgical treatment. Am J Dig Dis 1934,1:441-5.

Saarni H, Viikari J. A simple technique for continuous intravenous infusion under neuroleptic tranquillization. Lab Anim 1976,10:69-72.

Saba TM, Jaffe E. Plasma fibronectin (opsonic glycoprotein): its synthesis by vascular endothelial cells and role in cardiopulmonary integrity after trauma as related to reticuloendothelial function. Am J Med 1980,66:577-94.

Safrany L, Cotton PB. A preliminary report : Urgent duodenoscopic sphincterotomy for acute gallstone pancreatitis. Surgery 1981,89:424-8.

Safrany L, Neuhaus B, Krause S, Portocarrero G, Schott B. Endoskopische papillotomie bei akuter, biliar bedingter pankreatitis. Dtsh Med Wochenschr 1980,105:115-19.

Sahel J, Bourry J, Sarles H. Caroli's disease with acute pancreatitis and angiocholitis. Diagnosis and therapeutic value of endoscopic choledochowirsungography. Nouv Presse Med 1976,5:2067-9.

Saito A, Sinohara H. Rat plasma murinoglobulin : isolation, characterization and comparison with rat alpha-1 and alpha-2 macroglobulins. J Biochem 1985,98:501-16.

Saitoh Y. Clinical results with oral protease inhibitor FOY-305 in chronic pancreatitis. ed. Grozinger KH, Schrey A, Wabnitz RW. Proteinases - Inhibition. Munchen. Wolf and Sohn 1982,pp156-67.

Salt WB, Schenker S. Amylase - its clinical significance. A review of the literature. Medicine 1976,55:269-89.

Sanfey H, Bulkley GB, Cameron JL. The pathogenesis of acute pancreatitis. The source and role of oxygen-derived free radicals in three different experimental models. Ann Surg 1985,201:633-9.

Sakaran S, Walt AJ. The natural and unnatural history of pancreatic pseudocysts. Br J Surg 1975,62:37-44.

Sarles H. Pancreatitis. Symposium of Marseille 1963. Basel, Karger 1965.

Sarles H. Revised Classification of Pancreatitis - Marseille 1984. Dig Dis Sci 1985,30:573-4.

Sarner M, Cotton PB. Classification of pancreatitis. Gut 1984,25:756-9.

Satiani B, Store HH. Predictability of present outcome and future recurrence in acute pancreatitis. Arch Surg 1979,114:711-16.

Sauven P, Playforth MJ, Evans M, Pollock AV. Fluid sequestration: an early indicator of mortality in acute pancreatitis. Br J Surg 1986,73:799-800.

Schapira M, Scott CF, Colman RW. Protection of human plasma kallikrein from inactivation by C1 inhibitor and other protease inhibitors. The role of high molecular weight kininogen. Biochemistry 1981,20:2738-43.

Schechter I, Berger A. On the size of the active site in proteases. I.Papain. Biochim Biophys Res Commun 1967,27:157-62.

Schmid E, Blaich E. Acute pancreatitis in mycoplasma pneumoniae infections. Z Gastroenterol 1976,14:536-7.

Schmidt H, Creutzfeldt W. The possible role of phospholipase A in the pathogenesis of acute pancreatitis. Scand J Gastroenterol 1969,4:39-48.

Schmidt H, Creutzfeldt W. Akute und residivierende pankreatitis (ein schliesslich der sogenannten begleitpankreatitis). ed. Demling L. Klinische Gastroenterologie. Stuttgart. Thieme Verlag, 1973,pp929-69.

Schmieden V, Sebening W. Chirurgie des pancreas. Arch Klin Chir 1927,148:319-87.

Schult H, Lorenz W, Maroske D, Lange E, Luben L. Zur rolle des histamines bei der akuten pankreatitis. Chir Forum Exp Klin Forsch 1979,269-72.

Schultze HE, Heide K, Haupt H. Alpha$_1$ antitrypsin from human serum. Klin Wochenschr 1962,40:427-9.

Seelig R, Ehemann V, Tschabargane C, Seeling HP. The serum complement system - a mediator of acute pancreatitis. Virchows Arch [A] 1975,365:193-9.

Seelig R, Lankisch PG, Koop H, Kaboth U, Winckler K, Seelig HP. The serum complement system as an initiator and prognostic clue in acute pancreatitis. Ir J Med Sci 1977,146:Suppl 1:1.

Seifert G. Die Pathologie des Kindlinchen Pankreas. Leipzid. Thieme Verlag.1956. Cited by Goebell and Hotz 1976.

Senn N. The surgery of the pancreas. ed. Ewing Mears J. Trans Am Surg Assoc (4th volume) Philadelphia. P.Blakinston Son and Co. 1886,pp99-232.

Serafini AN, Al-Sheikh W, Barkin JS et al. Biliary scintigraphy in acute pancreatitis. Radiology 1982,144:591-5.

Shader AE, Paxon JR. Fatal pancreatitis. Am J Surg 1966,111:369-73.

Shannon HE. Stimulation of avoidance behavior by buprenorphine in rats. Psychopharmacology 1983,80:19-23.

Shatney CH, Lillihei RC. Surgical treatment of pancreatic pseudocysts. Analysis of 119 cases. Ann Surg 1979,189:386-94.

Shatney CH and Lillihei RC. The timing of surgical treatment of pancreatic pseudocysts. Surg Gynecol Obstet 1981,152:809-12.

Shaw JFL. Compact portable system for continuous central venous infusion in rats. Lab Anim 1982,16:331-4.

Shearer MG, Imrie CW. Parathyroid hormone levels, hyperparathyroidism and acute pancreatitis. Br J Surg 1986,73:282-4.

Shi ECP, Yeo BW, Ham JM. Pancreatic abscesses. Br J Surg 1984,71:689-91.

Shohl T, Machida R, Whitman N. Studies of experimental pancreatitis. Surgery 1960,48:1022-7.

Silverstein W, Isikoff MB, Hill MC, Barkin J. Diagnostic imaging of acute pancreatitis : prospective study using CT and sonography. AJR 1981,137:497-502.

Sim DN, Duprez A, Anderson MC. Alterations of lymphatic circulation during acute experimental pancreatitis. Surgery 1966,60:1175-82.

Skyring A, Singer A, Tornya P. Treatment of acute pancreatitis with trasylol : report of a controlled therapeutic trial. Br Med J 1965,2:627-9.

Smadja C, Bismuth H. Pancreatic debridement in acute necrotizing pancreatitis : an obsolete procedure? Br J Surg 1986,73:408-10.

Smith RB, Orahood RC, Wagensteen SL, Berkha GJ, Zarelis A. Effect of a trypsin inhibitor on experimentally induced pancreatitis in the dog. Surgery 1963,54:922-7.

Smith WI Jr, Lombardi B, Rabin BS, Van Thiel DH, Iammarino RM. C_3 and $alpha_1$-antitrypsin in human pancreatitis. Gastroenterology 1977,72:1134.

Sottrup-Jensen L, Petersen TE, Magnusson S. Mechanism of proteinase complex formation with $alpha_2$ macroglobulin. Three modes of trypsin binding. FEBS Lett 1981,128:127-32.

Spotti F, Holzknecht F. The influence of inhibitors of plasmin and plasminogen activation on the streptokinase-induced fibrinolytic state. Thromb Haemost 1970,24:100-12.

Stanbury JB, Wyngaarden JB, Fredrickson DS. The Metabolic Basis of Inherited Disease. 2nd Edn. New York, McGraw-Hill,1976.

Staritz M, Poralla T, Manns M, Meyer K-H. Effect of modern analgesic drugs (tramadol, pentazocine, and buprenorphine) on the bile duct sphincter in man. Gut 1986,27:567-9.

Steckman ML, Dooley MC, Jaques PF, Powell DW. Major gastrointestinal haemorrhage from peripancreatic blood vessels in pancreatitis. Treatment by embolotherapy. Dig Dis Sci 1984,29:486-97.

Steinbuch M, Loeb J. Isolation of an alpha$_2$ macroglobulin from human plasma. Nature 1961,192:1196.

Stone HH, Fabian TC. Peritoneal dialysis in the treatment of acute alcoholic pancreatitis. Surg Gynecol Obstet 1980,150:878-82

Stone HH, Fabian TC, Dunlop WE. Gallstone pancreatitis : biliary tract pathology in relation to time of operation. Ann Surg 1981,194:305-10.

Stone HH, Fabian TC, Morris ES. Failure of thoracic duct drainage to ameliorate life-threatening physiologic derangements of acute alcoholic pancreatitis. South Med J 1983,76:613-14.

Stroud WH, Cullom JW, Anderson MC. Haemorrhagic complications of severe pancreatitis. Surgery 1981,90:657-65.

Suda H, Aoyagi T, Hamada M, Takeuchi T, Umezawa H. Antipain; a new protease inhibitor isolated from actinomycetes. J Antibiot (Tokyo) 1972,25:263-6.

Sumi H, Takasugi S, Toki N. Studies on kallikrein-kinin system in plasma of patients with acute pancreatitis. Clin Chim Acta 1978,87:113-18.

Switz DM, Vlahcevic ZR, Farrar JT. The effect of anticholinergic and/or nasogastric suction on the outcome of acute alcoholic pancreatitis : A controlled trial. Gastroenterology 1975,68:994-5

Szabo D, Usadel KH. Cytoprotective-organoprotection by somatostatin; gastric and hepatic lesions. Experientia 1982,38:254-6.

Takahara H, Nakamura Y, Yamamoto K, Sinohara H. Comparative studies on the serum levels of alpha$_1$ antitrypsin and alpha-macroglobulin in several mammals.Tohoku J Exp Med 1983,139:265-70

Takahara H, Sinohara H. Mouse plasma trypsin inhibitors. Isolation and characterisation of alpha$_1$-antitrypsin and contrapsin,a novel trypsin inhibitor. J Biol Chem 1982,257:2438-46.

Takasugi S, Toki N. Inhibitory effects of native and synthetic protease inhibitors on plasma proteases in acute pancreatitis. Hiroshima J Med Sci 1980,29:189-94.

Tanaka N, Tsuchiya R, Ishii K. Comparative clinical study of FOY and Trasylol in acute pancreatitis. Adv Exp Med Biol 1979,120:367-78.

Terry TR, Grant D, Hermon-Taylor J. Specific intraperitoneal antiproteinase chemotherapy in acute necrotising pancreatitis - an experimental study. Digestion 1986,35:56-7.

Thal A. Studies on pancreatitis. II. Acute pancreatic necrosis produced experimentally by the Arthus sensitization reaction. Surgery 1955,37:911-17.

Thal A, Perry JF Jr, Egner W. A clinical and morphologic study of forty-two cases of fatal acute pancreatitis. Surg Gynecol Obstet 1957,105:191-202.

The TL. A review of the aetiology and pathogenesis of acute pancreatitis. Part III. Nutritional factors and metabolic disorders. West Indian Med J 1978,27:172-9.

Thomford NR, Jesseph JE. Pseudyocyst of the pancreas. A review of fifty cases. Am J Surg 1969,118:86-94.

Toffler AH, Spiro HM. Shock or coma as the predominant manifestation of painless acute pancreatitis. Ann Intern Med 1962,57:655-9.

Tondelli P, Stutz K, Harder F, Schuppisser JP, Allgower M. Acute gallstone pancreatitis : best timing for biliary surgery. Br J Surg 1982,69:709-10.

Topping RM, Seilman S. A four-straight-line model of proteinase binding characteristics of human blood serum. Biochem J 1979,177:493-9.

Trapnell JE. The natural history and prognosis of acute pancreatitis. Ann R Coll Surg Engl 1966,38:265-87.

Trapnell JE. Management of the complications of acute pancreatitis.Ann R Coll Surg Engl 1971,49:361-72.

Trapnell JE. Pathophysiology of acute pancreatitis. World J Surg 1981,5:319-27.

Trapnell JE, Duncan EHL. Patterns of incidence in acute pancreatitis. Br Med J 1975,2:179-83.

Trapnell JE, Rigby CC, Talbot CH, Duncan EHL. A controlled trial of Trasylol in the treatment of acute pancreatitis. Br J Surg 1974,61:177-82.

Trapnell JE, Talbot CH, Capper WM. Trasylol in acute pancreatitis. Am J Dig Dis 1967,12:409-12.

Trautschold I, Werle E, Zickgraf-Rudel G. Uber den kallikrein-trypsin inhibitor. Arzneimittelforschung 1966,16:1507-15.

Travis J, Salvesen GS. Human plasma proteinase inhibitors. Ann

Rev Biochem 1983,52:655-709.

Umezawa H. Structures and activities of protease inhibitors of microbial origin. Methods Enzymol 1979,45:678-95.

Umezawa H, Aoyagi T. Activation of proteinase inhibitors of microbial origin. ed. Dingle JT. Proteinases in Mammalian Cells. Holland. North 1977,pp637-62.

Usadel KH, Leuschner U, Uberla KK. Treatment of acute pancreatitis with somatostatin. A randomised study. ed. Hollender LF. Controversies in Acute Pancreatitis. Berlin/Heidelberg. Springer-Verlag 1982,pp167-70.

Usadel KH, Uberla KK, Leuschner U. Treatment of acute pancreatitis with somatostatin : results of the multicentre double-blind trial (APTS-study) Dig Dis Sci 1985,30:992.

Van Gossum A, Seferian V, Rodzynek JJ, Wetiendorff P, Cremer M, Delcourt A. Early detection of biliary pancreatitis. Dig Dis Sci 1984,29:97-101.

Van Heerden JA, ReMine WH. Pseudocysts of the pancreas. Review of seventy-one cases. Arch Surg 1975,110:500-5.

Van Leuven F. Human alpha$_2$ macroglobulin : primary amines and the molecular mechanisms of endoprotease inhibition and receptor-mediated endocytosis. Mol Cell Biochem 1984,58:121-8.

Van Leuven F, Cassiman JJ, Van den Berghe H. Demonstration of an alpha$_2$-macroglobulin receptor in human fibroblasts absent in tumour-derived cell lines. J Biol Chem 1979,254:5155-60.

Virgilio RW, Rice CL, Smith DE et al. Crystalloid vs. colloid resuscitation : Is one better ? A randomised clinical study. Surgery 1979,85:129-39.

Vogel R, Trautschold I, Werle E. Natural Protease Inhibitors - Biochemistry and Clinical Aspects. Stuttgart. Thieme 1966.

Von Reimann JF, Lux G. Therapeutische strategie bie der akuten pankreatitis (1). Fortschr Med 1984,102:179-82.

Walker L, Necheles H. Effect of glucagon on pancreatic secretion. Am J Physiol 1956,187:638.

Wall AJ. Peritoneal dialysis in the treatment of severe acute pancreatitis. Med J Aust 1965,2:281-3.

Wallensten S. Acute pancreatitis and hyperdiastasorra after partial gastrectomy. Acta Chir Scand 1958,115:182-8.

Wang CC, Grossman MI. Physiological determination of the release of secretin and pancreozymin from intestine of dogs with transplanted pancreas. Am J Physiol 1951,164:527-45.

Wanke M. Experimental acute pancreatitis. Curr Top Pathol 1970,52:64-142.

Warshaw AL. Pancreatic abscesses. N Engl J Med 1972,287:1234-6.

Warshaw AL. Inflammatory masses following acute pancreatitis. Phlegmon, pseudocyst and abscess. Surg Clin North Am 1974,54:621-36.

Warshaw AL. Serum amylase isoenzyme profiles as a differential index in disease. J Lab Clin Med 1977,90:1-3.

Warshaw AL. Surgical pros and cons. Surg Gynecol Obstet 1980,151:547-8.

Waterman NG, Walsky R, Moore R, Howell RS. Acute pancreatitis : an experimental model. Surgery 1969,66:746-50.

Waterworth TA, Bevan PG. Effect of glucagon on pain in acute pancreatitis. Ann R Coll Surg Engl 1976,58:33.

Watts GT. Total pancreatectomy for fulminant pancreatitis. Lancet 1963,2:384-5.

Weaver DW, Ledgerwood AM, Lucas CE, Higgins R, Bouwman DL, Johnson SD. Pulmonary effects of albumin resuscitation for severe hypovolemic shock. Arch Surg 1978,113:387-92.

Weaver DW, Walt AJ, Sugawa C, Bouwman DL. A continuing appraisal of pancreatic ascites. Surg Gynecol Obstet 1982,154:845-8.

Weiss AG, Hollender LF, Adloff M. Anti-enzymes in acute pancreatitis - first clinical trial. Mem Acad Chir 1960,85:664-7.

Wendt P, Fritsch A, Schullz F, Wunderlich G, Blumel G. Proteinases and inhibitors in plasma and peritoneal exudate in acute pancreatitis. Hepatogastroenterology 1984,31:277-81.

Wereszczynska-Siemiatkowska U, Nebendahl K, Pohl U, Otto J, Lankisch PG. Influence of buprenorphine in acute experimental pancreatitis. Digestion 1986,35:61.

Werle E. Zur biochemie des Trasylon. ed. Haberland GL, Matis P. Neue Aspekte der Trasylol-Therapie. Stuttgart/New York. Schattauer 1969,p49.

Werner MH, Hayes DF, Lucas CE, Rosenberg IK. Renal vaso-constriction in association with acute pancreatitis. Am J Surg 1974,127:185-90.

White TT. Acute Pancreatitis. ed. Conn HF. Current Therapy. London/Philadelphia/Toronto. Saunders 1974,pp363-4.

White TT, Magee DF. Perfusion of the dog pancreas with bile without the production of pancreatitis. Ann Surg 1960,151:245-50.

Williams HU, Busch FC. The aetiology and pathogenesis of acute pancreatitis. Trans Ass Am Physicians 1907,22:304-14.

Williams LF Jr, Byrne JJ. Natural variations in experimental haemorrhagic pancreatitis. Surg Gynecol Obstet 1967,124:531-5.

Williams LF Jr, Byrne JJ. The role of bacteria in hemorrhagic pancreatitis. Surgery 1968,64:967-72.

Williamson RCN. Early assessment of severity in acute pancreatitis. Gut 1984,25:1131-9.

Willingham MC, Maxfield FR, Pastan IH. Alpha$_2$ macroglobulin binding to the plasma membrane of cultured fibroblasts. Diffuse binding followed by clustering in coated regions. J Cell Biol 1979,82:614-25.

Wilson C, Carter DC, Imrie CW. Surgery for acute necrotising pancreatitis. Digestion 1986,35:63.

Wilson C, Imrie CW. Amylase and gut infarction. Br J Surg 1986,73:219-21.

Wiman B, Collen D. On the mechanism of the reaction between human alpha$_2$-antiplasmin and plasmin. J Biol Chem 1979,254:9291-7.

Winstone NE. Methaemalbumin in acute pancreatitis. Br J Surg 1965,52:804-8.

Wiznitzer T, Orda R, Bawnik JB, Bubis JJ. Acute necrotising pancreatitis in the guinea pig. Effect of chlorophyll-A on survival times. Dig Dis Sci 1976,21:459-64.

Wood J, Garcia R, Solomon TE. A simple model for pancreatitis : High dose caerulein injection in rats. Gastroenterology 1982,82:1213.

Worthington KJ, Cuschieri A. Estimation of plasma esterolytic activity and its in vitro inhibition by proteinase inhibitors during acute pancreatitis in the human. Br J Exp Pathol 1976,57:165-9.

Wu K, Wang D, Feinman RD. Inhibition of proteases by alpha$_2$-macroglobulin. The role of lysyl amino groups of trypsin in covalent complex formation. J Biol Chem 1981,256:10409-14.

Yajima H, Kiso Y, Okada Y, Watanabe H. Synthesis of the basic trypsin inhibitor from bovine pancreas (Kunitz and Northrop) by fragment condensation on a polymer support. J Chem Soc Chem Comm 1974,3:106-7.

Zajtchuk R, Amato JJ, Paloyan E, Baker RJ. Inhibition of pancreatic exocrine secretion by glucagon. Surg Forum 1967,18:410-11.

Zoepffel H. Das akute pankreasodem - eine vorstufe der akuten pankreasnekrose. Dtsch Z Chir 1922,175:301-12.