

Zymogen Activation Peptides and their Relationship to Disease  
Severity in Acute Pancreatitis.

By Dugal Heath © 1993

A Thesis Submitted to Leicester University for the Degree of Doctor of Medicine.

Work Performed in the Department of Surgery, Glasgow Royal Infirmary, Glasgow  
between October 1987 and July 1989.

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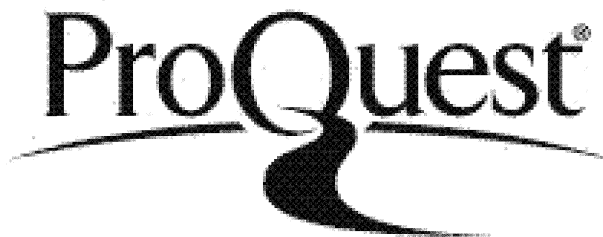
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Dedicated to my parents, Phillppa and Simon.



"Pancreatitis when acute is oft a mortal ill,  
It strikes the patient suddenly, and often strikes to kill".

From:-  
The acute abdomen in rhyme.  
by  
Zachary Cope (alias Zeta).  
5th Ed, 1972  
H K Lewis & Co. Ltd., London.

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#### DECLARATION OF ORIGINALITY AND COLLABORATION.

All the work included in this thesis is completely original and has not, to the best of my knowledge and belief, been performed or published previously.

The original idea that the measurement of urinary concentrations of Trypsinogen Activation Peptide (TAP) and Prothrombinase A<sub>2</sub> activation peptides (PLAP) might be useful in the severity prediction of acute pancreatitis was put forward by Professor Hermon-Taylor. At his invitation, M. Gudgeon, C. W. Imrie and myself met and produced a study protocol the result of which are displayed in chapter 3 of this thesis. I personally entered into this study 39 of the 54 patients with acute pancreatitis and 6 of the disease controls. I personally collected and processed all blood and urine samples from patients within the West of Scotland. I then personally transported 2 of the 4 aliquots of serum and urine to the Department of Surgery at St George's Hospital Medical School, London. Once the study had been completed I personally decanted aliquots of the collected specimens into 3 separate sets of tubes in preparation for the amylase, C-reactive protein,  $\alpha_2$ -macroglobulin and interleukin-6 assays. I personally transported the remaining aliquotes to the Department of Surgery at St. George's Hospital Medical School, London. All the TAP and PLAP assays were performed in the Department of Surgery at St. George's Hospital Medical School.

The study on interleukin-6 was performed by myself following discussions with Professor A Shenkin. Most patients were recruited from the Glasgow Hospitals. A small number from St. George's Hospital were also included. The interleukin-6 assay was performed by Dr. A Cruickshank.

The studies on the peritoneal fluid of patients with acute pancreatitis were conceived and conducted by myself. Mr C. Wilson contributed 4 specimens

I have personally analysed all of the data included in this thesis, utilising Minitab Accelerated version 8.1 <sup>™</sup> on an Apple Macintosh <sup>™</sup> II ci. Statistical advice was freely given Dr G Murray although at no time did he perform any of the statistical analysis.

The development of the TAP and PLAP assays and the studies on normal controls were conducted in the Department of Surgery at St. Georges' Hospital Medical School. The chapters on the development and validation of the PLAP assay have been included in this thesis for the sake of completeness, since at the time of writing, the details had not been published. This is not my own original work. It was conducted by Mr. M Gudgeon.

Ethical committee approval was obtained for the collection of blood and peritoneal fluid samples for the studies involved in this thesis.

### ACKNOWLEDGEMENTS.

It is a pleasure to be able to acknowledge the following people who kindly provided help with my research:

To Mr. C W imrie for his constant help, encouragement and support. To Professor J Hermon-Taylor for his enthusiastic support in our joint studies and for access to the TAP and PLAP assays in the Department of Surgery, St. George's Hospital Medical School. To Professor A Shenkin and the staff of the institute of Biochemistry, Glasgow Royal infirmary for finding time to discuss the biochemical aspects of my research and for providing unfailing biochemical back up. To Dr. A Cruickshank, also from the institute of biochemistry, for her help in the interleukin-6 study. To Dr. G Murray for invaluable statistical advice. To Mr. M Gudgeon, my opposite number at St. George's Hospital, for sharing in the collection of data in our joint studies.

I would also like to thank the Consultant Surgeons at the following Hospitals for allowing me to study patients under their care:- Glasgow Royal infirmary, Stobhill Hospital, the Royal Victoria Infirmary, Monklands District General Hospital, the Royal Alexandra Hospital, Paisley, the Southern General Hospital and Law Hospital, Carluke.

Finally i would like to thank Bayer UK Ltd., and in particular to Mr. D Lees, for providing financial support.

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## PRESENTATIONS.

The work included in this thesis has been presented to the following learned societies:-

1. American Pancreatic Association, Chicago 1988.

Trypsinogen activation peptide (TAP) assay in the assessment of acute pancreatitis.

A Gudgeon, D I Heath, P Hurley, A Shenkin, A Jehanli, G Patei, C Wilson, B Austen, C W Imrie, J Hermon-Taylor.

2. Pancreatic Society of Great Britain and Ireland, London 1988.

Trypsinogen activation peptide (TAP) assay in the assessment of acute pancreatitis.

M Gudgeon, D I Heath, P Hurley, A Shenkin, A Jehanli, G Patel, C Wilson, B Austen, C W Imrie, J Hermon-Taylor

3. European Pancreatic Club, Glasgow 1989.

Interleukin-6, a mediator of the acute phase response in acute pancreatitis?

D I Heath, A M Cruickshank, A Shenkin, C W Imrie.

4. European Pancreatic Club, Glasgow 1989.

Zymogen activation peptides (ZAP) assays in the severity prediction of acute pancreatitis.

A M Gudgeon, D I Heath, A Shenkin, A Jehanli, G Patei, C Wilson, B Austen, C W Imrie, J Hermon-Taylor.

5. European Pancreatic Club, Glasgow 1989.

TAP assay of peritoneal fluid in acute pancreatitis.

D I Heath, C Wilson, A M Gudgeon, A Jehanli, G Patei, C W Imrie, J Hermon-Taylor.

6. British Society of Gastroenterology, Dublin 1989.

interleukin-6, a mediator of the acute phase response in acute pancreatitis?

D I Heath, A M Cruickshank, A Shenkin, C W Imrie.

7. Pancreatic Society of Great Britain and Ireland, Leeds 1989.

Interleukin-6, a mediator of the acute phase response in acute pancreatitis?

D I Heath, A M Cruickshank, A Shenkin, C W Imrie.

8. Pancreatic Society of Great Britain and Ireland, Leeds 1989.

TAP assay of peritoneal fluid in acute pancreatitis.

D I Heath, C Wilson, A M Gudgeon, A Jehanli, G Patel, C W Imrie, J Hermon-Taylor.

9. Surgical Research Society, Liverpool 1990.

TAP assay of peritoneal fluid in acute pancreatitis.

D I Heath, C Wilson, A M Gudgeon, A Jehanli, G Patel, C W Imrie, J Hermon-Taylor.

10. International Association of Pancreatology, Nagasaki, Japan, 1990.

Interleukin-6, a mediator of the acute phase response in acute pancreatitis?

D I Heath, A M Cruickshank, A Shenkin, C W Imrie.

11. British Society of Gastroenterology, Southampton 1990.

The correlation between zymogen activation and tissue damage in acute pancreatitis.

D I Heath, A Cruickshank, M Gudgeon, C W Imrie and A Shenkin.

12. European Pancreatic Club, Basel, Switzerland 1990.

The correlation between zymogen activation and tissue damage in acute pancreatitis.

D I Heath, A Cruickshank, M Gudgeon, C W Imrie and A Shenkin.

13. Pancreatic Society of Great Britain and Ireland, Manchester 1990.

The correlation between zymogen activation and tissue damage in acute pancreatitis.

D I Heath, A Cruickshank, M Gudgeon, C W imrie and A Shenkin.

14. British Society of Gastroenterology, London 1991.

Immediate prognostic assessment of acute pancreatitis using assays to quantify pancreatic zymogen activation.

D I Heath, C Wilson, A M Gudgeon, A Jehanii, G Patel, C W imrie, J Hermon-Taylor.

#### PUBLICATIONS.

1. M Gudgeon, D I Heath, P Hurley, A Shenkin, A Jehanli, G Patel, C Wilson, B Austen, C W Imrie, J Hermon-Taylor.

Trypsinogen activation peptides assay in the early severity prediction of acute pancreatitis.

Lancet 1990; 335: 4-8.

2. D I Heath, A Cruickshank, M Gudgeon, A Jehanli, A Shenkin, C W Imrie.

The role of interleukin-6 in mediating the acute phase protein response and potential as an early means of severity assessment in acute pancreatitis.

Gut 1993; 34: 41-45.

3. D I Heath and C W Imrie. Diagnosis and severity assessment in acute pancreatitis. Chapter in Pancreatic Diseases: Progress and Prospects. C D Johnson and C W Imrie (Eds). Springer Verlag, London 1991.

**CHAPTER 1:**

**INTRODUCTION AND AIMS.**

### 1.1 HISTORICAL REVIEW.

The pancreas was probably named by Rufus of Ephesus around 100 AD, (Glazer and Ranson 1988) the term being derived from the Greek *pan* meaning ail and *kreas* flesh. Although references to the gland date back to 300 BC, (Glazer and Ranson 1988) little attention was paid to it until the 16th century, when Andreas Vesalius, in *De Humani Corporis Fabrica*, suggested that the pancreas was part of the greater omentum. Johann Wirsung named the main pancreatic duct in 1642, (Wirsung 1642) Abraham Vater the duodenal ampulla in 1728, (Vater 1728) Giovanni Santorini the accessory duct in 1724, (Santorini 1724) and Oddi the muscular sphincter at the lower end of the bile duct in 1887 (Oddi 1887).

The exocrine function of the gland was first noted by Claude Bernard who demonstrated that pancreatic juice was capable of digesting fat, starch and protein (Bernard 1856). Langerhans described the islets named after him in 1869 (Langerhans 1869). Von Mering and Minkowski (1889) made the connection between the pancreas and diabetes mellitus and it was suggested subsequently that the degeneration of the islets of Langerhans were responsible for its development (Opie 1901). Banting and Best (1922) were the first to lower blood glucose concentrations in a diabetic dog by the injection of a pancreatic extract. This was followed by the crystallisation of insulin in 1926 (Abel 1926) and the development of a radio immunoassay for insulin (Yalow and Berson 1960)

One of the earliest reports of pancreatic inflammatory disease was given by Diemerbroeck who reported a diffuse pancreatic abscess at post-mortem examination performed on a merchant from Leyden in 1672 (Diemerbroeck 1694). Further early descriptions of pancreatitis were given by Mayo, (1836), Classen (1842) and Senn (1886). In 1889 an important paper by Fitz reviewed a number of post-mortem examinations and proposed the first workable classification system for acute pancreatitis (Fitz 1889). He divided the cases into haemorrhagic, suppurative and gangrenous pancreatitis. Elman (1933) is credited with fully

recognising the entity of oedematous pancreatitis and Howard (1960) with noting that oedematous pancreatitis can proceed to haemorrhage and necrosis. Balsar (1882) provided the first description of fat necrosis associated with acute pancreatitis.

## **1.2 EXOCRINE PANCREATIC SECRETION.**

The pancreas has the greatest protein synthetic capacity of any organ in the body and has proved to be a paradigm for the investigation of protein synthesis (Howat and Sarles 1979). As a result the digestive enzymes and their secretion have been extensively investigated and are well understood (Palade 1975).

### **1.2.1 Pancreatic secretion.**

Pancreatic secretion comprises two separate secretory processes - enzyme secretion and electrolyte secretion. Pancreatic secretion is primarily under hormonal control although, as in gastric secretion, there is a nervous component (cephalic phase) mediated by the vagus (Case 1988).

Secretin, liberated from the duodenal mucosa, stimulates secretion of a watery alkaline fluid from the ductal cells, possessing a high bicarbonate ion concentration, but a poor enzyme content. Pancreozymin, also released from the duodenal mucosa, stimulates secretion of an enzyme-rich fluid by the discharge of zymogen granules from the acinar cells.

The rough endoplasmic reticulum is the site of production of pancreatic digestive enzymes, lysosomes and plasma membrane proteins (Palade 1975). This is composed of membranous sacs or cisternae, the outer surface of which are studded with ribosomes. Following translation on the ribosomal RNA the proteins are passed in a vectorial fashion into the cisternae (Biobel and Dobberstein 1975). All proteins undergo co-translational glycosylation of selected asparagine (Asn) residues with an oligosaccharide (Kornfeld 1985). Inside the endoplasmic



reticulum three glucose and one mannose residues are cleaved before vesicular transportation to the Golgi apparatus. From this point on, lysosomal enzymes are treated differently from the digestive enzymes, receiving a phosphomannosyl residue. This serves as a critical recognition site for mannose-6-phosphate receptors and leads to subsequent translocation to lysosomes, (Kaplan *et al* 1977) whilst the digestive enzymes are sent to zymogen granules. Secretion is achieved by the fusion of the zymogens granules with the apical acinar cell membrane.

#### 1.2.2 Pancreatic enzymes.

The enzymes produced by the pancreatic acinar cells play a major role in intraluminal digestion. The pancreatic enzymes are all hydrolases and are classed according to their substrate molecule: amylase - starch, proteolytic enzymes - proteins, lipolytic enzymes - fats and nucleolytic enzymes - nucleic acids (table 1.1). Pancreatic enzymes make up between 80 and 85% of the protein content of pancreatic juice (Desnuelle and Figarella 1979).

The digestive enzymes can be divided into 2 groups: those secreted as active enzymes and those secreted as pro-enzymes (zymogens).  $\alpha$  amylase, lipase, RNase and DNase are secreted as active enzymes and the remainder as inactive precursors (table 1.1).

##### 1.2.2.1 Amylase.

A "starch-splitting ferment" in the blood was first described in 1846 by Magendie (Elman *et al* 1939). This effect was due to the presence of amylase, an enzyme which hydrolyses the  $\alpha_{1,4}$  glycosidic linkage of branch chained starch molecules. The majority of the enzyme is derived from the pancreas (amylase P) and salivary glands (amylase S), each of which contribute approximately 50% to the total enzyme production.

Enzyme	Activator	Substrate	Function
$\alpha$ -amylase	NA	Starch	Hydrolysis of $\alpha_{1,4}$ glucosidic bonds
Lipase	NA	Triglycerides	Hydrolysis of C1 and C3 glycerol ester bonds
Trypsinogens (Trypsins)	Enterokinase	Entero-Proteins	Hydrolysis of kinase bonds at arginine and lysine
Chymotrypsinogen (Chymotrypsin)	Trypsin	Proteins	Hydrolysis of peptide bonds at amino acids with aromatic side chains
Procarboxypeptidase A (Carboxypeptidase A)	Trypsin	Proteins	Hydrolysis of peptide bonds at C terminal amino acids with aromatic or branched aliphatic side chains
Procarboxypeptidase-B (Carboxypeptidase-B)	Trypsin	Proteins	Hydrolysis of peptide bonds at C terminal amino acids with basic side chains
Prophospholipase-A <sub>2</sub> (Phospholipase A <sub>2</sub> )	Trypsin	Phospholipids	Hydrolysis of fatty acid lipids esters at 2-position of phospholipids
Carboxyl ester hydrolase	Trypsin	Cholesterol	Hydrolysis of esters of esters water soluble short chain fatty acids
Ribonuclease	NA	RNA	Hydrolysis of phosphate ester bonds in RNA
Deoxyribonuclease	NA	DNA	Hydrolysis of phosphate ester bonds in DNA bonds in DNA
Proelastases (Elastase)	Trypsin	Elastin	Hydrolysis of peptide bonds at amino acids with aliphatic side chains
Kallikreinogen (Kallikrein)	Trypsin	Kininogens	Cleavage of kininogen to active kinin

Table 1.1: Human pancreatic enzymes and zymogens.

#### 1.2.2.2 Lipase.

Lipase forms 1-3% of the total protein content of the pancreatic juice. It is a glycoprotein comprising a polypeptide chain of 470 amino acid residues, with a molecular weight of between 46,000 and 48,000 Daltons (Desnuelle and Figerella 1979). Lipase hydrolyses triglycerides to produce 2-monoglycerides, 1, 2 diglycerides and free fatty acids.

#### 1.2.2.3 Trypsinogens.

Trypsin is a very important zymogen, not just because it is the most abundant, forming 19% of the protein within the pancreatic juice, (Guy *et al*/1978) but also because it is capable of activating all of the other zymogens (Hermon-Taylor and Heywood 1985).

It was first isolated by Kuhne in 1874 and was shown to be secreted as an inactive enzyme by Heidenhain in 1875. Pavlov (1888) and Shepovainikow (1889) demonstrated the enhanced proteolytic capacity of trypsin in the presence of duodenal mucosa and suggested the presence of enterokinase.

There are 3 variants trypsinogen. Trypsinogen 1 has a molecular weight of 23,000 Daltons and is about twice as abundant as trypsinogen 2 (Guy *et al*/1978).

Trypsinogen 2 has a slightly higher molecular weight of 25,000 Daltons (Guy *et al*/1978). The most recently isolated trypsinogen 3 forms only 5% of the total trypsinogen (Rinderknecht *et al*/1984). Trypsin only hydrolyses basic bonds situated to the right of lysine and arginine residues (Case 1988). Each of the trypsinogens is inactive and only becomes capable of proteolysis once activated by enterokinase (Baratti and Maroux 1976). This endoprotease is secreted by the duodenal mucosa and hydrolyses amide linkages to give equimolar quantities of trypsin and its activation peptide (TAP) (Davie and Neurath 1955, Bricteaux *et al*/1970). Once some trypsinogen activation has occurred then trypsin can go on to activate rapidly the remainder of the trypsinogen (Hermon-Taylor and Heywood 1985, Rinderknecht 1986).

The amino acid sequence of the activation peptide has been determined in a number of species (table 1.2). In all except the lung fish the sequence of the terminal 5 amino acids is (Asp)<sub>4</sub> Lys.

In man the N-terminal 9 amino acids in trypsinogen 1 and 2 are identical (Guy *et al* 1978). Human trypsinogen 2 gives, by activation, a single peptide, the octapeptide Ala-Pro-Phe-(Asp)<sub>4</sub>-Lys (Guy *et al* 1978). In contrast trypsinogen 1 yields 2 activation peptides, one octapeptide, identical to the activation peptide of trypsinogen 2, and the pentapeptide (Asp)<sub>4</sub>-Lys (Guy *et al* 1978).

#### 1.2.2.4 Phospholipase A<sub>2</sub>.

Phospholipase A<sub>2</sub> is a zymogen of molecular weight 14,800 Daltons, which is secreted as the pro-enzyme pro-phospholipase A<sub>2</sub>, and catalyses the release of fatty acids from the 2 position on triglycerides (Hannah 1971). It is remarkably heat stable being able to tolerate heating to 98°C at pH 4.0 for 5 minutes (De Haas *et al* 1968). It has been found in all mammalian species except the Guinea pig. Until recently, it was thought to be produced exclusively by the pancreas. Recent research has demonstrated it in the lung, (O'Hare *et al* 1986) spleen (Tojo *et al* 1988a) and gastric mucosa (Tojo *et al* 1988b) of the rat and lung of man (Seilhamer *et al* 1986). In 1987 Matsuda *et al* demonstrated immunoreactive phospholipase A<sub>2</sub> activity in the lung, stomach, small intestine and kidney of man. Like trypsin, pancreatic phospholipase is produced as an inactive pro-enzyme: pro-phospholipase. It is activated by trypsin which releases different activation peptides depending on the species (table 1.3). The human pro-phospholipase A<sub>2</sub> activation peptide (PLAP) is a heptapeptide with an amino acid sequence Asp-Ser-Gly-Ile-Ser-Pro-Arg (Grataroli *et al* 1982). Unlike trypsin, there is no preservation of the amino acid sequence between species although human PLAP does retain some homology with the PLAP from the dog and horse (table 1.3).

Dogfish	Ala-Pro-Asp-Asp-Asp-Asp-Lys	Bradshaw <i>et al</i> /1970
Lungfish	Phe-Pro-Ile-Glu-Glu-Asp-Lys	Hermanson <i>et al</i> /1971
Horse	Ser-Ser-Thr-Asp-Asp-Asp-Asp-Lys	Harris and Hofmann 1969
Elephant Seal	Phe-Pro-Thr-Asp-Asp-Asp-Asp-Lys	Bricteux <i>et al</i> /1974
Lesser Porcine	Phe-Pro-Ile-Asp-Asp-Asp-Asp-Lys	Bricteux <i>et al</i> /1975
Dromedary	Val-Pro-Ile-Asp-Asp-Asp-Asp-Lys	Bricteux <i>et al</i> /1971a
Red deer	Phe-Pro-Val-Asp-Asp-Asp-Asp-Lys Val-Asp-Asp-Asp-Asp-Lys	Bricteux <i>et al</i> /1970
Roe deer	Phe-Pro-Val-Asp-Asp-Asp-Asp-Lys Val-Asp-Asp-Asp-Asp-Lys	Bricteux 1970
Sheep	Phe-Pro-Val-Asp-Asp-Asp-Asp-Lys Val-Asp-Asp-Asp-Asp-Lys	Bricteux <i>et al</i> /1966
Goat	Phe-Pro-Val-Asp-Asp-Asp-Asp-Lys Val-Asp-Asp-Asp-Asp-Lys	Bricteux <i>et al</i> /1971b
Bovine CT	Val-Asp-Asp-Asp-Asp-Lys	Davie and Neurath 1955
AT	Phe-Pro-Ser-Asp-Asp-Asp-Asp-Lys	Louvard and Pulgserver 1974
Porcine CT	Phe-Pro-Thr-Asp-Asp-Asp-Asp-Lys	Charles <i>et al</i> /1963
AT	Phe-Pro-Thr-Asp-Asp-Asp-Asp-Lys	Louvard and Pulgserver 1974
Human T1	Ala-Pro-Phe-Asp-Asp-Asp-Asp-Lys Asp-Asp-Asp-Asp-Lys	Guy <i>et al</i> /1978 Guy <i>et al</i> /1978
T2	Ala-Pro-Phe-Asp-Asp-Asp-Asp-Lys	Guy <i>et al</i> /1978

Table 1.2: The amino acid sequence of the activation peptides of trypsinogen. CT = cationic trypsin, AT = anionic trypsin, T1 = trypsinogen 1 and T2 = trypsinogen 2.

Species	Sequence	Reference
Porcine 1 & 2	Glu-Glu-Gly-Ile-Ser-Ser-Arg	Dutilh <i>et al</i> 1975
Bovine 1 & 2	Glu-Ala-Gly-Leu-Asn-Ser-Arg	" "
Ovine 1	Glu-Ala-Gly-Leu-Asn-Ser-Arg	" "
Equine 1	Gly-Ile-Ser-Pro-Arg	Evenberg <i>et al</i> 1977
Equine 2	Glu-Asn-Gly-Ile-Ser-Pro-Arg	" "
Dog	Glu-Gly-Gly-Ile-Ser-Pro-Arg	O'Hare 1986
Human	Asp-Ser-Gly-Ile-Ser-Pro-Arg	Grataroli <i>et al</i> 1982

Table 1.3: The amino acid sequence of the activation peptides of phospholipase A<sub>2</sub>.

#### 1.2.2.5 Chymotrypsinogen.

Man has two chymotrypsins: A and B which are secreted as the pro-enzymes chymotrypsinogen A and B. The A type constituting 90% of the total chymotrypsin activity. It has a molecular weight of 24,000 Daltons while chymotrypsin B is a larger molecule with a molecular weight of 27,000 Daltons. Chymotrypsinogen is activated by means of a double cleavage (Arg 15-Ile 16) (Desnuelle and Figerella 1979).

#### 1.2.2.6 Procarboxyl ester hydrolase.

This enzyme represents about 4% of the protein content of pancreatic juice. It is a glycoprotein and estimates of its molecular weight range from 100,000 to 300,000 Daltons (Desnuelle and Figerella 1979). Its role in intraluminal digestion has not been defined clearly.

#### 1.2.2.7 Procarboxypeptidases.

The carboxypeptidases are secreted as the pro-enzymes, procarboxypeptidases. Only the molecular weight of carboxypeptidase A has been determined at 35,000 Daltons. The binding site of carboxypeptidase A has no affinity for basic amino acid residues and the action of the enzyme therefore stops when a basic residue appears at the C-terminal end of the peptide chain. Carboxypeptidase B can attack basic residues and therefore the action of the two enzymes is complementary.

#### 1.2.2.8 Proelastase.

Two elastases, which are secreted as proelastases, have been isolated from pancreatic juice. They are immunologically identical but are electrophoretically different. They are activated by trypsin liberating a peptide of about 11 amino acid residues, the sequence of which remains to be fully elucidated.

### 1.3 BLOOD BORN DEFENCES AGAINST ACTIVE PANCREATIC ENZYMES.

The body contains a number of antiproteases (table 1.4).

#### 1.3.1 $\alpha_1$ -antiprotease.

$\alpha_1$ -antiprotease is a glycoprotein of molecular weight 52,000 Daltons (Careli *et al* 1982) which was known formerly as  $\alpha_1$ -antitrypsin or  $\alpha_1$ -proteinase inhibitor (Schultze *et al* 1962). It has the highest concentration of any of the antiproteases (5 and 10 times those of  $\alpha_2$ -macroglobulin). Whilst it is capable of binding a number of serine proteases including trypsin, chymotrypsin, pancreatic and polymorph neutrophil elastase, cathepsin B, thrombin, plasmin, acrosin, kallikrein, factor Xa, factor XIa, collagenase and urokinase, its primary function is as an inhibitor of elastase (Teitz 1986). It may in addition act as a carrier molecule for other proteases (Oda *et al* 1977). Trypsin is transferred to  $\alpha_2$ -macroglobulin which is then eliminated from the circulation (Oda *et al* 1977). Following this  $\alpha_1$ -antiprotease is unable to bind proteases again (Johnson and Travis 1976, Oda *et al* 1977). Enzymes are completely inhibited once complexed with  $\alpha_1$ -antiprotease (Vergani *et al* 1983).

#### 1.3.2 $\alpha_2$ -macroglobulin.

Whilst the congenital absence of  $\alpha_1$ -antiprotease results in a condition similar to cystic fibrosis, a corresponding deficiency state has never been identified for  $\alpha_2$ -macroglobulin molecule; presumably because such a deletion is incompatible with life.  $\alpha_2$ -macroglobulin is a glycoprotein with a molecular weight of approximately 725,000 Daltons which is produced by the hepatocytes and is present in the serum with a concentration of 1.4- 3.7 gm/l (Dunn and Spiro 1967). Its function is to bind and eliminate activated proteases and is principally directed against serine proteases (Barrett and Starkey 1973). It is capable of binding trypsin, chymotrypsin, elastase, collagenase, papain, cationic aspartate aminotransferase, subtilin A, plasmin, thrombin and kallikrein (Barrett and Starkey 1973). It will not bind phospholipase A<sub>2</sub>. It is composed of 4 subunits each with a molecular weight



Antiprotease	Mol. wt. (Daltons)	Enzymes Inhibited	Site of Antiprotease Synthesis
$\alpha_2$ -macroglobulin	725,000	Endopeptidases	Liver, Macrophages, Fibroblasts, Adherent lung cells
$\alpha_1$ -antiprotease	55,000	Serine proteases	Liver
Antichymotrypsin	68,000	Chymotrypsin, Cathepsin G	Liver
Inter- $\alpha$ -trypsin inhibitor	160,000	Trypsin, Plasmin, Chymotrypsin, Cathepsin G	Liver
C-1-inhibitor	104,000	C1, C1s, C1r, Factors Xla, Xlla, Kallikrein, Plasmin	Liver
Antithrombin III	65,000	Thrombin, Factors iXa, Xa, Xla, Xlla, Plasmin, Trypsin	Liver
$\alpha_2$ -antiplasmin	70,000	Plasmin, Trypsin, Kallikrein, Thrombin, Chymotrypsin, Factors Xa, Xla	Liver

Table 1.4: The major serum antiproteases.

The mechanism by which  $\alpha_2$ -macroglobulin binds proteases is unique and was described by Barrett and Starkey (1973). They named it "the trap hypothesis". At the bottom of each of the hollows, situated at the end of the molecule, lies a "bait region". This is composed of a sequence of amino acids which represent the substrate for each of the proteases it is capable of capturing. When a protease molecule enters the hollow and hydrolyses an amide linkage in the bait region, the molecule undergoes a rapid conformational change resulting in the irreversible trapping of the protease.  $\alpha_2$ -macroglobulin is capable of trapping 1 or 2 molecules of protease (one in each hollow) even though only 1 one of the "traps" has been sprung (Harpel 1973). An additional consequence of the conformational change is the exposure of a binding site on the "waist" of the molecule which is recognised by specific cell surface receptors on Kupffer cells. The complex is rapidly endocytosed and destroyed by the Kupffer cells (Ohlsson 1971), the half life for clearance in normal individuals being approximately 10 minutes.

Whilst the binding of  $\alpha_2$ -macroglobulin to a protease is irreversible the complex suffers the disadvantage of retaining some proteolytic activity towards small molecular weight substrates (Haverbacks *et al* 1969, Rinderknecht *et al* 1973). It has even been suggested that the hypocalcaemia of acute pancreatitis follows the destruction of parathyroid hormone by this mechanism (Hermon-Taylor *et al* 1981). Evidence from a large (90 patient) study makes this unlikely in the clinical situation (Imrie *et al* 1978). The most severely ill patients in this study had the highest parathyroid hormone (PTH) concentrations and another study has noted PTH concentrations in excess of those found in patients with parathyroid adenomas, during the first few days of an attack of acute pancreatitis (Croton *et al* 1981).

### 1.3.3 Inter- $\alpha$ -trypsin Inhibitor.

The serum concentration of inter- $\alpha$ -trypsin inhibitor is low and it contributes only about 3% of the trypsin inhibitory capacity (Laureli and Jeppsson 1975).

Furthermore, the complexes formed with proteases are loose and *in vivo* it

probably does not act as a physiological inhibitor (Lasson 1984).

Concentrations of inter- $\alpha$ -trypsin inhibitor are usually low early in the course of an attack, returning towards normal, in severe attacks, after about 4 days (Lasson and Ohlsson 1984).

#### 1.3.4 $\alpha_1$ -antichymotrypsin.

$\alpha_1$ -antichymotrypsin, also acts as an acute phase reactant, exhibiting a very rapid increase in response to injury, similar to that seen for C-reactive protein (Laurell and Jeppsson 1975). The affinity of  $\alpha_1$ -antichymotrypsin for chymotrypsin is not strong enough to compete favourably *in vivo* with  $\alpha_1$ -antiprotease, which is present in much higher concentrations (Laurell and Jeppsson 1975). It contributes little to the total antiprotease capacity.

#### 1.3.5 Lipocortins.

Lipocortins are a group of related glycoproteins whose synthesis or secretion is stimulated by glucocorticoids and which specifically inhibit phospholipase  $A_2$ , *in vivo* and *in vitro* (Di Rosa *et al* 1984). Lipocortin I specifically inhibits the production of arachadonic acid from membrane phospholipids (Hirata 1984). Their production is inhibited by protein kinase C, epidermal growth factor receptors and viral oncogenes linked to protein kinase activity (Pepinsky and Sinclair 1986). The evidence that they actually inhibit phospholipase  $A_2$  is scanty and initial studies flawed (Davidson and Dennis 1989). At present their physiological importance in the inhibition of phospholipase  $A_2$  is unclear (Davidson and Dennis 1989).

### 1.4 CLASSIFICATION OF PANCREATITIS.

If communications regarding acute pancreatitis are to be meaningful, and treatment of the condition logical, it is necessary that it should be defined in a universally accepted manner. Whilst the pathologically based classification (Fitz 1989) was a great step forward, it did not take account of mild cases. A number of further methods of classification were devised. These were based on clinical,

functional and pathological information, (White 1966, Gambill 1973) or upon the aetiology (Joske 1955, Howard 1960). In 1963 an important conference was held in Marseilles, to which an international collection of the most prominent names in the field were invited, in an attempt to produce definitions for the various types of pancreatitis (Sarles 1963). They defined 4 types as set out below:-

- "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 produce clinical and biological restitution of the gland.
3. Chronic relapsing pancreatitis: defined as a condition in which acute exacerbations occur against a background of chronic disease.
4. Chronic pancreatitis: defined as a condition in which anatomical and/or functional damage occurs to the gland."

The original Marseille classification was found to have a number of problems. In particular it was not possible to distinguish between acute relapsing and chronic relapsing pancreatitis. Episodes of acute pancreatitis could result in permanent functional and morphological changes and patients with "silent" chronic pancreatitis could suffer severe acute attacks. A further international conference in Cambridge in 1983 (Sarnar and Cotton 1984) recognized these problems and produced a new set of definitions. They omitted acute and chronic relapsing disease and took into account the advances in pancreatic physiology and imaging techniques and omitted relapsing acute and chronic acute pancreatitis. The modified definitions that emerged stated:-

- "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."

A subsequent revision of the Marseille classification in 1984, (Sarles 1984) was in agreement with the Cambridge classification in that it only recognised acute and chronic pancreatitis. The definitions provided were however more thorough than those of the Cambridge meeting since they included morphological information.

#### **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 acute episode or recur.

Morphologically there is a gradation of lesions in acute pancreatitis. In the mild form 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 intra-pancreatic 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. Only rarely does acute pancreatitis lead to chronic pancreatitis.

#### **Chronic pancreatitis.**

Clinically, chronic pancreatitis is characterised by recurrent or persistent abdominal pain, although chronic pancreatitis may be present without pain. Evidence of pancreatic insufficiency, e.g., steatorrhea 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 duct dilatation may be found, but most often it is associated with stricture of the ducts or intra-ductal 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 the ducts. 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 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."

In spite of the 1983 Cambridge and 1984 Marseille conference a number of problems in the classification of acute pancreatitis remain. These include: occasional disparity between the morphological changes and outcome, the inability to know exactly what morphological changes have occurred within the gland and a lack of agreement on the definition of complications. Glazer and Ranson (1989) have suggested a number of guidelines to help in producing a future classification system.

"1. Any system of classification should differentiate between clinical and pathological definitions.

2. There should be 'operational categories' as described for chronic pancreatitis (Frey 1986). In chronic pancreatitis there is structural change within the gland and hence a greater emphasis on pancreatic imaging and function and less on the multisystemic and local effects of the disease; slightly different categories will thus be generated. In acute pancreatitis, aetiology should be included and also whether the attack is a first or recurring event.

3. The effects of pancreatitis which cause the morbidity and mortality should be considered separately."

**"Clinical definitions of acute pancreatitis:**

**Acute pancreatitis.**

This is an acute condition typically presenting with abdominal pain and usually associated with raised pancreatic enzymes in blood or urine, due to inflammatory disease.

*Mild acute pancreatitis.* This is acute pancreatitis with no multisystemic failure and with an uncomplicated recovery.

*Severe acute pancreatitis.* This is acute pancreatitis with either derangement of one or more body systems or the subsequent development of one or more local complications.

1. *Fulminant acute pancreatitis.* This is severe acute pancreatitis with life-threatening multisystemic failure within 48 hours of onset.

2. *Complicated acute pancreatitis.* This is severe acute pancreatitis with local complications.

These subgroups are not mutually exclusive. Where possible the aetiology of the attack should be indicated as well as whether the attack is primary or recurrent.

**Pathological definition of acute pancreatitis.**

The pathological features are based on macroscopic and microscopic examination and on imaging techniques.

*Oedematous pancreatitis.* This is a condition in which a swollen pancreas with histologically demonstrates fluid accumulation, fibrin and often, but not always, polymorphonuclear leucocyte infiltration.

*Haemorrhagic pancreatitis (focal, segmental or total).* This is a condition in which the pancreas shows either focal, segmental or total areas of reddening which histologically are areas of red cell extravasation or haemorrhage.

*Necrotising pancreatitis (focal, segmental or total).* This is a condition in which the pancreas shows either focal, segmental or total necrosis. Histologically, necrosis is destruction of normal parenchyma, usually with evidence of vascular and lymphatic inflammation and intraluminal occlusion. It must again be stressed that as the macroscopic and histological appearances do not always correspond, the pathological definition should be preceded by one or other qualifying terms, and again aetiology should be inserted where known."

Glazer's definitions of fulminant and complicated acute pancreatitis are somewhat limiting in that he associates systemic complications with the former and local complications with the latter. Whilst there is a tendency for these complications to be separated temporally, (Ranson *et al* 1977) multisystem failure occurring during the first week and local complications thereafter, the two patterns of complications are not mutually exclusive. The definition of mild acute pancreatitis also failed to include the absence of local complications. It is less confusing to continue with the present method of classification (attacks being either mild or severe) and to list the complications which occur.



### 1.5 INCIDENCE AND MORTALITY.

There have been a number of studies examining the incidence and mortality of acute pancreatitis within a defined population. The first was reported from Rochester, Minnesota in 1972 and covered a 30 year period up to 1969 (O'Sullivan *et al* 1972). The average annual incidence of acute pancreatitis was 110 cases per million population, with an increasing incidence between the first and second decades. This was attributed to the increased use of serum and urinary enzyme measurements in establishing the diagnosis of acute pancreatitis and has been noted by other authors (Trapneli and Duncan 1975, Bourke 1975, Wilson and Imrie 1990).

In 1975 Trapneli and Duncan published a review of 551 cases of acute pancreatitis seen within the Bristol area between 1950 and 1969. The annual incidence of acute pancreatitis was 53.8 cases per million population with a mortality rate of 9 per million population. A recent update of the Bristol figures for the period between 1968 and 1979 has demonstrated an increase in the incidence of acute pancreatitis to 73 cases per million population and mortality rate to 15.7 cases per million population. In spite of the increasing mortality rate the case mortality rate was 19.6%, a figure comparable to the 20.5% observed between 1950 and 1969. A similar case mortality rate was noted in Scotland by Wilson and Imrie (1990).

Bourke (1975) studied 202 patients admitted to Nottingham hospitals between 1969 and 1974. The average incidence of acute pancreatitis was 47.5 cases per million population with an average mortality rate of 9.2 cases per million population. These results were similar to those produced by Trapneli and Duncan (1975). However, it should be noted that the annual incidence varied between 21.2 per million in 1970 and 83 in 1974 (Bourke 1975). Such a wide variation in the annual incidence was also noted by Trapneli and Duncan (1975) and suggested that it was necessary to follow a population for a number of years before drawing conclusions regarding changes in the incidence of acute pancreatitis.

Wilson and Imrie (1990) have recently published a review of Scottish hospital in-patient statistics, for patients with a discharge diagnosis of acute pancreatitis, admitted between 1961 and 1985. The incidence of acute pancreatitis increased 11 fold during the study period, whilst the case mortality fell from 17.8% in 1961-65 to 5.6% in 1981-85. In common with other reports, (O'Sullivan *et al* 1972, Trapnell and Duncan 1975) much of the change was attributed to the introduction of the Phadebas amylase test in 1971. However, the increasing proportion of alcohol related attacks was thought to represent a real increase in the incidence of the condition since it paralleled an increase in alcohol consumption within Scotland over the same period (Wilson and Imrie 1990).

The highest annual incidences of acute pancreatitis in the UK have been reported from Leeds (110 cases per million population, McMahon 1977) and Edinburgh (238 cases per million population, Graham 1977). Whilst McMahon (1977) has suggested that the incidence of acute pancreatitis is probably related to the thoroughness with which the diagnosis is sought, (McMahon 1977) there are other factors which could account for the variation in the annual incidence figures.

Patients who have acute pancreatitis diagnosed during life comprise only a proportion of the total number of patients with the condition (Trapnell and Duncan 1975, Wilson and Imrie 1988). Between 22% (Buggy *et al* 1983) and 86% (Whalen *et al* 1971) of all deaths from acute pancreatitis are diagnosed at post-mortem examination. A recent review of 126 deaths from acute pancreatitis, occurring in Glasgow between 1974 and 1984, has demonstrated that 42% were first diagnosed at post-mortem examination (Wilson and Imrie 1988). The presentation was atypical in 68% of patients and serum amylase concentrations measured in only 9%. This suggests that the principal reason for missing the diagnosis is failure to consider the diagnosis or to measure serum or urinary amylase concentrations.

The proportion of patients in whom the diagnosis is missed altogether is unknown. The best method of detecting this group of patients is through the wide application of serum and urinary amylase measurements and post-mortem examination of all patients suffering an unexplained death. This has already lead to an "increased incidence" of acute pancreatitis (Trapnell and Duncan 1975, O'Sullivan *et al* 1972, Bourke *et al* 1975 Wilson and imrie 1990).

Factors influencing the mortality of acute pancreatitis include the age of the patients and aetiology of the attack (Trapnell 1966, Imrie 1974, Ranson *et al* 1974, Imrie and Whyte 1975, Jacobs *et al* 1977). Gallstones pancreatitis is associated with a higher mortality than alcohol related disease (Imrie and Whyte 1975, Jacobs *et al* 1977). This may simply reflect the age difference between the groups, those with gallstone related disease tending to be older (Imrie and Whyte 1975, Jacobs *et al* 1977). A higher mortality is also associated with postoperative pancreatitis, pancreatitis secondary to trauma (White *et al* 1970, imrie *et al* 1974, Ranson *et al* 1976, Imrie *et al* 1978) and attacks where no aetiological factor is identified (Corfield *et al* 1985, Jacobs *et al* 1977, Mayer and McIlMahon 1985). The latter may be because patients died before investigations were completed, or else that they were not well enough to undergo investigation.

The first attack of acute pancreatitis, is thought by some, to be more likely to be fatal than the second or subsequent attack (Trapnell and Duncan 1975, Ranson *et al* 1976, Trapnell and Duncan 1977, Satiani and Stone 1979) although this has not been demonstrated by all authors (Corfield *et al* 1985).

## 1.6 AETIOLOGY.

The two principal causes of acute pancreatitis are gallstones and alcohol. In a cumulative review of 3,836 patients drawn from 24 published articles between 1972 and 1978 these two conditions were considered to have accounted for 68% of attacks (Durr 1979). The next most important group were those in whom no definite aetiological factor could be identified, accounting for 22% of attacks (Durr 1979). There are many other causes of acute pancreatitis (table 1.5).

1. Gallstones	Hypothermia
2. Alcohol	Malignant hypertension
3. Idiopathic	Emboli
4. Periapillary obstruction	Dissecting abdominal aortic aneurysm
Ampullary tumour	9. Drugs
Pancreas divisum	Azathioprine
Annular pancreas	Thiazide diuretics
Ampullary stenosis	Sulphonamides
Choledochal cyst	Valproic acid
Duodenal diverticulum	Oestrogens
Duodenal duplication	Tetracycline
Malrotation of gut	Corticosteroids
5. Infection	L-asparaginase
Mumps	Chlorthalidone
Cocksackie B virus	Phenformin
Echo virus	Ethacrinic acid
Congenital rubella	Procainamide
Hepatitis A virus	10. Neoplasia
Epstein-Barr virus	Primary pancreatic cancer
Ascaris lumbricoides	Carcinoma of the ampulla of Vater
Clonorchia sinensis	Duodenal neoplasia
Mycoplasma pneumonia	Lymphoma
Leptospirosis	Metastatic tumour of the pancreas
Typhoid	11. Hereditary disease
Malaria	Hereditary pancreatitis
Lamblasis	Cystic fibrosis
6. Trauma	$\alpha_1$ -antiprotease deficiency
Pancreatico-biliary and upper abdominal surgery	12. Miscellaneous
ERCP and ES	Post-renal transplant
Blunt and penetrating abdominal injuries	Afferent loop obstruction
Trans-lumbar aortography	Thrombocytopaenic purpura
7. Metabolic disorders	Acute intermittent porphyria
Hypercalcaemia	Tropical pancreatitis
Hyperlipidaemia	Henoch-Schönlein purpura
Protein-calorie malnutrition	Systemic lupus erythematosus
Diabetic ketoacidosis	Sting of the Trinidad scorpion
Reye's syndrome	Occupational exposure to hydrocarbon
8. ischaemia	Post cardiac bypass surgery
Circulatory shock	
Cardiac surgery	
Vasculitis	

Table 1.5: Causes of acute pancreatitis.

### 1.6.1 Gallstones.

Koerte (1898) noted, at post-mortem examination, that there was an association between the presence of gallstones and acute pancreatitis. It was Opie (1901) who popularised the aetiological link and proposed the mechanism by which gallstones cause acute pancreatitis.

The incidence of gallstone pancreatitis varies between countries. In the UK it has been reported to be between 34% and 65% (Gillespie 1973, Trapnell and Duncan 1975, McMahon 1978, Corfield *et al* 1985, Leese *et al* 1988, London *et al* 1989, Larvin and McMahon 1989). In the United States the incidence varies between rural and inner city areas. In rural areas the incidence is similar to that seen in the UK, being between 27% and 64% (Weisseberg *et al* 1972, Peterson and Brooks 1979, Kelly 1980, Howard 1983). In the inner city areas the incidence is generally lower (3% to 45%) (Jordan and Spjut 1972, Olsen 1974, Howes *et al* 1975, Satiani and Stone 1979, Jiminez and Alderete 1983). This difference is related to the higher incidence of alcohol related attacks in inner city areas (22.5% to 92%) (Jordan and Spjut 1972, Olsen 1974, Howes *et al* 1975, Satiani and Stone 1979, Jiminez and Alderete 1983) than rural areas (14% to 33%) (Berman *et al* 1961, Weissenberg *et al* 1972, Peterson and Brooks 1979, Kelly 1980, Howard 1983).

Gallstone pancreatitis is predominantly a disease of women; the incidence being between 58% to 83% (Edlung *et al* 1968, Imrie and Whyte 1975, Acosta *et al* 1980, Kelly 1980, Stone *et al* 1981). This increases with age, as does the overall incidence of gallstones (Bell 1958). Imrie and Whyte have demonstrated that this female predominance disappeared in patients over 60 years of age (Imrie and Whyte 1975). In an unselected group of patients undergoing post-mortem examination, the incidence of acute pancreatitis lay between 0.14% and 1.3%, (Durr *et al* 1979) whereas in patients with gallstones the incidence was 4.7% (Kozoll *et al* 1959). The incidence of gallstones in 8,540 unselected post-mortem examinations was 21.9%, compared with 56.2% in 178 patients with acute pancreatitis undergoing post-mortem examination (Bell 1958).

One study from Bristol has suggested that the incidence of gallstone related pancreatitis is increasing (Holland and Heaton 1972), the reason being the increased number of cholecystectomies being performed. This view has not been borne out by two epidemiological studies from Bristol (Trapnell and Duncan 1975, Corfield *et al* 1985) which have demonstrated that the proportion of gallstone related attacks has remained unchanged. Others have found no evidence of a rise in the standardised prevalence rate of gallstones, although a spurious increase in incidence had been noted as a consequence of an increasingly elderly population (Bateson and Bouchier 1975).

#### 1.6.2 Alcohol.

Acute alcoholic pancreatitis is a condition that tends to occur in young men, often in their early to mid-thirties, who have usually been drinking steadily for 8 to 10 years (Howard 1983, Ehrlich *et al* 1960). Many of these patients develop recurrent attacks and a number go on to develop chronic pancreatitis (Ammann *et al* 1972). Whilst it has been suggested that alcohol will only cause acute pancreatitis in the presence of pre-existing chronic pancreatitis (Strum and Spiro 1971), a post-mortem study of patients with a history of chronic alcoholism, who died of acute pancreatitis, failed to demonstrate any evidence of chronic pancreatitis (Kioppel *et al* 1984).

The incidence of alcohol related disease in the UK is equal to that seen in rural USA (Gillespie 1973, Trapnell and Duncan 1975, McMahon 1980, Corfield *et al* 1985, Leese *et al* 1988, London *et al* 1989, Larvin and McMahon 1991) and appears to be increasing (Svensson *et al* 1979). In Glasgow Royal infirmary the incidence of alcohol related attacks has risen from 12% to 33%, over a decade (Imrie *et al* 1974, Blamey *et al* 1983). This is probably related to the increasing consumption of alcohol, especially of wine and spirits, which has almost doubled between 1950 and 1980 (Scottish Health Co-ordination Committee 1985).

### 1.6.3 Idiopathic.

This forms a rather unsatisfactory group of patients in whom no recognised aetiological factor can be identified. Three retrospective studies from the 1970's reported the incidence of idiopathic pancreatitis to be around 30%, (Imrie *et al* 1974, Bourke 1975, Trapnell and Duncan 1975) and agree with a larger, more recent prospective study (Mayer *et al* 1985) which demonstrated an incidence of 27%. Some of these cases may be caused by viral infection, (Imrie *et al* 1977) ischaemia (Moberg *et al* 1968) or undiagnosed gallstones. ERCP has been shown to be of value in these patients, demonstrating previously undiagnosed gallstones, (Hamilton *et al* 1982) or delineate anatomical abnormalities such as pancreas divisum (Cooperman *et al* 1981).

### 1.6.4 Drugs.

Drugs are a recognised cause of acute pancreatitis, but in many instances the evidence for the association is scanty (Mallory and Kern 1980, Lendrum 1981). Drugs for which there appears to be a definite association include the thiazide diuretics, (chlorothiazide and hydrochlorothiazide) L-asparaginase, azathioprine, valproic acid, sulphonamides, oestrogens and tetracyclines (Mallory and Kern 1980). Other drugs for which the evidence is less convincing include corticosteroids and adrenocorticotrophic hormone, (Nakashima and Howard 1977) chlorthalidone, ethacrynic acid, phenformin, procainamide and agents causing hypercalcaemia (Mallory and Kern 1980). The mechanisms by which these drugs cause pancreatitis may include direct toxicity (L-asparaginase), or indirect, for example by causing hyperlipidaemia (oestrogens). In occasional cases it is impossible to be sure that it is the drug and not the underlying condition, for which the drug is being prescribed, which is important, e.g. the use of steroids and azathioprine in the treatment of systemic lupus erythematosus. Unless no alternative drug is available to the patient for future use, challenge testing with a smaller dose of the suspected drug has been considered to be unjustified on ethical grounds, (Mallory and Kern 1980) although this is often the only way of proving the association.

### 1.6.5 Infections.

Mumps has been considered to be a cause of acute pancreatitis for many years, but in early studies the diagnosis was based purely on clinical criteria (Brahdy and Scheffer 1931). Imrie *et al* (1977) found rising antibody titres to mumps virus in 3 patients with acute pancreatitis. Of 116 patients studied prospectively, a total of 5 had rising titres to either mumps or coxsackie B virus (Imrie *et al* 1977). Another study found evidence of an acute enterovirus infection (Coxsackie B2,3,5 and echovirus) in 8 of 91 patients with acute pancreatitis (Arnesjo *et al* 1976). It may be that a proportion of patients with idiopathic disease have attacks precipitated by viral infection.

A rising titre to mycoplasma pneumoniae has been demonstrated in 33% of 27 patients with acute pancreatitis (Freiman *et al* 1978). Six of these patients had other possible aetiologies and the significance of this finding, and in particular, whether the rising titre indicates an acute mycoplasma infection, is unknown.

Occasionally parasites such as *Ascaris lumbricoides* and *Clonorchis sinensis* have been reported to cause pancreatitis by ductal obstruction (Durr 1979).

### 1.6.6 Postoperative pancreatitis.

Postoperative pancreatitis most commonly complicates operations on the biliary tree (particularly common duct exploration), gastric resection and splenectomy (Imrie *et al* 1978, White *et al* 1974). In a collective review Durr found a 5.8% incidence of postoperative pancreatitis amongst 5,864 cases of acute pancreatitis drawn from 41 series (Durr *et al* 1979). The mechanisms responsible were either direct trauma to the gland, interference with the sphincter of Oddi or, in the case of pancreatitis following Polya gastrectomy, stasis in the afferent loop, or possibly duodeno-pancreatic reflux (Wallensten 1958). Acute pancreatitis may also occur following a number of seemingly unrelated operations such as transurethral resection of the prostate (White *et al* 1970). Transient hypotension under



anaesthesia is a possible aetiological factor but, in at least some of these cases, the pancreatitis may have been coincidental. Pancreatitis has also been reported to occur after cardiopulmonary by-pass (Feiner 1976). Hypoperfusion and ischaemia have been considered the likely cause, but the associated hypothermia is another possible explanation. Pancreatitis has also been reported following cardiac transplantation (Adisehiah 1983) and may be due to the above factors, or possibly to the use of immunosuppressive agents such as azathiaprine and steroids, which have themselves been implicated in causing pancreatitis (Malory 1980). These agents may also account for the attacks of pancreatitis which follow renal transplantation in between 2% and 5% of cases, (Woods *et al* 1972, Taft *et al* 1978, Fernandez and Rosenberg 1980) and which have been associated with a mortality approaching 83% (Taft *et al* 1978).

Hyperamylasaemia and acute pancreatitis may complicate endoscopic retrograde cholangiopancreatography (ERCP) in 20% and 7% of cases respectively (Ruppin *et al* 1974). The avoidance of pancreatic duct overfilling and parenchymal opacification has reduced the incidence of this complication to less than 1% in a large American series (Bilbao *et al* 1976). Endoscopic sphincterotomy is recognised as adding to the risk of ERCP and currently the incidence of pancreatitis following ERCP and sphincterotomy is between 1.7% and 3% (Reiter *et al* 1978, Frost 1984).

#### 1.6.7 Tumours.

Acute pancreatitis may be the first indication of an underlying ampullary or pancreatic malignancy (Gambili 1972, Durr *et al* 1979, Robertson and imrie 1987). In a review of 12 reported series, only 26 patients out of a total of 1,912 (1.4%) had pancreatitis associated with pancreatic carcinoma (Durr *et al* 1979). In a study of 255 patients with various pancreatic tumours, 2.7% of the patients gave a history suggestive of acute pancreatitis (Gambili 1972). Pancreatitis may also occur due to metastatic tumour (Baslov *et al* 1983). Underlying carcinoma should

be considered, particularly in older patients in whom no other aetiological factor is identified (Hermon-Taylor 1977). Ductal obstruction with zymogen activation has been postulated as a cause (Allan *et al* 1973).

#### 1.6.8 Hypothermia.

In a post mortem study, 23 of 28 patients with accidental hypothermia were found to have macroscopic evidence of acute pancreatitis, ranging in severity from focal fat necrosis to frank haemorrhagic pancreatitis (Mant 1969). Imrie and Whyte (1977) found two cases of hypothermia amongst 78 patients with acute pancreatitis. Seven of the 8 cases of hypothermia reported by Foulis showed perilobular acinar cell necrosis, an appearance which had previously been shown to represent ischaemia (Foulis *et al* 1982).

#### 1.6.9 Trauma.

Acute pancreatitis occurs as a complication in between 1% and 2% of all abdominal injuries (Durr *et al* 1979, Heyse-Moore 1976). The pancreas is particularly susceptible to injury in its mid portion, where it crosses the spinal column. Disruption of the pancreatic ducts and tissues, with extravasation of enzymes, presumably leads to the development of acute pancreatitis.

#### 1.6.10 Hyperlipidaemia.

Estimates of the incidence of hyperlipidaemia in acute pancreatitis have ranged from 4% to 53% (Wang *et al* 1959, Kessler *et al* 1967). Patients with the familial hyperlipoproteinaemias (types I and IV) may experience frequent bouts of abdominal pain which are usually attributed to pancreatitis (Fredrickson *et al* 1978). These attacks are typically characterised by marked hypertriglyceridaemia with lactescent serum and can be prevented by the reduction of serum triglyceride concentrations. This suggests that the hypertriglyceridaemia itself triggers the pancreatitis, or at least plays an intermediary role (Cameron *et al* 1974). Others consider that the pancreatitis may be the cause of the hyperlipidaemia, (Wang *et*

*a/1959*, Ehrlich *et al/1960*) increased serum lipid concentrations also being found in most cases of experimental pancreatitis (Wang *et al/1958*, Stackhouse *et al/1966*). In a study from Glasgow it was found that most cases of hyperlipidaemia with acute pancreatitis were secondary to alcohol abuse (Dickson *et al/1984*). As alcohol can cause both hyperlipidaemia and pancreatitis, it was unclear whether the hyperlipidaemia or alcoholism were causally related to the pancreatitis (Dickson *et al/1984*). Cameron and co-workers have shown that the lipid abnormalities persist in these patients many months after the acute attack of pancreatitis. They suggested that it was unlikely that the lipid abnormalities were secondary to the acute attack of pancreatitis (Cameron *et al/1974*). Dickson and co-workers found that, with the exception of one patient with familial hyperlipoproteinaemias, the lipid abnormalities only persisted in those patients who continued to drink alcohol (Dickson *et al/1984*).

#### 1.6.11 Hyperparathyroidism.

Following several case reports of pancreatitis complicating hyperparathyroidism, Cope and co-workers appeared to confirm the association in a report of two cases from Boston (Cope *et al/1957*). In a subsequent study the same group found evidence of acute or chronic pancreatitis in 7.1% of 155 patients with hyperparathyroidism (Mixer *et al/1962*). Other studies did not support this view of a more than incidental association. One large study found only a single case amongst 590 patients with acute pancreatitis (Trapnell and Duncan 1975). A recent large study of 1,153 patients with hyperparathyroidism from the Mayo Clinic has also questioned the relationship. Only 17 patients (1.5%) were found to have co-existing or prior pancreatitis, 11 of these patients having other possible aetiological factors such as gallstones or alcohol abuse (Bess *et al/1980*).

#### 1.6.12 Vascular factors.

Since the experiments of Popper and co-workers in 1948, it has been known that impairment of the pancreatic blood flow predisposes to the production of acute pancreatitis (Popper *et al/1948*). Embolisation to the pancreatic arteries has been reported as a cause of acute pancreatitis in man (Probstein *et al/1957*).

In an autopsy study, 9 of 12 patients with "idiopathic pancreatitis" were found to have died in direct or close relationship to an acute cardiovascular episode such as a myocardial infarction or pulmonary embolus (Moberg *et al* 1968). The concept of shock as a causative factor in acute pancreatitis has been supported by others (Warshaw and O'Hare 1978).

#### 1.6.13 Pancreas divisum.

Pancreas divisum occurs when the embryological ventral and dorsal primordia fail to fuse with the result that pancreatic drainage occurs mainly through the accessory papilla. Pancreas divisum has been found in around 3% of control patients with biliary tract disease, but in as many as 12% of patients with pancreatitis (Richter *et al* 1981) and in 25.6% of patients with unexplained recurrent pancreatitis (Cotton 1980). The accessory papilla and Santorini duct have been postulated to be too small to accept the total pancreatic secretion, resulting in obstructive symptoms and pancreatitis (Cotton 1980). Others have questioned this association considering that the occurrence of pancreas divisum is purely a coincidental finding (Mitchell *et al* 1979).

#### 1.6.14 Pregnancy.

Pregnancy has in the past been considered as a cause of acute pancreatitis but a recent study has shown that nearly all such cases are due to gallstones, 18 of the 20 cases identified having gallstones demonstrated (McKay *et al* 1980). In the remaining cases pancreatitis was attributed to alcohol abuse in one and familial hyperlipidaemia in the other (McKay *et al* 1980).

#### 1.6.15 Hereditary.

Hereditary pancreatitis is an extremely rare cause of pancreatitis and prior to 1975 only 20 affected families had been reported in the literature (Anonymous 1975). The disease, which usually begins in childhood, is inherited as an autosomal dominant with incomplete penetrance (Silbert 1975). It is sometimes associated with aminoaciduria but in other cases there appears to be no specific metabolic defect (Anonymous 1975).

### 1.7 PATHOGENESIS OF ACUTE PANCREATITIS.

Although the exact pathophysiological events surrounding the induction of an attack of acute pancreatitis are incompletely understood, a number of theories have been put forward in explanation. It is not possible to discuss one theory without making reference to the others and so it may be that the true situation reflects a combination of events.

#### 1.7.1 Common Channel.

The common channel theory has been attributed to Opie (1901) who observed a stone impacted in the ampulla of Vater in a patient dying of haemorrhagic pancreatitis. He concluded:

"A small stone impacted in the diverticulum of Vater may occlude the common orifice of the bile duct and duct of Wirsung and convert them into a continuous closed channel. Bile enters the pancreas by way of the pancreatic duct and the pancreas becomes the seat of inflammatory changes characterised by necrosis of the parenchymatous cells, haemorrhage and the accumulation of inflammatory products. Anatomical peculiarities of the diverticulum of Vater do not permit this sequence of events in all individuals".

He induced haemorrhagic pancreatitis in dogs by the injection of bile into the pancreatic duct, although subsequent experiments have shown that it is also necessary to stimulate pancreatic secretion in order to produce pancreatitis (Gamklou and Edlund 1966, Hansson 1967). The infusion of bile alone into the pancreatic duct of cats led to an increase in ductal permeability and marked structural changes. When 16, 16 dimethyl prostaglandin E<sub>2</sub> is infused, the histology changes from oedematous to haemorrhagic pancreatitis (Wedgwood *et al* 1986).

Following Opie's original report, a number of authors performed studies to assess the frequency of a common channel. In a post mortem study of 100 patients Opie noted a common channel in 89 patients (Opie 1903). Subsequent studies demonstrated a common channel in between 3.5% (Mann and Giordano 1923) and 66% (Cameron and Noble 1924) of patients. Sterling (1954) demonstrated a common channel in 58% of post mortem specimens although he doubted that the channel was long enough to allow biliary-pancreatic reflux in more than 3%. Cameron and Noble (1924) and Howard and Jones (1947) addressed the problem more directly by placing a stone in the ampulla of Vater, at autopsy, and then injecting dye into the bile duct. Cameron and Noble (1924) demonstrated reflux in 66% and Howard and Jones (1947) in 54% of cases. Radiological studies by Kelly (1976) demonstrated biliary-pancreatic reflux in 67% of patients with pancreatitis as against 18% of controls and Taylor and Rimmer (1980) 52.4% against 16.6%. In spite of the results of these studies, Anderson *et al* (1960) and Elmslie *et al* (1966) doubted their clinical significance, since the pancreatic ductal pressure was normally higher than that of the bile duct and it was unclear whether biliary-pancreatic reflux occurred under physiological circumstances.

A stone impacted at the ampulla of Vater, as postulated by Opie, is only seen in 5% of post mortem examinations performed on patients dying of acute gallstone pancreatitis (McCutcheon 1968). However, a dedicated examination of the stools of patients with gallstone pancreatitis showed that 34 of 36 patients passed gallstones per rectum during the first 10 days of their illness, whereas this only occurred in 3 of 36 controls with gallstones but not pancreatitis (Acosta and Ledesma 1974). One study compared patients who underwent cholecystectomy and transduodenal sphincteroplasty with septotomy within 72 hours of admission to those who underwent surgery after 3 months. A higher incidence of stones in the bile ducts (75% vs 28%) and lying in the duodenum (31% vs 0%) occurred in those operated on within 72 hours (Stone and Fabian 1981). The authors concluded that gallstone related attacks of pancreatitis were associated with

transient bile duct obstruction at the ampulla of Vater. These findings have been confirmed by a study in which 50% patients undergoing ERCP within 48 hours of admission were shown to have stones within the common bile duct (Neoptolomos *et al* 1986). Additional evidence comes from studies demonstrating that gallstones in patients with acute pancreatitis were typically smaller and more numerous than those from controls and that the cystic duct tended to be wider (McMahon and Shefta 1980, Armstrong *et al* 1985).

Other causes of ampullary obstruction include papillary spasm (Archibald 1919, Doubelet and Mulholland 1948) and oedema of the duodenal mucosa (The 1972). Observation of the ampulla of Vater after the passage of a stone shows that it is rather lax and pouting and possibly allows the reflux of duodenal contents into the pancreatic duct (Williams and Busch 1907, McCutcheon 1968).

#### 1.7.2 Duodenal reflux.

The duodenal reflux theory states that duodenal contents are able to pass in a retrograde manner through the sphincter of Oddi and into the pancreatic duct (McCutcheon 1968). Here enterokinase, and possibly a number of other substances from the duodenal juice, activate the zymogens within the gland, resulting in the development of acute pancreatitis (Wanke *et al* 1966).

Under physiological conditions, reflux is prevented by the sphincter of Oddi, the mucosal folds of the intramural portion of the common channel and the high pressure within the pancreatic duct (McCutcheon 1968). The effectiveness of these mechanisms is however disputed (Archibald 1919, Csendes *et al* 1979). Archibald (1919) was unable to force duodenal contents through the sphincter of Oddi in spite of a persistently elevated intraduodenal pressure and Csendes *et al* (1979) has demonstrated that cyclical phasic contractions of the sphincter are an efficient means of preventing reflux. However, severe acute pancreatitis can be produced in dogs by the formation of a closed duodenal loop which leads to high

intraduodenal pressures. This effect is abolished by prior ligation of the pancreatic duct or infusion of trypsin inhibitors (Pfeffer *et al* 1957). An analogous situation to the Pfeffer loop occurs in man if the afferent limb of a Polya gastrectomy becomes obstructed. Wallensten (1958) reported 12 deaths from acute pancreatitis in 1,769 Polya gastrectomies and none following 605 Billroth I gastrectomies. Acute pancreatitis may also appear following luminal duodenal obstruction.

Further evidence for the causative role of duodenal reflux in the initiation of acute pancreatitis is provided by the observation that the insertion of a plastic tube through the ampulla of Vater may result in acute pancreatitis (Tuzhilin *et al* 1981) (although sphincterotomy and sphincteroplasty are associated with a relatively low incidence of acute pancreatitis, (Reiter *et al* 1978, Frost 1984). Dilatation of the ampulla following the passage of a gallstone (Williams and Busch 1907) may well be an important factor in the production of gallstone pancreatitis. A nearby duodenal diverticulum may also allow reflux to occur but has also been thought to cause ampullary obstruction (Heath *et al* 1987).

It is unclear which component of the refluxing duodenal juice causes pancreatitis. It would be reasonable to assume that enterokinase is responsible since it would activate the zymogens under normal circumstances (Wanke *et al* 1966). However the injection of enterokinase alone into the pancreatic duct in rats does not produce acute pancreatitis (Wanke *et al* 1966). It is not known whether this is also true in patients.

### 1.7.3 Obstruction hypersecretion.

Ligation of the pancreatic duct alone does not result in the development of acute pancreatitis, but if it persists, will lead to glandular atrophy (Isaksson 1983). Subsequent stimulation of the gland will however, lead to oedematous pancreatitis (Creutzfeldt and Schmidt 1970). These findings fit well with clinical experience which suggests that pancreatic ductal obstruction alone rarely leads to acute pancreatitis (Robertson and Imrie 1987).



Hyperstimulation of the pancreas of rats with caerulein leads to mild pancreatitis (Lampel and Kern 1977). Food and the venom from the Trinidad scorpion are both capable of stimulating pancreatic secretion and are associated with acute pancreatitis (Eggers 1924, Rich and Duff 1936).

A combination of obstruction and stimulation of secretion may also be produced by alcohol and may explain alcohol related acute pancreatitis. Alcohol causes constriction of the sphincter of Oddi, producing obstruction, and by increasing secretin production (The 1978), stimulates pancreatic secretion (Menguy *et al*/1958, Piroia and Davis 1986). Alcohol is also capable of producing protein plugs which can block and lead to the rupture of the more peripherally placed pancreatic ducts at times of increased secretion (Saries *et al*/1984).

#### 1.7.4 Back diffusion.

In common with the gastric mucosa, which protects itself from the back diffusion of  $H^+$  ions, the pancreatic ductal mucosa protects itself against the back diffusion of digestive enzymes (Reber and Mosley 1979). The intraductal infusion of bile salts and administration of aspirin or alcohol can effect the integrity of this barrier. Reber *et al* (1981) have shown that following such damage molecules with masses less than 20,000 Daltons can penetrate the mucosa. Since this corresponds to the size of many of the digestive enzymes it would be possible for them to leak into the parenchyma. Present evidence suggests that pancreatitis does not develop as long as the pancreatic enzymes are not activated prior to diffusion.

#### 1.7.5 Other theories.

A further possible factor which may predispose patients who drink excessive quantities of alcohol to developing acute pancreatitis is the decreased secretion of pancreatic secretory trypsin inhibitor which has been described in the pancreatic juice of alcoholic rats (Singh 1983) and from rabbit pancreas incubated *in vitro* with varying concentrations of ethanol (Steer *et al*/1979). Alcohol is also capable of

increasing the amount of enterokinase excreted in the bile, via the enterohepatic circulation (Grant 1986). All these factors taken together may lead to the activation of trypsinogen and other zymogens within the gland (Grant 1986). Certainly the degeneration products of trypsinogen have been detected in the pancreatic precipitates of patients with chronic pancreatitis (Figarella *et al* 1984).

Attack of the acinar cells by viruses (Creutzfeldt and Schmidt 1970), the production of intracellular lipid droplets in hyperlipidaemia (Braunsteiner *et al* 1967), increased glandular secretion associated with hypercalcaemia (Goebell and Hotz 1976), and the effects of bacterial infection have all been described in relation to the aetiology of acute pancreatitis and pertain under specific circumstances.

### **1.8 POSSIBLE MECHANISMS OF GLANDULAR DAMAGE IN ACUTE PANCREATITIS.**

#### **1.8.1 Pancreatic enzyme activation.**

In 1886 Krebs suggested that the severe inflammatory changes seen in acute pancreatitis were produced by the "pancreatic ferments" (Glazer and Ranson 1988). In 1896 Chiari put forward the view that autodigestion of the gland, resulting from the inappropriate intraglandular activation of pancreatic zymogens, was the main cause of pancreatic necrosis (Glazer and Ranson 1988). Opie and Meakins (1909) went further and suggested that trypsinogen was the principal zymogen implicated in this process. Trypsinogen is found in a greater concentration than any other zymogen and once activated by enterokinase, has the capability to activate further trypsinogen as well as all the other zymogens (Hermon-Taylor and Heywood 1985, Rinderknecht 1986). In acute necrotising pancreatitis this activation is thought to escape inhibition (Hermon-Taylor and Heywood 1985). Both Hermon-Taylor and Heywood (1985) and Rinderknecht (1986) have postulated that the differences between oedematous and necrotising acute pancreatitis can be explained in terms of the biochemical events. During an attack of mild or oedematous acute pancreatitis, acinar cell damage leads to the

release of amylase and lipase. The patient experiences all the symptoms of acute pancreatitis and may in addition develop fat necrosis. There is however, no activation of the zymogens and pancreatic necrosis does not develop. During an attack of severe or necrotising pancreatitis, there is again a release of amylase and lipase, but in addition there is activation of the pancreatic zymogens. This leads to the features described above for oedematous pancreatitis but with pancreatic necrosis.

The validity of this theory is difficult to assess (Katz *et al* 1964) but doubts have been raised about the central role of proteases in the initiation of acute pancreatitis (Beck *et al* 1962, 1971, Schmidt and Creutzfeldt 1969). Schmidt and Creutzfeldt (1969) suggested that the principal zymogen involved is phospholipase A<sub>2</sub>. It has the potential to be particularly damaging to living cells since it digests lecithin and cephalin, which are essential components of cellular membranes. Phospholipase A<sub>2</sub> has been demonstrated in the pancreatic tissue, peritoneal fluid and blood of dogs in whom acute pancreatitis has been induced (Schmidt and Creutzfeldt, Hatao 1969) as well as in the peritoneal fluid of patients with acute pancreatitis (Schmidt and Creutzfeldt, Hatao 1969, Thouvenot *et al* 1974). In addition the histological appearances of pancreatic necrosis in rats, which have had acute pancreatitis induced by the intraductal injection of phospholipase A<sub>2</sub>, correspond closely with those found in man (Schmidt and Creutzfeldt 1969).

During autolysis of the gland phospholipase A<sub>2</sub> (Grossman 1958, Reeman and Shapiro 1959) and lysolecithin (Grossman 1958, Reeman and Shapiro 1959, Masshoff *et al* 1964) concentrations increase. Lysolecithin (the breakdown product of lecithin), when infused into the pancreatic duct of experimental animals, also leads to severe pancreatic necrosis (Schmidt and Creutzfeldt 1969, Wanke 1970) and fat necrosis throughout the abdominal cavity (Schmidt and Creutzfeldt 1969, Grossman 1958, Reeman and Shapiro 1959, Masshoff *et al* 1964, Poncelet and Thompson 1972). This is due to the fact that lecithin is itself toxic to cells.

Experimental studies in rats whose pancreatic ducts have been injected with bile demonstrated increased concentrations of lysolecithin Grossman 1958 and Reeman and Shapiro 1959 Masshoff *et al* 1964 Poncelet and Thompson 1972 (Arnesjo 1971). The demonstration of the enterohepatic recycling of enterokinase into the bile may be particularly important in this context since it can activate trypsinogen which in turn activates phospholipase A<sub>2</sub> (Grossman 1958 and Reeman and Shapiro, 1959 Masshoff *et al* 1964, Arnesjo 1971, Poncelet and Thompson 1972, Grant 1986). The presence of bile salts and lysolecithin, derived from the lecithin in bile, destabilise and dissolve the acinar cell membranes and zymogen granular membranes leading to autolysis (Grant 1986).

Whilst the above theories suggest that zymogen activation is extracellular, at least two types of pancreatitis, caerulein induced pancreatitis in rats (Lampe and Kern 1977) and diet induced pancreatitis in young female mice (Lombardi *et al* 1975) arise as the result of the abnormal production or storage of zymogens.

In diet induced pancreatitis in mice, microscopic studies have demonstrated fusion (crinophagy) of zymogen and lysosomal vacuoles (Lombardi 1975, Salto *et al* 1987). Subcellular fractionation has confirmed the mixing of these 2 enzyme types by demonstrating the presence of cathepsin B in the subcellular fraction that normally contain only zymogens (Saija *et al* 1987). Since cathepsin B is capable of activating trypsinogens, (Greenbaum *et al* 1961, Greenbaum and Hirschowitz 1961) it is presumed that the mixing of these enzymes is the primary pathological event (Steer *et al* 1984). Lombardi (1975) also demonstrated a breakdown in granular membranes and the release of zymogens into the cytoplasm and large autophagosomes. Although the structure of most zymogen granules was normal, a number showed degeneration proceeding from the periphery towards the centre, a finding also seen in surgical specimens from patients with acute pancreatitis (Willemer *et al* 1989). Similar changes have also been noted in spontaneous exocrine pancreatic insufficiency, which occurs in mice, and which has been

attributed to uncontrolled intraglandular trypsinogen activation (Eppig and Leiter 1977, Leiter *et al* 1977). The relationship of these findings to human acute pancreatitis is far from clear.

#### 1.3.2 The reversal of secretory polarity.

The segregation model of pancreatic secretion is widely accepted. However, it fails to explain the rapid discharge of pancreatic digestive enzymes (Rothman 1976) occurring within half an hour of stimulation of the gland, or their non parallel secretion (Rothman 1967). An alternative theory was therefore proposed (the equilibrium theory) which states that there is a pool of enzymes within the cytoplasm which is in equilibrium with those contained within the zymogen granules. This view is radical in that it goes against the accepted view that a cell can secrete either via a regulated or constitutive mechanism, but not both. However Arvan and Castle (1987) suggest that constitutive and regulated secretion could occur together in pancreatic acinar cells. If this is the case then it would be possible for digestive enzymes to be secreted across the basolateral membrane of the acinar cell.

The importance of this theory is that it might provide an explanation of the mechanism whereby pancreatic enzymes enter the circulation during an attack of acute pancreatitis (Janowitz and Hollander 1951, Palade 1975, Papp *et al* 1980) particularly if the apical discharge of pancreatic enzymes is blocked (Braganza 1988, Steer and Meldolesi 1988). Whilst the fusion of zymogen granules with the basolateral membrane of acinar cells had never been observed under physiological conditions, there is conflicting evidence regarding its occurrence in the caerulein model of acute pancreatitis (Lampel and Kern 1977, Adler *et al* 1982, Salto *et al* 1987, Scheele *et al* 1987).

A number of experiments were therefore devised to test the equilibrium theory. Preliminary experiments excluded the absorption of pancreatic enzymes from the small bowel or pancreatic ductal system as possible sources of circulating

enzymes (Rohr 1986, Musar and Case 1991). Male Sprague-Dawley rats were then injected with radiolabelled amino acids 48 hours before the collection of samples of pancreatic juice and blood. The delay ensured that these radiolabelled amino acids were incorporated into enzymes within the zymogen granules. Stimulation of the gland with optimal doses of cholecystokinin (CCK) produced a 3.3 fold increase in the concentrations of radiolabelled pancreatic juice but no change in the concentration within the blood. Hyperstimulation of the gland with CCK resulted in inhibition of pancreatic juice secretion and a 2.3 fold increase in the amount of amylase (which was not radiolabelled) in blood. This demonstrated that the zymogen granules were not the source of pancreatic enzymes found within the blood (Musa and Case 1991). Furthermore pancreatic secretion into the ductal system was inhibited by "high dose" CCK, probably via stimulation of the low affinity receptors (Sato *et al*/1989).

In further experiments involving the isolated perfused rat pancreas samples of pancreatic juice, venous effluent and gland exudate were collected following the intra-arterial injection of radiolabelled amino acids (Musa and Case 1991). During the first 30 minutes only 0.02% of the label was detected within the pancreatic juice, whilst 17% appeared in the exudate and 4.3% in the venous effluent. In the next 30 minutes 8.5% and 1.35% appeared in the exudate and venous effluent respectively. Hyperstimulation of the gland with CCK lead to the production of 23.5% in exudate and 23.2% in venous exudate. The concentrations decline after this time. These results are in agreement with the view that the enzymes in the exudate and venous blood are arising from a cytoplasm pool. Whilst these experiments are only preliminary, they provide an exciting new insight into the biochemical events surrounding acute pancreatitis.

### 1.3.3 Free radicals.

In spite of the variety of aetiological factors associated with the production of an attack of acute pancreatitis the clinicopathological expression of each is remarkably similar (Sanfey 1991). This suggests that there is a mechanism of injury common to them all and one possible explanation is that damage occurs through the production of free radicals (Sanfey 1991).

A free radical is a chemical species with an unpaired electron in its outer shell (Dormandy 1983). This makes it chemically unstable and therefore reactive. Molecular oxygen is the major biological source of free radicals and has been implicated in the production of endothelial damage in the heart, lungs, small bowel and pancreas (Bulkley 1983, Gardner *et al* 1983, Taylor 1983). Whilst most molecular oxygen is reduced tetravalently by intracellular reduction systems such as the cytochrome oxidase complex found within mitochondria, (Antonini *et al* 1970) a small proportion is metabolised univalently, through electron transport shuttles in the membranes of activated phagocytes, microsomes and mitochondria. This results in the rapid sequential production of three species: a superoxide anion free radical, hydrogen peroxide and a hydroxyl free radical. Under normal circumstances these species are metabolised by superoxide dismutase (SOD), an endogenously produced enzyme, that catalyses the conversion of oxygen free radicals to hydrogen peroxide (McCord and Fridovich 1969). Hydrogen peroxide is then reduced to water and oxygen by cytoplasmic peroxidases and peroxisomal catalases, whilst metal binding proteins inhibit the production of hydroxyl ions (Dei Maestro *et al* 1980). In pathological conditions oxygen free radicals production overwhelms the protective enzymes and oxidative stress results (Fridovich 1978, McCord and Fridovich 1978, Dei Maestro *et al* 1979, Dei Maestro *et al* 1980, Sies 1985). Cellular injury and death are produced by the destruction of hyaluronic acid and collagen in the extracellular matrix, the disruption of the cell membranes as well as membranes surrounding lysosomes and mitochondria (Halliwell 1978, Greenwald and Moy 1979). Lipid peroxide radicals are produced and lead to the production of further toxic products such as malondialdehyde (Kellogg and Fridovich 1975, Goldstein and Weissman 1977).

ischaemic injury is thought to be a common cause of free radical production, the effect being mediated by xanthine oxidase (Parks *et al* 1983). This enzyme is normally present in the inactive form, xanthine dehydrogenase, but is activated by ischaemia (Parks *et al* 1983). It also results in the conversion of adenosine 5 triphosphate to adenosine monophosphate and the accumulation of hypoxanthine, an important substrate for xanthine oxidase (Parks *et al* 1983). When oxygen becomes available again, as the tissue is reperfused, free radical production occurs and ischaemia injury supervenes (Parks *et al* 1983).

Early experimental studies, utilising the isolated canine model of acute pancreatitis, (Hermon-Taylor 1968) simulated three different aetiologies: alcoholism with hyperlipidaemia (free fatty acid infusion), gallstones (partial duct obstruction and secretion stimulation) and ischaemia (2 hours of total ischaemia). Pretreatment with the free radical scavengers SOD and catalase reduced the degree of gland oedema and serum amylase concentrations in each model (Sanfey 1984). SOD alone was as effective as SOD and catalase in the ischaemic model suggesting that oxygen free radicals were important. In the other models catalases were also required, suggesting that injury was also produced by hydroxyl ions (Sanfey 1984). Subsequent experiments demonstrated that allopurinol was as effective as SOD and catalase in reducing oedema and hyperamylasaemia and suggested that xanthine oxidase was the principal source of free radical production in oedematous pancreatitis (Sanfey 1985). It is currently suggested that chymotrypsin is somehow activated as an early event in the production of an attack of acute pancreatitis and leads to oxidative stress through the activation of xanthine dehydrogenase (Batelli *et al* 1973).

The administration of allopurinol shortly after the initiation of an attack of caerulein induced pancreatitis was also effective in reducing the degree of pancreatic oedema and hyperamylasaemia (Sarr *et al* 1978). This is not the case in the more severe forms of the disease. Both dimethylsulphoxide and allopurinol failed to



reduce the hyperamylasaemia and mortality associated with choline deficient ethionine supplemented acute pancreatitis (Rutledge *et al* 1987). Allopurinol alone was equally ineffective in sodium taurocholate induced pancreatitis (MacGowan *et al* 1987, Lankish *et al* 1989) as were antioxidant enzymes (Braganza *et al* 1988, Blind *et al* 1988). However subsequent studies have demonstrated that repeated doses of allopurinol will reduce the severity of the attack (Dergertekin *et al* 1984). Guyan *et al* (1988) have demonstrated that neither hydrogen peroxide, nor superoxide or hydroxyl radicals are capable of activating zymogens.

Another possible source of free oxygen radicals leading to pancreatic damage, is the leucocyte (Kakinuma 1974, Badwey *et al* 1981, Badwey *et al* 1984). However 98% depletion of circulating leucocytes has no effect on disease severity in isolated canine pancreas (Sarr *et al* 1987). On the basis of these findings Sanfey *et al* (1990) have suggested that xanthine oxidase within the pancreatic acinar cells is the primary source of oxidative stress. It is however possible that resident phagocytes, or those attracted to the gland as part of the inflammatory response, could be involved in sustaining injury (Sanfey *et al* 1990).

#### 1.3.4 Excessive leucocyte stimulation.

Recently, Rinderknecht (1991) has expressed doubts about the role of Intraglandular zymogen activation in the initiation of acute necrotising pancreatitis. He points out that there are a complex series of mechanisms designed to stop the inappropriate activation of zymogens (Rinderknecht 1986, Rinderknecht *et al* 1988) and that even if zymogens are inappropriately activated within the gland, they are not toxic unless associated with the presence of bile (Creutzfeldt and Schmidt 1970) or some other agent which produces acinar cell damage (Reber *et al* 1979). The fact that pancreatic extracts, obtained from patients with acute pancreatitis, are devoid of proteolytic activity (Rinderknecht 1988) and the failure of intravenous or intraperitoneal antiprotease therapy to improve the mortality or morbidity of the condition, reinforces the view that active zymogens are probably unimportant.

Whilst therapeutic trials of antiproteases have failed to demonstrate consistent benefit, this may be due to the delay involved in instituting therapy rather than the therapy itself (Steinberg 1988).

For these reasons Rinderknecht has postulated that there is an alternative mechanism of tissue injury and that this is caused by excessive leucocyte stimulation. During an attack of acute pancreatitis neutrophils and cells of the monocyte/ macrophage series are seen to move into the pancreas, possibly as the result of the release of reactive oxygen species (Rinderknecht 1988, Braganza and Rinderknecht 1988). Overactivity of the cells leads to "overt secretion" with the release of oxygen free radicals, polymorphonuclear elastase (PMN elastase), phospholipase A<sub>2</sub> and C, cathepsins, collagenase, DNase, RNase, phosphatase and glycosidases, (Rinderknecht 1988, Braganza and Rinderknecht 1988). The superoxide dismutase and catalase become overwhelmed and lead to further tissue damage, more superoxide release and further influx of leucocytes (Rinderknecht 1991). Acinar cell damage would lead to the release of lysosomes, further digesting leucocytes and worsening tissue damage (Rinderknecht 1991). Hydrogen peroxide produced by myeloperoxidase may damage  $\alpha_1$ -antiprotease, reducing its inhibitory capacity against PMN elastase (George 1984). Whilst evidence for this view is inconclusive it should be noted that granulocyte depletion of sheep prior to the induction of acute pancreatitis reduces pulmonary endothelial damage (Barie *et al* 1982). PMN elastase and phospholipase A<sub>2</sub>, which are released from damaged granulocytes, have been implicated in ARDS (Edelson *et al* 1991). Tumour necrosis factor (TNF), which is secreted by monocytes and polymorph leucocytes, has also been implicated in the production of septic shock (Tracey *et al* 1986). Support for its role in this context comes from the fact that the injection of anti-TNF antibodies protects experimental animals from normally fatal doses of endotoxin (Tracey *et al* 1986). High concentrations of TNF have been demonstrated in the serum of patients with severe acute pancreatitis, (Exley *et al* 1990, Banks *et al* 1991) although it is not possible to infer a causal relationship between elevated TNF concentrations and severe pancreatitis from these studies.

In spite of the above theories the true sequence of events surrounding the induction of an attack of acute pancreatitis in man remains unclear. Whilst animal models of pancreatitis are readily amenable to investigation, our understanding of the cellular events in acute pancreatitis in man is limited to biochemical investigations and histological examination of operative and post mortem specimens.

#### 1.9 PANCREATIC DEFENCE AGAINST AUTODIGESTION.

Pancreatic enzymes have the capacity to digest a wide variety of tissues including the pancreas itself. A number of mechanisms have therefore been evolved to reduce this risk to a minimum.

In order to ensure this, many of the pancreatic enzymes (zymogens) are secreted as inactive pro-enzymes (Hermon-Taylor and Heywood 1985, Rinderknecht 1986). Trypsinogen is unique amongst them in that it has a specific activator, enterokinase (Grant and Hermon-Taylor 1976) whilst all the others are activated by trypsin (Hermon-Taylor and Heywood 1985, Rinderknecht 1986). Since trypsin will also activate trypsinogen (the kinetics of activation following a sigmoidal curve) it is easy to see that even small quantities of enterokinase within the pancreas could lead to massive inappropriate zymogen activation and pancreatitis (Hermon-Taylor and Heywood 1985, Rinderknecht 1986). For this reason the zymogens and enterokinase are kept separate, the latter only being produced at the site of physiological activation of zymogens within the duodenum (Hermon-Taylor *et al* 1977). Pancreatic juice has a pH of between 8 and 9.5 and a low calcium concentration, both of which favour degradation rather than activation of trypsinogen (Colomb *et al* 1979). Should activation occur within the gland then the activated zymogens can to some extent be inhibited by pancreatic secretory trypsin inhibitor (PSTI) which is produced in parallel with the zymogens and is found within the zymogen granules. This is a protein with a molecular weight of 6,500 Daltons which makes up 1-3% of the protein in pancreatic juice (Pubols *et*

*et al* 1974). Small amounts are normally detectable in serum, ( $<10 \mu\text{g/l}$ ) although much higher concentrations have been recorded in acute pancreatitis (Eddeland and Ohlsson 1978). Available evidence suggests that PSTI acts purely as a local inhibitor in the pancreatic juice, as in the presence of the serum antiproteases  $\alpha_2$ -macroglobulin and  $\alpha_1$ -antiprotease, its complex with trypsin is immediately broken (Balldin *et al* 1984). Its action is specifically directed against trypsin and it is present in sufficient concentrations to be able to inhibit 20% of the available trypsin. The complex does however have a relatively high dissociation constant. If a sensitive assay is employed small amounts of trypsin can still be detected even when a large excess of pancreatic secretory inhibitor is present. This fits well with the observation that the addition of even small amounts of trypsin to pancreatic juice makes it unstable.

Pancreatic juice contains, in addition to trypsinogen 1 and 2, small quantities of mesotrypsin (Rinderknecht *et al* 1986). This variant of trypsin is completely resistant to pancreatic secretory trypsin inhibitor and might be thought to lead to runaway zymogen activation. Paradoxically it tends to have the opposite effect contributing to the stability of pancreatic juice by virtue of the fact it degrades zymogens (Rinderknecht *et al* 1986).

The pancreatic duct is resistant to the effects of activated zymogens, preventing their diffusion into the pancreatic parenchyma (Reber *et al* 1979).

#### 1.10 DIAGNOSIS OF ACUTE PANCREATITIS.

Acute pancreatitis can only be definitely diagnosed at laparotomy or post mortem examination. As most patients recover without the need for surgery the diagnosis is based on elevated concentrations of a pancreatic enzyme in the presence of a compatible clinical picture. The most commonly utilised enzyme is amylase.

#### 1.10.1 Blood amylase.

The value of amylase estimation in the diagnosis of acute pancreatitis was first reported in 1929 (Elman *et al* 1932).

The standard method of measuring serum amylase concentrations is the Phadebas test (a colourimetric assay which measures the amount of starch split by the enzyme) It does not distinguish between the iso-enzymes of amylase. In spite of this it is uncommon for diagnostic confusion to arise for this reason alone (O'Donnell *et al* 1977, Warshaw 1977, Kolars *et al* 1984). This has been confirmed by a number of studies which have failed to demonstrate consistently any major increase in diagnostic accuracy for a P iso-amylase assay (Warshaw 1977, O'Donnell *et al* 1977, Kolars *et al* 1984).

The normal range of the Phadebas test is 60-160 Somogyi units /100ml although there is considerable variation in the concentration accepted as diagnostic of acute pancreatitis. American groups have chosen cut off concentrations of 200 Somogyi units/100ml (Ranson *et al* 1976, Spechler *et al* 1983), 400 Somogyi units /100ml (Foster and Ziffren 1962) 800 Somogyi units /100ml (Kelly 1976) and a concentration of greater than 5 times normal, (White *et al* 1970) as criteria for making the diagnosis. Trapnell only diagnosed acute pancreatitis where the serum amylase concentration exceeded 1000 Somogyi units/100ml (Trapnell 1966). This probably reflects the higher incidence of gallstone related attacks in this country (Ranson *et al* 1976, MRC working party 1977). Most recent series from the USA have quoted the diagnostic range in international units per litre (IU/l). One Somogyi unit is equivalent to 1.85 IU/l (Salt *et al* 1976). The normal range is between 70 and 300 IU/l with diagnostic concentrations varying between 1000 IU/l (Thompson 1985), 1200 IU/l (Imrie *et al* 1978), and 2000 IU/l (MRC 1977).

The sensitivity of serum amylase measurements in the diagnosis of acute pancreatitis is difficult to assess since an elevated serum amylase is frequently the criteria on which the diagnosis is based. In a study by Steinberg *et al* (1985), in

which objective criteria were applied (ultrasound, computer tomography scanning, or laparotomy), the sensitivity of serum amylase measurements in diagnosing acute pancreatitis was 95%. This sensitivity is affected by factors such as an associated chronic pancreatitis (Nordestgaard *et al*/1988), hyperlipidaemia (Lesser and Warshaw 1975), the aetiology of the attack (Ranson *et al*/1976, MRC working party 1977) and the delay from the onset of symptoms (Ventrucci *et al*/1985).

The specificity of serum amylase estimation is adversely affected by a variety of conditions which can produce hyperamylasaemia (table 1.6). While few are likely to provide difficulty in the differential diagnosis of acute pancreatitis, other acute abdominal conditions, which can be associated with hyperamylasaemia, may. These include perforated peptic ulcer, mesenteric infarction, intestinal obstruction, biliary tract disease, ruptured ectopic pregnancy, afferent loop syndrome, dissecting aortic aneurysm, peritonitis, and acute appendicitis (Janowitz and Dreiling 1959, Salt *et al*/1976). The diagnostic confusion that they cause is affected by the cut off amylase concentration. Utilising a cut off concentration of 300 IU/l Pace *et al*/(1985) correctly diagnosed all cases of acute pancreatitis, but in addition incorrectly diagnosed acute pancreatitis in all patients with ruptured abdominal aortic aneurysms and 66% of patients with tubo-ovarian disease. With a cut off concentration of 1000 IU/l Thomson *et al*/(1987) achieved a specificity of 100% but at the expense of a reduced sensitivity of 61%.

#### 1.10.2 Urinary amylase.

Urinary amylase excretion has been considered to be a more sensitive index of acute pancreatitis than serum amylase estimations (Saxon *et al*/1957). Urinary amylase has been reported to rise earlier and higher and to persist for longer (Salt *et al*/1976). Hyperamylasuria, like hyperamylasaemia, is not necessarily diagnostic of acute pancreatitis, nor does a normal urinary amylase exclude its presence (Durr *et al*/1979). The normal half life of amylase in man is 130 minutes with 24% of the serum amylase being cleared through the kidneys and the rest being cleared

Pancreatic disease	Acute pancreatitis Chronic pancreatitis Trauma Pancreatic carcinoma Periapillary tumours
Hepatobiliary disorders	ERCP and ES
Gastrointestinal disorders	Perforated peptic ulcer Acute intestinal obstruction Mesenteric infarction Intestinal perforation Afferent loop syndrome
Gynaecological disorders	Salpingitis Torted ovarian cyst or tumour Ruptured ectopic pregnancy Endometriosis
Salivary gland disorders	Mumps Parotitis Sialadenitis Trauma Tumours Maxillo-facial surgery
Renal disease	Acute renal failure Chronic renal failure
Macroamylasaemia	
Neoplasia	Lung tumours Ovarian cystadenocarcinoma
Miscellaneous causes	Dissecting aortic aneurysm Post operative especially abdominal and cardiac surgery Diabetic ketoacidosis Intravenous steroids

Table 1.6: Causes of hyperamylasaemia. ERCP = endoscopic retrograde cholangiopancreatography, ES = endoscopic sphincterotomy.

via an extrarenal mechanism, possibly the reticuloendothelial system (Duane *et al* 1971). The renal clearance rate is 2-3 ml/hr against a creatinine clearance of 100 ml/hr (Levitt 1969). From this the amylase-creatinine clearance ratio (ACCR) can be calculated (Levitt 1969). The abnormal ACCR seen in patients with acute pancreatitis has been attributed to a direct effect of the disease on the kidney. This may be mediated by changes in glomerular permeability or tubular reabsorption (Warshaw and Fuller 1975) or through the production of a reversible renal tubular defect (Johnson *et al* 1976).

Whilst initial reports displayed a high diagnostic accuracy (Murray and McKay 1977) subsequent studies, in which patients were compared against those with other abdominal conditions rather than normal controls, have failed to maintain these good results (Farrer and Calkins 1976, McMahon *et al* 1982). This test is not widely used and is of doubtful clinical value (McMahon *et al* 1982, Moossa 1984).

#### 1.10.3 Lipase.

The measurement of serum lipase concentrations should be a more sensitive indicator of the presence of acute pancreatitis than serum amylase measurements since enzyme production is almost totally confined to the pancreas (Kasper and Sommer 1986). Beck (1967) noted that lipase concentrations were higher and persisted longer than those of amylase, as did Song *et al* (1970) and Lifton *et al* (1974). Patt *et al* (1970) found that on admission serum concentrations of lipase were elevated in 95% of 31 patients with acute pancreatitis whereas only 75% had elevated serum amylase concentrations. The duration of elevation was similar for both enzymes. Lifton *et al* (1974) found the converse, 70% of patients having elevated serum amylase concentrations, whilst only 63% had elevated concentrations of lipase. Increased concentrations of lipase are not seen within the serum of patients with salivary gland disease (Ticktin *et al* 1965) or macroamylasaemia, (Long and Kowlessor 1972). However, lipase measurements still suffer from the drawback that serum concentrations are elevated in a number of acute abdominal emergencies such as perforated small bowel, perforated peptic ulcer and mesenteric ischaemia (Warshaw and Feller 1977).



The assay has in the past been complex and fraught with technical problems (Zieve 1964, Calkins 1968). However, the recent development of turbidometric assays, has made its use in automated analysers possible (Vogel and Zieve 1963, Berk 1967, Zinterhofer *et al* 1973). It should probably replace amylase as the diagnostic test of choice (Kolars *et al* 1984). Unlike amylase, lipase is not normally excreted in urine (Rick 1972) although it may be detected where renal tubular damage has occurred (Muench 1987).

#### 1.10.4 Trypsin.

Most of the trypsinogen in the body is found within the pancreas (although small quantities have been demonstrated in the Paneth cells of the small intestinal mucosa, (Bohe *et al* 1986) and for this reason it was thought that serum concentrations of trypsin would correlate well with the degree of pancreatic damage. Measurement of the active trypsin within the plasma was however initially difficult because it was either complexed to  $\alpha_1$ -antiprotease or  $\alpha_2$ -macroglobulin, or in the inactive form, namely trypsinogen (Temler and Felber 1976). This problem was overcome by the development of an assay which utilised an antibody specific to trypsin (Temler and Felber 1976). This detected the inactive forms of the enzyme as well as that bound to  $\alpha_1$ -antiprotease, and  $\alpha_2$ -macroglobulin. Unfortunately the assay does not distinguish between these different forms of the enzyme and tended to underestimate the amount of trypsin bound to  $\alpha_2$ -macroglobulin by about 85% (Temler and Felber 1976). Normal serum concentrations of this immunoreactive trypsin (IRT) range between 20  $\mu\text{g/ml}$  and 400  $\mu\text{g/ml}$  and could be elevated by up to 40 times in pancreatic disease (Mero *et al* 1982, Ventrucci *et al* 1980, Poston *et al* 1987). Measurements of IRT have a specificity for acute pancreatitis of greater than 90% (Clavien *et al* 1989). Serum concentrations in patients with acute pancreatitis remain elevated for longer than those of amylase and it therefore has the potential to be useful in unmasking normoamylasaemic acute pancreatitis (Clavien *et al* 1989). In spite of this serum concentrations of IRT are still increased in a number of conditions other than acute

pancreatitis including choledocholithiasis, chronic renal failure, pancreatic cancer and following ERCP (Elias *et al* 1977, Ventrucci *et al* 1980, Mero *et al* 1982). It should be noted that current assays take 24 hours to perform (Moossa 1984, Mayer *et al* 1985).

#### 1.10.5 Phospholipase A<sub>2</sub>.

Phospholipase A<sub>2</sub> assays have not proved to be a reliable means of diagnosing acute pancreatitis (Hoffmann *et al* 1986, Kazmierczak *et al* 1991). Catalytically measured concentrations of phospholipase A<sub>2</sub> have failed to correlate with serum concentrations of amylase or lipase (Schrofer 1980, Hoffmann *et al* 1986, Kazmierczak *et al* 1991). Kazmierczak *et al* (1991) found that phospholipase A<sub>2</sub> concentration diagnosed acute pancreatitis with a sensitivity of only 13% compared to values of 48% for amylase and 60% for lipase.

#### 1.10.6 Clinical evaluation.

Clinical evaluation, whilst having a low sensitivity, may provide important indicators of the diagnosis. Almost all patients with acute pancreatitis experience severe epigastric pain passing through to the back, classically relieved by leaning forward and associated with vomiting (Jacobs *et al* 1977). This is not universally true. Rarely, acute pancreatitis can occur without any abdominal pain (Toffler and Spiro 1962, Dooner and Allaga 1965). There may be a recent history of jaundice, excessive alcohol, or drug ingestion prior to the onset of symptoms. The following may be present on examination: jaundice, cyanosis, cardiovascular collapse, renal failure, a tense distended abdomen with guarding, an ileus or peritoneal effusion (Foster and Ziffren 1962, Jacobs *et al* 1977) or abdominal wall ecchymosis (Cullen 1918, Grey-Turner 1919) .

#### 1.10.7 Plain X-rays.

Abnormalities noted on plain chest X-rays include raised hemidiaphragms, plate atelectasis, consolidation and pleural effusions (Durr *et al* 1979, Ranson 1979). Plain abdominal X-rays may display an adynamic ileus, sentinel loops, the "colonic

cut-off sign" (distension of the transverse colon with collapse of the descending colon), an increased epigastric soft tissue density, distortion of the gastric air bubble, increased gastrocolic separation, and loss of the psoas line. These signs may suggest acute pancreatitis, but are not diagnostic (Durr *et al*/1979). One or more of these may be present in 79% of patients (Ranson 1979).

#### 1.10 Ultrasound Scanning.

Ultrasound examination may demonstrate the presence of enlargement of the pancreas, perhaps with loss of density, a surrounding halo, more distant evidence of inflammatory infiltration, or a peritoneal effusion (McKay *et al*/1982, Moossa 1982). However a clear view of the gland is frequently obscured by the presence of overlying bowel gas (McKay *et al*/1982, Moossa 1982). In McKay *et al*'s series of 114 patients the pancreas was only visualised in 85 (74.5%). There was no correlation between the severity of the attack and the findings on ultrasound, although all patients who subsequently developed pseudocysts had evidence of pancreatic oedema. Of more value is the accuracy with which ultrasound is able to demonstrate the presence of associated gallstones (McKay *et al*/1982).

#### 1.10.9 Computerised Tomography (CT) Scanning.

The major advantage of CT scanning over ultrasound is that visualisation of the pancreas is not impaired by overlying bowel gas. Observed changes include: oedema of the gland, dilatation of the pancreatic duct, peripancreatic fluid collections and obliteration of the margins of the gland (Balthazar *et al*/1985). Whilst CT scanning can be used as a method of diagnosing acute pancreatitis, the low sensitivity, 10-29% of mild cases show a normal gland (Silverstein 1981, Nordestgaard *et al*/1986, Clavien *et al*/1988), and the expense involved preclude its use as a routine diagnostic tool. It may however, occasionally be useful where the diagnosis is in doubt, especially where a normal amylase measurement is associated with a delay in referral.

#### 1.10.10 Peritoneal aspiration and lavage.

Peritoneal aspiration and lavage have principally been utilised as a means of severity assessment, but have also proved valuable in diagnosis (Keith *et al* 1950, Pfeffer *et al* 1958). Whilst the presence of a high amylase concentration is consistent with a diagnosis of acute pancreatitis, blood or bile staining, a foul smell or organisms seen on Gram staining, suggest an alternative diagnosis (Pickford *et al* 1977). Under these circumstances an urgent laparotomy will be required (Pickford *et al* 1977).

#### 1.11 EARLY ASSESSMENT OF DISEASE SEVERITY.

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

##### 1.11.1 Clinical evaluation.

Clinical assessment tends to be very subjective and of limited value in the early detection of severe acute pancreatitis (McMahon *et al* 1980, Corfield *et al* 1985, Larvin and McMahon 1989, Wilson *et al* 1990). McMahon *et al* (1980) and Corfield *et al* (1985) reported the percentage of patients with mild disease correctly classified on admission (using clinical assessment alone) as 100% and 85% respectively. The percentage of severe attacks correctly predicted were a disappointing 39% and 34% (McMahon *et al* 1980, Corfield *et al* 1985). A delay of 48 hours increased the percentage of severe attacks correctly predicted to 73% and 83% respectively (McMahon *et al* 1980, Corfield *et al* 1985). However, two recent studies have suggested that clinical evaluation may not be as effective as previously demonstrated. Larvin and McMahon (1989) were only able to identify correctly 66% and Wilson *et al* (1990) only 44% of patients with severe attacks, 48 hours after admission.

Increasing age, (Jacobs *et al* 1977) cardiovascular collapse, (Jacobs *et al* 1977) renal impairment, (Baslov *et al* 1962, Imrie and Blumgart 1975) cyanosis, tetany, (Pollock 1959) body wall ecchymosis, (Cullen 1918, Grey-Turner 1919) abdominal guarding, (Durr 1979) abdominal masses, (Foster and Ziffren 1962, Jacobs *et al* 1977) prolonged paralytic ileus, (Foster and Ziffren 1962, Jacobs 1977) a peritoneal effusion, (Pickford *et al* 1977, McMahon *et al* 1980) fluid sequestration, (Ranson *et al* 1976, Sauven 1986) and hyperthermia (Jacobs *et al* 1977, Satiani and Stone 1979) are all correlated with a severe outcome. However many are either not present in patients with severe disease, or take a long time to assess (Cullen 1918, Grey-Turner 1919, Ranson *et al* 1976, McMahon *et al* 1980, Dickson and Imrie 1984, Corfield *et al* 1985, Larvin and McMahon 1989, Wilson *et al* 1990).

#### 1.11.1.1 Cardiovascular Collapse.

Cooper *et al* (1982) noted hypotension at the time of admission in 5 of 87 patients (6%) with acute pancreatitis, although only 3 of 21 patients were subsequently classified as suffering from a severe attack had hypotension. This resulted in a sensitivity of only 14%. These findings are not surprising in view of the fact that hypotension can be rapidly reversed by the intravenous infusion of fluids (Ranson *et al* 1974, Imrie and Whyte 1975). In contrast Jacobs *et al* (1977) noted an increased mortality associated with the presence of hypotension and tachycardia. A blood pressure less than 90 mm/Hg on admission was noted in 19% of patients dying of acute pancreatitis although only 40% of patients with a blood pressure of less than 90 mm/Hg died. Forty percent of those with a tachycardia of greater than 140 beats per minute died, but only 6% of patients who died had this degree of tachycardia on admission (Jacobs *et al* 1977).

#### 1.11.1.2 Respiratory Distress.

An increased respiratory rate (Romeo *et al* 1966, Ranson *et al* 1974, Jacobs *et al* 1977, Renner *et al* 1985), dyspnoea (Banks *et al* 1983) or respiratory distress (Satiani and Stone 1979) have all been associated with a poorer outcome.

#### 1.11.1.3 Abdominal Symptoms and Signs.

Jacobs *et al*(1977) noted that the presence of abdominal pain, back pain and abdominal tenderness were associated with mortality rates no higher than the those of the patient population as a whole. Trapnell found that pain during the course of the illness was an unreliable pointer to progress (Trapnell 1966). The presence of an abdominal mass on admission was associated with an increased risk of mortality (Jacobs *et al*1977). Romero and co-workers found, in addition, the presence of abdominal distension and ascites to be of value as did others (Romero *et al*1966, Bank *et al*1983). Trapnell considered the presence of a prolonged ileus probably to be the best basis on which to assess the severity of an individual attack (Trapnell 1966).

#### 1.11.1.4 Abdominal Wall Staining.

Two cases of fatal acute pancreatitis, one associated with flank ecchymosis and the other periumbilical ecchymosis, were reported by Grey-Turner in 1919. Whilst the former sign was named after him, periumbilical ecchymosis seen in a patient with a ruptured ectopic pregnancy was reported a year earlier by Cullen (1918) and the sign named after him. The appearance of either of these signs during an attack of acute pancreatitis has been reported to be associated with a grave prognosis (Hardy 1977). An authoritative review of 770 patients with acute pancreatitis showed an incidence of 3% (somewhat higher than previously reported) a mortality of 37% and complication rate of 96% (Dickson and Imrie 1984). Although associated with severe disease, the mortality of young patients with abdominal wall ecchymosis was not greater than those without it, whilst in patients over 70 years of age the appearance of either of these signs was frequently associated with an early death (Dickson and Imrie 1984).

#### 1.11.1.5 Hyperthermia.

Pyrexia on admission has been found to correlate with mortality, (Jacobs *et al*1977, Satiani and Stone 1979) Jacobs and co-workers finding that 20% of those with a temperature above 100° F died. Others have found no such relationship (Romeo *et al*1975).

#### 1.11.1.6 Fluid Sequestration.

During an attack of acute pancreatitis many patients with severe disease develop an abnormally large negative fluid balance (Ranson *et al* 1976, Sauven *et al* 1986). The cause of this is thought to be a combination of factors. Reduced oral fluid intake and vomiting, the sequestration of fluid within a small bowel ileus, oliguric renal failure and the leakage of fluid through the capillary bed into the peritoneum and retroperitoneal tissue have all been implicated (Sauven *et al* 1986). Ranson demonstrated that the sequestration of > 6 L during the first 48 hours after admission to hospital was related to a severe outcome, and was therefore included in his multifactorial scoring system. Sauven *et al* (1986) also noted this association but went further and suggested that it could be utilised as a means of severity prediction on its own. The sequestration of > 2 L per day for 48 hours predicted a severe attack of acute pancreatitis with a sensitivity of 76% and specificity of 96%. It is difficult to reconcile the effectiveness of fluid sequestration as a means of severity assessment as noted by Sauven *et al* (1986) with the relative inefficiency displayed by Ranson *et al* (1976).

#### 1.11.1.7 Tetany.

Tetany was noted by Pollock to be an adverse prognostic sign, all 6 patients in whom it was noted died (Pollock 1959).

#### 1.11.1.8 Nystagmus.

One particularly bad prognostic sign is the presence of nystagmus. It was detected in 11 of 26 fatal cases of acute pancreatitis, but in none of the survivors (Guillemin *et al* 1982).

#### 1.11.1.9 Clinical Scoring System.

Bank *et al* (1983) attempted to predict outcome using a clinical scoring system (table 1.7). His retrospective study of 75 patients was disappointing: it only detected 56% of the patients who died or had a complicated outcome. This scoring

Organ system	Example
Cardiac arrhythmias, ECG changes	Shock, tachycardia > 130 bpm,
Pulmonary PO <sub>2</sub> < 60 mm Hg, ARDS	Dyspnoea, crepitations,
Renal rising urea, or creatinine	Urine output < 50 ml hr <sup>-1</sup> ,
Metabolic albumin	Low or falling pH, Calcium,
Haematological DIC	Falling haematocrit,
Neurological localising signs	Irritability, confusion,
Haemorrhagic disease abdominal distension	On peritoneal tap, paralytic ileus,

Table 1.7: Banks criteria for severity assessment. A score of 0 was equated with a mild outcome and 1 in any system was associated with a severe outcome. DIC = disseminated Intravascular coagulopathy, Hg = mercury, ARDS = adult respiratory distress syndrome.



system has been modified and simplified by another group and now covers only 7 objective parameters as they relate to 4 different body systems; cardiac, pulmonary, renal and metabolic (Agarwal and Pitchumoni 1986). This may be an advance if confirmed to be as accurate as the more comprehensive multiple factor scoring systems.

#### 1.11.2 SINGLE FACTORS.

##### 1.11.2.1 Hypocalcaemia.

Edmondson and Berne (1944) were aware of the difficulties of severity prediction and were the first to try to detect severe attacks of acute pancreatitis using a variety of biochemical parameters. Like many other authors (Pollock 1959, Trapnell 1966, Ranson *et al* 1976) they noted the association between hypocalcaemia and severe pancreatitis. In their initial series a serum calcium <1.75 mmol/l indicated the presence of fatal pancreatic necrosis, although they later revised this initial estimate after a number of patients with serum calcium concentrations below this value survived (Edmondson *et al* 1952).

Hypocalcaemia has been attributed to the binding of calcium in areas of fat necrosis, (Edmondson *et al* 1952, Pollock 1959) increased serum glucagon concentrations (Paloyan *et al* 1967), parathyroid insufficiency (Condon *et al* 1975, Robertson *et al* 1976, McMahon 1978, ) or to a failure of bone to respond to parathyroid hormone, perhaps because of shock and reduced tissue perfusion (Hauser *et al* 1983). Parathyroid hormone degradation may also occur due to its proteolysis by circulating  $\alpha_2$ -macroglobulin-protease complexes (Hermon-Taylor *et al* 1981). Proteolysis of parathyroid hormone by sera from patients with acute pancreatitis has been demonstrated *in vitro* (Hermon-Taylor *et al* 1981, Broderick *et al* 1981). In spite of this much of the hypocalcaemia of acute pancreatitis is associated with hypoalbuminaemia and is of little physiological importance (Imrie *et al* 1976). When the serum values are corrected for the lower serum albumin only about 11% of all the results will show "true" hypocalcaemia of varying degrees

(Imrie *et al* 1976). Although serum calcium concentrations < 2.0 mmol/l provide useful predictive value as part of multifactorial scoring systems, (Ranson *et al* 1974b, Imrie *et al* 1978a) their measurement in isolation does not allow adequate discrimination between mild and severe attacks of acute pancreatitis (McMahon *et al* 1980, Croton *et al* 1981).

#### 1.11.2.2 C-reactive protein.

McMahon and colleagues (1984) measured CRP concentrations in 55 patients with acute pancreatitis and demonstrated that it provided good separation between complicated and uncomplicated attacks of acute pancreatitis. A more detailed analysis of these results was provided by Mayer *et al* (1984) who, in addition, concluded that a persistently elevated CRP suggested the presence of continuing inflammation, with the associated risk of a complication. These findings have been confirmed by other workers (Buchler *et al* 1986, Puolakkainen *et al* 1987). In the initial study by Mayer *et al* (1984) and a later study by Wilson *et al* (1989), CRP concentrations were noted to be low on admission. However in the studies conducted by Buchler *et al* (1986) and Puolakkainen *et al* (1987) high concentrations were noted at the time of admission. This finding can be explained by the fact that many of these groups' patients were referred from other centres and have therefore already undergone a delay of 48 hours. Buchler *et al* (1986) state that a CRP greater than 120 mg/l detected 95% of cases of necrotising pancreatitis confirmed at laparotomy. This resulted in an extraordinarily high incidence of pancreatic necrosis (22 of 35 or 63% of a consecutive series of patients). These results vary markedly with a recent paper from Glasgow (Wilson *et al* 1989) where the incidence of pancreatic necrosis was half this figure. Wilson and colleagues demonstrated that CRP levels of > 210mg/l were needed to detect severe disease reliably and that CRP was unable to discriminate between the types of complications: in particular CRP could not determine the presence of pancreatic necrosis. Buchler and co-workers have subsequently upgraded their estimation of the CRP concentration required to detect severe disease to 150 mg/l (personal communication).

#### 1.11.2.3 $\alpha_2$ -macroglobulin.

The consumption of  $\alpha_2$ -macroglobulin (Lasson and Ohlsson 1976, McMahon *et al* 1984) and the fact that it is not produced as part of the acute phase protein response, (Moshage *et al* 1988) means that its concentration tends to fall during attacks of acute pancreatitis (Lasson and Ohlsson 1976, McMahon *et al* 1984, Dubick *et al* 1987, Leese *et al* 1987 (Moshage *et al* 1988). A number of studies have demonstrated that this fall is significantly greater in patients with severe, (Lasson and Ohlsson 1976, Mero *et al* 1982, McMahon *et al* 1984, Buchler *et al* 1986, Dubick *et al* 1987, Leese *et al* 1987) or necrotising disease (Buchler *et al* 1986). In one study the lowest concentrations in severe attacks occurred 5 days after admission, (McMahon 1984) but concentrations can remain low for up to 14 days (Lasson and Ohlsson 1973). Significant differences in the serum concentrations of  $\alpha_2$ -macroglobulin, at the time of admission, have been noted by some authors, (Lasson and Ohlsson 1984, Buchler *et al* 1986) but not by others (Mero *et al* 1982, McMahon *et al* 1983, Leese *et al* 1987, Wilson *et al* 1989). Buchler *et al* (1986) demonstrated that  $\alpha_2$ -macroglobulin detected pancreatic necrosis with a sensitivity of 85%, although these findings have not been borne out by a more recent study (Leese *et al* 1987). The measurement of  $\alpha_2$ -macroglobulin is not routinely employed as a means of severity assessment (Howard and Jordan 1986, Glazer and Ranson 1988).

#### 1.11.2.4 $\alpha_1$ -antiprotease.

$\alpha_1$ -antiprotease is a positive acute phase protein, (Moshage *et al* 1988) whose concentration rises during attacks of acute pancreatitis (McMahon *et al* 1984, Leese *et al* 1987, Wilson *et al* 1989, Gross *et al* 1990). Serum concentrations increase from the time of admission, peaking between 3 and 7 days, (Mero *et al* 1982, McMahon *et al* 1984, Buchler *et al* 1986, Leese *et al* 1987, Wilson *et al* 1989). A number of studies have demonstrated that  $\alpha_1$ -antiprotease concentrations are significantly elevated in patients with severe disease (McMahon *et al* 1984, Buchler *et al* 1986, Leese *et al* 1988, Wilson *et al* 1989). In Buchler *et al*'s (1986) series elevated serum  $\alpha_1$ -antiprotease concentration detected 71% of patients with pancreatic necrosis.

#### 1.11.2.5 Fibrinogen.

Like CRP and  $\alpha_1$ -antiprotease, fibrinogen is a positive acute phase protein which has been evaluated as an early means of severity assessment in acute pancreatitis (Berry *et al*/1982, Shearer *et al*/1985). In experimental acute pancreatitis there is an initial fall in the plasma concentration of fibrinogen, which is thought to be due to the activation of the coagulation cascade by pancreatic enzymes (Kwaan *et al*/1971). Following this there is an increase in fibrinogen concentrations resulting in peak concentrations being produced on day 4 (Shearer *et al*/1985). Berry *et al*(1981) measured plasma concentrations of fibrinogen in 50 patients with acute pancreatitis. They were elevated in 24 of 31 patients with 43% of those with concentrations > 6.0 gm/l (normal range 1.5 - 4 gm/l) developing complications. Utilising the cut off value for fibrinogen of 6 gm/l they obtained 57% false positive results. These compared favourably with the Glasgow scoring system (Imrie *et al*/1978) (false positive rate 53%). The authors concluded that a plasma fibrinogen concentration > 6 gm/l provided comparable severity prediction to the Glasgow score (Imrie *et al*/1978). These findings were not borne out by a larger study, involving 181 patients, carried out by Shearer *et al*(1985). It should however be noted that the Shearer *et al*(1985) utilised the modified Glasgow score (Osborne *et al*(1981) and a different fibrinogen assay. Whilst they found that the mean daily fibrinogen concentrations were significantly higher in patients with severe disease, median fibrinogen concentrations were within the normal range on day 1 for those with severe, and days 1 and 2 for those with mild disease. In addition peak fibrinogen concentrations did not occur until day 4. Only 19% of the patients (9 mild and 4 severe) had admission fibrinogen concentrations > 6gm/l. The authors concluded that the measurement of plasma fibrinogen concentrations were not an effective means of severity assessment.

#### 1.11.2.6 Methaemalbuminaemia.

Methaemalbumin is a complex formed by the combination of albumin and haematin, (a product of the proteolytic digestion of haemoglobin). Northam and co-workers (1963), (Winstone 1965) and (Trapnell 1966) have described an

association between haemorrhagic pancreatitis and elevated serum concentrations of methaemalbumin. Experimental work in dogs has confirmed these observations. A number of clinical studies on peritoneal fluid (Geokas *et al* 1974, Lankisch *et al* 1978) and serum (Lankisch *et al* 1978) have supported the usefulness of methaemalbumin measurements, whilst others have not found it valuable (Ranson *et al* 1974, McMahon *et al* 1980, Berry *et al* 1981). In McMahon's series removal of methaemalbumin stained peritoneal fluid may have accounted for low serum concentrations. Both experimental work (Anderson *et al* 1969) and clinical studies (Goodhead 1970) have shown that methaemalbumin is not specific for acute haemorrhagic pancreatitis, increased concentrations being found in other acute abdominal conditions including intraperitoneal bleeding and strangulation or infarction of bowel. Furthermore, concentrations of methaemalbumin were not always raised in acute haemorrhagic pancreatitis and were not present early enough in an attack of acute pancreatitis to be of value in severity prediction (Battersby and Green 1971).

#### 1.11.2.7 Changes in the coagulation cascade.

Changes in the coagulation cascade, including occasional DIC, have been noted in association with acute pancreatitis (Goodhead 1969, Bryne *et al* 1971, Kwaan *et al* 1971, Murphy *et al* 1977, Ranson *et al* 1977a) and have been attributed to the effects of trypsin. Platelet and fibrinogen concentrations tend to rise during severe attacks. These have been correlated with renal, respiratory and hepatic impairment (Ranson *et al* 1977) but have not proved helpful in prognostication.

#### 1.11.2.8 Complement.

The complement system is a series of plasma enzymes which form part of the body's defence against injury and infection (Ganong 1981). They are involved in a variety of functions including the opsonisation of bacteria, cell lysis, the attraction of leucocytes and the release of histamine from mast cells and basophils (Ganong 1981). Complement is most commonly activated as part of the bodies defence

against infection but has been noted to occur in acute pancreatitis (Seellg 1975, Goldstein *et al*/1978, Balldin 1981, Foulis *et al*/1982, Whicher *et al*/1982, Roxvall *et al*/1989, Roxvall *et al*/1991), multisystem organ failure (Carey 1979) and adult respiratory distress syndrome (Horn *et al*/1980, Hammerschmidt *et al*/1980, Kellermann *et al*/1989). Bacteraemia, burn injury, mechanical trauma and hypotension all cause activation of the complement system via the alternative pathway (Heidemann *et al*/1979, Solomkin *et al*/1984, Bengtson *et al*/1996). In acute pancreatitis complement activation most probably occurs by the direct proteolytic action of trypsin on C3 (Molenaar *et al*/1974, Goldstein *et al*/1978, Whicher *et al*/1982, Lasson *et al*/1982, Roxvall *et al*/1989, Roxvall *et al*/1991). It has however been suggested that complement activation can take place via the alternative pathway (Foullis *et al*/1982) or as the result of C-reactive protein binding to damaged tissue, resulting in the activation of complement via the classical pathway (Pepys 1981, Wilson *et al*/1989). A number of studies have demonstrated reduced serum concentrations of C3 and increased concentrations in C3 breakdown products (Goldstein *et al*/1978, Whicher *et al*/1982, Foulis *et al*/1982, Roxvall *et al*/1990, Wilson *et al*/1989). Foulis *et al*/1982 demonstrated that the differences in concentrations between mild and severe attacks were large enough to allow the measurement of C3 to be utilised as a means of severity assessment. These findings have not been confirmed by other authors (Goldstein *et al*/1978, Whicher *et al*/1982, Roxvall *et al*/1990, Wilson *et al*/1989) and two have emphatically stated that the measurement of serum complement factors is not an effective means of severity assessment (Whicher *et al*/1982, Wilson *et al*/1989).

#### 1.11.2.9 Trypsin.

Few authors have examined the relationship between immunoreactive trypsin concentrations and disease severity. McMahon *et al*/1984 measured immunoreactive trypsin concentrations in 16 patients with acute pancreatitis. There was a wide variation in serum concentrations. Six of 7 patients with severe disease had concentrations >100 mg/ml 10 days after admission whilst only 1 of the 8

patients with mild disease did. Clearly such a delay is unacceptable. Poston *et al*(1987) found that 2 patients who developed severe acute pancreatitis had persistently elevated concentrations of Immunoreactive trypsin and suggested that it might be a useful indicator of severe disease. However, only 12 patients were studied and there was a wide variation in immunoreactive trypsin concentrations.

#### 1.11.2.10 Phospholipase A<sub>2</sub>.

A number of authors have examined the relationship between serum concentrations of phospholipase A<sub>2</sub> and the severity of acute pancreatitis. In a report involving 7 patients, Mero *et al*(1982) noted that phospholipase A<sub>2</sub> concentrations were elevated at the time of admission and remained so for a "few days". In 2 patients who died, concentrations were noted to be higher than those of the survivors. Whilst these differences did not reach statistical significance they confirmed the authors' view that a consistent rise in the serum concentrations of phospholipase A<sub>2</sub> activity were an ominous sign (Schroder *et al*/1980). In a subsequent study from the same group, involving 53 patients, the authors were able to demonstrate significant differences in serum phospholipase A<sub>2</sub> activity between patients with mild and severe acute pancreatitis (Puolakkainen *et al*/1987). These differences were significant at the time of admission (Puolakkainen *et al*/1987) and provided comparable severity prediction to CRP. However, in view of the fact that CRP measurements were already routinely available within the hospital they suggested that CRP concentrations should be measured in preference to phospholipase A<sub>2</sub>.

Matsuda *et al*(1986) employed 2 assays for phospholipase A<sub>2</sub>. They were a catalytic assay (in principle, similar to those employed by the Helsinki group) and a radioimmunoassay. The radioimmunoassay showed concentrations of phospholipase A<sub>2</sub> on admission to be 48 times greater than normal, in those with predicted severe disease, and 6 times normal, in those patients with predicted mild disease. In contrast the concentrations of catalytically measured phospholipase A<sub>2</sub>

were only 1 to 5 times greater than normal in those patients with predicted severe disease, and almost normal in those predicted as suffering from mild attacks. This difference between assays has been noted by other authors (Nevalainen *et al* 1985, Buchler *et al* 1989, Nordback *et al* 1989).

In Buchler *et al*'s (1989) series immunoreactive trypsin concentrations were unable to distinguish between necrotising and other forms of acute pancreatitis, whereas a concentration of catalytically active phospholipase A<sub>2</sub> >15 IU/l, during the first 5 days of the illness, detected pancreatic necrosis with a sensitivity and specificity of 79%. Nordback *et al* (1989) measured immunoreactive and catalytic phospholipase A<sub>2</sub> activity in the serum and homogenised, resected pancreas from 6 patients with pancreatic necrosis. They were unable to demonstrate any relationship between the extent of pancreatic necrosis and the concentrations of immunoreactive or catalytically active phospholipase A<sub>2</sub> in the serum or pancreatic tissue.

Bird *et al* (1989) measured catalytically active phospholipase A<sub>2</sub> in 73 patients with acute pancreatitis. Eleven patients had serum phospholipase A<sub>2</sub> concentrations above the cut off concentration of 85 IU/l. Ten of these had severe disease. From this they calculated that the positive predictive value of 91% and the percentage correct of 82%. However, only 10 of the 22 patients with severe disease were correctly identified, giving a sensitivity of only 45%!

Phospholipase A<sub>2</sub> is produced by a number of cells other than the pancreas (O'Hare *et al* 1986, Sellhammer *et al* 1986, Tojo *et al* 1988, Tojo *et al* 1988). One of the most important being the leucocyte and pulmonary macrophages in particular (Flesch *et al* 1989). The ability of phospholipase A<sub>2</sub> released from these cells to digest the cell walls of alveolar cells as well as surfactant mean that it has been implicated as an initiator of adult respiratory distress syndrome and the respiratory impairment seen in patients with acute pancreatitis (Nath and Warshaw 1982, Aho



*et al* 1983, Buchler *et al* 1989). Both Schroder *et al* (1980) and Buchler *et al* (1989) demonstrated significantly increased concentrations of catalytic phospholipase A<sub>2</sub> in the serum of patients with pulmonary complications associated with acute pancreatitis.

Increased concentrations of phospholipase A<sub>2</sub> have also been noted in a number of conditions other than acute pancreatitis including trauma, peritonitis and sepsis (Vadas *et al* 1984, Koeniger *et al* 1989, Schild *et al* 1989) as well as pancreatic cancer (Funakoshi *et al* 1991), thus reducing the sensitivity of the assay.

There are a number of reasons for the discrepancies noted between phospholipase A<sub>2</sub> concentrations as measured by radioimmunoassays and catalytic assays. Radioimmunoassays are unable to distinguish between the active or inactive forms of the enzyme and therefore concentrations measured by radioimmunoassays are greater (Nevalainen and Eskola 1989). However, this is not the whole explanation. Matsuda *et al* (1986) activated the pro-phospholipase A<sub>2</sub> in patients' serum by incubating it with trypsin and then reassayed the sample. Whilst this doubled the concentration of phospholipase A<sub>2</sub> detected by the catalytic assay it was, on average, 5 times less than that detected by the radioimmunoassay (Matsuda *et al* 1986). This may be related to the fact that there are 2 forms of the enzyme whose activity is pH dependent (Matsuda *et al* 1986). Hoffmann (1986) has demonstrated that the optimal pH at which the enzyme worked shifted during an attack of acute pancreatitis. Gel filtration separated 2 forms of the enzyme. The one with the lower molecular weight was thought to be pancreatic in origin, the other arising from other sources (Hoffmann 1986).

#### 1.11.2.11 Amylase.

Blood amylase elevation, although invaluable as a diagnostic test for acute pancreatitis, has been found to bear little relationship to the severity of acute pancreatitis (Edmondson *et al* 1952, Pollock 1959, Trapnell 1966, Ranson *et*

*al*/1974). Others have found that the magnitude of the serum amylase elevation was inversely related to the severity of the attack (Adams *et al*/1968, Satiani and Stone 1979). A persistent elevation of the serum amylase may be an indication of pseudocyst formation (Veith *et al*/1963).

#### 1.11.2.12 Nucleases.

Deoxyribonuclease was first reported as a valuable marker of pancreatic disease in 1956 (Kowlessar and McEvoy 1956). A marked and prolonged elevation was found only in patients with clinically severe acute pancreatitis. Patients with mild pancreatitis showed only a temporary elevation to the upper limit of normal (Kowlessar and McEvoy 1956). More recently ribonuclease has been studied and considered to be a reliable indicator of the presence of pancreatic necrosis (Warshaw and Lee 1979). In subsequent work they demonstrated the release of large amounts of ribonuclease during anoxic incubation of pancreatic tissue (Warshaw and Fournier 1984). More recent reports have failed to bear out its usefulness (Kemmer *et al*/1991).

#### 1.11.2.13 LDH.

Elevated serum concentrations of lactic dehydrogenase have been demonstrated, by a number of authors, to be correlated with outcome (Ranson *et al*/1974, Imrie *et al*/1978, Osborne *et al*/1981, Uhl *et al*/1991). Its measurement forms part of the Ranson and Glasgow scoring systems (Ranson *et al*/1974, Imrie *et al*/1978). Ranson *et al*/1974 demonstrated that 64% of patients with an LDH concentration > 600 IU/dl suffered a severe outcome. Blamey *et al*/1984 noted that LDH was one of the two factors that had independent significance in detecting severe attacks. More recently Uhl *et al*/1991 have examined the relationship between LDH and pancreatic necrosis. A serum LDH > 270 IU/l detected necrotising pancreatitis had an accuracy of 82%. This figure was better than that achieved by  $\alpha_1$ -antiprotease and  $\alpha_2$ -macroglobulin, but not as good as the results for PMN elastase or CRP. They did not recommend that it be utilised as a means of detecting pancreatic necrosis.

#### 1.11.2.14 Hyperglycaemia.

Hyperglycaemia and glycosuria may occur in acute pancreatitis, glycosuria being first described by Moynihan (Moynihan 1925). Pollock found that hyperglycaemia more commonly complicated the severe forms of the disease, 8 of 14 patients in whom it was present died (Pollock 1959).

#### 1.11.2.15 Hypoxaemia.

The association between early hypoxaemia and a severe outcome is well documented (Ranson *et al*/1974b, imrie *et al*/1977), the contribution to mortality being estimated as 30%. The age, obesity, cigarette intake and pre-existing respiratory function of the patient will affect the level of hypoxaemia, but where intubation and ventilation became necessary there was a 75% risk of death (Jacobs *et al*/1977). The presence of hypoxaemia forms an important part of the multifactorial scoring systems (Ranson *et al*/1974, imrie 1978).

#### 1.11.2.16 Haemoconcentration

Haemoconcentration on admission to hospital has been reported to be an adverse prognostic sign (Gray *et al*/1965). Trapnell considered that the haematocrit was high in the majority of patients on admission, reflecting the dehydration resulting from vomiting and internal shifts of body fluids and noted that this was corrected by intravenous fluids (Trapnell 1966). He and others considered a subsequent fall in haematocrit to be of adverse clinical significance (Trapnell 1966, Nugent *et al*/1967). A haematocrit of less than 35% (Romero *et al*/1975) during the course of the illness has been reported to be associated with an increased mortality (Romer and Carey 1966).

#### 1.11.2.17 Leucocytosis

The association of leucocytosis with a severe clinical course was described by Thal and co-workers in 1957 (Thal *et al*/1957). Trapnell reported that the white cell count was often elevated initially, usually falling to within normal limits by the end

of the first week (Trapnell 1966). Later elevation, or persistent elevation of the white cell count, appeared to indicate continuing disease activity or the development of a late complication (Trapnell 1966). Another group reported that the triad of tachycardia, leucocytosis, and fever was the normal response present in 90% of their patients. Six patients not developing this response died of acute haemorrhagic pancreatitis (Aibo *et al* 1963). The absolute lymphocyte count (Christophi *et al* 1985) has been shown to be of value in severity prediction, but these findings have not been confirmed in a large scale prospective clinical study.

### 1.11.3 MULTIFACTORIAL SCORING SYSTEM.

#### 1.11.3.1 Ranson and Glasgow scoring systems.

In 1974 Ranson produced the first of the multifactorial scoring systems. He prospectively measured 43 clinical and laboratory factors during the first 48 hours after admission in 100 patients with a diagnosis of acute pancreatitis. Thirteen of these factors correlated with outcome. However blood urea concentrations on admission and at 48 hours, and serum glutamic oxaloacetate transaminase and serum glutamic pyruvic transaminase concentrations at 48 hours paralleled each other so closely that they were considered to be reflecting a single function. The number of factors utilised in the scoring system were therefore reduced to 11 (table 1.8). The presence of 3 or more of these factors were taken to indicate a severe attack. Of the 79 patients with a score  $<3$ , 3% died and 11% underwent a complicated course, whilst 13 (62%) of those with a score  $\geq 3$  died and 8 (33%) suffered a complicated outcome. The efficacy of this scoring system has since been validated by further studies (Ranson *et al* 1976, Ranson 1982). A high proportion of Ranson's original study population (74%) had alcohol related attacks. While the scoring system worked well in these patients it was less effective in those with gallstone related disease. The system was therefore modified for gallstone disease (Ranson 1979). The measurement of arterial oxygen concentration was omitted and 8 of the 10 remaining parameters modified (table 1.9).

On admission.

Age	> 55 yrs	
WCC	> $16 \times 10^9/l$	
Blood glucose	> 10 mmol/l	
LDH	> 150 American U%	(>350 IU/l)
AST	> 250 Sigma-Frankel U%	(>120 IU/l)

During the first 48 hrs.

Fall in haematocrit of	> 10%	
Serum calcium	< 2.0 mmol/l	
Base deficit	> 4 mmol/l	
Increase in blood urea	> 5mg/dl	(>1.0 mmol/l)
Fluid sequestration	> 6 l	
Arterial pO <sub>2</sub>	< 60 mm Hg	(<8.0 kPa)

Table 1.8: The original Ranson criteria for severity assessment in acute pancreatitis. A severe attack is indicated by the presence of more than 3 positive factors. AST = Aspartate aminotransferase, and LDH lactate dehydrogenase.

**On admission.**

Age	>70 yrs	
WCC	>18 x 10 <sup>9</sup> /l	
Blood glucose	>220 mg/ml	(>12 mmol/l )
LDH	>400 IU/l	
AST	>240 Sigma-Frankel U%	(>250 IU/l)

**During the first 48 hrs.**

Fall in haematocrit of	>10%	
Serum calcium	<8 mg/dl	(<2.0 mmol/l)
Base deficit	>5.0 meq/l	(>5.0 mEqol/l)
Increase in blood urea	>2mg/dl	(>0.5 mmol/l)
Fluid sequestration	>6 l	

Table 1.9: The modified Ranson criteria for severity assessment in acute gallstone pancreatitis. A severe attack is indicated by the presence of 3 or more positive factors. AST = Aspartate aminotransferase, and LDH lactate dehydrogenase.

Ranson's original system was also modified and applied to patients within the UK by Imrie (Imrie *et al* 1978). The Glasgow system differed from Ranson's original criteria in that there were 9 rather than 11 parameters (table 1.10). Fluid sequestration, a fall in haematocrit and the base deficit were eliminated and a serum albumin of < 32 gm/l added. The cut off value for the white cell count was decreased to  $15 \times 10^9$  cells/l, the blood urea changed to a value of >16 mmol/l and the LDH and AST concentrations changed to the nearest corresponding international units (600 IU/l and 100 IU/l respectively). In addition any value obtained during the first 48 hours could be utilised in calculating the score. In both systems the greater the number of factors found to be positive in an individual patient, the greater the chance of major complications or death.

In this initial study, all 14 patients who died were correctly predicted as severe by the presence of 3 or more prognostic criteria (Imrie *et al* 1978). However the authors subsequently noted a tendency for patients with gallstone related attacks to be inappropriately predicted as suffering from a severe attack more frequently than those with alcohol related disease. The two factors which were most frequently implicated were age (those with gallstone related disease being older than those with alcohol related disease) and AST (which was readily elevated in association with common bile duct stones). Age was therefore eliminated from the scoring system and the cut off value for AST increased to >200 IU/l (table 1.11). In a retrospective review of 47 patients with gallstone pancreatitis, the prognostic accuracy of the original Glasgow criteria were compared with the modified system (Osborne *et al* 1981). The clinical course was uncomplicated in 37 and complicated in 10 patients (including 5 deaths). Utilising the original scoring system 27 patients with mild disease were correctly classified whilst 17 patients who also had mild disease were incorrectly classified as suffering from a severe attack. All 10 who had a complicated clinical course were correctly identified. Utilising the modified system all patients with severe disease were correctly identified but only 3 patients with mild disease were incorrectly classified as suffering from a severe attack.

Age	>55 yrs
Arterial Pa O <sub>2</sub>	<8.0 kPa
Serum albumin	<32 g/l
Serum calcium	<2.0 mmol/l
White cell count	>15 x 10 <sup>9</sup> /l
AST	>100 U/l
LDH	>600 IU/l
Blood glucose	>10 mmol/l in the absence of pre-existing diabetes mellitus.
Plasma urea	>16 mmol/l (following fluid replacement)

Table 1.10: The Glasgow criteria for severity prediction in acute pancreatitis. A severe attack is indicated by the presence of 3 or more positive criteria. AST = Aspartate aminotransferase, and LDH lactate dehydrogenase.



Arterial Pa O <sub>2</sub>	<8.0 kPa
Serum albumin	<32 g/l
Serum calcium	<2.0 mmol/l
White cell count	>15 x 10 <sup>9</sup> /l
(AST)	>200 U/l
(LDH)	>600 U/l
Blood glucose	>10 mmol/l (in the absence of diabetes mellitus)
Blood urea	>16 mmol/l (following fluid replacement)

Table 1.11: The modified Glasgow criteria for severity prediction in acute pancreatitis. A severe attack is indicated by the presence of 3 or more positive criteria. AST = Aspartate aminotransferase, and LDH lactate dehydrogenase.

The Glasgow system has subsequently been re-examined and further modifications suggested. Blamey *et al* (1984) reviewed 405 episodes of acute pancreatitis occurring during a seven year period in Glasgow. They examined the 9 prognostic factors. Only 2 of these, a serum calcium of  $<2.0$  mmol/l and LDH  $>600$  IU/l had independent predictive value. AST concentrations, included in the original scoring system, were not of predictive value. It was suggested that they should be dropped, leaving 8 factors including age. This has recently been utilised in a study of early endoscopic sphincterotomy in patients with gallstone pancreatitis (Neoptolomos *et al* 1986).

It should be noted that the sensitivity and specificity of these systems vary with the aetiology of the attack of acute pancreatitis. Both peritoneal aspiration (to be discussed shortly) and clinical assessment were significantly worse at predicting severe attacks in patients with gallstone as compared with alcohol related attacks (Corfield *et al* 1985). The modified Glasgow scoring system (Osborne *et al* 1981) was equally effective in both aetiological groups of patients within a multicentre study of severe acute pancreatitis in Leeds, Bristol and Glasgow (Corfield *et al* 1985).

Ranson (1982) improved the accuracy of prognostic assessment by employing multivariate analysis of 13 laboratory and clinical parameters. Using this technique it was possible to classify correctly 38.9% of patients using seven variables available at the time of admission or 95.7% of patients using 9 variables recorded within 48 hours. Whilst accurate, the methodology is somewhat complex and not well suited to everyday clinical practice.

There are a number of problems associated with the use of the disease specific multifactorial scoring systems in clinical practice. Whilst the death of a patient represents a very definite outcome, agreement on what constitutes a complicated attack is not universal. Groups can differ on their definition of respiratory and renal

failure, cardiovascular collapse, of a pseudocyst, of what constitutes pancreatic pseudocyst or a pancreatic abscess. Equally there may be differences in the indications for admission to and duration of stay in ITU, the need for oxygen therapy, insulin infusions, calcium therapy, renal dialysis or surgery, the timing of restarting oral fluids and food and the overall hospital stay. In addition data collection may be incomplete and can involve a delay of up to 48 hours or longer.

Both McMahon *et al* (1980) and Corfield *et al* (1985) have shown that by 48 hours after admission, clinical evaluation is as good as the disease specific scoring systems at predicting outcome. These studies were however performed by experienced clinical research workers and may not reflect the true situation in hospital practice. The scoring systems are more objective than clinical evaluation and may be especially useful when employed by a less experienced clinicians.

#### **1.11.3.2 The acute physiology and chronic health evaluation (APACHE II) and other illness scoring systems.**

Drawbacks of the disease specific multifactorial scoring systems for grading the severity of an attack of acute pancreatitis include: a delay of 48 hrs or longer, their inability to take account of pre-existing disease states, differences in their sensitivity and specificity depending upon the aetiology of an attack and their "once only" predictive ability. They can not be used to monitor the disease course or response to therapy.

One of the advantages of the injury and sepsis scoring systems is their ability to take account of pre-existing illnesses and to monitor the disease course. For this reason they have recently been investigated as alternatives to the Ranson and Glasgow scoring systems (Larvin and McMahon 1989, Wilson *et al* 1990). The best known, and most widely used, of them is the APACHE II scoring system.

In 1981 Knaus and colleagues set out to develop a scoring system which could be used to predict the risk of mortality and morbidity, in a group of medical and

# THE APACHE II SEVERITY OF DISEASE CLASSIFICATION SYSTEM

PHYSIOLOGIC VARIABLE	HIGH ABNORMAL RANGE					LOW ABNORMAL RANGE				
	+4	+3	+2	+1	0	+1	+2	+3	+4	
TEMPERATURE — rectal (°C)	≥ 41°	39°-40.9°		38.5°-38.9°	38°-38.4°	34°-35.9°	32°-33.9°	30°-31.9°	≤ 29.9°	
MEAN ARTERIAL PRESSURE — mm Hg	≥ 160	130-159	110-129		70-109		50-69		≤ 49	
HEART RATE (ventricular responses)	≥ 160	140-179	110-139		70-109		55-69	40-54	≤ 39	
RESPIRATORY RATE — (non-ventilated or ventilated)	≥ 50	35-49		25-34	12-24	10-11	6-9		≤ 5	
OXYGENATION: A-aDO <sub>2</sub> or PaO <sub>2</sub> (mm Hg)	≥ 500	350-499	200-349		≤ 200	PO <sub>2</sub> ≥ 70		PO <sub>2</sub> 55-60	PO <sub>2</sub> ≤ 55	
a. FIO <sub>2</sub> ≥ 0.5 record A-aDO <sub>2</sub>										
b. FIO <sub>2</sub> < 0.5 record only PaO <sub>2</sub>										
ARTERIAL pH	≥ 7.7	7.67-69		7.57-59	7.37-7.49		7.26-7.32	7.15-7.24	≤ 7.15	
SERUM SODIUM (mMol/L)	≥ 160	160-179	155-159	150-154	130-149		120-129	111-119	≤ 110	
SERUM POTASSIUM (mMol/L)	≥ 7	6.6-9		5.5-5.9	3.5-5.4	3-3.4	2.2-2.9		≤ 2.5	
SERUM CREATININE (mg/100 ml) (Double point score for acute renal failure)	≥ 3.5	2-3.4	1.5-1.9		0.6-1.4		≤ 0.6			
HEMATOCRIT (%)	≥ 60	50-59.9	40-49.9	30-39.9			20-29.9		≤ 20	
WHITE BLOOD COUNT (total/mm <sup>3</sup> ) (in 1,000s)	≥ 40		20-39.9	15-19.9	3-14.9		1-2.9		≤ 1	
GLASGOW COMA SCORE (GCS): Score = 15 minus actual GCS										
A) Total ACUTE PHYSIOLOGY SCORE (APS): Sum of the 12 individual variable points										
B) Serum HCO <sub>3</sub> (venous-mMol/L) (Not preferred, use if no ABGs)	≥ 52	41-51.9		32-40.9	22-31.9		18-21.9	15-17.9	≤ 15	

**AGE POINTS:**  
Assign points to age as follows:

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

## **CHRONIC HEALTH POINTS**

If the patient has a history of severe organ system insufficiency or is immunocompromised assign points as follows:

a. for nonoperative or emergency postoperative patients — 5 points

or

b. for elective postoperative patients — 2 points

## **DEFINITIONS**

Organ insufficiency or immunocompromised state must have been evident prior to this hospital admission and conform to the following criteria:

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

CARDIOVASCULAR: New York Heart Association Class IV.

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

RENAL: Receiving chronic dialysis.

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

## **APACHE II SCORE**

Sum of A + B + C

A) APS points

B) Age points

C) Chronic Health points

Total APACHE II

Figure 1.1: The APACHE II score card for assessing illness severity.

surgical patients, with a diverse collection of diagnoses admitted to the intensive care unit. They utilised 34 physiological and laboratory parameters each of which attracted a score of between 0 and 4 depending upon its degree of deviation from normal values. The higher the score the sicker the patient. Whilst it provided good stratification of disease severity it was unwieldy to use and was subsequently modified to give the APACHE II scoring system (Knaus *et al*/1984). This retains 12 of the original 34 parameters (figure 1.1). Its value has been confirmed on a series of 6,100 patients (Knaus *et al*/1985). We have applied this scoring system to 160 consecutive patients with acute pancreatitis (Wilson *et al*/1990). Utilising the peak APACHE II score of  $\geq 9$  we were able to distinguish mild and severe attacks of acute pancreatitis with a sensitivity of 82% and specificity of 76%, figures comparable to the Ranson and Glasgow scoring systems. No deaths occurred in patients with a score  $<10$  and only 6% developed a complication. Utilising a cut off value of  $>10$  Larvin and McMahon (1989) produced a comparable sensitivity of 72% and specificity of 92%. Interestingly a recent paper in which the APACHE II score was applied to patients with upper gastrointestinal hemorrhages utilised a value of 10 to distinguish between patients with a high and low risk of mortality (Schein and Gecelter 1989).

in Larvin and McMahon's paper they calculated the Medical Research Council (MRC) score and simplified acute physiology score (SAPS) in addition to the APACHE II score. Their sensitivity at 48 hrs was too low to be of use in clinical practice.

#### 1.11.4 Peritoneal aspiration.

The first account of paracentesis in acute pancreatitis was produced by Keith *et al* in 1950. The authors inserted a 20 gauge needle into the abdominal cavity of 15 patients with a presumptive diagnosis of acute pancreatitis and aspirated any free fluid.

Although they intended to use the technique to diagnose acute pancreatitis in patients with a normal amylase they noted that "turbid yellow fluid indicates the interstitial type (mild pancreatitis), whereas reddish brown fluid denotes haemorrhagic pancreatitis". They presented no histological evidence to support this hypothesis although their assumption that the discoloured fluid was associated with more severe disease has subsequently been confirmed. In 1957 Pfeffer *et al* reported a modified paracentesis technique which utilised a thoracentesis needle and trocar. They hoped that by using this technique they would be able to increase the likelihood of obtaining a fluid sample. In common with Keith *et al*, (1950) the authors employed this technique as an aid to diagnosis, noted the association between haemorrhagic peritoneal fluid and haemorrhagic pancreatitis (although again they do not present any evidence that this was the case) and failed to note the association between the fluid colour and severity. It was not until 1977 that the importance of paracentesis as a means of severity assessment was recognised. Pickford *et al* (1977) performed abdominal paracentesis with a one litre normal saline lavage on 27 patients with acute pancreatitis and then examined the fluid. The concentrations of albumin, AST, and total protein provided good discrimination between mild and severe groups and there were significant differences in the concentrations of urea, calcium, potassium, bilirubin, alkaline phosphatase, and white cell count between groups. Severe attacks were predicted on the basis of an aspirated fluid volume of greater than 10 ml and /or darkly discoloured fluid. Using these criteria 5 patients who had been predicted as mild by clinical assessment were correctly classified as severe.

These criteria were later refined as the result of a larger study conducted in Leeds (McMahon *et al* 1980) in which 96 patients had attempted paracentesis and lavage. The modified criteria were > 20 ml of free fluid and/or free fluid the colour of prune juice, and/or straw coloured return lavage fluid. The determination of fluid colour was made easier by the production of a fluid colour chart against which specimens could be compared (figure 1.2). Lavage was unsuccessful in 7 cases (5 due to

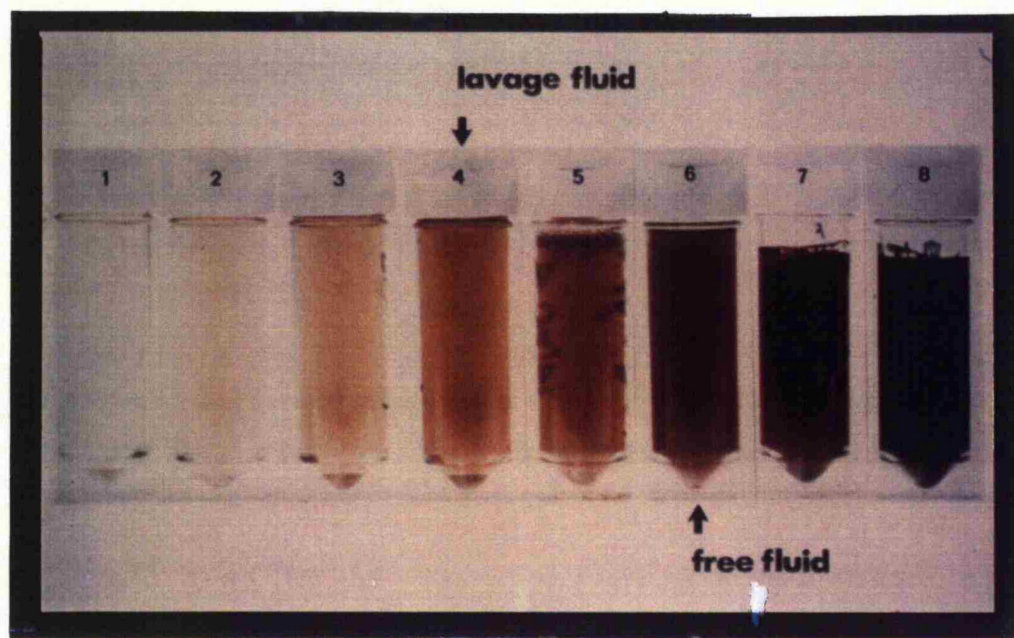


Figure 1.2: The Leeds colour chart for identifying the severity of acute pancreatitis from the colour of peritoneal fluid and lavage fluid obtained at peritoneal lavage.

incorrect placement of the cannula and 2 following suspected visceral perforation). There was only 1 visceral perforation; a stomach which had not been correctly emptied. This patient underwent laparotomy and subsequently survived. Seventy two percent of severe cases were correctly predicted as severe at a mean time after admission of 7 hours  $SD \pm 6.7$  hrs as against only 39% using clinical assessment. Clinical assessment, the Ranson and Glasgow criteria produced comparable prediction only after a delay of 48 hrs. A multicentre study of therapeutic peritoneal lavage conducted between Leeds, Glasgow and Bristol produced similar results (Corfield *et al* 1985). This study also showed that only 1 of 40 patients graded by this method required lavage. All others were graded by aspiration alone. It was especially valuable in patients with alcohol induced severe acute pancreatitis (Corfield *et al* 1985). It detected severe attacks with an accuracy of 90%, whilst in gallstone acute pancreatitis the accuracy was <50%.

The great advantage of paracentesis and peritoneal lavage is that it is capable of predicting the severity of an attack of acute pancreatitis at the time of admission. It has also been claimed that it reduces the amount of abdominal pain experienced by the patient although this has not been demonstrated in a controlled manner. A third advantage is the identification of causes of abdominal pain other than acute pancreatitis. Macroscopic examination of the fluid obtained will identify blood, bile or intestinal contents and Gram staining, organisms associated with visceral perforation (Mayer and McMahon 1985). Under these circumstances an urgent laparotomy should be performed. Disadvantages to this method include the fact that prediction is not as good for gallstone related as alcohol related attacks and the risk of visceral perforation.



#### 1.11.5 Imaging techniques.

##### 1.11.5.1 Computerised tomography (CT) scanning.

There have been a number of prospective studies addressing the usefulness of CT scanning as a means of severity assessment (Hill *et al* 1982, Grabbe *et al* 1982, Becker *et al* 1985, Balthazar *et al* 1985, Nordestgaard *et al* 1986, Takada *et al* 1986, Clavien *et al* 1988, London *et al* 1989). The factors looked at were the presence of pancreatic oedema as reflected by an increase in pancreatic size, the spread of inflammation and oedema outside the pancreas into the peripancreatic fat, "phlegmon" formation, the presence of peripancreatic collections and failure of enhancement of the gland with intravenous contrast.

Whilst a number of the studies included the presence of pancreatic swelling as part of their scoring systems only 3 have attempted to correlate pancreatic size directly with outcome (Becker *et al* 1988, Takada *et al* 1986, London *et al* 1989). Becker compared the sagittal diameter of the pancreas to the adjacent vertebral body and London the "pancreatic size index" by multiplying the maximum anteroposterior diameters of the pancreatic body and head. Using the latter London was able to distinguish mild from severe attacks with a sensitivity and specificity of 71% and 77% respectively. This compares favorably with the values of 85% and 79% for the modified Glasgow criteria. CT scanning is however, considerably more expensive and time consuming than the conventional methods and in addition may involve moving a seriously ill patient away from an intensive care setting.

The systems describing the extrapancreatic spread of inflammation tend to be complicated and were rationalised by London *et al* (1989) who simply suggested that the loss of the demarcation between the pancreas and the peripancreatic tissue indicated extrapancreatic spread. This gave a high sensitivity of 82% but an unacceptably low specificity of 45%. Likewise low dose contrast enhancement (which was not intended to detect pancreatic necrosis) failed to improve severity assessment (Grabbe *et al* 1982, London *et al* 1989).

The presence of inflammation extending into one or more of the peripancreatic spaces has been noted by other authors to be correlated with a high morbidity and mortality. Deaths in 2 American studies (Hill *et al*/1982, Balthazar *et al*/1985) only occurred where there was evidence of such extension.

The high mortality associated with pancreatic necrosis (Kivilaakso 1981, Nordback *et al*/1985, Hollander Edelman and Boutelier 1986, Block *et al*/1986) and our inability to detect its presence using the Ranson and Glasgow criteria (Teerenhovi *et al*/1988, Leese *et al*/1990), clinical assessment (Corfield *et al*/1985, Nordback *et al*/1985, Block *et al*/1986), visual inspection of the gland at the time of surgery (Nordback *et al* (1985) or biochemical parameters (Poulakkainen *et al*/1987, McMahon *et al*/1980, Nordback *et al*/1985, Leese *et al*/1988, Wilson *et al*/1991) has lead to a search for reliable means of detecting its presence. At present contrast enhanced CT scanning provides the best method of detecting pancreatic necrosis, an area of hypoperfusion being equated with the presence of necrotic tissue (Kivisaari *et al*/1984, Block *et al*/1986, Nuutinen *et al*/1988, Larvin *et al*/1990). Utilising these criteria Buchler *et al* (1986) have claimed that contrast enhanced CT scanning detects pancreatic necrosis with an accuracy of 85-90% and more recently Larvin *et al* (1990) have correctly identified 11 patients with histologically proven necrosis.

Whilst the multifactorial scoring systems correlate with the degrees of abnormality on CT examination (Clavien *et al*/1988) neither they nor CT scanning, used as described above, were capable of selecting those patients who developed local rather than systemic complications. It has been observed that patients with CT appearances suggesting a severe outcome have had an uncomplicated recovery, so it is not possible to base a decision for surgical intervention on these findings alone.

#### 1.11.5.2 Ultrasound.

McKay and co-workers found that the appearance of the pancreas on early ultrasound examination did not correlate significantly with objective assessment of severity by multiple factor scoring, although patients subsequently developing pseudocysts all had early localised pancreatic swelling (McKay *et al* 1982). Generalised swelling of the pancreas was frequently seen but appeared to be of no major clinical importance (McKay *et al* 1982). Furthermore in 22 patients (26%) the pancreas was unable to be visualised reducing the value of this technique which is itself very observer dependent.

#### 1.11.5.3 Nuclide scanning.

Interest has also been expressed in the technique of Indium<sup>111</sup> autologous leucocyte imaging of the pancreas and an initial study suggested that it may be as accurate as the multiple factor scoring systems (Anderson *et al* 1983).

Whilst it is hoped that early severity assessment will ultimately lead to a reduced morbidity and mortality, this has not been proven clearly.

### 1.12 THE ACUTE PHASE PROTEIN RESPONSE.

It was Abernathy and Avery who were the first to use the term "acute phase response" in 1941, although they were not the first to recognise the phenomenon. The earliest description of the acute phase protein response came from the Greeks (Fahraeus 1921) who observed an increase in the erythrocyte sedimentation rate due to what we now know to be an elevated plasma concentration of fibrinogen and other acute phase proteins (Sipe *et al* 1979). In man CRP and serum amyloid A undergo the greatest increases with lesser increases in  $\alpha_1$ -antitrypsin,  $\alpha_1$ -antichymotrypsin, haptoglobin and fibrinogen, no change in the concentration of  $\alpha_2$ -macroglobulin and a reduction in albumin and transferrin (table 1.12). In each case the alteration in the concentration of the acute phase proteins is preceded by a corresponding alteration in the

Increase	Human	Rat
10 fold	C-reactive protein	$\alpha_2$ -macroglobulin
	Serum Amyloid A	$\alpha_1$ -acid glycoprotein
2-100 fold	$\alpha_1$ -antiprotease	Fibrinogen
	$\alpha_1$ -antichymotrypsin	Haptoglobin
	Haptoglobin	Cysteine protease
	Fibrinogen	inhibitor
<2 fold	Caeruloplasmin	$\alpha_1$ -antiprotease
	C3 of Complement	Caeruloplasmin
	$\alpha$ -antiplasmin	Prekallikrein
	CP1-inhibitor	Haemopexin
No change		C-Reactive protein
	$\alpha_2$ -macroglobulin	$\alpha_2$ -macroglobulin
	Haemopexin	Antithrombin III
	Serum Amyloid P	Serum Amyloid P
Decrease	Prothrombin	Prothrombin
	Inter- $\alpha$ -antiprotease	$\alpha_1$ -inhibitor 3
	Transferrin	Transferrin
	$\alpha_1$ -lipoprotein	Prealbumin
	Prealbumin	Albumin
	Albumin	

Table 1.12: The acute phase protein response in man and the rat.

concentration of mRNA within the hepatocytes. In 1951 Miller *et al* were able to demonstrate that the liver was the major organ involved in the synthesis of acute phase proteins. In view of the fact that these changes resulted from injury at a distant site, it was presumed that the process must involve a hormone like mediator. Since leucocytes appeared quickly at the site of injury it was thought that they might be producing these substances. It was subsequently discovered that the supernatant from leucocytes stimulated the synthesis of a number of acute phase proteins in animals (Kampschmidt *et al* 1973, Powanda *et al* 1973, Wannemacher 1975) and that monocytes/ macrophages were the most important producers (Sipe *et al* 1979). Not surprisingly Interleukin-6 (IL-6) (the principal mediator of the acute phase protein response) was first identified as a product of leucocytes.

#### 1.12.1 C Reactive protein.

CRP is the most rapidly detectable of the acute phase proteins, appearing in the serum within 6 hours of an insult, usually reaching peak concentrations within 48 hours and which, in the absence of a continuing insult, is falling by 60 hours. The normal serum CRP level is <10mg/l.

C-reactive protein was discovered by Tillett and Francis (1930) whilst they were performing serological experiments with *Streptococcus pneumoniae* on patients suffering from pneumonia. One polysaccharide fraction of the organism, denoted fraction C, was found to precipitate when mixed with serum from acutely ill patients. This reaction was not seen in the serum of convalescing patients. CRP is, in addition to being able to bind fraction C, capable of binding a number of other substances. It undergoes calcium dependent binding to choline phosphatides (PC) such as lecithin, lysolecithin and sphingomyelin and to PC containing and non-PC containing microbial polysaccharides and peptidopolysaccharides found in many bacteria, fungi and parasites (Pepys 1981). It will also bind polyanions such as nucleic acids, heparin and dextran sulphate, the highest affinity binding occurring

with PC ( $K_m=10^{-7}$ ) (Pepys 1981). Non-calcium dependent binding also occurs to polycations such as histones, leucocyte cationic protein and protamine. The sites for calcium dependent and calcium independent binding are separate although they do interact (Tsujimoto *et al*/1980).

CRP is synthesised by the hepatocytes (Hurlimann *et al*/1966, Kushner and Feldmann 1978) as part of the acute phase response (Pepys 1981, Kushner *et al*/1981). It has a molecular weight of 105, 500 Daltons and is composed of five identical non-glycosylated polypeptide subunits which are non-covalently associated in a disc-like configuration with cyclic pentameric symmetry (Osmand *et al*/1977). Oliveira *et al* (1979) sequenced the molecule showing each subunit to be composed of 187 amino acids. Computer comparison of this sequence with other immune proteins has demonstrated only very distant homologies with the CH2 domain of IgG and the C3a fraction of complement (Oliveira *et al*/1979). The precise function of CRP is unknown although it is thought to be involved in the body's defence against "toxic substances" (Pepys 1981). CRP is capable, as noted by Kushner and Feldmann (1978), of binding ligands and agglutinating them. Once complexed, complement is activated via the classical pathway leading to lysis if the ligand is on a cell surface. Other activities ascribed to CRP include modification of T lymphocyte function, suppression of platelet aggregation and enhancement of the activity and motility of phagocytic cells (Pepys 1981). CRP has proved a useful marker of disease activity in a variety of inflammatory, infective and ischaemic conditions (Amos *et al*/1977, Kushner *et al*/1981). These include pneumonia, tuberculosis, pyelonephritis, Crohn's disease, ankylosing spondylosis, rheumatoid arthritis, myocardial infarctions and metastatic carcinoma of the breast. Serial measurements have been found useful in detecting the presence of postoperative complications (Mustard *et al*/1977), but have proved disappointing as a means of detecting the site or severity of intra-abdominal sepsis (Mustard *et al*/1977). Its importance as a marker of disease severity in acute pancreatitis has already been discussed in section 1.11.3.2.

### 1.12.2. Interleukin-6.

#### 1.12.2.1. History of Interleukin-6.

In 1973 Kishimoto *et al* described a product of T cells which induced antibody production in pre-activated normal and Epstein-Barr infected B cells, without first inducing cellular proliferation. They named it B cell stimulating factor (BSF-2). Seven years later Sehgal and Sagar (1980) described Interferon B2 (INF-B2), a protein produced by poly (I), poly (C) stimulated fibroblasts which displayed antiviral activity and has subsequently been shown to be identical to BSF-2 (Hirano *et al* 1986). Hybridoma growth factor (HGF) which has the capability of eliminating the need for feeder cells in the early stages of fusion experiments was identified in 1980. In 1983 Ritchie and Fuller described and partially characterised a product of activated macrophages, hepatocyte stimulating factor (HSF), which was capable of stimulating fibrinogen synthesis by rat hepatocyte cell cultures. Baumann *et al* (1984) showed that HSF was also produced by keratinocytes and that it was capable of modulating protein synthesis in tissue cultures of hepatocytes and human hepatoma cell lines. Finally all of the above factors were shown to be identical (Gauldie *et al* 1987), (table 1.13). Following this Poupart and Yasukawa proposed that this molecule be named interleukin-6. In spite of the fact that the nomenclature is somewhat confusing, since it suggests that the molecule is primarily produced by and acts upon leucocytes, the name was adopted at a conference of the New York Academy of Science and the National Foundation for Cancer Research in New York in December 1988 (Seghal *et al* 1988).

#### 1.12.2.2 Discrimination of Interleukin-6 from Interleukin-1 and tumour necrosis factor.

Since monocytes/ macrophages stimulated with endotoxin are capable of producing IL-1 and TNF as well as IL-6 it was necessary to investigate which of these substances was producing the acute phase protein response. The availability of recombinant IL-1, IL-6 and TNF meant that it was possible to observe the effect of these cytokines on the production of acute phase proteins in isolation. The results of these experiments are given in table 1.14 (although only the effects of IL-1 and TNF will be discussed here).

Name	Abbreviation	Reference
Hepatocyte stimulating factor	HSF	Ritchie and Fuller 1983 Andus <i>et al</i> 1987 Gauldie <i>et al</i> 1987 Baumann and Muller -Eberhard 1987 Koj <i>et al</i> 1988
B cell stimulating factor-2	BSF-2	Hirano <i>et al</i> 1985 1986
Interferon B2	INF-B2	Sehgal and Sagar 1988 Weissenbach <i>et al</i> 1980
26 KDa protein		Content <i>et al</i> 1982 Haegeman 1986
Hybridoma-plasmocytoma growth factor	HPGF	Van Damme <i>et al</i> 1987
Myeloid blood cell differentiation inducing factor	MGI-2A	Shabo <i>et al</i> 1988

Table 1.13: Synonyms for interleukin-6.



Acute phase protein	IL-6	IL-1	TNF	Reference
C-reactive protein	+++	0+	0	Castell <i>et al</i> 1988, 1989 Moshage <i>et al</i> 1988
Serum Amyloid A	+++	0+	0	Castell <i>et al</i> 1988 Moshage <i>et al</i> 1988
Haptoglobin	++	0	0	" "
$\alpha_1$ -antichymotrypsin	+	0	0	" "
Fibrinogen	++	-	-	" "
$\alpha_1$ -antiprotease	+	0	0	" "
$\alpha_1$ -acid glycoprotein	+	0	0	" "
$\alpha_2$ -macroglobulin	0	0	-	" "
Fibronectin	-	0	0	" "
Transferrin	-	-	-	" "
Albumin	-	-	-	" "

Table 1.14: The regulation of acute phase protein synthesis by IL-6, IL-1 and TNF in normal human hepatocyte culture. +++, ++ and + represent increased synthesis, 0 no change, - inhibition of synthesis and + augmentation of the action of IL-6.

IL-1 was capable of stimulating the production of rat haemopexin, haptoglobin,  $\alpha_1$ -glycoprotein, albumin, murine serum amyloid A and P and human metallothionein (Darlington *et al*/1986, Andus *et al*/1988, Geiger *et al*/1988a). It did not stimulate the production of the majority of acute phase proteins (Woloski *et al*/1985, Darlington *et al*/1986, Baumann *et al*/1987, Goldmann *et al*/1987, Northoff *et al*/1987, Andus *et al*/1988). TNF had a very limited effect on a subset of acute phase proteins. It has been demonstrated to stimulate the production of C3 component of complement in the Hep 3B and Hep G2 hepatoma cell line (Darlington *et al*/1986, Andus *et al*/1988) and in addition to produce  $\alpha_1$ -antichymotrypsin (Perlmutter *et al*/1986). However these effects on Hep G2 hepatoma cells were not seen by Baumann and Muller-Eberhard (1987) or Goldmann *et al*(1987), or in rat hepatocyte culture (Andus *et al*/1988). It is clear from these results that neither IL-1 nor TNF was capable of producing the acute phase protein response seen in man.

#### 1.12.2.3 The Induction of the acute phase protein response in the rat by Interleukin-6.

Utilising recombinant IL-6 Gauldie *et al* were able to stimulate the production of  $\alpha_2$ -macroglobulin and cysteine protease inhibitor in rat hepatocytes (Gauldie *et al*/1987). Further experiments in rat hepatocyte primary culture (Andus *et al*/1988) and in the rat hepatoma cell lines Fao and H-35 (Andus *et al*/1988, Baumann *et al*/1987) showed that the administration of human recombinant IL-6 led to a dose and time dependent regulation of  $\alpha_2$ -macroglobulin,  $\beta$  fibrinogen, cysteine protease inhibitor,  $\alpha$  acid glycoprotein, angiotensin and albumin. Thirty pmol of human recombinant IL-6 (rIL-6) produced a half maximal acute phase response in hepatocyte cultures. A maximal response was achieved with 1 nmol of human rIL-6 (Andus *et al*/1988). IL-6 also had a stimulatory effect on the acute phase proteins. *In vivo* experiments, in which rats were given recombinant IL-6, confirmed the findings of the earlier *in vitro* studies. Increases in the hepatic mRNA for  $\alpha_2$ -macroglobulin,  $\beta$  fibrinogen, cysteine protease inhibitor and acid glycoprotein, as well as reductions in mRNA for albumin were observed (Geiger *et*

*et al* 1988). Supportive evidence for the hypothesis that IL-6 is released from the site of inflammation comes from studies on the time course of the response. When recombinant IL-6 is injected into the rat it produces a more rapid acute phase protein response than when an inflammatory response is artificially induced through the intramuscular injection of turpentine (Geiger *et al* 1988). Similar changes in the acute phase proteins have been observed in mice (Ramadori *et al* 1988, Neta *et al* 1989).

#### **1.12.2.4 The induction of the acute phase protein response in man by Interleukin-6.**

Initial experiments in this area involved the use of the hepatoma cell lines Hep G2 and Hep 3B. Stimulation of Hep G2 cells by rIL-6 resulted in the production of a fibrinogen,  $\alpha_1$ -antichymotrypsin, caeruloplasmin, haptoglobin,  $\alpha_1$ -acid glycoprotein, complement factor B and  $\alpha_1$ -antiprotease. There was no increase in the major acute phase proteins CRP and serum amyloid A (Perlmutter *et al* 1986, Baumann *et al* 1987, Gauldie *et al* 1987, Daveau *et al* 1988, Ganapathi *et al* 1988, Koj *et al* 1988, Mackiewicz *et al* 1989). The stimulation of these proteins was only seen when IL-1 was present (Ganapathi *et al* 1988). Only NPLC/PRF/5 cells would produce CRP and serum amyloid A with IL-6 alone (Ganapathi *et al* 1988). It was not until IL-6 was administered to human hepatocyte cultures that the importance of IL-6 as a major inducer of the acute phase protein response was demonstrated. Both CRP and serum amyloid A were produced in a dose and time dependent manner (Castell *et al* 1989).

#### **1.12.2.5 Discrimination of the effects of IL-6 from those of IL-1 and TNF.**

Having examined the effects of IL-1, IL-6 and TNF separately it became necessary to assess the interaction between them. This has considerable importance since they are usually secreted together. Studies in animals and hepatoma cell lines showed that IL-1 was capable of inhibiting the IL-6 induced synthesis of  $\alpha_2$ -macroglobulin, cysteine protease inhibitor and fibrinogen in rats hepatocyte

cultures (Gauldie *et al* 1987, Koj *et al* 1987 and Andus *et al* 1988) and of potentiating the production of CRP and serum amyloid A (Moshage *et al* 1988, Castell *et al* 1989). TNF does not modulate the effects of IL-6 (Gauldie *et al* 1987, Koj *et al* 1987 and Andus *et al* 1988). The work comparing the effects of a combination of IL-6 and either IL-1 or TNF was repeated in the human hepatocytes culture system (table 1.12). IL-6 continued to produce a full spectrum of positive and negative acute phase proteins whilst IL-1 and TNF had only a moderate effect on the positive acute phase proteins (Castell *et al* 1989). Both inhibited the synthesis of  $\beta$  fibrinogen, albumin, and transferrin and failed to induce CRP or serum amyloid A (Castell *et al* 1989). Interestingly IL-1 potentiated the effects of IL-6 on the production of CRP and serum amyloid A (table 1.12).

#### 1.12.2.6 Regulation of Interleukin-6 biosynthesis.

IL-6 is produced by a wide variety of cells including monocytes/ macrophages (Navarro *et al* 1989), fibroblasts (Content *et al* 1982), endothelial cells, keratinocytes, B and T lymphocytes, synoviocytes, amnion cells, endometrial stromal cells, bone marrow stromal cells, astrocytes, and microglial cells. However monocytes/ macrophages, fibroblasts and endothelial cells are probably the most important source during inflammation since they are abundant and widely distributed, have a high capacity to synthesise and secrete IL-6 and can be triggered by a wide variety of stimuli. Monocytes/ macrophages are preferentially stimulated by endotoxin, and to a lesser extent by IL-1 (May *et al* 1988) whilst fibroblasts and endothelial cells respond better to the endogenous cytokines IL-1 and TNF (Van Damme *et al* 1987). These are both secreted in large quantities by monocytes/macrophages and suggests that these cells play an important role in IL-6 production. In support of these results, obtained from *in vitro* studies, are a number of human studies. Elevated concentrations of IL-6 have been observed in human volunteers following the intravenous injection of lipopolysaccharide (Coule *et al* 1987) and TNF (Brouckaert *et al* 1989) and following infections (Helfgott *et al* 1989).

#### 1.12.2.7 Hepatocytes as targets for Interleukin-6.

It has been noted above that the primary site of acute phase protein synthesis is the liver. In rats injected intravenously with  $^{125}\text{I}$  labelled human rIL-6 both radioactivity and biological activity disappeared rapidly from the circulation. The kinetics of clearance are biphasic with a rapid initial clearance corresponding to a half life of 3 minutes, followed by a slower second decrease corresponding to a half life of 55 minutes (Castell *et al* 1988). Twenty minutes after the Injection of  $^{125}\text{I}$  labelled recombinant IL-6 80% had disappeared from the circulation and was found to have been taken up by the liver (Castell *et al* 1988). The labelled IL-6 was localised to the surface of the parenchymal cells suggesting the existence of cell surface receptors (Castell *et al* 1988). Since then receptors have been found on the surface of a variety of cells (Coulie *et al* 1987).

#### 1.12.2.8 Interleukin-6 concentration in disease states in man (clinical studies).

To date the number of clinical studies involving IL-6 is limited by the very recent nature of most of the discoveries and the problems associated with setting up and running an IL-6 assay. In a letter to the Lancet Nijsten *et al* (1987) reported the results of IL-6 and acute phase proteins measurements (CRP,  $\alpha_1$ -antiprotease and  $\alpha_2$ -macroglobulin) in 13 patients with burns. Peak IL-6 concentrations were seen within a few hours of admission and were 2 to 100 times normal. Peak CRP and  $\alpha_1$ -antiprotease concentrations were seen on the 1st and 2nd post burn days respectively. This time lag was explained by the fact that IL-6 was directly produced by stimulated monocytes, whereas the acute phase proteins were synthesised in the liver after induction by mediators. They then went on to suggest that CRP was positively correlated ( $r = 0.44$ ,  $p < 0.0001$ ) and total protein negatively correlated ( $r = -0.48$ ,  $p < 0.001$ ) with IL-6 concentrations. These conclusions are not supported by the evidence. Each patient had three serum samples taken: on admission, day 1 and 2 months after the burn injury. In analysing the results they should have correlated results for each of these times

separately. Instead they grouped all 3 groups together and plotted the results on a single graph. Such a practice merely increases the numbers spuriously and leads to invalid conclusions. In the final paragraph they suggest that IL-6 plays a causal role in the acute phase response. Whilst it undoubtedly does, this conclusion is not supported by the evidence presented: i.e. an association does not imply causation. In summary the data are consistent with the hypothesis that IL-6 was the mediator of the acute phase protein response but did not prove it.

In 1988 the same group published a paper in which they examined serum and urinary concentrations of IL-6 in patients undergoing renal transplantation (Van Oers *et al* 1988). Their aims were to examine the possible significance of IL-6 in inflammation and to see whether IL-6 could be utilised as a means of detecting episodes of rejection in renal transplant patients. There is no mention in the paper of attempts to correct the IL-6 concentrations with reference to creatinine clearance. IL-6 concentrations in serum and urine were comparable to the control group prior to transplantation but rose following surgery to peak on day 2. In those patients who did not develop rejection, IL-6 concentrations fell towards normal, whilst in those patients who developed rejection (based on fine needle aspiration cytology of the transplanted kidney) concentrations rose again. A rise in IL-6 concentrations were seen 48 hours before rejection became evident and subsided when the episode was controlled with steroids. Urinary concentration of IL-6 tended to be higher than that in serum. This is not surprising in view of the fact that the site of inflammation is within the kidney. The authors conclude that IL-6 measurements may have a place in the monitoring of renal transplant patients but do not set any concentrations which might allow us to reliably detect these episodes. Neither did they tell us about CRP or the other acute phase protein concentrations so we have no idea of the time course of IL-6 or acute phase protein production. The relative cheapness and wide availability of the CRP assay may have made it a more sensible means of monitoring for rejection.

Houssiau *et al*(1988) measured IL-6 concentrations in the CSF from 218 patients with a variety of neurological disorders. The most striking fact was that high concentrations were seen in patients with infective meningitis or encephalitis with no significant differences between those with viral or bacterial disease. They also demonstrated that concentrations were higher in patients sampled early in the disease course. Whilst the authors suggest that it may provide an indicator of the presence of meningitis it does not provide any information that could not be more easily, usefully and quickly obtained by Gram staining and culture of the CSF.

Helgott *et al* (1989) examined the CSF and serum of 4 patients with meningitis (2 of whom had a bacteraemia), serum from 3 patients with bacteraemia from other causes, as well as joint effusion from a patient with septic arthritis. High concentrations of IL-6 were found in the CSF of the patients with meningitis (up to 500 ng/ml) whilst lesser concentrations were noted in the 2 patients with bacteraemia (5-70 ng/ml). The 3 other patients with bacteraemia and the joint fluid had high concentrations. IL-6 was not detected in the serum or CSF of controls. These findings are in agreement with the work of Houssiau *et al*(1988) described above.

More recently Shenkin *et al*(1989) have examined the relationship between IL-6 and surgical trauma. They studied 6 patients undergoing routine cholecystectomy. Venous blood samples were taken 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, 36, 48 and 96 hours post surgical incision. Increases in serum concentrations of IL-6 were seen between 0.5 and 1.5 hrs after the skin incision and peaked between 1.5 and 4 hours. CRP concentrations were not detectable until between 8 and 12 hrs and peaked between 36 and 48 hrs. Whilst there was a significant correlation between the IL-6 concentration and the duration of surgery ( $r = 0.95$ ) there was no significant correlation between peak IL-6 and CRP concentrations or between CRP concentration and the duration of surgery.

A similar study involving 26 patients undergoing a range of elective operations from minor through to major measured IL-6 and CRP concentrations over the first 48 post operative hours. Serum IL-6 concentrations became detectable at between 2 and 4 hours and peaked at between 6 and 12 hours. This is somewhat later than the study conducted by Shenkin *et al* (1989). Whilst there was no detectable rise in CRP concentrations in those undergoing minor surgery, in all other patient groups concentrations rose after 8-12 hrs and peaked between 24 and 48 hours (Cruickshank *et al* 1990). Comparison of the areas under the CRP and IL-6 curves showed a correlation co-efficient of 0.67. The authors concluded that this weak correlation reflected the fact that IL-6 is not the only stimulator of the acute phase protein response. This is reasonable in view of the fact that Baumann *et al* (1987) have shown that maximal CRP production only occurs in the presence of IL-6, IL-1 and dexamethasone.

To date there have not been any attempts to use IL-6 concentrations as a means of severity prediction in any medical or surgical condition.

#### 1.13 AIMS OF THE THESIS.

The principal aim of this thesis is to develop a reliable means of detecting severe attacks of acute pancreatitis at an early a stage as possible. Such early discrimination has a number of potential advantages. It would allow the selection of patients who require intensive monitoring and therapy, help in the early prediction of complications, allow comparison of groups of patients from different centres, and select those suitable for inclusion in therapeutic trials (Larvin and McMahon 1989) and for expensive monitoring by contrast enhanced CT scanning.

In spite of the large number of systems employed in an attempt to select patients with severe disease each has its drawbacks and at present none can be considered ideal.



The importance of zymogen activation in the production of complicated attacks of acute pancreatitis has been discussed in section 1.8 as have the problems associated with the measurement of parent zymogen concentrations (sections 1.5.4 and 1.11.3.10). This thesis describes the development of an immunoassay against the C terminal end of the PLAP molecule and correlates urinary PLAP and TAP concentrations with the disease severity of acute pancreatitis, through clinical studies.

Synthetic PLAP (CYPLAP) will be haptenised to bovine thyroglobulin and used to immunise rabbits. These sera was harvested, purified and used in the development of an immunoassay which will detect the presence of PLAP, but not the parent zymogen. The latter assertion will be tested by assaying trypsinised and untrypsinised homogenates of human pancreas for PLAP. Once the reliability of the assay has been established it will be employed in investigating the stability of PLAP in urine and serum.

Finally a number of clinical studies will performed to establish the usefulness of TAP and PLAP assays in assessing disease severity in acute pancreatitis. More specifically these will take the form of a study measuring urinary TAP and PLAP concentrations in patients with acute pancreatitis, disease controls, who have "acute abdominal conditions" other than acute pancreatitis and healthy controls. The second clinical study will measure the concentrations of TAP and PLAP in the peritoneal fluid of patients with acute pancreatitis and examine the relationship between their concentration and pancreatic necrosis. The final study will examine the relationship between the severity of acute pancreatitis, markers of the acute phase protein response (IL-6 and CRP) and urinary concentrations of TAP and PLAP.

## **CHAPTER 2:**

### **THE DEVELOPMENT AND CHARACTERISATION OF AN IMMUNOASSAY FOR THE FREE ACTIVATION PEPTIDES OF HUMAN PROPHOSPHOLIPASE**

***A<sub>2</sub>***

## 2.1. INTRODUCTION AND AIMS.

The hypothesis, laid out in section 1.8.1, that zymogen activation occurs only in the necrotising, but not in the oedematous form of acute pancreatitis, provides us with a potential means of detecting patients at risk of developing severe acute pancreatitis (Hermon-Taylor and Heywood 1985, Rinderknecht 1986). The problems associated with the measurement of serum trypsin and phospholipase A<sub>2</sub> concentrations, as laid out in sections 1.5.4 and 1.11.3.10, mean that we are still searching for a better means of quantifying their concentrations in patients with acute pancreatitis.

The activation peptides of pancreatic zymogens are produced in equimolar quantities to the active enzymes and are rapidly excreted in the urine, in which they can be detected readily (Hurley *et al* 1988). By directing the antibody against the C-terminal end of the activation peptide (the end attached to the active zymogen) it will be possible to quantify the degree of zymogen activation, without reporting the presence of the parent zymogen.

Trypsinogen was considered an ideal subject for initial study. It is quantitatively the most abundant zymogen (Guy *et al* 1978), its activation is considered to be the earliest event in the production of an attack of necrotising acute pancreatitis (Hermon Taylor and Heywood 1985, Rinderknecht 1986) and there is good preservation of the amino acid sequence of trypsinogen activation peptide (TAP) between species and between isoenzymes of trypsinogen (Davie and Neurath 1955, Charles *et al* 1963, Bricteux *et al* 1966, Bricteux *et al* 1970, Bricteux *et al* 1971, Bricteux *et al* 1971, Bricteux *et al* 1974, Louvard and Puigserver 1974, , Bricteux *et al* 1975, Guy *et al* 1978). The development of such an assay has been successfully achieved (Hurley *et al* 1988).

The strong implication of phospholipase A<sub>2</sub> in the pathogenesis of acute pancreatitis (Massenhoff *et al* 1964, Hatao 1969, Schmidt and Creutzfeldt 1969, Poncelet and Thompson 1972) made it the obvious candidate for the development of the next immunoassay.

## **2.2. DEVELOPMENT OF THE ASSAY FOR FREE PLAP.**

### **2.2.1. MATERIALS.**

Prophospholipase A<sub>2</sub> activation peptide (PLAP) and PLAP with cysteine and tyrosine residues attached to the N-terminal end (CYPLAP) were synthesised at Cambridge Research Biochemical Ltd. (Harston, England). Other peptides were synthesised, purified and characterised by the Peptide Chemistry Unit, Department of Surgery, St. George's Hospital Medical School, London. Bovine thyroglobulin, Freund's complete and incomplete adjuvant were obtained from Sigma (Poole, England) and dialysis tubing from Marathon laboratories (London, England). All other chemicals were of analytical grade, or highly purified, and obtained from BDH (Dagenham, England) or Sigma (Poole, England).

#### **2.2.1.1. Amino acid analysis and column chromatography.**

Amino acid analysis was performed using an LKB 4400 amino acid analyser (Milton-Keynes, England). Sephadex G-15, G-25 and G-50 were obtained from Pharmacia Ltd. (Milton-Keynes, England) and activated 6-aminohexanoic acid Sepharose-4B from Sigma (Poole, England). Cytochrome C and Chymotrypsinogen A, which were used to calibrate the Sephadex G-50 column, were obtained from Cambiotech (Mannheim, W. Germany).

An LKB 2138 Uvicord S detector, LKB 2210 channel recorder and 2112 Redirac fraction collector were used in column chromatography (Milton Keynes, England). Concentrations of protein and peptide solutions were measured using a Perkin-Elmer Lambda 5 uv/vis spectrophotometer (Oakbrook, USA).

#### **2.2.1.2. Radioimmunoassays.**

Radiolabelled Na <sup>125</sup>I was obtained from the Radiochemical Centre (Amersham, England) and RiA grade bovine serum albumin from Sigma (Poole, England). Donkey anti-rabbit serum was initially employed as a second antibody (Wellcome Reagents Ltd., Beckenham, England) until production was discontinued. Thereafter goat anti-rabbit serum from Calbiochem (San Diego, USA) was used.

#### 2.2.1.3. Pancreas extract studies.

Equine pancreas acetone powder, TPCK treated bovine trypsin, and bovine lung Aprotinin were purchased from Sigma (Poole, Dorset). Human pancreas was obtained from freshly excised surgical specimens. These were obtained from patients undergoing Whipple's procedure for carcinoma of the head of pancreas. Only macroscopically normal tissue was used.

#### 2.2.2. METHODS.

##### 2.2.2.1. Coupling of CYPLAP to bovine albumin.

CYPLAP (Cys-Tyr-Asp-Ser-Gly-Ile-Ser-Pro-Arg) was coupled to bovine thyroglobulin (Tg) using the heterobifunctional linker m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as described by Liu *et al* (1979). This method relies on the coupling of the N-hydroxysuccinimide group to the free amino groups in the thyroglobulin molecule and the m-maleimidobenzoyl to the cysteine residue of CYPLAP.

Thirty three µg of bovine thyroglobulin was dissolved in 2.06 ml of 10 mM sodium phosphate buffer at pH 7.4 and activated by the dropwise addition of a solution of 5.8 mg of MBS in 100 µl of dimethyl formamide (DMF). The mixture was stirred at room temperature for 30 minutes, centrifuged at 1400 g for 3 minutes and the supernatant decanted.

Free MBS was removed by passing the supernatant down a Sephadex G-25 column (28 x 0.75 cms) equilibrated with 50 mM of sodium phosphate buffer (PBS) at pH 6.0. A flow rate of 1.3 ml per minute was used and 2.5 ml aliquots collected and their absorbance measured at 280 nm. The fractions containing the first peak contained the Tg-MBS complex and were therefore saved. Twenty five mg of CYPLAP was dissolved in 5 ml of deoxygenated phosphate buffered saline and mixed at room temperature with 6 ml of the Tg-MBS complex. The solution was

continuously flushed with nitrogen to prevent oxidation of the thiol group on CYPLAP. The product was then dialysed overnight at 4°C using 2 L of PBS and then for a further 24 hours using 2 L of 0.15 M sodium chloride solution. Five hundred µl of the final solution was hydrolysed for amino acid analysis and the remainder aliquoted and stored.

#### 2.2.2.2. Modified Lowry assay.

A modified Lowry assay was used to measure the amount of protein in a solution (Markwell *et al* 1978).

The following reagents were used:-

Solution A: 2% w/v sodium bicarbonate, 0.4% w/v sodium hydroxide, 0.16% potassium tartrate and 1% w/v SDS.

Solution B: 4% w/v copper sulphate

Solution C: A freshly prepared mixture of solution A and 0.2 ml of solution B.

Solution D: Folin reagent diluted 50:50 with distilled water.

A protein solution containing 0.25 mg of BSA was utilised as a standard. 0.2 ml of the solution containing 10-100 mg/ml of protein was mixed with 0.6 ml of solution C and left to stand at room temperature for 15 minutes. This was then mixed with 60 µl of solution D and left for a further 45 mins. The optical density of the resulting solution was read at 660 nm on a Perkin-Elmer Lambda 5 uv/vis spectrophotometer and the protein concentration read by comparison against the standard curve constructed using the BSA.

#### 2.2.2.3. Amino acid analysis.

The solutions utilised in this assay were:

Solution A: 50 mg of phenol in 1 ml of 6 N hydrochloric acid.

Solution B: 20  $\mu$ l of mercaptoacetic acid in 1 ml of 6 N hydrochloric acid.

Solution C: 15 ml of 6 N hydrochloric acid plus 30  $\mu$ l of solution A plus 37.5  $\mu$ l of solution B.

One hundred mg of bovine thyroglobulin, and a similar quantity of the thyroglobulin CYPLAP conjugate were added to separate hydrolysis tubes. The amount of protein in each was then measured using the modified Lowry method described above. Half a ml of solution C was added to each the hydrolysis tubes which were then sealed under a vacuum and placed in an oven at 110°C for 24 hours. The seals were then broken and the samples placed in a desiccator, under vacuum, until dry. Two hundred  $\mu$ l of citric acid buffer at pH 2.2 was added to each tube through a 0.2  $\mu$ m pore filter and centrifuged at 2500 rpm for 5 minutes. The amino acid analyser capsule was washed in buffer and then 20  $\mu$ l of norleucine, followed 50  $\mu$ l of the above filtrate and finally 10  $\mu$ l of the buffer, added. The capsule was then placed in the LKB 4400 amino acid analyser and the amount of peptide bound to the thyroglobulin calculated from the amino acid profiles produced.

#### 2.2.2.4. Generation of antisera.

Twenty ml of blood was removed from each of the 2.5-3.0 kg New Zealand White rabbits prior to immunization. Each rabbit was then immunised by the subcutaneous or intramuscular injection of 1 ml of a 50:50 mixture of Freund's complete adjuvant and 1.0 mg/ml of Tg-CYPLAP in 0.15 M sodium chloride. Monthly booster injections of 1 ml of a 50:50 mixture of Freund's incomplete adjuvant with Tg -CYPLAP were given. After the first 4 months these booster injections were performed every 2 months. Blood was taken 14 days after each inoculation. Approximately 10 ml of antiserum was obtained at each venesection. This was aliquoted and stored at -20°C until required.

#### 2.2.2.5. Radioiodination of CYPLAP.

This was performed using the chloramine T method (Hunter and Greenwood 1962). Ten ml of 0.5 M phosphate buffer at pH 7.4 and 5  $\mu$ l of a 0.5  $\mu$ g/ml solution of CYPLAP in 0.05 M phosphate buffer were added to a microcentrifuge tube and thoroughly mixed. One mCi of Na  $^{125}$ I and 5  $\mu$ l of a 4 mg/ml solution of chloramine-T in 0.1 M phosphate buffer (pH 7.4) was then added, the solution mixed and allowed to stand for 3 minutes. The reaction was stopped by the addition of 20  $\mu$ l of a 2 mg/ml solution of sodium metabisulphate and 2 minutes later 10  $\mu$ l of sodium iodide (1 M). This mixture was run down a G-15 Sephadex column (25 x 0.325 cms) which had been equilibrated with 0.1 M tris hydrochloride buffer containing 0.1% BSA at pH 7.4. Half ml fractions were collected, 10  $\mu$ l aliquots removed from each, and their radioactivity determined. Fractions from the first peak were pooled, counted on an LKB 1282 gamma counter, aliquoted and the radioiodinated CYPLAP stored at -20°C until needed.

#### 2.2.2.6. Radioimmunoassay for phospholipase A<sub>2</sub> activation peptide.

Antisera from rabbits immunised using Tg-CYPLAP were diluted (depending upon the concentration of the antibody) in 0.05% tris hydrochloride buffer at pH 7.4 which contained 2.5 mg/ml of BSA, 0.15 M sodium chloride and 0.05% NaH<sub>2</sub>PO<sub>4</sub> (RIA buffer), plus 2% w/v normal rabbit serum.  $^{125}$ I-CYPLAP was diluted in RIA buffer to give 10<sup>4</sup> cpm in 100  $\mu$ l. 1 vial of goat anti-rabbit Ig G was made up in 10 ml of RIA buffer.

One hundred  $\mu$ l of diluted specific antiserum was mixed with 100  $\mu$ l of standard solution, unknown or RIA buffer and 100  $\mu$ l of  $^{125}$ I- CYPLAP solution in LP4 tubes. Fifty  $\mu$ l of goat anti-rabbit serum was then added, the tubes mixed and incubated overnight at 4°C. They were then centrifuged at 600g for 30 minutes at 4°C, the supernatant aspirated and the pellet counted in an LKB 1282 gamma counter. A concentration of antisera sufficient to give 30% binding of  $^{125}$ I-CYPLAP was chosen as that which provided optimum conditions for the competitive assay. A standard curve was constructed by plotting the percentage of  $^{125}$ I-CYPLAP bound against



the log of the concentration of competing ligands. The affinity constants ( $K_D$ ) were determined using the Longmuir equation. The intra assay coefficient of variation is calculated by repeating points on the standard curve 8 times in the same run. It was calculated from the formula:-

$$\text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{mean}} \times 100$$

The limit of detection of the assay was the concentration corresponding to 2 standard deviations from the blank assay or the concentration causing 90% inhibition of binding. Peptides, both related and unrelated to the amino acid sequence of PLAP and CYPLAP were assayed in order to determine specificity. Once a reproducible assay had been produced it was possible to calculate the concentration of PLAP in unknown samples using the LKB 1282 computer program.

#### **2.2.2.7. Purification of the antisera.**

Specific anti-PLAP antibodies were isolated from the rabbit serum by affinity purification on immobilised PLAP. Three g of activated 6-aminohexanoic acid Sepharose-4B was suspended in 60 ml of 1 mM hydrochloric acid for 1 hour. The swollen gel was then washed on a sintered glass filter with 500 ml of 1 mM hydrochloric acid followed by 10 ml of coupling buffer (0.1 M sodium bicarbonate and 0.5 M sodium chloride). Two mg of PLAP was dissolved in 5 ml of coupling buffer, added to a stoppered bottle, containing activated gel, and rotated at room temperature for 90 mins. One ml of 3 M tris at pH 8.0 was then added to block the excess carboxylate groups on the gel and rotation continued for a further hour. The gel was then washed alternately in 50 ml of acetate in 0.5 M sodium chloride buffer at pH 4.5 and tris hydrochloric acid buffer at pH 8.0 through sintered glass, on four occasions. The first 2 washes were collected for assay.

The substitution of PLAP onto the gel was demonstrated by amino acid analysis and radioimmunoassay.

Amino acid analysis involved hydrolysis of the gel. Two hundred  $\mu$ l of 50:50 mixture of activated Sepharose gel in 50 mM tris buffer was placed in a microcentrifuge tube and spun for 5 minutes. The supernatant was decanted, 100  $\mu$ l of distilled water added and the gel mixed by hand. A 100  $\mu$ l aliquot of the final suspension was added to the hydrolysis tube and assayed as previously described. A similar assay was performed on Sepharose gel without any attached PLAP.

The radioimmunoassay was performed on:-

1. PLAP plus buffer prior to the addition of gel (diluted 1:1000, 1: 5000, 1: 10000).
2. The initial filtrate which contained any unbound PLAP (diluted 1: 2000).
3. The first washing of the tris buffer (diluted 1:2000).
4. The first washing of the acetate buffer (diluted 1:1000).

The assay demonstrated that the majority of the PLAP had bound to the gel. The affinity column was packed and equilibrated with 50 mM tris hydrochloride buffer at pH 7.4. Pooled antisera was then passed through a 0.65 micrometer millipore filter and then added to the column. A flow rate of 1 ml per minute was used and 2 ml aliquots collected. Their absorbance was measured at 280 nm. Once the absorbance had returned to the baseline following the first peak 50 mM tris buffer with 0.5 M sodium chloride was run through the column to elute the second peak which contained all electrostatically bound contaminants. Following a return to baseline a propionic acid gradient was run through the column whilst 1 ml aliquots were collected into tris hydrochloride. Fractions of the individual peaks were dialysed against 50 mM tris at pH 7.4 for 36 hours with 2 changes of buffer. The separately pooled fractions were then subjected to photospectrophotometry

using an extinction co-efficient of 1.4 for 1 mg/ml solution to determine the protein concentration, gel electrophoresis to determine the immunoglobulin status and radioimmunoassay to determine the antibody titre and affinity. The affinity purified antisera was concentrated using the B15 Minicon concentrator, aliquoted and stored at -20°C until required.

#### **2.2.2.8. SDS polyacrylamide gel electrophoresis (PAGE).**

This was performed using 10% acrylamide slab gels (18 X 16 cms) according to the method of Laemmli (1970). Five  $\mu$ g samples were reduced, prior to electrophoresis, with mercaptoethanol, the proteins electrophoresed for 6h at 150 Volt. The gel was then stained with 0.05% Coomassie brilliant blue (R250) N 12.5% w/v trichloroacetic acid. Standard proteins were human Ig M, m chain, BSA, human Ig G heavy chain, bovine aldolase, bovine carbonic anhydrase, bovine chymotrypsinogen A and human immunoglobulin light chain.

#### **2.2.2.9. Preparation of Cys-Tyr-Asp-Ser-Gly-Ile-Ser-Pro-Arg rabbit serum albumin adduct (RSA).**

The method used was that of Bassiri and Utiger (1971) which utilises bis-diazotised benzidine (BDB).

To make the BDB 30 mg of benzidine dichloride was dissolved in 6 ml of 0.2 M hydrochloric acid and 0.6 ml of sodium nitrite solution (200 mg/ 5.7 ml of distilled water) added. The solution turned orange immediately and the reaction was allowed to continue at 4°C for 1 hour whilst being stirred. Two ml of BDB solution in 2.5 ml of 0.6 M borate and 0.13 M sodium chloride at pH 9.0 were added to 12.5 mg RSA and 5 mg of CYPLAP. A dark brown colour appeared immediately which later turned dark green. The reaction was allowed to continue at 4°C for 2 hours. The mixture was then dialysed at 4°C, against 2 L of saline, for 48 hours with one change of buffer. The protein concentrations were then determined by the Lowry assay.

#### **2.2.2.10. Preparation of human pancreas homogenate.**

Surgically excised human pancreas was homogenised in 10 mM tris hydrochloride containing 5 mM calcium chloride (at pH 7.4) in a ratio of 1 g of tissue to 1.5 ml of buffer. The homogenate was centrifuged for 15 minutes at 1400 g, the supernatant decanted and diluted 1: 10 with tris hydrochloride buffer and recentrifuged. Two ml of Aprotinin was then added to the supernatant which was stored at -70°C until needed.

#### **2.2.2.11. Determination of the presence of PLAP in untrypsinised and trypsinised human pancreatic homogenate.**

The homogenate of human pancreas were subjected to RIA for PLAP both before and after trypsinisation. Trypsinisation was achieved by incubating 0.2 ml of 10 mg/ml trypsin/RIA buffer solution with 1 ml of homogenate for 40 minutes at 37°C. The reaction was stopped by placing the mixture over boiling water for 5 minutes. One ml of trypsinised and untrypsinised homogenate was subjected to G-25 column chromatography and the eluted fractions assayed for free PLAP. One ml of the homogenates was also subjected to G-50 column chromatography, followed by trypsinisation of the fraction, to demonstrate that the peptide is eluted where phospholipase A<sub>2</sub> would be expected.

### **2.3. DETERMINATION OF THE STABILITY OF PLAP .**

#### **2.3.1. INTRODUCTION AND AIMS.**

These studies were performed in order to determine the conditions under which clinical samples would have to be taken and stored.

#### **2.3.2. METHODS.**

##### **2.3.2.1. Stability of PLAP to boiling.**

Two sets of serial dilutions of PLAP were prepared with concentrations from 10<sup>-5</sup> to 10<sup>-12</sup> Mol/l. One set was suspended over boiling water for five minutes, centrifuged for a further 5 minutes and the supernatant subjected to radioimmunoassay. The second set were assayed untreated.

#### 2.3.2.2. Stability of PLAP in urine.

A mid stream specimen of urine was obtained from a healthy male. An aliquot was frozen and stored at  $-20^{\circ}\text{C}$  whilst the remainder had PLAP added to give a final concentration of  $5 \times 10^{-8}$  Mol/l. The urine was then added to 3 tubes. One containing (EDTA), one 0.05% sodium azide and the third no additives. The samples from each tube were split one set being stored at room temperature and the second at  $4^{\circ}\text{C}$ . Samples from each set of tubes were assayed for PLAP immediately, and at 12, 24, 48, 72 and 96 hours.

#### 2.3.2.3. Stability of PLAP in blood.

Thirty ml of blood was taken from a normal healthy male volunteer and PLAP added to give a concentration of  $10^{-8}$  mol/l. Ten ml were transferred to an EDTA tube (final concentration of EDTA 5 mmol/l) and stored for up to 24 hours at  $4^{\circ}\text{C}$ . The remainder were allowed to clot, was centrifuged and the serum removed and stored at room temperature,  $4^{\circ}\text{C}$  and  $-20^{\circ}\text{C}$  for increasing intervals up to 24 hours. Each sample was boiled for 5 minutes and centrifuged prior to PLAP assay in order to remove any non-specific activity.

### 2.4. RESULTS.

#### 2.4.1. DEVELOPMENT OF THE ASSAY FOR FREE PLAP

##### 2.4.1.1. Radiolodination of CYPLAP

The elution profile following column chromatography on Sephadex G-15 is shown in figure 2.1. The first peak represents the radiolabelled peptide and the second smaller peak free  $\text{Na } ^{125}\text{I}$ . Most of the radiiodinated CYPLAP was contained in fractions 7-11 which when pooled were shown to have a radioactivity of  $2.12 \times 10^6$  cpm/ mg of peptide. The maximum shelf life of the radiiodinated peptide was 2 months.

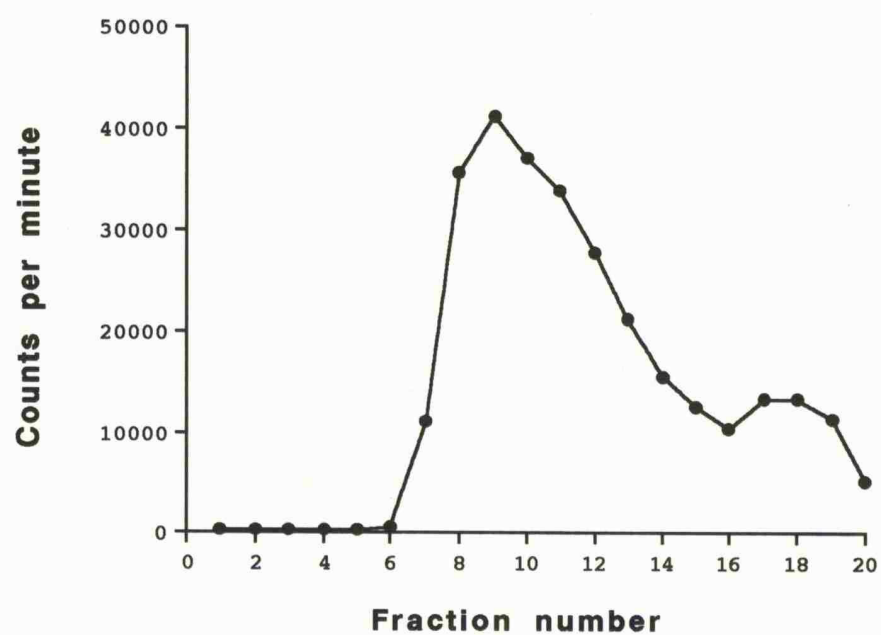


Figure 2.1: The elution profile of  $^{125}\text{I}$  CYPLAP passed down a Sephadex G-15 column.

#### **2.4.1.2. Generation of anti-PLAP antibody.**

CYPLAP bound to thyroglobulin was very effective in generating an immune response in each of the four rabbits immunised. Radioimmunoassay detected significant antibody production after one month with maximum concentrations appearing one month after their first booster injection (figure 2.2). Titres fell after this to remain constant with 50% binding of antisera diluted to 1 in 250. Antisera from each rabbit was pooled and diluted to allow 30% binding of  $^{125}\text{I}$ -CYPLAP in the immunoassay. The range of dilution required was between 1 in 1000 and 1 in 6000.

#### **2.4.1.3. Construction of the standard curve for the PLAP assay.**

Standard solutions of PLAP and CYPLAP were prepared and their concentrations checked by amino acid analysis. Serial dilutions were then performed to give concentrations between  $10^{-5}$  and  $10^{-12}$  M. The percentage binding of  $^{125}\text{I}$  CYPLAP was then plotted against the log of concentration of the standard peptide dilutions for each of the 4 rabbit's antisera. The plot for each rabbit's serum are shown in figure 2.3. The affinity constants were then calculated from the straight line portion of each curve. These ranged between  $4.73 \times 10^{-9}$  mol/l and  $2.00 \times 10^{-9}$  mol/l for CYPLAP and  $1.00 \times 10^{-7}$  mol/l and  $7.69 \times 10^{-9}$  mol/l for PLAP. The differences in the affinity of the various sera for CYPLAP and PLAP were due to the development of antibodies directed against different parts of the CYPLAP and PLAP peptides. The sera from 2 rabbits (R1707 and R1708) had similar binding constants and were therefore particularly suitable for use in a PLAP assay.

#### **2.4.1.4. Antibody specificity.**

In order to determine that the antisera really was specifically directed against CYPLAP and PLAP several related and unrelated peptides were assayed. The unrelated peptides showed no inhibition of binding. There was no binding to the related peptides (Equine and canine PLAP, human TAP, and human procolipase activation peptide, (CLAP) shown in table 2.1.

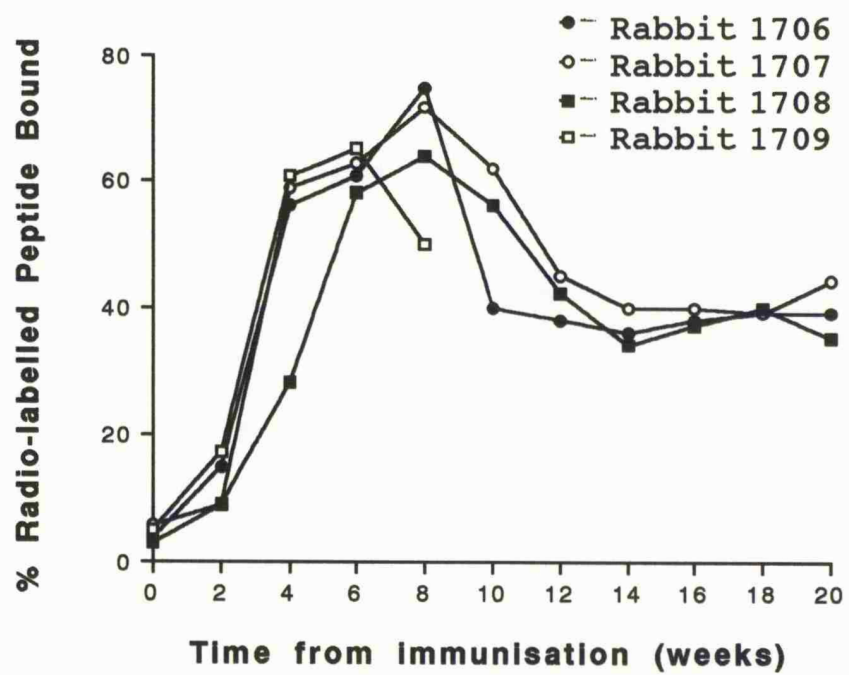


Figure 2.2: The degree of antibody production by the 4 rabbits' sera following immunization expressed as a percentage binding of  $^{125}\text{I}$  CYPLAP at 1/250 dilution.



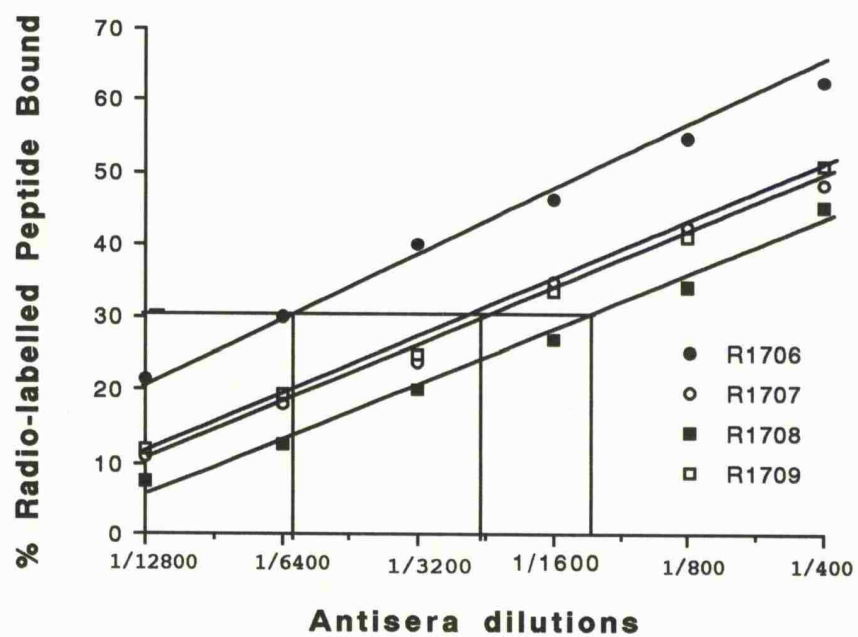


Figure 2.3: The percentage binding of  $^{125}\text{I}$  CYPLAP at differing dilutions for each of the 4 rabbits' sera.

Asp-Ser-Gly-Ile-Ser-Pro-Arg	(Human PLAP)
Gly-Ile-Ser-Pro-Arg	(Equine and canine PLAP)
Ile-Ser-Pro-Arg	
Ser-Pro-Arg	
Pro-Arg	
Ala-Pro-Gly-Pro-Arg	(Human CLAP)
Arg-Gly-Asp-Val	
(Asp) <sub>4</sub> -Lys	(TAP)

Table 2.1: Amino acid sequences utilised in determining the antibodies specificity.

There was no inhibition with dipeptides or tripeptides but there was gradually increasing inhibition as the amino acid sequence approached that of PLAP (figure 2.4).

#### **2.4.1.5. The limit of detection and inter-assay variation.**

The detection limit of the RiA was calculated as the concentration of peptide sufficient to cause 10% inhibition of binding. This is  $3.35 \times 10^{-9}$  mol/l (figure 2.5).

The inter-assay coefficient of variation was calculated by repeatedly measuring the concentration of PLAP in a standard. This was 11%.

#### **2.4.1.6. Affinity purification of antisera.**

The antisera from individual rabbits was purified by passage down an affinity purification column. The antisera were eluted and the absorbance measured at an optical density of 280 nm. Four antibody peaks were noted (A, B, C and D) which were further characterised by immunoassay. Figure 2.6 shows the profiles for 2 pooled sera. The type of antibody produced varied as did its affinity for CYPLAP and PLAP. Peak A had a low affinity was PLAP and was not investigated further. There was however no difference in the inhibition of binding by the 3 purified as against unpurified antisera (figure 2.7). SDS PAGE electrophoresis demonstrated that the antibodies were predominantly Ig G. The terminal amino acid sequence of CYPLAP is Pro-Arg but inhibition of binding in the immunoassay could not be achieved by this dipeptide alone or using CLAP which has Pro-Arg as its C-terminal amino acid sequence suggesting that the antibody recognition site is greater than 2 amino acids long.

#### **2.4.1.7. Determination of the antibody binding specificity.**

The major drawback in previous attempts to quantify the degree of zymogen activation has been the inability of antibodies to distinguish between non activated and activated zymogens. The following experiments were therefore conducted to demonstrate that the antibodies are directed against the C-terminal end of PLAP and that they did not bind the proenzyme.

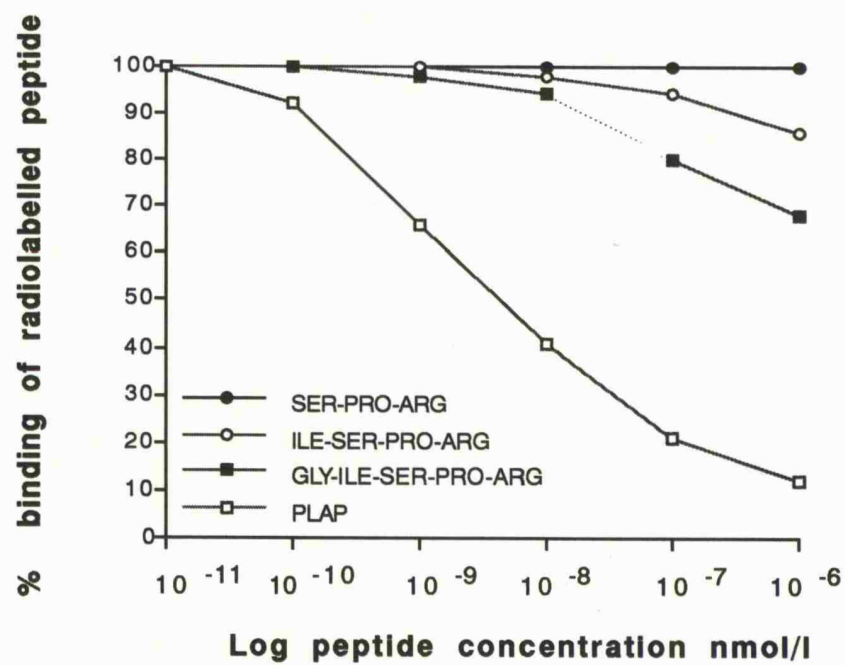


Figure 2.4: The percentage binding of  $^{125}\text{I}$  CYPLAP to variety of peptides related to PLAP.

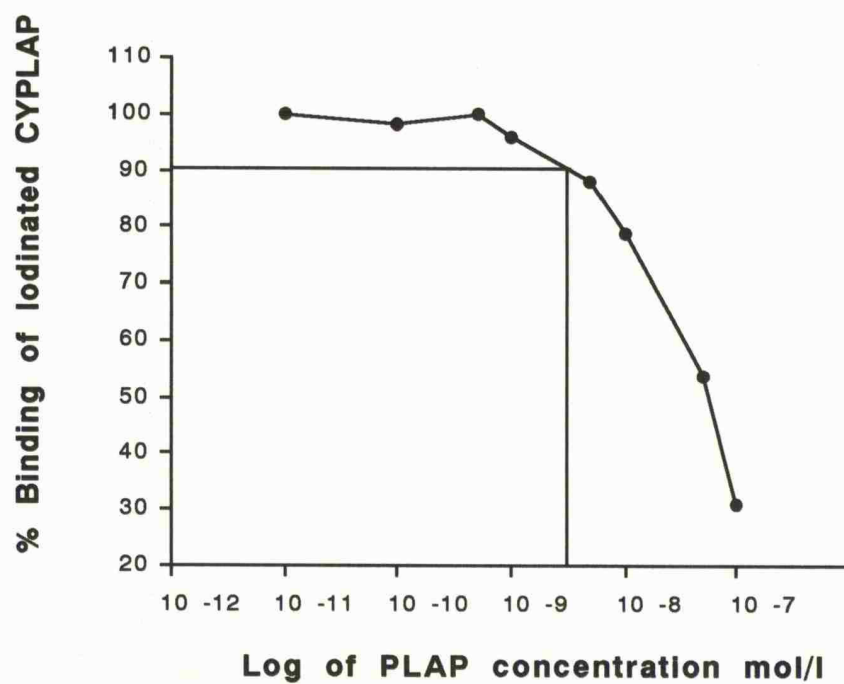


Figure 2.5: The standard curve for the radioimmunoassay of PLAP. The limit of detection is  $3.35 \times 10^{-9}$  mol.

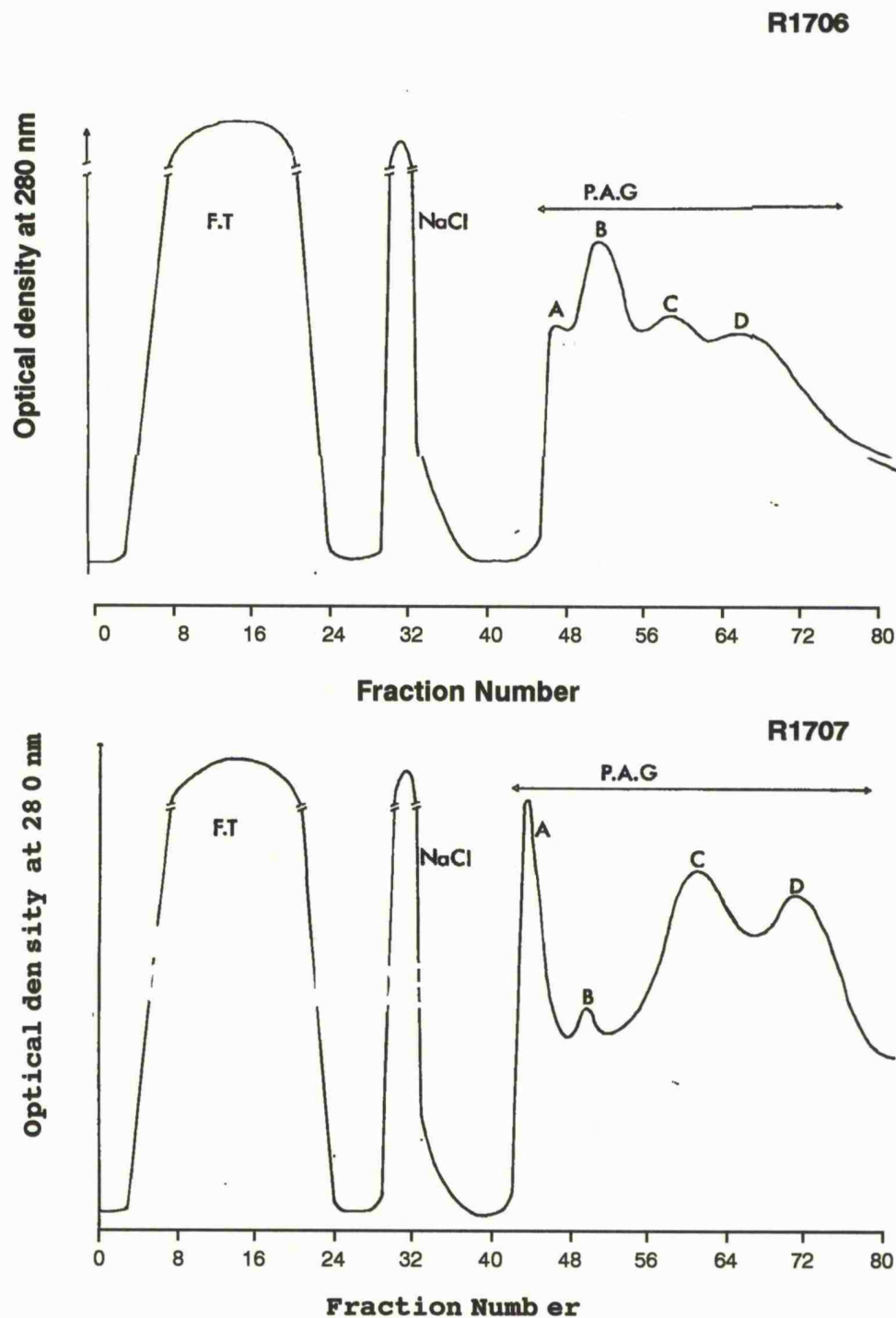


Figure 2.6: The affinity chromatography elution profile for 2 antisera (R1706 and R 1707) utilising PLAP substituted Sephadex G-15. A-D represent peaks produced by differing antibodies, Na Cl = sodium chloride. FT = flow through, PAC = propionic acid gradient.

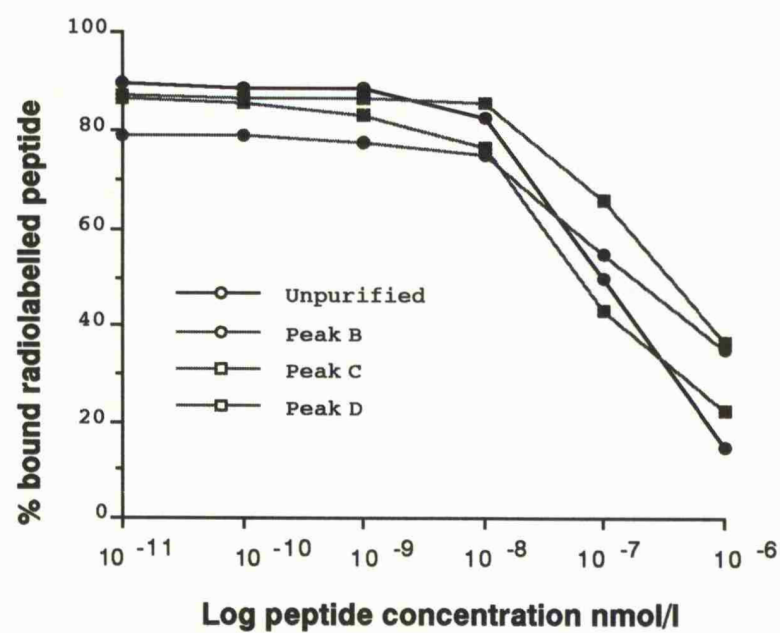


Figure 2.7: The inhibition of  $^{125}\text{I}$  CYPLAP binding by purified and unpurified rabbit sera.

The following experiments were conducted with human pancreatic extracts.

#### **2.4.1.8. Trypsinisation of pancreatic extracts.**

The pancreatic extracts were assayed for PLAP both before and after trypsinisation and the amount of PLAP per microgram of protein calculated (figure 2.8). The absence of any PLAP within the extract before, and the appearance of PLAP after, trypsinisation confirms that the antibody directed against PLAP does not recognise the non activated zymogen. Further confirmation is provided by column chromatography.

#### **2.4.1.9. Sephadex G-25 separation of the pancreatic extracts.**

The column was calibrated by eluting a solution of synthetic PLAP. Individual fractions were assayed for PLAP (Figure 2.9). Peak concentrations were seen in fractions 24 to 26. Homogenised non trypsinised human pancreas was passed down the column and then eluted and assayed, followed by trypsinised human pancreas. Figure 2.10 clearly demonstrates that there was no PLAP activity in the non trypsinised pancreas but was found at a position corresponding to a molecular weight of 14-18 Kd (figure 2.11).

#### **2.4.1.10. Sephadex G-50 fractionation of pancreatic extracts.**

The column was calibrated to show the elution positions of the standard proteins (figure 2.12). Homogenised human pancreas was subjected to chromatography. Following chromatography the fractions were assayed for PLAP before and after trypsinisation. PLAP was not detected by radioimmunoassay in any of the fractions

### **2.5. DETERMINATION OF THE STABILITY OF PLAP**

#### **2.5.1. Stability of PLAP to boiling.**

Dilution curves for PLAP in RIA buffer were performed before and after boiling the peptide solution for 5 minutes. It is clear from the curve that boiling neither destroyed nor altered the immunogenicity of the PLAP molecule figure 2.13.



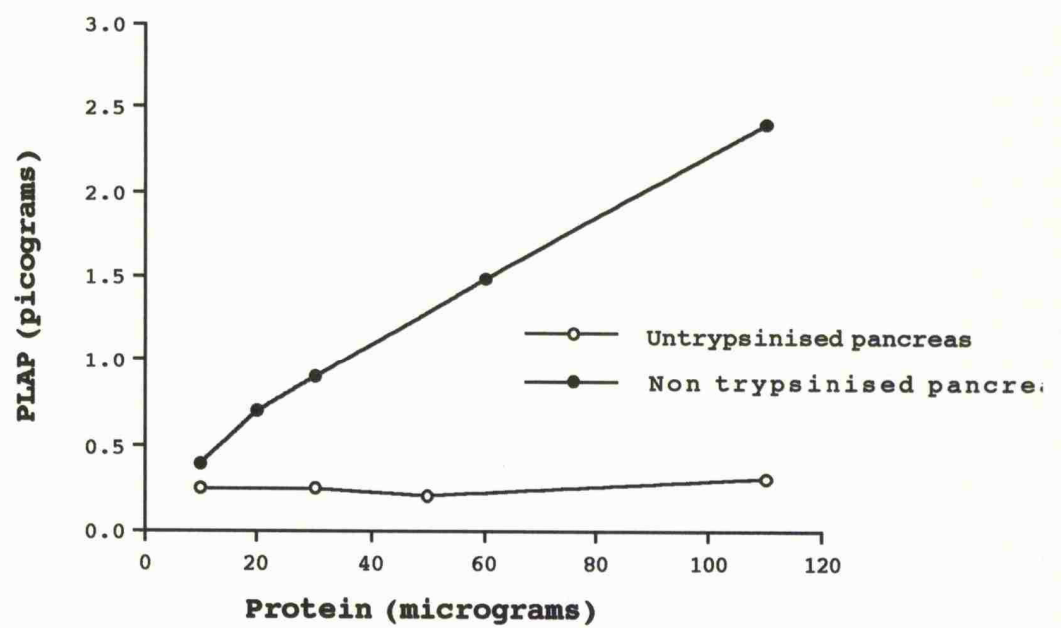


Figure 2.8: PLAP concentrations in homogenised human pancreas before and after trypsinisation.

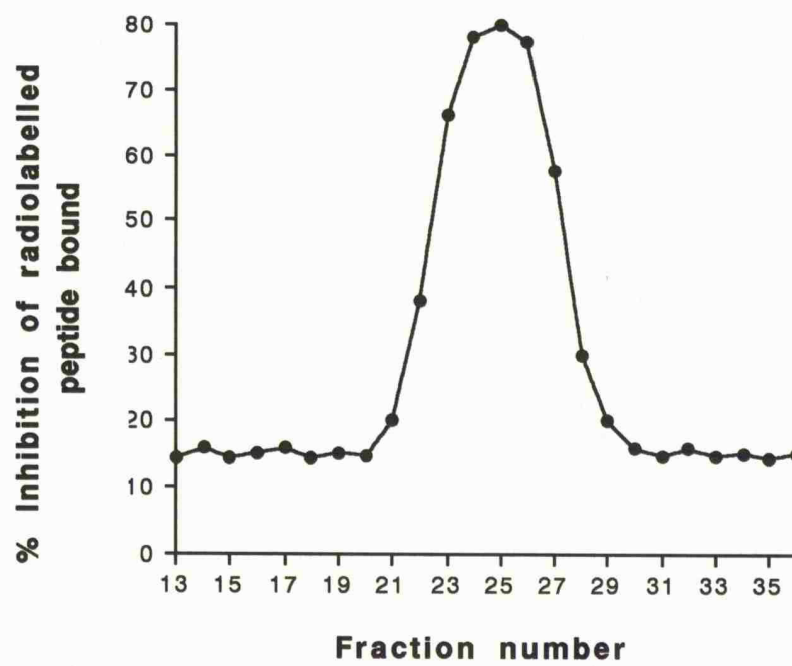


Figure 2.9: Calibration of the Sephadex G-25 chromatography column with synthetic PLAP.

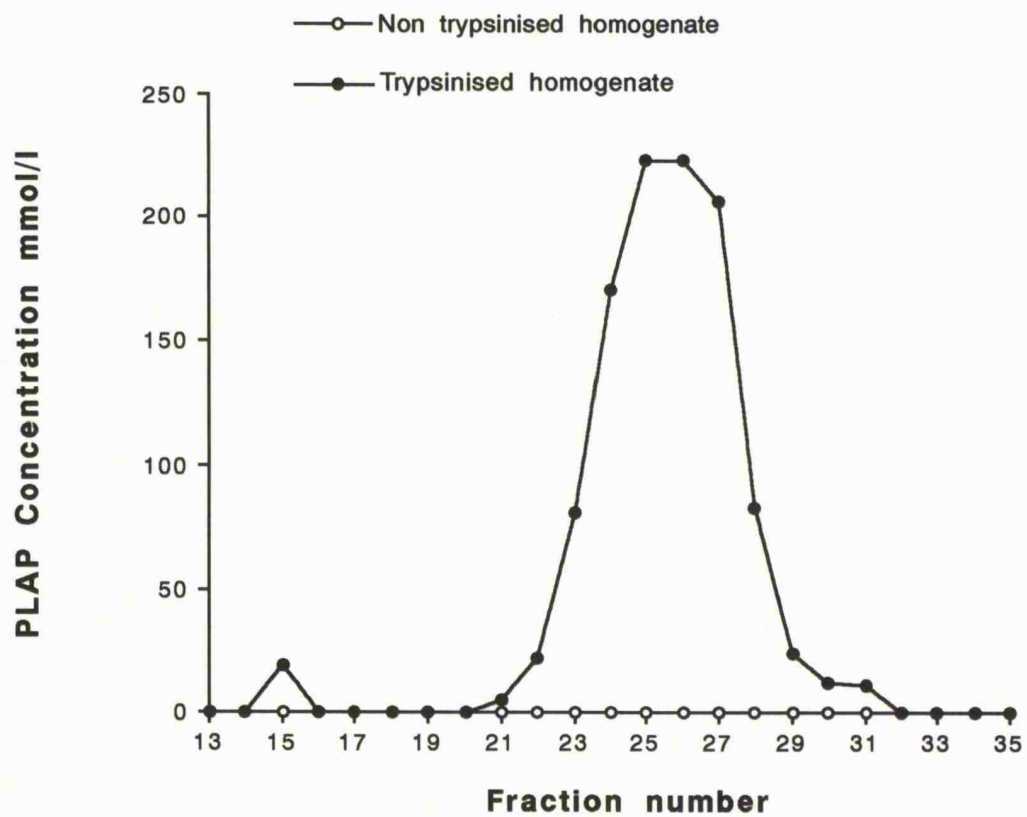


Figure 2.10: The elution profile of human pancreatic homogenate passed through Sephadex G-25 before and after trypsinisation.

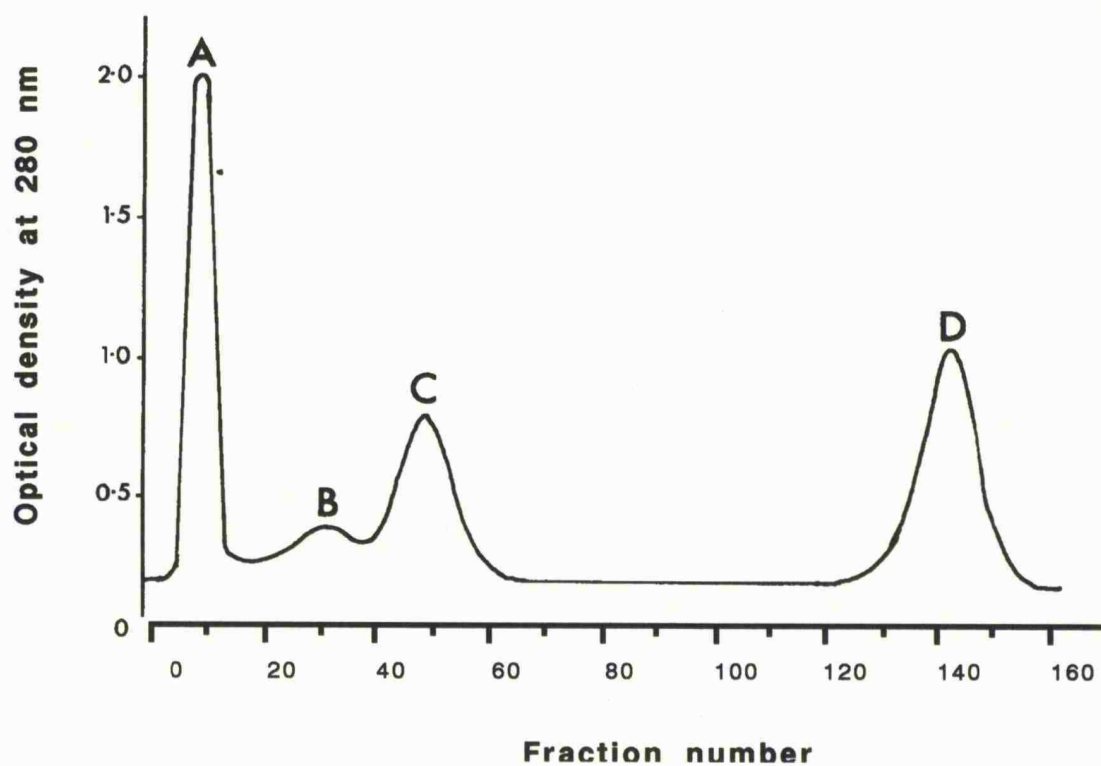


Figure 2.11: The elution profile of a Sephadex G-50 column calibrated with standard proteins. A, B, and C are the elution positions of protein standards: dextran blue (30 Kd), chymotrypsinogen (25 Kd) and cytochrome C (12.5 Kd) respectively.

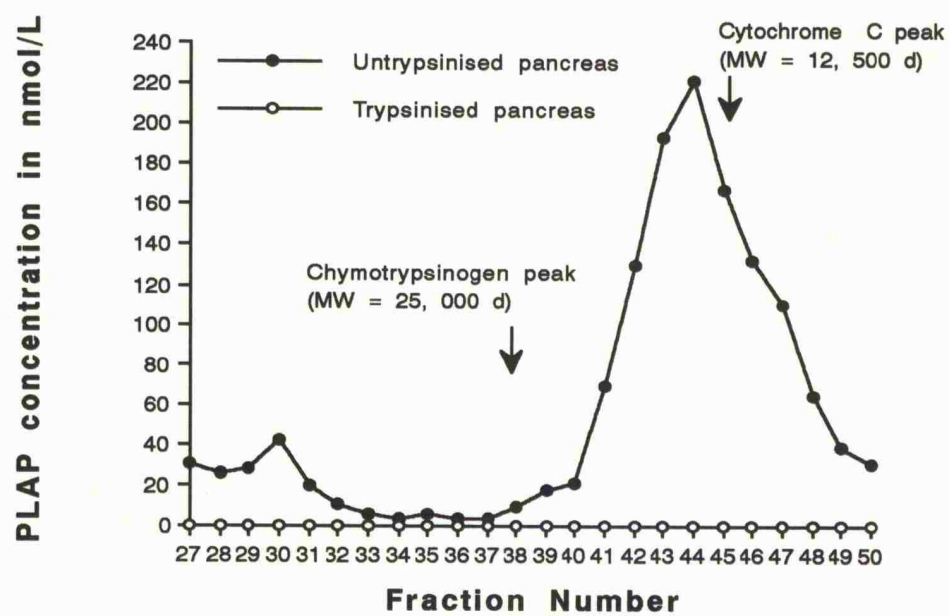


Figure 2.12: Sephadex G-50 gel chromatography of human pancreatic homogenate. Column fractions were assayed for PLAP by radioimmunoassay, before and after trypsinisation.

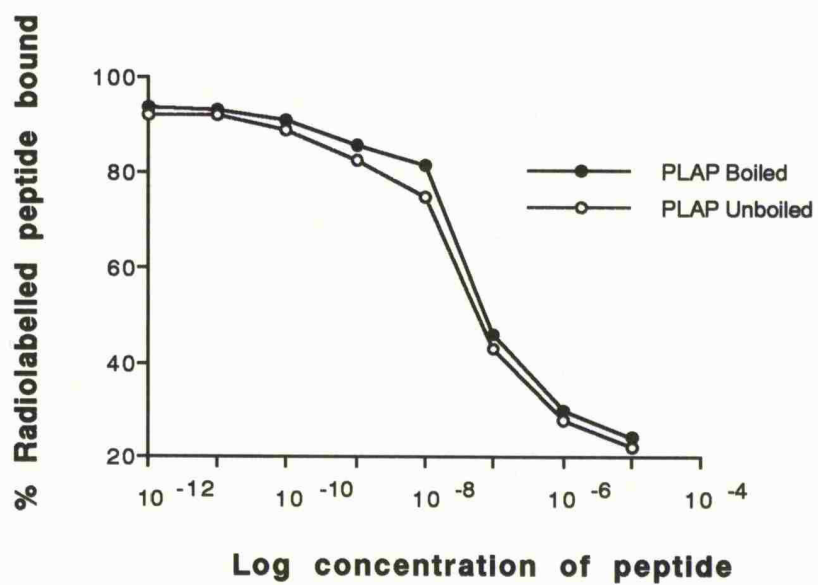


Figure 2.13: Concentration of PLAP in boiled and unboiled specimens of urine.

### 2.5.2. Stability of PLAP in urine.

The concentration of PLAP measured in the urine following storage at room temperature, 4°C and -20°C is shown in figure 2.14. The PLAP concentrations did not appreciably decrease in samples stored at room temperature or 4°C as long as EDTA was present. Where EDTA was not present appreciable degradation trypsinised, but peak activity in the trypsinised specimen, corresponding to the previously noted peaks found with synthetic PLAP occurred. In addition samples stored for several months at -20°C showed no appreciable reduction in PLAP concentrations, or after repeated freezing and thawing.

### 2.5.3. Stability of PLAP in blood, serum and plasma.

PLAP was not detected in the blood or serum after storage for 6 hours at room temperature or 4°C (in spite of it being initially present in a concentration of  $10^{-8}$  mol/l). If EDTA was added then there was no observable fall in the concentration of PLAP in specimens stored for up to 24 hours at 4°C. EDTA specimens may be safely stored for several months at -20°C without any detectable reduction in the PLAP concentration.

## 2.6. DISCUSSION.

On activation, prothymosin A<sub>2</sub> yields one molecule of the active enzyme (prothymosin A<sub>2</sub>) and one of PLAP (Rinderknecht 1986). By directing an antibody against the C-terminal end of PLAP (which is attached to prothymosin A<sub>2</sub> prior to activation) it should be possible to detect prothymosin A<sub>2</sub> activation without falsely reporting the presence of the parent zymogen. Synthetically produced CYPLAP was haptenised to bovine thyroglobulin by its N-terminal end, prior to injection into the rabbits. This ensured that the C terminal end of the molecule was exposed as an antigenic site. Antibodies have successfully been produced against the C-terminal end of TAP using this method (Hurley 1988) and was successful on this occasion.

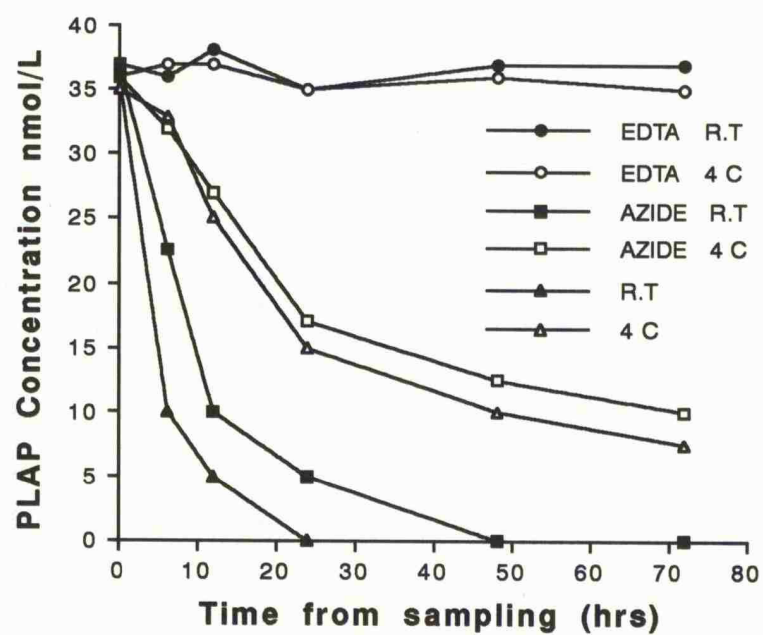


Figure 2.14: The stability of PLAP in urine under various storage conditions. EDTA = ethylene diamine tetracetic acid, R T = room temperature. 4 °C = 4 degrees centigrade.



There were a number of different antisera produced by the rabbits immunised with Tg- CYPLAP. Some showed high affinity to CYPLAP whilst others showed equal affinity for PLAP and CYPLAP. Competitive studies, using synthesised peptides, showed that a minimum of 5 amino acids (Asp-Ser-Gly-Ile-Ser-Pro-Arg) at the C-terminal end of the peptide were needed for antibody recognition.

Having confirmed the specificity of the assay to a variety of peptides related to PLAP it was necessary to demonstrate that the antibody would not bind phospholipase A<sub>2</sub>. This was achieved by assaying non trypsinised and trypsinised homogenised human pancreas. The absence of PLAP in the non trypsinised pancreas, with its appearance following trypsinisation, (trypsin activating the phospholipase and releasing PLAP) suggest that the antibody is exclusively directed against the C-terminal end of the PLAP molecule. Further evidence was provided by the results of Sephadex G-25 and G-50 column chromatography. Following passage of untrypsinised pancreatic homogenate down a Sephadex G-25 column fractions were assayed for PLAP and none found. However, in the pancreas that had been trypsinised, PLAP was found in the same fractions as those in which the PLAP, used to calibrate the column, had been detected. Pancreatic homogenate was eluted down a Sephadex G-50 column which had been previously calibrated with dextran blue (molecular weight (mw) 30,000 daltons), chymotrypsin (mw 25,000 daltons) and cytochrome C (mw 12,000 daltons). Phospholipase A<sub>2</sub> has a molecular weight of 14,500 daltons. Following elution all fractions were assayed for PLAP. PLAP was not detected in any aliquots that were not trypsinised but was present in the fractions corresponding to the molecular weight of phospholipase A<sub>2</sub> (between chymotrypsin and cytochrome C).

The detection limit of the radioimmunoassay was taken as the concentration of PLAP required to produce 90% inhibition of binding and was  $3.35 \times 10^{-9}$  mol/l.

These results demonstrate that the first aim of the thesis, the development of a sensitive and specific immunoassay for PLAP, has been achieved.

The stability of PLAP to boiling is useful since it allows specimens of urine, serum and peritoneal fluid to be heated sufficiently to precipitate proteins, and remove any non-specific activity, without denaturing the analyte. In addition it allowed trypsin, used to digest pancreatic homogenates, to be inactivated by heating.

It is clear that where urine specimens are stored at room temperature or 4°C, either with or without azide, there is a significant degradation of PLAP within a few hours. The addition of EDTA to the samples overcomes this problem and allows specimens to be stored at room temperature for up to 72 hours without any appreciable reduction in PLAP concentrations. This effect is probably due to the ability of EDTA to chelate zinc and thus inhibit zinc dependent aminopeptidases responsible for the degradation.

PLAP is almost certainly removed from the serum by a similar mechanism, although much more rapidly than for urine, PLAP being undetectable after 6 hours storage at 4°C. The effect is abolished by the addition of EDTA which allows specimens to be stored at 4°C for up to 24 hours without any appreciable reduction in PLAP concentrations.

Both serum and urine samples are stable when stored at -20°C for several months. In addition PLAP is resistant to freeze thawing.

Whilst the immediate freezing of urine and serum specimens to -20°C will prevent degradation it is recommended that all samples be collected into EDTA.

**CHAPTER 3:**

**THE MEASUREMENT OF URINARY CONCENTRATIONS OF TRYPSINOGEN  
ACTIVATION PEPTIDE (TAP) AND PROPHOSPHOLIPASE A<sub>2</sub> ACTIVATION  
PEPTIDE (PLAP) IN ACUTE PANCREATITIS, OTHER ABDOMINAL  
CONDITIONS AND HEALTHY CONTROLS.**

### 3.1 INTRODUCTION.

I have already discussed in section 1.11 the reasons why objective severity assessment, in acute pancreatitis, is considered desirable and outlined the methods currently available for its prediction. The fact that there are so many suggests that none is ideal.

The hypothesis, laid out in section 1.8.1, that trypsinogen activation only occurs in severe pancreatitis, provides a possible early means of detecting those patients with severe disease. All that is required is a method of quantifying accurately the degree of trypsinogen activation. Until now its measurement has been associated with a number of major problems (section 1.5.4). However, work in the Department of Surgery at St. George's Hospital Medical School has led to the development of an immunoassay directed against the C-terminal end of TAP. This reports the activation of trypsinogen without reporting the presence of the parent zymogen (Hurley *et al* 1988). Phospholipase A<sub>2</sub>, which is activated by trypsin, may also play an important role in the production of tissue damage in acute pancreatitis (section 1.8.1). Its quantification by immunoassays has also been associated with a number of problems (section 1.5.5) which have been overcome by the development of an assay utilising an antibody directed against the C-terminal end of PLAP (chapter 2).

Preliminary experiments have demonstrated that TAP and PLAP are not detected in the serum or plasma of most patients with acute pancreatitis but are readily detected in their urine (Hurley 1988). For this reason urinary TAP and PLAP concentrations have been measured in the following studies.

### 3.2 AIMS.

1. To examine the ability of urinary concentrations of TAP and PLAP to distinguish between severe and mild attacks of acute pancreatitis and to compare their predictive ability against previously described methods of severity assessment.

2. To examine the concentrations of TAP and PLAP in the urine of healthy individuals.
3. To examine the concentrations of TAP and PLAP in the urine of patients with acute abdominal conditions other than acute pancreatitis.

### **3.3 THE MEASUREMENT OF URINARY TAP AND PLAP CONCENTRATIONS IN HEALTHY CONTROLS.**

#### **3.3.1 INTRODUCTION AND AIMS.**

In order to evaluate the usefulness of TAP and PLAP measurements in the assessment of disease severity in acute pancreatitis it is important to know whether or not these peptides are present in the urine of healthy subjects. The time they are most likely to appear is following a meal, when large quantities of zymogens are liberated into the upper small bowel lumen. The aim of this study was to assess the degree to which healthy individuals excreted TAP and PLAP in their urine both pre and post prandially.

#### **3.3.2 PATIENTS AND METHODS.**

Ten ml of urine were collected from each of 40 healthy individuals. In 10 patients samples were taken before and 1, 2 and 3 hours after a meal.

##### **3.3.2.1 Patients.**

The median age of the 40 subjects was 33.5 yrs (range 20 to 70 yrs) and the ratio of M:F 23:17 (appendix 1).

#### **3.3.3 STATISTICAL ANALYSIS.**

Data were analysed using medians and the Friedman's rank sum test as appropriate. The statistical analyses were performed by Minitab Accelerated version 8.1™.

### **3.3.4 ASSAYS**

#### **3.3.4.1 Trypsinogen Activation Peptide (TAP).**

The materials and methods utilised were the same as for the PLAP radioimmunoassay (sections 2.2.1 and 2.2.2.6), with the exception of TAP which was synthesised by the Peptide Chemistry Unit, St George's Hospital Medical School and the antibody which was directed against the C-terminal end of TAP rather than PLAP. Urinary TAP concentrations were determined in duplicate using a radioimmunoassay after the method of Hurley *et al*(1988).

One hundred  $\mu$ l of diluted anti-TAP rabbit antiserum were added to each of 2 LP4 tubes. Urine specimens were centrifuged at 3000 rpm for 5 minutes, the supernatant removed, boiled for 5 minutes (to remove any non specific activity) and 100  $\mu$ l added to each of the LP4 tubes. This was followed by the addition of 100  $\mu$ l of  $^{125}$ I TAP (having a specific activity of 1500 cpm per 100  $\mu$ l of labelled  $^{125}$ I TAP) and 50  $\mu$ l of goat anti-rabbit ig G. A set of tubes containing known concentrations of TAP were also set up to provide a standard curve against which the samples could be read. All the tubes were mixed and allowed to stand overnight at 4°C. The following morning they were centrifuged at 3000 rpm for 30 min, the supernatant discarded and the radioactivity of the pellet counted on an LKB 1284 gamma counter. The concentrations of the samples were read from the standard curve, the results being expressed in nmol/l. The limit of detection of the assay was  $10^{-11}$  mol.

#### **3.3.4.2 Prophospholipase A<sub>2</sub> activation peptide (PLAP).**

The PLAP assay was performed as described in section 2.2.2.6.

### **3.4 THE MEASUREMENT OF URINARY CONCENTRATIONS OF TRYPSINOGEN ACTIVATION PEPTIDE (TAP) AND PROPHOSPHOLIPASE A<sub>2</sub> ACTIVATION PEPTIDE (PLAP) IN ACUTE PANCREATITIS.**

#### **3.4.1 MATERIAL AND METHODS.**

##### **3.4.1.1 Patient documentation.**

Patients admitted to the following hospitals were considered for inclusion in the study: St. George's Hospital (London), the Royal Infirmary, Stobhill General Hospital, Southern General Hospital and the Victoria Infirmary (Glasgow), the Royal Alexandra Hospital (Paisley), Law Hospital (Carlisle) and Monklands General Hospital (Airdrie) (figure 3.1). All patients admitted to hospitals in the West of Scotland were seen by myself, whilst those admitted to St. George's Hospital, London were assessed by Mr Mark Gudgeon. Patients were examined on a daily basis throughout the study. The biochemical and clinical course of the disease, as well as the outcome, was recorded for all patients. Patients from the West of Scotland also had up to 6,000 pieces of additional information recorded on a proforma (appendix 2). This allowed the assessment of the following "clinical factors" as means of severity assessment. The severity of abdominal pain, tachycardia, hypotension, abnormal temperature, abdominal distension, peritonism, peritoneal effusion, the presence and duration of an ileus, clinical assessment of disease severity and fluid sequestration. It was also possible to examine the predictive ability of laboratory parameters. These were serum amylase, CRP,  $\alpha_2$ -macroglobulin, LDH concentrations and white cell count. A combination of clinical and laboratory parameters allowed the calculation of the modified Glasgow (Osborne *et al*/1981) and Ranson (Ranson *et al*/1977) scores and the APACHE II score (Knaus *et al*/1985).

##### **3.4.1.2 Sample processing.**

Samples of blood and urine were taken into plain containers at the time of admission, 6 hourly for 48 hours and then twice daily for a further 3 days.

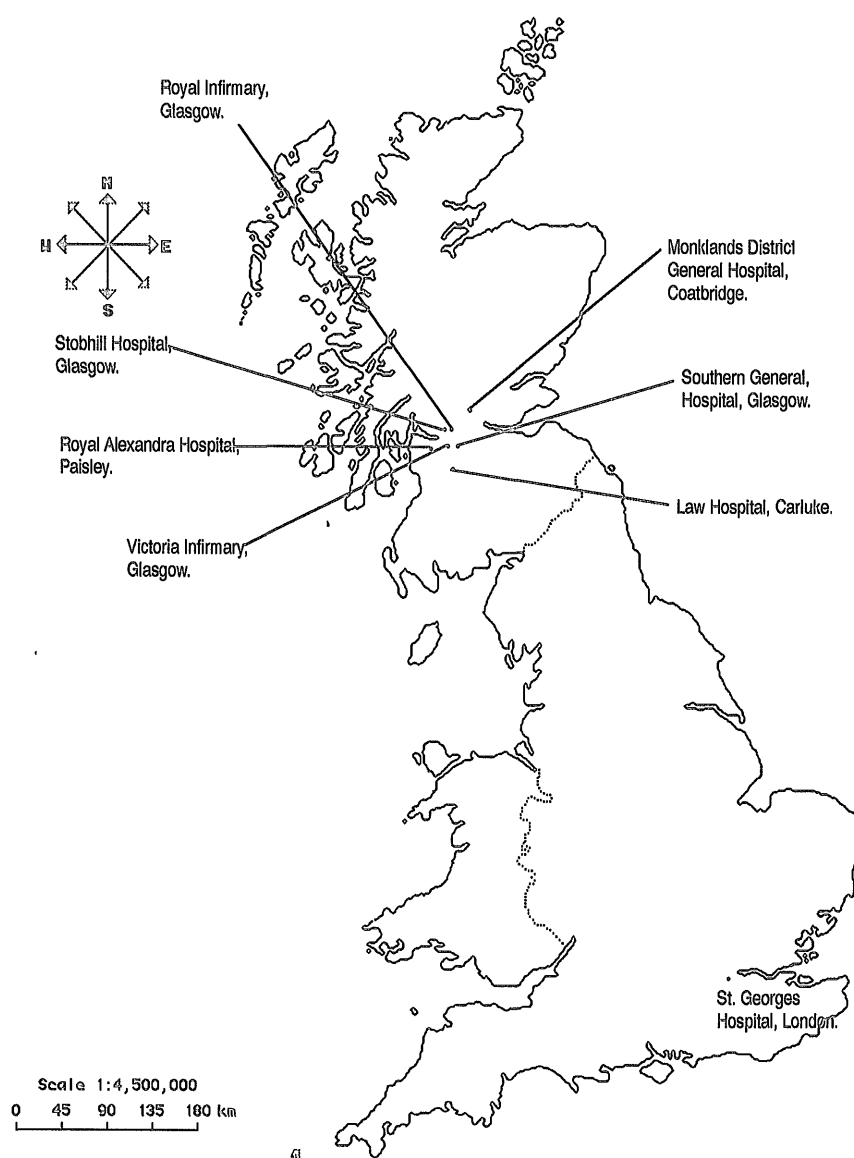


Figure 3.1: Map of the United Kingdom to show the situation of the hospitals from which patients were entered into the study.



Specimens were centrifuged within 1 hour of being taken, separated, aliquoted and stored at -20°C until analysis. The bloods required to calculate the 8 factor Glasgow (Osborne *et al*/1981) and Ranson (Ranson *et al*/1977) scores were collected on a daily basis and analysed as part of the routine laboratory service.

### 3.4.2 ASSAYS.

#### 3.4.2.1 TAP and PLAP

The TAP and PLAP assays were performed as in sections 3.3.4.1 and 2.2.2.6.

#### 3.4.2.2 Amylase.

Measurements of serum amylase concentrations were made on a Hitachi 737 random access discrete analyser (Mannheim, W. Germany) using an enzymatic colourimetric assay ( $\alpha$ -amylase PNP (Mannheim, W. Germany). The normal range was 0-200 IU/l and the within batch precision was 1.01% at an amylase of 1129 IU/l.

#### 3.4.2.3 CRP.

CRP concentrations were measured using a competitive binding, fluorescence polarisation immunoassay (TDX reagents and analyser, Abbott Diagnostic, England). The sensitivity was 0.3 mg/dl (95% confidence). The specificity with a CRP concentration of 10 mg/l was found to be less than the sensitivity. At a CRP concentration of 10 mg/l the within run coefficient of variance (CV) was 1.65% and between runs CV 2.65%. The average recovery from serum samples was  $100.6 \pm 3.0\%$ .

#### 3.4.2.4 $\alpha_2$ -macroglobulin.

The  $\alpha_2$ -macroglobulin assay was an antibody antigen, turbidimetric assay performed on an Encore centrifugal analyser (Baker Instruments Amersham, England). Absorbance was measured at 292 nm. Anti- $\alpha_2$ -macroglobulin antibody and the  $\alpha_2$ -macroglobulin standard were obtained from Atlantic antibodies (Berkshire, England).

#### **3.4.2.5 LDH.**

Measurements of LDH concentrations were made on a Hitachi 737 random access discrete analyser (Mannheim, Germany) using an enzymatic rate reaction assay, with absorbance being measured at 340 nm (Mannheim, Germany)

#### **3.4.3 DIAGNOSIS OF ACUTE PANCREATITIS.**

A diagnosis of acute pancreatitis was made on the basis of a serum amylase greater than 720 IU/l (equivalent to a value of 1200 IU/l as measured by the Phadebas method), in the presence of a compatible clinical picture of the disease.

#### **3.4.4 DEFINITIVE OUTCOME.**

Disease severity was graded retrospectively depending upon the clinical outcome. Patients were considered to have suffered a severe attack of acute pancreatitis if they developed one or more of the following.

- (a) A pancreatic collection: A collection of fluid or necrotic tissue, of  $\geq 5$  cm diameter within or adjacent to the pancreas, identified by ultrasound or CT scanning or at laparotomy or autopsy.
- (b) Pseudocyst: A pancreatic collection containing non-viscous fluid, lined by a well-defined capsule. This definition includes a pseudocyst complicated by either haemorrhage or infection, detected by microscopic examination and culture of cyst fluid.
- (c) Pancreatic abscess: A pancreatic collection in which pus is the major element, although necrotic tissue may also be present. The presence of necrotic tissue was confirmed by histological examination.
- (d) Pancreatic or peripancreatic necrosis: A pancreatic collection in which necrotic pancreatic or peripancreatic tissue, as opposed to fluid, is the dominant component. This diagnosis is supported by histological examination.
- (e) Infective cholangitis: The finding of pus within the biliary tree at operation or post-mortem examination.

- (f) Septicaemia: A positive blood culture with bacterial counts  $>10^5$  organisms per litre.
- (g) Acute respiratory failure: Arterial  $pO_2 < 8\text{KPa}$  requiring oxygen therapy or ventilation for a minimum of 24 hours, in the absence of pre-existing respiratory failure.
- (h) Adult respiratory distress syndrome: Respiratory failure with radiologically demonstrated pulmonary infiltration. Pulmonary oedema, as a result of left ventricular failure, was excluded by the measurement of the pulmonary wedge pressure using a Swan Ganz catheter.
- (i) Acute renal failure: A urine volume of less than 400 ml/24 hours or plasma urea  $> 16\text{ mmol/l}$ , with no response to 24 hours of intravenous fluid therapy.
- (j) Refractory systemic hypotension: Failure to maintain a blood pressure  $> 90\text{ mm Hg}$  in the presence of an adequate circulating volume.
- (k) Gastrointestinal haemorrhage: A haematemesis or melaena accompanied by signs of hypovolaemia (pulse rate  $> 120$  beats per minute and blood pressure  $< 90\text{ mmHg}$ ) or a subsequent fall in haemoglobin of  $> 2\text{ gm/dl}$ .
- (l) Coagulation failure: The association of abnormal coagulation indices (partial thromboplastin time or prothrombin time greater than twice normal or a platelet count a platelet count  $< 40 \times 10^9/\text{l}$ ) with abnormal bleeding.
- (m) Duodenal obstruction: Complete or partial obstruction of the duodenum resulting in the development of mechanical obstruction demonstrated on barium meal or endoscopic examination.

#### 3.4.5 STATISTICAL ANALYSIS.

Data were analysed using medians, the Mann Whitney U test, and Fisher's exact test as appropriate. To avoid repetition in the text the Mann Whitney U test has been utilised unless otherwise stated. Confidence intervals were used in preference to p values in judging the relevance of differences, (Gardener and Altman 1989). The statistical analyses were performed by Minitab Accelerated version 8.1™ and Epistat statistical packages.

#### 3.4.6 PATIENTS .

Fifty seven consecutive patients with a diagnosis of acute pancreatitis were prospectively entered into the study between December 1987 and July 1988. Two patients were excluded from the study: one patient with pancreatitis secondary to hypercalcaemia, associated with advanced metastatic disease from carcinoma of the lung, died on day three from bronchopneumonia; and a second patient who died of a myocardial infarction following coronary artery bypass grafting.

The severe and mild groups of patients were comparable in terms of the age, sex and aetiology of attacks (table 3.1 and 3.2). There was a greater proportion of male patients with severe disease. This was related to the fact that a large number of these attacks were alcohol related. The hospital stay was significantly greater in those with severe disease. Alcohol was the commonest aetiological factor making up 47.3% of patients, with gallstones the second most common, with an incidence of 40%. The most frequent systemic complication was respiratory failure which affected 6 patients (table 3.3). Of the two patients with renal failure 1 died of fulminant acute pancreatitis within a few hours of admission. The second died on day 15 following the development of respiratory failure and a pancreatic abscess. All the pancreatic collections resolved spontaneously, whilst only 2 of the pseudocysts did. One was effectively treated with repeated percutaneous aspiration under ultrasound control and the other 2 with surgical cystogastrostomy. The associated duodenal obstructions resolved following treatment of the pseudocysts.

A further more detailed analysis was performed on 39 patients documented within the West of Scotland. Twenty nine patients suffered a mild and 10 a severe outcome. These groups were comparable in terms of age, sex and aetiology (table 3.4). The duration of hospital stay was significantly longer, and the Glasgow and Ranson scores significantly higher in patients with severe disease.

Severe	Mild	Comparison	
Median age	51 yrs (23 - 88 yrs)	59.5 yrs (21 - 90 yrs)	95% CI = -16 to 6 yrs p <0.4
Sex M : F	12 : 3	22 : 18	p = 8.02, p = 0.8 (NS) (Fisher's exact test).
Delay to 1st sample (median)	12 hrs (1 - 48 hrs)	12 hrs (1 - 48 hrs)	95% CI = -8 to 2 p <0.4
Glasgow score (median)	3 (0 - 5)	0 (0 - 3)	95% CI = 1 to 3 p <0.001
Hospital stay days (days)	18 (1 - 50)	8 (2 - 44)	95% CI = 4 to 12 p <0.001

Table 3.1: The clinical details of 15 patients with severe and 40 patients with mild acute pancreatitis. CI = Confidence interval.

<b>Aetiology</b>	<b>Severe</b>	<b>Mild</b>	<b>Overall</b>
Alcohol	9 (60%)	17 (42.5%)	26 (47.3%)
Gallstones	4 (26.7%)	18 (45%)	22 (40%)
ERCP	0	3 (7.5%)	3 (5.5%)
Unknown	2 (13.3%)	2 (5%)	4 (7.2%)

Table 3.2: The aetiology of attacks of acute pancreatitis in 15 patients with severe and 40 patients with mild acute pancreatitis. ERCP = endoscopic retrograde cholangiopancreatography.

Complication	Number of patients
<b>Local</b>	
Pancreatic collection	4
Pancreatic pseudocyst	5
Pancreatic abscess	1
Duodenal obstruction	2
Upper Gi haemorrhage	1
<b>Systemic</b>	
Respiratory failure	6
Renal failure	2
Death	2

Table 3.3: Complications occurring in 15 patients with severe acute pancreatitis.

NB: some patients had more than 1 complication.

	<b>Severe</b>	<b>Mild</b>	<b>Comparison</b>
Median age 15, (range)	51.5yrs (23 - 68 yrs)	50 yrs (29 - 79 yrs)	95.2% CI = -8.99 to yrs, p = 0.75
Sex M : F	8 : 2	16 : 13	p = 0.16 (NS) (Fisher's exact test).
Median Glasgow score (range)	3 (0 - 5)	0 (0 - 3)	95% CI = 1 to 3, p <0.0002
Median Ranson score (range)	3 (1 - 7)	1 (0 - 5)	95.25 CI = -1 to 3, p = 0.0028
Hospital stay days, (days)	18 (1 - 27)	9 (5 - 28)	95.2% CI = 2 to 11 p <0.017

Table 3.4: The clinical details of 10 patients with severe and 29 patients with mild acute pancreatitis documented within the West of Scotland. CI = Confidence interval.



### **3.5 MEASUREMENT OF URINARY TAP AND PLAP CONCENTRATIONS IN PATIENTS WITH ACUTE ABDOMINAL CONDITIONS OTHER THAN ACUTE PANCREATITIS.**

#### **3.5.1 INTRODUCTION AND AIMS.**

The appearance of TAP and PLAP in the urine of patients with acute abdominal conditions, other than acute pancreatitis, will lead to a reduction in their specificity in predicting disease severity. For this reason it is important to know whether or not these peptides appear in the urine of patients with conditions other than acute pancreatitis. The aim of this study was to assess the degree to which this group of patients excreted TAP and PLAP in their urine.

#### **3.5.2 MATERIALS AND METHODS.**

Patients were admitted to the study if they had severe abdominal pain (requiring opiate analgesia) associated with a condition that required specific therapeutic intervention (e.g. perforated peptic ulcer, cholecystitis, appendicitis, etc.) or had conditions affecting the biliary tree or pancreas (e.g. obstructive jaundice or exploration of the common bile duct). In addition patients were only included if they presented within 48 hours of the onset of symptoms.

#### **3.5.3 ASSAYS.**

##### **3.5.3.1 TAP and PLAP**

The TAP and PLAP assays were performed as in sections 3.3.4.1 and 2.2.2.6.

#### **3.5.4 STATISTICAL ANALYSIS.**

Data were analysed using medians, Mann Whitney U,  $\chi^2$  and Friedman's rank sum tests as appropriate. To avoid repetition in the text the Mann Whitney U test has been utilised unless otherwise stated. The statistical analyses were performed by Minitab Accelerated version 8.1™.

### **3.5.5 PATIENTS.**

Twenty five patients were included in this study (table 3.5). Their median age was 45 yrs (range 18 - 80 yrs) as against 52 yrs (range 21 - 90 yrs) for the patients with acute pancreatitis. The disease control group was significantly younger (95% CI = -0.01 to 18,  $p < 0.04$ ). The ratio of male to female patients in the disease control group was 15:10 and of the patients with acute pancreatitis 34:21. These differences were not significant ( $\chi^2 = 0.0009$ , 95% CI = 0.3 to 2.7,  $p < 0.9$ ).

## **RESULTS.**

### **3.6 THE MEASUREMENT OF URINARY TAP AND PLAP CONCENTRATIONS IN HEALTHY CONTROLS.**

#### **3.6.1 Urinary TAP concentrations.**

TAP was detected in the urine of 12 subjects (30%), the median concentration being 0.28 nmol/l (range 0 to 1.68 nmol/l) (appendix 1). There was no significant difference in the urinary concentrations of TAP before or after food ( $s = 4.10$ ,  $df = 3$   $p = 0.251$ , Friedman rank sum test) (table 3.6).

#### **3.6.2 Urinary PLAP concentrations.**

PLAP was detected in the urine of all but 1 subject (appendix 1). The median concentration measured in the urine was 1.2 nmol/l (range 0.1 to 3.22 nmol/l). There was no significant difference in the urinary concentrations of PLAP before or after food ( $s = 1.67$ ,  $df = 3$ ,  $p = 0.644$ , Friedman rank sum test) (table 3.6).

Patient	Age	Sex	Diagnosis	TAP (nmol/l)	PLAP (nmol/l)
1	62	M	Appendicitis	0	7.61
2	18	M	Perf' Appendix	1.56	14.11
3	23	M	Perf' Appendix	0	3.20
4	52	M	Cholangitis	2.20	24.6
5	26	M	Biliary Colic	0	9.71
6	43	M	Biliary Colic	0	0.65
7	62	F	Cholecystitis	0.08	1.26
8	47	M	Cholecystitis	0	0
9	35	F	Cholecystitis	0.16	1.84
10	40	M	Cholecystitis	0	5.58
11	62	M	Obst' jaundice	0	8.05
12	54	F	Obst' Jaundice	1.56	2.14
13	80	M	Obst' Jaundice	0	0.96
14	39	F	Post ECBD	0	26.04
15	73	F	Perf' Gallbladder	1.20	2.65
16	41	M	Peptic Ulcer	0.20	3.32
17	58	M	Perf' DU	1.65	4.06
18	28	F	Perf' DU	0.20	46.34
19	22	F	2° Haemorrhage	0.10	18.59
20	45	F	Intest' Obst'	0	0.45
21	54	M	Intest' Obst	0.90	0.96
22	66	M	SB Infarction	0.48	1.19
23	55	M	Str' Hernia	1.86	17.86
24	24	F	Lymphoma	0.21	0.83
25	27	F	Pyelonephritis	0.48	0.23

Table 3.5: Admission urinary TAP and PLAP concentrations in 25 patients with a variety of surgical emergencies other than acute pancreatitis. Perf' = perforated, Intest = Intestinal, Obst' = obstruction, DU = duodenal ulcer, 2° = secondary and SB small bowel, ECBD = exploration of the common bile duct.

Patient	Fasting	1hr	2hr	3hr
TAP (nmol/l)	0.00	0.00	0.00	0.00
PLAP (nmol/l)	0.55	0.42	0.50	0.38
TAP (nmol/l)	0.22	0.16	0.20	0.18
PLAP (nmol/l)	1.32	1.48	1.30	1.42
TAP (nmol/l)	0.00	0.08	0.05	0.00
PLAP (nmol/l)	0.00	0.18	0.00	0.23
TAP (nmol/l)	0.00	0.00	0.00	0.00
PLAP (nmol/l)	0.96	0.84	0.98	0.88
TAP (nmol/l)	1.20	1.05	1.18	1.10
PLAP (nmol/l)	2.68	2.18	1.96	1.96
TAP (nmol/l)	0.56	0.48	0.32	0.52
PLAP (nmol/l)	1.98	1.65	1.72	1.87
TAP (nmol/l)	0.00	0.00	0.00	0.00
PLAP (nmol/l)	0.00	0.00	0.00	0.00
TAP (nmol/l)	0.00	0.00	0.00	0.00
PLAP (nmol/l)	0.67	1.23	0.98	0.38
TAP (nmol/l)	0.00	0.00	0.00	0.00
PLAP (nmol/l)	0.35	0.52	0.42	0.82
TAP (nmol/l)	1.30	0.80	0.34	0.56
PLAP (nmol/l)	1.70	0.99	0.78	0.80

Table 3.6: The urinary concentrations of TAP and PLAP before, 1, 2 and 3 hours after food in 10 normal subjects.

### **3.7 THE MEASUREMENT OF URINARY CONCENTRATIONS OF TRYPSINOGEN ACTIVATION PEPTIDE AND PROPHOSPHOLIPASE A<sub>2</sub> ACTIVATION PEPTIDE IN ACUTE PANCREATITIS.**

The data analysed in this section are displayed fully in appendix 3.

#### **3.7.1 Amylase.**

The median admission serum amylase concentration in the mild group of patients was 2259 IU/l (range 782 to 8030 IU/l) and for those with severe disease 2670 IU/l (range 342 to 8030 IU/l). These differences were not significant (95.2% CI = -666 to 1135 IU/l,  $p < 0.58$ ). The median peak amylase concentration in the mild group was 2279 IU/l (range 270 to 8030 IU/l) and in the severe group 2670 IU/l (range 696 to 8030 IU/l). These differences were not significant (95.2% CI = -462 to 1352 IU/l,  $p = 0.36$ ).

#### **3.7.2 Glasgow Score.**

The median Glasgow score was 3 in patients with severe and 0 in those with mild disease. These differences were significant (table 3.7). Nine of 15 patients with severe and 3 of 40 patients with mild disease had Glasgow scores of  $\geq 3$ . This gave the Glasgow score a sensitivity of 60% and specificity of 93%. Seventy five per cent of those with a Glasgow score  $\geq 3$  developed at least one major complication whereas 86% of those with a score of 2 or less had a mild outcome.

#### **3.7.3 CRP.**

The overall pattern of CRP production can be seen in figure 3.2. The median CRP concentration on admission for the severe and mild groups were 74 mg/l (range 10 to 234 mg/l) and 50.5 mg/l (range 10 to 256 mg/l) respectively. These differences were not significant (95.2% CI = -63 to 2 mg/l,  $p < 0.15$ ).

The median peak values during the first 48 hours were 250 mg/l (range 76 to 492 mg/l) and 129 mg/l (range 16 to 366 mg/l) for the severe and mild groups respectively. The differences at this time were significant (95.2% CI = 61 to 185 mg,  $p = < 0.001$ ). Utilising a 'cut off' value of  $\geq 200$  mg/l (this value gave the best balance of sensitivity and specificity) during the first 48 hours severe disease could be detected with a sensitivity of 74% and specificity of 75% (figure 3.3).

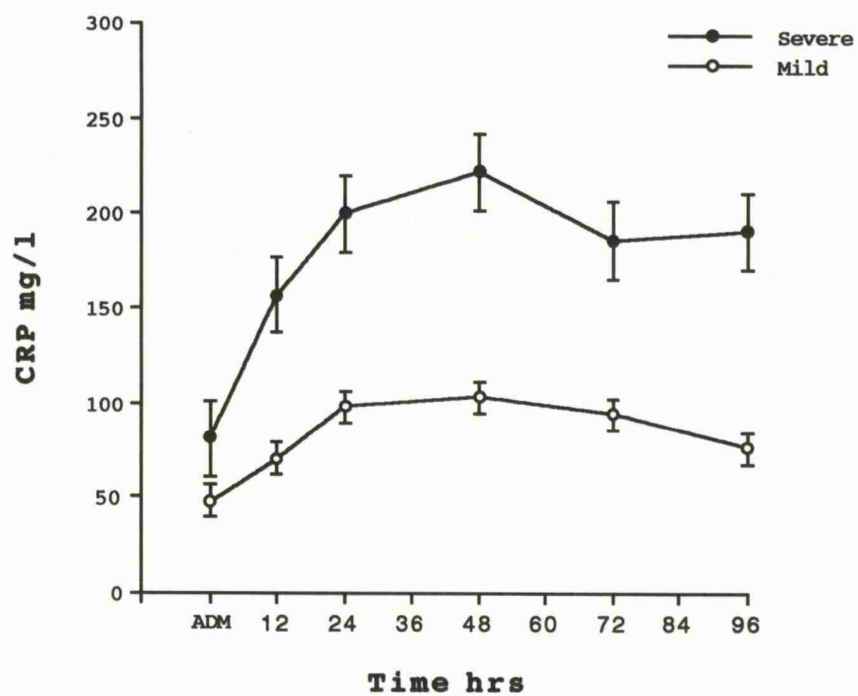


Figure 3.2: The median serum concentrations of CRP ( $\pm$  standard error of the mean) in 15 patients with severe and 40 patients with mild acute pancreatitis. CRP = C-reactive protein, ADM = admission.

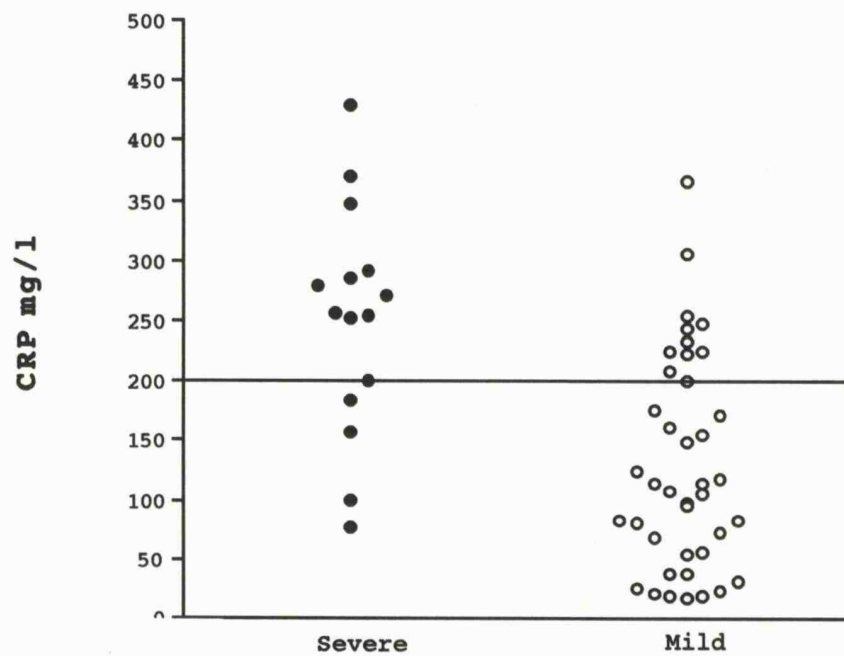


Figure 3.3: Scattergram to show the peak serum CRP concentrations during the first 48 hours of the study in 15 patients with severe and 40 patients with mild acute pancreatitis.

#### 3.7.4 $\alpha_2$ -macroglobulin.

The overall pattern of serum  $\alpha_2$ -macroglobulin concentrations are shown in figure 3.4. The median admission serum concentration of  $\alpha_2$ -macroglobulin for patients in the severe group was 1.1 mg/l (range 0.6 - 3.1 mg/l) and for the mild group 1.5 mg/l (range 0.8 - 3.7 mg/l). The differences between groups were significant (95.2% CI = 0.001 to 0.7 mg/l,  $p < 0.03$ ). The median minimum serum concentration of  $\alpha_2$ -macroglobulin for patients in the mild group was 1.2 mg/l (range 0.6 - 2.8 mg/l) and for the severe group 0.8 mg/l (range 0.4 - 1.3 mg/l). The differences in the minimum values were significant (95.2% CI = 0.1 to 0.6 mg/l,  $p < 0.001$ ).

An admission  $\alpha_2$ -macroglobulin of  $\leq 1.4$  mg/l separated mild and severe disease with a sensitivity of 73% and specificity of 65%. Whilst utilising the values at 24 hours made a minor improvement in the sensitivity and specificity the best results were obtained when the minimum  $\alpha_2$ -macroglobulin concentration over the 5 days of the study was examined (figure 3.5). Utilising a cut off value of  $\leq 0.9$  mg/l produced a sensitivity of 80% and specificity of 73% with 73% of patients correctly predicted.

#### 3.7.5 TAP

The overall pattern of TAP production is displayed in figure 3.6. Peak median concentrations were seen on admission, concentrations falling rapidly thereafter in association with clinical resolution. In 7 of the 15 patients in the severe group, the admission TAP concentrations were the highest values recorded throughout the study. The median admission concentration of TAP was 3.23 nmol/l (range 0 to 10.3 nmol/l) for the severe and 0 nmol/l (range 0 to 5.55 nmol/l) for the mild group. The difference between the groups was highly significant with a 95% CI of 1.93 to 4.08 nmol/l,  $p < 0.001$ . The median peak TAP concentration within the first 24 hours was 4.03 nmol (range 0.17 to 16.19 nmol/l) for the severe group and 0.29 nmol/l (range 0 to 5.55 nmol/l) for the mild group. The differences between these



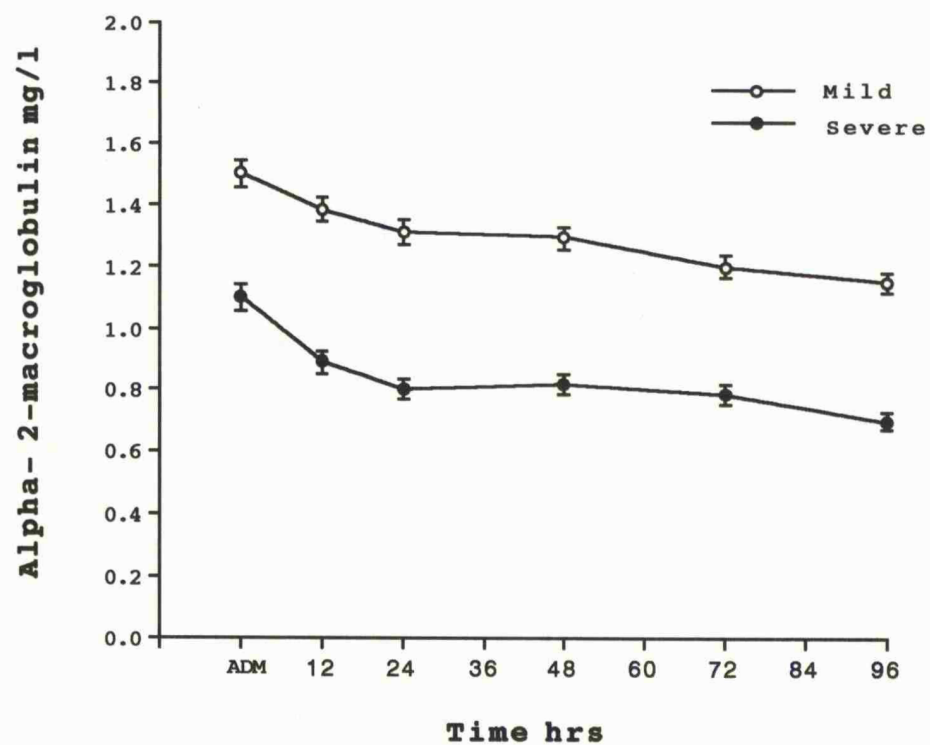


Figure 3.4: The median serum concentrations of  $\alpha_2$ -macroglobulin ( $\pm$  standard error of the mean) in 15 patients with severe and 40 patients with mild acute pancreatitis, ADM = admission.

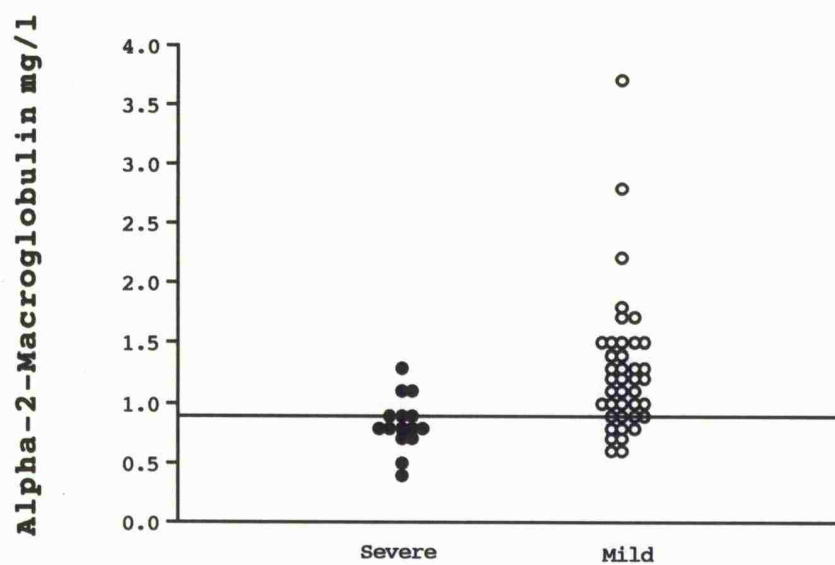


Figure 3.5: Scattergram to show the minimum admission serum  $\alpha_2$ -macroglobulin concentrations in 15 patients with severe and 40 patients with mild acute pancreatitis.

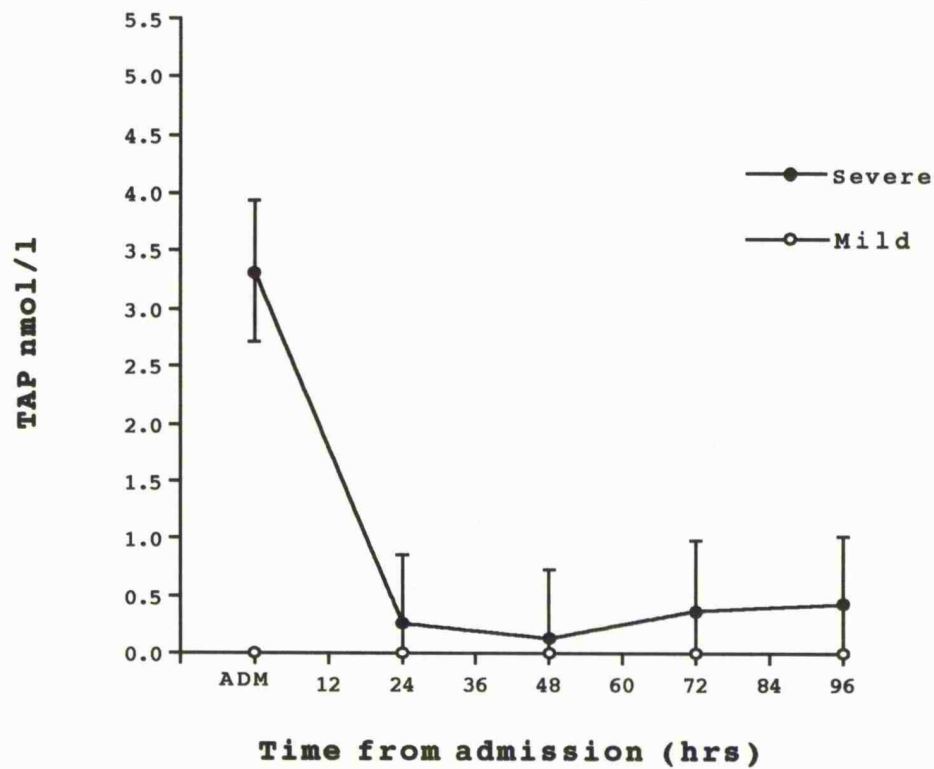


Figure 3.6: The median urine concentrations of TAP ( $\pm$  standard error of the mean) in 15 patients with severe and 40 patients with mild acute pancreatitis.

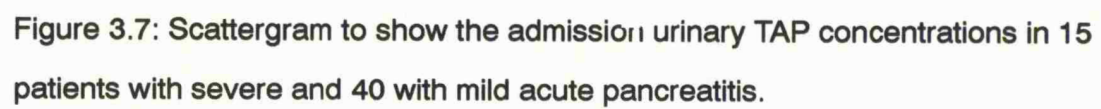
groups were again highly significant (95% CI = 2.27 to 4.45 nmol/l,  $p < 0.001$ ).

The admission and peak TAP concentrations during the first 24 hours, for each group of patients, are displayed in the form of scattergrams in figures 3.7 and 3.8. An admission TAP concentration  $\geq 2$  nmol/l distinguishes between mild and severe disease with a sensitivity of 80% and specificity of 90%. Utilising the same cut off the sensitivity and specificity for peak TAP concentrations during the first 24 hours were 80% and 85% respectively.

Of the 55 patients in the trial, 28 had no TAP in their urine on admission and 16 had no TAP at all in their urine during the 5 days of the study. Only 1 patient without TAP in his urine on admission suffered a severe outcome (he developed respiratory failure). None of the 16 patients who had no TAP in their urine during the 5 days of the study developed complications.

Four patients who suffered a mild attack of acute pancreatitis had urinary TAP concentrations  $\geq 2$  nmol/l suggesting that there had been a significant degrees of trypsinogen activation. It is possible that they developed a small focus of pancreatic necrosis which resolved spontaneously.

Three patients with severe disease had admission urinary TAP  $\leq 2$  nmol/l and were therefore incorrectly classified as mild. The first patient was a 50 year old male with alcohol related disease. The delay between the onset of symptoms and the first urine sample was 9 hours and the highest urinary concentration of TAP 0.57 nmol/l was achieved 12 hours after the onset of symptoms. At 48 hours his Glasgow score was 3. The patient subsequently developed a pancreatic collection, which spontaneously resolved, and had a hospital stay of 19 days. The second patient was a 54 year old male with viral pancreatitis. In spite of the delay between the onset of symptoms and the first urine sample being only 1 hour no TAP was detected in his urine throughout the 5 days of the study. His Glasgow score was 3 and he was classified as severe on the basis of a peripancreatic collection which



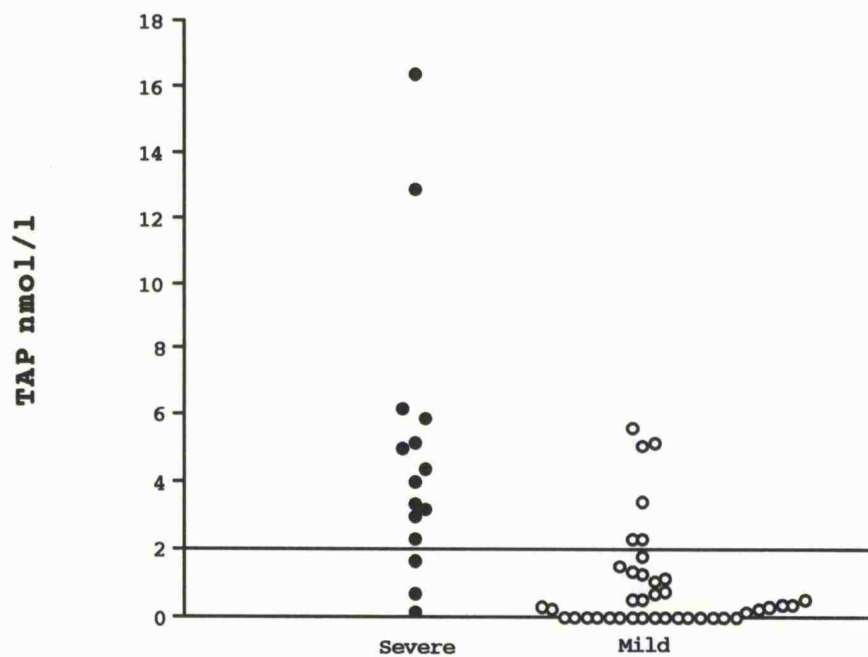


Figure 3.8: Scattergram to show the peak urinary TAP concentrations during the first 24 hours in 15 patients with severe and 40 patients with mild acute pancreatitis.

resolved spontaneously. The final patient was a 69 year old female with gallstone pancreatitis. She presented with a 12 hour history of pain and had an admission urinary TAP concentration of 1.04 nmol/l. The highest concentration recorded was 1.6 nmol/l at 8 hours. She developed a pancreatic pseudocyst and had a hospital stay of 15 days.

Comparison of admission urinary TAP concentrations between patients with alcoholic and gallstone pancreatitis and pancreatitis of other aetiologies, failed to demonstrate any significant differences ( $H = 1.77$ ,  $df = 2$ ,  $p = 0.413$ , Kruskal Wallis test).

### 3.7.6 PLAP

Figure 3.9 shows the pattern of PLAP excretion during the 5 days of the study. Significant differences between the groups were only demonstrated on the first 3 days. The median PLAP concentration on admission in the severe group was 4.31 nmol/l (range 0 to 69.67 nmol/l) and in the mild group 2.58 nmol/l (range 0 to 8.24 nmol/l). These differences were highly significant (95.2% CI = 0.51 to 3.34 nmol/l,  $p < 0.006$ ). An admission urinary PLAP concentration of  $\geq 3$  nmol/l was able to distinguish between mild and severe attacks of acute pancreatitis with a sensitivity and specificity of 73% (table 3.10).

Eleven of the 40 patients who suffered an mild attack of acute pancreatitis had admission urinary PLAP concentrations  $\geq 3$  nmol/l. Of these, 4 had TAP in their urine on admission, and one had a urinary TAP concentration  $\geq 2$  nmol/l. The latter had alcohol related disease and was discharged home well on day 7.

Four patients with severe disease had admission urinary PLAP concentrations  $< 3$  nmol/l and were therefore incorrectly classified as mild. Two of these patients had admission TAP concentrations  $< 2$  nmol/l. They were the 54 year old man and 69 year old woman documented in section 3.7.5 above. Of the 2 patients who were

resolved spontaneously. The final patient was a 69 year old female with gallstone pancreatitis. She presented with a 12 hour history of pain and had an admission urinary TAP concentration of 1.04 nmol/l. The highest concentration recorded was 1.6 nmol/l at 8 hours. She developed a pancreatic pseudocyst and had a hospital stay of 15 days.

Comparison of admission urinary TAP concentrations between patients with alcoholic and gallstone pancreatitis and pancreatitis of other aetiologies, failed to demonstrate any significant differences ( $H = 1.77$ ,  $df = 2$ ,  $p = 0.413$ , Kruskal Wallis test).

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Four patients with severe disease had admission urinary PLAP concentrations  $< 3$  nmol/l and were therefore incorrectly classified as mild. Two of these patients had admission TAP concentrations  $< 2$  nmol/l. They were the 54 year old man and 69 year old woman documented in section 3.7.5 above. Of the 2 patients who were



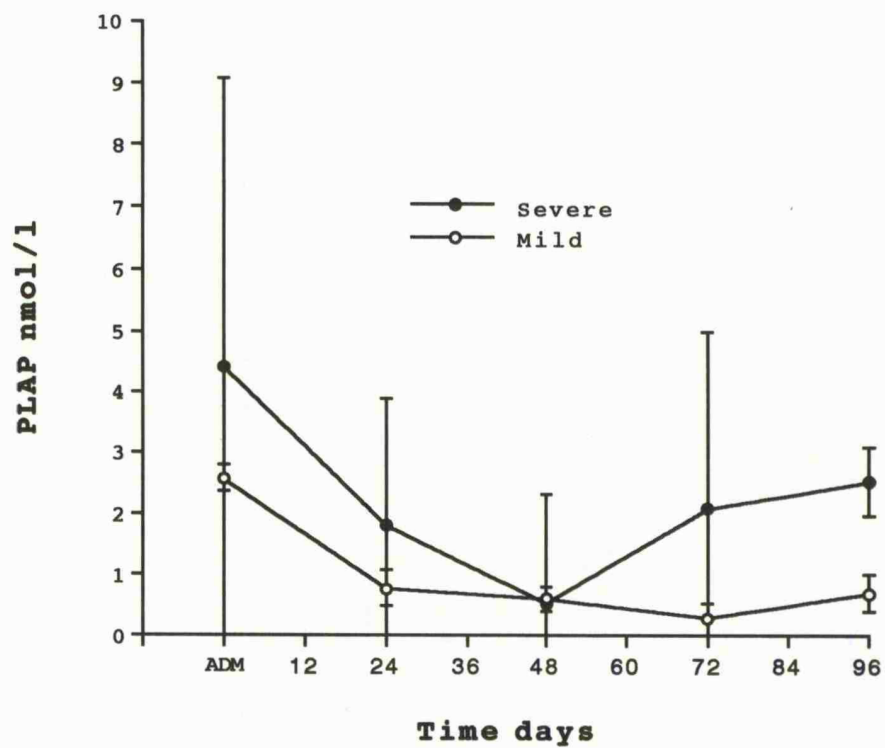


Figure 3.9: The median urine concentrations of PLAP ( $\pm$  standard error of the mean) in 15 patients with severe and 40 patients with mild acute pancreatitis.

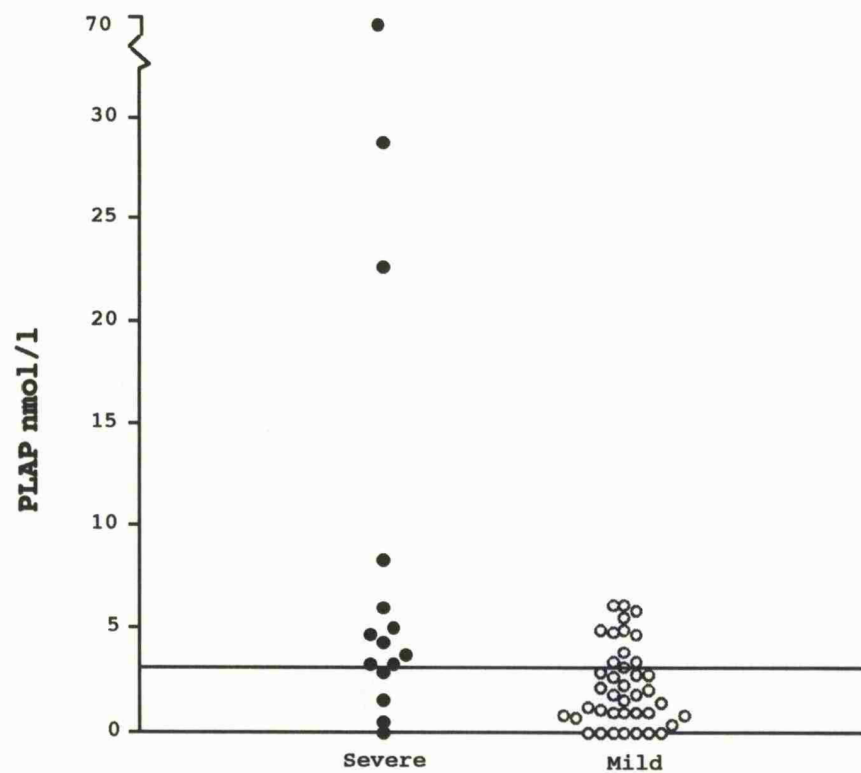


Figure 3.10 Scattergram to show the admission urinary PLAP concentrations in 40 patients with mild and 15 with severe acute pancreatitis.

correctly classified as severe on the basis of an admission TAP concentration  $>2$  nmol/l, one with gallstone pancreatitis developed respiratory failure and died and the other developed a pancreatic pseudocyst and survived.

Comparison of admission urinary PLAP concentrations between patients with alcoholic and gallstone pancreatitis and pancreatitis of other aetiologies failed to demonstrate any significant differences ( $H = 2.83$ ,  $df = 2$ ,  $p = 0.243$ , Kruskal Wallis test).

The median peak PLAP concentration in the severe group was 9.16 nmol/l (range 3.23 to 132.39 nmol/l) and in the mild group 3.78 nmol/l (range 0 to 160.58 nmol/l). These differences were significant (95.2% CI = 1.7 to 10.59 nmol/l,  $p < 0.0021$ ). A peak PLAP concentration of  $\geq 6$  nmol/l distinguished between mild and severe acute pancreatitis with a sensitivity of 67% and specificity of 75% (Figure 3.11).

#### 3.7.7 Combined TAP and PLAP measurements.

The TAP and PLAP concentrations for individual patients were added together. The median admission TAP + PLAP concentration for those with severe disease was 9.26 nmol/l (range 1.04 to 80.2 nmol/l) and for those with mild disease 2.99 nmol/l (range 0 to 8.3 nmol/l). These differences were highly significant (95.2% CI = 2.69 to 7.68 nmol/l,  $p < 0.0005$ ). Utilising a value of  $\geq 6$  nmol/l it was possible to distinguish between mild and severe disease with a sensitivity of 67% and specificity of 88% (Figure 3.12).

A summary of the sensitivity and specificity for each of the methods of severity prediction described above are given in Table 3.7.

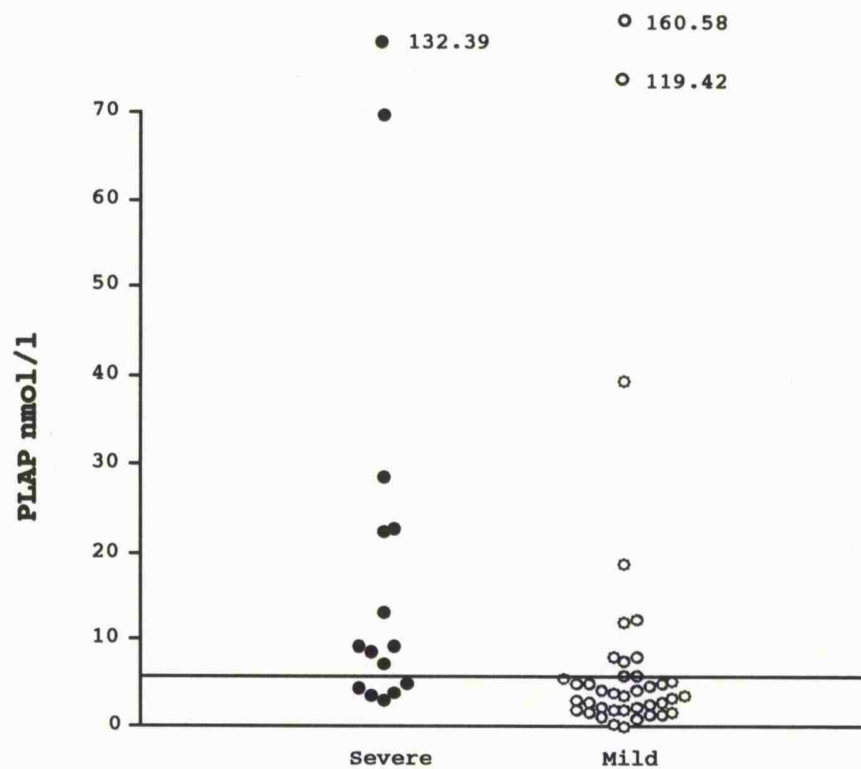


Figure 3.11: Scattergram to show the peak urinary concentrations of PLAP during the first 48 hours of the study in 15 patients with severe and 40 patients with mild acute pancreatitis.

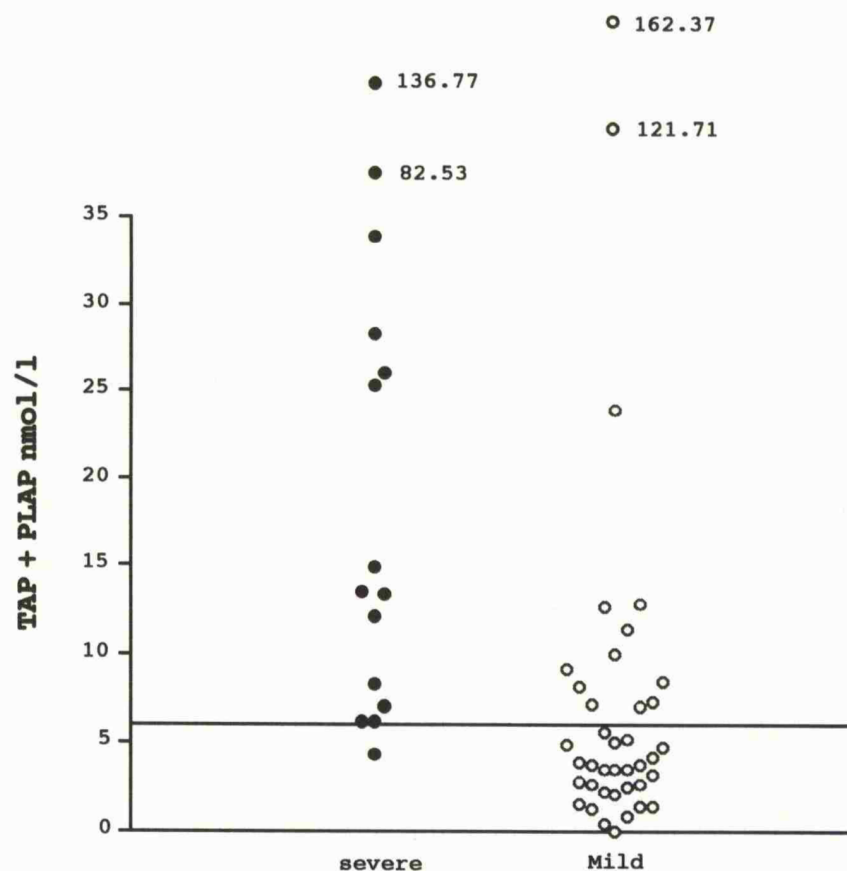


Figure 3.12: Scattergram to show peak urinary concentrations of TAP+ PLAP during the first 48 hours of the study in 15 patients with severe and 40 patients with mild acute pancreatitis.

Parameter Correct	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	%
Glasgow Score $\geq 3$	60	93	75	88	84
CRP $\geq 100$ mg/l (1st 24 hrs)	60	75	87	71	71
CRP $\geq 200$ mg/l (1st 48 hrs)	73	75	75	62	75
TAP $\geq 2$ nmol/l (Admission)	80	90	75	92	87
TAP $\geq 2$ nmol/l (1st 24 hrs)	80	85	67	92	84
PLAP $\geq 3$ nmol/l (Admission)	73	73	50	88	73
PLAP $\geq 6$ mmol/l (peak)	67	75	88	86	75
TAP + PLAP $\geq 8$ nmol/l (peak)	67	88	66	88	82

Table 3.7: The sensitivity and specificity of each of the methods of severity assessment.

### **3.8 SEVERITY ASSESSMENT IN A SUBPOPULATION OF 39 PATIENTS DOCUMENTED WITHIN THE WEST OF SCOTLAND.**

The data displayed in this section is displayed fully in appendices 4 and 5.

#### **3.8.1 Presenting symptoms.**

All patients in the study had abdominal pain at the time of presentation with no difference in the severity reported by patients with mild and severe disease ( $p < 0.73$ , Fisher's exact test). The commonest sites of abdominal pain were the epigastrium and right upper quadrant and the least common sites the hypogastrium and left iliac fossa (table 3.8).

Sixty two per cent of patients described pain radiating through to the back.

Collapse, which is customarily associated with the perforation of a peptic ulcer, and pain radiating to the shoulder, associated with diaphragmatic irritation secondary to peritonitis, were only reported by a small number of patients (5% and 10% respectively).

A history vomiting prior to admission was present in 77% of patients. Vomiting was not significantly more common in patients with severe disease ( $p < 0.58$ , Fisher's exact test).

#### **3.8.2 Physical signs.**

Abdominal distension on admission ( $p < 0.57$ , Fisher's exact test) was not more common in patients with severe disease. However moderate or severe dehydration ( $p < 0.003$ , Fisher's exact test), abnormal peripheral perfusion ( $p < 0.0026$ , Fisher's exact test) marked abdominal tenderness or the presence of localised peritonism ( $p < 0.03$ , Fisher's exact test) or of abnormal or absent bowel sounds ( $p < 0.0007$ , Fisher's exact test) and paralytic ileus (95.2% CI = 0.001 to 3.99 days,  $p = 0.001$ ) were more common in patients who were subsequently demonstrated to have suffered a severe attack of acute pancreatitis (table 3.9).

Epigastrium	35 (90%)
Right hypochondrium	26 (67%)
Central area	18 (46%)
Left hypochondrium	14 (36%)
Right lumbar area	8 (21%)
Left lumbar area	7 (18%)
Right iliac fossa	3 (8%)
Hypogastrium	1 (3%)
Left iliac fossa	1 (3%)

Table 3.8: The site of abdominal pain in 39 patients with acute pancreatitis.



	Severe	Mild
Vomiting	8	22
nausea	2	2
neither	0	5
Dehydration		
none	4	24
mild	2	7
moderate	3	0
severe	1	0
Abdominal distension		
present	4	26
absent	6	3
Bowel sounds		
normal	3	26
abnormal	4	1
absent	3	2
Peritoneal effusion	2	0
Abdominal tenderness		
none	0	3
minimal	4	19
marked	1	5
local peritonism	5	2
Median duration of ileus (range)	3 days (0-7 days)	0 days (0-2 days)
Fluid sequestration (range)	3412 ml (2017-8812 ml)	2175 ml (383-4726 ml)

Table 3.9: The clinical features in 10 patients with severe and 29 with mild acute pancreatitis.

Seven of the patients with severe disease and 4 of those with mild disease had on admission pulse rates  $\geq 100$  bpm. This detected severe attacks of acute pancreatitis with a sensitivity of 70% and specificity of 86%. The 2 patients who died had admission pulse rates of 120 bpm and 75 bpm.

One patient with severe and 1 with mild disease had an admission blood pressure  $\leq 100$  mm Hg. This resulted in an admission blood pressure  $\leq 100$  mm Hg detecting severe attacks of acute pancreatitis with a sensitivity of 10% and specificity of 97%.

Four patients with severe disease (40%) had an admission temperature  $\geq 37.2^{\circ}\text{C}$  whereas only 4 of the patients with mild disease (14%) did. The 2 patients who died had admission temperatures of  $36.5^{\circ}\text{C}$  and  $38.4^{\circ}\text{C}$ . An admission temperature  $\geq 37.2^{\circ}\text{C}$  predicted a severe attack of acute pancreatitis with a sensitivity of 40% and specificity of 86%.

### **3.8.3 Clinical evaluation.**

Clinical evaluation on admission correctly classified 27 of 29 patients (93%) as suffering from mild and 7 of 10 patients (70%) from severe disease.

Of the 3 patients with severe disease incorrectly classified on admission 1 had a Glasgow score of 3, and developed cystic collections within the head of the pancreas. These resolved spontaneously, and he was discharged home well on day 12. The second patient also had a Glasgow score of 3 and was similarly classified as severe on the basis of a cystic collection within the head of the pancreas (which resolved spontaneously). The final patient had a Glasgow score of 5. She was classified as severe on the basis respiratory failure. She survived. Only the first patient was still considered to have mild disease when reassessed at 48 hours.

Of the patients incorrectly classified by clinical assessment on admission as suffering a severe attack of acute pancreatitis. One developed a peak CRP of 256 mg/l on day 2 although he did not develop any complications, and was allowed home on day 7. He was correctly classified as mild by clinical assessment at 24 hours. The other patient was still considered severe when reassessed at 48 hours.

#### **3.8.4 Fluid sequestration.**

The median volume of fluid sequestered during the first 48 hours after admission in patients with severe and mild disease were 3412 ml and 2175 ml respectively. These differences were highly significant (95.2% CI = 504.9 ml to 2064 ml,  $p = 0.0072$ ). Only 1 patient (classified as suffering from a severe attack) sequestered greater than 6000 ml of fluid. Four patients with mild disease sequestered greater than 2000 ml of fluid a day for the first 48 hours after admission, whereas only 1 patient with severe disease sequestered a similar amount of fluid. This resulted in the sequestration of greater than 2000 ml of fluid a day for 48 hours predicting a severe attack of acute pancreatitis with a sensitivity of only 10% and specificity of 86%.

#### **3.8.5 APACHE II Score.**

The median APACHE II scores, throughout the 5 days of the study, are shown in figure 3.13. The most abnormal values were seen at the time of admission, values falling thereafter as the patients' conditions improved. Differences in the APACHE II score between patients with a severe and mild disease were statistically significant at the time of admission and remained so for the first 3 days of the study (figure 3.13). An admission APACHE II score  $>4$  detected a severe attack of acute pancreatitis with a sensitivity of 80% and specificity of 62% (figure 3.14) whilst a peak value  $>9$  detected severe attacks with a sensitivity of 60% and slightly higher specificity of 69% (figure 3.15).

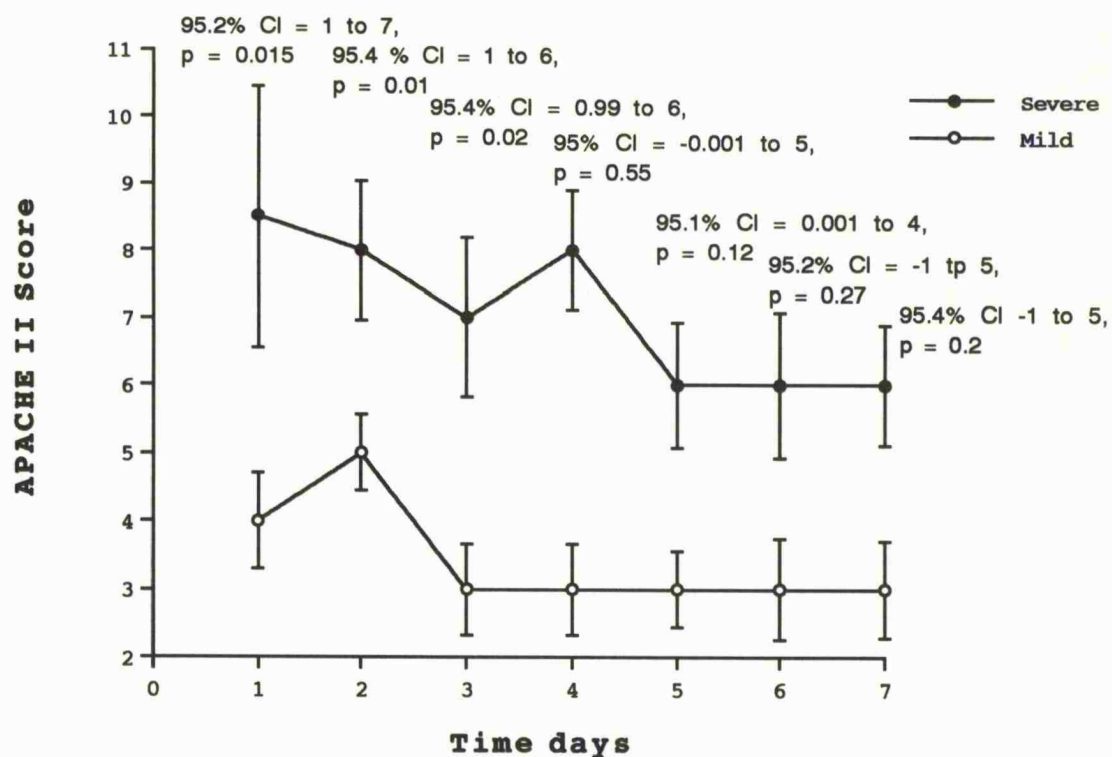


Figure 3.13: The median APACHE II scores ( $\pm$  standard error of the mean) in 10 patients with severe and 29 patients with mild acute pancreatitis.

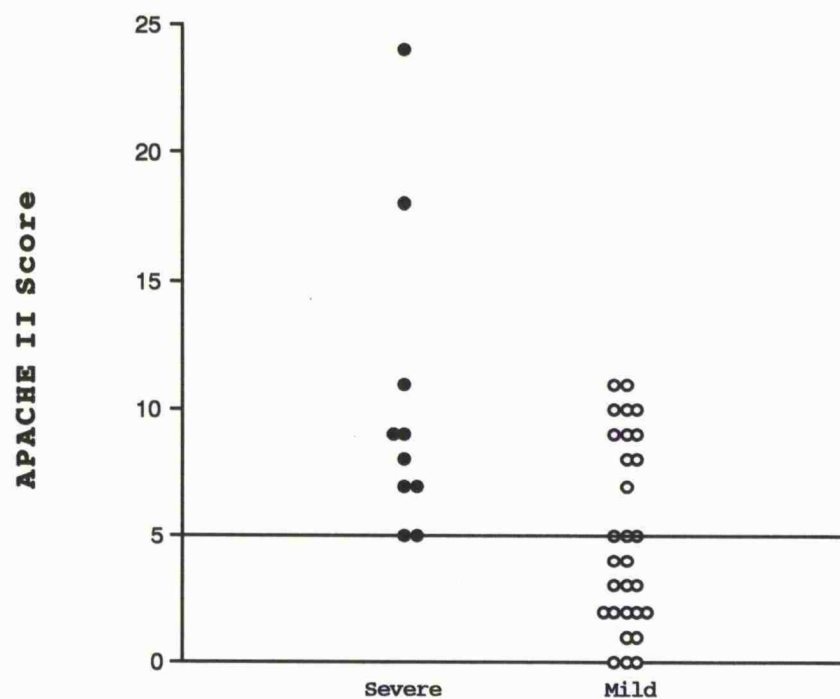


Figure 3.14: Scattergram to show the admission APACHE II score in 10 patients with severe and 29 patients with mild acute pancreatitis.

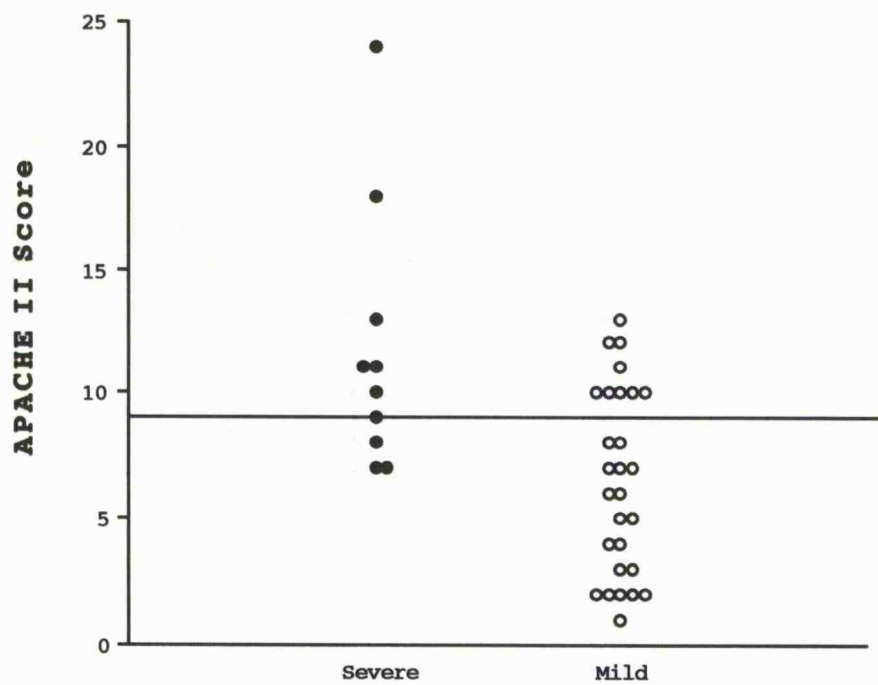


Figure 3.15: Scattergram to show the peak APACHE II score in 10 patients with severe and 29 patients with mild acute pancreatitis.

The median APACHE II physiology scores, throughout the 5 days of the study, are shown in figure 3.16. As with the total APACHE II score, most abnormal values were seen at the time of admission, values falling thereafter as the patients' conditions improved. Differences in the APACHE II physiology score between patients with severe and mild disease were statistically significant at the time of admission and remained so throughout the 5 days of the study. An admission APACHE II physiology score  $>4$  detected a severe attack of acute pancreatitis with a sensitivity of 70% and specificity of 90% (figure 3.17) whilst a peak value  $>5$  only detected severe attacks with a sensitivity of 70% and specificity of 83% (figure 3.18). The sensitivity, specificity, positive and negative predictive values and percentage correct for clinical evaluation and the APACHE II score are displayed in table 3.10 and percentage correct for clinical severity assessment in 39 patients with acute pancreatitis.

When the 14 individual components of the admission APACHE II score were examined creatinine, arterial pH, white cell count and mean arterial blood pressure were found to separate mild and severe attacks of acute pancreatitis (table 3.11). None of the patients had an abnormal serum sodium concentrations or Glasgow coma score and only 1 patient scored because of chronic ill health. Whilst there was no significant difference in the number of patients scoring for age there was a tendency for patients with mild disease to have a higher proportion of patients scoring higher values than those with severe disease.

The APACHE II scores for individual patients can be found in appendix 5.

### **3.3.6 White cell Count.**

The admission median white cell count was significantly higher in patients with severe than in those with mild disease (figure 3.19). The median white cell count peaked on day 2 and declined until day 6 when it started to rise again. In patients with mild disease the median white cell count fell from the time of admission.

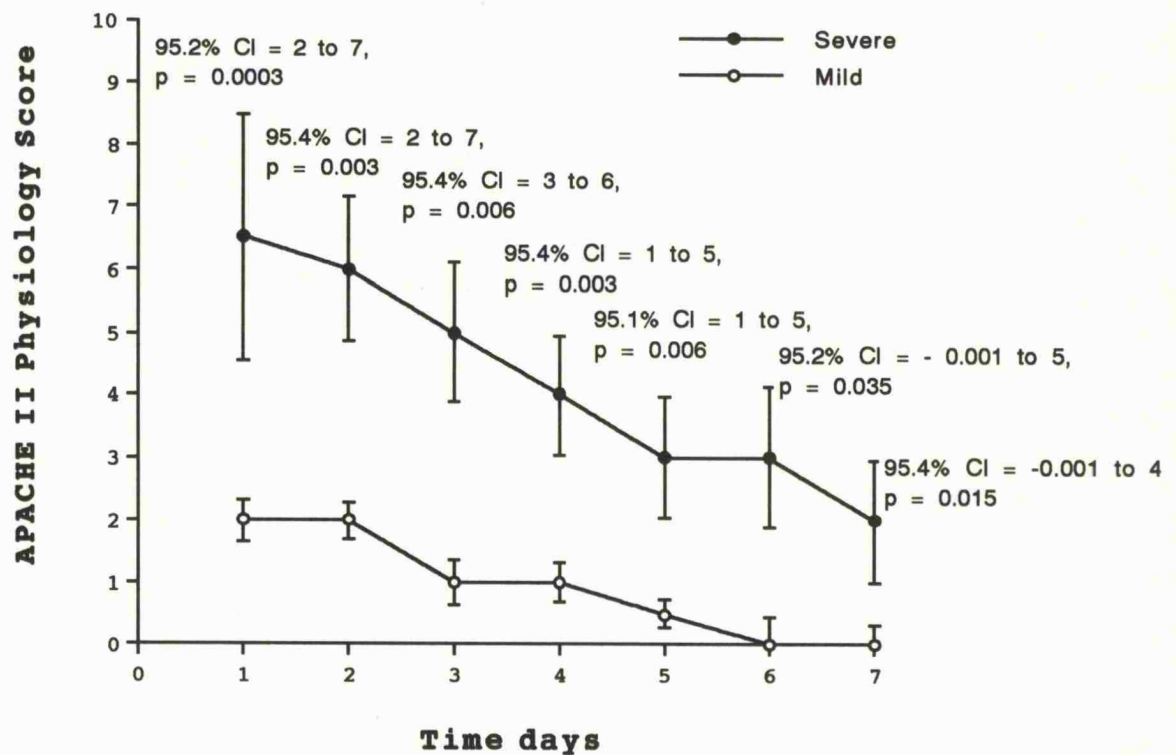


Figure 3.16 The median APACHE II physiology scores ( $\pm$  standard error of the mean) in 10 patients with severe and 29 patients with mild acute pancreatitis.





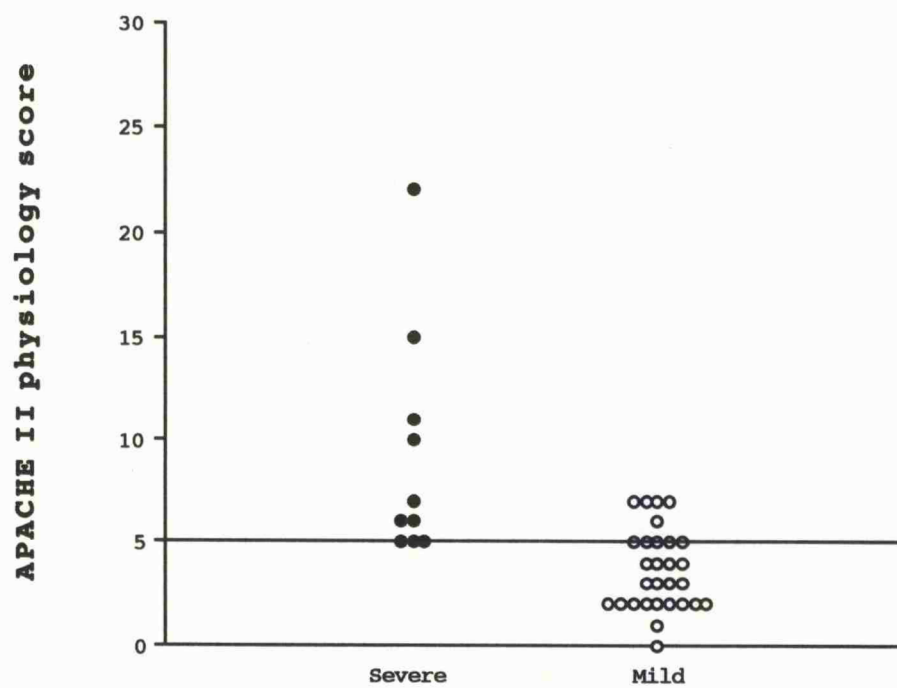


Figure 3.18: Scattergram to show the peak APACHE II physiology score in 15 patients with severe and 40 patients with mild acute pancreatitis.

	<b>Sensitivity %</b>	<b>Specificity %</b>	<b>Positive predictive value %</b>	<b>Negative predictive value %</b>	<b>% correct</b>
<b>On admission</b>					
Clinical assessment	70	93	78	90	87
APACHE II (>5)	80	62	42	90	67
APACHE II Physiology Score (>4)	70	90	70	90	85
<b>24 hrs after admission</b>					
Clinical assessment	80	97	89	93	92
<b>48 hrs after admission</b>					
Clinical assessment	90	97	90	96	90
Peak APACHE II (>9) score	60	69	40	77	77
APACHE II Physiology Score (>5)	70	83	58	89	80

Table 3.10: Sensitivity, specificity, positive predictive value, negative predictive value and percentage correct of clinical assessment and the APACHE II score in assessing the severity of acute pancreatitis in 29 patients with mild and 10 patients with severe acute pancreatitis.

Parameter	Disease severity	Number of patients scoring	Weighting score distribution						p value
			1	2	3	4	5	6	
Sodium	Severe	0							NS
	Mild	0							
Potassium	Severe	4	4						NS
	Mild	2	1			1			
Creatinine	Severe	0							p 0.05
	Mild	2		2					
Arterial pH	Severe	0							p <0.005
	Mild	5	2		2	1			
Arterial pO <sub>2</sub>	Severe	3	2			1			NS
	Mild	3	2			1			
Haematocrit	Severe	5	4	1					NS
	Mild	5	2	3					
White cell count	Severe	3	3						p <0.005
	Mild	6	4		2				
Temperature	Severe	3	3						NS
	Mild	0							
Heart rate	Severe	9	9						NS
	Mild	6	6						
Mean BP (mm Hg)	Severe	12	11	1					p < 0.005
	Mild	9	7	2					
Respiratory rate	Severe	1	1						NS
	Mild	1	1						
Glasgow coma score	Severe	0							NS
	Mild	0							
Age	Severe	20	6	5		5	4		NS
	Mild	8	5	2		1			
Chronic health evaluation	Severe	1					1		NS
	Mild	0							

Table 3.11: The distribution of the 14 individual components of the APACHE II score between 10 patients with severe and 29 with mild acute pancreatitis.

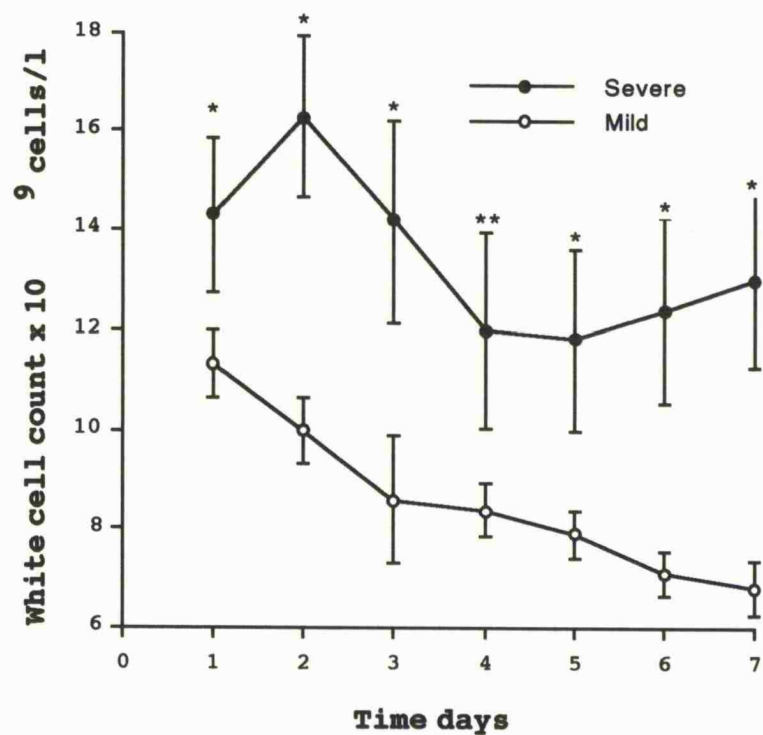


Figure 3.19: The median white cell count ( $\pm$  standard error of the mean) in 10 patients with severe and 29 patients with mild acute pancreatitis.

Statistically significant differences were noted between the 2 groups of patients on all days. The median peak white cell count was also significantly higher in patients with severe disease (95.2% CI = 1.1 to 7.7 x10<sup>9</sup> cells/l, p = 0.007). An admission white cell count of >12 x 10<sup>9</sup> cells/l detected severe attacks of acute pancreatitis with a sensitivity of 80% and specificity of 55% (figure 3.20).

#### **3.8.7 LDH.**

The median admission LDH concentration was significantly higher in patients with severe disease at the time of admission and rose to peak on the 4th day, falling thereafter (figure 3.21). Serum concentrations of LDH in patients with mild disease followed a similar pattern with peak median concentrations being seen on day 4. Differences between these groups were significant on all days except the sixth, the p value just failing to reach significance. The peak LDH concentration was significantly higher in patients with severe disease (95.2% CI = 202 to 426.8 IU/l, p = 0.0004). The admission and peak LDH concentrations for individual patients are displayed in figures 3.22 and 3.23.

The sensitivity and specificity of the white cell count and serum LDH concentrations in assessing disease severity are displayed in table 3.12.

### **3.9 URINARY TAP AND PLAP CONCENTRATIONS IN PATIENTS WITH ACUTE ABDOMINAL CONDITIONS OTHER THAN ACUTE PANCREATITIS.**

#### **3.9.1 Urinary TAP concentrations.**

The median admission TAP concentration for the disease control group was 0.16 nmol/l (range 0 to 2.2 nmol/l) compared with values of 0 (range 0 to 5.55 nmol/l) for patients with mild and 3.23 nmol/l (range 0 to 10.3 nmol/l) for patients with severe acute pancreatitis. These differences were highly significant (H = 25.41, df = 2, p = 0.000, Kruskal-Wallis test). Further analysis demonstrated that there was no significant difference between the median concentrations for the disease

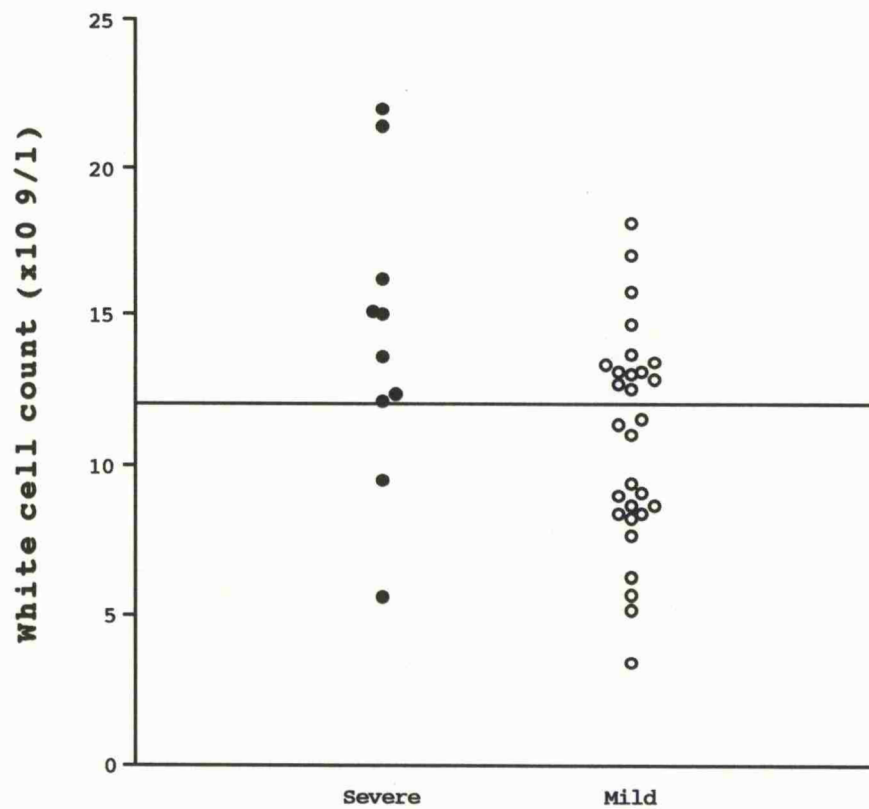


Figure 3.20: Scattergram to show the admission white cell count in 10 patients with severe and 29 patients with mild acute pancreatitis.

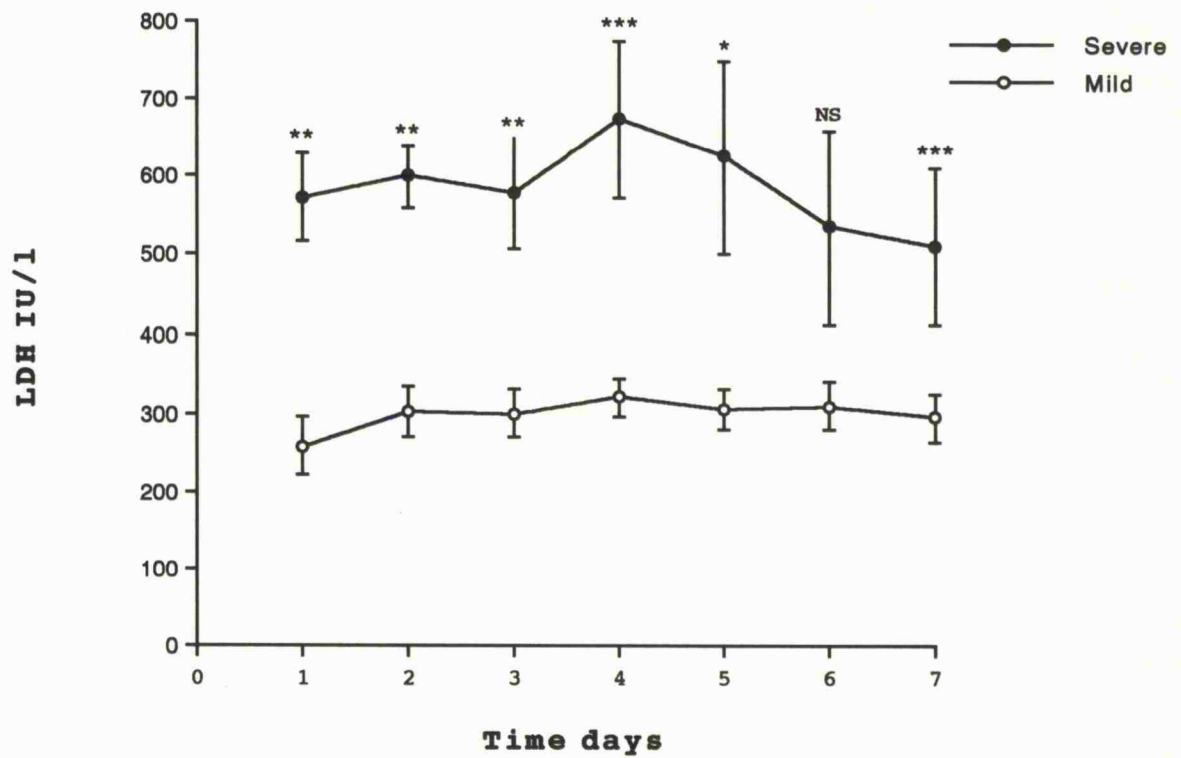


Figure 3.21: The median daily LDH concentration ( $\pm$  standard error of the mean) in 10 patients with severe and 29 patients with mild acute pancreatitis.



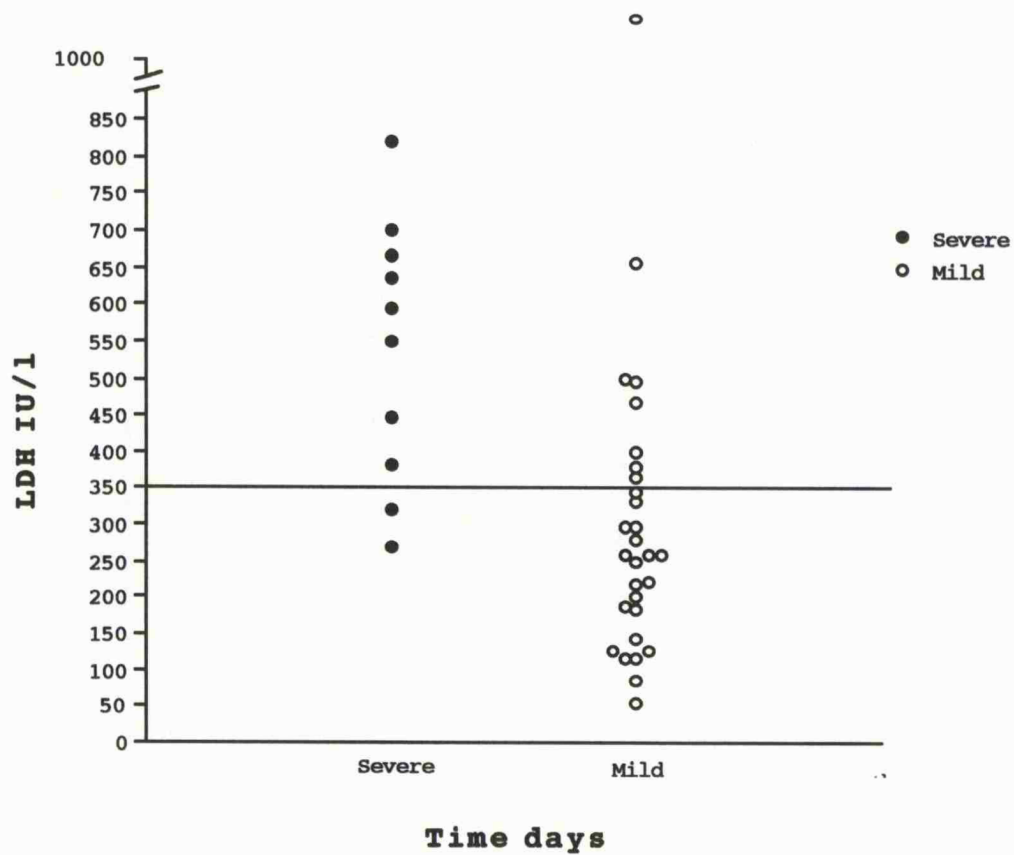


Figure 3.22: Scattergram to show the admission LDH concentration in 10 patients with severe and 29 patients with mild acute pancreatitis.

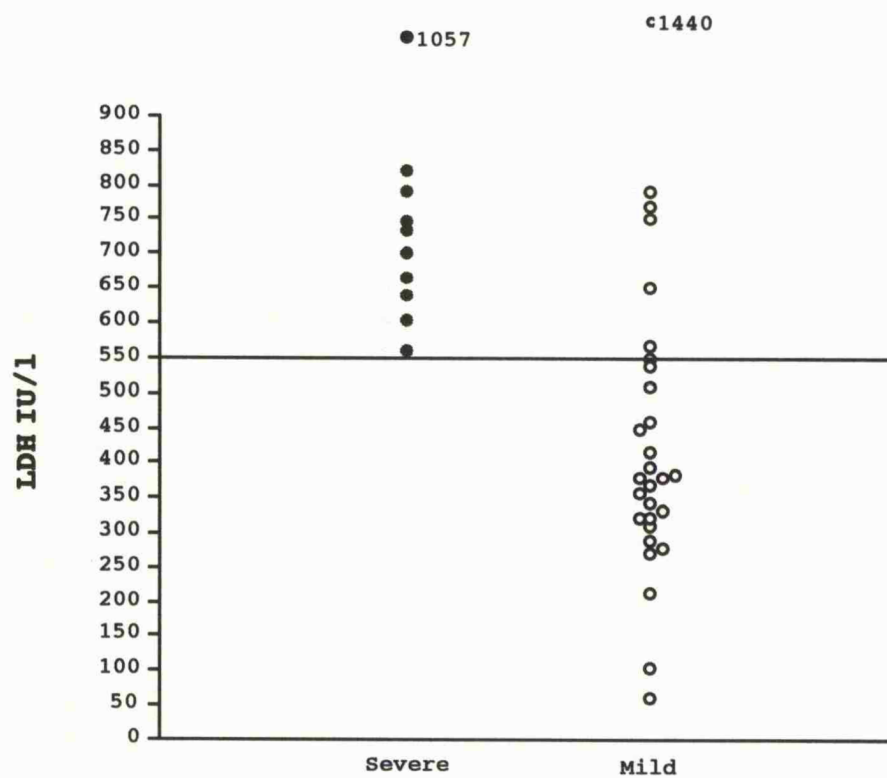


Figure 3.23: Scattergram to show the peak LDH concentration in 10 patients with severe and 29 patients with mild acute pancreatitis.

	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	% Correct
Admission white cell count > $12 \times 10^9/l$	80	55	38	89	62
Peak white cell count > $12 \times 10^9/l$	90	48	38	93	59
Admission LDH > 350 IU/l	80	74	50	97	74
Peak LDH > 550 IU/l	100	79	63	59	85

Table 3.12: Sensitivity, specificity, positive predictive value, negative predictive value and percentage correct of the white cell count and serum LDH concentrations in assessing the severity of acute pancreatitis in 29 patients with mild and 10 patients with severe acute pancreatitis.

control and mild groups (95% CI = -0.18 to 0,  $p < 0.24$ ) although highly significant differences were observed between the disease control and the severe group (95.3% CI = 1.9 to 4.3,  $p < 0.001$ ). TAP was present in 15 of the 25 disease controls although only 1 patient (number 4 in table 5.1) displayed a value  $\geq 2$  mmol/l. He had ascending cholangitis associated with a stone in the common bile duct and made a full recovery following endoscopic removal of the stone).

Nine (40%) of the 25 patients in the disease control group did not have TAP detected in their urine. Two of these had appendicitis, 2 biliary colic, 2 cholecystitis, 2 obstructive jaundice, 1 an exploration of his common bile duct and 1 intestinal obstruction.

### 3.9.2 Urinary PLAP concentrations.

The admission PLAP concentration for the disease control group was 3.2 nmol/l (range 0 to 46.3 nmol/l) compared with values of 1.9 (range 0 to 5.2 nmol/l) for patients with mild and 4.4 nmol/l (range 0 to 69 nmol/l) for patients with severe acute pancreatitis. These differences were significant ( $H = 6.37$ ,  $df = 2$ ,  $p = 0.042$ , Kruskal-Wallis test). There was no significant difference in the concentrations of PLAP between the control and mild or the control and severe groups (95.3% CI = -3.73 to 4.15,  $p < 0.2$  and 95% CI = -3.7 to 0.01,  $p < 0.06$  respectively). Thirteen of the 25 disease control patients would have been classified as suffering from severe acute pancreatitis on the basis of a PLAP concentration  $\geq 3$  nmol/l.

### 3.10 DISCUSSION.

#### TAP and PLAP

The results of this study demonstrate that the measurement of urinary TAP concentrations provide better severity prediction in acute pancreatitis than any of the following methods evaluated in this and previous studies: clinical assessment (Corfield *et al* 1984), the Glasgow (Imrie *et al* 1978) and APACHE II scores (Larvin *et al* 1989), CRP (Wilson *et al* 1989),  $\alpha_2$ -macroglobulin (Howard and Jordan 1986, Glazer and Ranson 1988), and peritoneal aspiration (McMahon *et al* 1980). Furthermore the best severity prediction was based on measurements made in urine samples taken at the time of admission to hospital.

The studies involving normal subjects and disease controls have validate the use of the TAP assay in the clinical setting, where patients with conditions other than acute pancreatitis will be encountered. None of the normal subjects had a background urinary TAP concentrations high enough to interfere with the clinical usefulness of the test (the highest concentration was 1.68 nmol/l as against an admission concentration of  $\geq 2$  nmol/l required to predict a severe attack) and only 1 disease control had a urinary TAP  $> 2$  nmol/l.

The measurement of admission urinary PLAP or combined of TAP and PLAP concentrations failed to improve on the severity prediction provided by urinary TAP measurements alone or the following methods of severity assessment evaluated in this and previous studies: clinical assessment (Corfield *et al* 1984), the Glasgow (Imrie *et al* 1978) and APACHE II scores (Larvin *et al* 1989), CRP (Wilson *et al* 1989),  $\alpha_2$ -macroglobulin (Howard and Jordan 1986, Glazer and Ranson 1988), and peritoneal aspiration (McMahon *et al* 1980). This failure is probably because phospholipase A<sub>2</sub> is not confined to the pancreas: a number of other cell lines are known to contain this enzyme including peritoneal (Wightman *et al* 1981) and hepatic (Birmelin *et al* 1984) cells, leukocytes, lung, stomach, small intestine and kidney (Matsuda *et al* 1987). Gudgeon (1990) has recently demonstrated that the

trypsinisation of human leucocytes leads to the release of PLAP and it seems likely that leucocytes from other sources possess a similar capacity. If this is the case it is not difficult to see how conditions such as peritonitis, septicaemia, adult respiratory distress syndrome (ARDS) (Vadas 1984), and any condition with deranged hepatic function can lead to the appearance of PLAP in urine. Certainly PLAP concentrations of  $> 3$  nmol/l were noted in 13 of the disease controls whilst only one of the same group had a urinary TAP  $> 2$  nmol/l.

The sensitivity of the Glasgow scoring system in the present study is comparable to those previously reported (Imrie *et al* 1978, Osborne *et al* 1981, Blamey *et al* 1984) as was the failure of serum amylase concentrations to provide separation between mild and severe attacks of acute pancreatitis (Pollock 1957, Trapnell 1966, Corfield *et al* 1985).

The effectiveness of severity prediction provided by CRP was comparable to a number of previous reported studies (Mayer *et al* 1984, Poulakkainen *et al* 1987), but not as good as those of Wilson *et al* 1989, Buchler *et al* (1986) and Poulakkainen *et al* (1987) who noted significant differences in the serum concentrations of CRP between mild and severe disease at the time of admission. However, in the present study, as well as those of Wilson *et al* (1989) and Mayer *et al* (1984), peak differences were not noted until 48 hours. This reflects the fact that patients entered into the present study were seen shortly after the onset of symptoms and were not tertiary referrals from other centres as occurs in Ulm (Buchler *et al* 1986). Whilst CRP concentration measurements are cheap, quick and simple to perform prediction in patients within the West of Scotland and St. George's Hospital, London, involved a delay of 48 hours.

An admission  $\alpha_2$ -macroglobulin concentration of  $\leq 1.4$  mg/l distinguished between patients with mild and severe disease with a reasonably sensitivity but had an inadequate specificity. It only provided a good balance of sensitivity and specificity

where the minimum concentration over 5 days was considered. Such a delay makes this test of no value in clinical practice and agrees with previously published views (Howard and Jordan 1986, Glazer and Ranson 1988).

The number of patients presenting with abdominal was comparable to the 98% reported by Bockus *et al* (1955) and greater than the 85% reported by Jacobs *et al* (1977). However, the true incidence of painless acute pancreatitis is difficult to judge accurately since a number of these patients may not be diagnosed (Wilson and Imrie 1991).

The severity of the abdominal pain was not related to outcome, a finding in agreement with that of Jacobs *et al* (1977). Whilst the small proportion of patients experiencing pain in their lower abdomen are in agreement with previous reports (Bockus *et al* 1955, Romer and Carey 1966, Foster 1962) a greater proportion experienced epigastric and right upper quadrant pain than previously noted (Bockus *et al* 1955, Foster 1962, Albo *et al* 1963, Romer and Carey 1966, Cogbill and Song 1970). Abdominal pain, radiating to the back, was also more common than noted previously (Foster 1962, Bockus *et al* 1955). The incidence of vomiting was comparable to previous reports (Jacobs *et al* 1977, Pollock 1959).

In common with other studies the features of ileus was more common in patients with severe disease (Trapnell 1966, Foster and Ziffren 1962, Bockus *et al* 1955). Pyrexia and the admission blood pressure failed to provide effective discrimination between mild and severe attacks and the sensitivity of on admission hypotension was poor, as previously published by Cooper *et al* (1982). Surprisingly, and in contrast to the finding of Jacobs *et al* (1977), an admission pulse rate >100 bpm distinguished between mild and severe attacks with a high sensitivity and specificity.

The sensitivity of clinical assessment in distinguishing mild and severe attacks of acute pancreatitis at the time of admission was approximately twice the values reported by McMahon *et al* (1980) and Corfield *et al* (1984). The reason for this is unclear. Both of the quoted studies and the present one were conducted by interested researchers with an extensive exposure to acute pancreatitis and we might therefore expect their degree of clinical skill to be comparable. If the differences are simply the result of an eagerness on the part of the present researcher to detect all patients with severe disease then we would expect a large number of patients with mild disease to be incorrectly classified as suffering from severe disease. However, this is not the case. The specificity of admission clinical assessment in the present study slightly lower than the 100% quoted by McMahon *et al* (1980) but better than the 85% quoted by Corfield *et al* (1984). Whilst the present study involved only a small number of patients the findings are in agreement with a larger study involving 122 patients, recently presented by the author. Admission severity assessment achieved a sensitivity of 64% and specificity of 87% (Heath and Imrie 1990).

By 48 hours the sensitivity and specificity of clinical assessment were comparable to previously reported results (McMahon *et al* 1980, Corfield *et al* 1984).

An admission serum LDH concentration > 500 IU/l provided better severity prediction than previously demonstrated with a very high sensitivity and specificity (Ranson *et al* 1974). The finding of an elevated white cell count (Thal *et al* 1957, Trapnell 1966, Ranson *et al* 1974, Imrie *et al* 1977) and a persistently elevated value at the end of the first week (Trapnell 1966) agree with previous reports. The specificity of a peak white cell count >12 x10<sup>9</sup> cells/l was too low to be of value in clinical practice.

One of the advantages of the APACHE II score over the other disease specific scoring systems is its ability to take account, not only of the physiological disturbance caused by the disease process, but also factor that will affect the



patients' ability to recover i.e.: age and pre-existing chronic ill health. The admission severity prediction of the APACHE II scoring system provided a similar balance of sensitivity and specificity to the study of Larvin and McMahon (1989) and was slightly better than that of Wilson *et al* (1990). In contrast to the studies of Larvin and McMahon (1989) and Wilson *et al* (1990) peak scores failed to improve severity prediction. The admission physiology score was better providing clinically useful separation between groups at the time of admission. This was surprising since it fails to take account of mortality associated with chronic ill health and increasing age, although only one patient scored for chronic ill health and there was a tendency for those with mild disease collect a larger proportion of high scores for age.

The laboratory tests needed to calculate the APACHE II score are simple, routine and readily available as part of the "out of hours" service, which is not the case for the calcium, albumin and the enzyme assays required for the Glasgow and Ranson scoring systems. In addition it is not necessary to wait for 48 hours before prediction: the APACHE II score can be calculated at the time of admission. Further advantages are that it may allow the continuing monitoring of the patients' progress and may prove useful in assessing response to therapy.

Several components of the APACHE II score contributed to the total score in only a few patients and others failed to provide effective discrimination between patient groups.

#### **CHAPTER 4:**

**TRYPSINOGEN ACTIVATION PEPTIDE (TAP) CONCENTRATIONS IN THE  
PERITONEAL FLUID OF PATIENTS WITH ACUTE PANCREATITIS, AND  
THEIR RELATIONSHIP TO THE PRESENCE OF PANCREATIC NECROSIS.**

#### 4.1 INTRODUCTION AND AIMS.

One of the principal factors determining the outcome of an attack of acute pancreatitis is the presence of pancreatic necrosis (Uhl *et al* 1991, Bradley *et al* 1989, Leger *et al* 1981, Hollender *et al* 1986). This occurs in 10% to 15% of patients with acute pancreatitis and is associated with a mortality rate of 30% to 40% (Nordback *et al* 1985, Hollender *et al* 1986 Edelman and Boutelier 1974, Kivlaakso 1981, Beger *et al* 1986). For this reasons its early identification and prompt treatment remains an important priority in the management of acute pancreatitis.

Whilst the Ranson and Glasgow criteria provide effective separation between mild and severe attacks of acute pancreatitis they are unable to identify accurately patients with necrotising disease (Teerenhovi *et al* 1988, Leese *et al* 1988). Clinical assessment (Corfield *et al* 1985, Nordback *et al* 1985, Block *et al* 1986) and visual inspection of the gland at the time of surgery have also proved ineffective (Nordback *et al* 1985). Nordback *et al* (1985) also failed to demonstrate any correlation between the extent of histologically proven pancreatic necrosis and blood glucose, serum sodium, potassium, creatinine, calcium, triglycerides, bilirubin or transaminases, haematocrit, white cell or platelet counts. Whilst a number of studies have suggested that RNAse (Warshaw *et al* 1983),  $\alpha_1$ -antiprotease,  $\alpha_2$ -macroglobulin (Buchler *et al* 1986), calcium and CRP are effective in detecting pancreatic necrosis, others (Poulakkainen *et al* 1987) have failed to confirm these findings (McMahon *et al* 1980, Nordback *et al* 1985, Leese *et al* 1988, Kemmer *et al* 1991, Wilson *et al* 1991). Ultrasound has also proved ineffective (Swobodnick *et al* 1985, Block *et al* 1986)

At present contrast enhanced computerised tomography (CT) scanning provides the best method of detecting pancreatic necrosis, an area of hypoperfusion being equated with the presence of necrotic tissue (Kivisaari *et al* 1984, Block *et al* 1986, Nuutinen *et al* 1988, Larvin *et al* 1990). Utilising these criteria Buchler *et al* (1986)

have claimed that contrast enhanced CT scanning detects pancreatic necrosis with an accuracy of 85-90% and more recently Larvin *et al* (1990) have correctly identified 11 patients with histologically proven necrosis. Forty patients with equivocal or normal enhancement recovered without the need for surgery. CT scanning is however, not universally available, involves moving a sick patient away from intensive care and is relatively expensive. For these reasons alternative methods of detecting pancreatic necrosis have been sought.

It has been suggested that pancreatic necrosis only develops where intraglandular trypsinogen activation occurs (section 1.8.1). If this is the case then we would expect to see a correlation between the presence and extent of pancreatic necrosis and the serum concentration of trypsin in patients with acute pancreatitis. Failure to demonstrate such a relationship in the past may be due to the failure of the immunoreactive trypsin assay to differentiate between the active enzyme (trypsin) and the inactive parent zymogen (trypsinogen) (section 1.5.4). This problem has been overcome recently by the development of an immunoassay utilising antibodies directed against the C-terminal end of TAP (Hurley *et al* 1988a)). The studies described in chapter 3 demonstrate the effectiveness of urinary TAP measurements in determining the severity of acute pancreatitis. In this chapter we examine the ability of TAP concentrations, measured in peritoneal fluid, to detect histologically proven pancreatic necrosis.

#### **4.2 DIAGNOSIS OF ACUTE PANCREATITIS.**

The diagnosis of acute pancreatitis was made as described in section 3.4.3.

#### **4.3 ASSAYS.**

##### **4.3.1 TAP and PLAP**

The TAP and PLAP assays were performed as in described in sections 2.2.2.6 and 3.3.4.1.

#### **4.3.2 Amylase.**

Serum amylase concentrations were measured as set out in section 3.4.2.2.

#### **4.4 DIAGNOSIS OF ACUTE PANCREATITIS.**

The diagnosis of acute pancreatitis was made as described in section 3.4.3.

#### **4.5 DEFINITIVE OUTCOME.**

Disease severity was graded retrospectively depending upon the clinical outcome as set out in section 3.4.4.

Pancreatic necrosis was only considered to be present when histologically proven.

#### **4.6 PERITONEAL ASPIRATION.**

The stomach was emptied with a nasogastric tube and the bladder with a urinary catheter. Five ml of 1% lignocaine was infiltrated into the skin of anterior abdominal wall 2 cm below the umbilicus and an 8 French gauge peritoneal dialysis catheter (Kimal Scientific Products Ltd., Uxbridge, England) inserted into the peritoneal cavity through a small stab incision. Its tip was directed towards the pelvis, any free fluid aspirated and its volume and colour recorded (see figure 1.2). Peritoneal lavage was then performed using one litre of normal saline. Once as much of the saline as possible had been drained out 1l of isotonic saline, containing 700 mg of Aprotinin was instilled into the peritoneal cavity. The fluid was drained out 8 hours later, a further 1l of the Aprotinin solution instilled and the intraperitoneal catheter removed.

Any free fluid aspirated from the peritoneal cavity was centrifuged at 4°C within 1 hour of being taken, frozen and stored at -20°C until analysis.

#### 4.7 STATISTICAL ANALYSIS.

Data were analysed using medians, the Mann Whitney U test, Fisher's exact test and Kruskal Wallis test as appropriate. To avoid repetition in the text the Mann Whitney U test has been utilised unless otherwise stated. Confidence intervals were used in preference to p values in judging the relevance of differences, (Gardener and Altman 1989). The statistical analyses were performed by Minitab Accelerated version 8.1™ and Epistat statistical packages.

The total amount of TAP contained within the peritoneal fluid was calculated by multiplying the free fluid TAP concentration by the total volume of fluid aspirated

#### 4.8 PATIENTS.

A consecutive series of 309 patients with acute pancreatitis were prospectively documented between February 1986 and July 1989. Ninety eight patients, considered on clinical assessment, to have moderate or severe disease were entered into a randomised controlled trial of standard conservative therapy plus intraperitoneal Aprotinin therapy. Of all patients documented 72 (23%) suffered a severe outcome. Of those not randomised to the trial 23 (11%) suffered a severe and 182 (89%) a mild attack. One hundred and four patients fulfilled the entry criteria. Fifty of these (49%) were randomised to the treatment group. One refused aspiration following randomisation and was withdrawn from the study. The remaining 49 underwent a diagnostic peritoneal aspiration followed by the intraperitoneal instillation of Aprotinin. The remaining patients did not undergo peritoneal aspiration and received standard therapy alone. Intraperitoneal Aprotinin therapy failed to influence the mortality or morbidity of the condition (Larvin *et al* 1992) : 49 (48%) patients suffered a mild and 54 (52%) a severe outcome.

The median delay between the onset of symptoms and peritoneal aspiration was 27.5 hours (range 16 to 102 hours). Two patients had a diagnoses other than acute pancreatitis made following aspiration. In one patient frank blood was returned and in the other foul smelling fluid. Both underwent a laparotomy. The former was found to have a traumatic transection of the pancreas and the latter extensive mesenteric venous infarction. Free peritoneal fluid was aspirated from 26 (55%) of the remaining 47 patients and a sufficient volume of fluid was available for analysis in 22 of these. There was insufficient fluid available for analysis in the 4 remaining patients.

Nine patients were classified as suffering from a severe attack with proven pancreatic necrosis (5 from specimens obtained at post-mortem examination and 4 from specimens obtained at the time of surgery), 7 as having severe disease without proven necrosis and 6 as having mild disease, also without proven necrosis (table 4.1).

The three patient groups were comparable in terms of age, sex, aetiology and hospital stay (table 4.2). The prolonged median stay and lack of differences in the length of hospital stay between the patient groups, was due to 2 patients with mild disease who remained in for a cholecystectomy and another who had a delayed discharge for social reasons.

#### 4.9 RESULTS.

##### 4.9.1 TAP concentration.

The distribution of TAP concentrations within the three groups was not uniform ( $H = 13.10$ ,  $df$  (degrees of freedom) = 2,  $p = 0.001$ , Kruskal Wallis test) (table 4.3). Significantly higher TAP concentration were seen in the peritoneal fluids of patients with severe pancreatitis associated with proven necrosis when compared to patients with severe disease but without proven pancreatic necrosis (95.6% CI = 0.4 to 9.0 nmol/l,  $p < 0.4$ ) or those with mild disease (96.1% CI = 0.68 to 10.4 nmol/l,  $p < 0.006$ ). Significant differences were not seen between patients with severe attacks without proven necrosis and mild attacks (96.2% CI = -0.07 to 1.4).

	<b>Necrotising 9 Patients</b>	<b>Severe 7 Patients</b>	<b>Mild 6 Patients</b>
<b>Median age</b>	55 yrs (29-62 yrs)	34 yrs (31-74 yrs)	53 yrs (37-68 yrs)
<b>Sex M: F</b>	8:1	7:0	4:2
<b>Aetiology</b>			
Alcohol	5	5	4
G'stones	2	1	2
Unknown	2	1	0
<b>Median Glasgow score</b>	5 (3-6)	2 (2-6)	1 (0-5)
<b>Median stay</b>	50 days (1-90 days)	17 days (12-64 days)	12 days (6-27 days)

Table 4.1: The clinical details on 22 patients with acute pancreatitis who underwent diagnostic peritoneal lavage. G'stones = gallstones.



Complications	Necrotising 9 Patients	Severe 7 Patients
Respiratory failure	2	5
Renal failure	1	1
Proven pancreatic necrosis	9	-
Pancreatic collection	2	-
Pancreatic pseudocyst	1	-
Pancreatic abscess	2	-
Died	5	-

Table 4.2: Complications in 22 patients with acute pancreatitis who underwent diagnostic peritoneal lavage. The number of complications was greater than the number of patients since one patient may suffer more than one complication.

	<b>Necrotising</b>	<b>Severe</b>	<b>Mild</b>
Median fluid volume	140 mls (60 - 1120 mls)	120 mls (11 - 900 mls)	21.5 mls (10 - 210 mls)
Median fluid colour	8 (6-8)	6 (3-8)	4 (3-5)
Median TAP	4.08 nmol/l (0.42-21 nmol/l)	0.33 nmol/l (0-1.68 nmol/l)	0.00 nmol/l (0-0.4 nmol/l)
Median TAP x fluid volume	442 nmol (41- 4570 nmol)	17 nmol (0-840 nmol)	0 nmol (0-84 nmol)

Table 4.3: Details of the peritoneal fluids from 22 patients with acute pancreatitis who underwent diagnostic peritoneal lavage. The ranges are enclosed in brackets.

nmol/l,  $p < 0.5$ ). Utilising a peritoneal fluid TAP concentration of  $\geq 2$  nmol/l (figure 4.1) we were able to distinguish between necrotising and other forms of acute pancreatitis with a sensitivity of 67% (6 of 9 patients) and specificity of 100% (13 of 13 patients).

The distribution of the total TAP content of the free peritoneal fluid within the three groups was not uniform ( $H = 11.75$   $df = 2$   $p = 0.003$  (adj for ties), Kruskal Wallis test) (table 4.3). Significantly higher total TAP concentrations were seen in the peritoneal fluids of patients with severe pancreatitis associated with proven necrosis when compared to patients with severe disease but without proven pancreatic necrosis (95.6% CI = 25.8 to 2100.0 nmol,  $p = 0.026$ ) or those with mild disease (96.1% CI = 96.1 to 2444.0 nmol,  $p = 0.0024$ ). Significant differences were not seen between patients with severe attacks without proven necrosis and mild attacks (96.2% CI = -8.6 to 630.0 nmol,  $p = 0.15$ ). Utilising a cut off value of 96 nmol we were able to detect pancreatic necrosis with a sensitivity of 89% and specificity of 85% (figure 4.2).

#### 4.9.2 Fluid volume.

The distribution of fluid volumes within the three groups were not uniform ( $H = 6.47$ ,  $df = 2$ ,  $p = 0.04$ , Kruskal Wallis test) (table 4.3). There were no significant differences in the volume of fluid obtained from patients with severe attacks with proven necrosis and severe attacks without proven necrosis (95.6% CI = -390.1 to 189.1 ml,  $p < 0.75$ ) or between those with severe attacks without proven necrosis and mild attacks (96.2% CI = -4.1 to 490 ml,  $p < 0.22$ ). However, significant differences were seen between patients with severe attacks with proven necrosis and mild attacks (96.1% CI = 34.9 to 225 ml,  $p < 0.05$ ). A free fluid volume of  $>20$  ml predicted pancreatic necrosis with a sensitivity of 100% (9 of 9 patients) but the specificity of only 38% (4 of 13 patients).

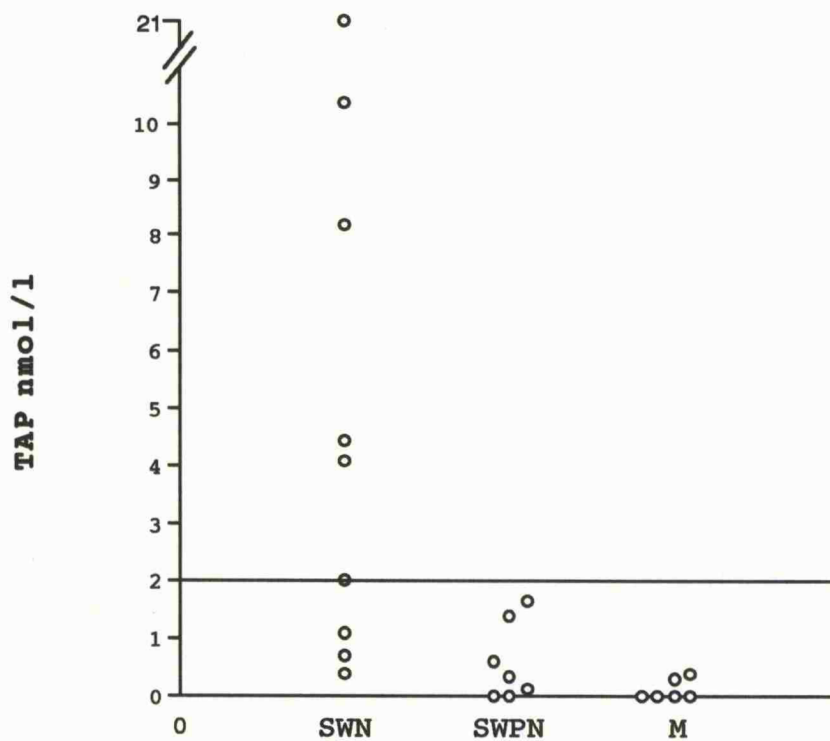


Figure 4.1: Free peritoneal fluid TAP concentrations in 9 patients with severe pancreatitis with histologically proven necrosis (SWN), 7 patients with severe acute pancreatitis without histologically proven necrosis (SWPN) and 6 patients with mild disease (M).

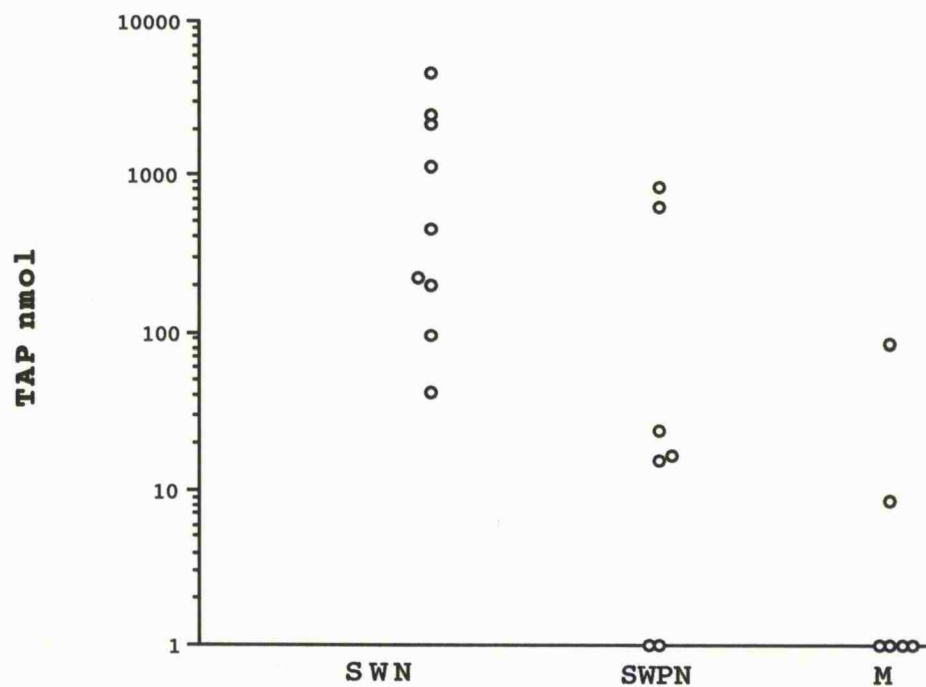


Figure 4.2: Total free peritoneal fluid TAP concentration in 9 patients with severe pancreatitis with histologically proven necrosis (SWN), 7 patients with severe acute pancreatitis without histologically proven necrosis (SWPN) and 6 patients with mild disease (M).

#### 4.9.3 Fluid colour.

The distribution of fluid colour between the three groups was not uniform ( $H = 13.62$ ,  $df = 2$ ,  $p = 0.001$ , Kruskal Wallis test) (table 4.3). Significant differences were noted in the colour of fluid samples between those patients with severe attacks with and without proven necrosis (95.6% CI = 0 to 5,  $p < 0.03$ ) and severe attacks with proven necrosis and mild attacks of acute pancreatitis (96.1% CI = 3 to 5,  $p < 0.003$ ). However, significant differences were not seen in the fluid colour between patients with severe attacks but without proven necrosis and mild attacks of acute pancreatitis (96.2% CI = -1.0 to 3,  $p = 0.3$ ). A free fluid colour of greater than grade 5 selected patients with pancreatic necrosis with a sensitivity of 100% (9 of 9 patients) and a specificity of only 46% (6 of 13 patients).

Where a patient had >20 ml of free fluid aspirated and/or free fluid colour darker than grade 5, pancreatic necrosis as detected with a sensitivity of 100% and specificity of 31%.

A summary of the sensitivity, specificity, predictive values and percentage correct for the above parameters are set out in table 4.4.

#### 4.10 DISCUSSION.

The current study used clinical evaluation on admission to select those patients who would undergo peritoneal aspiration. The closest similarity to the current selection criteria was provided by the multicentre trial of peritoneal aspiration reported by Corfield *et al* (1985) in which patients with predicted severe disease underwent peritoneal aspiration. Twenty seven per cent of Corfield *et al*'s (1985) patients had either >20 ml of free fluid or fluid darker than grade 5 compared with 38% in the current series. The presence of >20 ml of free peritoneal fluid, fluid of colour > grade five or a combination of the two were unable to detect histologically proven pancreatic necrosis with a specificity high enough to make them of clinical value. This failure agrees with the findings of a study by Larvin and McMahon

	Sensitivity	Specificity	+ve PV	-ve PV	% correct
TAP $\geq$ 2nmol/l	67%	100%	100%	81%	86%
Free fluid volume > 20 ml	100%	38%	50%	100%	59%
Fluid colour > grade 5	100%	62%	64%	100%	77%
> 20 ml of free fluid or colour > grade 5	100%	31%	50%	100%	59%
Median TAP x fluid volume	89%	85%	80%	92%	86%

Table 4.4: Summary of the sensitivity, specificity, predictive values and percentage correct of peritoneal free fluid volume, colour, TAP concentrations and total TAP content in determining the presence of pancreatic necrosis in 22 patients with acute pancreatitis.

(1986) who noted that a free fluid volume >40 ml or fluid the colour of "prune juice" was only able to detect pancreatic necrosis with a sensitivity of 72% and specificity of 84%.

Whilst we have demonstrated previously the presence of TAP in the urine of patients with acute pancreatitis (Chapter 3), this is the first time that TAP has been demonstrated in their peritoneal fluid. TAP concentrations were significantly higher in patients with severe pancreatitis with proven necrosis than in either of the other 2 groups, results which agree with the findings of a study involving the induction of experimental acute pancreatitis in rats (Fernandez-del Castillo *et al* 1992). The sensitivity of peritoneal fluid TAP concentrations in detecting histologically proven pancreatic necrosis was too low to be clinically useful. However, a positive predictive value of 100% meant that it did identify correctly all patients with histologically proven necrosis, information which is valuable in the management of patients. When the peritoneal fluid TAP concentration is multiplied by the free peritoneal fluid volume, a TAP concentration > 96 nmol detected pancreatic necrosis with a sensitivity and specificity comparable to the "gold standard": contrast enhanced CT scanning (Block *et al* 1986, Buchler *et al* 1986, Larvin *et al* 1990).

The elevated concentrations of TAP found in those patients without proven necrosis could be present for a number of reasons. Part of the explanation may be that these values represent background activity due to non-specific binding of salts within the urine. It is also possible that a degree of trypsinogen activation occurs in oedematous acute pancreatitis. Recently Yamaguchi (1992) has described, for the first time, trypsinogen activation in rats with caerulein induced pancreatitis put under stress. Fernandez-del Castillo *et al* (1992) has confirmed these findings by detecting TAP in the urine of rats with caerulein induced pancreatitis. It may be that a similar situation arises in man. A third possibility is that a number of those patients without histologically proven necrosis had undetected pancreatic necrosis.



Contrast enhanced CT scanning was unavailable during the study period and so it is not possible to exclude pancreatic necrosis in these patients. In spite of this theoretical deficiency it should be remembered that the presence of pancreatic necrosis demonstrated on contrast enhanced CT scanning is not an absolute indication for surgical intervention *per se*. A number of authors have demonstrated that even patients with extensive areas of pancreatic hypoperfusion can recover with conservative therapy alone (Teerenhovi *et al* 1988, Larvin 1990, White and Heimbach 1976, Smadja and Bismuth 1984). In addition it is generally agreed that the decision to operate should be based upon a combination of CT findings and assessment of the patients general condition; those with CT necrosis and a deteriorating clinical condition or evidence of pancreatic or peripancreatic sepsis being considered suitable for debridement (Beger 1989). Therefore, we can see that what we need is to be able to identify correctly are those patients with pancreatic necrosis who will require surgery rather than simply those with pancreatic necrosis. In the current study total peritoneal fluid a TAP concentration >96 nmol/l identified correctly 8 of 9 patients who either underwent or might have benefited from surgery.

In common with other authors experience (Corfield *et al* 1985, Edmondson *et al* 1952, Larvin and McMahon 1989, Wilson *et al* 1990) we failed to identify correctly a number of patients who were subsequently demonstrated to have suffered a severe attack of acute pancreatitis. This failure will reduce the sensitivity of peritoneal fluid TAP measurements in detecting histologically proven pancreatic necrosis at the time of, or shortly after, admission to hospital. This may however, not be a major drawback since very few of the patients coming to surgery undergo an operation during the first week after admission to hospital. If peritoneal fluid TAP concentrations were being utilised as a means of selecting those patients requiring surgery, then a delay of 48 hours or longer before prediction became available would be acceptable. Such a strategy would also have the advantage of restricting the number of mild cases undergoing peritoneal aspiration. In spite of these theoretical considerations it is unlikely that this assay

will be adopted into clinical practice. The anxiety regarding the risk of viscreal perforation (Corfield *et al* 1985) and the recent statement by a major proponent of peritoneal aspiration that it should no longer be used as a means of severity prediction (Mc Mahon, personal communication) mean that clinical evaluation and CT scanning will almost certainly remain the major determinant of the need for surgery.

## **CHAPTER 5:**

**THE ROLE OF INTERLEUKIN-6 IN MEDIATING THE ACUTE PHASE PROTEIN  
RESPONSE, ITS RELATIONSHIP TO ZYMOGEN ACTIVATION AND  
POTENTIAL AS AN EARLY MEANS OF SEVERITY ASSESSMENT.**

### 5.1 INTRODUCTION AND AIMS.

Our inability to identify severe acute pancreatitis correctly on admission to hospital, using clinical examination alone (McMahon *et al* 1980, Larvin and McMahon 1989, Wilson *et al* 1989), has led to the development of a number of more objective means of severity assessment. These include the Ranson (Ranson *et al* 1974) and Glasgow (Imrie *et al* 1978) scoring systems, APACHE II scoring system (Larvin and McMahon *et al* 1980, Wilson *et al* 1990) and a variety of single factors measured in the serum (Berry *et al* 1981, McMahon *et al* 1984). Recently the effectiveness of serum C- reactive protein (CRP) concentration measurements in determining the severity of acute pancreatitis has been demonstrated (McMahon *et al* 1980, Mayer *et al* 1984, Puolakkainen *et al* 1987, Buchler *et al* 1986). However, in common with the other methods described above, CRP measurements involve a delay of 48 hours or longer before prediction. One possible means of avoiding this delay is by measuring the serum concentrations of the principal mediator of the acute phase protein response: interleukin-6 (IL-6) (Moshage *et al* 1988, Castell *et al* 1988, Castell *et al* 1989). The first aims of this study were to examine the ability of IL-6 to provide early severity prediction in acute pancreatitis and in addition examine the relationship between IL-6 and the acute phase protein CRP.

It has been suggested that pancreatic necrosis only develops where intraglandular trypsinogen activation occurs (1.8.1). If is the case then we would expect to see a correlation between the presence and extent of pancreatic necrosis and the serum concentrations of trypsin in patients with acute pancreatitis. Failure to demonstrate such a relationship in the past may be due to the failure of the immunoreactive trypsin assay to differentiate between the active enzyme (trypsin) and the parent zymogen (trypsinogen) (section 1.5.4). This problem has been overcome recently by the development of an immunoassay utilising antibodies directed against the C-terminal end of TAP (chapter 2). The studies described in chapter 3 demonstrate the effectiveness of urinary TAP measurements in determining the

severity of acute pancreatitis. In chapter 4 I examined the relationship between TAP concentrations in peritoneal fluid and histologically proven pancreatic necrosis. In this chapter I examine the relationship between urinary concentrations of TAP and PLAP, the serum concentrations of the acute phase proteins CRP and  $\alpha_2$ -macroglobulin and the principal mediator of the acute phase protein response; IL-6.

## **5.2 PATIENTS.**

Twenty four patients with acute pancreatitis were prospectively entered into the study between December 1987 and July 1989. The clinical and aetiological details of the 24 patients entered into the study (14 mild and 10 severe attacks) are summarised in table 5.1. The groups were comparable in terms of age, sex and aetiology with hospital stay being significantly longer in patients with severe disease. Samples of blood and urine were taken at the time of admission, 6 hourly for 48 hours and then twice daily for a further 3 days. Specimens were centrifuged, separated, aliquoted and stored at -20°C prior to analysis for  $\alpha_2$ -macroglobulin, CRP, IL-6 and amylase, TAP and PLAP.

## **5.3 ASSAYS.**

### **5.3.1 TAP and PLAP**

The TAP and PLAP assays were performed as in described in sections 2.2.2.6 and 3.3.4.1.

### **5.3.2 Amylase.**

Serum amylase concentrations were measured as set out in section 3.4.2.2.

### **5.3.3 CRP.**

The CRP assay was performed as described in section 3.4.2.3.

### **5.3.4 $\alpha_2$ -macroglobulin.**

The  $\alpha_2$ -macroglobulin assay was performed as described in section 3.4.2.4.

	Severe Pancreatitis	Mild Pancreatitis	Differences
Median Age (range)	51.5 yrs (23-69 yrs)	44.5 yrs (21-77 yrs)	95% CI = -13 to 20 yrs, p < 0.79
Ratio of sexes M: F	8:2	11:3	NS p< 0.67
Aetiology of attack			
Alcohol	6	6	NS p < 0.3
Gallstones	3	7	
unknown	1	1	
Median hospital stay (days) (range)	19 days (7-25 days)	7.5 days (6-22 days)	95% CI = 9.6 to 12 days p < 0.001
Complications			
Respiratory failure	2	0	-
Renal failure	1	0	-
Hospital stay >14 days	5	0	-
Pancreatic pseudocyst	4	0	-

Table 5.1 : The clinical details of 10 patients with severe and 14 with mild acute pancreatitis. GS = gallstones. There are more complications than patients due to the fact that a patient may have more than 1 complication. CI = confidence interval, NS = not significant.

### 5.3.5 Creatinine.

Serum creatinine concentration were measured on a Beckman Synchron CX<sup>™</sup> system, Galway Ireland. Creatinine concentrations were determined by the Jaffé rate method.

### 5.3.6 Interleukin-6 assay.

At present the only method available for measuring iL-6 concentrations is a bioassay. Whilst the sensitivity is high and only biologically active IL-6 detected, it suffers the disadvantages of being very time consuming, labour intensive and expensive. The assay outlined below is an in-house modification of a technique reported by Van Snick *et al* (1986).

#### 5.3.6.1 Materials.

The assay was performed using the 7TDI mouse-mouse hybridoma cell line which was kindly provided by Dr. Van Damme, Brussels. The standard preparations of rIL-6 and anti-IL-6 antibody were obtained from Dr. Aarden, Amsterdam.

Recombinant iL-6 had been assigned a specific activity of  $10^6$  units per microgram, i.e. 1 picogram of iL-6 was equivalent to 1 u/ml. Triton-X (10% in 0.5 M hydrochloric acid) was obtained from BDH chemicals (Dagenham, England) and (3,-[4,5-Dimethylthiazol-2-yl]-2,5 Diphenyltetrazolium Bromide) (MTT) from Sigma (Pool, Dorset). Dulbecco's modified Eagle's medium (DMEM) (Gibco, Heidelberg, FRG) was supplemented with 10% foetal calf serum, 1.5 mM L-glutamine, 0.24 mM L-asparagine, 0.55 mM L-arginine, 50 mM 2-mercaptoethanol, 0.1 hypoxanthine and 16 mM thymidine. Absorbance was measured on a Titretrek micro plate reader (Guernsey, Channel islands).

#### 5.3.6.2 Methods.

The success of the assay revolves around the fact that the 7DTI cell line is dependent on IL-6 for growth and that its degree of growth is proportional to the concentration of IL-6 within the specimen (Van Snick *et al* 1986). Each serum

sample was heated to 56°C for half an hour prior to assay to remove any cytotoxic inhibitors. Following this it underwent doubling dilutions to give a total of 8 samples with a range of dilutions from 1 in 4 to 1 in 512 (Dilutions were carried out in DMEM). The assays were carried out on 96 well microtitre plates with each sample being run in triplicate. Fifty µl of DMEM was added to each well on the plate. Into wells 1, 2 and 3 (numbered 1 to 12 from left to right) were placed 50 µl of the highest concentration of rIL-6 (the standard). Fifty µl of 3 separate specimens diluted 1 in 4 were then placed into wells 4-6, 7-9 and 10-12 respectively. A 50 µl aliquot from each was transferred from the first well in each column to the second. Following mixing a similar aliquot was transferred from the second well to the third and so on throughout all 8 wells, thus producing the doubling dilution described above. Fifty µl was discarded from the last row of wells so that each contained a final volume of 50 µl. The 7TDI cell line was washed 3 times in DMEM, to remove any IL-6, and then corrected to a concentration of  $2 \times 10^3$  cells per 50 µl. A 50 µl aliquot was added to the first two columns of each of the triplicated samples. A further 50 µl of DMEM was added to the third column that acted as a sample blank. A reagent blank, in which only DMEM and cells were added, was run on a separate plate. The plates were then incubated at 37°C, in 8% CO<sub>2</sub> for 4 days. Viable cell numbers were evaluated by measurement of intra-mitochondrial activity of dehydrogenases, a group of enzymes found only in living cells. Ten µl of MTT (a tetrazolium salt that is cleaved by dehydrogenase) was added to each well and incubated at 37°C for 4 hours. The reaction was stopped and the colour developed by the addition of Triton-X. The absorbance is measured at a wavelength of 540 nm (the optimal absorbance of the coloured product) and with a blank at 690 nm (a wavelength not absorbed by the coloured product and which therefore corrects for any turbidity within the suspension). The latter value was subtracted from the former. Figure 2.1 displays the appearance of a typical plate following development by Triton-X and figure 5.2 the standard curve. Since the standard curve was only linear over a limited range it was necessary to dilute samples with higher IL-6 concentrations in order to allow readings to be made in the linear part of the curve.



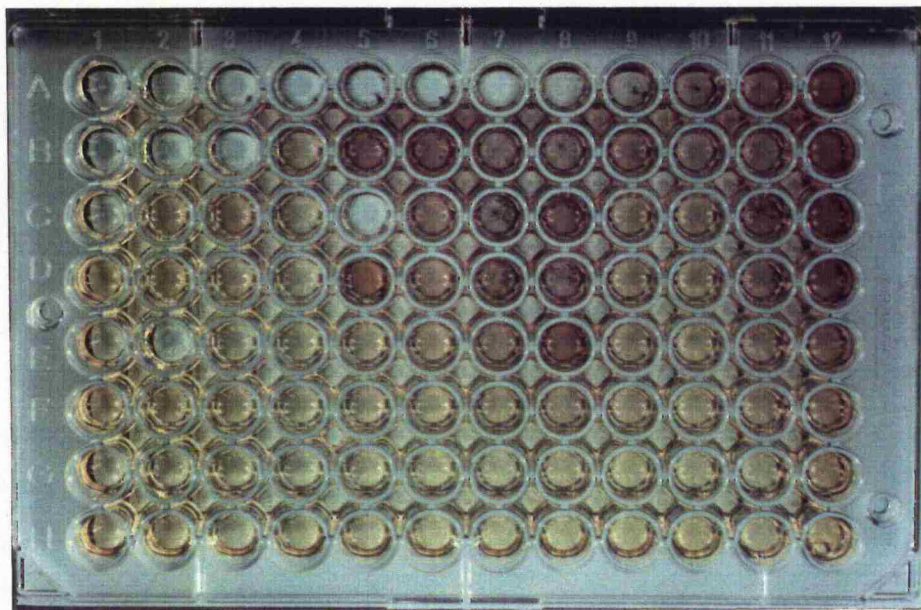


Figure 5.1: A typical microtitre plate following development of the colour with Triton-X. The rows (1-12) are displayed horizontally and the columns (A-H) vertically. The standard rIL-6 is in rows 1-3. It can be seen that the density of the colour developed diminishes as the dilution increases. The remaining rows contain specimens with an unknown concentration of IL-6

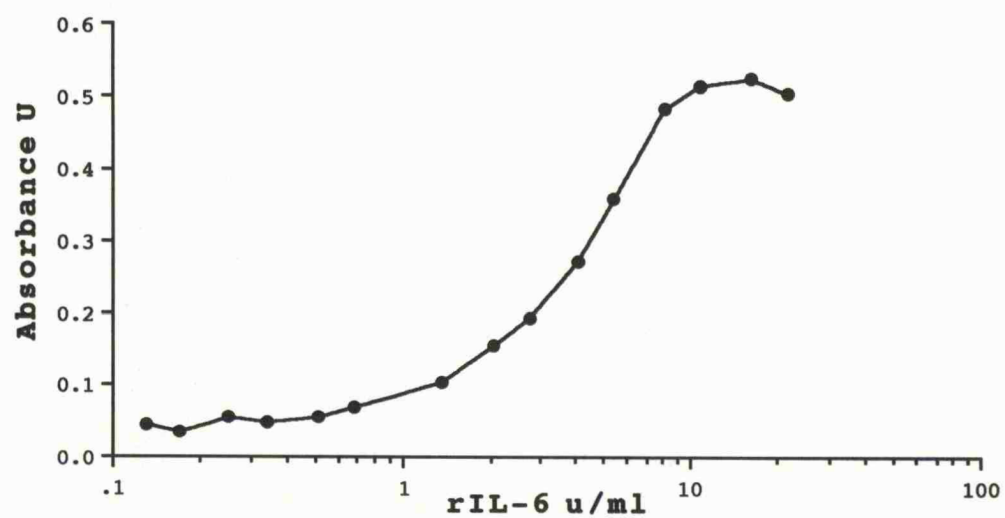


Figure 5.2: The standard curve relating IL-6 concentration and the absorbance of Triton-X.

#### **5.3.6.3 Assay characteristics.**

Sensitivity- This was variable, but usually the limit of detection was 10 u/l or less.

Specificity- Anti-IL-6 anti-serum was added to both the rIL-6 standard and samples and effectively inhibited growth of the hybrid cells confirming the specific nature of the assay.

Precision- Within batch 10%. Between batch 20%.

Recovery- 85 -129%.

#### **5.4 DIAGNOSIS OF ACUTE PANCREATITIS.**

The diagnosis of acute pancreatitis was made as described in section 3.4.3.

#### **5.5 DEFINITIVE OUTCOME.**

Disease severity was graded retrospectively depending upon the clinical outcome as set out in section 3.4.4.

#### **5.6 DATA ANALYSIS.**

in order to examine the time course of IL-6, CRP,  $\alpha_2$ -macroglobulin, amylase TAP and PLAP production in patients with acute pancreatitis it was necessary to correlate measured concentrations not from the time of admission but rather from the time of onset of symptoms. Since it is not possible to control the delay between the onset of symptoms and admission to hospital it was not possible to produce samples from different patients with exactly corresponding time points. Samples were therefore allocated to one of nine groups. These were <12 hr 13-24 hr, 25-36 hr, 37-48 hr, 49-60 hr, 61-72 hr, 73-96 hr, 97-120 hr and >121 hr and represent the range of times between the onset of symptoms and the sample being taken.

The urinary concentrations of TAP and PLAP were divided by the urinary creatinine concentrations to correct for the effects of dilution.

Data were analysed using medians, the Mann Whitney U test, Fisher's exact test and the Spearman rank correlation test as appropriate. Confidence intervals were used in preference to p values in judging the relevance of differences (Gardner and Altman 1989).

The area under the curve (AUC) of IL-6, CRP, TAP and PLAP representation the magnitude of production during the study period.

The areas under curves (AUC) were calculated from the formula:-

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (t_{i+1} - t_i) (y_i + y_{i+1})$$

Where t = the time after admission, y = the concentration at time t, i = the initial time points and n represents the last time point.

The areas under the curves were divided by the number of hours in order to take account of the different times for which samples had been collected from individual patients.

The area under the TAP and PLAP curves of individual patients underwent a logarithmic transformation (in order to display the data on the scattergrams). It was in addition necessary to add 1 to each value in order to avoid having to plot the log of 0 (minus infinity) where there was no TAP or PLAP produced. This mathematical manipulation is purely for display purposes and did not affect data analysis.

## 5.7 RESULTS.

### 5.7.1 Acute Phase Response.

#### 5.7.1.1 IL-6

Serum concentrations of IL-6 were seen to be rising during the first 24 hours after the onset of symptoms in all patients and peaked between 24 and 36 hours (figure 5.3). The magnitude of the IL-6 response (represented by the area under the time response curve) differed between patient groups. In patients with severe disease the median response was 236.5 u/ml/hr (range 72-560 u/ml/hr) and in the mild group 38 u/ml/hr (range 18-140 u/ml/hr). These differences were highly significant (95% Confidence interval (CI) = 92 to 232 u/ml/hr,  $p < 0.001$ ). Peak IL-6 concentrations correlated well with the magnitude of the IL-6 response ( $r = 0.94$ ,  $p = 0.01$ ) and can therefore be considered to represent accurately the overall response.

#### 5.7.1.2 CRP

CRP concentrations rose during the first 24 hours to peak between 36 and 48 hours (figure 5.3). The magnitude of the CRP response differed between patient groups. In patients with severe disease the median response was 147.5 mg/l/hr (range 69-273 mg/l/hr) and in the mild group 47.5 mg/l/hr (range 2-120 mg/l/hr). These differences were highly significant (95% CI = 59 to 147 mg/l/hr,  $p < 0.001$ ). Peak CRP concentrations correlated well with the magnitude of the CRP response ( $r = 0.93$ ,  $p = 0.01$ ).

#### 5.7.1.3 $\alpha_2$ -macroglobulin.

The median serum concentrations of  $\alpha_2$ -macroglobulin over the 5 days of the study are displayed in figure 5.4. The magnitude of the  $\alpha_2$ -macroglobulin response did not differ significantly between groups (median values in the mild group were 1.33 mg/l/hr and 1.385 mg/l/hr in the severe group, CI = -0.46 to 0.58 mg/l/hr,  $p = 0.40$ ).

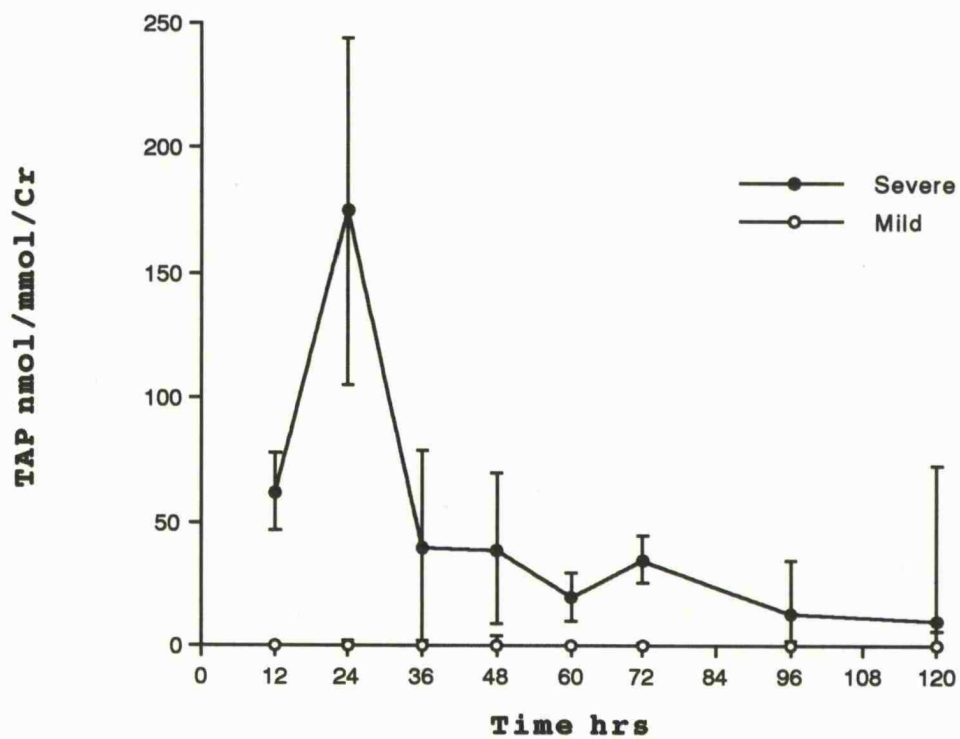


Figure 5.10: Median urinary TAP/creatinine ratio ( $\pm$  standard error of the mean) throughout the 5 days of the study in 10 patients with severe and 14 with mild acute pancreatitis.

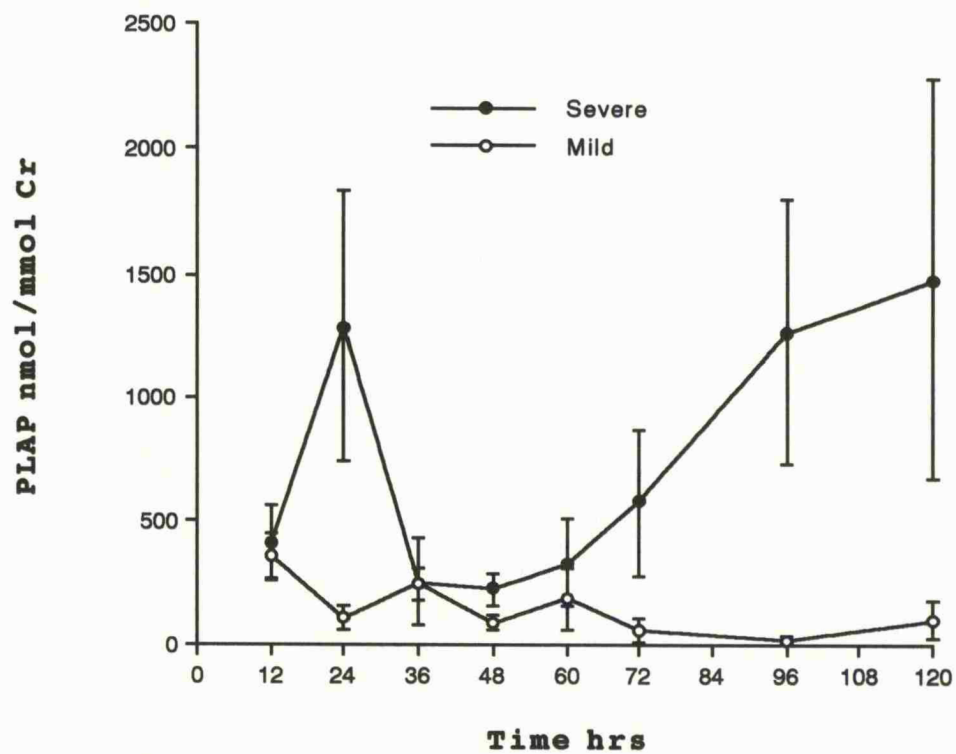
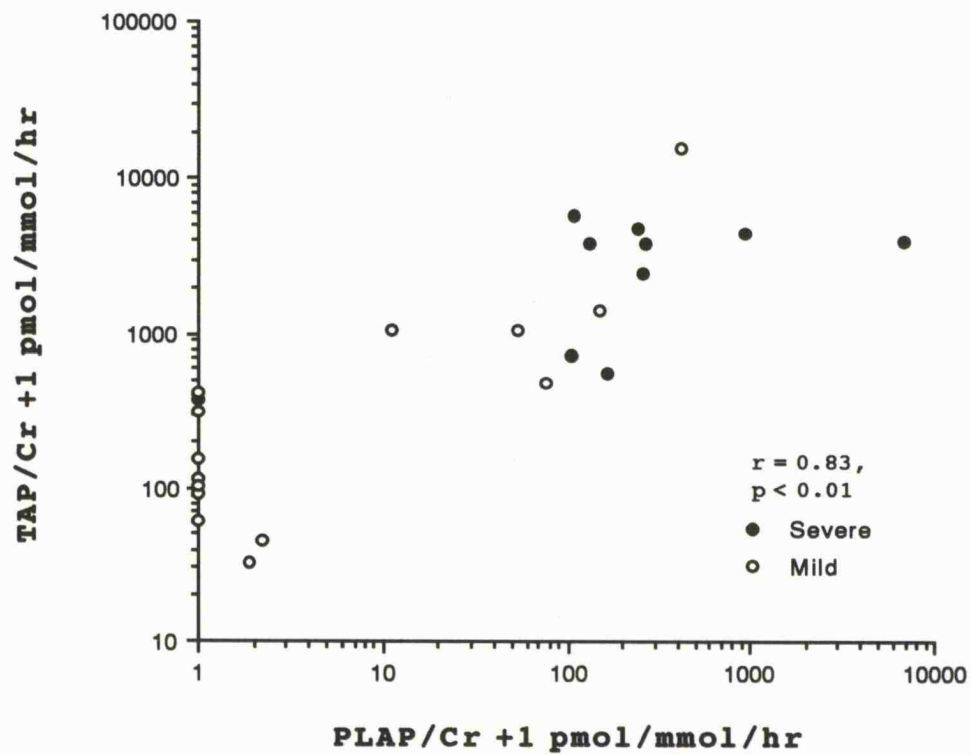


Figure 5.11: Median urinary PLAP/creatinine ratio ( $\pm$  standard error of the mean) throughout the 5 days of the study in 10 patients with severe and 14 with mild acute pancreatitis.





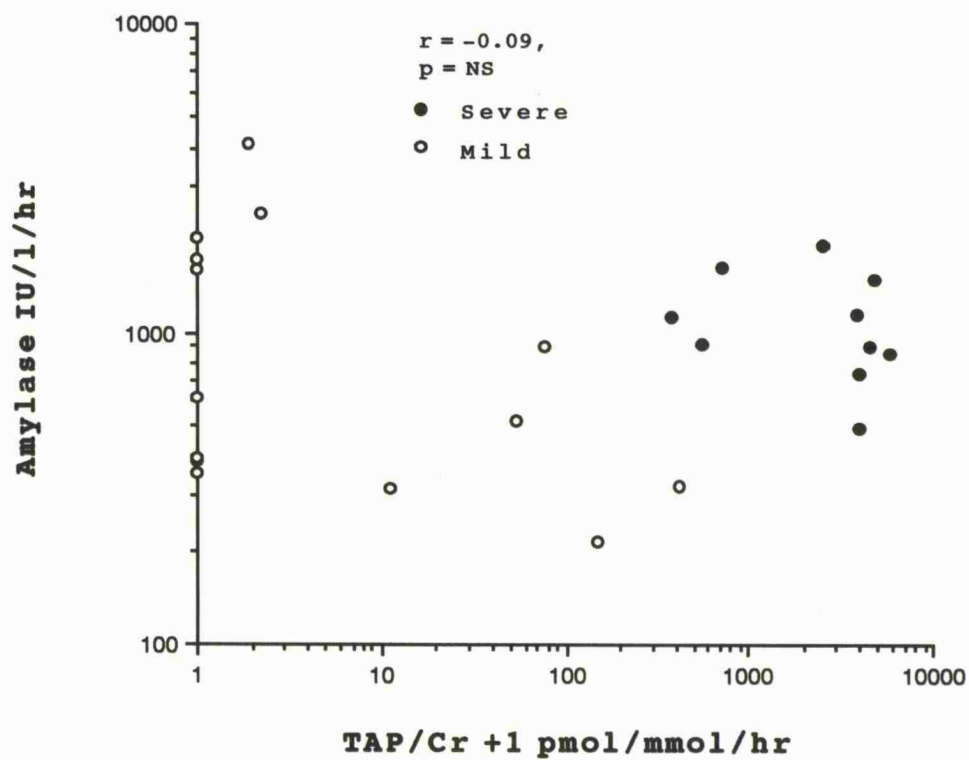


Figure 5.13: Integrated TAP/Cr and amylase responses in 10 patients with severe and 14 with mild acute pancreatitis.

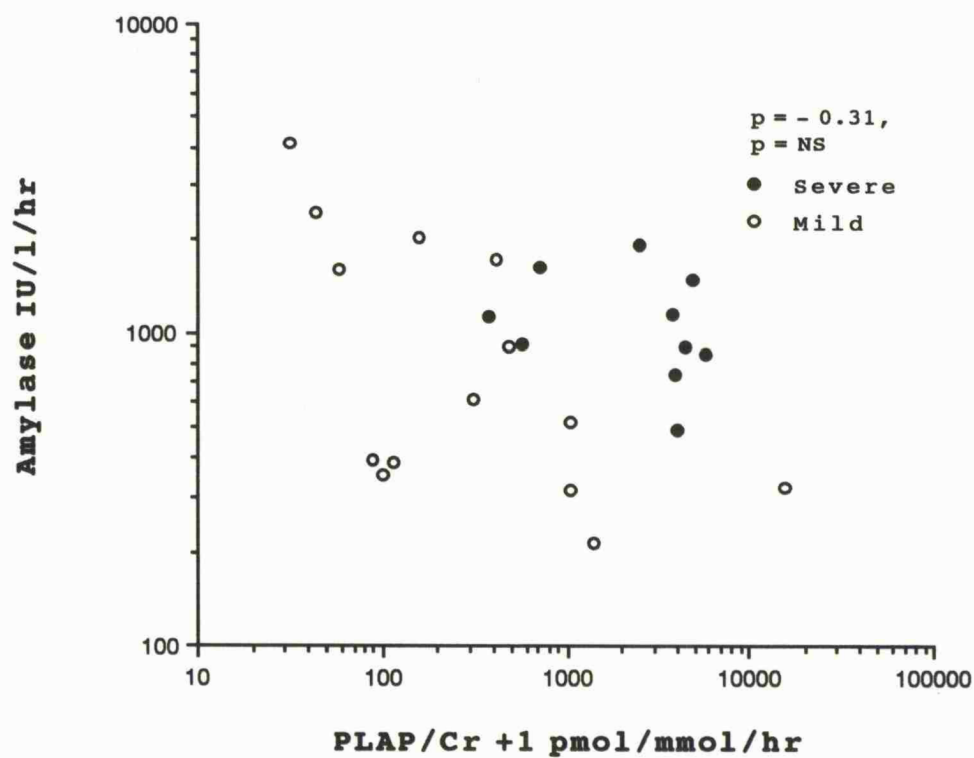


Figure 5.14: Integrated PLAP/Cr and amylase responses in 10 patients with severe and 14 with mild acute pancreatitis.

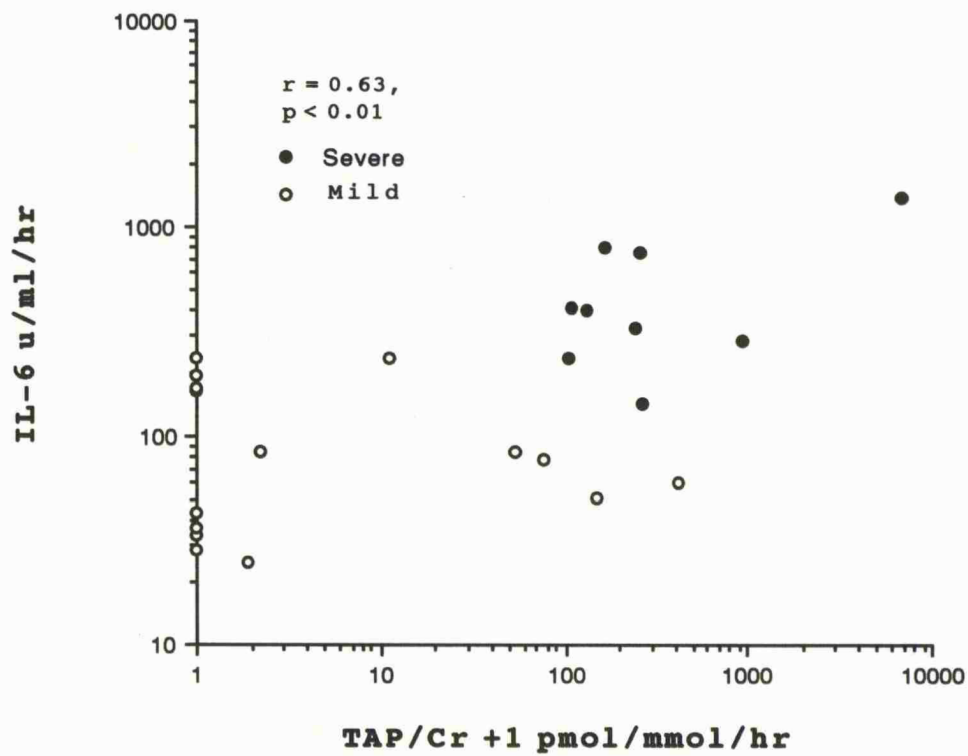


Figure 5.15: Integrated IL-6 and TAP/Cr responses in 10 patients with severe and 14 with mild acute pancreatitis.

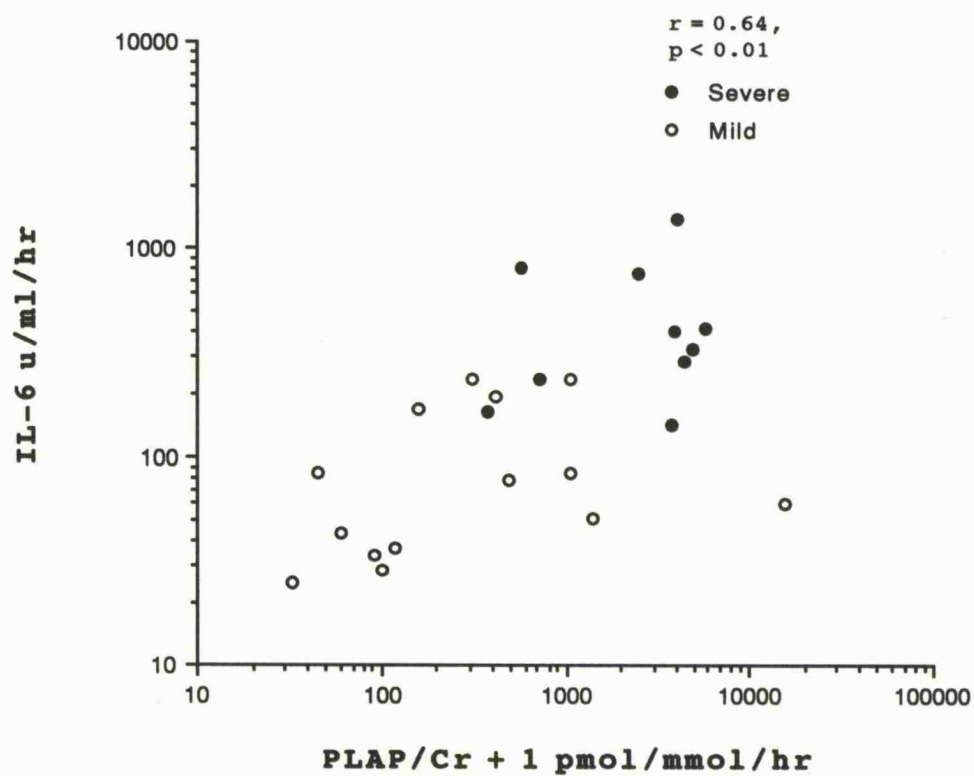


Figure 5.16: Integrated IL-6 and PLAP/Cr responses in 10 patients with severe and 14 with mild acute pancreatitis.

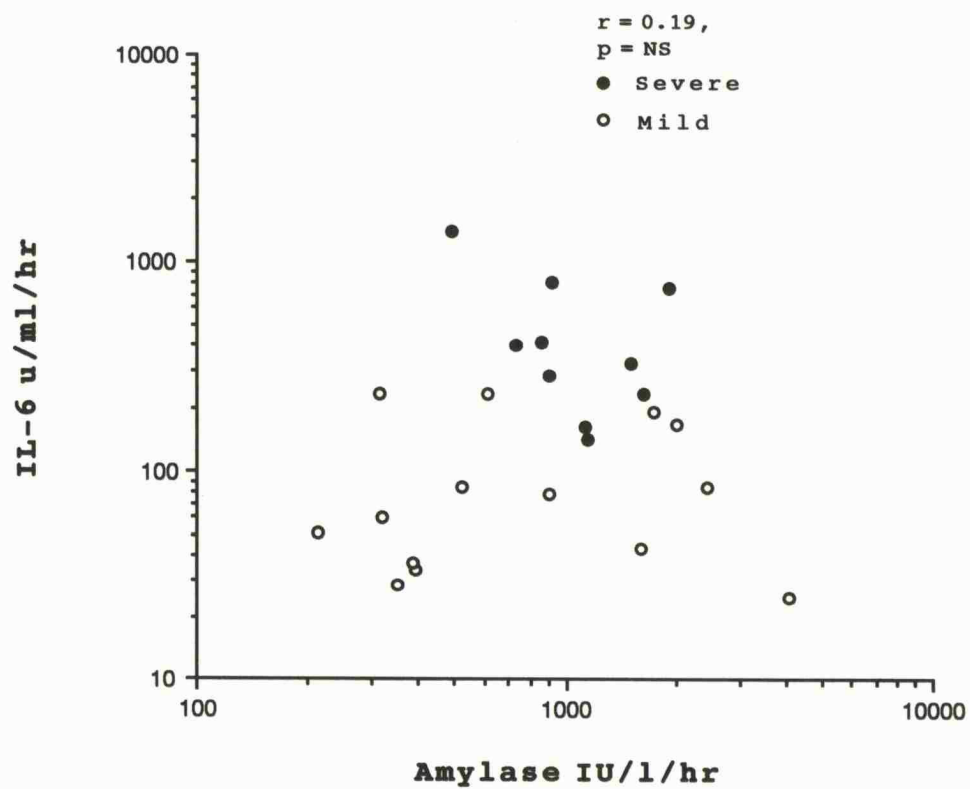


Figure 5.17: Integrated IL-6 and amylase responses in 10 patients with severe and 14 with mild acute pancreatitis.

## DISCUSSION.

Experimental studies conducted in animals, hepatoma cell lines and normal human hepatocyte cultures have demonstrated that IL-6 is the principal mediator of the acute phase protein response, of which CRP is an important component (Moshage *et al* 1988, Castell *et al* 1988, Castell *et al* 1989).

In the present study the median delay between the onset of symptoms and peak IL-6 concentrations was between 24 and 36 hours and are in agreement with the clinical studies of Van Oers *et al* (1988) but are somewhat later than those of Nijsten *et al* (1987), Shenkin *et al* (1989) and Cruickshank *et al* (1990). Van Oers *et al* (1988) noted peak IL-6 concentrations on the second day post renal transplantation (presumed delay between transplantation and the measurement of IL-6 concentrations 36-48 hr) and Nijsten *et al* (1987) noted peak concentrations "on admission" in burns patients (presumed delay <6 hr). Shenkin *et al* (1989) noted peak concentrations of IL-6 between 1.5 and 4 hours after the skin incision was made in a group of patients undergoing elective cholecystectomy and Cruickshank *et al* (1990) 6 to 12 hours after the skin incision for a variety of surgical procedures. Since Nijsten *et al* (1987) tell us little about the severity of the burns (if patients had only mild burns the peak IL-6 concentration would be small and short lived) and only took samples on a daily basis, it is probable that peak IL-6 concentrations have been missed. Further evidence that peak IL-6 concentrations may occur later in inflammatory disease comes from animal work. Geiger *et al* (1988) injected one group of rats with human rIL-6 and one with turpentine (a means of inducing an acute inflammatory reaction) and then compared the time to maximal mRNA production for a number of acute phase proteins. In those injected with IL-6 peak concentrations of acute phase protein mRNA's were seen within 4 hours, whereas there was a delay of 16-24 hours in those injected with turpentine. Whilst peak mRNA concentrations will precede those of the proteins for which they code these findings are in better agreement with those of the present study and of Van Oers *et al's* (1988) than of Nijsten *et*

*al's* (1987) and the other workers quoted above. On the basis of this and the studies quoted above the timing of peak IL-6 concentrations appears to be somewhat variable. The late peaks probably relate to a continuing inflammatory process, in contrast to operative trauma which was short lived.

Of more importance than the exact time of peak IL-6 concentrations is the relationship between peak IL-6 and CRP concentrations. In the present study the delay to peak CRP concentrations was 36 to 48 hours. In Nijsten *et al's* (1987) study peak CRP concentrations were noted on the first post admission day (presumed delay between IL-6 and CRP peak 24 hours) and are in keeping with the results for this study population. Similar results were achieved by Shenkin *et al* (1989) who noted peak CRP concentrations between 36 and 48 hours and Cruickshank *et al* (1990) who noted peak CRP concentrations between 24 and 48 hours. These results are also in keeping with the findings of laboratory based studies. The administration of human rIL-6 to hepatoma cell lines has been shown to produce a maximal acute phase protein response between 20 and 36 hours (Ritchie and Fuller 1983, Baumann and Muller-Eberhard 1987). When IL-6 was added to adult rat hepatocyte cultures the delay was 24 hours (Andus *et al* 1988) and when injected into rats 16 -24 hours (Geiger *et al* 1988). The delay to peak CRP concentrations in patient with acute pancreatitis in the present study are in agreement with the work of the group from Leeds (McMahon *et al* 1984) and our own previous study (Wilson *et al* 1989). However both Buchler *et al* (1986) and Puolakkainen *et al* (1987), demonstrated peak CRP concentrations at the time of admission. Many of their referrals were tertiary and probably had already undergone a delay of 48 hours or longer prior CRP measurements.

The strong correlation noted between the integrated IL-6 and CRP responses ( $r=0.73$ ) and between peak IL-6 and CRP concentrations ( $r=0.75$ ), coupled with the fact that the median peak IL-6 concentrations preceded those of CRP provides support for the hypothesis that IL-6 is acting as a mediator of CRP production.

However IL-6 may not be the only mediator of the acute phase protein response. When Morrone *et al* (1988) added rIL-6 to Hep 3B cell line they were able to induce partially the gene governing CRP production. Complete induction was only achieved when monocyte supernatant was added as well. Baumann *et al* (1987) have demonstrated that the optimal acute phase protein response in Hep G2 human hepatoma cell lines is only produced in the presence of IL-1 and dexamethasone and both Castell *et al* (1988, 1989) and Moshag *et al* (1988) have demonstrated that IL-1 augments the action of IL-6 in producing CRP in human hepatocyte cultures. It therefore seems likely that at least one of the factors inducing CRP production in Morrone *et al*'s monocyte supernatant was IL-1 (Morrone *et al* 1988).

CRP has been widely adopted as a non-specific indicator of inflammation because serum concentrations rise more rapidly and to a greater degree than any other acute phase protein. Its usefulness in this role has been demonstrated in medical (Amos *et al* 1977) and surgical (Mustard *et al* 1977) conditions, including acute pancreatitis (Wilson *et al* 1989, Mayer *et al* 1984, Puolakkainen *et al* 1987, Buchler *et al* 1986). It does however have a major drawback in that it entails a similar delay to the other methods of severity assessment in acute pancreatitis (Wilson *et al* 1989, Mayer *et al* 1984, Puolakkainen *et al* 1987, Buchler *et al* 1986). The measurement of IL-6 concentrations may overcome this problem and facilitate earlier severity prediction. Serum IL-6 concentrations rose more rapidly than those of CRP. This earlier rise in IL-6 concentrations resulted in significant differences in on-admission IL-6 concentrations between patients, an on admission serum concentration >120 u/ml separated severe and mild attacks with a sensitivity of 70% and specificity of 79%. These figures are comparable to figures previously reported for the multifactorial scoring systems and provide clinically useful separation between patient groups (Ranson *et al* 1974, Imrie *et al* 1978). Such differences were not seen for serum CRP on admission, a serum concentration of >120 mg/l being unable to distinguish effectively between patient groups. These



findings are in agreement with the studies of Mayer (1984) and Wilson *et al* (1989), as discussed above. Better separation between the patient groups was noted when peak concentrations were considered, the sensitivity and specificity of IL-6 and CRP being comparable, and somewhat better than previous reports of the multifactorial scoring systems (Ranson *et al* 1974, Imrie *et al* 1978).

Speculation that IL-6 may be useful in clinical practice, as a severity indicator in acute pancreatitis, is dependent on results being available within a few hours of sample collection. The major drawback of the IL-6 bioassay used here is the long turn around time of five days. However several commercial manufacturers have recently developed and marketed immunoassays for IL-6 (Quantikine Human IL-6, British Bio-technology Ltd, Abingdon, Oxon; Biokine IL-6 test kit, T Cell Sciences, Cambridge; Co-eliza IL-6, Kabi Diagnostica, Sweden). These assays have turnaround times of less than six hours and consequently, the methodology is now available to produce IL-6 results within the time scale necessary for them to be of value clinically. It should be noted however, that we have no direct experience of any of these kits.

Whilst serum amylase concentrations were at their peak <12 hours after the onset of symptoms, their failure to display significant differences between the integrated response of patients with mild and severe acute pancreatitis agree with previous reports (Edmondson and Berne 1952, Pollock 1959, Trapnell 1966, Ranson *et al* 1974). These facts combined with a lack of correlation with the integrated IL-6 response agree with the view that amylase is not an Initiator of tissue damage in acute pancreatitis (Hermon-Taylor and Heywood 1985, Rinderknecht 1986).

The marked elevation of the TAP/Cr and PLAP/Cr ratios between 12 and 24 hours of the onset of symptoms and the significant correlation between their integrated responses are consistent with the view that these zymogens are of pancreatic origin and that their activation is an early event in an attack of acute pancreatitis.

The significant correlation between the integrated responses of these parameters and IL-6, and the fact that their peak concentrations preceded those of IL-6 is in agreement with, but not proof of, the hypothesis that trypsinogen and phospholipase A<sub>2</sub> activation are important factors in determining the degree of the acute phase protein response and the severity of acute pancreatitis.

Interestingly, and in contrast to the results displayed in figure 3.6, PLAP/Cr concentrations were seen to rise from 48 hours until the end of the study, in those patients with severe disease. The failure to note a parallel rise in the median TAP/Cr concentration suggests that the PLAP may be of extrapancreatic origin. Certainly phospholipase A<sub>2</sub> is known to be present in a wide variety of extrapancreatic cell types including leukocytes. Gudgeon *et al* (1990) have demonstrated recently that PLAP can be detected in the filtrate of trypsinised peripheral blood leukocytes and it is possible that phospholipase A<sub>2</sub> from other extrapancreatic sources also exists as the proenzyme. It is therefore possible that this secondary rise in the PLAP/Cr ratio is caused by PLAP released from leukocytes.

## **CHAPTER 6:**

### **SUMMARY AND FINAL DISCUSSION.**

I have already described in section 1.8.1 the theory that intrapancreatic trypsinogen activation leads to the development of severe acute pancreatitis, whereas its absence is associated with the development of the mild form of the disease. Since almost all the mortality and morbidity is confined to the 20% of patient classified as suffering from severe disease, quantification of the degree of trypsinogen activation should identify accurately this high risk group of patients. The inability of the antibody used in immunoassays to distinguish between the active enzyme and parent zymogen and the rapid binding of trypsin to antiproteases, means that the immunoassay is unable to quantify accurately the degree of trypsinogen activation (section 1.10.4). An alternative approach is to measure the concentration of the activation peptide (TAP). This is released in equimolar concentrations to the active enzyme and can be detected readily in the patient's urine (Hurley *et al* 1988). A recently developed radioimmunoassay utilising an antibody directed against the C-terminal end of the molecule is able to measure TAP concentrations without reporting the presence of the parent zymogen (Hurley *et al* 1988).

Trypsin activates all other pancreatic zymogens including phospholipase A<sub>2</sub>, phospholipase A<sub>2</sub> having been implicated in the production of tissue damage in acute pancreatitis (section 1.8.1). Immunoassays for phospholipase A<sub>2</sub>, in common with those for trypsin, utilise antibodies that are unable to distinguish between the active enzyme and parent zymogen and are therefore not able to quantify accurately the presence of the active enzyme (section 1.10.5). Phospholipase A<sub>2</sub> was therefore thought to be a suitable candidate for the development of an immunoassay utilising an antibody directed against the C-terminal end of PLAP.

Antibodies directed against the C-terminal end of PLAP were generated following the immunisation of rabbits with Tg- CYPLAP. The specificity of the antibodies generated were confirmed by competitive binding studies, using synthesised peptides. This demonstrated that a minimum of 5 amino acids (Asp-Ser-Gly-Ile-Ser-Pro-Arg) at the C-terminal end of the peptide were needed for antibody recognition.

The absence of PLAP in the non trypsinised human pancreas, with its appearance following trypsinisation, demonstrated that the antibody is exclusively directed against the C-terminal end of the PLAP molecule. Following passage down a Sephadex G-25 column untrypsinised pancreatic homogenate was assayed for PLAP and none found. However, in the pancreas that had been trypsinised, PLAP was found in the same fractions as those in which the PLAP, used to calibrate the column, had been detected. Pancreatic homogenate eluted down a Sephadex G-50 column, that had been previously calibrated with dextran blue, chymotrypsinogen and cytochrome C. Following elution, all fractions were assayed for PLAP. It was not detected in any aliquots that were not trypsinised but were present in the fractions corresponding to the molecular weight of phospholipase A<sub>2</sub> (between chymotrypsinogen and cytochrome C).

The limit of detection of the radio immunoassay (the concentration of PLAP required to produce 90% inhibition of binding) and was  $3.35 \times 10^{-9}$  mol/l.

The stability of PLAP to boiling means that in the experiments where the pancreatic extracts were boiled to inactivate the trypsin we could be confident that PLAP had not been destroyed. Whilst TAP and PLAP taken into plain containers, centrifuged, aliquoted and frozen within an hour of being taken did not suffer significant degradation, in future studies it would be preferable to take samples into EDTA containers since the EDTA chelates the metal in the zinc dependent amino peptidase that is thought to lead to degradation of TAP and PLAP.

The initial clinical studies measured TAP and PLAP concentrations in normal individuals and was designed to see whether or not they excrete these peptides in their urine and to quantify the degree of background activity associated with each assay. Based on this study, a urinary TAP concentration  $>2$  nmol/l or PLAP  $>3$  nmol/l were considered abnormal. None of the normal controls had a urinary TAP concentration  $>2$  nmol/l and only one had a urinary PLAP concentration  $>3$  nmol/l. The main study examined the relationship between urinary TAP and PLAP concentrations and disease severity in patients with acute pancreatitis. Peak concentrations of TAP were seen within 12 hours of admission and peak PLAP concentrations at the time of admission. An admission urinary TAP concentrations  $> 2$  nmol/l provided better discrimination between mild and severe attacks of acute pancreatitis than previously described methods (section 1.11 to 1.12.6). A urinary PLAP concentration  $> 3$  nmol/l or combined TAP and PLAP measurement failed to improve on this. Recently Fernandez-del Castillo *et al* (1992) have demonstrated that polymorphonuclear (PMN) elastase provided equally good early severity prediction although such good results have not been demonstrated by other groups (Gross *et al* 1990, Fenton-Lee *et al* 1992). Gross *et al* (1990) found that a PMN elastase concentration of  $> 400$   $\mu$ g/l during the first 2 days detected severe attacks of acute pancreatitis with a sensitivity and specificity of 83% and 77%. In a recent series from Glasgow (Fenton-Lee *et al* 1992) a PMN elastase concentration  $\geq 400$   $\mu$ g/l 24 hours after admission only separated mild and severe attacks with a sensitivity of 39% although the specificity was 92%. Whilst peritoneal aspiration can provide prediction within a similar time period, the sensitivity and specificity are inferior to those provided by urinary TAP measurements (Pickford *et al* 1977, McMahon *et al* 1980, Mayer and McMahon 1985, Corfield *et al* 1985). This combined with the interventional nature of the technique and association risk of visceral perforation (Corfield *et al* 1984) has recently lead McMahon to suggest that it should no longer be used in severity prediction (personal communication). Unfortunately CT scanning was not available at the time the study was undertaken and so the relationship between urinary TAP and PLAP concentrations and the degree of hypoperfusion on contrast enhanced CT scanning is unknown. Now that a CT scanner is available, examination of the relationship is possible.

With the recent development of an ELISA assay, which can measure TAP concentrations within a few hours of admission to hospital, it should now be possible to select patients, with predicted severe disease, for inclusion in therapeutic trials. This will allow the targeting of potentially dangerous therapies to those who need it most.

The measurement of urinary TAP and PLAP concentrations in patients with acute abdominal conditions other than acute pancreatitis was performed in order to ensure that they did not have TAP and PLAP in their urine and therefore reduce their specificity in severity prediction. TAP was reported in 15 of the 25 disease controls although only 1 patient displayed a value > 2 nmol/l. Thirteen of the 25 disease control patients would have been classified as suffering from severe acute pancreatitis on the basis of a PLAP concentration >3 nmol/l.

The volume, colour and TAP concentration of peritoneal fluid from patients with acute pancreatitis were measured and the findings related to the presence of histologically proven pancreatic necrosis. The volume and colour of the free peritoneal fluid were unable to identify accurately the patients with histologically proven pancreatic necrosis, a finding in agreement with the previous report of (Larvin and McMahon 1986). Urinary TAP concentrations were little better. However the total TAP concentration did provide severity prediction comparable with contrast enhanced CT scanning.

The findings of a strong correlation between the integrated IL-6 and CRP response and the weak correlation between the integrated IL-6 and  $\alpha_2$ -macroglobulin responses are in keeping with previous studies which have demonstrated that CRP production is stimulated and that  $\alpha_2$ -macroglobulin production is unaffected by IL-6 (Moshage *et al* 1988, Castell *et al* 1988, Castell *et al* 1989).

There was a poor relationship between the integrated IL-6 and amylase responses suggesting that the former is not causing the latter. This finding agrees with previous reports which have suggested that there is no significant difference in

amylase concentrations between patients with mild and severe acute pancreatitis (Edmondson *et al* 1952, Pollock 1959, Ranson *et al* 1974, Trapnell 1966). There was however a significant correlation between the integrated IL-6 and TAP and PLAP responses. Such findings agree with the view that trypsinogen and phospholipase A<sub>2</sub> activation are important initial events related to the production of tissue damage in an attack of acute pancreatitis.

One of the premises examined in this thesis is that trypsinogen activation, followed by activation of the other pancreatic zymogens, is one of the earliest events in an attack of acute pancreatitis. Certainly the appearance of peak urinary concentrations of TAP and PLAP within a few hours of the onset of symptoms are in agreement with this hypothesis, although it is not possible to infer a causal relationship between their appearance and the development of acute pancreatitis. The second premise is that the differences between mild and severe attacks of acute pancreatitis can be explained by the presence or absence of trypsinogen activation. The data presented in this thesis does not provide clear cut evidence in favour of such a relationship and requires further examination and explanation.

Since almost all the body's trypsinogen is confined to the pancreas (only small quantities being present in the Paneth cells (Bohe *et al* 1986) and no other human protein contains the same amino acid sequence as TAP, it is reasonable to assume that most of the TAP detected in patients' urine is of pancreatic origin. This is not to say that a positive result from the TAP assay is necessarily due to the presence of TAP. A degree of background activity is expected from any immunoassay, even when it is fully characterised. In the case of the TAP assay one possible cause of false positive results is due to a non-specific reaction between the antibody and salts within the urine. The highly charged nature of the TAP molecule, with the 4 negatively charged Asp residues, means that antibodies directed against it are also highly charged. These may inappropriately bind salts within the urine, producing false positive results.



The presence of low concentrations of TAP in the urine of normal and disease controls, as well as patients with mild acute pancreatitis, may be accounted for by the above mechanism. This however can not be the whole explanation since significantly fewer normal controls than disease controls had TAP in their urine ( $\chi^2 = 5.9$ , 95% Ci = 0.007 to 0.8,  $p < 0.001$ ). This suggests that TAP concentrations may have increased in response to these abdominal conditions. The cause of this remains unclear. It may be that the TAP had entered the circulation from its normal physiological site, been absorbed from the upper small bowel lumen, or in the case of patients with perforated small bowel, transperitoneally. Alternatively it may be that the disease process has in some way lead to the inappropriate activation of trypsinogen, possibly as the result of ischaemic damage to the gland. It would be possible to investigate this question further by conduct experimental studies in rats, whose TAP is identical to that in man. It would be possible to simulate a number of conditions including perforated peptic ulcer, ischaemic small bowel, intestinal perforation and generalised peritonitis. TAP and amylase concentrations in peritoneal fluid, blood and urine could then be related to the experimentally induced conditions. It would also be necessary to repeat these experiments following the diversion of pancreatic juice away from intestinal lumen. Histological examination of the pancreas would be essential in all cases to exclude pancreatitis as a possible cause of TAP production.

Significantly higher admission urinary concentrations of TAP and integrated TAP / Cr response in patients with severe acute pancreatitis and a good separation between those patients with and without histologically proven pancreatic necrosis are in agreement with the view that inappropriate trypsinogen activation occurs in patients with severe disease. Further support for this view is provided by the significant correlation between the integrated TAP and IL-6 response: the degree of TAP production being correlated with a marker of the magnitude of the acute phase protein response. However, 3 patients with a severe attack of acute pancreatitis had no TAP detected in their urine at the time of admission and 4 with

a mild attack had urinary TAP concentrations  $> 2$  nmol/l. In addition a number of patients without histologically proven pancreatic necrosis had significant quantities of TAP in their peritoneal fluid. These facts are not in agreement with the view that trypsinogen activation is confined to those patients with severe disease. It is therefore possible that a degree of trypsinogen activation occurs even in oedematous acute pancreatitis. Recently Yamaguchi (1991) has described trypsinogen activation in rats with caerulein induced pancreatitis put under stress. Fernandez-dei Castillo *et al* (1992) has confirmed these findings by detecting TAP in the urine of rats with caerulein induced pancreatitis. It may be that a similar situation arises in man.

Urinary PLAP concentrations  $> 3$  nmol/l were seen in 11 of the 40 patients with mild acute pancreatitis and 13 of 25 disease controls. This was a significantly higher proportion than had a TAP concentration  $> 2$  nmol/l. Whilst trypsinogen is confined almost completely to the pancreas (Bohe *et al* 1986) phospholipase  $A_2$  is known to be present in a number of other cell lines including peritoneal (Wightman *et al* 1981) and hepatic (Birmelin *et al* 1984) cells, leukocytes, lung, stomach, small intestine and kidney (Matsuda *et al* 1987). Gudgeon (1990) has recently demonstrated that the trypsinisation of human leukocytes leads to the release of a substance that is reported by the PLAP assay. It is not possible to say that this substance has the same amino acid sequence as PLAP until it has been characterised. However, it is possible that leukocyte phospholipase  $A_2$ , as well as the phospholipase  $A_2$  in other cell types, are secreted as proenzymes and are reported by the PLAP assay. If this is the case it is not difficult to see how conditions such as peritonitis, septicaemia, adult respiratory distress syndrome (ARDS) (Vadas 1984), and any condition with deranged hepatic function may lead to the appearance of PLAP in urine.

Whilst median peak urinary concentrations of both TAP and PLAP were seen within a few hours of admission, when urinary TAP and PLAP concentrations were corrected for the effects of dilution, a rise in the median urinary PLAP

concentrations in patients with severe acute pancreatitis were noted 60 hours after the onset of symptoms. It is possible that this PLAP is arising from an extrapancreatic sources, possibly leucocytes. At present PLAP from leukocytes has not been characterised. It may be that it is not the same as PLAP from pancreatic prothymosin A<sub>2</sub> and that in the future it will be possible to distinguish between PLAP from the differing sources.

One of the most interesting aspects of the TAP and PLAP assays is their potential to improve our understanding of the biochemical events surrounding an attack of acute pancreatitis. There are a number of possible avenues for further research.

The most direct approach to examining the site of inappropriate intraglandular activation of trypsinogen is by staining pancreas by using an immunoperoxidase technique employing the anti-TAP antibody. Initial experiments, by the author, in which specimens of human pancreas taken at post mortem examination were stained, did not display any specific activity. This failure was unlikely to be due to the fixative because Asp and Lys residues are not cross linked by formalin. In addition I was unable to demonstrate any specific staining in unfixed specimens stored at -70°C. This may be because the TAP was not present in the tissue some days after the onset of symptoms or that it was washed out during processing. A better approach may be to repeat this work on specimens from rats with experimentally induced pancreatitis, tissue being fixed soon after the induction of acute pancreatitis. By altering the technique used to induce an attack of acute pancreatitis in rats Schmidt *et al* (1992) demonstrated that the serum and urinary concentrations of TAP correlated well with outcome and the histological changes. This knowledge should allow the production of experimental pancreatitis with a known and controllable severity, for use in experimental studies.

Prothymosin A<sub>2</sub> may also be important in the pathogenesis of acute pancreatitis (section 1.8.1). Unfortunately the amino acid sequence of rat PLAP is different from that in man and therefore can not be used in experimental studies.

However it should be possible to produce an antibody to rat PLAP and apply it to the studies outlined above. Phospholipase  $A_2$  has also been implicated in the production of adult respiratory distress syndrome because of its ability to digest surfactant and the membranes of pulmonary cells (Vadas *et al* 1984). The recent discovery of "PLAP" in the supernatant of trypsinised leucocytes means that it is now possible to examine the relationship between the degree of phospholipase  $A_2$  activation and pulmonary impairment.

Finally, following the production of assays against TAP, PLAP and more recently the activation peptide of procolipase (Bowyer *et al* 1991), it should be possible to develop antibodies against the C-terminal end of the remaining zymogens. Their use in immunoradiometric assays will hopefully improve our understanding of the biochemical events in acute pancreatitis.

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Appendix 1: Urinary TAP and PLAP concentrations in 40 normal subjects.					
Patient Number	Age	Sex	TAP (nmol/l)	PLAP (nmol/l)	
1	43	female	3.22	0	
2	23	male	0.86	0	
3	42	female	0.55	0	
4	30	male	0.8	0	
5	31	male	1.51	0	
6	38	male	0.23	0	
7	39	female	0.67	0	
8	52	male	2.04	0	
9	60	male	0.4	0	
10	25	female	0.65	0	
11	37	male	2.05	0	
12	22	female	3.16	0	
13	22	male	0.46	0	
14	24	male	2.04	0	
15	21	male	0.16	0	
16	22	female	2.06	0	
17	45	male	2.15	0	
18	37	male	0.35	0	
19	41	female	0.12	0	
20	34	male	0.96	0	
21	20	female	1.43	0	
22	20	male	0.6	0	
23	30	male	0.95	0	
24	26	female	0.46	0	
25	54	male	1.7	1.3	
26	34	female	0.47	0	
27	43	male	0.27	0	
28	21	male	0.23	0	
29	20	female	1.6	1.68	
30	21	female	0.95	0.29	
31	51	male	1.32	0.22	
32	23	male	2.68	1.2	
33	42	male	1.9	0.56	
34	48	female	1.05	1.65	
35	29	male	0.33	0.27	
36	38	female	1.92	0.38	
37	28	male	1.44	1.28	
38	55	female	3.06	0.63	
39	36	female	1.2	1.6	
40	33	female	0	0.21	

## Appendix 2: Data collection sheet

TRIAL OF INTRA-PERITONEAL TRASYLOL THERAPY IN ACUTE PANCREATITIS									
INSTRUCTIONS									
1. Ring one of a set of alternative responses:	<table border="1"> <tr> <td>Male</td> <td>0</td> </tr> <tr> <td>Female</td> <td>2</td> </tr> <tr> <td>mmet 1</td> <td>4</td> </tr> <tr> <td></td> <td>33</td> </tr> </table>	Male	0	Female	2	mmet 1	4		33
Male		0							
Female		2							
mmet 1	4								
	33								
2. Print clearly into the boxes, numeric data, or text (start at left), or dates/times:									
3. A '*' symbol requests further specific details. Print these in the space to the left of the boxes, or at the foot of the page.									

QUESTIONNAIRE	
TRIAL NUMBER	0 1
SURNAME	01 02 03 04 05 06
INITIALS	07 08 09 10 11 12 13 14 15 16 17 18
DATE OF BIRTH	19 20
AGE	21 22 23 24 25 26
SEX	1 Male 2 Female
MARITAL STATUS	1 Married 2 Single 3 Div/cop 4 Widowed
DOMESTIC STATUS	1 Living alone 2 With others
ETHNIC ORIGIN	1 Caucasoid 2 Negroid 3 Other
NATIONALITY	1 British 2 Irish 3 Other
HOSPITAL INITIALS	34 35
CONSULTANT INITIALS	36 37
HOSPITAL NUMBER	38 39 40 41 42 43 44
HOSPITAL ADMISSION DATE	45 46 47 48 49 50
TIME	51 52 53 54
OCCUPATION	
HUSBAND'S OCCUPATION (Father's if single female)	

PRESENTING SYMPTOMS	
ABDOMINAL PAIN Ring sites on Abdomen	
RADIATION to back	No 1 Yes 2
to shoulder tip	No 1 Yes 2
DURATION of PRESENTING PAIN (hours before admission)	33 37
Subjective SEVERITY	Inapplicable 1 None 2 Mild 3 Moderate 4 Severe 5
OPIATE given before admission	No 1 Yes 2
NAUSEA/VOMITING	None 1 Nausea only 2 Vomiting 3
DURATION OF NAUSEA/VOMITING (hours before admission)	71 72
Did patient 'COLLAPSE'?	No 1 Yes 2
Recent JAUNDICE	No 1 Yes 2
DURATION in days	73 74
TIME INTERVALS	Accurate 1 Estimated 2

Appendix 2: Data collection sheet

TRIAL NUMBER									
0	1	0	2	0	3	0	3	0	0
									A 2

CHRONIC ILLNESS EVALUATION			
CVS:	Ischaemic Heart Disease	None	1
		Has had MI	2
			3
	Heart Failure	No	07
		Yes	1
			2
	Hypertension	No	08
		Yes	1
			2
	Other CVS disease	No	09
	Yes	1	
		2	
RS:	Airways Disease	None	1
		Asthma	2
		COPD	3
	Other RS disease	No	11
	Yes	1	
NS:	Cerebro-Vascular Disease	None	1
		Had CVA	2
			3
	Other NS disease	No	13
	Yes	1	
PVS:	Peripheral Vascular Disease	None	1
		PVD	2
	Varicose Veins	No	10
		Yes	1
		2	
DVT		No	11
		Yes	1
	Pulmonary Embolus	No	12
		Yes	1
		2	
AS:	Liver Disease	No	1
		Yes	2
	Other AS disease	No	10
		Yes	1
		2	
DIABETES		None	1
		Yes - no drug PK	2
		Yes - oral drugs	3
		Yes - on Insulin	4
OTHER ILLNESS		No	21
		Yes	1
EFFECTS	Unaffected		22
	Social limits		1
	Wheelchair		2
	Housebound		3
			4
			5

PREVIOUS SURGERY & PROCEDURES			
CHOLECYSTECTOMY	No		1
	Yes - no details		2
	CBD NOT explored		3
	CBD explored		4
	Other procedure		5
			26
	No POC		1
	Satisfactory POC		2
	Abnormal POC		3
			27
Date			28
			29
Number of E.R.C.P. examinations			30
			31
			32
			33
Endoscopic Sphincterotomy	No		1
	Yes		2
			3
			4
Date			5
Date most recent ERCP			6
Surgery for GASTRO-OESOPHAGEAL REFLUX	No		1
	Yes		2
			3
			4
VAGOTOMY	None		1
	Truncal		2
	Selective/HSV		3
			4
Year			5
GASTRIC DRAINAGE	None		1
	Pyloroplasty		2
	Gastrojejunostomy		3
	Gastric segment		4
	Other		5
			6
Year			7
GASTRECTOMY	None		1
	Antrectomy/B-I		2
	Polya/B-11		3
	Other		4
Year			5
PREGNANCY			1
			2
			3
			4
Number of pregnancies			5
Miscarriages			6
Date delivered if recent			7
			8
LIST OTHER RELEVANT OPERATIONS BELOW			

Appendix 2: Data collection sheet

TRIAL NUMBER				A	3
01	02	03	04	05	06

SYSTEMATIC ENQUIRY			
PREVIOUS SIMILAR PAIN	None	1	
	Undiagnosed	2	
	*Other diagnosis	3	
	Acute Pancreatitis	4	
	Number of episodes	07	
	Months since last episode	08	
PREVIOUS GALLSTONES	None known	1	
	Previously noted	2	
	Year	10	11
PREVIOUS JAUNDICE	Number of attacks	12	
	Gallstones	13	
	*Other causes	14	
CHOLECYSTITIS	Number of attacks	15	
	Year began	16	
BILIARY COLIC	No	17	
	Yes	18	
	Year began	19	
CHOLECYSTECTOMY	*Not planned	20	
	On waiting list	21	
	Waste on waiting list	22	
DYSPEPSIA	None significant	23	
	Undiagnosed	24	
	Proven D.U.	25	
	*Other	26	
ESOPHAGEAL REFLUX	No	27	
	*Yes	28	
DIVERTICULAR DISEASE	No	29	
	*Yes	30	
METABOLIC ABNORMALITY	None known	31	
	Hypercalcaemia	32	
	Hyperlipidaemia	33	
CALCULOUS DISEASE	None	34	
	Renal calculi	35	
	Biliary calculi	36	
	*Other	37	
AVERAGE ALCOHOL INTAKE PER WEEK	Pints beer etc	38	39
	Glasses wine etc	40	41
	Spirit measures (70 cl = 30)	42	43

ACUTE EXCESS OF ALCOHOL	No	1
	Yes	2
Hours before symptom onset		30
Amount	Pints beer etc	31
	Glasses wine etc	40
	Spirit measures	42
CURRENT SMOKING	Cigarettes/day	43
	Oz/tobacco/week	44
	Cigars/day	45
DIET	Normal	1
	Reducing	2
	Low-Fat	3
	*Other	4
PRECIPITATING MEAL	Hours before symptom onset	32
TRAUMA	None	1
	*External	2
	*Operative	3
	*Endoscopic	4
VIRAL ILLNESS	Not relevant	1
	*Relevant	2
FAMILY HISTORY	No pancreatic disease	1
	*Pancreatitis	2
	*Other pancreatic disease	3
	No gallstones	1
	*Gallstones	2
	No metabolic abnormality	1
	*Hyperlipidaemia	2
	*Hypercalcaemia	3
Drugs: THIAZIDE DIURETIC	No	1
	Yes	2
STERIODS	No	1
	Yes	2
ORAL CONTRACEPTION	No	1
	*Yes	2
AZOTHIAPRINE	No	1
	Yes	2
TRASYLOL	Never	1
	*Yes	2
PHYSICAL DATA AT ADMISSION	Height in cm	05
	Weight in kg	06

Appendix 2: Data collection sheet

TRIAL NUMBER					A	4
	01	02	03	04	05	06

LIST ALL PRE-ADMISSION DRUGS							
No.	DRUG	DOSE/FREQ.	INDICATION	No.	DRUG	DOSE/FREQ.	INDICATION
1				5			
2				6			
3				7			
4				8			
DETAILS OF INITIAL ASSESSMENTS							
				A-> E Officer	Surgeon	Investigator	
HOURS ASSESSED AFTER ADMISSION							
PULSE RATE				per minute	07 08	09 10	11 12
SYSTOLIC BLOOD PRESSURE				mmHg	13 14 15	16 17 18	19 20 21
TEMPERATURE				degree Celsius	22 23 24	25 26 27	28 29 30
PERIPHERAL PERFUSION				Not assessed	31 32 33	34 35 36	37 38 39
				Normal	1	2	3
				Abnormal	4	5	6
DEHYDRATION				Not assessed	7	8	9
				None	1	2	3
				Mild	4	5	6
				Moderate	7	8	9
				Gross	10	11	12
ABDOMINAL DISTENSION				Not assessed	13	14	15
				None	1	2	3
				Present	4	5	6
ABDOMINAL TENDERNESS				Not assessed	7	8	9
				None	1	2	3
				Minimal	4	5	6
				Marked	7	8	9
				Localised peritonism	10	11	12
				Generalised peritonism	13	14	15
				Rigid	16	17	18
BOWEL SOUNDS				Not assessed	19	20	21
				Normal	1	2	3
				Abnormal	4	5	6
				Absent	7	8	9
MAIN DIAGNOSIS				None made	10	11	12
				Acute Pancreatitis	1	2	3
				Perforated Viscus	4	5	6
				Cholecystitis	7	8	9
				Peptic Ulcer	10	11	12
				*Other	13	14	15
WAS AP CONSIDERED?				Not at all	16	17	18
				Yes - later on	1	2	3
				Yes - initially	4	5	6
ESTIMATED SEVERITY OF ACUTE PANCREATITIS				None made	7	8	9
				Clinically mild	1	2	3
				Clinically severe	4	5	6

Appendix 2: Data collection sheet

SEVERITY OF ACUTE PANCREATITIS			
SOURCE OF REFERRAL TO SURGEONS	G.P. direct	1	
	A & E Dept.	2	
	Physician	3	
	*Other	4	
			07
ASSESSING SURGEON	House Surgeon	1	
	SHO	2	
	Registrar	3	
	Senior Reg.	4	
	Consultant	5	
			08
SUBSTANTIATED DIAGNOSIS A.P.			
Hours after admission			09 10
CLINICAL SEVERITY			
	Entry	24hrs	48hrs
Mild	1	1	1
Moderate	2	2	2
Severe	3	3	3
	11	12	13
RANSON CRITERIA		IMRIE CRITERIA	
Age > 65y	14	V/x	
WBC > 16 x 10 <sup>9</sup> /l	15	WBC > 16 x 10 <sup>9</sup> /l	22
BS > 11mmol/l	16	BS > 10mmol/l (not diabetic)	23
LDH > 350 iu/l	17	LDH > 600u/l	24
AST > 120 iu/l	18	AST > 200u/l	25
Urea ↑ > 0.6mmol/l	19	Urea > 16mmol/l (after fluids)	26
Calc. < 2.00mmol/l	20	Calc. < 2.00mmol/l	27
pO <sub>2</sub> < 8kPa	21	pO <sub>2</sub> < 8kPa	28
PCV ↓ > 10%	22	Alb < 32g/l	29
Bases def > 4mmol/l	23		
Fl. seq > 0l	24		
Items available		Items available	
RANSON Score	25	IMRIE Score	30
	26		31
DELAY IN ASSESSING SEVERITY			
Hours after admission		DPL	
		Ranson	32 33
		Imrie/R'	34 35
			40 41

TRIAL NUMBER					A	6
	01	02	03	04	05	06

INVESTIGATION & COMPLICATIONS											
CLINICAL	No mass felt	1	on day								
	Mass palpable	2									
		27									
AXR	No gallstones	1									
	Gallstones	2									
		10									
USS performed on days											
		11	12	13	14	15	16				
Pancreas not seen		1	2	1	2	1	2				
Pancreas normal		1	2	1	2	1	2				
Pancreatitis		3	3	3	3	3	3				
*Pancreatic collection		4	4	4	4	4	4				
*Other collection		5	5	5	5	5	5				
Collection size (cm)		17		10		19					
		20	21	22	23	24	25				
No free ascitic fluid		1		1		1					
Free fluid noted		2		2		2					
		26		27		28					
No gallstones seen		1		1		1					
Gallstones seen		2		2		2					
CBD gallstones seen		3		3		3					
		29		30		31					
Diameter of CBD (mm)		32	33	34	35	36	37				
CT performed on day											
				38	39	40	41				
Plain cuts				1	2	3	4				
IV contrast enhanced				1	2	3	4				
				42	43	44	45				
Normal pancreas				1	2	3	4				
Pancreatitis				1	2	3	4				
*Pancreatic necrosis				1	2	3	4				
*Pancreatic collection				1	2	3	4				
*Pancreatic abscess				1	2	3	4				
*Other collection				5	6	7	8				
Size of collection (cm)				9	10	11	12				
				46	47	48	49				
Pancreatic duct not seen				1	2	3	4				
Pancreatic duct visualised				5	6	7	8				
				50	51	52	53				
OCG on day											
		54	55	IVC on day				56	57	58	59
Normal		1	2	Normal		1	2				
NFGB only		1	2	NFGB only		1	2				
Gallstones		3	4	Gallstones		3	4				
		60	61			62	63				
PTC performed on day											
				64	65	66	67				
Findings:	Failed			1	2	3	4				
	No gallstones			1	2	3	4				
	GB unfilled			1	2	3	4				
	GB stones			1	2	3	4				
	CBD stones			1	2	3	4				
	Diameter (mm) CBD			5	6	7	8				
				68	69	70	71				

ERCP	performed on	day	83	67
	Ampulla normal		1	2
	*Ampulla abnormal		2	3
	Ampulla stone		3	3
	*RPG not obtained		1	1
	Panc duct normal		2	2
	*Panc duct abnormal		3	3
	*RCG not obtained		1	1
	No bill tract calculi		1	1
	Biliary tract calculi		3	3
			6	6
	CBD diameter (mm)		58	61
	No procedure		1	1
	Endos. sphincterotomy		2	2
	Lithopap. achieved		3	3
			7	7
Not fully investigated		No	1	1
		*Yes	2	2
			7	7
AETIOLOGY				
GALLSTONES		No	1	1
		Yes	2	2
			7	7
ALCOHOL		No	1	1
		Yes	2	2
			7	7
EXTERNAL TRAUMA		No	1	1
		Yes	2	2
			7	7
IATROGENIC		No	1	1
		*Endos.	2	2
		*Op.	3	3
			7	7
DRUG RELATED		No	1	1
		Yes	2	2
			7	7
METABOLIC		No	1	1
		*Calc.	2	2
		*Lipids	3	3
			7	7
HYPOTHERMIA		No	1	1
		*Yes	2	2
			7	7
OTHER		No	1	1
		*Yes	2	2
			7	7
Specify details in space below.				

Appendix 2: Data collection sheet

TRIAL NUMBER				1
01	02	03	04	05

OUTCOME		
Recovery uncomplicated		1
Survived complication		2
Died - PM held		3
Died - no PM held		4
* Total number of operations		07
Total number of days in I.T.U.		04
Hospital discharge/death	on day	02 14
Reason if more than 14d. stay	*Social etc. Cholecystectomy Slow recovery Complication	11 12 1 2 3 4 15
THERAPY AND PROGRESS		
N.G. TUBE	days	14 16
I.V. FLUIDS	days	14 17
T.P.N.	days	10 10
ILEUS PRESENT	days	10 10
ORAL FLUIDS	began on day	20 21
DIET	began on day	22 23
MASK OXYGEN	days	23 24
VENTILATED	days	24 27
ANTI-COAGULANT	None Heparin Prophylaxis Full anti-coagulation	1 2 3 4 5
STERIODS	No Yes	1 2 3
H2 ANTAGONISTS	No Yes	1 2 3
DIURETICS	No Yes	1 2 3
PRESSORS	No Yes	1 2 3
PLASMA	No Yes	1 2 3
FRESH FROZEN PLASMA	No Yes	1 2 3

COMPLICATIONS		
Proven DVT	on day	37 38
Proven MI	on day	39 40
Proven PE	on day	41 42
Proven SEPTICAEMIA	on day	43 44
ACUTE RENAL FAILURE	on day	45 46
MAJOR G.I. HAEMORRHAGE	on day	47 48
Insulin Treated HYPERGLYCAEMIA	on day	49 50
Symptomatic HYPOCALCAEMIA	on day	51 52
PLEURAL EFFUSION	on day	53 54
Major CHEST INFECTION	on day	55 56
A.R.D.S.	on day	57 58
Pancreatic FISTULA	on day	59 60
Proven D.I.C.	on day	61 62
A.P. RECURRENCE	on day	63 64
TOXIC CONFUSION	on day	65 66
Infected SPUTUM	on day	67 68
Infected URINE	on day	69 70
Infected BILE	on day	71 72
Infected PANC. COLLECTION	on day	73 74
Infected OTHER COLLECTION	on day	75 76
Positive BLOOD CULTURE	on day	77 78
CAUSE OF DEATH		
MODE	Not ascertained Fulminant A.P. - 1st week Pancreatic complication *Non-pancreatic complication *Therapeutic misadventure	1 2 3 4 5 6
CERTIFIED	a. b. c. d.	1 2 3 4
Enter details below and on P.M. sheet		



TRIAL NUMBER					A	8
	01	02	03	04	05	06

OPERATIVE OR AUTOPSY FINDINGS									
POST MORTEM carried out by Hospital Coroner								1	2
									07
OPERATION No.		on day		by				1A	1B
Indication		Diagnostic Emergency Elective cholecystectomy Other elective surgery						1	2
Incision		Midline Paramedian Transverse Kocher Other						1	2
FREE FLUID		Amount in ml Colour Chart No. Other appearance						10	11
FAT NECROSIS		None Localised to pancreas Localised to lesser sac More widespread						1	2
ECCHYMOSIS		None Pancreas only Retroperitoneum More widespread						1	2
Appearance of PANCREATIC Head Body Tail								23	24
Normal								1	2
Oedematous only								2	3
Extensive haemorrhagic								3	4
Frank necrosis								4	5
'Pseudocyst'								6	7
Abscess = pus								8	9
COLLECTION		Max. diam. (cm)		Min. diam. (cm)				25	26
DISTANT COLLECTION		None Supra-colic Intra-colic/pelvic						1	2
LIVER		Normal Cirrhotic						1	2
GALL BLADDER		Serosa Inflamed Dilated Stones						No Yes Yes No	1 2 3 4
USE FURTHER SHEETS AS REQUIRED									

DUCTS	Cholangitis	No Yes	1 2 3 4
	Common duct stones	No Yes	1 2 3 4
	C.B.D. diameter (mm)		37 38 39 40
AMPULLA	Normal Oedematous Stone impacted		1 2 3 4
ANATOMY	Normal appearance Variant anatomy		1 2 3 4
P.O.C.	Not performed Cholangiogram only Pans. duct filling noted		1 2 3 4
GALL BLADDER	No procedure Cholecystectomy Cholecystotomy		1 2 3 4
C.B.D. EXPLORED	Supra-duodenally Trans-duodenally	No Yes No Yes	1 2 3 4
AMPULLA	No procedure Dilated only Sphincterotomy/plasty		1 2 3 4
Y-TUBE	Not inserted Inserted		1 2 3 4
PANCREATIC PROCEDURE	None Neurosect/cecostomy Partial pancreatectomy Total pancreatectomy		1 2 3 4
DRAINAGE	No internal drainage Pancr./Cycto-pancreatectomy Cycto-gastrostomy No external drainage Simple drains only Sump type drain Exteriorisation		1 2 3 4 5 6 7
POST-OP	No irrigation Saline irrigation only Tresylol irrigation Tresylol mega-units / 24hrs Number of days treatment		1 2 3 4 5 6 7
Y-TUBE	Removed on day		56 57 58 59 60
WOUND INFECTION		Minor Major	1 2 3 4
SOMATOSTATIN (or analogues)	days	DRAIN	days

Appendix 2: Data collection sheet

TRIAL NUMBER					0	1
	01	02	03	04	05	06

OBSERVATIONS	01	02	03	04	05	06	07
PULSE RATE	Max 07 08 09	07 08 09	07 08 09	07 08 09	07 08 09	07 08 09	07 08 09
	Min 10 11 12	10 11 12	10 11 12	10 11 12	10 11 12	10 11 12	10 11 12
BP SYSTOLIC	Max 13 14 15	13 14 15	13 14 15	13 14 15	13 14 15	13 14 15	13 14 15
	Min 16 17 18	16 17 18	16 17 18	16 17 18	16 17 18	16 17 18	16 17 18
TEMP. CORE	Max 19 20 21	19 20 21	19 20 21	19 20 21	19 20 21	19 20 21	19 20 21
	Min 22 23 24	22 23 24	22 23 24	22 23 24	22 23 24	22 23 24	22 23 24
No. PEAKS > 33.4 C	25	25	25	25	25	25	25
INTAKE	Oral ml 26 27 28 29	26 27 28 29	26 27 28 29	26 27 28 29	26 27 28 29	26 27 28 29	26 27 28 29
	Cryotalloid ml 30 31 32 33	30 31 32 33	30 31 32 33	30 31 32 33	30 31 32 33	30 31 32 33	30 31 32 33
	Plasma ml 34 35 36 37	34 35 36 37	34 35 36 37	34 35 36 37	34 35 36 37	34 35 36 37	34 35 36 37
	Other colloid ml 38 39 40 41	38 39 40 41	38 39 40 41	38 39 40 41	38 39 40 41	38 39 40 41	38 39 40 41
	Lavage in ml 42 43 44 45	42 43 44 45	42 43 44 45	42 43 44 45	42 43 44 45	42 43 44 45	42 43 44 45
	Whole blood ml 46 47 48 49	46 47 48 49	46 47 48 49	46 47 48 49	46 47 48 49	46 47 48 49	46 47 48 49
OUTPUT	Urino ml 50 51 52 53	50 51 52 53	50 51 52 53	50 51 52 53	50 51 52 53	50 51 52 53	50 51 52 53
	NG asp./vomit ml 54 55 56 57	54 55 56 57	54 55 56 57	54 55 56 57	54 55 56 57	54 55 56 57	54 55 56 57
	Lavage out ml 58 59 60 61	58 59 60 61	58 59 60 61	58 59 60 61	58 59 60 61	58 59 60 61	58 59 60 61
	*Other ml 62 63 64 65	62 63 64 65	62 63 64 65	62 63 64 65	62 63 64 65	62 63 64 65	62 63 64 65
FLUID BALANCE	ml 66 67 68 69 70	66 67 68 69 70	66 67 68 69 70	66 67 68 69 70	66 67 68 69 70	66 67 68 69 70	66 67 68 69 70
PERFUSION	Normal 1	1	1	1	1	1	1
	Reduced 2	2	2	2	2	2	2
DEHYDRATION	None 3	3	3	3	3	3	3
	Mild 4	4	4	4	4	4	4
	Moderate 5	5	5	5	5	5	5
	Gross 6	6	6	6	6	6	6
DISTENSION	None 7	7	7	7	7	7	7
	Present 8	8	8	8	8	8	8
TENDERNESS	None 9	9	9	9	9	9	9
	Mild 10	10	10	10	10	10	10
	Marked 11	11	11	11	11	11	11
	Loc. peritonism 12	12	12	12	12	12	12
	Gen. peritonism 13	13	13	13	13	13	13
	Rigid 14	14	14	14	14	14	14
ILEUS	None 15	15	15	15	15	15	15
	Present 16	16	16	16	16	16	16
SPUTUM	None 17	17	17	17	17	17	17
	Present 18	18	18	18	18	18	18
RIGORS	None 19	19	19	19	19	19	19
	Present 20	20	20	20	20	20	20
ECCHYMOSIS	None 21	21	21	21	21	21	21
	*Port-peritoneal 22	22	22	22	22	22	22
	Distant 23	23	23	23	23	23	23
	24	24	24	24	24	24	24

Appendix 2: Data collection sheet

TRIAL NUMBER	01	02	03	04	05	06

THERAPY	D1	D2	D3	D4	D5	D6	D7
DIURETICS							
No	1	1	1	1	1	1	1
Yes	2	2	2	2	2	2	2
PRESSORS							
No	1	1	1	1	1	1	1
Yes	2	2	2	2	2	2	2
INSULIN							
units	01 10 11	02 10 11	03 10 11	04 10 11	05 10 11	06 10 11	07 10 11
IV CALCIUM	mmol	12 13	12 13	12 13	12 13	12 13	12 13
IV BICARB.	mmol	14 15	14 15	14 15	14 15	14 15	14 15
PPP GIVEN	pccto	16 17	16 17	16 17	16 17	16 17	16 17
PACKED CELL	unit	18 19	18 19	18 19	18 19	18 19	18 19
CHEST RADIOLOGY							
CXR Collapse No.	1	1	1	1	1	1	1
Basal only	2	2	2	2	2	2	2
Pneumonia	3	3	3	3	3	3	3
EFFUSION No.	1	1	1	1	1	1	1
Angles only	2	2	2	2	2	2	2
Signif. unilat	3	3	3	3	3	3	3
Signif. bilat	4	4	4	4	4	4	4
PULM. OEDEMA no	1	1	1	1	1	1	1
Yes	2	2	2	2	2	2	2
A.R.D.S. No	1	1	1	1	1	1	1
Yes	2	2	2	2	2	2	2
MISCELLANEOUS							
G.I. BLEED							
None	1	1	1	1	1	1	1
Minor	2	2	2	2	2	2	2
Major	3	3	3	3	3	3	3
CONFUSION							
None	1	1	1	1	1	1	1
Mild	2	2	2	2	2	2	2
Marked	3	3	3	3	3	3	3
GASES 09.00							
pH	20 27 28	20 27 28	20 27 28	20 27 28	20 27 28	20 27 28	20 27 28
pO2 kPa	29 30 31	29 30 31	29 30 31	29 30 31	29 30 31	29 30 31	29 30 31
pCO2 kPa	32 33 34	32 33 34	32 33 34	32 33 34	32 33 34	32 33 34	32 33 34
Std. Dev. mmol	35 36	35 36	35 36	35 36	35 36	35 36	35 36
Baco shift +/-	37 38 39	37 38 39	37 38 39	37 38 39	37 38 39	37 38 39	37 38 39
% O2 Inspired	40 41	40 41	40 41	40 41	40 41	40 41	40 41
GASES 21.00							
pH	42 43 44	42 43 44	42 43 44	42 43 44	42 43 44	42 43 44	42 43 44
pO2 kPa	45 46 47	45 46 47	45 46 47	45 46 47	45 46 47	45 46 47	45 46 47
pCO2 kPa	48 49 50	48 49 50	48 49 50	48 49 50	48 49 50	48 49 50	48 49 50
Std. Dev. mmol	51 52	51 52	51 52	51 52	51 52	51 52	51 52
Baco shift +/-	53 54 55	53 54 55	53 54 55	53 54 55	53 54 55	53 54 55	53 54 55
% O2 Inspired	56 57	56 57	56 57	56 57	56 57	56 57	56 57

Appendix 2: Data collection sheet

TRIAL NUMBER					
02	03	04	05	06	07

CRITICAL CARE		D1	D2	D3	D4	D5	D6	D7
Best	pO2 kPa	07 08 09	07 08 09	07 08 09	07 08 09	07 08 09	07 08 09	07 08 09
Worst	pO2 kPa	10 11 12	10 11 12	10 11 12	10 11 12	10 11 12	10 11 12	10 11 12
Best	pCO2 kPa	13 14 15	13 14 15	13 14 15	13 14 15	13 14 15	13 14 15	13 14 15
Worst	pCO2 kPa	16 17 18	16 17 18	16 17 18	16 17 18	16 17 18	16 17 18	16 17 18
Lowest	pH	19 20 21	19 20 21	19 20 21	19 20 21	19 20 21	19 20 21	19 20 21
Highest	pH	22 23 24	22 23 24	22 23 24	22 23 24	22 23 24	22 23 24	22 23 24
Highest	Mean B.P.	25 26 27	25 26 27	25 26 27	25 26 27	25 26 27	25 26 27	25 26 27
Lowest	mmHg	28 29 30	28 29 30	28 29 30	28 29 30	28 29 30	28 29 30	28 29 30
CVP cm H2O	Max	31 32	31 32	31 32	31 32	31 32	31 32	31 32
	Min	33 34	33 34	33 34	33 34	33 34	33 34	33 34
Highest	PEEP mcd	35 36	35 36	35 36	35 36	35 36	35 36	35 36
RESP RATE	Max	37 38	37 38	37 38	37 38	37 38	37 38	37 38
	Min	39 40	39 40	39 40	39 40	39 40	39 40	39 40
VENTILATED	No	1	1	1	1	1	1	1
	Yes	2	2	2	2	2	2	2
ARF	No	3	3	3	3	3	3	3
	Conservative	4	4	4	4	4	4	4
Dialysed	No	5	5	5	5	5	5	5
	Yes	6	6	6	6	6	6	6
URINE	Na	7	7	7	7	7	7	7
	Urea	8	8	8	8	8	8	8
COAG.	PT s	9	9	9	9	9	9	9
	control s	10	10	10	10	10	10	10
KPTT s	control s	11	11	11	11	11	11	11
	control s	12	12	12	12	12	12	12
FDP/FIBRINOGEN g/dl	control s	13	13	13	13	13	13	13
	control s	14	14	14	14	14	14	14
PCWP mmHg	Max	15	15	15	15	15	15	15
	Min	16	16	16	16	16	16	16
Max % FIO2/PaO2		17	17	17	17	17	17	17
ARRHYTHMIA (by initials)		18	18	18	18	18	18	18
GLASGOW COMA SCALE 15 items		19	19	19	19	19	19	19
research		20	20	20	20	20	20	20
research		21	21	21	21	21	21	21
research		22	22	22	22	22	22	22

## Appendix 2: Data collection sheet

TRIAL NUMBER					B	4
	01	02	03	04	05	06

LABORATORY DATA				D1				D2				D3				D4				D5				D6				D7			
FBC WCC				x 10 <sup>9</sup> /l																											
Hb				g/dl				07 08 09				07 08 09				07 08 09				07 08 09				07 08 09				07 08 09			
PCV				%				10 11 12				10 11 12				10 11 12				10 11 12				10 11 12				10 11 12			
Plt				x 10 <sup>9</sup> /l				13 14				13 14				13 14				13 14				13 14				13 14			
Lymphs				%				15 16 17				15 16 17				15 16 17				15 16 17				15 16 17				15 16 17			
Monos				%				18 19 20				18 19 20				18 19 20				18 19 20				18 19 20				18 19 20			
ESR				mm/hour				21 22 23				21 22 23				21 22 23				21 22 23				21 22 23				21 22 23			
CHEMISTRY (mmol/l if unstable) at 09.00				Na <sup>+</sup>				24 25 26				24 25 26				24 25 26				24 25 26				24 25 26				24 25 26			
				K <sup>+</sup>				27 28 29				27 28 29				27 28 29				27 28 29				27 28 29				27 28 29			
				Cl <sup>-</sup>				30 31				30 31				30 31				30 31				30 31				30 31			
				Bic				32 33 34				32 33 34				32 33 34				32 33 34				32 33 34				32 33 34			
				Urea				35 36				35 36				35 36				35 36				35 36				35 36			
Creat				μmol/l				37 38				37 38				37 38				37 38				37 38				37 38			
Spot Glucose								39 40 41				39 40 41				39 40 41				39 40 41				39 40 41				39 40 41			
Amylase				iu/l				42 43 44				42 43 44				42 43 44				42 43 44				42 43 44				42 43 44			
Alk. Phosp				iu/l				45 46 47 48				45 46 47 48				45 46 47 48				45 46 47 48				45 46 47 48				45 46 47 48			
AST				iu/l				49 50 51				49 50 51				49 50 51				49 50 51				49 50 51				49 50 51			
ALT				iu/l				52 53 54				52 53 54				52 53 54				52 53 54				52 53 54				52 53 54			
LDH				iu/l				55 56 57				55 56 57				55 56 57				55 56 57				55 56 57				55 56 57			
T. Bilrub.				μmol/l				58 59 60 61				58 59 60 61				58 59 60 61				58 59 60 61				58 59 60 61				58 59 60 61			
T. Protein				g/l				62 63 64				62 63 64				62 63 64				62 63 64				62 63 64				62 63 64			
Albumin				g/l				65 66				65 66				65 66				65 66				65 66				65 66			
								67 68				67 68				67 68				67 68				67 68				67 68			
Calcium								69 70 71				69 70 71				69 70 71				69 70 71				69 70 71				69 70 71			
Phosphate								72 73 74				72 73 74				72 73 74				72 73 74				72 73 74				72 73 74			
CRP				mg/l				75 76 77				75 76 77				75 76 77				75 76 77				75 76 77				75 76 77			
γ-GT				iu/l				78 79				78 79				78 79				78 79				78 79				78 79			

**LIST ALL ANTIBIOTIC TREATMENTS**

No	DRUG	START	DURN	INDICATION	No	DRUG	START	DURN	INDICATION
1					6				
2					7				
3					8				
4					9				
5					10				

Appendix 3: Urinary TAP and PLAP concentrations in 55 patients with acute pancreatitis.										
Patient Number	Age	Sex	Aetiology	Hospital stay (days)	Glasgow Score	Outcome	Admission TAP (nmol/l)	Peak TAP	Admission PLAP (nmol/l)	Peak PLAP (nmol/l)
1	21	male	ALC	1.00	1	mild	0.00	0.00	0.90	0.90
2	37	male	ALC	19.00	3	mild	0.00	0.00	0.00	1.45
3	26	male	ALC	18.00	3	mild	0.00	0.72	3.14	3.14
4	49	male	ALC	50.00	3	mild	0.00	0.00	2.10	2.10
5	49	male	ALC	18.00	3	mild	0.00	0.00	0.95	2.72
6	61	male	ALC	18.00	5	mild	0.00	0.21	0.86	2.55
7	45	male	ALC	20.00	2	mild	0.00	0.35	1.05	2.17
8	29	male	ALC	24.00	3	mild	0.00	0.00	0.00	4.24
9	74	male	ALC	12.00	2	mild	0.00	1.09	2.91	8.08
10	30	female	ALC	23.00	0	mild	0.00	0.00	1.40	1.40
11	77	male	ALC	15.00	1	mild	0.00	0.66	3.36	2.90
12	61	female	ALC	14.00	5	mild	0.89	0.54	2.61	7.63
13	48	female	ALC	27.00	3	mild	0.90	0.53	0.00	4.24
14	78	male	ALC	18.00	3	mild	1.80	1.80	2.76	5.30
15	46	male	ALC	14.00	0	mild	1.90	3.59	3.40	3.40
16	37	male	ALC	6.00	6	mild	3.10	5.04	2.32	4.89
17	36	male	ERCP	6.00	6	mild	5.10	5.10	2.79	18.84
18	41	male	ERCP	12.00	12	mild	0.00	0.29	0.99	12.48
19	65	male	ERCP	14.00	14	mild	0.00	0.00	4.96	4.96
20	43	male	GS	44.00	44	mild	1.30	1.30	4.90	5.99
21	58	male	GS	8.00	8	mild	0.00	0.00	0.00	0.00
22	80	male	GS	23.00	23	mild	0.00	0.16	3.81	3.81
23	72	male	GS	7.00	7	mild	0.00	0.29	4.66	4.95
24	79	male	GS	5.00	5	mild	0.00	0.00	1.21	1.21
25	36	male	GS	6.00	6	mild	0.00	0.00	0.00	1.58
26	66	female	GS	13.00	13	mild	0.00	0.00	0.32	3.74
27	63	male	GS	5.00	5	mild	0.00	0.00	0.76	3.54
28	69	male	GS	7.00	7	mild	0.00	0.00	0.00	0.38
29	68	female	GS	12.00	12	mild	0.00	0.00	5.59	5.59
30	69	male	GS	8.00	8	mild	0.00	0.23	2.22	8.24
31	72	male	GS	7.00	7	mild	0.00	0.52	1.75	12.19
32	90	male	GS	7.00	7	mild	0.00	0.50	6.19	39.43
33	77	female	GS	10.00	10	mild	0.00	0.00	0.99	2.28
34	50	female	GS	11.00	11	mild	1.37	1.37	1.83	1.83
35	64	male	GS	10.00	10	mild	1.46	1.46	0.00	2.05
36	67	female	GS	8.00	8	mild	1.47	1.02	0.94	1.62
37	69	female	GS	8.00	8	mild	2.13	0.00	4.80	5.07
38	32	male	IDIO	12.00	12	mild	5.55	5.55	5.90	5.90
39	41	female	IDIO	17.00	17	mild	0.00	2.29	1.53	119.42
40	49	female	GS	22.00	22	mild	1.79	1.79	6.10	160.58
41	57	female	ALC	7.00	7	severe	10.35	12.86	69.67	69.67
42	54	female	Viral	20.00	20	severe	0.00	0.17	1.62	13.30
43	50	female	ALC	5.00	5	severe	0.66	0.68	3.71	3.71
44	34	female	ALC	23.00	23	severe	2.03	2.98	0.43	3.98
45	51	female	ALC	5.00	5	severe	2.33	3.37	22.64	22.64
46	39	male	ALC	9.00	9	severe	2.80	16.19	8.28	9.16
47	33	female	ALC	10.00	10	severe	3.23	3.23	5.06	5.06
48	36	male	ALC	18.00	18	severe	4.38	4.38	6.00	132.39
49	23	male	GS	5.00	5	severe	4.95	4.95	4.31	7.17
50	64	male	GS	8.00	8	severe	6.15	6.15	3.31	8.68
51	69	female	GS	16.00	16	severe	1.04	1.60	0.00	4.61
52	88	female	IDIO	23.00	23	severe	4.08	4.03	2.80	9.39
53	68	female	IDIO	14.00	14	severe	5.12	5.12	28.74	28.74
54	49	male	IDIO	5.00	5	severe	3.23	3.23	3.23	3.23
55	52	female	ALC	2.00	2	severe	5.88	5.88	4.74	22.37

A2-M = alpha2-macroglobulin. Other abbreviations are as previously described.

Appendix 3 continued: Urinary TAP and PLAP concentrations in 55 patients with acute pancreatitis.					
Admission TAP + PLAP (nmol/l)	Admission CRP (mg/l)	Peak CRP (mg/l)	Admission A2-M (mg/l)	Peak A2-M (mg/l)	
0.90	256.00	256	0.70	0.50	
1.45	16.00	270	1.10	0.80	
2.52	20.00	285	1.30	1.10	
2.10	18.00	76	1.10	0.80	
2.27	79.00	253	1.60	0.90	
2.55	37.00	429	0.60	0.40	
2.17	13.00	254	1.10	0.70	
2.80	5.00	100	1.50	0.90	
3.10	48.00	200	3.10	1.30	
1.40	20.00	157	1.30	1.10	
2.90	12.00	279	1.00	0.90	
4.09	135.00	348	1.50	0.80	
5.14	10.00	184	1.00	0.70	
4.58	73.00	291	1.10	0.80	
5.30	5.00	369	1.20	0.90	
6.06	5.00	83	0.90	0.90	
8.30	5.00	23	3.20	2.80	
2.61	5.00	175	1.30	0.90	
2.01	12.00	19	1.50	1.60	
3.98	43.00	155	2.50	1.50	
0.00	26.00	114	1.10	1.10	
3.81	119.00	201	1.40	1.20	
4.95	15.00	37	1.90	1.70	
1.21	11.00	248	2.50	1.50	
1.58	5.00	30	0.90	0.90	
3.74	61.00	223	2.30	1.20	
3.54	5.00	80	2.40	1.30	
0.38	5.00	68	0.80	0.70	
5.59	215.00	254	1.90	0.80	
8.24	108.00	171	1.30	1.10	
2.85	11.00	234	0.80	0.60	
2.80	99.00	308	1.00	0.80	
2.28	10.00	97	1.10	1.00	
3.20	16.00	125	2.30	1.00	
3.51	10.00	117	0.90	0.90	
3.08	10.00	82	1.90	1.00	
6.13	41.00	208	1.10	0.90	
7.75	117.00	148	1.90	1.70	
2.24	10.00	24	1.50	1.30	
3.39	10.00	366	1.50	0.60	
80.20	62.00	106	0.90	0.80	
1.62	17.00	243	1.60	1.30	
4.37	112.00	113	2.30	1.50	
2.46	34.00	73	3.70	2.20	
24.97	10.00	162	1.50	0.90	
11.08	10.00	226	2.80	1.80	
8.29	48.00	107	2.10	1.50	
10.38	10.00	56	1.30	1.00	
9.26	10.00	19	1.70	1.40	
9.46	33.00	53	1.50	1.40	
1.04	96.00	96	1.40	1.20	
6.98	130.00	226	2.00	1.50	
33.86	10.00	38	1.60	1.10	
6.17	13.00	20	1.30	1.80	
10.62	10.00	16	1.10	1.00	

A2-M = alpha2-macroglobulin. Other abbreviations are as previously described.

Appendix 4: Clinical and laboratory data in 39 patients with acute pancreatitis										
Patient Number	Sex	Age	Stay (days)	Imrie Score	Ranson Score	Outcome	Site of Abdominal Pain	Pain Radiated to back	Pain Radiated to Shoulder	Severity of Pain
4	male	49	14	2	2	mild	EPI	no	no	severe
6	male	61	8	0	1	mild	CEN	no	no	severe
7	male	45	28	0	0	mild	EPI	yes	no	severe
8	male	29	7	0	0	mild	EPI+CEN	yes	no	severe
9	male	74	5	3	5	mild	EPI	no	no	severe
10	male	30	6	0	0	mild	EPI	yes	no	severe
11	male	77	13	0	1	mild	EPI+R+LUQ	yes	no	mild
12	male	61	5	0	1	mild	EPI	no	no	severe
13	male	48	7	1	1	mild	EPI+CEN	no	no	severe
14	female	78	12	2	5	mild	EPI+RUQ	yes	no	severe
15	male	46	8	1	2	mild	EPI+R+LUQ	yes	yes	mild
16	male	37	7	1	1	mild	EPI	yes	no	severe
17	female	36	7	0	0	mild	EPI+R+LUQ+CEN	yes	yes	severe
18	female	41	10	0	0	mild	EPI+RUQ+RIF	yes	no	severe
19	female	65	11	3	4	mild	EPI+R+LUQ+CEN	yes	no	severe
20	male	43	10	0	0	mild	RUQ+RIF	yes	no	severe
21	female	58	9	1	1	mild	ALL	yes	yes	severe
23	male	72	12	0	1	mild	EPI+R+LUQ	yes	no	severe
24	female	79	17	2	3	mild	EPI	yes	no	severe
25	female	36	22	0	0	mild	EPI+RUQ	no	no	severe
27	female	63	20	0	0	mild	EPI+RUQ	yes	no	severe
29	female	68	23	1	2	mild	RUQ+CEN	no	no	moderate
30	female	69	5	2	4	mild	EPI	no	no	severe
33	male	77	18	0	1	mild	EPI+RUQ	no	no	severe
34	male	50	5	0	0	mild	EPI+R+LUQ+CEN	no	no	severe
35	male	64	8	0	2	mild	RUQ+CEN	yes	no	severe
38	female	32	14	2	3	mild	CEN+RUQ	yes	no	severe
39	male	41	5	0	0	mild	EPI+R+LUQ	no	no	severe
40	female	49	9	1	1	mild	CEN+RUQ	yes	no	severe
41	male	57	1	3	4	severe	EPI+R+LUQ	yes	yes	severe
42	male	54	19	3	2	severe	EPI+R+LUQ+CEN	no	no	severe
43	male	50	18	3	3	severe	EPI+R+LUQ	yes	no	severe
45	male	51	18	3	3	severe	EPI	yes	no	severe
46	male	39	18	5	7	severe	EPI+RUQ	yes	no	severe
49	male	23	12	2	2	severe	EPI+RUQ+CEN	no	no	severe
50	female	64	23	0	1	severe	EPI+RUQ	yes	no	severe
53	female	68	27	4	5	severe	EPI+R+LUQ	no	no	severe
54	male	49	18	3	5	severe	EPI+CEN+L+RUQ	no	no	severe
55	male	52	14	2	2	severe	EPI+CEN	yes	no	moderate



Appendix 4: continued							
Collapse	Vomiting	Admission	Admission	Admission	Admission	Admission	Admission
		Heart Rate	Systolic Blood	Temperature	Peripheral	Dehydration	Abdominal
			Pressure (mm	Degrees C	Perfusion		Distension
			Hg)				
no	yes	75	126	37.10	normal	none	absent
no	yes	60	170	37.00	normal	none	present
no	yes	80	140	37.20	normal	none	absent
no	no	78	140	37.00	normal	none	absent
no	yes	100	160	36.60	normal	mild	absent
no	yes	78	150	36.90	normal	none	absent
no	yes	110	85	36.50	normal	none	absent
no	no	80	110	37.60	normal	none	absent
no	nausea	80	120	37.00	normal	none	absent
no	yes	95	120	37.10	normal	mild	absent
no	yes	100	145	36.00	normal	none	absent
no	no	90	120	36.40	normal	none	absent
no	no	80	140	36.40	normal	mild	present
no	yes	85	125	37.00	normal	none	absent
no	yes	80	150	35.50	normal	none	absent
no	yes	70	100	36.50	normal	none	absent
no	yes	80	140	37.00	normal	mild	absent
yes	yes	80	110	36.40	normal	none	absent
no	yes	80	135	36.00	normal	none	absent
no	yes	80	11	37.00	normal	none	absent
no	yes	78	160	36.20	normal	none	absent
no	nausea	100	120	36.40	normal	none	present
no	yes	95	155	37.30	normal	none	absent
no	no	70	170	37.00	normal	none	absent
no	yes	68	135	36.50	normal	none	absent
no	yes	80	100	35.60	normal	mild	absent
no	yes	80	135	37.20	normal	none	absent
no	yes	80	125	36.80	normal	none	absent
no	yes	85	125	38.20	normal	none	absent
no	yes	160	120	38.40	abnormal	none	present
yes	yes	104	20	36.50	normal	none	absent
no	yes	125	105	36.80	abnormal	moderate	present
no	yes	100	150	36.00	abnormal	moderate	present
no	nausea	120	17	37.50	normal	mild	present
no	nausea	110	110	37.00	normal	moderate	absent
no	yes	110	130	37.20	normal	none	absent
no	yes	90	135	37.20	normal	mild	absent
no	yes	90	70	34.00	abnormal	severe	absent
no	yes	60	155	36.20	normal	none	absent

Appendix 4: continued							
Admission	Admission	Admission	Duration of	Fluid	Day 1	Day 1	Day 1
Peritoneal	Abdominal	Bowel	Ileus (days)	sequestration	White Cell	Haemoglobin	Haematocrit
Effusion	Tenderness	sounds		48 hrs Post	Count	(gm/dl)	
				Admission (ml)	(x10 <sup>9</sup> /l)		
none	minimal	normal	0	2145	5.70	15.30	0.43
none	minimal	normal	0	2350	12.70	10.50	0.35
none	minimal	normal	0	2369	8.40	17.20	0.45
none	minimal	normal	0	1186	7.60	12.80	0.36
none	minimal	normal	0	2970	17.00	13.50	0.50
none	minimal	normal	0	4278	13.70	14.80	0.43
none	minimal	normal	0	383	15.80	12.30	0.34
none	none	normal	0	4726	11.00	14.30	0.47
none	none	normal	0	2751	13.30	14.60	0.44
none	local peritonism	absent	1	2108	13.10	14.20	0.43
none	minimal	normal	0	3332	12.50	16.00	0.47
none	minimal	normal	0	1175	13.10	15.20	0.44
none	marked	abnormal	1	4370	13.40	16.60	0.47
none	minimal	normal	0	2175	9.00	15.50	0.46
none	local peritonism	absent	2	1118	18.10	12.30	0.32
none	marked	normal	0	2325	11.50	13.60	0.38
none	minimal	normal	0	1085	6.30	13.20	0.39
none	marked	normal	0	3186	9.40	138.00	0.41
none	minimal	normal	0	1010	5.20	11.20	0.33
none	minimal	normal	0	1586	8.60	12.20	0.38
none	minimal	normal	0	3192	9.10	12.70	0.36
none	minimal	normal	0	1289	8.60	16.20	0.48
none	marked	normal	0	4135	14.70	13.80	0.40
none	minimal	normal	0	2250	13.00	12.80	0.37
none	minimal	normal	0	1300	12.80	14.90	0.42
none	minimal	normal	0	2030	8.40	18.20	0.46
none	minimal	normal	0	2078	11.30	13.10	0.40
none	none	normal	0	1006	8.20	15.80	0.38
none	marked	normal	0	2395	3.40	11.30	0.33
none	local peritonism	absent	1	2650	12.30	16.30	0.56
none	minimal	absent	4	2017	21.40	15.90	0.47
none	local peritonism	absent	5	3275	15.60	13.50	0.38
none	local peritonism	abnormal	3	3776	12.10	16.80	0.49
present	local peritonism	abnormal	7	3795	15.10	19.30	0.55
none	marked	abnormal	4	3065	22.00	18.10	0.52
present	minimal	normal	0	3830	9.50	13.20	0.36
none	minimal	normal	0	3250	16.20	14.80	0.42
none	local peritonism	abnormal	3	8812	15.00	9.70	0.24
none	minimal	normal	0	3549	13.60	13.00	0.45

Appendix 4: continued									
Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
Platelet	Serum	Serum	Serum	Serum	Blood	Serum	Blood	Alkaline	AST
Count	Sodium	Potassium	Chloride	Bicarbonate	Urea	Creatinine	Glucose	Phosphatase	(IU/l)
10 9/l	(mmol/l)	(mmol/l)	(mmol/l)	(mmol/l)	(mmol/l)	(umol/l)	(mmol/l)	(U/l)	
227	142	3.70	102.00	26	3.50	75	7.00	95	249
221	141	3.80	98.00	28	4.30	88	6.21	175	24
340	138	4.10	101.00	26	4.60	81	4.60	135	14
185	140	4.40	104.00	2.6	4.90	85	6.90	157	11
290	136	5.00	98.00	23	5.00	95	9.30	235	49
229	139	4.00	100.00	26	2.90	84	5.20	65	38
275	136	3.70	100.00	20	8.00	130	5.30	65	12
189	147	3.80	102.00	28	4.20	82	6.80	455	186
250	139	3.60	103.00	27	5.30	70	5.80	125	16
274	135	3.70	98.00	21	5.20	75	9.80	245	61
170	135	4.10	95.00	24	3.90	65	6.00	290	187
326	142	4.00	104.00	20	3.10	95	6.50	140	12
356	140	3.90	104.00	19	2.20	100	5.30	175	28
360	140	4.10	101.00	25	3.10	60	6.00	165	18
350	139	4.00	105.00	22	5.50	50	6.30	395	256
230	140	3.60	104.00	24	5.70	120	5.80	85	24
364	137	4.10	97.00	25	4.80	110	5.80	225	45
352	138	3.60	106.00	20	5.60	94	6.00	1258	85
232	143	4.00	107.00	20	7.70	90	6.80	410	445
313	141	3.90	103.00	20	3.30	80	7.90	340	45
436	135	3.90	98.00	21	3.20	70	8.60	1270	39
392	140	4.10	93.00	23	9.40	85	4.50	990	35
193	139	3.30	99.00	25	5.20	100	7.40	285	445
254	140	3.40	102.00	27	6.50	85	6.20	135	42
271	140	3.90	106.00	23	4.10	83	6.30	60	19
173	137	3.00	92.00	27	5.60	110	6.70	125	12
415	138	3.80	106.00	23	4.00	68	6.00	289	39
292	135	5.00	108.00	22	10.00	24	7.30	98	34
171	137	3.30	106.00	23	4.70	85	6.40	295	330
421	142	4.10	103.00	23	6.90	250	15.10	115	74
281	137	3.90	101.00	26	6.50	119	9.30	194	25
274	138	3.90	106.00	21	7.00	90	10.50	390	105
360	137	3.80	95.00	21	7.70	95	7.70	115	37
205	135	3.70	97.00	20	4.30	75	6.70	648	78
327	135	3.20	100.00	17	5.60	95	9.90	170	18
284	137	3.40	99.00	22	3.30	87	9.00	372	112
235	140	3.60	102.00	23	5.60	110	12.10	765	295
164	136	9.00	94.00	4	20.70	295	93.00	380	112
227	135	0.36	109.00	21	6.80	85	5.60	125	85

Appendix 4: continued											
Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 2	Day 2	Day 2	Day 2
ALT	LDH	Billrubin	Total	Serum	Serum	Serum	GGT	White cell	Haemoglobin	Haematocrit	Platelet
(U/l)	(U/l)	(umol/l)	Protein	Albumin	Calcium	Phosphate	(U/l)	Count	(gm/dl)		Count
			(g/l)	(g/l)	(mmol/l)	(mmol/l)	(U/l)	10 <sup>9</sup> /l			10 <sup>9</sup> /l
198	1057	18	72	34	2.32	1.03	147	6.00	13.30	0.39	317
14	344	17	69	43	2.02	0.98	118	13.80	14.70	0.40	229
15	221	20	69	38	2.04	0.97	109	11.70	13.00	0.39	282
13	126	12	64	47	2.21	0.95	13	8.40	11.70	0.35	174
49	500	8	72	44	2.35	0.85	100	16.20	12.40	0.48	296
22	468	6	76	47	2.28	1.20	81	11.60	14.30	0.42	251
15	145	8	69	35	2.33	0.99	114	12.90	11.70	0.33	249
142	219	32	73	35	2.23	1.15	26	10.70	15.70	0.46	163
35	297	58	76	42	2.14	1.20	33	8.70	12.50	0.37	201
97	400	41	66	35	2.02	0.78	98	12.80	13.70	0.39	257
260	364	29	76	44	2.65	1.20	206	10.90	14.70	0.42	155
23	258	5	70	45	2.26	0.95	37	13.90	15.40	0.44	358
45	260	10	68	48	2.35	1.05	179	13.20	15.40	0.45	344
21	185	8	65	42	2.45	1.35	48	8.90	14.30	0.43	334
208	495	22	60	31	2.20	1.10	152	18.30	11.00	0.33	321
22	125	17	68	44	2.30	0.86	147	12.20	13.60	0.39	285
23	250	7	62	37	2.23	0.99	23	7.10	12.50	0.38	340
118	258	115	52	32	2.05	1.10	750	10.00	13.60	0.39	313
360	115	12	62	39	2.20	0.95	100	4.50	12.10	0.35	290
86	280	10	62	37	2.20	1.20	42	7.40	11.80	0.36	283
27	380	245	64	36	2.20	1.00	12	8.20	12.80	0.36	435
72	189	6	73	34	2.15	1.30	156	10.70	15.60	0.45	371
240	655	33	66	42	2.20	0.86	224	13.80	12.50	0.37	171
118	55	18	62	38	2.20	1.10	70	9.30	12.60	0.36	251
72	86	9	66	40	2.20	0.96	85	9.70	14.90	0.42	234
9	115	33	66	38	2.20	1.30	63	8.60	18.00	0.46	184
53	298	28	70	43	2.15	1.10	173	8.00	11.70	0.35	349
28	200	12	64	34	2.20	1.20	50	8.00	14.00	0.35	235
335	330	24	63	43	2.15	1.10	185	3.60	11.20	0.23	161
44	820	25	62	45	2.10	0.65	80	*	*	*	*
28	383	10	76	48	2.33	1.05	91	17.40	17.00	0.50	286
138	664	41	73	45	2.36	1.05	456	16.30	11.90	0.38	365
35	320	27	67	41	1.95	0.80	135	11.30	16.50	0.48	340
50	635	22	64	42	1.90	1.15	*	16.30	18.70	0.54	113
11	271	15	62	40	2.31	0.75	35	24.30	17.00	0.50	267
67	448	44	73	39	2.20	1.10	820	8.70	11.20	0.32	352
1750	700	42	61	42	2.20	0.75	295	17.70	14.10	0.43	228
71	595	25	47	34	2.35	1.05	70	16.70	9.80	0.28	151
96	550	15	68	36	2.01	1.15	130	9.40	13.10	0.43	235

Appendix 4: continued										
Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2
Serum	Serum	Serum	Serum	Blood	Serum	Blood	Alkaline	AST	ALT	LDH
Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)	Bicarbonate (mmol/l)	Urea (mmol/l)	Creatinine (umol/l)	Glucose (mmol/l)	Phosphatase (U/l)	(U/l)	(U/l)	(U/l)
137	3.50	104	22	1.60	88	8.00	94	198	116	530
135	3.50	98	25	3.80	95	7.10	165	26	9	303
141	3.90	99	23	4.90	76	7.60	150	11	15	322
139	4.40	104	25	4.40	82	5.30	158	16	18	22
135	4.20	96	24	6.00	100	11.30	2105	37	39	500
142	4.00	99	28	2.70	79	5.00	59	44	20	382
134	3.60	100	22	1.90	86	4.60	197	27	26	155
140	3.70	103	26	3.60	75	5.20	510	156	168	280
141	4.00	104	22	4.20	92	5.90	153	28	40	379
138	3.60	102	24	7.50	85	8.20	210	34	68	340
133	3.90	99	25	3.20	65	7.40	240	80	151	355
139	4.40	105	23	3.50	85	6.50	150	19	27	300
136	3.70	103	24	1.60	85	5.60	135	27	28	300
140	3.90	103	28	2.40	65	5.40	185	20	36	270
138	4.00	106	23	4.90	50	7.10	59	280	227	610
138	3.70	104	26	3.80	110	6.10	110	39	28	221
140	3.90	98	30	4.20	90	64.00	250	59	33	340
137	3.80	104	24	4.50	70	5.90	1330	99	122	280
142	4.10	113	21	6.70	80	6.00	570	570	460	1270
141	3.90	106	26	2.70	70	5.30	440	90	124	330
131	3.60	97	24	2.50	55	7.70	1080	33	27	280
135	3.90	90	29	7.40	100	3.30	1080	31	61	569
141	4.40	105	20	6.90	95	7.70	370	430	200	750
138	4.00	104	27	4.10	90	5.30	165	51	109	105
139	4.20	105	23	2.50	76	5.40	73	26	91	137
141	3.50	91	27	7.00	103	7.20	125	13	9	190
141	3.60	107	26	3.00	61	5.30	266	172	275	393
138	4.90	106	22	6.90	100	168.00	90	36	20	215
135	3.80	105	25	3.40	75	5.50	290	320	231	345
*	*	*	*	*	*	*	*	*	*	*
140	4.00	102	26	7.00	135	14.30	197	27	26	635
184	3.30	98	25	4.10	86	10.50	267	62	99	369
137	3.80	95	21	7.70	95	11.90	115	37	40	420
133	3.30	91	23	5.20	80	15.40	240	74	46	650
135	3.20	98	20	6.20	80	9.50	175	23	14	460
138	3.30	105	21	3.30	78	9.20	254	108	68	403
138	3.70	104	22	6.00	100	13.10	760	300	1700	660
146	4.60	113	8	22.30	280	34.00	320	92	66	600
136	3.70	106	22	7.00	87	5.30	130	62	105	105

Appendix 4: continued										
Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 3	Day 3	Day 3	Day 3	Day 3
Bilirubin	Total	Serum	Serum	Serum	GGT	White Cell	Haemoglobin	Haematocrit	Platelet	Serum
(umol/l)	Protein	Albumin	Calcium	Phosphate	(U/l)	Count	(gm/dl)		Count	Sodium
	(g/l)	(g/l)	(mmol/l)	(mmol/l)		10 <sup>9</sup> /l			10 <sup>9</sup> /l	(mmol/l)
31	70	35	2.23	0.79	99	7.50	12.60	0.34	234	140
24	69	43	2.08	0.71	102	12.50	10.90	0.35	235	139
37	73	33	2.05	0.82	104	11.80	13.40	0.31	267	137
9	68	38	2.10	0.95	13	6.40	12.50	0.37	193	138
19	67	40	1.90	0.60	95	16.10	14.50	0.42	220	134
6	66	47	2.27	1.19	*	10.90	12.30	0.37	246	136
12	71	35	2.20	1.22	98	15.10	11.60	0.44	256	138
20	68	33	2.32	0.99	50	8.20	14.80	0.44	144	137
78	74	44	2.19	0.95	42	8.80	12.40	0.40	214	132
35	60	34	2.10	0.65	101	12.60	11.40	0.36	244	137
21	72	42	2.56	1.16	228	8.50	13.50	0.39	200	138
7	68	45	2.23	0.80	60	15.70	14.70	0.46	382	138
20	61	42	2.11	1.05	172	15.00	12.00	0.43	326	135
10	62	40	2.20	1.00	*	8.60	13.80	0.40	317	138
29	59	33	2.10	1.10	164	8.60	10.60	0.32	286	136
31	63	42	2.25	0.75	186	11.80	13.40	0.36	255	138
10	58	35	2.24	0.95	35	10.30	12.40	0.37	314	140
118	52	32	2.05	1.00	828	7.40	14.30	0.41	282	137
17	61	38	2.15	1.00	135	7.40	12.10	0.35	175	134
14	62	37	2.20	1.30	62	7.80	11.30	0.34	264	143
300	60	33	2.25	1.00	18	6.30	11.50	0.30	420	136
8	48	38	2.05	1.25	489	10.50	14.70	0.43	392	132
48	60	40	2.10	1.10	213	12.40	12.10	0.35	154	139
17	62	37	2.20	1.05	60	7.70	12.80	0.36	241	140
14	67	43	2.23	0.92	112	8.50	14.30	0.41	220	141
33	66	37	2.25	1.25	75	4.50	18.30	0.45	172	141
36	60	38	2.06	0.80	123	7.80	11.00	0.34	314	137
14	64	36	2.20	1.19	67	6.50	14.50	0.39	218	140
23	61	41	2.25	1.10	172	3.80	11.40	0.34	167	138
*	*	*	*	*	*	*	*	*	*	*
12	71	39	2.20	1.15	89	21.50	15.10	0.44	194	132
68	68	47	2.05	0.84	291	14.50	12.40	0.36	367	133
27	67	41	1.95	0.75	155	12.90	14.10	0.41	241	131
28	63	34	1.90	1.10	*	14.20	9.20	0.40	115	133
18	62	36	2.45	0.75	50	28.00	16.40	0.48	259	130
87	62	34	2.15	0.95	722	8.30	10.50	0.31	302	137
40	60	41	2.20	0.75	21	17.00	14.30	0.41	205	137
18	40	32	2.40	0.15	41	11.10	10.80	0.30	141	144
25	65	36	1.95	1.05	122	10.20	12.50	0.42	230	137

Appendix 4: continued											
Day 3	Day 3	Day 3	Day 3	Day 3	Day 3	Day 3	Day 3	Day 3	Day 3	Day 3	Day 3
Serum	Serum	Serum	Blood	Serum	Blood	Alkaline	AST	ALT	LDH	Bilirubin	Total
Potassium	Chloride	Bicarbonate	Urea	Creatinine	Glucose	Phosphatase	(U/l)	(U/l)	(U/l)	(umol/l)	Protein
(mmol/l)	(mmol/l)	(mmol/l)	(mmol/l)	(umol/l)	(mmol/l)	(U/l)					(g/l)
3.90	99	24	1.8	78	7.00	103	161	114	381	38	66
4.00	101	26	3.8	97	5.20	150	25	11	417	30	74
3.80	103	24	2.6	85	4.90	175	35	16	340	33	63
4.40	103	27	4.4	80	4.00	285	16	16	250	11	60
4.00	97	23	5.3	95	9.20	165	36	22	550	24	57
3.70	99	25	2.9	73	4.10	48	71	21	320	10	57
3.80	104	23	5.1	82	5.30	155	24	35	220	8	72
4.20	105	25	2.4	75	4.80	345	44	133	224	32	69
3.80	100	23	3.5	85	6.50	140	20	23	260	19	70
3.50	100	23	7.2	81	9.00	216	23	54	327	29	59
3.80	102	20	3.4	70	4.90	250	78	128	250	34	69
3.70	102	24	3.1	110	5.30	150	30	16	650	28	72
3.80	102	24	2.1	83	5.90	142	29	28	345	12	63
4.00	104	27	2.2	55	4.40	165	25	30	320	6	60
3.40	103	23	3.6	45	5.00	510	107	140	770	12	56
3.40	101	24	3.4	71	6.00	110	41	28	290	33	65
4.30	101	23	3.8	95	6.00	200	22	14	400	16	56
3.80	102	24	3.8	65	5.90	1270	46	85	290	54	59
4.50	107	18	4.2	75	6.30	500	208	390	790	7	62
3.80	110	25	2.6	95	4.00	390	51	90	300	13	60
3.70	97	25	2.6	60	7.10	956	21	23	245	333	60
4.30	95	26	8.3	94	3.60	440	23	47	510	9	72
3.90	105	21	5.2	90	3.90	330	124	152	380	32	64
4.40	107	26	4.7	90	4.90	175	47	91	96	14	61
4.50	103	22	3.4	96	4.50	80	23	70	161	11	65
3.60	105	26	6.9	90	8.10	165	15	11	230	29	64
3.90	106	26	1.5	60	4.60	244	73	170	290	32	65
4.80	1.3	23	6	108	16.40	95	33	20	180	13	69
3.70	104	28	1.6	65	5.30	250	78	155	270	13	57
*	*	*	*	*	*	*	*	*	*	*	*
4.30	94	25	11.5	156	7.20	216	26	18	579	24	62
4.10	101	24	2.6	79	8.00	250	33	76	415	38	60
3.10	93	25	7	85	16.30	95	39	25	530	22	60
3.60	97	27	5.6	100	8.50	115	64	79	950	38	57
3.70	95	22	4.4	70	7.30	165	40	13	840	20	57
3.90	112	19	2.8	70	5.50	237	71	56	312	63	59
3.50	104	22	5.4	110	6.40	455	76	300	450	26	59
5.10	114	16	22.6	250	18.20	290	111	59	820	7	45
3.20	1.6	23	6.9	80	6.30	160	125	130	720	41	66

Appendix 4: continued										
Day 3	Day 3	Day 3	Day 3	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4
Serum	Serum	Phosphate	GGT	White Cell	Haemoglobin	Haematocrit	Platelet	Serum	Serum	Serum
Albumin	Calcium	(mmol/l)	(U/l)	Count	(gm/dl)		Count	Sodium	Potassium	Chloride
(g/l)	(mmol/l)			10 9/l			10 9/l	(mmol/l)	(mmol/l)	(mmol/l)
36	2.20	1.00	54	8.9	11.80	0.35	256	139	3.90	105
46	2.17	0.96	102	9.4	12.00	0.36	248	139	4.00	101
33	2.15	0.92	67	11	12.80	0.39	286	142	3.70	104
39	2.20	0.90	18	6.7	14.30	0.40	223	141	3.90	103
32	1.80	0.50	83	15.6	13.50	0.39	254	131	3.60	97
36	2.18	1.10	*	8.4	11.80	0.37	283	137	3.70	98
36	2.11	0.95	41	5	10.60	0.30	174	142	4.00	98
33	2.25	1.06	*	6.8	14.20	0.43	154	140	4.10	104
46	2.33	0.96	22	13	8.90	0.38	229	134	3.90	101
34	1.93	0.98	63	9.5	12.20	0.35	213	135	3.40	99
39	2.35	0.70	223	6.6	12.80	0.38	325	135	3.70	101
46	2.23	0.53	65	12.3	15.00	0.44	380	138	3.60	101
41	2.20	0.96	175	9.1	14.50	0.41	313	134	3.60	101
39	2.20	1.16	*	8.4	13.60	0.42	325	141	3.70	102
32	2.10	0.90	89	7.6	12.20	0.33	360	135	4.30	102
44	2.30	0.75	255	10.5	13.20	0.35	263	135	4.20	1.5
34	2.20	0.85	45	9.2	12.00	0.36	332	139	5.10	105
33	2.15	0.90	740	7.2	14.50	0.42	380	143	4.10	104
39	2.10	1.00	195	8.6	11.50	0.33	251	142	3.80	106
37	2.10	1.15	53	5	11.20	0.33	238	143	4.10	107
31	2.20	1.05	85	4.3	10.40	0.29	425	138	3.70	98
33	2.05	1.05	572	11	14.60	0.44	385	130	6.20	97
42	2.30	0.65	180	9.3	12.00	0.35	169	133	3.40	105
37	2.15	0.80	51	6.1	12.60	0.36	271	138	3.90	102
41	2.33	1.25	98	7.1	13.90	0.40	235	141	4.30	105
38	2.20	1.20	77	15.4	9.20	0.44	188	142	3.90	106
39	2.15	0.83	105	8.2	11.50	0.39	370	139	3.40	105
34	2.20	1.20	70	6	15.00	0.40	223	136	5.00	106
39	2.20	0.85	160	4.3	11.60	0.33	183	138	3.50	107
*	*	*	*	*	*	*	*	*	*	*
44	2.05	1.31	60	19.7	14.30	0.41	197	129	3.90	94
40	2.05	0.41	273	15.1	12.50	0.37	377	136	3.70	104
37	1.75	0.60	159	12	12.20	0.35	236	134	3.00	95
41	1.70	0.37	*	7.5	12.60	0.36	114	134	4.00	98
38	2.15	0.70	40	25.3	15.80	0.44	266	132	3.90	97
32	2.15	0.80	639	10.5	10.50	0.31	366	137	4.10	109
42	2.05	0.75	176	13.8	12.70	0.37	174	134	4.70	105
27	2.45	0.60	49	8.4	11.10	0.32	115	141	4.70	109
35	1.95	0.95	145	9.6	12.40	0.40	247	136	3.10	105



Appendix 4: continued										
Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4
Serum	Blood	Serum	Blood	Alkaline	AST	ALT	LDH	Bilirubin	Total	Serum
Bicarbonate	Urea	Creatinine	Glucose	Phosphatase	(U/l)	(U/l)	(U/l)	(umol/l)	Protein	Albumin
(mmol/l)	(mmol/l)	(umol/l)	(mmol/l)	(mmol/l)					(g/l)	(g/l)
24	3.10	70	7.00	98	147	109	456	35	70	37
26	3.80	97	5.20	150	25	11	417	30	74	46
26	2.30	79	6.30	121	28	12	384	33	69	38
25	3.40	85	4.80	290	22	15	310	13	69	44
22	4.00	95	8.10	160	26	18	520	24	61	34
23	4.80	75	4.30	50	67	20	273	8	51	33
24	6.20	80	5.20	152	52	43	159	12	70	33
22	4.00	76	5.10	323	37	95	164	23	70	35
22	3.60	86	6.00	144	20	22	226	25	74	49
21	3.10	75	10.90	220	18	27	340	23	62	35
24	2.90	70	8.80	250	76	118	370	20	67	39
25	3.80	90	5.80	260	89	70	500	27	64	41
24	3.50	80	4.80	156	48	42	358	13	62	40
28	2.30	58	4.60	150	20	25	320	5	62	29
24	2.40	45	4.80	470	48	87	532	13	61	33
22	4.10	112	5.20	110	35	31	285	35	62	43
23	3.50	80	4.20	205	12	9	280	78	56	35
26	3.20	75	5.00	1400	47	65	333	110	62	34
23	4.30	90	7.40	520	109	290	403	75	65	42
26	2.70	75	4.00	375	44	75	340	14	61	38
27	2.60	62	6.00	830	18	18	200	360	54	28
22	5.10	80	3.70	1010	23	47	470	14	72	32
21	4.70	80	5.60	275	38	87	310	28	61	40
24	3.70	85	4.70	165	30	66	35	15	63	36
25	3.50	52	5.50	75	35	55	124	9	67	43
24	4.10	100	11.40	175	15	12	270	22	65	40
22	2.20	66	3.70	266	38	129	329	30	75	45
23	11.00	75	11.00	96	29	15	130	11	63	36
24	1.50	80	6.50	230	27	102	230	12	58	39
*	*	*	*	*	*	*	*	*	*	*
23	8.00	128	8.00	220	29	18	674	32	60	36
20	2.80	76	5.70	245	17	44	491	25	63	40
23	5.40	75	7.40	110	49	24	640	21	59	36
22	4.20	75	11.90	17	97	27	1440	37	56	36
25	4.30	65	5.60	165	45	18	730	11	59	39
19	3.20	68	5.00	217	32	40	357	23	57	31
19	6.00	70	7.50	350	31	174	600	17	49	32
19	17.20	180	9.90	430	89	67	820	11	46	28
23	69.00	76	6.00	210	185	183	735	46	67	34

Appendix 4: continued									
Day 4	Day 4	Day 4	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5
Serum	Serum	GGT	White Cell	Haemoglobin	Haematocrit	Platelet	Serum	Serum	Serum
Calcium	Phosphate	(U/l)	Count	(gm/dl)		Count	Sodium	Potassium	Chloride
(mmol/l)	(mmol/l)		10 9/l			10 9/l	(mmol/l)	(mmol/l)	(mmol/l)
2.17	0.90	103	6.00	10.40	0.36	284	141	3.60	96
2.17	0.96	102	9.60	13.00	0.37	267	141	4.10	102
2.13	0.97	106	11.50	11.50	0.33	345	138	3.90	98
2.40	0.85	19	6.20	13.70	0.39	229	141	4.40	104
1.95	0.95	61	11.80	12.00	0.35	250	132	3.70	98
2.13	1.20	150	6.90	13.50	0.40	296	139	3.90	102
2.23	1.14	*	4.50	11.00	0.31	215	140	4.00	100
2.32	1.08	*	5.40	14.80	0.43	172	143	4.20	107
2.18	0.86	49	6.60	12.80	0.37	231	137	4.20	102
1.95	1.10	*	6.20	11.90	0.34	317	135	3.30	101
2.25	0.80	*	6.20	12.80	0.38	325	137	4.10	101
2.20	0.55	265	10.70	14.20	0.42	385	137	3.60	101
2.20	1.10	156	7.60	13.00	0.39	348	134	4.00	99
2.12	1.05	*	8.30	13.70	0.41	341	143	4.40	102
2.10	0.90	101	6.20	11.30	0.34	320	134	4.40	101
2.20	0.70	380	9.60	11.80	0.35	250	137	4.10	107
2.02	0.80	41	8.30	12.50	0.41	366	138	4.50	102
2.15	1.15	707	7.40	14.60	0.42	385	136	4.00	102
2.25	1.00	286	8.50	11.50	0.34	238	141	4.10	109
2.15	1.30	44	6.30	11.30	0.34	234	139	4.30	103
2.20	0.95	91	8.20	10.70	0.30	427	138	3.10	103
2.20	2.05	563	12.40	15.60	0.45	381	133	5.20	97
2.20	0.25	161	8.40	12.20	0.34	167	137	4.10	107
2.15	1.05	40	8.40	12.30	0.36	263	139	4.70	106
2.32	1.17	107	*	*	*	*	*	*	*
2.25	0.85	62	10.00	14.70	0.43	195	142	4.00	106
2.35	1.15	99	5.60	11.30	0.33	378	140	3.50	105
2.15	0.60	35	15.60	5.00	0.45	249	137	4.60	103
2.20	0.60	150	3.80	11.60	0.33	192	139	3.70	104
*	*	*	*	*	*	*	*	*	*
2.03	0.95	59	18.20	14.80	0.44	278	135	3.70	97
2.34	1.13	169	15.00	12.50	0.37	371	126	3.60	354
1.85	0.40	166	11.80	11.40	0.34	327	132	3.20	100
1.85	0.30	*	8.60	11.40	0.33	136	140	3.00	102
1.85	0.60	37	22.10	14.00	0.40	265	136	3.70	96
2.15	0.95	571	7.80	11.40	0.34	309	135	4.20	107
1.80	0.50	125	12.00	12.00	0.35	205	132	4.80	104
2.40	1.05	64	5.60	9.80	0.30	114	144	4.50	111
2.05	0.65	118	9.20	12.40	0.39	228	135	3.60	1.3

Appendix 4: continued										
Day 5	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5
Serum	Blood	Serum	Blood	Alkaline	AST	ALT	LDH	Bilirubin	Total	Serum
Bicarbonate	Urea	Creatinine	Glucose	Phosphatase	(U/l)	(U/l)	(U/l)	(umol/l)	Protein	Albumin
(mmol/l)	(mmol/l)	(umol/l)	(mmol/l)	(mmol/l)					(g/l)	(g/l)
24	0.70	70	6.90	168	451	244	454	76	73	36
26	4.10	82	5.80	178	28	14	275	16	67	42
24	2.10	71	6.40	134	22	11	460	34	70	33
26	3.40	90	4.80	275	21	16	280	8	68	43
22	4.10	85	6.40	150	28	20	420	34	56	30
23	1.60	70	5.60	69	136	52	628	7	75	46
24	5.50	94	6.30	144	23	28	103	14	70	32
27	5.30	80	5.70	315	20	82	164	15	72	33
24	4.60	72	6.50	140	21	21	244	18	73	47
23	3.00	73	10.10	234	19	25	366	20	612	33
25	3.10	68	7.20	255	70	123	384	18	57	34
24	4.00	80	7.10	320	104	151	571	14	66	40
25	2.30	90	3.70	170	55	43	530	14	61	37
29	4.40	60	4.90	130	18	24	155	9	60	32
22	2.90	45	10.00	485	34	68	320	10	64	35
23	3.40	110	5.80	120	4	26	280	37	62	39
24	2.50	78	4.60	202	18	13	365	14	57	33
24	3.10	75	7.10	1680	118	100	300	145	58	82
22	5.60	70	8.10	650	98	236	440	10	65	42
24	2.90	70	5.20	420	63	89	400	17	62	38
25	2.10	55	7.50	77	20	16	260	310	51	27
28	4.40	80	4.20	1100	21	32	480	8	44	24
22	4.10	80	8.60	270	20	64	290	19	65	60
22	3.50	80	5.00	165	31	56	66	11	65	39
*	*	*	*	*	*	*	*	*	*	*
24	4.60	110	8.40	170	15	13	235	18	64	38
23	2.60	67	4.10	24	28	93	309	23	73	45
23	48.00	110	13.40	102	22	26	130	12	60	36
23	2.00	85	5.30	245	27	84	280	11	61	41
*	*	*	*	*	*	*	*	*	*	*
21	11.00	124	8.50	221	27	15	690	37	60	36
22	3.00	77	7.50	185	12	15	280	13	63	40
22	4.40	80	10.00	113	56	53	550	18	64	37
24	4.40	80	9.00	220	127	38	1330	34	54	34
27	3.60	70	6.10	160	29	17	790	15	56	34
18	2.20	64	6.80	190	19	32	256	24	63	32
21	5.30	86	7.40	290	16	115	46	13	50	32
20	16.00	170	12.60	425	56	48	630	13	42	26
22	75.00	75	5.20	190	170	190	625	53	67	36

Appendix 4: continued									
Day 5	Day 5	Day 5	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6
Serum	Phosphate	GGT	White Cell	Haemoglobin	Haematocrit	Platelet	Serum	Serum	Serum
Calcium		(U/l)	Count	(gm/dl)		Count	Sodium	Potassium	Chloride
(mmol/l)	(mmol/l)		10 9/l			10 9/l	(mmol/l)	(mmol/l)	(mmol/l)
2.17	0.99	*	5.80	10.00	0.36	286	140	3.90	97
2.14	1.03	126	7.70	12.40	0.35	317	139	4.20	100
2.15	0.86	44	11.30	11.40	0.39	268	135	4.30	96
2.45	0.95	20	*	*	*	*	141	4.50	104
2.00	0.45	53	*	*	*	*	*	*	*
2.43	0.81	141	5.90	12.90	0.39	293	135	3.80	101
2.11	0.98	50	5.40	11.70	0.33	240	138	3.60	10.4
2.33	1.08	*	*	*	*	*	*	*	*
2.41	1.08	49	8.30	13.30	0.38	263	136	4.10	102
2.03	1.02	*	5.60	11.40	0.33	222	136	3.40	102
2.31	0.89	125	6.30	12.70	0.37	409	141	4.20	104
2.20	0.65	345	12.00	13.60	0.39	384	141	3.80	105
2.20	1.05	162	6.50	12.50	0.36	337	140	3.80	100
2.40	1.25	*	8.60	13.20	0.38	352	136	3.90	102
2.10	1.00	153	9.80	11.60	0.34	384	135	4.40	101
2.20	0.60	399	6.40	12.00	0.33	267	140	4.20	103
2.30	0.85	36	6.30	13.20	0.40	417	139	4.60	103
2.10	1.05	901	7.70	14.30	0.41	395	137	4.60	103
2.20	1.20	280	6.80	11.30	0.33	227	135	4.30	107
2.05	1.30	36	7.40	11.10	0.34	272	140	4.20	106
2.05	0.85	90	7.10	11.00	0.30	457	140	3.30	106
2.10	1.25	563	12.30	14.80	0.44	378	134	5.00	99
2.35	0.65	92	*	*	*	*	*	*	*
2.25	1.20	37	9.00	12.30	0.37	259	142	5.30	102
*	*	*	*	*	*	*	*	*	*
2.25	1.10	50	9.30	13.80	0.40	212	140	3.90	107
2.36	1.75	97	4.80	11.30	0.34	386	139	3.40	104
2.27	0.81	50	*	*	*	*	*	*	*
2.30	0.95	134	4.60	12.40	0.38	229	139	3.60	106
*	*	*	*	*	*	*	*	*	*
2.28	0.95	76	16.90	13.40	0.41	336	135	3.50	97
2.37	0.95	130	13.00	11.50	0.35	324	136	3.90	104
2.10	0.60	170	12.70	11.10	0.33	274	136	2.50	103
2.05	0.05	*	9.90	11.10	0.32	154	141	3.50	107
2.10	0.55	43	22.20	13.80	0.40	301	135	3.80	94
2.00	0.86	511	8.10	11.60	0.33	351	131	4.50	1.6
2.00	1.05	96	12.40	12.20	0.35	238	130	4.90	103
2.05	0.35	58	2.70	8.90	0.25	111	143	3.70	110
2.10	0.80	125	10.00	11.90	0.40	231	135	3.70	1.3

Appendix 4: continued											
Day 6	Day 6	Day 6	Serum	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6
Serum	Blood	Serum	Blood	Alkaline	AST	ALT	LDH	Bilirubin	Total	Serum	Serum
Bicarbonate	Urea	Creatinine	Glucose	Phosphatase	(U/l)	(U/l)	(U/l)		Protein	Albumin	Calcium
(mmol/l)	(mmol/l)	(umol/l)	(mmol/l)	(mmol/l)					(g/l)	(g/l)	(mmol/l)
24	1.80	74	7.10	267	320	167	208	44	69	35	2.11
25	3.90	88	8.40	181	29	13	270	17	72	40	2.15
23	1.10	73	4.30	135	12	389	389	41	66	27	2.15
23	3.90	75	4.40	290	37	30	280	6	68	43	2.45
*	*	*	*	*	*	*	*	*	*	*	*
23	2.30	75	4.60	73	126	50	538	8	72	43	2.50
29	3.30	80	6.60	265	14	27	320	20	69	39	2.30
*	*	*	*	*	*	*	*	*	*	*	*
20	4.20	90	4.60	137	21	20	248	18	72	47	2.50
23	2.00	70	10.20	250	27	32	40	18	62	32	2.10
23	1.40	65	5.30	260	108	145	430	13	68	38	2.30
26	2.80	85	5.30	345	77	171	450	10	65	39	2.15
23	24.00	85	3.80	41	46	490	7	60	37	2.25	1.25
25	5.40	65	5.60	120	18	23	105	8	63	34	2.35
23	4.60	45	5.40	475	23	52	360	8	68	36	2.30
28	3.90	120	6.30	124	43	22	187	30	63	40	2.20
21	1.90	90	5.20	200	21	12	450	12	56	34	2.30
23	3.80	86	5.10	1200	48	69	300	57	65	36	2.25
23	4.60	75	5.30	550	78	205	425	14	62	35	2.25
27	2.90	75	3.80	500	280	310	490	15	63	37	2.25
25	2.00	35	4.60	760	32	24	276	205	49	26	2.05
25	5.30	85	3.70	1240	29	33	590	7	69	28	2.05
*	*	*	*	*	*	*	*	*	*	*	*
28	3.20	100	6.20	150	29	48	40	13	66	34	2.10
*	*	*	*	*	*	*	*	*	*	*	*
24	4.60	95	8.00	160	11	11	240	13	64	39	2.30
26	2.80	65	5.80	210	20	73	273	16	72	45	2.43
*	*	*	*	*	*	*	*	*	*	*	*
24	2.20	76	5.90	380	60	101	360	7	65	43	2.30
*	*	*	*	*	*	*	*	*	*	*	*
28	13.00	128	7.30	243	24	15	747	24	62	35	2.17
23	2.80	78	7.90	157	8	9	213	12	67	38	2.37
21	4.40	80	9.30	115	113	78	490	17	62	35	2.10
29	3.80	70	9.50	235	134	53	1330	34	54	34	2.05
24	3.80	75	6.30	195	83	19	750	13	57	34	2.20
17	2.30	68	2.50	275	17	33	278	19	64	33	2.23
22	4.10	80	7.20	360	18	95	48	15	5	32	2.05
22	14.20	180	19.50	420	22	34	610	7	41	24	2.05
23	6.00	77	5.80	175	135	155	535	40	66	34	2.10

Appendix 4: continued									
Day 6	Day 6	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7
Phosphate	GGT	White Cell	Haemoglobin	Haematocrit	Platelet	Serum	Serum	Serum	Serum
(mmol/l)	(U/l)	Count	(gm/dl)		Count	Sodium	Potassium	Chloride	Bicarbonate
		10 <sup>9</sup> /l			10 <sup>9</sup> /l	(mmol/l)	(mmol/l)	(mmol/l)	(mmol/l)
0.90	55	5.20	11.70	0.35	257	142	3.50	100	24
1.03	134	6.80	12.00	0.34	325	134	3.90	101	26
0.85	37	7.40	9.60	0.37	283	136	3.80	103	24
0.96	22	8.50	13.20	0.37	222	*	*	*	*
*	*	*	*	*	*	*	*	*	*
0.96	*	*	*	*	*	*	*	*	*
1.10	62	5.60	11.30	0.32	241	133	3.60	105	26
*	*	*	*	*	*	*	*	*	*
1.30	50	9.00	13.00	0.35	266	139	3.80	103	22
1.00	*	6.40	12.60	0.35	295	138	3.40	102	24
0.94	136	7.40	13.00	0.38	506	141	4.50	104	26
0.75	329	11.50	13.20	0.38	355	139	3.60	102	27
*	137	9.90	13.20	0.40	446	140	4.20	101	25
1.05	*	6.00	13.40	0.39	289	135	4.50	102	25
1.50	*	7.30	10.70	0.31	432	135	4.50	100	24
1.02	310	5.30	12.50	0.36	283	138	4.10	102	24
0.95	56	6.60	12.20	0.37	376	140	4.50	102	22
0.90	837	7.90	14.40	0.41	489	143	4.10	104	28
0.95	145	6.30	11.30	0.35	225	138	4.40	105	25
1.15	24	6.30	11.20	0.34	278	132	3.90	97	25
0.80	*	6.30	10.70	0.31	467	132	3.40	96	26
1.35	558	12.40	14.20	0.40	366	135	5.60	97	29
*	*	*	*	*	*	*	*	*	*
1.15	15	15.40	12.00	0.35	254	141	5.20	102	26
*	*	*	*	*	*	*	*	*	*
1.10	44	9.20	13.70	0.40	2.4	136	4.20	99	23
1.28	92	4.90	11.20	0.35	366	140	3.60	106	26
*	*	*	*	*	*	*	*	*	*
1.05	120	4.80	12.20	0.36	239	138	3.50	105	24
*	*	*	*	*	*	*	*	*	*
1.25	75	16.40	13.80	0.42	337	136	4.00	96	27
0.90	115	12.50	12.60	0.36	325	133	3.80	102	22
0.75	179	14.00	10.30	0.30	394	138	2.90	102	23
0.80	*	13.10	10.90	0.32	197	136	3.20	99	27
0.85	42	21.40	13.60	0.41	476	135	3.70	93	23
0.87	479	8.50	12.30	0.35	435	137	4.20	1.5	24
0.75	100	13.00	12.40	0.35	268	133	4.20	98	22
1.45	54	3.10	9.20	0.37	160	142	3.80	110	21
0.85	197	8.40	11.50	0.41	234	136	4.00	102	23

Appendix 4: continued											
Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7
Blood	Serum	Blood	Alkaline	AST	ALT	LDH	Serum	Total	Serum	Serum	Serum
Urea	Creatinine	Glucose	Phosphatase	(U/l)	(U/l)	(U/l)	Bilirubin	Protein	Albumin	Calcium	Phosphate
(mmol/l)	(umol/l)	(mmol/l)	(mmol/l)				(umol/l)	(g/l)	(g/l)	(mmol/l)	(mmol/l)
1.30	75	5.90	14	168	188	288	23	72	32	2.10	0.90
4.00	78	7.50	23	43	20	*	20	79	44	2.32	1.00
2.00	77	6.30	12	12	10	189	16	76	34	2.09	0.96
*	*	*	280	34	38	320	4	70	44	2.43	1.05
*	*	*	*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*	*	*	*
5.30	88	5.90	145	18	16	122	26	71	33	2.34	1.09
*	*	*	*	*	*	*	*	*	*	*	*
4.50	85	5.10	12	19	19	224	12	72	45	2.45	1.36
1.90	60	9.00	265	24	29	370	19	64	33	2.10	1.10
19.00	65	4.30	260	108	145	430	10	70	40	2.40	0.90
2.60	90	6.40	340	67	170	320	7	65	37	2.11	0.76
2.50	95	4.70	180	30	40	465	7	64	39	2.30	1.25
5.60	75	5.60	86	12	25	103	8	62	34	2.26	1.20
4.20	45	5.90	405	20	37	340	7	64	34	2.10	1.50
3.60	110	7.10	195	35	22	105	25	62	39	2.30	0.70
2.30	85	5.00	122	55	28	420	10	56	33	2.10	1.05
3.60	80	4.90	1660	72	78	300	56	67	38	2.35	1.20
4.30	82	4.90	440	32	117	410	13	60	39	2.25	1.15
1.90	90	4.00	510	265	325	510	16	63	37	2.10	0.95
1.10	50	*	750	48	36	195	145	48	25	1.90	1.15
8.20	95	3.90	1200	26	32	470	8	63	28	2.01	1.36
*	*	*	*	*	*	*	*	*	*	*	*
3.30	100	6.00	405	380	420	32	43	67	35	2.30	0.95
*	*	*	*	*	*	*	*	*	*	*	*
4.70	85	10.30	150	9	9	125	12	65	38	2.20	1.00
2.30	69	4.80	192	15	60	225	13	70	43	2.30	1.15
*	*	*	*	*	*	*	*	*	*	*	*
3.00	76	5.50	350	50	120	30	6	57	41	2.30	1.05
*	*	*	*	*	*	*	*	*	*	*	*
12.60	136	6.70	236	24	14	687	23	61	34	2.30	1.15
2.90	76	8.00	155	12	11	198	16	64	39	2.33	1.25
3.80	65	7.60	130	77	74	470	14	64	37	2.15	0.80
4.40	70	8.20	265	111	58	1290	16	55	33	2.00	0.65
3.00	80	5.90	205	24	18	720	12	57	35	2.25	1.75
54.00	75	4.70	170	17	25	502	18	68	37	2.26	0.84
5.00	90	7.20	455	20	70	510	17	52	32	2.00	0.95
9.30	150	11.60	390	16	33	560	4	41	24	2.05	1.05
6.00	7.3	4.00	160	105	140	440	36	67	35	2.15	0.95

Appendix 4: continued									
Day 7	Day 1	Day 1	Day 2	Day 2	Day 3	Day 3	Day 4	Day 4	Day 5
GGT	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	maximum
(U/l)	Pulse Rate	Pulse Rate	Pulse Rate	Pulse rate	Pulse Rate	Pulse Rate	Pulse rate	Pulse Rate	Pulse rate
*	105	74	105	76	100	70	84	72	90
150	110	85	110	80	105	80	96	80	94
*	110	87	90	85	90	80	88	70	86
19	80	68	75	70	85	75	80	68	80
*	100	86	98	82	100	83	100	90	94
*	80	72	85	82	96	76	88	88	80
*	95	80	70	84	100	80	78	71	81
*	79	68	64	60	64	62	70	60	78
41	120	96	115	90	108	80	108	75	106
*	95	90	100	88	95	83	88	80	92
118	120	104	108	100	85	80	85	80	82
334	85	80	100	93	105	88	97	80	98
89	102	70	120	116	113	104	119	108	110
*	82	80	82	80	76	75	94	83	82
84	80	80	105	82	95	87	100	78	130
260	80	48	80	55	98	78	80	78	78
35	90	80	100	85	100	76	99	79	90
758	85	70	80	72	72	68	100	68	82
133	120	90	80	72	105	95	98	90	85
*	94	90	92	80	89	80	90	71	88
*	86	70	96	78	92	80	83	75	84
478	100	95	104	90	109	87	88	75	88
*	96	95	93	80	95	75	80	76	94
16	98	64	98	65	100	68	104	60	85
*	68	56	77	67	72	62	78	63	*
36	76	68	82	75	88	80	90	75	85
82	88	80	85	80	85	80	86	75	84
*	85	72	98	80	95	82	90	90	102
115	90	85	80	70	80	75	78	72	80
*	*	*	*	*	*	*	*	*	*
83	82	70	120	95	120	95	122	96	112
105	110	92	98	90	100	80	105	85	95
185	120	100	124	100	125	94	104	98	114
*	120	110	124	119	128	115	120	100	130
41	120	80	170	133	105	70	100	95	103
345	84	73	120	85	124	94	108	95	102
111	90	70	96	72	100	80	95	82	90
53	110	66	105	78	92	65	98	80	84
88	95	60	120	95	120	100	104	90	101



Appendix 4: continued								
Day 5	Day 6	Day 6	Day 7	Day 7	Day 1	Day 1	Day 2	Day 2
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Pulse Rate	Pulse rate	Pulse Rate	Pulse rate	Pulse rate	Mean Bp	Mean Bp	Mean Bp	Mean Bp
					(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)
70	88	72	90	74	78	72	86	78
78	96	70	96	70	110	76	106	87
72	80	70	85	70	83	70	80	76
70	85	70	85	70	103	80	103	80
80	*	*	*	*	120	80	107	85
70	90	80	*	*	103	77	97	93
68	83	68	84	80	120	50	77	67
64	*	*	*	*	103	83	93	85
76	112	78	108	80	104	86	108	76
82	92	80	90	88	95	93	96	86
80	90	90	85	85	130	121	103	97
82	98	80	97	76	108	87	100	92
100	130	102	104	85	113	109	107	97
75	85	70	85	75	88	87	93	77
100	128	104	120	100	107	92	100	67
60	80	73	83	77	95	63	97	87
72	97	78	83	75	107	97	107	93
73	70	65	82	70	83	73	80	73
80	95	83	80	75	93	80	97	81
78	80	72	75	70	96	80	80	60
70	88	63	81	62	120	77	83	72
78	90	75	90	75	87	73	83	63
80	93	77	87	75	110	108	123	103
65	*	*	*	*	119	90	115	90
*	*	*	*	*	119	63	87	73
75	80	72	82	72	113	103	90	80
75	85	75	80	72	76	73	80	75
80	*	*	*	*	116	83	106	70
67	83	77	80	72	103	93	77	67
*	*	*	*	*	*	*	*	*
100	140	100	120	100	156	101	146	51
80	90	80	85	75	103	97	105	97
105	120	105	120	108	130	123	123	100
100	127	100	120	100	130	97	127	108
84	100	87	90	80	111	93	130	63
84	112	100	124	100	107	90	107	87
80	84	80	87	80	115	93	105	87
80	82	78	80	78	63	53	61	48
93	109	87	105	90	113	90	106	82

Appendix 4: continued								
Day 3	Day 3	Day 4	Day 4	Day 5	Day 5	Day 6	Day 6	Day 7
Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Mean Bp	Mean Bp	Mean Bp	Mean Bp	Mean Bp	Mean Bp	Mean Bp	Mean Bp	Mean Bp
(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)	(mm Hg)
94	70	93	70	87	73	85	72	84
104	78	105	76	104	81	96	87	96
80	72	82	73	79	73	78	77	83
109	78	104	78	103	75	103	78	97
93	82	93	77	86	72	*	*	*
103	80	97	77	103	80	93	78	*
120	77	87	75	90	70	93	87	96
100	80	100	95	96	77	*	*	*
104	83	100	80	96	70	112	73	96
103	90	95	75	90	83	91	88	100
101	83	95	95	95	83	96	77	87
103	90	103	90	100	77	97	73	97
107	87	90	87	93	80	90	87	93
85	83	86	73	87	77	83	73	88
77	50	99	80	96	80	93	73	93
97	93	95	83	95	83	93	83	87
109	86	94	76	96	75	96	75	107
103	83	121	87	101	85	97	85	87
97	83	97	81	104	88	104	83	97
72	66	70	60	82	63	93	60	75
82	77	75	67	90	75	101	72	103
80	63	72	63	72	57	67	60	73
116	100	123	116	120	93	*	*	*
108	84	118	85	105	87	114	90	108
100	77	103	77	*	*	*	*	*
103	91	103	90	97	80	93	78	93
80	76	83	73	80	70	80	73	80
93	76	115	86	117	97	*	*	*
93	78	87	83	87	78	87	83	83
*	*	*	*	*	*	*	*	*
136	112	150	110	132	77	120	100	111
97	84	97	83	103	87	97	83	87
120	98	113	100	113	93	106	93	110
113	100	133	106	129	110	130	110	113
130	97	116	61	96	96	98	93	96
110	83	103	97	107	100	105	87	87
103	83	103	87	97	77	101	73	87
88	33	80	60	70	50	70	60	70
110	93	100	90	103	93	97	87	97

Appendix 4: continued							
Day 7	Day 1	Day 1	Day 2	Day 2	Day 3	Day 3	Day 4
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Mean Bp	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature
(mm Hg)	Degrees C	Degrees C	Degrees C	Degrees C	Degrees C	Degrees C	Degrees C
70	37.10	36.60	37.00	36.20	37.00	36.10	36.00
76	37.00	36.20	37.80	36.00	37.00	36.00	36.80
72	37.30	36.80	37.20	36.40	37.00	36.40	36.70
72	37.00	36.00	36.80	36.50	36.30	36.30	36.80
*	36.50	36.20	37.50	36.30	37.80	35.80	37.80
*	36.30	36.00	36.70	36.50	36.80	36.30	36.70
77	37.80	36.80	38.20	36.80	39.20	37.00	36.90
*	37.80	36.00	32.00	35.70	36.40	35.50	37.00
77	37.00	36.40	36.90	36.30	37.10	36.50	37.20
96	37.60	36.80	38.00	36.90	38.20	37.00	37.80
63	37.10	35.80	37.00	36.50	36.90	36.80	37.80
73	37.10	36.20	38.40	36.40	37.80	36.60	37.70
83	37.30	36.40	38.00	36.70	38.20	37.00	38.00
73	36.80	36.60	36.70	36.00	36.90	36.80	36.70
73	38.00	35.50	36.80	36.20	37.80	36.80	38.00
77	37.50	36.00	37.40	36.90	37.30	36.70	37.20
73	37.10	36.40	37.00	36.20	37.00	36.50	37.20
73	36.80	36.00	36.80	36.30	36.80	36.00	37.60
80	36.50	36.00	36.80	35.70	36.00	35.80	36.80
70	38.30	37.10	37.50	36.60	37.50	36.60	37.50
82	38.50	35.70	38.20	36.80	37.70	36.90	37.40
80	36.50	36.20	37.00	36.20	36.90	36.00	37.70
*	37.20	36.50	37.50	36.00	37.30	36.00	37.70
65	38.00	36.00	38.30	36.00	37.80	36.20	37.50
*	36.60	35.50	36.10	35.80	35.90	35.70	36.90
78	37.00	36.80	37.10	36.70	37.20	36.50	36.90
73	37.00	37.00	36.90	36.50	36.90	36.30	36.50
*	36.50	36.20	36.60	35.60	36.00	35.90	36.70
73	37.00	36.60	37.00	36.10	36.90	36.40	37.20
*	*	*	*	*	*	*	*
93	36.50	36.40	37.80	36.00	37.40	36.60	37.30
83	36.60	36.50	37.30	36.50	38.00	36.40	38.20
100	36.00	36.00	37.60	36.00	37.00	36.00	37.50
90	37.50	37.00	37.00	36.30	37.40	36.50	37.50
87	37.60	36.50	38.00	36.80	38.00	36.50	38.20
83	37.20	37.00	37.20	37.00	36.90	36.80	37.00
73	37.60	36.40	37.80	36.90	37.50	36.50	37.80
61	36.30	36.20	37.30	36.50	37.30	36.20	37.30
93	36.30	36.00	37.90	36.80	38.40	37.10	38.20

Appendix 4: continued							
Day 4	Day 5	Day 5	Day 6	Day 6	Day 7	Day 7	Day 1
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Temperature	Temperature	Temperature	Temperature	Temperature	Temperature	Temperature	Respiratory
Degrees C	Degrees C	Degrees C	Degrees C	Degrees C	Degrees C	Degrees C	Rate
36.30	37.30	36.00	36.90	36.10	37.00	36.80	20
36.20	36.80	36.20	37.00	36.50	37.20	36.30	20
36.70	36.40	36.10	36.80	36.00	36.70	36.00	20
36.20	36.40	36.10	36.80	36.40	36.80	36.50	20
36.50	36.90	36.70	*	*	*	*	20
36.70	37.10	36.70	37.00	36.80	*	*	22
35.90	36.80	36.00	37.00	35.80	36.30	36.00	20
36.20	36.90	36.10	*	*	*	*	22
36.40	37.00	36.90	37.10	36.20	37.00	36.40	20
37.20	37.70	37.20	37.80	36.50	37.00	36.50	22
36.20	36.50	36.50	36.70	36.70	36.80	36.00	20
37.10	37.60	36.00	37.10	36.80	36.40	36.20	20
37.00	38.00	37.70	37.50	37.00	37.30	36.50	20
36.40	36.80	36.20	36.90	36.30	36.80	36.40	20
36.00	37.30	37.00	37.00	35.70	36.50	36.10	20
36.40	37.20	36.80	37.00	36.40	36.90	36.20	22
36.40	37.00	36.60	37.30	36.20	36.80	36.20	22
35.50	36.50	36.00	36.90	36.10	36.80	36.30	22
36.10	37.00	36.40	36.80	36.30	36.80	36.00	20
36.50	37.00	36.50	36.60	36.50	37.00	36.20	22
36.40	37.10	36.50	37.30	36.60	37.30	36.40	20
36.00	37.50	36.00	37.70	36.20	37.50	36.60	28
36.50	36.50	36.10	*	*	*	*	20
36.50	37.50	36.30	37.00	36.80	37.00	36.00	22
36.00	*	*	*	*	*	*	18
36.50	36.80	36.40	36.80	36.30	37.00	36.40	20
36.30	36.30	36.30	36.90	36.40	37.00	36.60	18
36.70	36.50	36.50	*	*	*	*	18
36.60	37.10	36.20	37.10	36.50	37.20	36.70	20
*	*	*	*	*	*	*	*
36.50	37.00	36.70	36.80	36.50	37.00	36.30	22
37.00	37.70	36.60	37.40	36.10	37.30	36.30	22
36.00	38.10	37.70	38.20	37.00	38.50	36.20	22
36.50	38.30	37.20	38.70	37.40	38.20	37.00	22
36.40	37.90	37.30	38.00	37.60	38.40	36.30	20
36.70	37.00	36.70	37.20	36.50	37.00	36.40	22
36.10	37.80	36.00	37.00	36.30	37.60	36.50	22
36.40	36.90	36.00	37.80	36.20	37.50	36.00	36
37.70	37.40	36.40	38.30	37.30	38.20	36.90	20

Appendix 4: continued							
Day 1	Day 2	Day 2	Day 3	Day 3	Day 4	Day 4	Day 5
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Respiratory	Respiratory	Respiratory	Respiratory	Respiratory	Respiratory	Respiratory	Respiratory
Rate	Rate	Rate	Rate	Rate	Rate	Rate	Rate
18	20	18	20	18	20	18	20
16	20	20	18	18	20	18	20
18	18	14	22	18	18	14	20
18	20	18	20	18	20	18	20
18	20	18	20	16	20	18	18
18	20	16	18	16	18	16	18
20	29	24	26	22	20	20	20
20	22	18	20	16	18	16	18
18	20	18	20	16	18	14	18
20	28	23	24	20	22	20	21
18	20	18	22	18	22	20	20
18	20	18	18	16	18	14	18
16	28	24	22	20	22	20	20
18	20	18	18	16	16	14	18
18	20	16	18	16	18	16	18
18	20	16	20	14	22	16	18
18	22	16	20	18	22	16	20
18	22	20	20	18	20	16	18
18	20	18	18	16	18	14	18
22	20	18	20	18	22	20	20
18	20	16	20	16	18	16	18
26	30	24	27	22	24	20	23
18	20	18	20	16	18	16	16
20	22	20	20	18	20	18	20
14	18	16	20	16	22	16	*
18	20	18	20	16	22	18	18
14	22	15	20	16	18	14	16
20	18	16	22	16	18	16	20
20	20	18	22	18	20	20	20
*	*	*	*	*	*	*	*
20	26	20	26	20	26	18	26
20	20	18	20	18	20	18	20
22	22	16	22	20	24	22	26
20	25	22	22	20	36	20	42
18	20	18	22	20	22	20	22
20	24	20	20	20	20	18	20
20	22	20	26	20	22	20	20
34	36	30	28	24	28	24	24
20	24	20	22	20	20	20	20

Appendix 4: continued				
Day 5	Day 6	Day 6	Day 7	Day 7
Minimum	Maximum	Minimum	Maximum	Minimum
Respiratory	Respiratory	Respiratory	Respiratory	Respiratory
Rate	Rate	Rate	Rate	Rate
18	22	18	20	16
16	20	16	22	18
18	20	18	20	14
18	20	18	20	18
16	*	*	*	*
16	20	16	*	*
16	18	14	16	14
16	*	*	*	*
14	18	14	18	16
20	21	20	20	18
16	20	16	18	14
16	18	14	20	16
16	18	16	18	16
16	18	14	20	14
14	18	16	20	14
14	18	14	18	14
16	20	14	18	14
16	18	16	18	16
16	20	16	18	14
20	20	18	28	18
16	25	22	33	23
20	23	20	21	14
14	*	*	*	*
18	22	18	18	16
*	*	*	*	*
16	18	16	20	18
12	20	16	18	16
18	*	*	*	*
18	20	18	18	16
*	*	*	*	*
20	24	18	24	20
16	18	16	18	16
20	22	20	34	20
20	40	32	40	33
18	22	18	20	18
18	20	18	20	18
18	20	18	20	18
22	22	20	22	20
18	22	20	22	18

Appendix 5: APACHE II scores.									
Patient	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1
Number	Temperature	Mean	Pulse Rate	Respiratory	Serum	Serum	Serum	pH	FiO2
	Degrees C	Blood Pressure		Rate	Sodium	Potassium	Creatinine		
4	0	0	0	0	0	0	0	0	0
6	0	2	2	0	0	0	0	0	0
7	0	0	2	0	0	0	0	0	0
8	0	2	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0
10	0	3	0	0	0	0	0	0	0
11	0	0	2	0	0	0	0	0	0
12	0	0	2	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0
14	0	2	2	0	0	0	0	0	0
15	1	0	0	0	0	0	0	0	0
16	0	2	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0
19	0	2	2	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0
24	0	0	2	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0
27	1	2	0	0	0	0	0	0	0
29	0	0	0	1	0	0	0	0	3
30	0	2	0	0	0	1	0	0	1
33	0	2	2	0	0	1	0	0	0
34	1	2	0	0	0	0	0	0	0
35	0	2	2	0	0	1	0	0	1
38	0	0	0	0	0	0	0	0	0
39	0	2	0	0	0	0	0	0	0
40	0	0	0	0	0	1	0	0	0
								0	
41	0	3	0	0	0	0	3	3	4
42	0	0	2	0	0	0	0	0	0
43	0	2	2	0	0	0	0	0	0
45	0	2	2	0	0	0	0	1	1
46	0	2	2	0	0	0	0	0	0
49	0	2	2	0	0	1	0	3	0
50	0	2	0	0	0	0	0	0	0
53	0	2	0	0	0	0	0	0	1
54	0	3	2	3	0	4	3	4	0
55	0	2	0	0	0	0	0	1	0

Appendix 5 continued: APACHE II scores.									
Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 2
1FiO2/O2	Bicarbonate	Haematocrit	White cell	Glasgow	Physiology	Age	Chronic	Total	Temperature
Ratio			count	Coma	Score		Health	Score	
				Score			Score		
0	0	0	0	0	0	2	0	2	0
0	0	0	0	0	4	3	0	7	0
0	0	0	0	0	2	2	0	4	0
0	0	0	0	0	2	0	0	2	0
0	0	2	1	0	3	5	0	8	0
0	0	0	0	0	3	0	0	3	0
0	0	0	1	0	3	6	0	9	0
0	0	0	0	0	2	3	0	5	0
0	0	0	0	0	0	2	0	2	1
0	0	0	0	0	4	6	0	10	0
0	0	1	0	0	2	2	0	4	0
0	0	0	0	0	2	0	0	2	0
0	0	1	0	0	1	0	0	1	0
0	0	1	0	0	1	0	0	1	0
0	0	0	1	0	5	5	0	10	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	3	0	3	0
0	0	0	0	0	0	5	0	5	0
0	0	0	0	0	2	6	0	8	1
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	3	3	5	11	0
0	0	0	0	0	4	5	0	9	0
0	0	0	0	0	4	5	0	9	0
0	0	0	0	0	5	6	0	11	0
0	0	0	0	0	3	2	0	5	1
0	0	1	0	0	7	3	0	10	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	2	0	0	2	1
0	0	0	0	0	1	2	0	3	0
0	0				0			0	
0	0	2	0	0	15	3	0	18	*
0	0	1	3	0	6	2	0	8	0
0	0	0	1	0	5	2	0	7	0
0	0	1	0	0	7	2	0	9	0
0	0	2	1	0	7	0	0	7	0
0	0	0	3	0	11	0	0	11	0
0	0	0	0	0	2	3	0	5	0
0	0	0	1	0	4	5	0	9	0
0	0	2	1	0	22	2	0	24	0
0	0	0	0	0	3	2	0	5	0



Appendix 5 continued: APACHE II scores.									
Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2
Median	Blood Pressure	Respiratory	Serum	Serum	Serum	pH	FI02	1FI02/O2	Bicarbonate
Pulse Rate		Rate	Sodium	Potassium	Creatinine			Ratio	
0	0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	3	0	0	0	0	0	0	0	0
2	0	2	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	0	0
0	3	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
2	2	2	0	0	0	0	0	0	0
0	0	0	0	0	2	0	0	0	0
0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	2	0	0	0	0
0	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	3	0	0
0	2	0	0	0	0	0	1	0	0
2	2	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0
0	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
2	3	2	0	0	0	0	0	0	0
0	3	0	0	0	0	0	0	0	0
0	3	0	0	0	0	0	1	0	0
2	3	2	0	0	0	0	0	0	0
2	2	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	1	0	0
0	3	3	0	0	3	0	0	0	0
2	2	0	0	0	0	0	0	0	0

Appendix 5 continued: APACHE II scores.									
Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 3	Day 3	Day 3
Haematocrit	White cell count	Glasgow Coma Score	Physiology Score	Age	Chronic Health Score	Total Score	Temperature	Blood Pressure	Median Pulse Rate
0	0	0	0	2	0	2	0	0	0
0	0	0	4	3	0	7	0	0	0
0	0	0	0	2	0	2	0	0	0
1	1	0	2	0	0	2	0	0	0
0	0	0	2	5	0	7	1	0	0
0	0	0	0	0	0	0	0	0	0
1	0	0	4	6	0	10	3	2	0
0	0	0	4	3	0	7	0	0	2
0	0	0	3	2	0	5	0	0	0
0	0	0	2	6	0	8	0	0	0
0	0	0	3	2	0	5	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	6	0	0	6	0	0	0
0	0	0	2	0	0	2	0	0	0
0	1	0	1	5	0	6	0	2	0
0	0	0	2	0	0	2	0	0	0
0	0	0	0	3	0	3	0	0	0
0	0	0	0	5	0	5	0	0	2
0	0	0	1	6	0	7	1	0	0
0	0	0	2	0	0	2	0	0	0
0	0	0	2	3	5	10	0	0	0
0	0	0	3	5	0	8	0	2	0
0	0	0	3	5	0	8	0	2	0
0	0	0	4	6	0	10	0	0	2
0	0	0	5	2	0	7	1	0	2
1	0	0	3	3	0	6	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	3	0	0	3	1	0	0
2	0	0	2	2	0	4	0	0	0
			0						
*	*	*	*	*	*	*	*	0	*
2	1	0	10	2	0	12	0	3	2
0	1	0	4	2	0	6	0	0	0
1	0	0	5	2	0	7	0	2	2
2	1	0	10	0	0	10	0	2	2
2	2	0	8	0	0	8	0	3	0
0	0	0	2	3	0	5	0	2	2
0	1	0	6	5	0	11	0	0	0
2	1	0	12	2	0	14	0	4	2
0	0	0	4	2	0	6	0	2	2



Appendix 5 continued: APACHE II scores.									
Day 3	Day 3	Day 3	Day 3	Day 3	Day 4	Day 4	Day 4	Day 4	Day 4
Glasgow	Physiology	Age	Chronic	Total	Temperature	Blood Pressure	Median	Respiratory	Serum
Coma	Score		Health	Score			Pulse Rate	rate	Sodium
Score			Score						
0	0	2	0	2	0	0	0	0	0
0	0	3	0	3	0	0	0	0	0
0	0	2	0	2	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	2	5	0	7	0	0	2	0	0
0	0	0	0	0	0	0	0	0	0
0	7	6	0	13	1	0	0	0	0
0	2	3	0	5	0	0	2	0	0
0	3	2	0	5	0	0	0	0	0
0	0	6	0	6	0	0	0	0	0
0	0	2	0	2	0	0	0	0	0
0	1	0	0	1	0	0	0	0	0
0	1	0	0	1	0	0	3	0	0
0	0	0	0	0	0	0	0	0	0
0	3	5	0	8	0	0	0	0	0
0	1	0	0	1	0	0	0	0	0
0	0	3	0	3	0	0	0	0	0
0	2	5	0	7	1	2	2	0	0
0	1	6	0	7	0	0	0	0	0
0	0	0	0	0	0	2	0	0	0
0	0	3	5	8	0	2	0	0	0
0	7	5	0	12	0	2	0	0	0
0	3	5	0	8	0	2	0	0	0
0	2	6	0	8	0	2	2	0	0
0	3	2	0	5	0	0	2	0	0
0	0	3	0	3	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	1	0	0	1	0	2	0	0	0
0	0	2	0	2	0	0	0	0	0
0	0			0					
*	*	*	*	*	*	*	*	*	*
0	11	2	0	13	0	3	2	1	2
0	0	2	0	2	0	0	0	0	0
0	6	2	0	8	0	2	0	0	0
0	4	0	0	4	0	3	2	3	0
0	6	0	0	6	0	2	0	0	0
0	4	3	0	7	0	0	0	0	0
0	4	5	0	9	0	0	0	0	0
0	10	2	0	12	0	2	0	1	0
0	5	2	0	7	0	0	0	0	0

Appendix 5 continued: APACHE II scores.									
Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4
Serum	Serum	pH	FI02	1FI02/O2	Bicarbonate	Haematocrit	White cell	Glasgow	Physiology
Potassium	Creatinine			Ratio			count	Coma	Score
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	3
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	2
0	0	0	4	0	0	0	0	0	4
0	0	0	0	0	2	0	0	0	2
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	3
0	2	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	5
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	2	0	0	0	4
0	0	0	3	0	0	0	0	0	5
1	0	0	0	0	2	0	0	0	5
0	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	1	0	1
1	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0
		0	0	0					0
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	1	0	9
0	0	0	0	0	2	0	1	0	3
1	0	0	3	0	0	0	0	0	6
0	0	0	0	0	0	0	0	0	8
0	0	0	0	0	0	0	2	0	4
0	0	0	0	0	2	0	0	0	2
0	0	0	1	0	2	0	0	0	3
0	2	0	0	0	2	0	0	0	7
1	0	0	0	0	0	0	0	0	1

Appendix 5: APACHE II scores.									
Day 4	Day 4	Day 4	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5	Day 5
Age	Chronic	Total	Temperature	Blood Pressure	Median	Respiratory	Serum	Serum	Serum
Health	Score	Score			Pulse Rate	rate	Sodium	Potassium	Creatinine
Score									
2	0	2	0	0	0	0	0	0	0
3	0	3	0	0	0	0	0	0	0
2	0	2	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
5	0	8	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
6	0	7	0	0	2	0	0	0	0
3	0	5	0	0	2	0	0	0	0
2	0	6	0	0	0	0	0	0	0
6	0	8	0	0	0	0	0	1	0
2	0	2	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	3	0	0	2	0	0	0	0
0	0	2	0	0	0	0	0	0	0
5	0	5	0	0	2	0	0	0	0
0	0	0	0	0	2	0	0	0	0
3	0	3	0	0	0	0	0	0	0
5	0	10	0	0	0	0	0	0	0
6	0	6	0	0	0	0	0	0	0
0	0	2	0	2	0	0	0	0	0
3	5	12	0	0	0	0	0	1	2
5	0	10	0	0	0	0	0	0	0
5	0	10	0	2	0	0	0	0	0
6	0	10	0	0	2	0	0	0	0
2	0	4	*	*	*	*	*	*	*
3	0	4	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0
0	0	2	0	2	0	0	0	0	0
2	0	2	0	0	2	0	0	0	0
		0							
*	*	*	*	*	*	*	*	*	*
2	0	11	0	3	2	0	0	0	0
2	0	5	0	0	0	0	2	0	0
2	0	8	0	2	2	0	0	1	0
0	0	8	0	2	2	2	0	1	0
0	0	4	0	0	0	0	0	0	0
3	0	5	0	0	0	0	0	0	0
5	0	8	0	0	0	0	0	0	0
2	0	9	0	2	0	0	0	0	2
2	0	3	0	0	0	0	0	0	0

Appendix 5 continued: APACHE II scores.										
Day 5 pH	Day 5 FIO2	Day 5 1FIO2/O2 Ratio	Day 5 Bicarbonate	Day 5 Haematocrit	Day 5 White cell count	Day 5 Glasgow Coma Score	Day 5 Physiology Score	Day 5 Age	Day 5 Chronic Health Score	Day 5 Total Score
0	0	0	0	0	0	0	0	2	0	2
0	0	0	0	0	0	0	0	3	0	3
0	0	0	0	0	0	0	0	2	0	2
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	5	0	5
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	2	6	0	8
0	0	0	0	0	0	0	2	3	0	5
0	1	0	0	0	0	0	1	2	0	3
0	0	0	0	0	0	0	1	6	0	7
0	0	0	0	0	0	0	0	2	0	2
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	2	0	0	2
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	2	5	0	7
0	0	0	0	0	0	0	2	0	0	2
0	0	0	0	0	0	0	0	3	0	3
0	0	0	0	0	0	0	0	5	0	5
0	0	0	0	0	0	0	0	6	0	6
0	0	0	0	0	0	0	2	0	0	2
0	0	0	0	0	0	0	3	3	5	11
0	4	0	0	0	0	0	4	5	0	9
0	0	0	0	0	0	0	2	5	0	7
0	0	0	0	0	0	0	2	6	0	8
*	*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	3	0	3
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	3	0	0	3
0	0	0	0	0	0	0	2	2	0	4
0	0	0					0			0
*	*	*	*	*	*	*	*	*	*	*
0	0	0	2	1	0	0	8	2	0	10
0	0	0	0	1	0	0	3	2	0	5
1	1	0	0	0	0	0	7	2	0	9
0	0	0	0	0	0	0	7	0	0	7
0	0	0	0	2	0	0	2	0	0	2
0	0	0	2	0	0	0	2	3	0	5
0	1	0	0	0	0	0	1	5	0	6
0	0	0	0	0	0	0	4	2	0	6
0	0	0	0	0	0	0	0	2	0	2

Appendix 5 continued: APACHE II scores.									
Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6
Temperature	Blood Pressure	Median	Respiratory	Serum	Serum	Serum	pH	FiO2	1FiO2/O2
		Pulse Rate	rate	Sodium	Potassium	Creatinine			Ratio
0	0	0		0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
0	2	2	0	0	0	0	0	3	0
0	0	2	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	0	2	0	0	0	2	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	2	3	0	0	0	0	0	0	0
0	0	0	1	0	1	2	0	0	0
0	2	0	0	0	0	0	0	4	0
*	*	*	*	*	*	*	*	*	*
0	2	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
0	2	3	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	1	0
1	3	2	3	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0
0	2	0	0	0	0	2	0	0	0
0	0	0	0	0	0	0	0	0	0



Appendix 5 continued: APACHE II scores.								
Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 6	Day 7
Bicarbonate	Haematocrit	White cell count	Glasgow Coma Score	Pysiology Score	Age	Chronic Health	Total	Temperature
			Score			Score		
0	0	0	0	0	2	0	2	0
0	0	0	0	0	3	0	3	0
0	0	0	0	0	2	0	2	0
*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	0
0	0	0	0	1	6	0	7	0
*	*	*	*	*	*	*	*	*
0	0	0	0	7	2	0	9	0
0	0	0	0	3	6	0	9	0
0	0	0	0	0	2	0	2	0
0	0	0	0	0	0	0	0	0
0	0	0	0	2	0	0	2	0
0	0	0	0	0	0	0	0	0
0	0	0	0	5	5	0	10	0
0	0	0	0	0	0	0	0	0
2	0	0	0	4	3	0	7	0
0	0	0	0	0	5	0	5	0
0	0	0	0	0	6	0	6	0
0	0	0	0	5	0	0	5	0
0	0	0	0	4	3	5	12	0
0	0	0	0	6	5	0	11	0
*	*	*	*	*	*	*	*	*
0	0	0	0	2	6	0	8	0
*	*	*	*	*	*	*	*	*
0	0	0	0	0	3	0	3	0
0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*
0	0	0	0	0	2	0	2	0
0				0			0	
*	*	*	*	*	*	*	*	*
0	0	1	0	6	2	0	8	0
0	0	0	0	0	2	0	2	0
0	0	0	0	3	2	0	5	1
0	0	0	0	9	0	0	9	0
0	0	2	0	2	0	0	2	0
3	0	0	0	5	3	0	8	0
0	0	0	0	1	5	0	6	0
0	2	2	0	8	2	0	10	0
0	0	0	0	0	2	0	2	0

Appendix 5 continued: APACHE II scores.									
Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7
Blood Pressure	Median	Respiratory	Serum	Serum	Serum	pH	FI02	1FI02/O2	Bicarbonate
	Pulse Rate	rate	Sodium	Potassium	Creatinine			Ratio	
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	1	0	0
0	0	0	0	1	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	0	0	0	0
0	2	3	0	0	0	0	0	0	0
0	0	0	0	1	2	0	0	0	0
0	0	0	0	0	0	0	4	0	0
*	*	*	*	*	*	*	*	*	*
2	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
*	*	*	*	*	*	*	*	*	*
0	0	0	0	0	0	0	0	0	0
						0	0	0	0
*	*	*	*	*	*	*	*	*	*
2	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
2	2	1	0	0	0	0	1	0	0
2	2	3	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	2	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0
2	0	0	0	0	2	0	0	0	0
0	0	0	0	0	0	0	0	0	0

Appendix 5 continued: APACHE II scores.						
Day 7	Day 7	Day 7	Day 7	Day 7	Day 7	Day 7
Haematocrit	White cell	Glasgow	Physiology	Age	Chronic	Total
count	count	Coma	Score		Health	
		Score			Score	
0	0	0	0	2	0	2
0	0	0	0	3	0	3
0	0	0	0	2	0	2
*	*	*	*	*	*	*
*	*	*	*	*	*	*
0	0	0	0	0	0	0
0	0	0	0	6	0	6
*	*	*	*	*	*	*
0	0	0	1	2	0	3
0	0	0	1	6	0	7
0	0	0	2	2	0	4
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	4	5	0	9
0	0	0	0	0	0	0
0	0	0	0	3	0	3
0	0	0	0	5	0	5
0	0	0	2	6	0	8
0	0	0	5	0	0	5
0	0	0	3	3	5	11
0	0	0	4	5	0	9
*	*	*	**	*	*	*
0	1	0	3	6	0	9
*	*	*	*	*	*	*
0	0	0	0	3	0	3
0	0	0	0	0	0	0
*	*	*	*	*	*	*
0	0	0	0	2	0	2
			0			0
*	*	*	*	*	*	*
0	1	0	5	2	0	7
0	0	0	0	2	0	2
0	0	0	7	2	0	9
0	0	0	8	0	0	8
0	2	0	2	0	0	2
0	0	0	2	3	0	5
0	0	0	1	5	0	6
0	0	0	4	2	0	6
0	0	0	0	2	0	2