THE AMYLASE CREATININE CLEARANCE RATIO

by

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SUMMARY

In the majority of cases the diagnosis of acute pancreatitis in man requires confirmation from biochemical estimations and serum amylase is at present the commonest single biochemical test used to confirm this diagnosis. Hyperamylasaemia is regarded as the biochemical yardstick for acute pancreatitis despite very definite limitations which may not be recognised by all clinicians. It has been clearly demonstrated that acute pancreatitis may be present without hyperamylasaemia, either due to a very severe inflammatory response which renders the pancreas unable to excrete amylase or, conversely, to a mild attack in which serum amylase is never elevated or returns to normal rapidly. It is also well recognised that hyperamylasaemia is not specific for acute pancreatitis, having been documented in several other acute abdominal conditions which may enter the differential diagnosis of acute pancreatitis. This significantly reduces the accuracy of serum amylase estimations in making the diagnosis of acute pancreatitis and also severely limits its sensitivity in detecting mild or sub-clinical attacks of pancreatic inflammation associated with small elevations in serum amylase.

In 1969 the amylase creatinine clearance ratio (ACCR) was proposed as a simple diagnostic test for acute

pancreatitis. The ACCR is the renal clearance of amylase expressed as a percentage of the simultaneous creatinine clearance and can be calculated from 10 ml samples of venous blood and urine. The ACCR gives the information of an amylase clearance study without requiring a timed collection of urine and therefore can be used as a screening test for pancreatitis in the acute situation. The initial evidence from the United States suggested that the ACCR may be an accurate and sensitive diagnostic test for acute pancreatitis and that a significant elevation of this ratio might be specific for this condition. This evidence was based on only three clinical studies with one further study questioning the specificity of an elevated ACCR for acute pancreatitis. At this time no work had been published from Britain or Europe, both areas of the world where the aetiology of acute pancreatitis often differs from that found in the United States. The aims of the studies in this thesis were to evaluate the role of the ACCR as a diagnostic test in British patients with acute pancreatitis and to attempt to demonstrate whether or not a significant elevation in the ACCR is related to pancreatic inflammation.

In two studies in this thesis a significant elevation in the ACCR was found to be 94% accurate in diagnosing acute pancreatitis confirmed by other methods and the

ACCR was found to be the most successful single non-invasive diagnostic test for this condition. No patient without acute pancreatitis was found to have an ACCR above the upper limit of normal, including patients with acute abdominal conditions other than acute pancreatitis who had hyperamylasaemia. The ACCR was also found to have prognostic significance in acute pancreatitis being significantly related to the severity of each attack as defined by established prognostic factors and retrospective analysis of each patient's illness. An admission ACCR of over 10% in a patient with acute pancreatitis was found to be highly suggestive of a severe attack with a mortality greater than 50%. The ACCR was found to remain abnormal for longer than serum amylase in acute pancreatitis and was judged to be of value in diagnosing acute pancreatitis in patients presenting to hospital with abdominal pain of over 48 hours duration.

Post-cholecystectomy pancreatitis is well documented and significant ACCR elevations have been recorded after biliary surgery. A study was designed to confirm significant ACCR elevations after biliary surgery and demonstrate that this elevation was related to pancreatic inflammation. Intravenous Trasylol, a proteolytic enzyme inhibitor, was infused before, during and after

biliary surgery in a group of patients and was found to significantly reduce the incidence of significant ACCR elevation on the first postoperative day. It has been shown in animal experiments that Trasylol can significantly modify the severity of acute pancreatitis when given prior to the induction of the pancreatitis and its failure to significantly influence the course of established acute pancreatitis in humans is almost certainly due to the fact that it is given once the inflammatory process is established. In this study it was possible to administer Trasylol prophylactically with results in keeping with the hypothesis that post-biliary surgery ACCR elevation is due to pancreatic inflammation.

Acute pancreatitis and elevated ACCR values have also been recorded after cardiac surgery performed using non-pulsatile cardiopulmonary bypass. The pancreatitis developing in this situation is thought to be ischaemic in origin and it has recently been shown that pulsatile cardiopulmonary bypass can improve tissue perfusion and the functioning of several organs during bypass. ACCR changes during and after cardiac surgery performed using either non-pulsatile bypass or pulsatile bypass were studied. Significant ACCR elevation was observed in 9 out of 10 patients using non-pulsatile bypass but

only 1 out of 10 patients using pulsatile bypass. The ACCR changes associated with cardiac surgery performed using cardiopulmonary bypass can therefore be significantly altered by the mode of perfusion employed. The evidence from this study suggests that the significant reduction in ACCR elevation during and after pulsatile bypass is related to normalisation of pancreatic function resulting from improved tissue perfusion.

In the light of the preceding evidence suggesting that an elevated ACCR in the absence of hyperamylasaemia may detect sub-clinical pancreatic inflammation, it was decided to screen the administration of Cimetidine for a possible relationship with acute pancreatitis as suggested in the literature. Acute and chronic administration of Cimetidine was tested and a small but significant elevation in the mean ACCR of patients was observed during the first 24 hours of intravenous Cimetidine therapy. All other factors detrimental to the pancreas appeared to be matched with the control group in this study and this finding may represent a genuine short term effect of Cimetidine on the pancreas.

The well documented increase in amylase clearance during the recovery phase of acute pancreatitis, when serum amylase levels are normal, has been shown to occur as a result of reduced amylase reabsorption in the renal tubules which is thought to be secondary to

an as yet unidentified factor released by the pancreas. It has been demonstrated in this thesis that the ACCR, which measures amylase clearance, is significantly correlated with complement activation via the classical pathway in acute pancreatitis. Trypsin is known to activate complement via the classical pathway and it is therefore probable that Trypsin is responsible for the change in renal tubular reabsorption of amylase seen in acute pancreatitis.

The ACCR has therefore been shown to be a simple, accurate and sensitive test for pancreatic inflammation which has a definite role to play in the diagnosis of acute pancreatitis at the present time. The ACCR should complement serum amylase values in the diagnosis of acute pancreatitis and allow a more accurate interpretation of hyperamylasaemia both above and below the diagnostic level for acute pancreatitis. This thesis has provided evidence that the ACCR can detect the presence of pancreatitis in the absence of hyperamylasaemia and this may lead to an increased use of the ACCR as a screening test for pancreatitis in situations where it may arise as a complication of treatment unrelated to the pancreas. Care must be taken, however, in the interpretation of ACCR values in the absence of hyperamylasaemia since there is no

evidence that a significantly elevated ACCR is specific for acute pancreatic inflammation alone. The ACCR has been shown to be of prognostic value in an established attack of acute pancreatitis and its use alone, or in combination with other prognostic factors, may lead to patients with potentially severe acute pancreatitis receiving appropriate therapy at an earlier stage in their illness.

C H A P T E R O N E

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1. THE DIAGNOSIS OF ACUTE PANCREATITIS

In 1929 the criteria for accepting a diagnosis of acute pancreatitis began to change from clinical judgement to clinical suspicion confirmed by laboratory results. In that year Elman and his associates published a paper entitled "Value of blood amylase estimations in the diagnosis of pancreatic disease; a clinical study" and convincingly demonstrated an association between an abnormal elevation of serum amylase and acute pancreatitis (1). Not surprisingly this lead to a progressive increase in the frequency with which acute pancreatitis was diagnosed and to a lesser extent this increase in diagnostic frequency continues today (2,3). In 1833 Payen and Persoz first precipitated a starch splitting product from malt which they named diastase (4). A similar substance was found in the blood in 1846 by Magendie and named amylase after the Greek word 'amylon' meaning starch (5). Amylase was first measured quantitatively in animals by Foster in 1867 (6) and in 1916 Stocks suggested that amylase activity in blood and urine might be a sensitive and reliable test for various pancreatic disorders (7).

Since 1929 numerous diagnostic tests for acute pancreatitis have been proposed and evaluated to a lesser or greater extent. Despite this, serum amylase remains the single test most commonly used to diagnose acute pancreatitis throughout the World. It is clear, however, that the measurement of serum amylase in acute pancreatitis has been so valuable that clinicians have frequently lost sight of its limitations (8). To date these limitations have not been fully overcome by any other test. The search for a simple test to take over from serum amylase in the diagnosis of acute pancreatitis has been hindered however by the stranglehold serum amylase estimation has over this diagnosis. Without a separate "gold standard" diagnosis which can be readily applied it is difficult to show that any one test is better than serum amylase alone when serum amylase is the normal yardstick used to diagnose acute pancreatitis. This thesis sets out to examine the role of the amylase—creatinine clearance ratio (ACCR) as a simple diagnostic test for acute pancreatic dysfunction including clinically evident acute pancreatitis. Before setting out the evidence accumulated in these studies it is relevant to discuss the presently accepted biochemical tests considered diagnostic for acute pancreatitis in order to highlight their merits and weaknesses.

2. SERUM AMYLASE

Serum amylase is usually elevated within 2-12 hours of the onset of acute pancreatitis and commonly returns to normal within 3 or 4 days. The response is more prompt and of greater magnitude if the gland is secreting at the time of injury (8). Pancreatic amylase reaches the blood stream via three routes; venous, lymphatic and peritoneal, the total absorption of amylase being greatest through the lymphatics (9,10,11). It has been shown to be impossible to predict the extent of pancreatic damage from the degree of abnormality of the serum amylase, (12).

Although serum amylase is usually elevated in acute or relapsing acute pancreatitis, it is also often elevated in conditions other than acute pancreatitis, a fact which limits the specificity of the test (13). Table 1 lists 20 documented causes of hyperamylasaemia occurring without evidence of acute pancreatitis. Because of these findings the minimum serum amylase level acceptable as diagnostic of acute pancreatitis is usually 4-5 times the upper limit of the normal range. This creates a 'grey area' between these two values in which a diagnosis of acute pancreatitis cannot be made or discarded with any certainty.

TABLE 1

Causes of hyperamylasaemia without acute pancreatitis.

1. Biliary tract disease
2. Perforated peptic ulcer
3. Intestinal obstruction
4. Ruptured ectopic pregnancy
5. Mesenteric infarction
6. Afferent loop syndrome
7. Dissecting aortic aneurysm
8. Peritonitis
9. Acute appendicitis
10. Pancreatic carcinoma
11. Cerebral trauma
12. Burns
13. Post-operative hyperamylasaemia
14. Diabetic ketoacidosis
15. Renal transplantation
16. Pneumonia
17. Acquired bisalbuminaemia
18. Prostatic disease
19. Pregnancy
20. Drugs

Serum amylase values confirm the diagnosis of acute pancreatitis in only 75-80% of cases (13). In some patients with acute pancreatitis the serum amylase rapidly falls to normal, or within the grey area, before a blood specimen is taken; in others gland destruction is so complete that the serum amylase never rises, while in a third group of patients the presentation of acute pancreatitis may be so atypical that the serum amylase is not estimated. The lack of specificity of hyperamylasaemia for acute pancreatitis undoubtedly reduces the tests sensitivity in making this diagnosis. It must be remembered that hyperamylasaemia does not provide a "yes" or "no" diagnosis of acute pancreatitis in 100% of cases but simply indicates the statistical probability of the presence of acute pancreatitis. At the upper end of the normal range of serum amylase values this probability is around 5%, rising to 75% or 80% at the lowest level considered diagnostic for acute pancreatitis. The probability of the presence of acute pancreatitis rises from there but due to the lack of specificity of hyperamylasaemia never reaches 100%.

3. URINE AMYLASE

Hyperamylasuria per se is not diagnostic of acute pancreatitis and may be seen in most of the disorders

associated with hyperamylasaemia listed in Table 1. In some of these conditions only urine amylase may be elevated with values in the range expected in acute pancreatitis (14). The level of amylase in the urine is significantly influenced by the excretory function of the kidneys and levels may vary considerably from hour to hour (13). These changes are accentuated in acute illnesses such as acute pancreatitis and result in a wide scatter of results in both normal and abnormal patients. Thus the sensitivity as well as the specificity of a single urine amylase estimation for the diagnosis of acute pancreatitis is poor and urine amylase results have been used mainly to calculate amylase clearance values. It has been noted however that the elevation of urine amylase in acute pancreatitis usually lags behind the serum level, since urinary levels may be still elevated for 7-10 days after serum levels have returned to normal (15,16,17).

4. AMYLASE ISOENZYMES

The amylase in serum and urine can be fractionated into two principle isoenzymes, the pancreatic type (p-type isoamylase) and the salivary type (s-type isoamylase) (18). There is now excellent evidence that the p-type isoamylase in serum and urine comes from the pancreas (18,19,20) and isoenzyme determination

holds considerable promise for the future (19,21,22). At present separation of isoenzymes requires polyacrylamide gel electrophoresis, chromatographic techniques or isoelectric focusing, all of which are sophisticated and complicated techniques not readily available in most hospitals. This therefore limits the clinical application of isoamylase estimations at the present time and renders them useless in the acute situation where a rapid result is required. The theoretical advantages of measuring the pancreatic isoenzyme of amylase are clear and the full investigation of the clinical relevance of these measurements only awaits the development of simplified routine analytical procedures.

5. AMYLASE CLEARANCE

Numerous investigators have demonstrated that the measurement of urine amylase excretion in acute pancreatitis is a more sensitive index of the disease than the serum amylase, rising relatively earlier and higher and persisting at abnormal levels for longer (15,16,17,23,24). Amylase clearance per se may be elevated above the normal range of 2.7 - 3.1 ml/min in hyperamylasaemia from any source although a level greater than 4 ml/min is usually associated with acute

pancreatitis (25). Amylase clearance measurements do have a place in helping to confirm the diagnosis of acute pancreatitis in patients whose serum amylase values just fail to reach the minimum diagnostic level for the disease (26). The main drawback to the routine use of amylase clearance is the fact that a timed urine collection is required to calculate the results. There is evidence that a short period of urine collection, i.e. 2-4 hours is just as accurate as a 12 or 24 hour collection in acute pancreatitis (27), but even this imposes definite restrictions on the use of the test in the acute situation. For this reason amylase clearance has not been used as a diagnostic screening test in many units treating acute pancreatitis. The amylase creatinine clearance ratio (ACCR) provides a means of calculating the renal clearance of amylase without the need for a timed collection of urine and will be discussed in the next chapter.

6. LIPASE

Serum lipase has long been recognised as a useful clinical measurement in acute pancreatitis and it has been appreciated for years that serum lipase tends to remain abnormal in acute pancreatitis longer than does serum amylase (28). Lipase determination in

pancreatic disease has, however, not been used extensively because of methodological problems associated with the Cherry-Crandall assay which requires an incubation period of 24 hours, thereby rendering the test totally unsuited to the acute situation. It has been recognised that, like amylase, abnormal serum lipase values occur in various peripancreatic diseases and it is not yet clear whether the pancreas is the source of the elevated lipase in these cases (8). In 1974 a modification of a 5 minute turbidimetric determination of lipase was described and used to evaluate the diagnostic value of lipase in acute pancreatitis (29). Serum lipase was found to be elevated in 63% of the patients with acute pancreatitis, amylase was elevated in 70%, while one or other of the two enzymes was elevated in 83%. The diagnostic yield in acute pancreatitis may therefore be increased by measuring both serum amylase and lipase. More evidence to support or refute this finding should be available in the near future following the sophistication of improved assay techniques for lipase.

7. PHOSPHOLIPASE, TRYPSIN AND DEOXYRIBONUCLEASE

These pancreatic enzymes are not commonly measured and changes occurring in acute pancreatitis have not been well documented. Serum phospholipase rises in

acute pancreatitis along with serum lipase and amylase but the particular situations in which one enzyme might be preferred to another for diagnostic use awaits further evaluation (8). Serum trypsin activity also increases during an attack of acute pancreatitis but its specificity awaits evaluation in studies in various clinical states that enter into the differential diagnosis of acute pancreatitis. Its diagnostic sensitivity must also be compared with other established enzyme measurements in a large number of patients with acute pancreatitis. These steps in the evaluation of the usefulness of trypsin estimation in acute pancreatitis await a simplification of the analytical technique (8). Serum deoxyribonuclease at present also requires a complicated analysis for its estimation but interest in this enzyme has been stimulated by claims that it is only elevated in pancreatic disease (30), and that elevated levels specifically reflect pancreatic necrosis (31). These claims require full investigation and at present deoxyribonuclease estimation is not regarded as a routine diagnostic test in acute pancreatitis.

The aim of the work presented in this thesis was to evaluate the amylase creatinine clearance ratio in a clinical context and assess its role as a diagnostic test for acute pancreatic dysfunction and clinically evident acute pancreatitis. The studies have been

presented in chronological order and each project has been discussed separately using the knowledge available in the literature at the time of completing the study. In the final chapter (Chapter 8) the results and implications of each study are discussed in the light of present knowledge and the findings from all the studies integrated to produce an assessment of the role of the amylase creatinine clearance ratio in acute pancreatic dysfunction.

CHAPTER TWO

THE AMYLASE CREATININE CLEARANCE RATIO

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The indirect nature of the diagnosis of the majority of cases of human acute pancreatitis presents a problem. If the correct diagnosis is to be reached in every case then a test is required which is specific for acute pancreatic inflammation and 100% reliable at indicating the presence of that disease. The ideal test should also be simple and quick to perform, and the analytical procedures required should be readily available in any hospital laboratory. None of the diagnostic tests mentioned in the last chapter meet up to all these requirements and it seems unlikely that any diagnostic test for acute pancreatitis will do so in full in the near future. However, there is room for improvement in the diagnosis of acute pancreatitis in practice and a challenge exists to find a diagnostic test to fulfil more of the criteria stated above than those already available.

Dr. Michael Levitt and his colleagues, working in Minneapolis, first described the concept of the amylase creatinine clearance ratio (ACCR) in a paper published in 1969 (32). This study set out to examine the relationship between serum amylase, urine amylase and the renal clearance of amylase in patients with

various conditions including acute pancreatitis and chronic renal failure. The renal clearance of creatinine was also estimated and this stimulated the authors to express amylase clearance as a percentage of the simultaneous creatinine clearance (ACCR). It was observed that the ACCR could be calculated from spot samples of venous blood and urine, thus simplifying the clearance estimation by removing the need for a timed urine collection thus:

$$\frac{Cam}{Ccr}\% =$$

$$\frac{\frac{(Am)\ urine}{(Am)\ serum} \times \frac{Volume}{Time}}{\frac{(Cr)\ urine}{(Cr)\ serum} \times \frac{Volume}{Time}} \times 100$$

$$= \frac{(Am)\ urine}{(Am)\ serum} \times \frac{(Cr)\ serum}{(Cr)\ urine} \times 100$$

where C = clearance
Am = amylase
Cr = creatinine

volume and time = urine collection

this leaves a formula which can be solved quickly and easily from a 10 ml sample of venous blood and a 10 ml sample of urine collected at around the same time as the blood.

In Levitt's study the ACCR was noted to be markedly and consistently elevated in 12 patients with alcohol induced acute pancreatitis, giving a mean value of 6.6% and a minimum value of 4.5% within the first four days of the illness (32). Eighteen hospitalised patients with a variety of acute and chronic diseases thought not to involve the pancreas had a mean ACCR of 3.1% with two of this group having an ACCR of $> 4.5\%$. Pancreatitis, however, could not be excluded in these two cases after more detailed examination of their records. Fifty-two healthy medical students had a mean ACCR of $2.3\% \pm 0.1$ s.e.m., the highest value being less than 4.5%. The ACCR of 13 patients with stable chronic renal insufficiency (mean creatinine clearance = 28 ml/min) was found to be similar to that of the control patients despite a highly significant reduction in amylase clearance per se. This finding lead the authors to suggest that the ACCR might be an index of amylase clearance which could be used to compare the renal handling of amylase in patients with various degrees of renal impairment. They noted that this feature of the ACCR might also be of value in the diagnosis of acute pancreatitis in some patients. The ACCR was also found to remain elevated for 3-4 days in acute pancreatitis before falling towards normal. The serum amylase levels, on the other hand,

tended to fall precipitously towards normal in 24-48 hours.

Little was heard of the ACCR after Dr. Levitt's publication until 1974 when Dr. Dreiling and his group from New York (33) and Dr. Warshaw and his group from Boston (34) published independent ACCR studies on a total of 215 patients with acute pancreatitis, mainly due to alcohol abuse. Both groups concluded that in acute pancreatitis the ACCR was superior to the diagnostic significance of serum or urine amylase determinations alone, with a diagnostic accuracy for acute pancreatitis of around 88%. Warshaw's group suggested in 1975 that the ACCR could accurately determine between pancreatic and non-pancreatic hyperamylasaemia, taking the upper limit of normal for the ACCR as being 5.3% as opposed to Dr. Dreiling's value of 4% (35,36). Warshaw reported that the ACCR remained below 5.3% in 44 patients with hyperamylasaemia due to disease other than pancreatitis, but was over 5.3% in all but 3 of 42 patients with acute pancreatitis. This suggested that the renal handling of amylase was specifically altered in acute pancreatitis and provided a basis for using the ACCR in the differential diagnosis of hyperamylasaemia.

In 1976 Orda et al published the first ACCR study in animals (37). Using a guinea pig model, and inducing acute pancreatitis by injecting sodium taurocholate into the pancreatic parenchyma, they found a significant elevation in the ACCR in guinea pigs with acute pancreatitis compared to controls, animals with acute duodenal perforations and animals with small bowel infarction. The ACCR was found to be superior to serum amylase alone in identifying animals with acute pancreatitis and was also noted to rise significantly in animals with acute pancreatitis but without hyperamylasaemia. In short this animal study confirmed the main findings of the human ACCR studies and the authors concluded without reservation that the ACCR should be a valuable diagnostic test in acute pancreatitis in man. Between 1975 and 1976 Dr. Levitt and his colleagues, Dr. Warshaw and his colleagues and Drs. Long and Grider from Philadelphia all published evidence to explain why the renal clearance of amylase is increased during an attack of acute pancreatitis (38,39,40,41,42). There were three possible explanations for this observation, i.e.

1) an increased serum concentration of a very rapidly cleared isoamylase in acute pancreatitis;

2) an increased glomerular permeability to amylase in acute pancreatitis;

3) a decreased renal tubular reabsorption of filtered amylase in acute pancreatitis.

All three groups of workers came to the firm conclusion that no new isoenzymes of amylase were formed during an attack of acute pancreatitis and that the permeability of the glomerulus to amylase was unaltered. That left reabsorption of amylase in the renal tubules as the most likely site for the defect and this was supported by Johnston et al who showed that the excretion of B2-microglobulin, a low molecular weight protein similar to amylase and known to be reabsorbed in the renal tubules, was greatly increased in acute pancreatitis (39). There was presumed to be a 'tubular dysfunction factor' which comes into play in acute pancreatitis and inhibits the tubular handling of amylase and other low molecular weight proteins. No evidence was available concerning the nature of this 'factor' or whether it functioned in conditions other than acute pancreatitis.

In 1975 Levine et al reported significant elevation of the ACCR during 10 episodes of diabetic ketoacidosis and in 6 patients with burns (43). No patient studied had an elevated serum amylase level and although there

was no proof that these patients did not have acute pancreatitis, these observations cast doubt on the specificity of an elevated ACCR for acute pancreatitis. A further note of caution appeared in the literature in September 1976 from Cape Town when Berger et al reported a significant elevation in the ACCR in 2 out of 5 patients with an acute duodenal perforation as well as 6 out of 8 patients with acute pancreatitis (44). No good evidence was put forward in this small study however to show that the patients with acute duodenal perforations did not also have pancreatitis. The normal ACCR value in this study, which was not actually quoted, was obtained from 10 control patients, an insufficient number from which to determine an accurate normal range. The results of this paper must therefore be regarded as suspect although they cannot be overlooked.

In the studies reported in this thesis the ACCR was always calculated according to the formula described by Levitt (32). Amylase activity in serum and urine was measured using the Phadebas amylase test kit (Pharmacia diagnostics, Uppsala, Sweden) which for all studies except that reported in Chapter 3 had bovine serum albumin added to increase the sensitivity for amylase, especially in the urine. The addition of bovine serum albumin to the test

has been shown to slightly increase the value of the ACCR from the same samples of blood and urine (45). The substrate in the test kit is a water insoluble cross-linked starch polymer carrying a blue dye. This polymer is hydrolysed by α-amylase to form water soluble blue fragments. The absorbance of the blue solution on a photometer measuring 620 $\pm$ 5 nm is a function of the α-amylase activity of the sample. Serum and urine creatinine levels were all measured using a Technicon A11 autoanalyser which operates using a modified Jaffe reaction (46) based on the fact that creatinine forms a red complex in alkaline picrate solution. Amylase activity has been expressed in international units per litre, while serum and urine creatinine levels have been expressed as micromoles per litre and millimoles per litre respectively.

The original paper describing the ACCR suggested calculating the ratio from simultaneous samples of venous blood and urine (32). In practice this can be difficult unless the patient has a bladder catheter in place and this problem becomes most obvious in the first few hours after admission to hospital when the diagnostic accuracy of the ACCR for acute pancreatitis should be most useful. The ACCR was therefore estimated in 6 patients with no known

pancreatic disease who had indwelling bladder catheters in place. The 10 ml urine samples were taken from the catheter tubing at the time of venous blood sampling and again one hour later. Two ACCR values were calculated for each patient using the results from the two urine samples. The mean ACCR values for each urine sample time were not significantly different (1.42% $\pm$ 0.2 s.e.m. stat and 1.49% $\pm$ 0.25 s.e.m. after one hour) and statistical evaluation of the individual pairs of results for each patient showed no significant difference (Paired Student's t test). To test the reproducibility of the ACCR over a 12 hour period in a patient without acute pancreatitis venous blood samples were taken from one 21 year old male admitted electively with a sternal deformity at 9 a.m., 1 p.m., 5 p.m. and 9 p.m. the same day. A urine sample was obtained within an hour of each blood sample and the ACCR results were as follows; 0.9%, 1%, 1.1%, 0.95% respectively. These results indicate that the ACCR is a reproducable test within a short period of time in a normal person and that the urine sample required for ACCR estimation can be taken during the hour following venous blood sampling without significantly altering the ACCR results.

C H A P T E R T H R E E

THE AMYLASE CREATININE CLEARANCE RATIO IN ACUTE PANCREATITIS

CHAPTER THREE

THE AMYLASE CREATININE CLEARANCE RATIO IN ACUTE PANCREATITIS

1. INTRODUCTION

The literature on the ACCR up to 1976 was in general encouraging. No clinical publications, however, had appeared from Great Britain or Europe and it was felt that the first step in determining the usefulness of this ratio in the diagnosis of acute pancreatitis in this country should be to confirm the basic findings from the U.S.A., i.e. the sensitivity and specificity of the ACCR as a diagnostic test in acute pancreatitis. An important difference in studies of acute pancreatitis in Great Britain and North America lies in the aetiology of the disease. The majority of cases of acute pancreatitis reported from large centres in North America are related to alcohol abuse whereas in Glasgow and the remainder of the United Kingdom gallstones are the commonest related factor (51%) with alcohol abuse only accounting for up to one quarter of the patients (26, 47). The aetiology of acute pancreatitis had not been taken into account in previous studies involving the ACCR. It was therefore decided to attempt to verify the changes in the ACCR previously noted in

the U.S.A. during an attack of acute pancreatitis since this seemed to be the logical first step in evaluating the use of the ACCR as a diagnostic test in patients with acute pancreatitis in the United Kingdom.

2. PATIENTS AND METHODS

i) Patients Studied

One hundred and twenty two patients admitted to the Western Infirmary, Glasgow were studied. Venous blood and urine samples for analysis and calculation of the ACCR were obtained within 24 hours of admission to hospital in all cases. Particular attention was paid to this point with patients admitted suffering from acute surgical conditions. The 122 patients were divided into four groups for analysis of the ACCR data based on the diagnosis reached in each patient's case by the surgical team responsible for his or her management without prior knowledge of the ACCR results. Two groups of patients had chronic conditions that were either related or unrelated to the gastrointestinal tract. The remaining two groups had acute abdominal conditions including acute pancreatitis. The diagnostic details of the

four groups of patients studied were as follows -

Group 1 - 40 elective patients acting as controls with chronic conditions unrelated to the gastrointestinal tract.

Breast lumps	- 8
Inguinal hernia	- 8
Varicose veins	- 5
Stitch sinus	- 4
Soft tissue injury	- 4
Scrotal swelling	- 4
Neck lump	- 2
Undescended testis	- 1
Ingrowing toenail	- 1
Adrenal adenoma	- 1
Phimosis	- 1
Ovarian cyst	- 1

Group 2 - 30 patients admitted electively to the Western Infirmary, Glasgow, with chronic gastrointestinal conditions but with no evidence of past or present involvement of the pancreas.

Chronic duodenal ulceration	- 9
Upper gastrointestinal neoplasms (excluding the pancreas)	- 5
Cholelithiasis	- 5

Simple anorectal conditions - 5

Lower gastrointestinal neoplasms - 4

Chronic gastric ulcer - 1

Diverticular disease - 1

Group 3 - 35 patients admitted to the Acute Surgical Receiving Unit of the Western Infirmary, Glasgow, with acute abdominal conditions diagnosed independently as being -

Acute cholecystitis - 9

Acute peptic ulceration - 7

Perforated viscus - 7

Alcohol induced 'gastritis' - 4

Ureteric colic - 3

Acute appendicitis - 2

Acute diverticulitis - 1

Mesenteric adenitis - 1

Pelvic inflammatory disease - 1

The diagnosis of cholelithiasis, peptic ulceration, diverticular disease and ureteric colic was confirmed at a later date by appropriate radiology. The diagnosis of perforated viscus, acute appendicitis and mesenteric adenitis was made at laparotomy which post-dated the collection of samples for calculation of the

ACCR. Two cases of acute gastritis were confirmed at upper gastrointestinal endoscopy while a gynaecologist supported the diagnosis of acute pelvic inflammatory disease.

Group 4 - This group comprised 17 patients admitted to the Acute Surgical Receiving Unit of the Western Infirmary, Glasgow and diagnosed as having acute pancreatitis without prior knowledge of ACCR values.

ii) <u>The Diagnosis of Acute Pancreatitis</u>

The diagnostic criteria for acute pancreatitis used in the Western Infirmary, Glasgow, were a compatable history and clinical findings plus a serum amylase of $>$ 1200 iu/l. The diagnosis could of course also be made at laparotomy. Fourteen of the 17 patients had a serum amylase $>$ 1200 iu/l within the first hour of their hospital admission. In 4 of these 14 patients however the serum amylase subsequently fell to $<$ 1200 iu/l when a blood sample was taken for ACCR estimation within the first 24 hours in hospital.

The diagnosis of acute pancreatitis was made at laparotomy in 3 patients, all of whom had serum amylase values within the normal range for the Western Infirmary on admission (upper limit of normal: 300 iu/l). A laparotomy was carried out in all 3 cases within 24 hours of admission to hospital and post-dated the collection of blood and urine for ACCR estimation. A laparotomy diagnosis was made in each case by an experienced surgeon of consultant or senior registrar grade. No histology, however, is available to confirm the macroscopic findings at operation. ACCR estimations were carried out daily where possible in patients with acute pancreatitis.

iii) Calculation of the ACCR

The ACCR was calculated using the formula of Levitt et al described earlier (32). This ratio of clearances allows urine volume and collection times to be cancelled out of the clearance equation leaving only simultaneous concentrations of amylase and creatinine in blood and urine to be estimated. In practice a urine sample (10 ml) obtained within one hour of a venous blood sample (10 ml) was regarded as satisfactory for ACCR estimation. Amylase was estimated using a Phadebas reagent kit,

while creatinine was measured on a Technicon A11 autoanalyser as described in Chapter 2.

3. RESULTS

i) Patient Details

Table 2 shows the number, mean age and sex distribution of the patients in each of the four groups studied. No statistically significant difference was found between the four groups with regard to these factors. Table 3 shows the aetiology of the acute pancreatitis in the 17 cases studied. Viral titres were not carried out in the patient labelled as having idiopathic acute pancreatitis and therefore this not unknown form of the condition (5 out of 116 cases in Glasgow) may have explained the aetiology in this case (48).

ii) Creatinine Results

Table 4 shows the mean serum and urine creatinine values used to calculate the ACCR in the four groups of patients studied. There was no statistically significant difference between the four groups in either mean serum or urine creatinine values. Two patients in group 2, 4 patients in group 3, and 1 patient in group 4 had a serum creatinine value above the upper limit of normal (115 μmol/l) at the time of ACCR

TABLE 2

Age and sex distribution of the groups of patients studied.

	Groups			
	1	2	3	4
Number of Patients	40	30	35	17
Mean Age (Yr)	44	54	48	54
Male (M)	22	17	20	12
Female (F)	18	13	15	5
M/F Ratio	1.2	1.3	1.3	2.4

TABLE 3

The aetiology of acute pancreatitis in the 17 cases studied.

Cholelithiasis	-	9	(53%)
Alcohol Abuse	-	6	(35%)
Hyperparathyroidism	-	1	
Idiopathic	-	1	

TABLE 4

Serum and urine creatinine values used to calculate the ACCR in the four groups of patients (mean ± s.e.m.).

Group	Serum Creatinine (μmol/l)	Urine Creatinine (μmol/l)
1	86.7 ± 2.5	11,400 ± 80
2	86.4 ± 4.5	12,600 ± 1,300
3	86.2 ± 4.4	12,900 ± 1,400
4	90.1 ± 5.3	10,900 ± 1,700

calculation. None of these patients had established renal failure or subsequently developed renal failure during their admission and it was felt at the time that the elevated serum creatinine values could be explained by dehydration.

iii) Amylase Results

The mean serum amylase values used to calculate the ACCR in the four groups of patients are shown in Table 5 and plotted individually in Figure 1. The mean serum amylase of patients with acute pancreatitis (1751 iu/l) was significnatly higher in the mean serum amylase values in the other three groups ($p < 0.001$). It is interesting to note that the mean serum amylase for group 4 patients within an hour of admission to hospital was 2893 iu/l which was considerably greater ($p < 0.02$) than the mean of the serum amylase values for group 4 used to calculate the ACCR during the first 24 hours in hospital. This illustrates the precipitous fall in serum amylase levels which is not infrequently observed in acute pancreatitis. A repeat blood sample was usually necessary in these patients in order to correspond with the urine sample and therefore two serum amylase values were often available within the

TABLE 5

Serum and urine amylase values used to calculate the ACCR in the four groups of patients (mean ± s.e.m.). Unpaired Student's t test.

	Serum Amylase (I.U./L)		Urine Amylase (I.U./L)
Group			
1	190 ± 10		338 ± 32
2	209 ± 15		444 ± 63
3	230 ± 19	+	590 ± 68
4	1,751 ± 374	*	10,669 ± 3,308

* 4 > 1,2,3 for serum and urine amylase $p < 0.001$

\+ 3 > 1 serum amylase $p < 0.05$

urine amylase $p < 0.02$

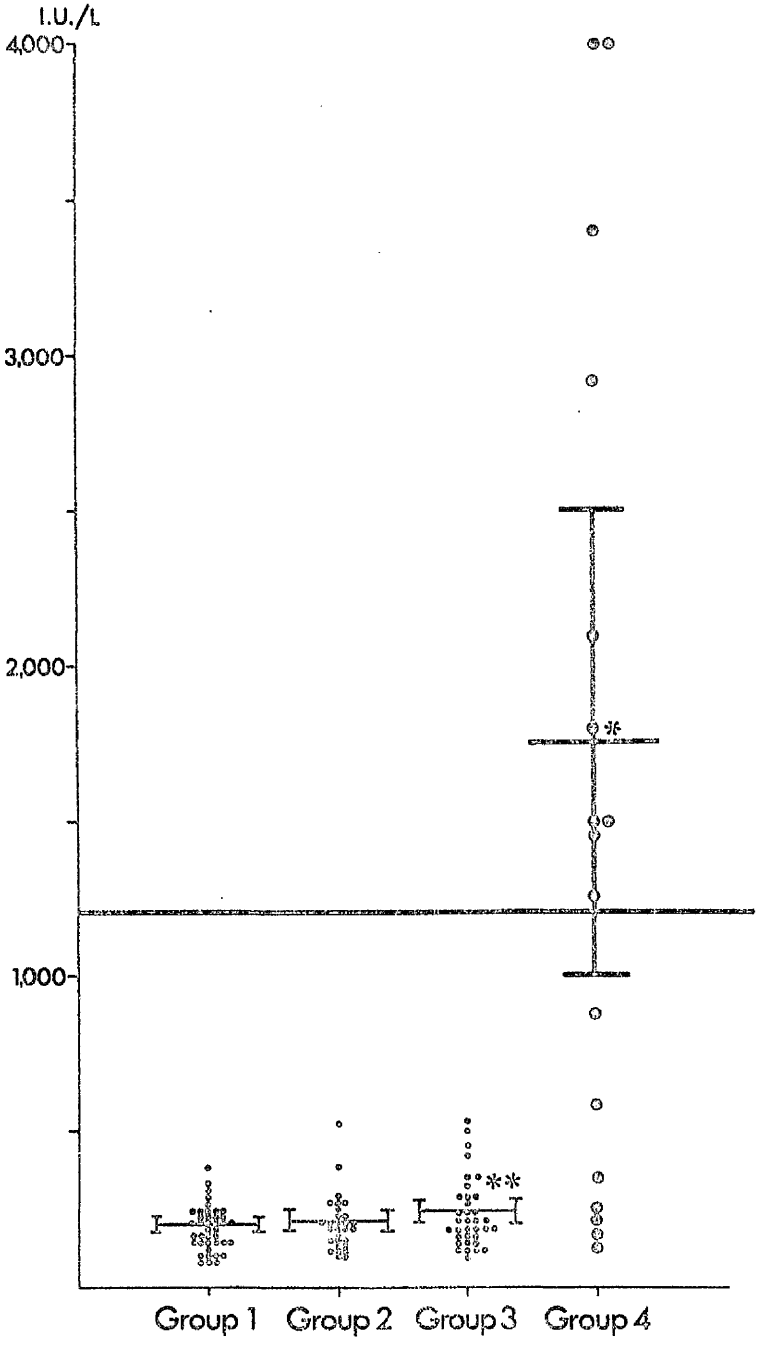

Figure 1. Serum amylase values for the 4 groups of patients studied. Mean ± 2 s.e.m. Unpaired Student's t test.

* 4 > 1, 2 & 3 - $p < 0.001$
** 3 > 1 - $p < 0.05$

first 24 hours of hospital admission. Figure 1 shows that at the time of sampling for ACCR calculation (within the first 24 hours in hospital) only 10 patients in group 4 had serum amylase values $>$ 1200 iu/l. Three out of the remaining 7 patients were diagnosed at laparotomy without hyperamylasaemia leaving 4 patients in whom the serum amylase had fallen to $<$ 1200 iu/l within a few hours of admission.

Patients with acute abdominal conditions of non-pancreatic origin (Group 3) were found to have a significantly higher mean serum amylase than the control patients in group 1 ($p < 0.05$: Table 5). Seven per cent of the patients in group 1 (3) and group 2 (2) had a serum amylase value above the upper limits of normal for the Western Infirmary Biochemistry Laboratory (300 iu/l). In group 3, however, 20% of the patients (7) had a serum amylase value in excess of 300 iu/l.

The changes in urine amylase concentration essentially mirrored the findings with serum amylase (Table 5: Figure 2). The mean urine amylase value for group 4 was found to be significantly greater than the mean values for groups 1, 2 and 3 ($p < 0.001$), despite a large scatter round the mean in group 4. The mean urine amylase concentration

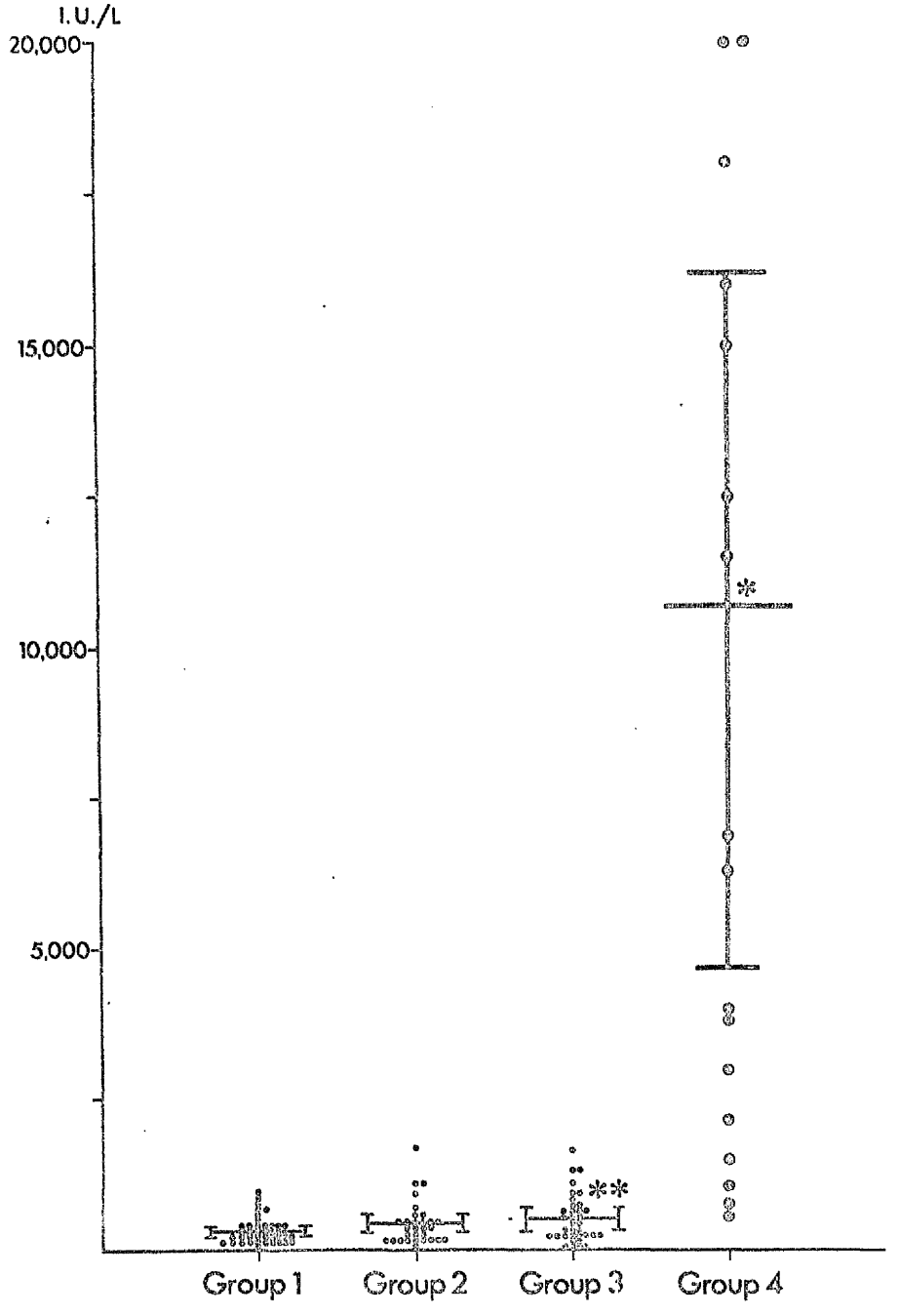

Figure 2. Urine amylase values for the 4 groups of patients studied. Mean ± 2 s.e.m. Unpaired Student's t test.

* 4 > 1, 2 & 3 - $p < 0.001$
** 3 > 1 - $p < 0.025$

for group 3 was found to be significantly greater than the mean concentration for control patients (Group 1) - $p < 0.02$.

The ratio of serum amylase to urine amylase was calculated for each patient using the data employed to calculate the ACCR. The mean values of this ratio for each group of patients is shown in Table 6. No statistically significant difference was noted between the mean serum to urine amylase ratios of the four groups of patients. Patients with acute pancreatitis (Group 4) tended to have a lower ratio indicating a relative increase in excretion of amylase. This was, however, of little diagnostic use due to the wide range of values seen in patients with no evidence of acute pancreatitis.

iv) ACCR Results

The ACCR values for the four groups of patients within 24 hours of admission are plotted in Figure 3. The mean ACCR in patients with acute pancreatitis (6%) was significantly higher than the mean ACCR values found in groups 1, 2 and 3 ($p < 0.001$). There was no significant difference between the mean ACCR values of patients without acute pancreatitis (Groups 1, 2 and 3), each being 1.5%. The 7 patients (20%) with acute abdominal conditions other than acute pancreatitis (Group 3) who had a

TABLE 6

Ratio of serum to urine amylase for the four groups of patients (mean ± S.D.)

Group	SA/UA
1	0.74 ± 0.5
2	0.72 ± 0.4
3	0.95 ± 1.4
4	0.21 ± 0.12

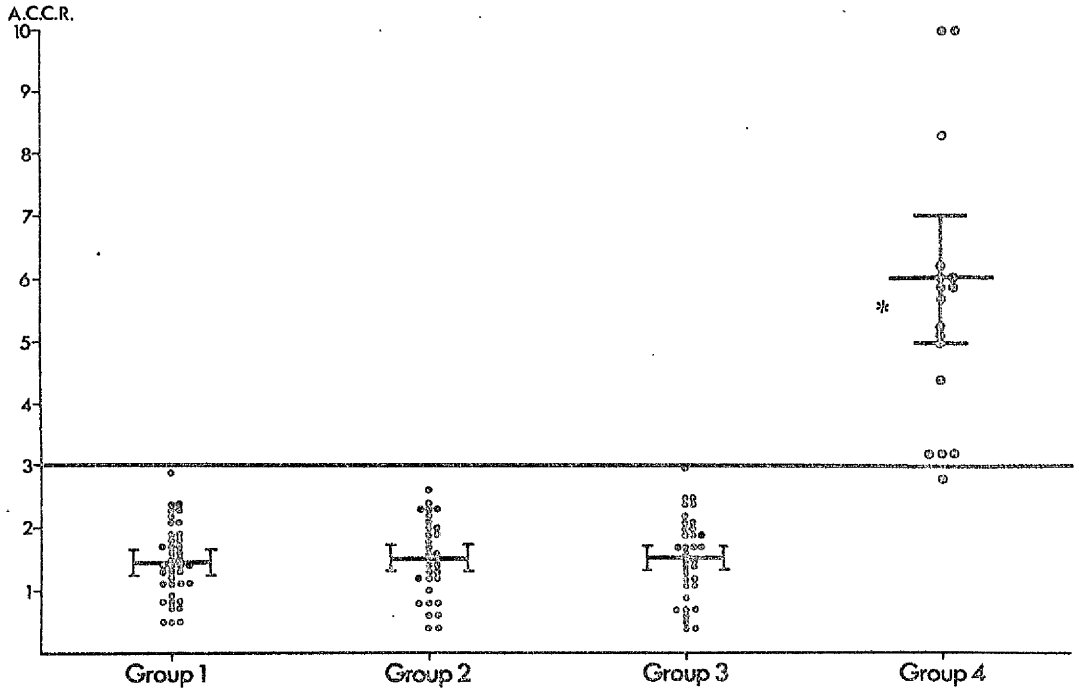

Figure 3. ACCR values for the 4 groups of patients studied. Mean ± 2 s.e.m. Unpaired Student's t test.

* 4 > 1, 2 & 3 - $p < 0.001$

serum amylase value > 300 iu/l had a mean ACCR of 1.34%. This was not significantly different from the mean ACCR value for the control patients in group 1 (1.5%).

The mean ACCR value for the 105 patients in groups 1, 2 and 3 with no evidence of acute pancreatitis was found to be 1.498% with a standard deviation of 0.64. The 95% upper confidence limit of this mean based on the standard deviation was taken to be 2.8%. The upper limit of normal for the ACCR in the Western Infirmary, Glasgow was therefore judged to be 3%. Figure 3 shows that no patient in groups 1, 2 or 3 had an ACCR value over 3%, whereas 16 out of 17 (94%) of the patients diagnosed independently as having acute pancreatitis had an ACCR value > 3%. One 63 year old male patient had an ACCR of 2.8% but was diagnosed at laparotomy as having mild pancreatitis secondary to cholelithiasis. His serum amylase on admission to hospital was within the normal range at 130 iu/l.

The diagnostic accuracy in acute pancreatitis of various biochemical parameters used individually or to calculate the ACCR are shown in Table 7. The ACCR shows an encouraging diagnostic accuracy of 94% in this small study and betters serum

TABLE 7

Diagnostic accuracy of various biochemical estimations in patients with acute pancreatitis.

ACCR	=	94%	(> 3% within 24 hours)
Serum amylase	=	83%	(> 1200 i.u./l on admission)
Urine amylase	=	76%	(> 1700 i.u./l)
Serum amylase	=	59%	(> 1200 i.u./l within 24 hours of admission)
SA/UA	=	35%	(< 0.125 within 24 hours of admission)

amylase alone which shows a maximum accuracy of 83% within one hour of admission.

Figure 4 shows daily serum amylase and ACCR results obtained from a 89 year old woman admitted with acute pancreatitis and entered into the study as a group 4 patient. It can be seen that her serum amylase value rapidly fell (within 48 hours) to below the diagnostic level (1200 iu/l). The ACCR however remained elevated for significantly longer and did not fall below 3% until the eighth day. This pattern was observed in the majority of the 17 cases of acute pancreatitis studied, the ACCR usually returning to normal between the fifth and tenth days.

4. DISCUSSION

i) Diagnostic Accuracy

The results of the study were encouraging in as much as they confirmed most of the findings of the American workers in this field to date. The findings in this study also indicate that ACCR changes in acute pancreatitis are independent of the aetiology of the disease (i.e. alcohol $\gg$ gallstones in U.S.A. and gallstones $\gg$ alcohol in G.B.). In this study the mean admission ACCR of patients with pancreatitis secondary to gallstones

Author: Murray, William R.

Title: The amylase creatinine clearance ratio

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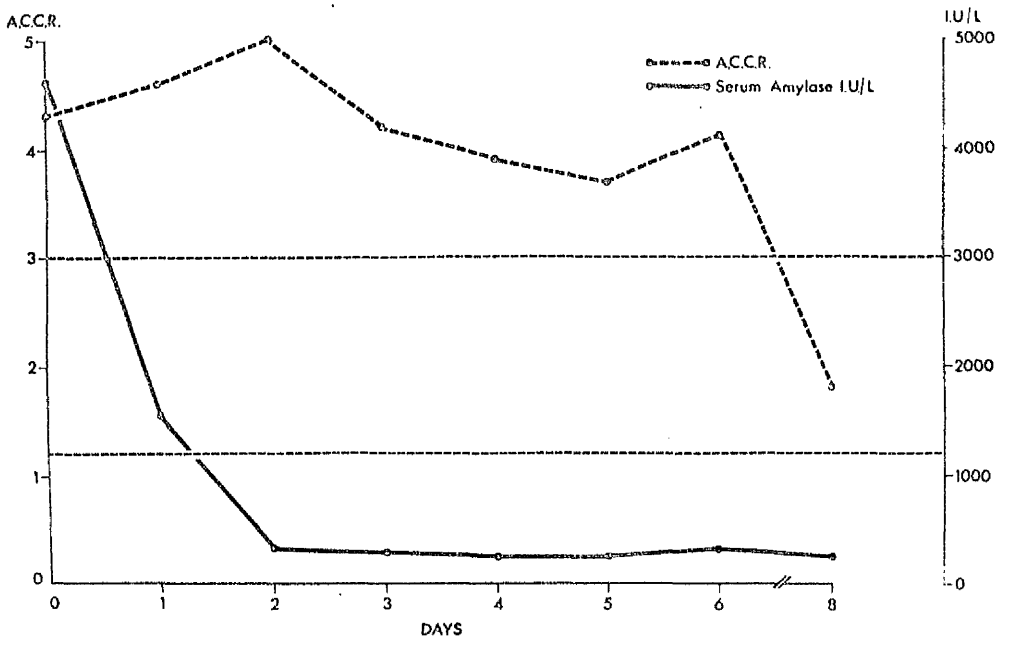


Figure 4. ACCR and serum amylase values in a patient with acute pancreatitis.

(6.4% ± 2.7 S.D.) was not significantly different from the value found in patients with alcohol abuse as the cause for their attack of acute pancreatitis (5.6% ± 1.7 S.D.).

The study confirmed that the ACCR is a simple test to perform and that its great advantage over amylase clearance per se lies in the ability to calculate the ratio using a single sample of urine only. The ability to calculate an expression of amylase clearance in this way allows a clearance value such as the ACCR to be used as a screening test in an acute situation if so desired. All four estimations required to calculate the ACCR can be performed rapidly in most biochemical laboratories and the ACCR could be available within an hour of taking the blood and urine samples.

A serum amylase value and the ACCR could be used together as a biochemical screen for acute pancreatitis. The results of this study support the conclusions of Dreiling et al (33) that simultaneous evaluation of the serum amylase and the ACCR is superior to the diagnostic significance of amylase determinations alone. In this study an ACCR performed during the first 24 hours in

hospital was 94% accurate in diagnosing acute pancreatitis. Over the same period of time serum amylase alone was only 59% accurate in making this diagnosis. Time was found to be an important factor when considering serum amylase estimations. The diagnostic accuracy of serum amylase alone rose to 83% when the blood sample was taken during the first hour of admission as opposed to some time during the first 24 hours of admission. No other single biochemical parameter estimated in this study was found to be superior to the ACCR in terms of diagnostic accuracy for acute pancreatitis.

ii) Normal Range of the ACCR

The upper limit of normal for the ACCR in the Western Infirmary, Glasgow was calculated to be 3%, based on results from 105 patients with no evidence of acute pancreatitis. Levitt et al found the upper limit of normal for the ACCR in 52 healthy volunteers to be 4% (32). A similar figure was obtained by Dreiling et al for 125 normal controls (33). Warshaw obtained an upper limit of normal for the ACCR of 5.3% from 46 normal subjects in Boston (35). The figure of 3% calculated from study is lower than previously recorded in humans

but similar to the value obtained in a guinea pig study by Orda et al - normal < 3.2% (37). Because of possible variations in analytical technique it has been suggested that a normal range of ACCR values should be established in each centre using the ratio (32). This is obviously important in early work with this ratio since if upper limits of normal for the ACCR of 4 and 5.3% were adopted in this study the diagnostic accuracy of the test in acute pancreatitis would be reduced to 77% and 53% respectively.

iii) Sensitivity of the ACCR

In this study an ACCR of over 3% (mean 5.2%) was obtained within the first 24 hours of admission from 3 patients without significant hyperamylasaemia (mean serum amylase = 233 iu/l) who were all found to have acute pancreatitis at laparotomy. In these 3 patients the history of abdominal pain was of greater than 48 hours duration and it is therefore probable that initially abnormal serum amylase levels had fallen to within the normal range prior to admission. This hypothesis is supported by the results of serial estimations of serum amylase and the ACCR carried out in this study on patients

with acute pancreatitis. The ACCR in these cases always returned to the normal range after serum amylase had done so and therefore this demonstrated that an elevated ACCR without hyperamylasaemia could indicate resolving acute pancreatic inflammation. This observation also indicates that the ACCR may be a more sensitive diagnostic test for acute pancreatic inflammation than is serum amylase alone. It is therefore possible that the ACCR will be of particular diagnostic value for acute pancreatitis in cases presenting to hospital with abdominal pain of over 48 hours duration. These patients may have serum amylase values within the normal range or within the 'grey area' between the upper limit of normal (300 iu/l) and 1200 iu/l and it is in these cases particularly that the ACCR could be of extreme value.

iv) Specificity of the ACCR

In this study an ACCR value of $>$ 3% was specific for acute pancreatitis. The mean ACCR values of the other three groups of patients were identical, an encouraging finding in the presence of statistically significant hyperamylasaemia in the group of patients with acute abdominal conditions excluding acute pancreatitis (Group 3). There

cannot be conclusive proof that no patient in this group had acute pancreatitis, but the highest serum amylase recorded was 520 iu/l in a patient with a perforated anterior duodenal ulcer, one of several conditions represented in this group which have been associated with non-pancreatic hyperamylasaemia (14,35,49,50,51,52,53,54).

v) Urine Amylase

The changes in urine amylase observed in this study broadly mirrored the changes in serum amylase. Mean urine amylase values were significantly elevated in patients with acute pancreatitis (Group 4) and also, but to a lesser extent, in patients with non-pancreatic acute abdominal conditions (Group 3). Great variability, however, was noted in the urine amylase results and the diagnostic discrimination of this test for acute pancreatitis was found to be poor. These findings are in agreement with the conclusions relating to urine amylase estimations in a recent review article (13). The ratio of serum to urine amylase was found to be decreased in acute pancreatitis but these changes were found not to be statistically significant due to the wide scatter of results in patients without acute pancreatitis. Like urine

amylase alone this ratio was found to be of poor diagnostic specificity for acute pancreatitis.

vi) Conclusions

In conclusion therefore the main findings of this study were interpreted as follows:

1. The ACCR is simple to estimate and could be used as a screening test in a patient presenting with acute abdominal pain.

2. The ACCR is a reliable and sensitive index of acute pancreatic inflammation in humans.

3. The diagnostic accuracy of the ACCR in acute pancreatitis does not appear to be significantly influenced by the aetiology of the disease.

4. In this study a significant ACCR elevation was only observed in patients with acute pancreatitis and not in patients with other acute and chronic gastrointestinal conditions.

5. The sensitivity of the ACCR for acute pancreatic inflammation may give a diagnostic advantage over serum amylase alone in patients who present to hospital more than 48 hours after the onset of abdominal pain.

CHAPTER FOUR

THE AMYLASE CREATININE CLEARANCE RATIO FOLLOWING CHOLECYSTECTOMY

CHAPTER FOUR

THE AMYLASE CREATININE CLEARANCE RATIO FOLLOWING CHOLECYSTECTOMY

1. INTRODUCTION

i) Diagnostic Problems in Acute Pancreatitis

The study outlined in Chapter 3 confirmed the earlier finding that the ACCR was very likely to be significantly elevated in the presence of acute pancreatic inflammation. In only 3 out of the 17 cases of pancreatitis studied, however, was a direct diagnosis of acute pancreatitis made at laparotomy. In the remaining 14 cases the diagnosis of acute pancreatitis was made indirectly on the basis of appropriate clinical findings and hyperamylasaemia, the two factors that lead to the diagnosis of acute pancreatitis in the majority of cases throughout the World. It is well established, however, that acute pancreatitis is not the only intra-abdominal source for hyperamylasaemia (14). Despite this knowledge the clinical impression of acute pancreatitis is seldom documented by pathological examination of tissue or by some other unequivocal 'gold standard' technique. How then can one prove a new test is better than amylase measurements when amylase

values remain the usual yardstick used to diagnose acute pancreatitis?

This question poses a difficult practical problem in the evaluation of the ACCR in humans. In the ideal situation ACCR values should be estimated before, during and after anticipated attacks of acute pancreatitis with all other factors equal. This is obviously not possible in the human, to whom this test is most applicable, and therefore some other method of approach has to be sought in an attempt to demonstrate a relationship between the ACCR and acute pancreatic inflammation.

ii) Post-Operative Pancreatitis

Operative trauma to the upper abdomen is a well established cause of acute pancreatitis, especially when the operation involves the biliary tract (55,56,57,58). Interference with the Sphincter of Oddi is said to be the most important single factor in promoting this form of acute pancreatitis (59). Post-operative acute pancreatitis is notoriously difficult to diagnose clinically and carries a high mortality (47). In 1977 Donaldson et al published a study of 25 patients undergoing elective cholecystectomy

in whom the ACCR was estimated before and after surgery in an attempt to further define the incidence of acute pancreatic dysfunction following cholecystectomy (60). Twenty eight percent of all patients were found to have a significantly elevated ACCR (> 5.5%) 24 hours after cholecystectomy while 70% of patients who underwent exploration of the common bile duct had a significantly elevated ACCR. None of the patients, however, developed clinical acute pancreatitis and only one had an abnormal serum amylase level. Did these findings represent 'sub-clinical' pancreatic inflammation detected by a rising ACCR or was the correlation spurious?

It seemed that an attempt could be made to answer this question by making use of the fact that the incidence of pancreatic inflammation following cholecystectomy could be manipulated in at least two ways, i.e.

1. through the presence or absence of common bile duct exploration, or -
2. by using the drug Trasylol (Aprotinin) as a prophylaxis against acute pancreatitis.

Trasylol is a proteolytic enzyme inhibitor which has been advocated as being beneficial in the treatment of established acute pancreatitis. An initially encouraging study (61) was followed by good evidence of the drug's ineffectiveness in established acute pancreatitis (62,63). It should not be forgotten, however, that animal studies have shown clearly that Trasylol is very effective at modifying the inflammatory response of the pancreas when given prior to the insult inducing the inflammation (64,65,66). In the majority of cases of human acute pancreatitis prophylaxis is not possible. In post-cholecystectomy acute pancreatitis, however, prophylaxis is eminently possible and this situation presents a genuine indication for the use of Trasylol in the operative and peri-operative period, especially in patients with a high risk of developing acute pancreatitis i.e. patients with known common bile duct stones or a past history of acute pancreatitis.

It was therefore decided to study ACCR changes in a group of patients undergoing cholecystectomy, half of whom had been randomised to receive Trasylol during and after surgery. It was hoped that this study would confirm Donaldson's finding of

significant ACCR elevation following cholecystectomy and also extend this finding by showing that Trasylol prophylaxis significantly modifies the ACCR changes. A result of this sort would add weight to the evidence that an elevated ACCR was in fact related to pancreatic inflammation.

iii) Thermolability of Amylase

In 1974 Warshaw demonstrated that over 90% of the amylase activity in saliva and pancreatic juice was destroyed by heating at 65°C for 15 minutes (67). In normal serum only 44% of total amylase was thermolabile under these conditions but this rose significantly to 84% in acute pancreatitis (68). These observations suggested that the estimation of thermolabile amylase in blood and urine may be a simple way to monitor changes in the pancreatic isoenzyme of amylase which would otherwise require sophisticated techniques for identification (14). Thermolabile amylase estimation was therefore requested on all samples of venous blood and urine obtained during this study in an attempt to demonstrate that any significant ACCR changes observed were related to pancreatic dysfunction.

2. PATIENTS AND METHODS

i) Patient Details

Eighty patients admitted to wards G6 and 7 of the Western Infirmary, Glasgow, were studied. All patients were admitted for elective surgery with either cholelithiasis or minor non-abdominal conditions requiring surgery under general anaesthesia. These patients were divided into three groups for analysis of amylase and ACCR data.

Group 1

This group was made up of 29 patients admitted for elective non-abdominal surgery under general anaesthesia. These patients acted as a control group for the study and were suffering from the following conditions:

Inguinal hernia	10
Varicose veins	7
Breast lump	5
Pilonidal sinus	3
Ganglion	2
Stitch sinus	2

Group 2 (N.T.)

This group contained 22 patients who underwent cholecystectomy with or without operative cholangiography and common bile duct exploration.

Cholelithiasis was confirmed at surgery in each case and all operations were performed by surgeons working in one surgical unit at the Western Infirmary (Professor Sir Andrew Watt Kay's Unit).

Group 3 (T)

In this group were 29 patients similar in all respects but one to patients in Group 2. These 29 patients were randomly selected to receive Trasylol before, during and for 24 hours after their cholecystectomy. The use of this drug was approved by the Ethical Committee of the Western Infirmary, Glasgow, and each patient in this group gave informed consent to its use. Trasylol was administered intravenously in a dosage of 1,200,000 units/24 hours added to either 5% dextrose, normal saline or Ringer's lactate infusions. Administration was started at least one hour prior to induction of anaesthesia and was continued for 24 hours after surgery.

No significant difference was noted in either age or sex distribution between the control patients (Group 1) and the patients in Groups 2 and 3 with gallstones (Table 8).

TABLE 8

Age and sex distribution of the patients studied - Mean ± s.e.m.

	SEX		AGE	
	♂	♀	♂	♀
Controls	10 (35%)	19 (65%)	52 ± 6	48 ± 3
Group 2	6 (26%)	16 (74%)	50 ± 3	56 ± 3
Group 3	7 (33%)	22 (66%)	53 ± 3	50 ± 2
Groups 2 & 3	13 (25%)	38 (75%)	51 ± 4	52 ± 2

No significant difference in these parameters was noted between the two groups of gallstone patients. Table 9 details the grade of surgeon who performed the cholecystectomies in Groups 2 and 3, while Table 10 details the incidence of intra-operative radiology, common bile duct exploration and previous pancreatitis.

ii) Experimental Protocol

A 10 ml sample of venous blood and a 10 ml sample of urine were collected within one hour of each other from each patient in the study on the day before surgery and again on the morning of the first post-operative day. Samples were stored and analysed in one run for total amylase and creatinine as already described in Chapter 2. The ACCR was calculated using the formula described by Levitt (32). Amylase thermolability was assessed by repeating a Phadebas amylase estimation after heating the serum or urine sample at 65^{o} for 15 minutes. The thermolabile amylase content of the sample was calculated by subtracting the result of the second estimation from the first and then expressing this figure as a percentage of the total amylase content, i.e.

TABLE 9

Grade of surgeon who performed cholecystectomy.

	Group 2	Group 3
Consultant	8	9
Senior Registrar	4	7
Registrar	8	10
Senior House Officer	2	3

TABLE 10

Incidence of operative cholangiography, duct exploration, and previous pancreatitis.

	Group 2	Group 3
Simple cholecystectomy	17	22
+Operative cholangiogram	3	2
+Common bile duct exploration	2	5
Previous pancreatitis	2	3

$$\text{Thermolabile amylase \%} = \frac{\text{Result A - Result B}}{\text{Result A}} \times \frac{100}{1}$$

where Result A = preheated value

Result B = postheated value

3. RESULTS

i) Pre-Operative Data

The pre-operative values for serum amylase, thermolabile serum amylase, urine amylase, thermolabile urine amylase, serum creatinine, urine creatinine and the ACCR for study Groups 1, 2 and 3 are shown in Table 11. No statistically significant difference was noted between the groups in these pre-operative values and therefore the data from the 80 patients was combined to provide a pre-operative control value for each estimation derived from 80 observations (Table 12).

A mean pre-operative, and therefore presumably normal, ACCR value of 2% $\pm$ 0.24 s.e.m. was obtained from the 80 patients in this study. In order to increase the population sampled and thus the accuracy of this mean and its distribution, the ACCR results from 105 patients with no evidence of acute pancreatitis detailed in the previous study. This gave 185 patients with no evidence of acute pancreatitis and produced a mean ACCR of 1.75%

TABLE 11

Pre-operative values from the three groups of patients studied.

Mean ± s.e.m.

	GROUP 1	GROUP 2	GROUP 3
Serum amylase iu/l	190 ± 8	220 ± 20	202 ± 12
Thermolabile serum amylase %	32 ± 1.8	30 ± 2.1	28 ± 1.4
Urine amylase iu/l	285 ± 20	300 ± 35	314 ± 50
Thermolabile urine amylase %	61.5 ± 6	59.8 ± 8	60.4 ± 4
Serum creatinine µmol/l	96 ± 4	99 ± 5	98.5 ± 4
Urine creatinine µmol/l	8685 ± 658	8210 ± 723	8101 ± 516
ACCR %	1.84 ± 0.2	1.96 ± 0.3	2.26 ± 0.4

TABLE 12

Preoperative mean values derived from all 80 patients studied - mean ± s.e.m.

1.	Serum amylase iu/l	=	203 ± 12
2.	Thermolabile serum amylase %	=	29.4 ± 2.4
3.	Urine amylase iu/l	=	307 ± 48
4.	Thermolabile urine amylase %	=	60.9 ± 4
5.	Serum creatinine µmol/l	=	98 ± 3.6
6.	Urine creatinine µmol/l	=	8221 ± 879
7.	ACCR	=	2.0 ± 0.24

with a standard deviation of 1.53 and a standard error of the mean of 0.12. The upper limit of normal of this mean ACCR and the distribution around it was calculated to be 5%.

None of the variables measured in the study was significantly altered by general anaesthesia and surgery carried out on control patients in Group 1 (Table 13). This finding suggests that the ACCR is not influenced by general anaesthesia per se, nor by minor tissue injury at a site distant from the pancreas.

ii) Amylase Results

The changes in serum and urine amylase values recorded after cholecystectomy in Groups 2 and 3 are shown in Table 14 and Figures 5 and 6. No significant change was noted in mean serum amylase and only two patients in each cholecystectomy group had a serum amylase value above the upper limit of normal (300 iu/l) on the first post-operative day. One cholecystectomy patient not receiving Trasylol (Group 2) had a serum amylase of 1668 iu/l on the first post-operative day. This patient had undergone a cholecystectomy plus removal of one stone from the common bile duct and it must be assumed that post-operative pancreatitis occurred

TABLE 13

Pre and post-operative results for the control patients (Group 1: n = 29) mean ± s.e.m.

		Pre-op	Post-op
1.	Serum amylase iu/l	190 ± 8	194 ± 6
2.	Thermolabile serum amylase %	32 ± 1.6	32.8 ± 2
3.	Urine amylase iu/l	285 ± 20	259 ± 38
4.	Thermolabile urine amylase %	61.5 ± 6	63.8 ± 5.5
5.	Serum creatinine µmol/l	96 ± 4	99 ± 8
6.	Urine creatinine µmol/l	8685 ± 658	9976 ± 1210
7.	ACCR %	1.84 ± 0.2	1.61 ± 0.26

TABLE 14

Serum and urine amylase values before and after cholecystectomy (mean ± s.e.m.).

Unpaired Student's t test.

	Pre-op	Post-op	
		Group 2	Group 3
Serum amylase iu/l	203 ± 12	174 ± 28	156 ± 18
Urine amylase iu/l	307 ± 48	752 ± 96*	613 ± 84+

Post-op > pre-op

* $p < 0.001$

\+ $p < 0.01$

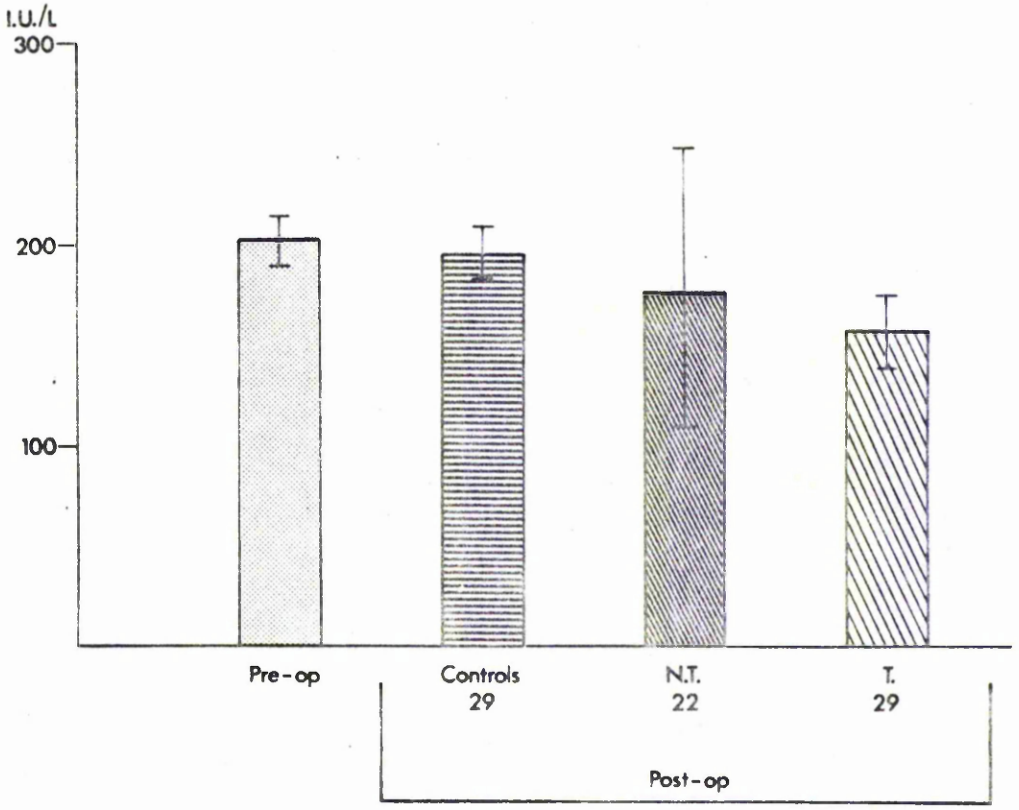


Figure 5. Mean serum amylase of pre-operative and post-operative study groups ± s.e.m.

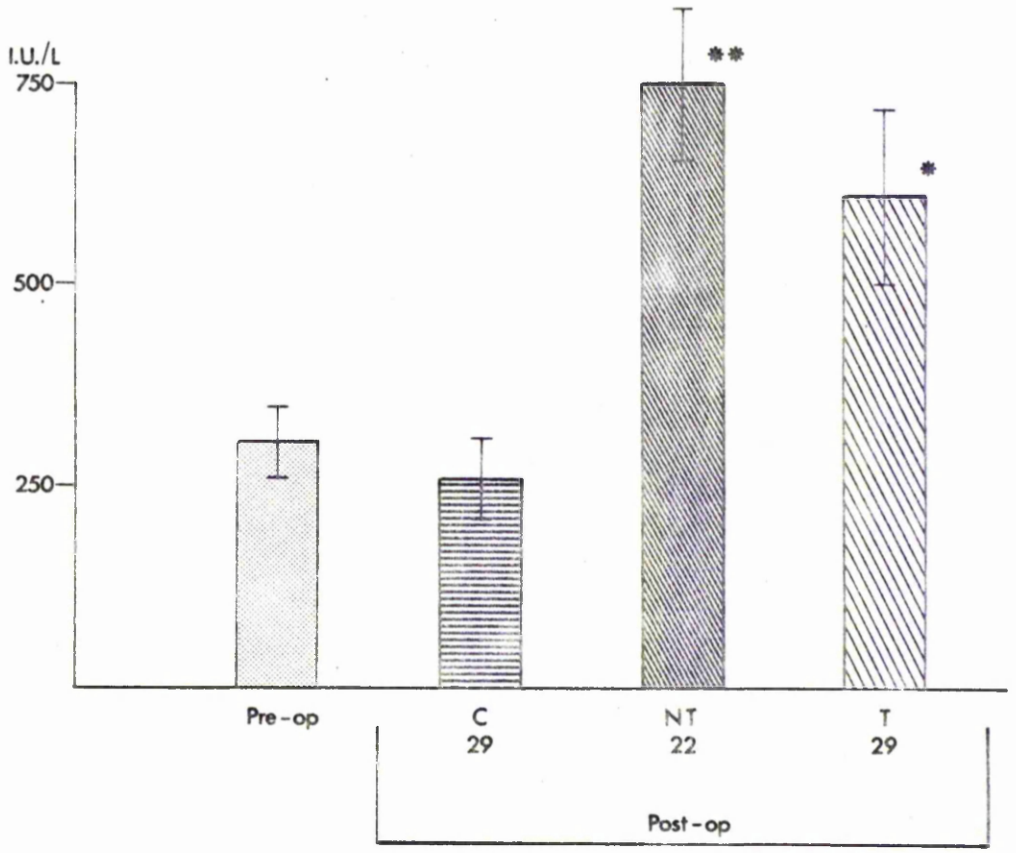


Figure 6. Mean urine amylase of pre-operative and post-operative study groups ± s.e.m. Unpaired Student's t test.

* T > pre-op - $p < 0.01$
** NT > pre-op - $p < 0.001$

in this case. This patient's ACCR on the first post-operative day was 8.6%, in keeping with a diagnosis of acute pancreatitis.

A significant elevation in urine amylase concentration, indicating increased amylase clearance by the kidneys, was noted from the post-operative samples obtained from patients in both cholecystectomy groups. This effect was most marked, however, in patients who had not received Trasylol (Group 2). Figure 6 illustrates this change in urine amylase which was striking in the absence of significant hyperamylasaemia (Figure 5).

iii) Thermolabile Amylase Results

The changes in thermolabile serum and urine amylase values recorded after cholecystectomy in patients in Groups 2 and 3 are shown in Table 15 and Figures 7 - 9. A statistically significant increase in thermolabile serum amylase was observed after cholecystectomy in patients not receiving Trasylol ($p < 0.01$). In these patients (Group 2) mean thermolabile serum amylase increased from 29% to 53% following surgery, similar to the figure of 55% obtained by Donaldson from 14 patients with resolving acute pancreatitis (68). In

TABLE 15

Serum and urine thermolabile amylase values before and after cholecystectomy (mean ± s.e.m.). Unpaired Student's t test.

	Pre-op	Post-op	
		Group 2	Group 3
Thermolabile serum amylase %	29.4 ± 2.4	53 ± 6.4*	40 ± 9.6
Thermolabile urine amylase %	60.9 ± 4	63 ± 5	62 ± 4

* Post-op > pre-op: $p < 0.01$

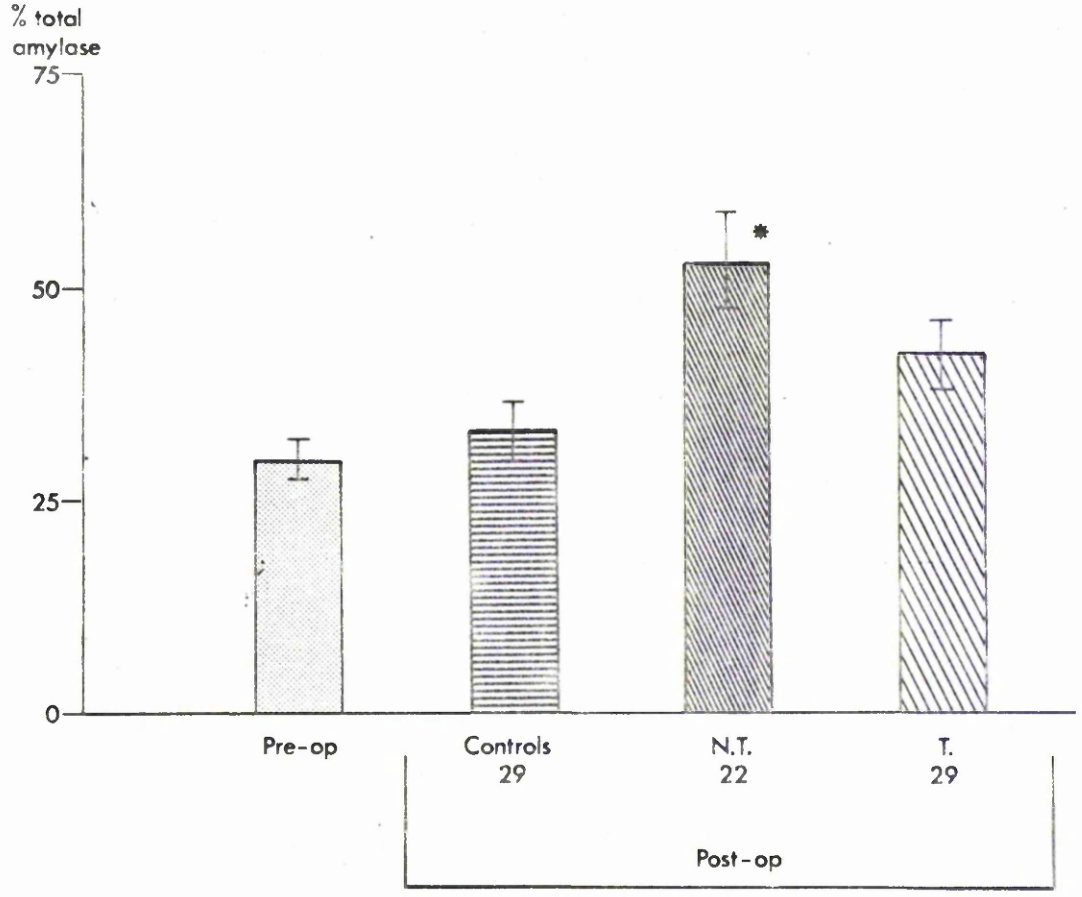


Figure 7. Mean thermolabile serum amylase in pre-operative and post-operative study groups ± s.e.m. Unpaired Student's t test.

* NT > pre-op - $p < 0.01$

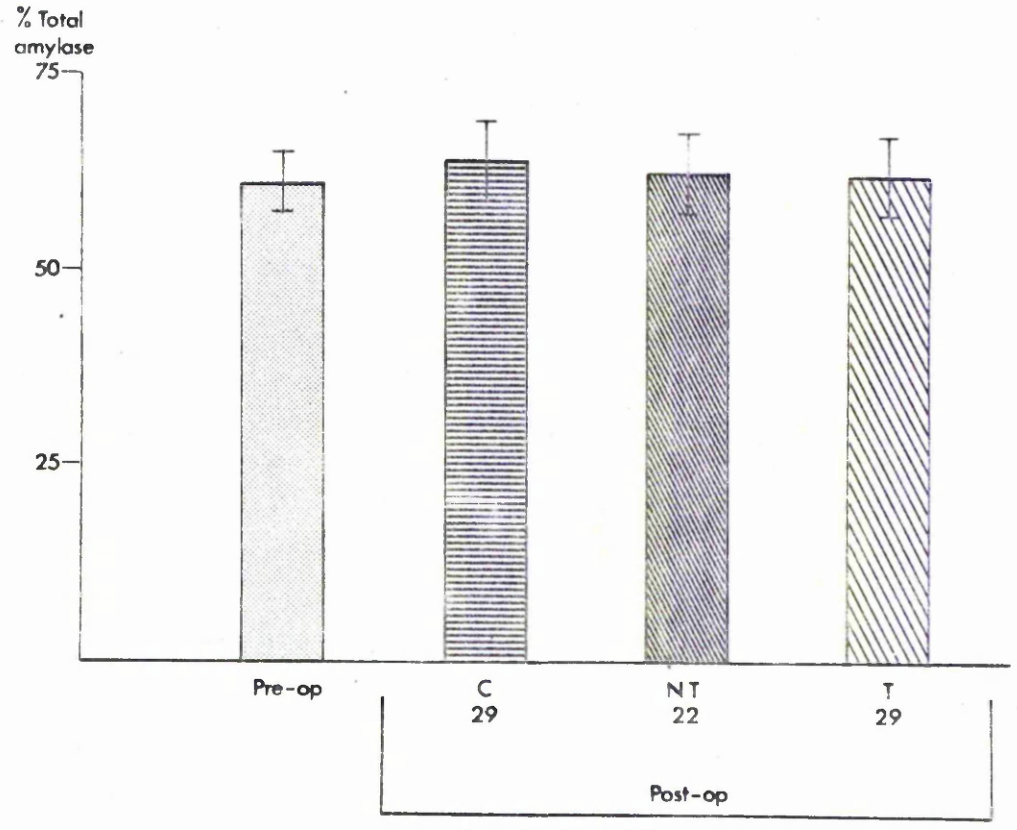


Figure 8. Mean thermolabile urine amylase in pre-operative and post-operative study groups ± s.e.m.

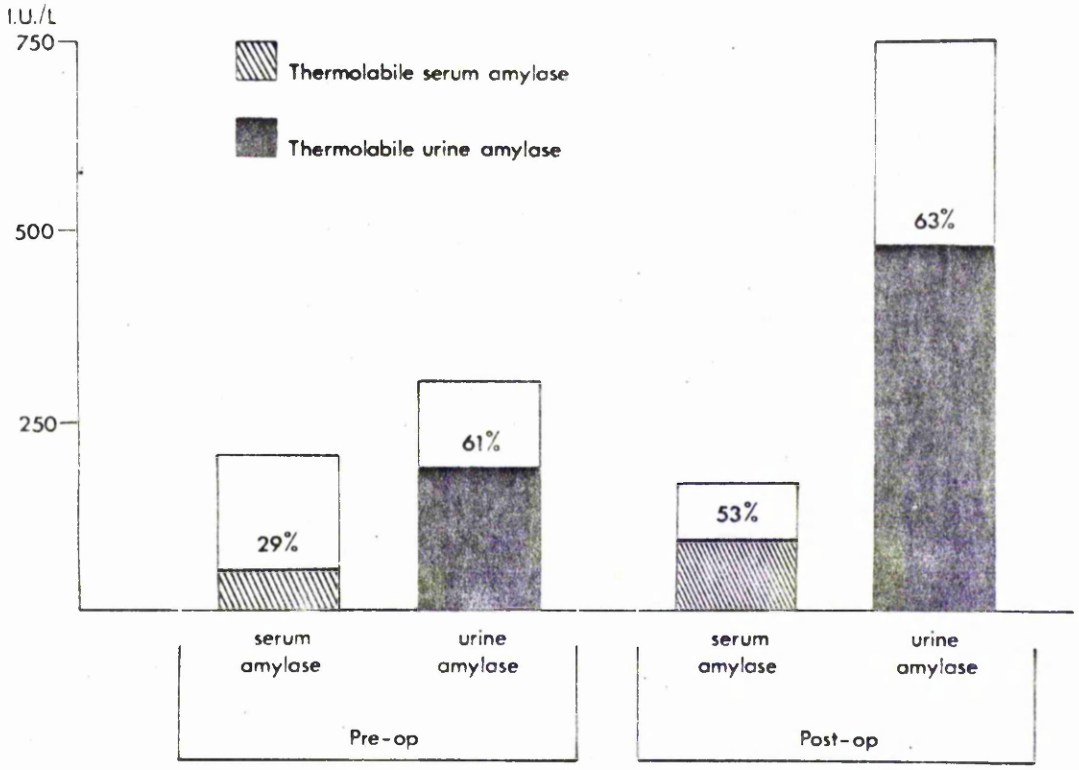


Figure 9. Serum and urine amylase composition before and after cholecystectomy.

patients receiving Trasylol (Group 3) the mean thermolabile serum amylase rose to 40% following surgery, but this increase was not found to be statistically significant. These changes are illustrated in Figure 7.

The percentage of thermolabile urine amylase remained remarkedly constant and was uninfluenced by cholecystectomy (Figure 8). The absolute amount of thermolabile urine amylase excreted, however, increased following cholecystectomy in keeping with the increase in total urine amylase observed. Changes in total and thermolabile serum and urine amylase in patients undergoing cholecystectomy without Trasylol, (Group 2) are shown in Figure 9.

iv) ACCR Results

No significant changes were noted in either serum or urine creatinine values following cholecystectomy with or without the use of Trasylol. A significant rise in the mean post-operative ACCR value was seen in Groups 2 and 3 after cholecystectomy (Table 16: Figure 10). This rise was more marked in those patients who had not received Trasylol (Group 2) and the mean ACCR value in this group (9.5%) was significantly greater than the

TABLE 16

ACCR changes following cholecystectomy with and without Trasylol cover - mean ± s.e.m. Unpaired Student's t test.

	Pre-op	Post-op	
		Group 2	Group 3
ACCR %	2.0 ± 0.24	9.5 ± 2.4*	5.6 ± 1.6+

Post-op > pre-op: * $p < 0.001$

+ $p < 0.01$

Group 2 > Group 3: $p < 0.05$

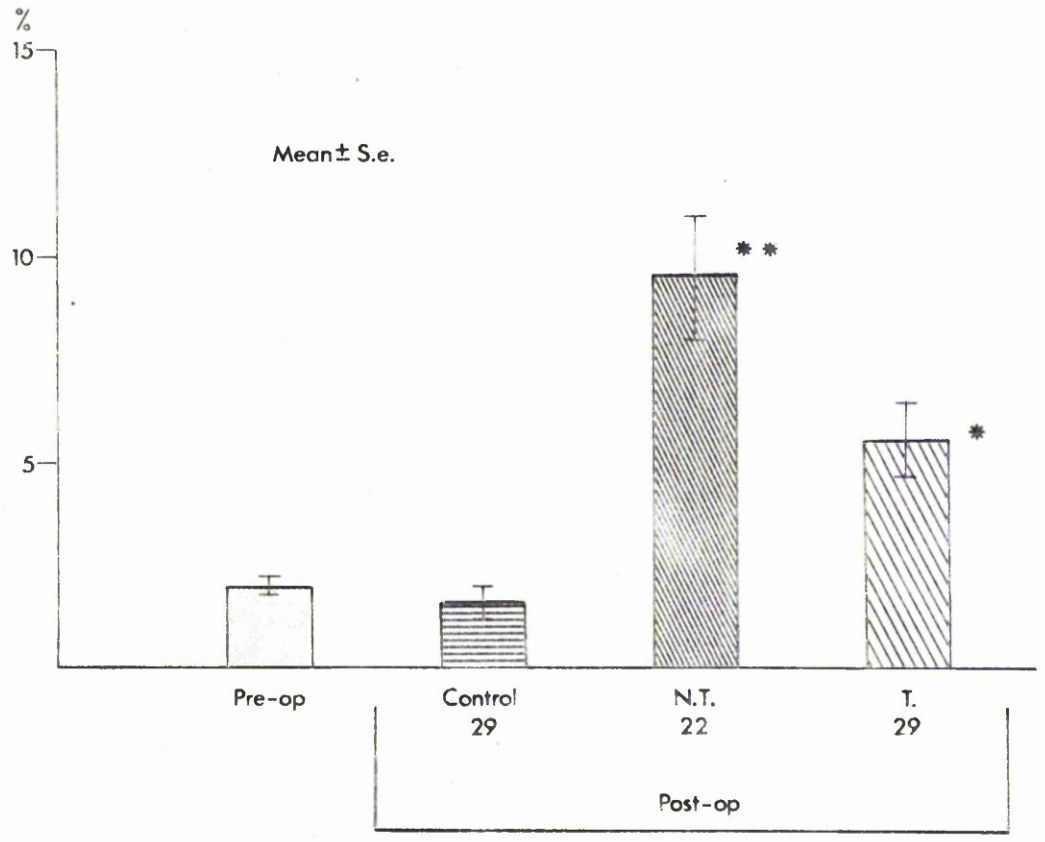


Figure 10. Mean ACCR values for pre-operative and post-operative study groups ± s.e.m. Unpaired Student's t test.

* T > pre-op - $p < 0.01$
** NT > pre-op - $p < 0.001$
NT > T - $p < 0.05$

mean value (5.6%) of patients in Group 3 who had received Trasylol ($p < 0.05$).

A post-operative ACCR of greater than 5% was recorded in a total of 24 patients (47%) who underwent cholecystectomy. Sixteen out of 22 patients (73%) not receiving Trasylol (Group 2) had a post-operative ACCR greater than 5% whereas only 8 patients out of 29 (28%) who received Trasylol showed this rise in the ACCR. The difference in ACCR elevation between the two groups is statistically highly significant ($p < 0.005$). Cholecystectomy alone lead to a post-operative ACCR of greater than 5% in 61% of patients in Group 2 but only 18% of patients in the Trasylol group (Group 3). This difference is statistically significant ($p < 0.025$). Cholecystectomy plus common bile duct exploration lead to a post-operative ACCR of greater than 5% in 100% of patients in Group 2 but only 57% of patients in the Trasylol group. This difference just fails to reach statistical significance. An ACCR of over 5% was not recorded in any of the 80 pre-operative samples and did not occur in the control group (Group 1) post-operatively.

4. DISCUSSION

i) Pre-Operative Data

This study has confirmed that the ACCR may be significantly elevated in the first 24 hours after cholecystectomy; demonstrated that these ACCR changes may be significantly reduced by the use of the proteolytic enzyme inhibitor Trasylol, and provided evidence via thermolabile amylase measurements that the ACCR changes observed were related to pancreatic dysfunction.

The pre and post-operative studies on the 29 control patients in Group 1 demonstrated that general anaesthesia plus minor surgery at an extra abdominal site had no significant influence on either serum or urine amylase in total or thermolabile form or on the ACCR itself (Figures 5 - 8, 10). The pre-operative results obtained from Groups 2 and 3 showed no statistically significant difference for each value estimated and when means were calculated for all gallstone patients these were similar to the means obtained in the control patients (Table 11). A mean for each test in the study was therefore calculated from the 80 pre-operative samples (Table 12). There was no reason to suspect the presence of pancreatic dysfunction

in any of the patients prior to surgery. Two patients in Group 2 and 3 patients in Group 3 had suffered a previous attack of acute pancreatitis but on admission for elective surgery all were asymptomatic with normal serum amylase values (Table 10).

The mean pre-operative serum thermolabile amylase value was found to be 29.4%. This is lower than Donaldson's value of 44% (68) but in keeping with Warshaw's finding of between 10 and 30% (67). The corresponding percentage for urine in this study was found to be 60.9%. Donaldson's mean value was 87% and although higher, our two studies agree on a significant increase in the percentage of thermolabile amylase in the urine compared to serum. The difference in our figures probably reflects the smaller numbers in Donaldson's study (serum = 23; urine = 11). It is well recognised that the pancreatic isoenzyme of amylase is preferentially excreted by the kidneys (38). Isoelectric focusing techniques have demonstrated that pancreatic amylase represents about one third of the amylase in normal serum but about 70-80% of the total urine amylase. Pancreatic

amylase is thus excreted three to four times more rapidly than is the remainder of the serum amylase. Thermolabile amylase also demonstrates this point and it is tempting to suggest that the thermolabile component of amylase as defined in this study is related to the pancreatic isoenzyme of amylase. Donaldson has produced further evidence to support this concept by showing that patients with pancreatic pseudocysts maintain a high percentage thermolabile amylase in their serum until the cysts resolve or are drained (69).

ii) Amylase Data

Cholecystectomy and its related procedures did not significantly alter the mean serum amylase concentration of the patients studied within the period of observation. One patient who did not receive Trasylol, however, developed biochemical evidence of acute pancreatitis on the first post-operative day. An overall incidence of 4% for acute pancreatitis following biliary tract surgery has been quoted in the literature (55). Based on this figure two attacks of acute pancreatitis could have been expected during

this study and the one attack documented using the standard diagnostic criteria was therefore consistent with the phenomenon under study.

Although total serum amylase concentration was not altered significantly, cholecystectomy lead to a significant increase in thermolabile serum amylase in patients not receiving Trasylol (Group 2). This change was marked and closely resembled the change in thermolabile serum amylase seen in resolving acute pancreatitis (68). If one postulates that this represents an increase in pancreatic amylase in the serum secondary to pancreatic inflammation, then this helps explain the findings with urine amylase in this study. Cholecystectomy lead to a significant increase in total urine amylase 24 hours after surgery which was most marked in patients who had not received Trasylol. Any increase in pancreatic amylase in the serum would be preferentially cleared by the kidneys resulting in some increase in the urine amylase concentration. Since urine normally contains 70-80% pancreatic amylase this relatively small change in serum amylase composition would be unlikely to be reflected in urine amylase

composition which did in fact remain stable in this study. The significant increase in amylase in the urine observed in this study cannot simply be explained by an increase in pancreatic amylase production and excretion. Overall renal excretion of amylase must have been increased to explain the amount of urine amylase excreted in the absence of hyperamylasaemia. This situation clearly occurs during the resolution of an attack of acute pancreatitis when there is good evidence that the renal excretion of amylase is increased due to a reduction in amylase reabsorption in the proximal renal tubules (39). This would be a logical explanation for the amylase findings in this study and would link these observations with pancreatic inflammation. Figure 9 outlines the changes in serum and urine amylase values in patients undergoing cholecystectomy without Trasylol cover and highlights the following points:

1. No change in total serum amylase concentration after cholecystectomy.

2. Significant increase in thermolabile serum amylase percentage after cholecystectomy.

3. Significant increase in total urine amylase concentration after cholecystectomy.

4. No change in thermolabile urine amylase percentage after cholecystectomy.

5. Significant increase in thermolabile urine amylase concentration after cholecystectomy.

iii) ACCR Data

The mean ACCR value for the 80 patients in the study was combined with the value from the 105 patients without acute pancreatitis in the study presented in Chapter 3 to give a mean ACCR of 1.75%. The mean was not used in this study for comparative purposes but was used to calculate an upper limit of normal for the test based on 185 observations. This was found to be 5% and this figure was used in this study when assessing a significant ACCR rise for an individual.

The mean ACCR of patients undergoing cholecystectomy was found to be significantly elevated on the first post-operative day. This finding was entirely in keeping with the amylase excretion data discussed above. There is therefore little doubt that amylase excretion was increased in the

first 24 hours following cholecystectomy. The question remains, however, as to whether or not this increased amylase excretion was related in any way to pancreatic inflammation.

iv) Effect of Trasylol

In this study the use of the proteolytic enzyme inhibitor Trasylol was found to:

1. Significantly diminish the rise in percentage thermolabile serum amylase after cholecystectomy.
2. Diminish the rise in urine amylase concentration after cholecystectomy.
3. Significantly diminish the mean ACCR after cholecystectomy.
4. Significantly reduce the number of patients with an ACCR greater than 5% after cholecystectomy.
5. Reduce the number of patients with an ACCR greater than 5% after cholecystectomy plus biliary radiology and common duct exploration.

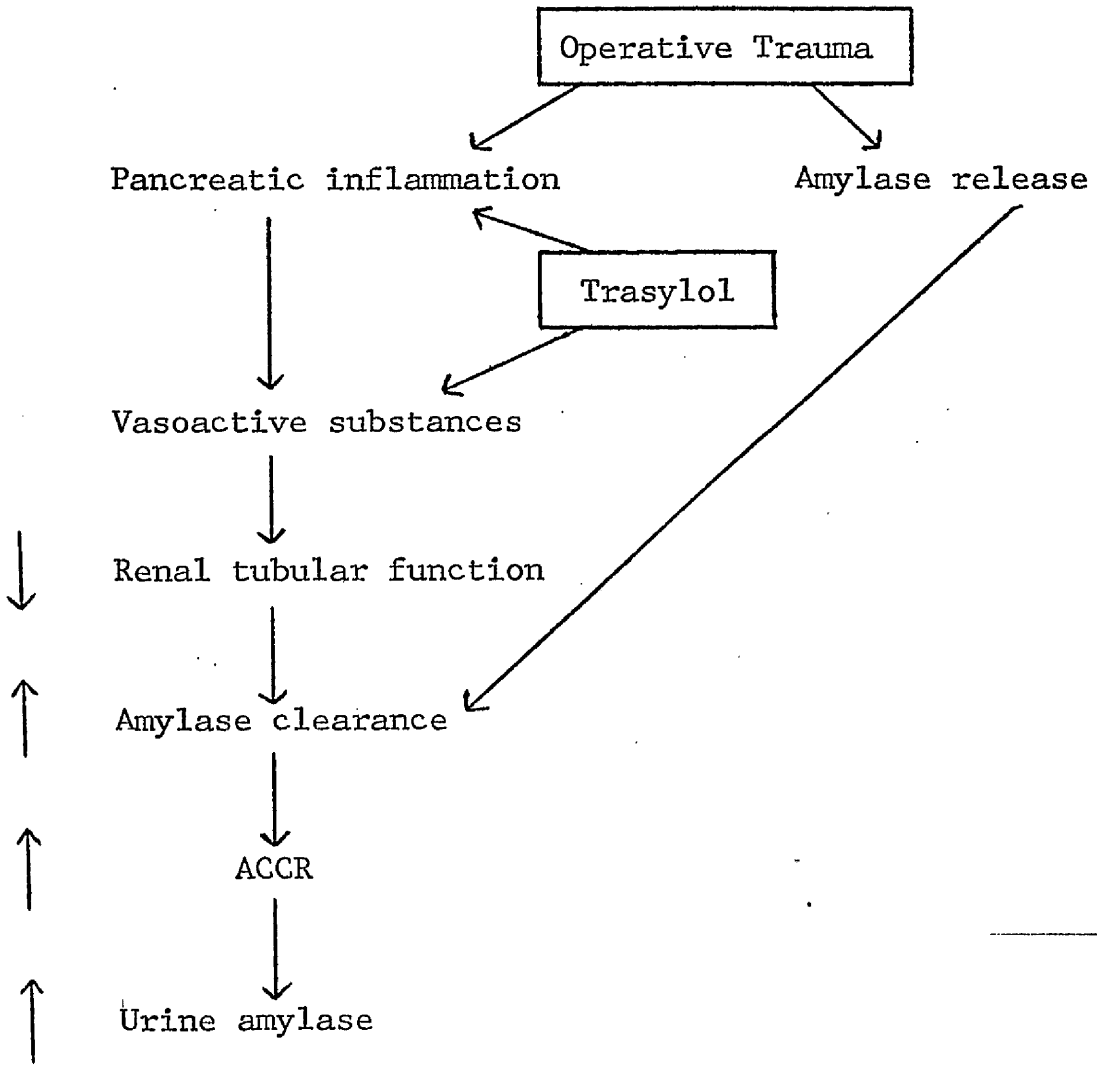
The significant effects resulting from the use of Trasylol suggest that pancreatic inflammation was responsible for the observed changes in amylase

excretion and the ACCR. Trasylol, when present in an adequate concentration in the blood prior to induction of acute pancreatitis, can modify the acute pancreatic inflammatory process by the partial inhibition of trypsin and other related proteolytic enzymes. This protective role has been well documented in animal models (64,65,66). The opportunity to give Trasylol before the induction of pancreatitis seldom arises in the human. Two exceptions to this rule are prior to cholecystectomy and before ERCP in high risk cases.

The postulated mechanisms to explain the changes observed in this study are shown in Figure 11. Trasylol is believed to act both directly on the pancreas and indirectly on the enzyme 'broth' released by the inflamed gland. One of the systemic effects of acute pancreatitis appears to be to reduce the reabsorption of amylase from the renal tubules. This in turn leads to an increase in amylase excretion as measured by the ACCR and an increase in urine amylase concentration. These changes in acute pancreatitis should be reduced, although obviously not abolished, by the action of Trasylol. The

FIGURE 11

Suggested mechanism of action of Trasylol in reducing the ACCR after cholecystectomy.



stronger the stimulus to acute pancreatitis, the less should be the protective effect of Trasylol. The findings in the study have supported this hypothesis by showing a reduced protective effect of Trasylol on the ACCR in patients undergoing common bile duct exploration as well as cholecystectomy.

It may be of course that acute pancreatic inflammation had no part to play in the changes in amylase excretion observed in this study. It is possible that general anaethesia plus cholecystectomy per se resulted in the changes seen. It would however be difficult to explain the observed effects of Trasylol in the absence of any pancreatic involvement. The pancreatic dysfunction which may have been detected by the ACCR changes in the study is of course essentially sub-clinical. There can be no absolute proof from the study that this postulated pancreatic inflammation actually existed, and the evidence in favour of ACCR changes being related to pancreatitis accumulated from this study remains indirect. This will probably remain the case in human studies at least until a gold standard diagnosis of pancreatic inflammation other than histology is described.

The evidence in favour of ACCR elevation being related to pancreatic inflammation is however increasing, albeit mainly indirect. From the first two studies it can be stated that:

1. The ACCR is significantly elevated in acute pancreatitis.
2. No significant elevation in the ACCR has been found in acute abdominal conditions other than acute pancreatitis.
3. The ACCR is a more sensitive diagnostic test for pancreatic inflammation than serum amylase.
4. Significant ACCR elevation occurs following cholecystectomy.
5. Post-cholecystectomy ACCR elevation can be significantly reduced by Trasylol given before, during and after surgery.
6. Exploration of the common bile duct added to cholecystectomy produces the greatest post-cholecystectomy ACCR changes.

The ACCR may therefore be a sensitive index of pancreatic inflammation which could be applied as a monitor and might be of use after biliary surgery to detect patients developing pancreatic inflammation. The ACCR might also be used in other situations where pancreatic inflammation has been shown to occur occasionally in order to assess the incidence of sub-clinical pancreatitis.

C H A P T E R F I V E

THE AMYLASE CREATININE CLEARANCE RATIO FOLLOWING CARDIOPULMONARY BYPASS

CHAPTER FIVE

THE AMYLASE CREATININE CLEARANCE RATIO FOLLOWING CARDIOPULMONARY BYPASS

1. INTRODUCTION

Evidence has been cited in the last chapter to show that acute pancreatitis is a well documented complication of biliary tract surgery and is thought to be related to operative trauma to the pancreas or sphincter of Oddi (55, 56,57,58,59). Post-operative acute pancreatitis has more recently been documented following cardiac surgery performed using conventional non-pulsatile cardiopulmonary bypass (CPB) (57,70,71,72). Feiner has reported a 16% incidence of unexplained pancreatitis at autopsy in patients dying after cardiac surgery (73) and this figure is far higher than the 0.1% incidence of acute pancreatitis expected after procedures not involving the area of the pancreas (58).

Operative trauma to the upper abdomen cannot readily be implicated in the aetiology of pancreatitis in cardiac cases. Evidence from Feiner (73), Warshaw (74) and Foulis (75) suggests that pancreatic tissue ischaemia may be an important aetiological factor in post-bypass pancreatitis. The ACCR has been found to be significantly elevated without clinical signs of acute pancreatitis after non-pulsatile CPB (76,77). Patients undergoing thoracotomy without CPB did not exhibit a significant

rise in the ACCR. The studies so far outlined in this thesis have provided indirect evidence that the ACCR may be a sensitive index of acute pancreatic dysfunction. A significant ACCR elevation observed following cardiac surgery may therefore be indicative of pancreatic ischaemia during non-pulsatile CPB.

There is now convincing evidence of organ system dysfunction during non-pulsatile CPB, including a progressive rise in peripheral vascular resistance associated with renin-angiotensin activation and tissue hypoperfusion (78,79,80,81). During the last five years reliable systems have been developed to deliver pulsatile CPB. Improved metabolic and haemodynamic responses have been documented during and after pulsatile CPB, e.g. improved tissue oxygen consumption (82,83), reduced activation of the renin-angiotensin system (84,85), and reduced indices of kidney and brain damage consistent with improvement in overall tissue perfusion (83,86,87,88,89).

If pancreatic tissue ischaemia during non-pulsatile CPB is an important aetiological factor in post-bypass pancreatitis, then pulsatile CPB might be expected to reduce the incidence of this complication. It was decided to use the ACCR to test this hypothesis in view of the fact that significant ACCR elevation had already been demonstrated following non-pulsatile

CPB (76,77). Moreover the apparent sensitivity of the ACCR for subclinical pancreatic dysfunction makes it a suitable screening test in a situation such as this where the expected incidence of clinical acute pancreatitis is low. The ACCR was therefore measured before, during and after cardiac surgery performed using both non-pulsatile and pulsatile CPB in an attempt to further elucidate the nature of post-bypass acute pancreatitis.

2. PATIENTS AND METHODS

i) Patients

Twenty adult patients admitted to Glasgow Royal Infirmary for elective open heart surgery were arbitrarily allocated to pulsatile or non-pulsatile study groups. No patient had a past history of pancreatic disease and all had normal renal function prior to surgery. In the pulsatile group, 5 patients underwent valve replacement and 5 had coronary artery bypass grafting. In the non-pulsatile group 7 patients underwent valve replacements while 3 had coronary artery bypass grafting.

ii) Bypass Details

The pre-operative and operative details for both groups are shown in Table 17. There was no

TABLE 17

Preoperative and operative details in pulsatile and non-pulsatile groups. Unpaired Student's t test. (Mean ± s.e.m.)

	Non-pulsatile Group N = 10	Pulsatile Group N = 10	p
Age (yrs)	42.8 ± 4.2	51.6 ± 2.5	N.S.
Weight (kg)	65.3 ± 5.3	70.52 ± 4.0	N.S.
B.S.A. (M^2)	1.72 ± 0.009	1.81 ± 0.006	N.S.
Perfusion Details Pump Flow (L/min)	3.35 ± 0.14	3.43 ± 0.09	N.S.
Perfusion Pressure (mmHg)	54.5 ± 2.54	49.0 ± 2.58	N.S.
Haematocrit (%)	22.3 ± 0.97	23.4 ± 1.2	N.S.
Total Bypass Time (Mins)	73.2 ± 9.75	96.8 ± 13.5	N.S.
Aortic Cross Clamp Time (Mins)	38.0 ± 7.3	41.3 ± 9.51	N.S.

Legend : B.S.A. = body surface area N.S. = not significant

significant difference between the groups in the factors documented. In all cases general anaethesia was induced with sodium thiopentone and maintained with nitrous oxide, oxygen and intravenous morphine. The bypass circuit was primed with 2 litres of Ringer's lactate solution and a Cobe-Stöckert cardiopulmonary bypass pump was used in both groups. This pump is a modified roller pump based on a large 'stepping' motor with low pump-head inertia which allows rapid acceleration and deceleration of the pump-head. The pump motor is driven via a control module which allows the operator to set the frequency, amplitude and duration of pulsation delivered by the pump-head. The pump can also run as a standard roller pump, delivering non-pulsatile blood flow. A digital record for instantaneous mean or phasic pump flow is displayed on the pump module while the radial artery pressure profile is relayed to a pen recorder to record the production of a true pulsatile arterial waveform. This pump was used in both groups according to the following protocol:

Non-Pulsatile Group

The pump in non-pulsatile mode throughout the period of perfusion. In the non-pulsatile group the classical ripple pattern was seen in

the radial artery waveform and at no time was pressure fluctuation greater than 10 mmHg.

Pulsatile Group

The pump in non-pulsatile mode until left ventricular ejection ceased. Thereafter pump in pulsatile mode at 72 pulses/minute until left ventricular ejection restarted. Pump reverted to non-pulsatile mode until end of perfusion. Pump settings for pulsatile flow were as previously described (84). Pulse run time was 55% of the total cycle length, delay time was 0 and the automatic control was set to 80% to ensure a small constant forward flow through the system. In each patient pulse pressure in the radial artery was greater than 25 mmHg with a rise time in the pressure wave less than 20% of the total cycle length.

Normothermic bypass was used in both groups, myocardial protection being achieved using topical hypothermia and aortic root flush cardioplegia.

iii) ACCR Calculation

Spot samples (10 ml) of venous blood and urine were obtained simultaneously at the following times:

1. before surgery

2. during surgery but before bypass
3. during bypass (approximately midpoint)
4. during surgery but after bypass
5. daily, for the first five post-operative days.

Amylase was estimated using a Phadebas reagent kit while creatinine was measured on a Technicon A11 auto-analyser. The ACCR was calculated using the standard formula described by Levitt (32). The mean ACCR of 185 patients admitted to the Western Infirmary, Glasgow, with no evidence of pancreatic disease has previously been shown to be 1.75%. The upper limit of normal for the ACCR for the purposes of this study was accepted as 5%, a figure based on the 95% confidence limit of the mean just described. The criterion for a significant elevation in the ACCR in this study was taken to be two consecutive ACCR values greater than 5% in any patient.

3. RESULTS

i) Amylase Data

No patient in this study developed clinical signs of post-operative acute pancreatitis. Two patients in the pulsatile group had a serum amylase value greater than the upper limit of normal (300 iu/1)

but neither value approached the diagnostic level for acute pancreatitis (1200 iu/l). Five patients in the non-pulsatile group (50%) had a serum amylase value of greater than 300 iu/l and in one of these patients a serum amylase level of 1224 iu/l was recorded during bypass. No significant difference however was found between the mean serum amylase values of the pulsatile and the non-pulsatile groups at each sample time (Figure 12). On the first post-operative day the mean urine amylase concentration of patients in the non-pulsatile group (969 iu/l) was found to be significantly higher ($p > 0.01$) than the corresponding value for the pulsatile group patients (252 iu/l) - Figure 13.

ii) ACCR Data

The ACCR was found to be significantly elevated, as defined above, in 9 patients in the non-pulsatile group (90%) and 1 patient in the pulsatile group (10%). This difference was found statistically (Fisher's exact test) to be highly significant ($p < 0.001$). Figure 14 shows the mean ACCR values for the 10 patients in the non-pulsatile group. The mean ACCR for these patients was found to be significantly greater than the

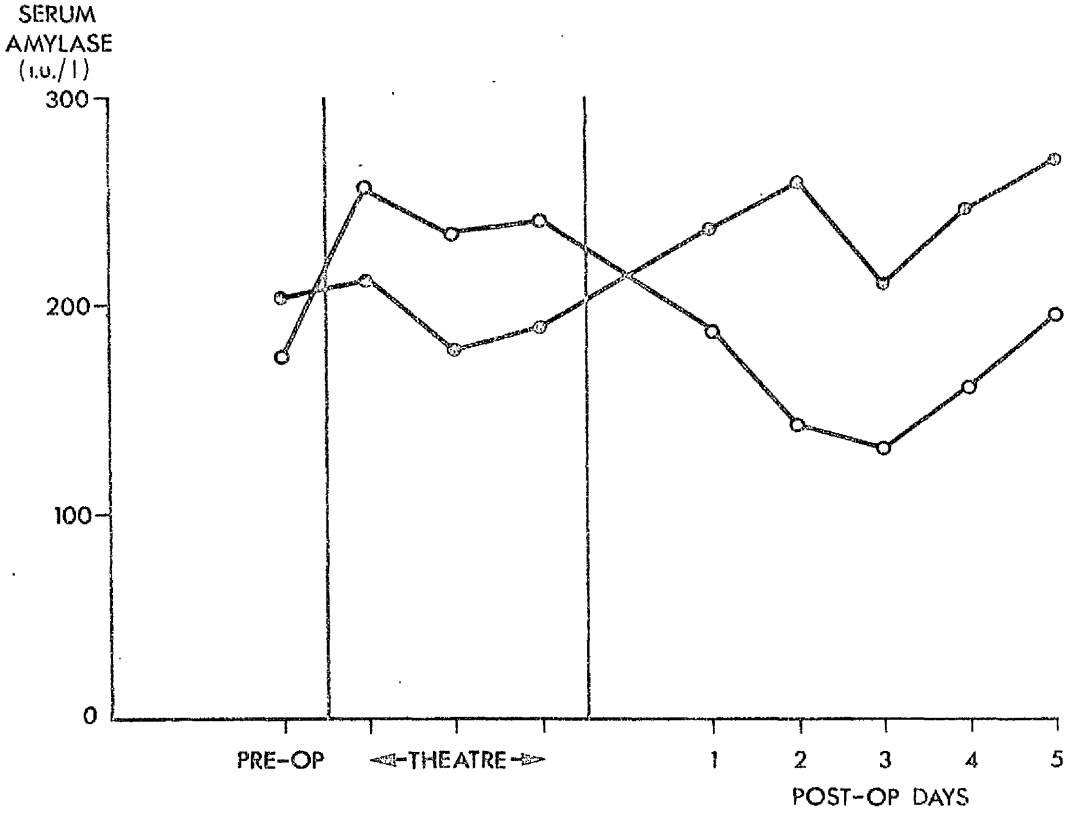


Figure 12. Mean serum amylase values for pulsatile group (open circles) and non-pulsatile group (closed circles).

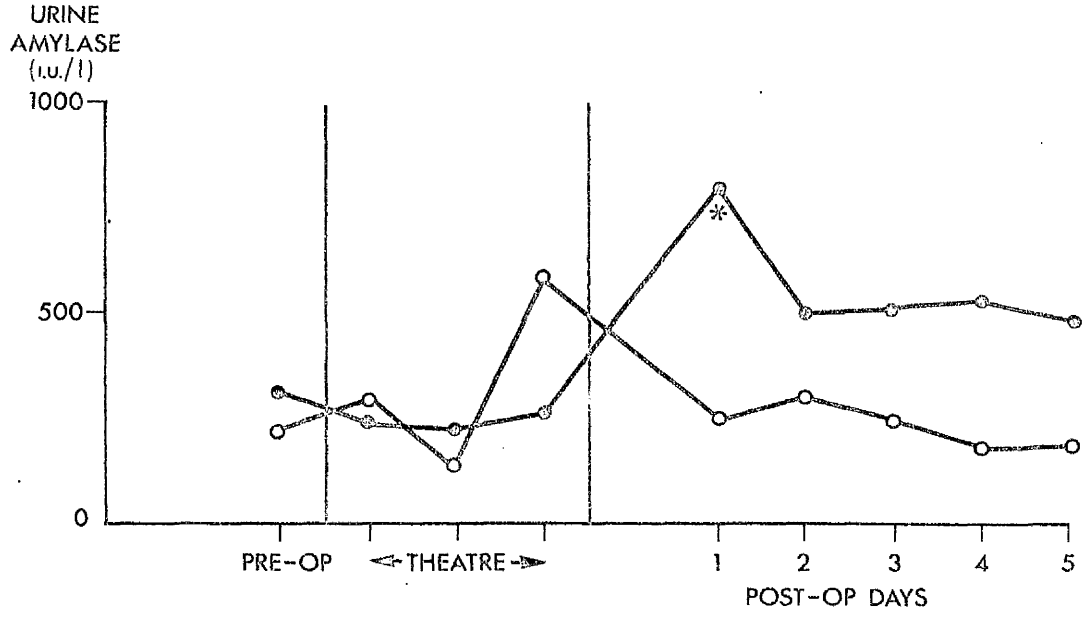


Figure 13. Mean urine amylase values for pulsatile group (open circles) and non-pulsatile group (closed circles).
Unpaired Student's t test.

* Non-pulsatile > pulsatile - $p < 0.01$

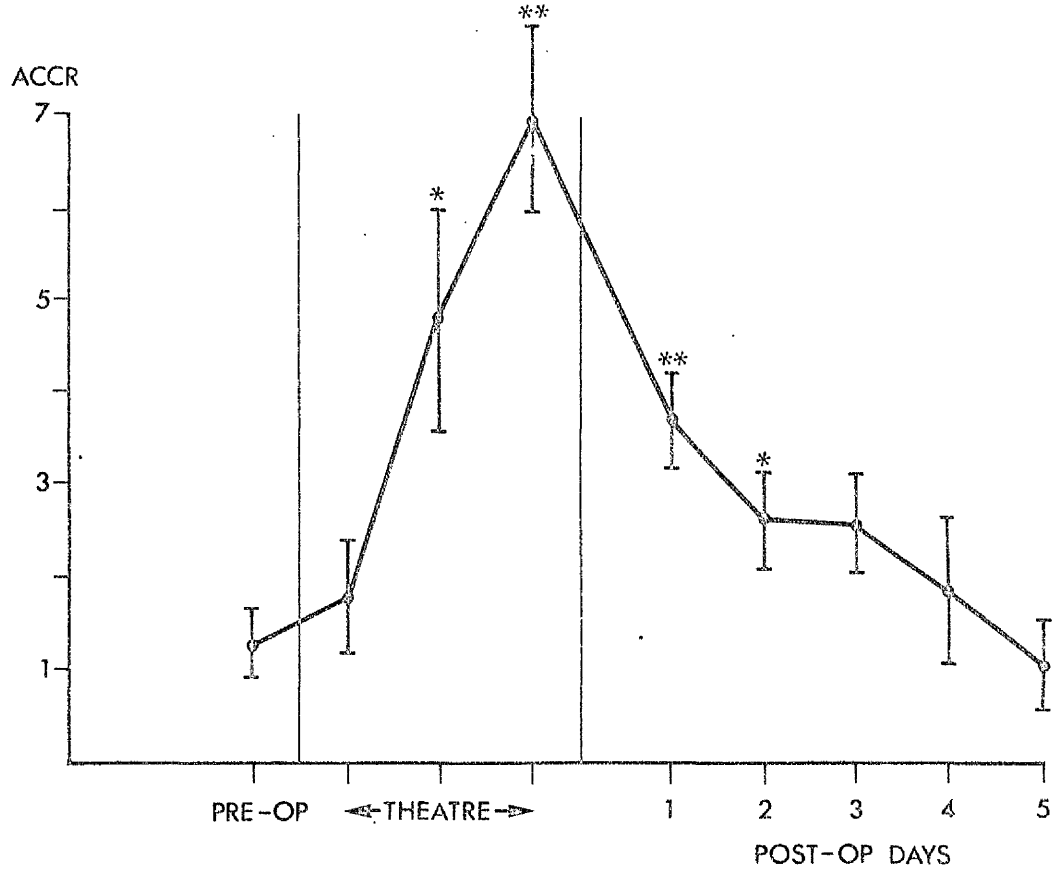


Figure 14. Mean ACCR ± s.e.m. for non-pulsatile group. Paired Student's t test.

* value > pre-op - $p < 0.05$
** value > pre-op - $p < 0.005$

pre-operative value during bypass, just after bypass and on the first two post-operative days. The 10 patients in the pulsatile group did not show a significant rise in ACCR during CPB and their mean post-bypass ACCR values were not significantly greater than the pre-operative value (Figure 15). The mean ACCR results for the non-pulsatile group and the pulsatile group are combined in Figure 16. The patients in the non-pulsatile group had significantly higher mean ACCR values during bypass, just after bypass and on the first post-operative day. Pre-operative and pre-bypass mean ACCR values were similar for both groups.

iii) Clinical Progress

The post-operative clinical course was uneventful in all patients studied. In particular post-bypass haemodynamic status was stable, no forms of circulatory assistance was required, and all patients were extubated and breathing spontaneously within 24 hours of surgery. Significant post-operative renal insufficiency was not encountered in any patient studied. The mean post-operative maximum value for serum creatinine was 102 μmol/l in the non-pulsatile group and 109 μmol/l in the pulsatile group.

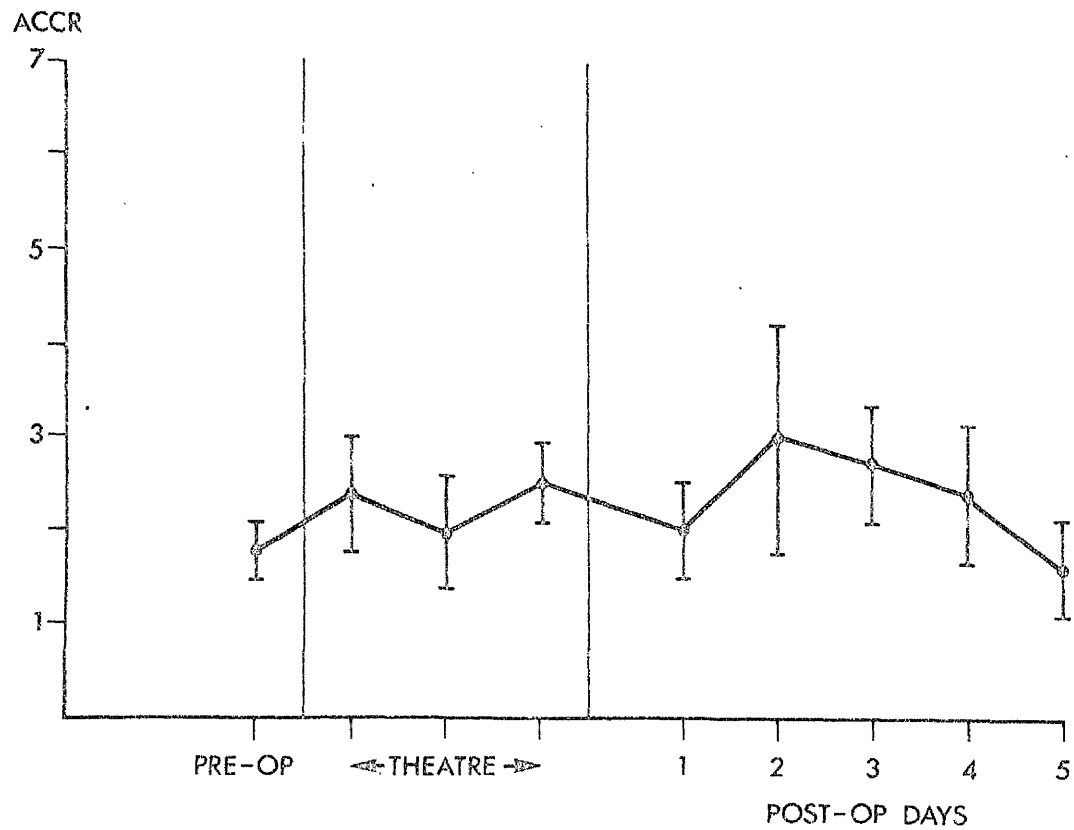


Figure 15. Mean ACCR ± s.e.m. for pulsatile group.

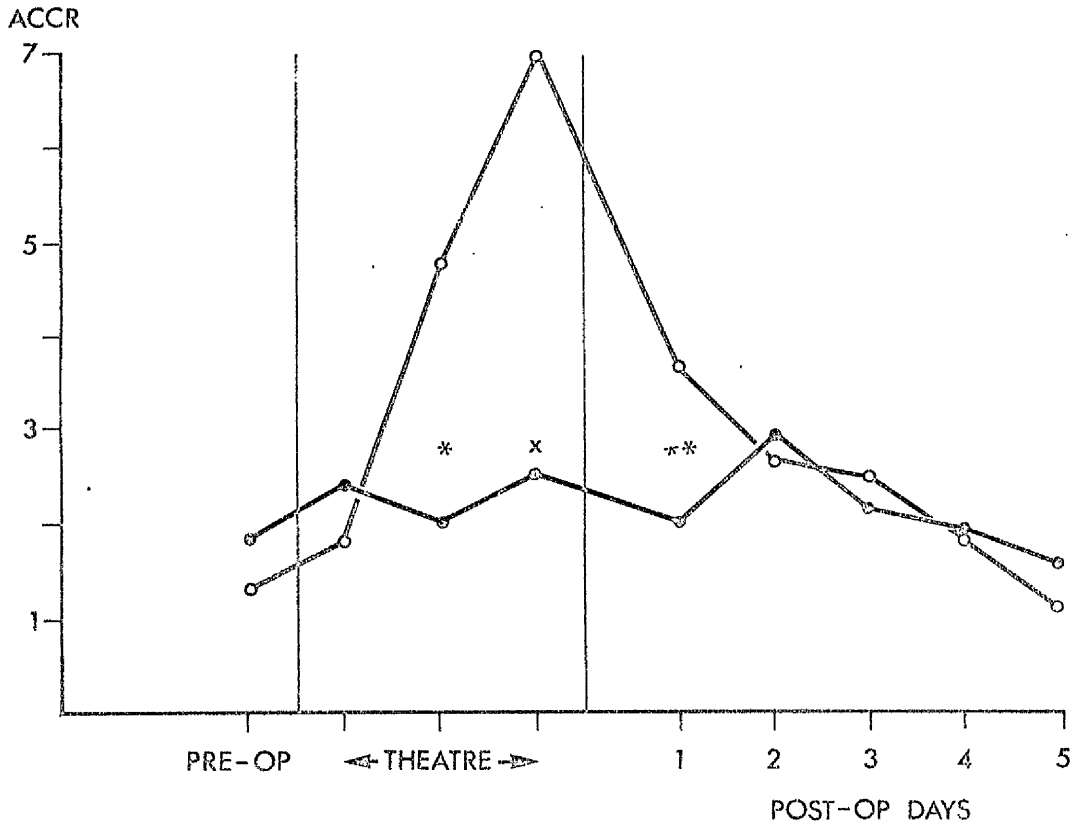


Figure 16. Mean ACCR values for pulsatile group (closed circles) and non-pulsatile group (open circles). Unpaired Student's t test.

Non-Pulsatile > Pulsatile

X - $p < 0.001$
* - $p < 0.05$
** - $p < 0.025$

4. DISCUSSION

i) Amylase Excretion

This study has confirmed previous reports that the renal clearance of amylase may be significantly increased during and immediately following non-pulsatile CPB (76,77). This is reflected in this study firstly by a significant increase in urine amylase concentration on the first post-operative day and secondly by a significant increase in the ACCR during non-pulsatile CPB and on the first two post-operative days. Increased amylase excretion in the presence of stable serum amylase levels, such as is seen in the resolution phase of acute pancreatitis, is thought to result from a reduction in amylase reabsorption from the renal tubules rather than a change in amylase isoenzyme structure or an increased filtration of amylase at the glomerular level (39,40,90). The precise mechanism leading to the reduction in the reabsorption of amylase in the renal tubules secondary to pancreatic inflammation is as yet unknown.

This general theory, however, is supported by the findings of Hennings and Jacobsen (77) who noted increased renal excretion of β2 micro-

globulin, a low molecular weight protein known to be reabsorbed in the renal tubules, following non-pulsatile CPB. There is some evidence that competitive inhibition of amylase reabsorption secondary to non-specific low molecular weight proteinuria may also occur in the renal tubules (90). This may explain the raised ACCR values reported in conditions apparently unrelated to acute pancreatitis (43,90,91), although, due to the problems in making a certain diagnosis of acute pancreatitis discussed earlier it is impossible to be sure that acute pancreatitis was not coexistant in the majority of these cases. While the changes in renal clearance of amylase observed in this study are entirely in keeping with those expected in mild acute pancreatitis, the possibility of non-specific low molecular weight proteinuria causing these changes cannot be excluded.

ii) Effect of Pulsatile CPB

The study has shown that the abnormal amylase excretion observed following non-pulsatile CPB can be almost totally normalised by the use of pulsatile perfusion. Only 1 out of the 10 patients undergoing cardiac surgery using pulsatile CPB

showed a significant ACCR elevation and the mean ACCR of the 10 patients in the pulsatile group at each sample point did not rise significantly above the pre-operative value. The post-bypass mean ACCR of 3.1% for this group of patients was well below the ACCR level considered to be the upper limit of normal (5%). Comparing the two groups of patients confirmed that the renal excretion of amylase was significantly reduced in the pulsatile CPB group during bypass and on the first post-operative day. Since there was no other significant difference between the two groups of patients it may be assumed that the normalisation of the renal excretion of amylase was related to pulsatile perfusion itself. The similarity of the mean serum amylase levels in the two groups indicates that pulsatile perfusion normalises renal excretion by largely preventing the reduced amylase reabsorption which has been shown to exist following non-pulsatile CPB (77). Whether or not this effect of pulsatile perfusion is mediated through a protective action on pancreatic function remains open to discussion.

The study presented in Chapter 4 demonstrated a protective effect on amylase excretion following

cholecystectomy by the use of Trasylol given before, during and after surgery. The results suggested that ACCR elevation following cholecystectomy was related to pancreatic dysfunction and that pancreatic inflammation had been diminished by the use of Trasylol prophylactically. Ischaemia has been implicated in the aetiology of post-bypass pancreatitis (77) and pulsatile perfusion may act prophylactically by protecting the pancreas through an improvement in its microcirculation. Warshaw (74) has demonstrated that the pancreas is highly vulnerable to ischaemic necrosis and has reported a 9% incidence of major pancreatic necrosis at autopsy in patients dying after hypovolaemic shock without evidence of acute renal tubular necrosis (ATN), compared with a 50% incidence of major pancreatic necrosis in patients dying with concomitant ATN. Studies have shown that pulsatile CPB prevents the progressive rise in peripheral resistance which occurs with non-pulsatile perfusion thereby improving cardiac output and peripheral circulation (92,93,94). Improved tissue perfusion during pulsatile CPB is reflected in restoration of normal pituitary adrenal stress responses (95) and

reduction in the renin-angiotensin activation seen during non-pulsatile CPB (83,84). There is therefore good evidence to suggest that pulsatile perfusion may protect tissues from ischaemic damage during CPB.

If the increase in amylase excretion following non-pulsatile CPB were secondary to low molecular weight proteinuria following non-specific tissue damage, one might expect to find increased amylase excretion after operations of a similar magnitude. This has not been reported to date and in the study where amylase excretion was measured in patients undergoing thoracotomy without CPB no significant increase in amylase output was observed (77). It is possible that non-pulsatile CPB per se might result in significant low molecular weight proteinuria secondary to non-specific tissue breakdown with a resultant increase in amylase excretion. Pulsatile CPB may therefore decrease amylase excretion by reducing non-specific tissue breakdown secondary to ischaemia during bypass. There is however no evidence as yet to support this hypothesis. The amylase clearance changes observed in the non-pulsatile bypass group cannot be explained by changes in overall renal function.

No gross abnormalities in renal function were observed and any minor changes would have been compensated for by the use of ratio of amylase clearance to creatinine clearance (ACCR).

ii) Conclusion

It can be concluded therefore that pulsatile flow during CPB can significantly reduce the increased renal excretion of amylase, as measured by the ACCR, observed during surgery and in the early post-operative period following non-pulsatile bypass. This finding may indicate that pulsatile CPB will protect the pancreas against ischaemic damage and hence reduce the incidence of post-bypass pancreatitis. Pulsatile bypass is now starting to be used commonly in Great Britain and the United States and over the next five to seven years it should be possible to assess its effect on the incidence of post-bypass acute pancreatitis. The prediction from this ACCR study is that the incidence should fall significantly if other factors are matched. If this is in fact verified, then it will provide substantial evidence that the elevated ACCR values observed in this study were related to pancreatic inflammation.

CHAPTER SIX

THE AMYLASE CREATININE CLEARANCE RATIO AND CIMETIDINE THERAPY

CHAPTER SIX

THE AMYLASE CREATININE CLEARANCE RATIO AND CIMETIDINE THERAPY

1. INTRODUCTION

In 1972 Professor JW Black and his colleagues published the first paper on an H_2-receptor antagonist named Burimamide and started an explosion of interest into this group of compounds (96). Cimetidine (Tagamet) was the first of these compounds approved for general use in the United Kingdom and to date it has been most successful in the treatment of acute duodenal ulceration (97). Cimetidine has also been used to treat simple gastric ulceration, peptic oesophagitis, the Zollinger Ellison syndrome and upper gastrointestinal haemorrhage, both as a prophylaxis and as treatment for established bleeding (98).

In 1978 two reports appeared in the literature which gave some cause for concern that Cimetidine therapy may be causally related to acute pancreatitis. The first report was of a patient who developed biochemically confirmed acute pancreatitis while receiving oral Cimetidine therapy in a dosage of 1 gm/day for duodenal ulceration (99). The correlation with Cimetidine therapy may well have been spurious, and perhaps the pancreatitis was more likely to have been associated with the acute

duodenal ulceration than with Cimetidine therapy. However, at the same time, Joffe and Lee reported histologically proven pancreatic inflammation in rats receiving Cimetidine to treat experimentally induced duodenal ulcers (100).

It was felt that the ACCR would be an appropriate screening test to evaluate any relationship between Cimetidine therapy and pancreatic inflammation, in as much as if the ACCR was not altered significantly by Cimetidine therapy a causal relationship with acute pancreatitis would be extremely unlikely. It was decided to test both acute and chronic administration of Cimetidine and this was done by:

1. studying patients receiving Cimetidine acutely as a treatment for upper gastro-intestinal haemorrhage and
2. studying patients attending the peptic ulcer clinic of the Western Infirmary, Glasgow, who were receiving oral Cimetidine regularly for duodenal ulceration.

2. PATIENTS AND METHODS

i) The Acute Study

At the time this study was conducted (1980) a trial was underway at the Western Infirmary, Glasgow, to assess the value of Cimetidine in the

management of upper gastrointestinal haemorrhage. All patients admitted to the acute medical receiving unit of the Western Infirmary with this diagnosis were started on either -

a) Cimetidine - 1.6 gm/day intravenously in normal saline or 5% dextrose or

b) a placebo in the same crystalloid solutions.

The study was double-blind and the drug packs for intravenous and then oral administration were identical under the study name of Cimetex. The code was held centrally and not broken until analysis of the data or at the specific request of a clinician. Cimetidine therapy was continued until definite evidence of recurrent haemorrhage, surgery or discharge from hospital. In the Cimetex study the site of bleeding was identified in 88% of the patients at endoscopy performed within 24 hours of admission in 90% of cases.

Spot samples (10 ml) of venous blood and urine were taken for amylase and creatinine estimation within 24 hours of starting Cimetidine therapy and again 48 hours later (third day of admission) if the patient was still in the unit

and receiving Cimetex. Patients were managed by members of the Haematemesis Management Team using conventional medical and surgical principles and detailed records were kept to facilitate analysis of the Cimetex study.

Patients were chosen from the Cimetex study for the ACCR study if they fulfilled the following criteria:

1. 24 hour ACCR estimation available
2. no past history of pancreatitis
3. no hospital admission in the last five years
4. not taking Cimetidine prior to admission
5. no regular medications from the General Practitioner
6. the site of bleeding defined at endoscopy.

Sixty patients admitted to the Cimetex study fulfilled the above criteria and on breaking the code after the ACCR study had been completed it was found that 32 had received Cimetidine and 28 had received the placebo. Assuming effective randomisation, the only significant difference between the two groups of patients should have been the use of Cimetidine and therefore any significant difference in the mean ACCR of the two groups should have been the result of Cimetidine therapy.

ii) The Chronic Study

Thirty-two out-patients with confirmed duodenal ulceration but otherwise in good health were studied. No patient had a past history of acute or chronic pancreatitis and none had ingested alcohol within the 48 hours prior to ACCR estimation. The only drugs taken by the patients, apart from Cimetidine, were simple antacids in a few cases. The patients in the study were being treated with Cimetidine in a mean daily dose of 863 mg (range 400-1600 mg) at the time of ACCR estimation and had consumed this drug for a mean period of 18 weeks (range 2-52 weeks). Venous blood and urine samples were taken from each patient for amylase and creatinine estimation and the ACCR calculated using the formula described by Levitt (32).

3. RESULTS

i) The Acute Study

Of the 60 patients studied 32 had received Cimetidine acutely while 28 had received the placebo. All 60 patients had suffered an acute gastrointestinal haemorrhage and it is therefore mandatory to demonstrate that factors which may have been detrimental to pancreatic function other than Cimetidine were evenly distributed between

the two groups of patients. Patients with a history of pancreatitis, recent significant illness and chronic drug ingestion were excluded from the study.

Table 18 shows the mean age and sex distribution of the 60 patients along with the number whose blood alcohol was found to be greater than 80 mg percent on admission. Blood alcohol estimation was performed routinely as part of the Cimetex study. There was no significant difference between the two groups in these factors. Table 19 shows the source of blood loss defined at endoscopy in the two groups of patients. The lesions diagnosed were distributed evenly between the two groups. Table 20 shows that there was no significant difference between the Cimetidine and placebo groups with respect to the incidence of hypotension, blood loss, further haemorrhage, surgery and overall mortality.

Table 21 shows the mean amylase, creatinine and ACCR values obtained within 24 hours of admission to hospital for the Cimetidine and placebo patients. There was no significant difference in the mean values of either serum or urine amylase although the mean urine amylase

TABLE 18

Number, age, sex distribution and admission blood alcohol levels in acute study patients.

	Cimetidine	Placebo
Number	32	28
Age (yrs)	46	49
Male	22	20
Female	10	8
Admission alcohol > 80 mg %	7	6

TABLE 19

The source of blood loss identified at endoscopy.

	Cimetidine	Placebo
Acute duodenal ulcer	12	10
Acute posterior duodenal ulcer	4	5
Gastritis	8	7
Acute gastric ulcer	6	5
Oesophagitis	4	4
Mallory-Weiss tear	2	2

TABLE 20

Severity and outcome of upper gastrointestinal haemorrhage.

	Cimetidine N = 32	Placebo N = 28
Admission systolic BP < 100 mmHg	3	3
Mean admission Hb (g/l)	9.2	8.7
Mean blood transfusion (units)	3.6	4.2
Further haemorrhage	6	6
Surgery	4	3
Overall mortality %	3.1%	3.6%

TABLE 21

Amylase, creatinine and ACCR values within 24 hours of admission to hospital (mean ± s.e.m.). Unpaired Student's t test.

	Cimetidine N = 32	Placebo N = 28	P
Serum amylase iu/l	199 ± 15	218 ± 22	NS
Urine amylase iu/l	708 ± 100	600 ± 113	NS
Serum creatinine μmol/l	97 ± 5	94 ± 3	NS
Urine creatinine μmol/l	1251 ± 114	1370 ± 196	NS
ACCR %	2.9 ± 0.3	1.89 ± 0.2	< 0.025

value of the Cimetidine group was greater than that of the placebo group. The wide scatter of urine amylase values however prevented this difference reaching statistical significance. Serum and urine creatinine values were similar for both groups but the mean ACCR of patients receiving Cimetidine was found to be significantly greater than that of patients receiving the placebo ($p < 0.025$). Four patients (12.5%) in the Cimetidine group had an ACCR value greater than 5% while this degree of rise was not seen in the placebo group.

The significant change in the mean ACCR of the Cimetidine group appeared to be short lived since when the ACCR was re-estimated in 40 of the 60 patients (20 Cimetidine and 20 placebo) 72 hours after admission no significant difference in the mean ACCR values was found (Table 22). The mean ACCR value of the placebo group at 24 hours (1.89%) and of both groups at 72 hours (1.84% Cimetidine: 1.76% placebo) do not differ statistically from the normal mean ACCR of 1.75% derived from earlier studies.

ii) The Chronic Study

The mean ACCR of the 32 outpatients studied

TABLE 22

ACCR values 24 and 72 hours after admission to hospital. (mean ± s.e.m.). Unpaired Student's t test.

	Cimetidine	Placebo	P
24 hr ACCR %	2.9 ± 0.3	1.89 ± 0.2	< 0.025
72 hr ACCR %	1.84 ± 0.2	1.76 ± 0.2	NS

ACCR at 24 hr $>$ 72 hr for Cimetidine group: $p < 0.025$

Paired Student's t test.

was found to be 1.79% ± 0.31 s.e.m. which was not significantly different from the normal mean ACCR of 1.75%.

4. DISCUSSION

On face value the results of this study indicate that the intravenous administration of Cimetidine in a dosage of 1.6 gm/day may lead to mild pancreatic dysfunction which will settle within 72 hours of starting the drug and is unlikely to recur on prolonged administration. If this statement is to hold water then two points must be accepted:

1. a significant ACCR elevation is likely to be related to pancreatic inflammation and
2. the two groups of patients in the acute study were equally matched for risk factors of pancreatic inflammation excluding Cimetidine therapy.

The first point is the main theme of this thesis and evidence to support this statement has been presented in Chapters 3-5 with further evidence to be presented in Chapter 7.

i) Matching of the Acute Study Groups

The second point can be answered from the data collected in this study which has provided evidence heavily in favour of the two acute groups

being well matched. The age and sex distribution of the two acute groups was similar with patients slightly younger than the overall population of patients admitted with upper gastrointestinal haemorrhage. This undoubtedly reflects the exclusion clauses in the ACCR study which biased against the elderly and chronic sick. Alcohol ingestion may certainly induce an attack of acute pancreatitis and it was therefore gratifying to find that the consumption of alcohol just prior to admission was evenly distributed between the two groups in the acute study. In the chronic study all patients denied alcohol intake in the 48 hours prior to ACCR estimation.

The pathologies resulting in upper gastrointestinal haemorrhage were found to be evenly distributed between the two acute groups of patients. Only patients in whom a confident diagnosis of the source of blood loss had been made at endoscopy were accepted for the ACCR study. The incidence of posterior duodenal ulceration, which may be directly related to acute pancreatitis, was similar in both groups. No malignant conditions were included in the study and in the seven patients who came to surgery the endoscopic diagnosis was confirmed in all cases.

Warshaw has documented that acute pancreatitis can be associated with hypovolaemic shock especially in the presence of acute tubular necrosis of the kidneys (74). In the acute part of this study the incidence of shock on admission was 9% for the Cimetidine group and 11% for the placebo group. This amounted to six patients in all, none of whom developed acute tubular necrosis. Warshaw's incidence of acute pancreatitis in this situation was only 9% which means that statistically 11 patients in this study would need to have been shocked before one case of acute pancreatitis would be expected. The mean admission haemoglobin and the mean blood transfusion required by each group of patients were similar, indicating similar blood loss in both groups. The incidence of further haemorrhage and surgery and the overall mortality were again similar for both groups of patients. The low overall mortality for a group of patients with acute upper gastrointestinal haemorrhage reflects the patient selection for this study.

ii) ACCR Results and Implications

The mean ACCR of the acute Cimetidine group was found to be significantly higher than that of the placebo group within 24 hours of starting

Cimetidine therapy. The mean ACCR of the Cimetidine group at this time (2.9%) was not in the same range as the mean value for patients with acute pancreatitis (6%) described in Chapter 3. Only 4 out of 32 patients in the Cimetidine group had an ACCR greater than 5% on the first sample and this change is certainly less marked than that seen after cholecystectomy or cardio-pulmonary bypass using the non-pulsatile pump. In this study the placebo group, on the other hand, had a normal mean ACCR value on the first sample (less than 24 hours after admission) and no patient in this group had an ACCR value greater than 5%. It is therefore likely that the ACCR change observed in patients received Cimetidine for less than 24 hours is meaningful and may well represent an effect of Cimetidine on pancreatic function. It may well be that in the acute phase of an illness such as upper gastrointestinal haemorrhage the pancreas is more susceptible to damage by an agent such as Cimetidine and this may well be the point demonstrated by this study.

At 72 hours after admission and on chronic Cimetidine therapy there was no evidence of any effect of Cimetidine on the ACCR. This indicates

that any effects of Cimetidine on pancreatic function only act in the acute phase of administration and may well be related to any underlying debility at the time of starting Cimetidine administration. No patient in the Cimetidine group of this study developed acute pancreatitis as defined by serum amylase values, and from this study it would appear that the risk of developing acute pancreatitis as a result of Cimetidine therapy is a theoretical rather than practical possibility. The possibility of acute pancreatitis at the start of Cimetidine therapy should be borne in mind, however, in patients with a past history of acute pancreatitis and patients with acute debilitating conditions receiving the drug.

CHAPTER SEVEN

THE PROGNOSTIC VALUE OF THE AMYLASE CREATININE CLEARANCE RATIO IN ACUTE PANCREATITIS AND ITS RELATIONSHIP TO ENDOTOXAEMIA AND COMPLEMENT ACTIVATION

CHAPTER SEVEN

THE PROGNOSTIC VALUE OF THE AMYLASE CREATININE CLEARANCE RATIO IN ACUTE PANCREATITIS AND ITS RELATIONSHIP TO ENDOTOXAEMIA AND COMPLEMENT ACTIVATION

1. INTRODUCTION

i) Prognostic Factors in Acute Pancreatitis

An MRC Multicentre study involving 10 centres in Great Britain and Northern Ireland, including the Western Infirmary, Glasgow, has recently documented an overall mortality of 11% in acute pancreatitis as defined in this thesis (62). During the decade 1960-1969 the mortality from acute pancreatitis in a single Glasgow hospital was recorded as 18.9% (47). From 1971 to 1974 the mortality from acute pancreatitis in the same Glasgow hospital fell to 11% in agreement with the MRC figures obtained two years later (101). This reduction in mortality was achieved by careful conservative management of acute pancreatitis and by the early recognition and treatment of the known complications of the disease. It is unlikely that the existing mortality rate in acute pancreatitis will be significantly reduced further until a means of specifically inhibiting pancreatic inflammation is discovered. In the

meantime some workers have attempted to identify those patients who do badly at an early stage in their illness in an attempt to be more aggressive with their initial management in order to 'pre-treat' anticipated complications (102,103, 104,105,106). Ranson and his group have used 11 objective findings which correlated with the occurrence of serious illness or death (103), while Imrie in Glasgow has employed nine objective findings obtained within the first 48 hours of admission to predict outcome in acute pancreatitis (105). Neither of these workers has included the ACCR in their prognostic evaluation.

The debate in the literature since 1974 concerning the ACCR has almost exclusively pertained to the tests relationship to pancreatic inflammation and the mechanisms which may produce the increase in the renal clearance of amylase. No publication to date has prospectively examined the prognostic role of the ACCR in acute pancreatitis. This seemed to be an omission in the evaluation of the ACCR, especially in view of the findings presented in this thesis suggesting that the ACCR may be a very sensitive index of pancreatic inflammation. In only one paper has a possible prognostic role for the ACCR been suggested with some evidence

to back up the claim. In 1979 Van Hee and Hubens published a study of 18 patients with acute pancreatitis as defined in this thesis, 63 patients with intra abdominal diseases apart from acute pancreatitis and 80 control patients (107). The mean ACCR for the group with acute pancreatitis was 7.4% as against 2.4% for the control patients ($p < 0.001$). Twenty-six patients with peripancreatic disorders, i.e. common duct stones, gastric cancer and peptic ulcers, were selected from the group of 63 patients with intra abdominal diseases other than acute pancreatitis. At operation 85% of these 26 patients were noted to have a mild form of oedematous pancreatitis and the mean ACCR of all 26 on admission was found to be significantly elevated at 4.1%. This level was significantly lower, however, than that for those patients diagnosed as having acute pancreatitis as their primary disorder. These observations led the authors to suggest that the extent of the rise in the ACCR may help to discriminate between mild and severe attacks of acute pancreatitis.

The aim of this study was therefore to examine the role of the ACCR as a prognostic index in acute pancreatitis in a prospective study using death

and Imrie's prognostic factors to judge the severity of each attack of acute pancreatitis.

ii) Endotoxaemia and Acute Pancreatitis

In order to maximise the returns from a prospective study of acute pancreatitis it was decided to combine the ACCR study with a study of endotoxaemia and complement activation in acute pancreatitis. In 1974 it was reported that three patients with peritonitis due to acute pancreatitis had endotoxin detected in their circulation only when they had peritonitis and not when the disease had resolved (108). Cuevas et al (109) have shown that in experimental sterile peritonitis in rabbits there is a release of endotoxin from the gut into the systemic circulation and it has been proposed that this may be the source of endotoxin in patients with acute pancreatitis (108,110). Endotoxaemia could itself explain some of the systemic complications of acute pancreatitis, i.e. shock lung, disseminated intravascular coagulation and renal failure (110, 111).

iii) Complement Activation in Acute Pancreatitis

There is also evidence of complement catabolism in acute pancreatitis (112) and

experimental animal work has suggested that complement depleted animals develop less severe pancreatitis (113,114). Recently systemic activation of complement has been implicated in the development of shock lung (115) which may help to explain the hypoxia of acute pancreatitis. Since endotoxin is known to activate complement through the alternative pathway (116) it seemed possible that endotoxaemia, resulting from the peritonitis associated with acute pancreatitis, may be instrumental in activating complement in this disease. The aims of this part of the study were to test this hypothesis and to see whether the presence of endotoxaemia and the degree of complement activation correlated with the severity and mortality of acute pancreatitis. This study also allowed for the evaluation of the relationship, if any, between the ACCR, endotoxaemia and complement activation. Only the aspects of the endotoxin and complement results that relate to the ACCR will be presented in detail in this thesis. The remainder of the work on endotoxaemia and complement activation will be referred to where necessary but not presented in depth as it is judged to be outwith the remit of this thesis.

2. PATIENTS AND METHODS

Thirty-one patients admitted to the acute surgical receiving unit of the Western Infirmary, Glasgow, with a diagnosis of acute pancreatitis were accepted for the study. The diagnosis of acute pancreatitis was made in one patient at laparotomy, while in the other 30 the diagnosis was made using the criteria described in Chapter 3. No cases of post-operative or post-traumatic acute pancreatitis were included and where possible the patients progress was followed until a cause for the attack of acute pancreatitis was identified.

The severity of each attack of acute pancreatitis was assessed using Imrie's criteria (105) which had been evolved from studies on patients with acute pancreatitis in a Glasgow teaching hospital. A patient was judged to have potentially severe acute pancreatitis if, within the first 48 hours of admission, three or more of the following nine factors were present;

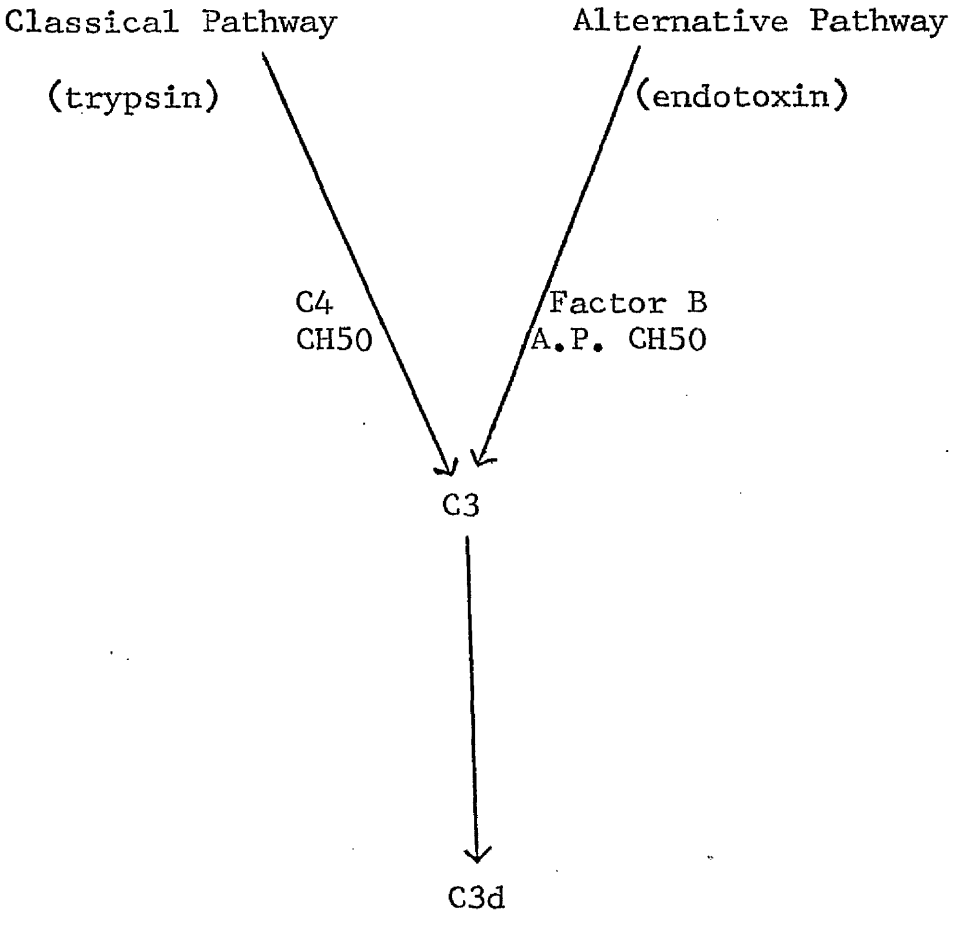
1. $PaO_2 < 60$ mmHg
2. Serum albumin < 32 g/l
3. Serum calcium < 2.0 mmol/l
4. W.B.C. $> 15 \times 10^9$/l
5. SGOT/PT > 100 u/l
6. LDH > 600 u/l
7. Plasma glucose > 10 mmol/l
8. Blood urea > 16 mmol/l
9. Age > 55 years.

The ACCR was estimated within 48 hours of admission using samples of venous blood and urine analysed for amylase and creatinine as previously described. Venous blood was assessed daily for the presence of endotoxin and blood cultures were carried out routinely in conjunction with this estimation. The complement components C_3, C_4, factor B, C_3d and complement haemolytic activity, as measured by CH_{50} and alternative pathway CH_{50}, were estimated daily for up to five days. Endotoxaemia was assessed using the limulus lysate technique and the reactions were graded from 0 (no endotoxin) to 3 (marked endotoxin). The technique used by Dr. Christine McCartney in the Western Infirmary and the techniques used by Dr. Keith Whaley to estimate the complement components have been fully described recently in the literature (117).

The complement system may be activated through either the classical pathway, through which Trypsin works, or the alternative pathway through which endotoxin acts (Figure 17). Once activated, complement, as measured by C_3, is broken down into a number of degradation products including C_3d. Some of these products are biologically active and are responsible for the systemic effects of complement activation. Complement activation has been assessed in this study

FIGURE 17

The complement system.



by using the $\frac{C_3d}{C_3}$ ratio. As complement activation increases the $\frac{C_3d}{C_3}$ ratio will rise due to the breakdown of C_3 and increase of its degradation product C_3d. Whether it is the classical pathway or the alternative pathway or both that has caused complement activation can be determined by measuring markers for each pathway. C_4 and CH_{50} will fall if the classical pathway is activated while factor B and alternative pathway CH_{50} will fall if the alternative pathway is activated. All these components were measured in this study to allow a comprehensive assessment of the functioning of the complement system in acute pancreatitis.

3. RESULTS

i) Patient Details

There were 31 patients with acute pancreatitis in this study and of these 8 were predicted to have potentially severe disease by having a positive factor count of $\geqslant 3$ within 48 hours of admission to hospital. This left 23 patients with a prognostic factor count of less than 3 and these two groups were compared in an attempt to relate the admission ACCR to the predicted severity of each attack of acute pancreatitis. The mean age of the two groups of patients was similar (Table 23)

TABLE 23

Comparison of patient details based on predicted severity of acute pancreatitis.

	Factor count < 48 hrs	
	⩾ 3	< 3
Number	8	23
Mean Age (yrs)	54	51
Males/females	0.6	1.9
Gallstones %	63	35

but there were more females in the potentially severe group than in the mild to moderate group (factor count < 3). This difference can be explained by the 63% incidence of gallstone induced acute pancreatitis in the potentially severe group compared to 35% in the mild to moderate group which contained more alcohol induced attacks (Table 23).

No attempt was made to assess the morbidity of each attack of acute pancreatitis other than through the purely quantitative biochemical and haematological measurements made (Table 24). Subjective factors such as severity of pain and frequency of bowel sounds were not assessed, while management dependent variables such as duration of nasogastric suction and total stay in hospital were not recorded because patients with acute pancreatitis managed by all the surgical teams in the Western Infirmary, Glasgow, were included in the study. There was therefore no absolute uniformity of management in this study. Significant hypoxia ($PaO_2 < 60$ mmHg) occurred in 5 patients in the potentially severe group and 3 of these patients died. This complication was not noted in the mild to moderate group. Five patients in the potentially severe group only

TABLE 24

Comparison of morbidity and mortality based on predicted severity of acute pancreatitis.

	Factor count <48 hrs	
	⩾ 3	< 3
Significant hypoxia	5	0
Intravascular coagulation	5	0
Renal failure	3	0
Mortality %	25	0

were found to have coagulation abnormalities in that either their platelet count fell to less than 50,000/mm^3 or serum fibrin degradation products were raised above 40 mm/l. Only 3 patients developed renal failure and all died. The mortality in the potentially severe group was 25% compared with no deaths in patients judged to have only mild or moderate disease (factor count < 3). Out of 38 attacks of acute pancreatitis studied in the project as a whole, 11 patients were judged to have potentially severe disease using the factor count and 4 of these died (36%), with no deaths in the remaining 27 patients judged to have mild to moderate disease. These findings support the use of the factor count as a predictor of severity in acute pancreatitis. The ACCR values were only available in 31 of the 38 attacks of acute pancreatitis mentioned above and therefore only these patients are reported in detail.

The mean values of three of the four estimations used to calculate the ACCR within 48 hours of admission are shown in Table 25 along with the mean values for blood urea. The values for the patients with predicted severe acute pancreatitis are compared to those of the patients with predicted mild to moderate attacks. As expected there was

TABLE 25

Comparison of biochemical tests based on predicted severity of acute pancreatitis - mean ± s.e.m.

Unpaired Student's t test.

	Factor Count < 48 hrs		P
	⩾ 3	< 3	
Serum amylase i.u./l	5089 ± 1165	4834 ± 906	N.S.
Urine amylase i.u./l	8393 ± 1614	8753 ± 1307	N.S.
Serum creatinine μmol/l	117 ± 14	91 ± 3	N.S.
Blood urea mmol/l	7.7 ± 1.8	4.7 ± 0.4	N.S.
ACCR %	10.1 ± 0.9	5.7 ± 0.3	< 0.001

no significant difference between the serum and urine amylase values for each group. It is generally accepted that although diagnostic of acute pancreatitis in the majority of cases, the serum amylase level per se has no prognostic value (14). The mean values for both serum creatinine and blood urea were raised in the potentially severe group of patients although the differences were not statistically significant. In no patient was the level of creatinine in the serum on admission such that an alteration in the ACCR on account of impaired renal function would have been expected.

ii) ACCR Results

The mean ACCR of patients predicted to have severe attacks of acute pancreatitis using the factor count was found to be significantly greater than the mean value of the 23 patients predicted to have mild to moderate attacks (Table 25). The individual ACCR values obtained from all 31 patients within 48 hours of admission are shown in Figure 18. In this figure the patients have been divided into those with potentially mild acute pancreatitis (factor count < 2), those with potentially moderate acute pancreatitis (factor count = 2) and those with potentially

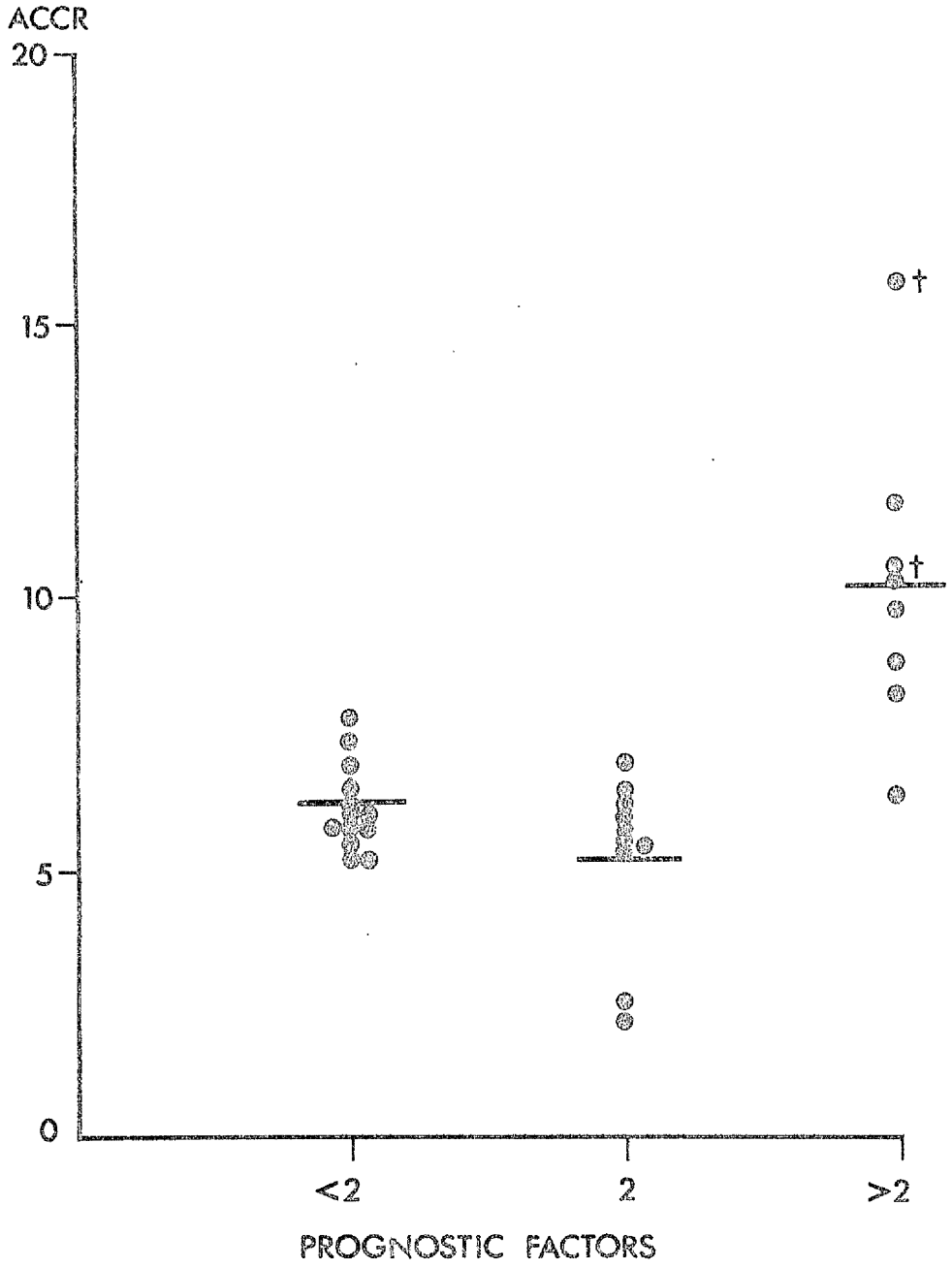


Figure 18. ACCR values in mild (< 2) moderate (2) and severe (> 2) acute pancreatitis.

severe acute pancreatitis (factor count > 2). It can be seen from the scattergram that the distribution of ACCR values in the first two groups of patients (mild and moderate) is similar and these two groups have been combined for further analysis of results. The ACCR values of patients with a factor count of > 2 are higher than the other two groups with only one patient in the potentially severe group having an ACCR value lower than the highest value in the other two groups. Both patients who died in the study were identified as having potentially severe acute pancreatitis by the factor count and both had ACCR values over 10% within 48 hours of admission. These represented two of the three highest ACCR values recorded in this study. Only 2 of the 31 patients had an ACCR of less than 5% within 48 hours of admission and this represents a 94% diagnostic accuracy which is exactly the same figure that was obtained in the first study presented in Chapter 3.

iii) Endotoxin Results

A patient was considered to have significant endotoxaemia when two consecutive venous blood samples were positive for endotoxin on limulus lysate assay. Twenty-one patients in this study

had both endotoxin and ACCR estimated and of these 10 (40%) were found to be endotoxin positive as defined above. The mean ACCR within 48 hours of admission for the endotoxin positive patients was found to be 6.85% compared to 6.71% for the endotoxin negative patients. There is no significant difference between these values and it therefore appears that the ACCR is not influenced by the presence or absence of endotoxin in the blood during an attack of acute pancreatitis.

iv) Complement Results

The relationship between the ACCR and the activity of the complement system within 48 hours of admission to hospital with an attack of acute pancreatitis was examined by plotting the ACCR against the $\frac{C_3d}{C_3}$ ratio for each patient. The result is shown in Figure 19 and a significant positive correlation between the two factors is demonstrated ($p < 0.001$). As the $\frac{C_3d}{C_3}$ ratio increases, indicating increasing complement activation, so too does the ACCR. The next step was to test this correlation of the ACCR with complement activity against the activation pathways for the complement system to see if the ACCR correlated with classical or

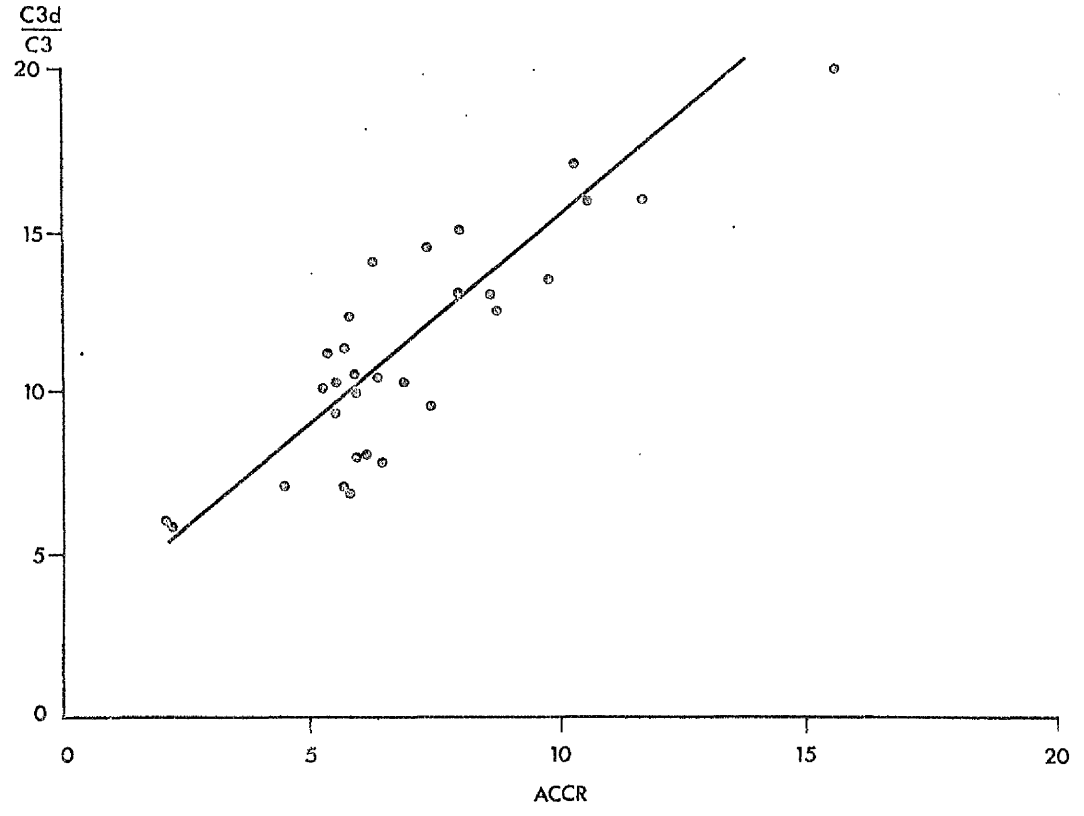


Figure 19. Relationship between total complement activation and the ACCR.
Linear regression.

Correlation coefficient = 0.85 : $p < 0.001$

alternative pathway activity or both. Figures 20 and 21 show a highly significant negative correlation between the ACCR and both C_4 and CH_{50}, two markers of classical pathway activity. As these factors fall, indicating increasing consumption and activation of the classical pathway, so the ACCR rises. When the corresponding markers for the alternative pathway (factor B and alternative pathway CH_{50}) were plotted against the ACCR, no significant correlation was found (Figure 22 and 23). It therefore appears that in acute pancreatitis the ACCR is positively related to the activation of the complement system via the classical pathway only.

4. DISCUSSION

i) Prognostic Factors

The prognostic factor count used in this study of patients with acute pancreatitis was 100% reliable with respect to mortality and accurate in the prediction of the significant short term complications of the disease. Professor Ranson from New York described 11 prognostic factors in acute pancreatitis and stated that to have three or more of these factors present within 48 hours of admission indicated potentially severe acute

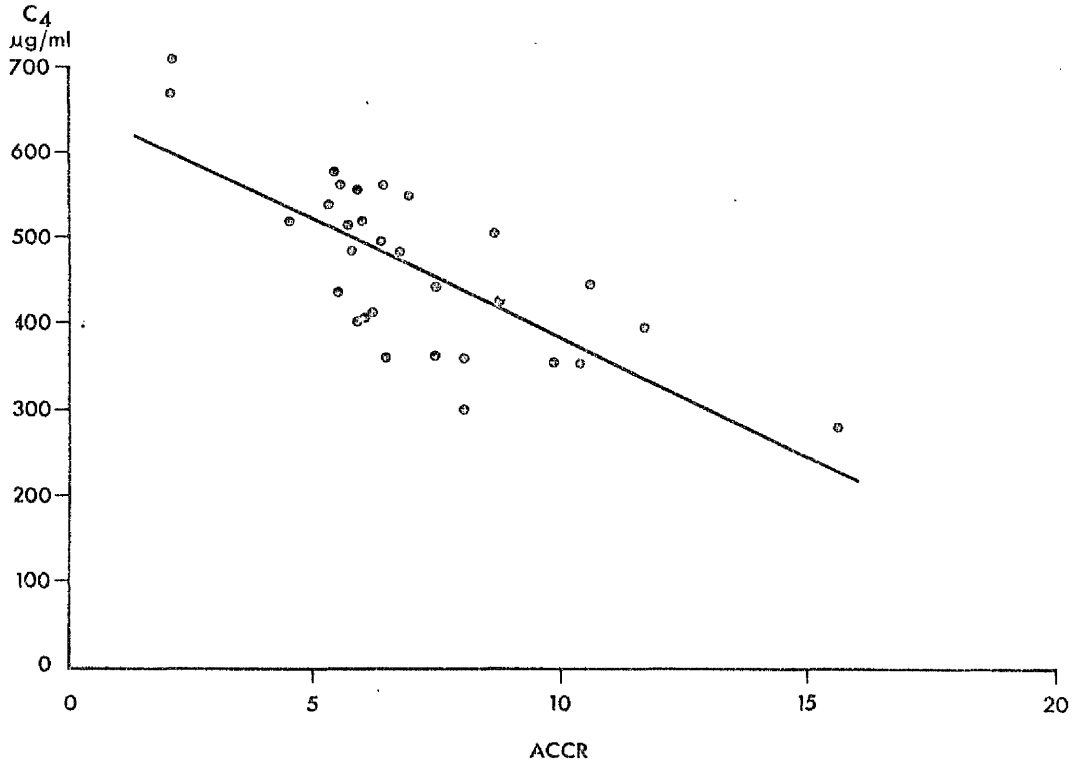


Figure 20. Relationship between classical pathway complement activation (C4) and the ACCR.
Linear regression.

Correlation coefficient = -0.73 : $p < 0.001$

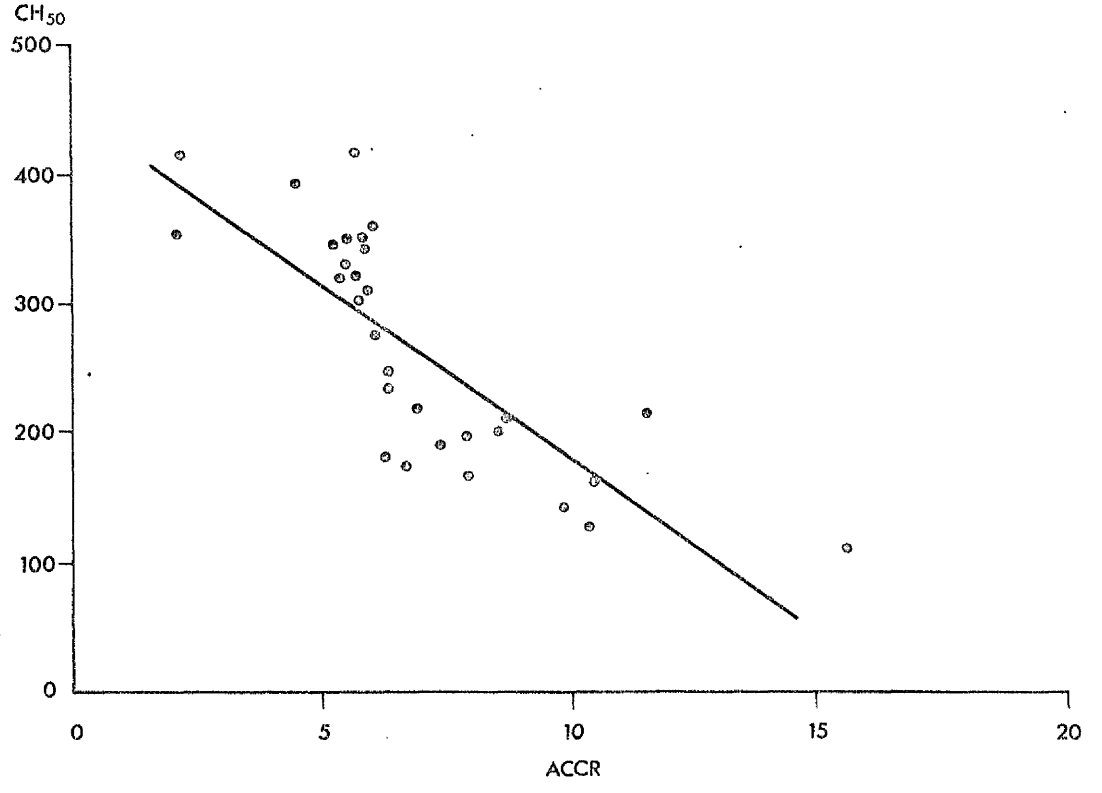


Figure 21. Relationship between classical pathway complement activation (CH50) and the ACCR.
Linear regression.
Correlation coefficient = -0.79 : $p < 0.001$

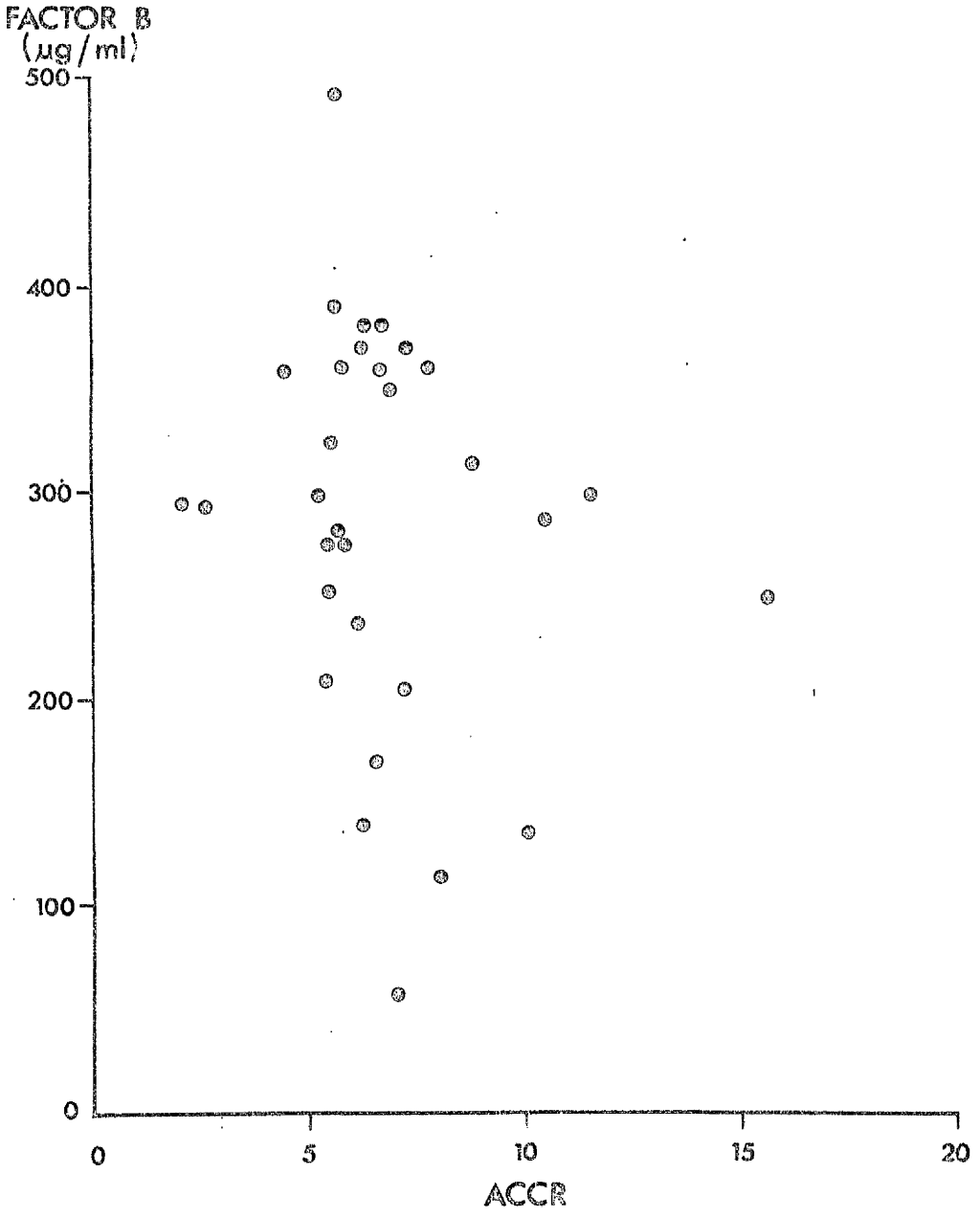


Figure 22. Relationship between alternative pathway complement activation (Factor B) and the ACCR.
Linear regression.
Correlation coefficient = -0.2

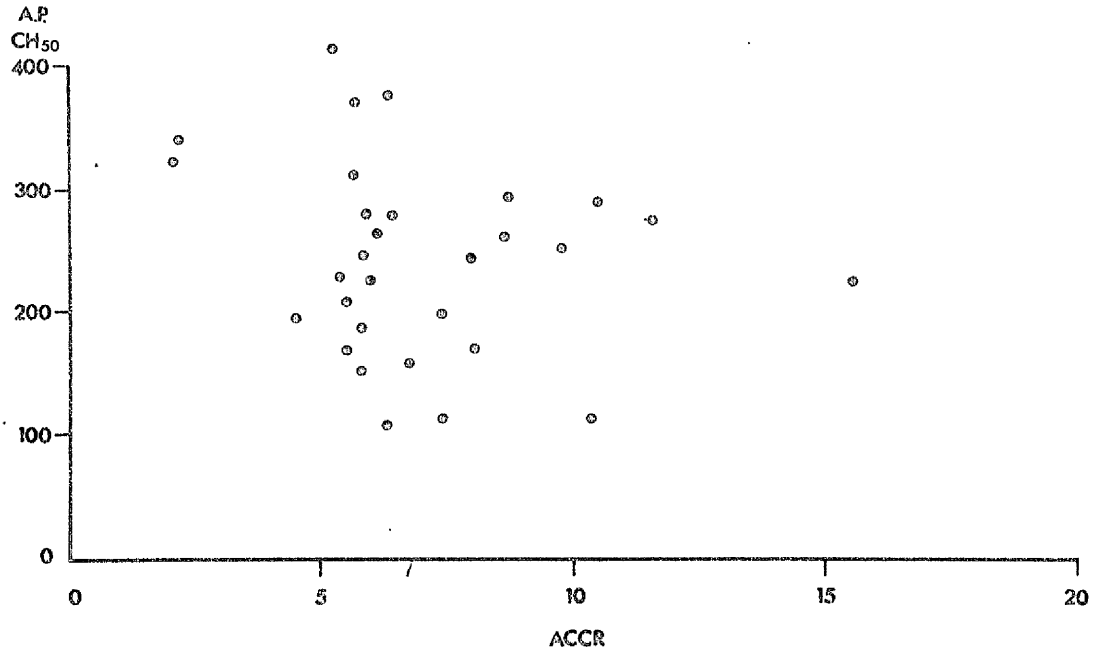


Figure 23. Relationship between alternative pathway complement activation (A.P. CH50) and the ACCR.
Linear regression.

Correlation coefficient = -0.28

pancreatitis. He reported 100 cases of acute pancreatitis of which 21 fell into the potentially severe group. These patients had a mortality of 62% and a serious morbidity of 95% in the survivors compared with 3% and 12% respectively with patients with less than three prognostic factors (103). Imrie has assessed the value of these factors using the results of his studies of patients with acute pancreatitis in Glasgow (105). As a result of this he discarded -

1. a haematocrit decrease of $>$ 10% in 48 hours
2. a base deficit of $>$ 4 mequiv/l and
3. an estimated fluid sequestration of over 6.1 in 48 hours from Ranson's criteria and added -

a. a serum albumin of $<$ 32 g/l, giving nine factors which did not require to be repeated for full interpretation.

These factors were used in this study since it was felt to be more appropriate to use data derived in Glasgow for a Glasgow study. Retrospective analysis of the severity of each attack of acute pancreatitis in this study (Table 24) shows that the factor count used gave an accurate prediction

of both mortality and serious morbidity if the presence of three or more prognostic factors was taken to indicate the likelihood of a severe attack of acute pancreatitis. It seems that the use of this discriminant as a means of expressing the severity of an attack of acute pancreatitis is justified since its predictive accuracy is excellent. It was therefore used in the study to assess the prognostic role of the ACCR in acute pancreatitis.

ii) ACCR Data

All but 2 of the 31 patients diagnosed as having acute pancreatitis in this study had an admission ACCR value $> 5\%$, the upper limit of normal defined in Chapter 4. The 8 patients with three or more prognostic factors within 48 hours of admission, however, had a significantly higher ACCR ($p < 0.001$) than the 23 patients whose factor count was less than 3. The difference between the two groups of patients was striking with only 1 $\geqslant$ 3 factor patient overlapping with the other group. The results of this study suggest that in acute pancreatitis an ACCR value within 48 hours of admission does have a prognostic value with a direct relationship between the

probable severity of the acute attack and the ACCR result. Moreover, the results of this study suggest that a patient with acute pancreatitis who has an ACCR $\geqslant$ 10% within 48 hours of admission to hospital is extremely likely to suffer from a severe attack of the disease.

iii) Endotoxin Data

Forty percent of patients with acute pancreatitis in this study were discovered to have significant endotoxaemia as defined above at some stage during the first five days in hospital. Endotoxaemia was not related to the insertion of an indwelling urethral catheter and a septic source was only found in two patients. Significant endotoxaemia per se was found to have no effect on the ACCR which was the same for both endotoxin positive and endotoxin negative patients. This finding is relevant since Dr. Levitt has suggested that endotoxaemia may be the cause of the impaired renal tubular reabsorption of amylase seen in acute pancreatitis (personal communication).

iv) Complement Data

The full study showed that some degree of increased complement activation was universal in patients with acute pancreatitis. The mean $\frac{C_{3^d}}{C_3}$ level on day 1 was 12.47×10^{-3}, with an

upper limit of normal of 6.03×10^{-3} ($p < 0.001$). Complement activation was found to be related to the predicted severity of the disease and was most marked in the patients who died. There was evidence of both classical and alternative pathway activation of the complement system, but in the potentially severe group, with the greatest incidence of significant endotoxaemia, alternative pathway activation was predominant.

The ACCR was found to be directly related to complement activation and this relationship was found to be statistically highly significant. This correlation supports the evidence that the ACCR may be of prognostic value since complement activation was itself found to be related to severity in acute pancreatitis. The ACCR was found to correlate specifically with complement activation via the classical pathway and not the alternative pathway. This suggests strongly that the relationship between the ACCR and complement activation is indirect rather than direct. It is probable that both the ACCR and the complement system are influenced in acute pancreatitis by a third factor rather than complement activation directly influencing the ACCR. If that were the case one would have expected the ACCR to correlate

with both classical and alternative pathway activation instead of classical pathway activation alone. This finding suggests that some other factor, which stimulates complement activation through the classical pathway, also acts to alter the ACCR in acute pancreatitis. The most likely candidate for this role in acute pancreatitis is Trypsin which is known to activate complement through the classical pathway (118,119). Thus Trypsin, and perhaps other proteolytic pancreatic enzymes, may be the link in the chain and explain why the ACCR appears to be related to complement activation in acute pancreatitis. The evidence relating an elevated ACCR to pancreatic inflammation has therefore been strengthened by this study. For the first time there is some evidence that Trypsin and possibly other proteolytic enzymes from the pancreas are directly responsible for producing the renal tubular phase of the increase in amylase excretion seen in acute pancreatitis.

C H A P T E R E I G H T

DISCUSSION AND CONCLUSIONS

CHAPTER EIGHT

DISCUSSION AND CONCLUSIONS

1. SUMMARY OF MAIN FINDINGS

The studies presented in this thesis have produced both conclusive findings and findings which are open to interpretation and discussion. The conclusive findings are listed as follows:

1. The ACCR is a simple test to perform in an acute or elective situation and the result can be available within an hour of taking the venous blood and urine samples.

2. The ACCR was found to be an accurate diagnostic test for acute pancreatitis in 48 patients diagnosed by hyperamylasaemia or laparotomy. Ninety-four percent of these patients had an ACCR value above the appropriate upper limit of normal within 48 hours of admission to hospital.

3. The ACCR was significantly elevated in 7 out of 48 patients with confirmed acute pancreatitis who had either normal serum amylase levels or levels below the minimum considered diagnostic for acute pancreatitis at the time of testing.

4. The ACCR, estimated within 48 hours of admission to hospital, has been shown to be directly related to the severity of an attack of acute pancreatitis.

5. The ACCR was found to be significantly correlated with the activation of complement via the classical pathway in acute pancreatitis.

6. The ACCR was not significantly elevated in 267 patients admitted to hospital with acute or chronic conditions outwith the pancreas despite significantly elevated mean serum and urine amylase levels in 35 patients with acute intra-abdominal diseases excluding acute pancreatitis.

7. Significant ACCR elevation was documented following cholecystectomy, cardiac surgery using cardiopulmonary bypass and the acute administration of Cimetidine intravenously in patients with upper gastrointestinal haemorrhage.

8. The significant ACCR elevation following cholecystectomy was reduced by Trasylol infusion before, during and after surgery.

9. The significant ACCR elevation following non-pulsatile cardiopulmonary bypass was reduced by using pulsatile cardiopulmonary bypass.

2. SUMMARY OF MAIN DISCUSSION POINTS

The following points arising from this work are not clear-cut conclusions and require either support from the literature, confirmation from repeat studies or further evaluation through a different approach.

1. Is a significantly elevated ACCR in the absence of hyperamylasaemia specific for acute pancreatitis?

2. Is the significant ACCR elevation following cholecystectomy, cardiopulmonary bypass and acute Cimetidine therapy related to pancreatic inflammation?

3. Is the relationship between the ACCR and the complement system in acute pancreatitis secondary to Trypsin activity?

3. DISCUSSION

Up to 1966 the literature relating to the ACCR referred to in Chapter 2 was generally favourable, with authors regarding a significantly elevated ACCR as an accurate index of acute pancreatic inflammation

which might possibly be specific for that disease. Since then the picture has become more confusing. There have been favourable reports on the diagnostic accuracy of the ACCR in acute pancreatitis from Great Britain (120,121), Sweden (122), Belgium (107), U.S.A. (123,124,125) and China (126). The upper limit of normal for the ACCR in these studies varied from 3% to 5.3% and Levitt has suggested that the results of amylase clearance measurements vary with the technique of amylase assay employed (127). One must therefore know the normal ACCR for the technique employed in one's own laboratory in order to make a meaningful interpretation of the measurement on a patient. This is illustrated in this thesis by the difference between the upper limit of normal for the ACCR obtained in the study presented in Chapter 3 and the subsequent studies (3% and 5% respectively). This difference is explained by the addition of bovine serum albumin to the Phadebas test kit used in all but the first study. This addition increases the sensitivity of the Phadebas test for amylase, especially in the urine, and therefore tends to slightly increase the value of the ACCR for any one specimen (45, 128).

The specificity of a raised ACCR for acute pancreatitis has been challenged in some studies.

Marten et al, from London, noted significant ACCR elevation in patients with diabetic ketoacidosis, fulminant alcoholic liver disease and advanced renal failure (121). They were however unable to exclude acute pancreatitis in all these cases. The paper by Berger et al (44), mentioned in Chapter 2, suggesting that the ACCR was significantly raised in 2 out of 5 patients with an acute perforated duodenal ulcer was soon followed by a paper from West Germany questioning the value of the ACCR in acute pancreatitis (129). The West German authors studied 190 patients with hyperamylasaemia with the aim of documenting the incidence of macroamylasaemia and used the ACCR among other tests to screen for this. Fifty-six patients were retrospectively judged to have acute or chronic pancreatitis, 27 patients were suspected but not proven to have acute pancreatitis and in 107 patients there was no evidence of pancreatic disease apart from an elevated serum amylase. This classification of the patients in this study is clearly unacceptable since no criteria for the diagnosis or exclusion of pancreatitis in the presence of hyperamylasaemia was given and the number of patients without pancreatitis in the presence of hyperamylasaemia was very high. No control group was studied and no local normal range for the ACCR established, the

authors accepting a value of $< 4\%$ as normal. Nineteen out of 46 patients with acute pancreatitis had an ACCR $< 4\%$ while 48 out of the 134 patients in the non-pancreatitis groups had an ACCR $> 4\%$. However 27 patients in the non-pancreatitis groups were suspected of having acute pancreatitis and they were not excluded from these results. Although these results raised doubts about the accuracy and specificity of the ACCR in the diagnosis of acute pancreatitis, the study itself was criticised in several areas and the relevance of the results questioned (M Levitt - personal communication).

In 1977 Lankisch and his co-workers from Sweden published a paper reporting a normal ACCR in 11 out of 33 patients diagnosed as having acute pancreatitis by hyperamylasaemia (130). The mortality for this group was high at 24% and acute pancreatitis was confirmed at all seven post-mortem examinations. The mean ACCR in their laboratory for 21 healthy volunteers was $1.8\% \pm 1.0$ 2S.D. This gave an upper limit of normal for the ACCR in the author's hospital of 3%, as found in the study presented in Chapter 3. For no good reason, however, the authors chose 5.3% as their upper limit of normal and, based on this figure, the ACCR was normal in 36% of the patients with acute

pancreatitis and hyperamylasaemia on their first day of admission. If an ACCR of over 3% had been accepted as abnormal for this study then 88% of patients with acute pancreatitis would have had an abnormal value on admission, including 5 patients with acute pancreatitis without hyperamylasaemia diagnosed at laparotomy. This totally changes the message from the study and emphasises the importance of establishing and using a normal range for the ACCR in one's own hospital.

In 1978 Farrar and Calkins from Kansas City reported that in 29 male patients with alcohol induced acute pancreatitis diagnosed by hyperamylasaemia the ACCR was only significantly elevated ($>$ 5.3%) in 59% (131). The authors were unable to explain the discrepancy between their results and others regarding the ACCR. They stated that, apart from one case who died, all their patients had mild acute pancreatitis with no evidence of shock or renal failure. They also admit that they cannot prove that their patients had acute pancreatitis since there was no biopsy or autopsy evidence of the disease. The results in this thesis support a relationship between ACCR elevation and the severity of an attack of established acute pancreatitis. On the other hand significant elevations of the ACCR have been recorded in studies

in this thesis when only sub-clinical pancreatic inflammation was presumed to be present. Warshaw has shown that the ACCR may discriminate between pancreatic and non-pancreatic hyperamylasaemia (35), and it may be that in Farrer and Calkins study of heavy alcohol drinkers with apparent mild acute pancreatitis some of the patients with normal ACCR values may have had other causes for their hyperamylasaemia.

The concensus of opinion in the literature to date remains favourable to the concept of the ACCR as a useful diagnostic test in patients suspected of having acute pancreatitis. This has been supported in two studies in this thesis with a 94% accuracy for the ACCR in reaching a diagnosis of acute pancreatitis in 48 patients of whom 7 did not have significant hyperamylasaemia. It has been suggested that the ACCR may be most useful as a diagnostic test for acute pancreatitis in patients with high serum levels of amylase of uncertain origin (127). A low ACCR strongly supports a non-pancreatic cause for the hyperamylasaemia, whereas a high ACCR indicates a strong probability of the presence of acute pancreatitis. The interpretation of an abnormal ACCR in the presence of a normal serum amylase is much more difficult and there appears to be no fixed opinions in the literature

regarding this. Can mild, resolving or sub-clinical pancreatic inflammation be detected by an elevated ACCR and does an elevated ACCR in the absence of hyperamylasaemia always indicate pancreatic inflammation? These are the crutial questions that the work presented in this thesis has gone some way towards answering.

There is no doubt that an elevated ACCR commonly occurs in mild or resolving acute pancreatitis in the absence of hyperamylasaemia. The delay in ACCR values returning to normal compared to serum amylase following an attack of acute pancreatitis was noted in the first ACCR paper in 1969 (32) and confirmed in the patients studied and presented in Chapters 3 and 7 in this thesis. This suggests that the ACCR is a sensitive test for pancreatic inflammation but may in fact only demonstrate that the renal tubular changes induced by a clinically evident attack of acute pancreatitis are slow to resolve. If this were the case then the ACCR would not necessarily be a sensitive test for mild or sub-clinical pancreatic inflammation. The most convincing direct evidence in the literature supporting the former view that the ACCR is a sensitive test for pancreatic inflammation comes from Van Hee (107). His group studied 26 patients with acute peripancreatic disorders who underwent emergency laparotomy and found a mild form of oedematous

pancreatitis, previously unsuspected clinically, in 85% of these patients. The mean ACCR value for this group on admission (4.1%) was significantly higher than for patients with acute intra-abdominal diseases not involving the pancreas (2.6%) and significantly lower than for patients diagnosed conventionally as having acute pancreatitis (7.4%). This study indicates that significant ACCR changes in the absence of hyperamylasaemia can reflect unsuspected pancreatic inflammation and suggests that the degree of ACCR elevation may reflect the severity of the disease process. In 31 patients with acute pancreatitis in this study (Chapter 7), the ACCR almost completely differentiated between 8 patients with potentially severe disease and 23 patients with only mild to moderate disease. An ACCR $>$ 10% always occurred in patients with severe disease in whom two out of three died. Seven of the patients in the two studies of acute pancreatitis in this thesis had a serum amylase value below the diagnostic level for the disease at the time of ACCR estimation within 24 hours of admission. The ACCR, however, was significantly elevated in all 7 and the diagnosis of acute pancreatitis was reached independently by laparotomy in 3 cases and an initial serum amylase value above

the lower diagnostic level in 4. The 3 cases in whom acute pancreatitis was independently diagnosed in the presence of a significantly elevated ACCR and a normal serum amylase level again demonstrate that the ACCR can detect pancreatic inflammation in the absence of significant hyperamylasaemia. It is probable that in these cases the serum amylase had fallen below the diagnostic level for acute pancreatitis prior to admission to hospital and it was the prolonged effect of acute pancreatitis on amylase excretion which allowed the ACCR to confirm the diagnosis. In Van Hee's patients, on the other hand, a mild and unsuspected pancreatitis was demonstrated and correlated with a significant increase in ACCR (107). There was no suggestion that the pancreatitis observed in these patients was resolving from a more severe attack which had been misdiagnosed.

The ACCR was found to be significantly elevated in this thesis in patients following cholecystectomy and during and after cardiac surgery performed using non-pulsatile cardiopulmonary bypass. These basic observations have been made by other workers both before and after the work presented in this thesis (55,56,57,58,59,76,77,132,133,134). In the studies presented in Chapters 4 and 5 there were only 2 cases

of acute pancreatitis diagnosed by hyperamylasaemia out of 71 patients studied and this is in agreement with the general experience in the literature (55,73). It is recognised, however, that cholecystectomy, especially with the addition of exploration of the common bile duct, and cardiac surgery using cardio-pulmonary bypass both carry a significantly higher risk of post-operative acute pancreatitis than surgery at other sites distant from the pancreas (55,73). All studies in which the ACCR has been estimated after cholecystectomy have shown more striking changes in the ACCR in patients who have also undergone common bile duct exploration, a finding in keeping with clinical studies of acute pancreatitis following biliary surgery (55,57,58,59).

In this thesis an attempt was made to modify the ACCR response after biliary and cardiac surgery by using a drug and a technique respectively to protect the pancreas against the operative insults. In the case of biliary surgery Trasylol was used prophylactic-ally as a proteolytic enzyme inhibitor, while in cardiac surgery pulsatile cardiopulmonary bypass was used in order to improve the tissue blood flow through the pancreas and protect the gland against pancreatitis of ischaemic origin. In both instances these manouevres were successful in significantly reducing

the ACCR response following biliary and cardiac surgery. These results provide strong indirect evidence that a significantly elevated ACCR in the absence of hyperamylasaemia or clinical signs of acute pancreatitis can indicate the presence of sub-clinical pancreatic inflammation. The evidence however is not indisputable and it remains possible, although very unlikely, that both Trasylol and pulsatile cardiopulmonary bypass act to reduce the ACCR following biliary and cardiac surgery by mechanisms not involving the pancreas.

The postulated ability of the ACCR to detect sub-clinical pancreatic inflammation was used to monitor the effects of Cimetidine therapy on the pancreas. A small but significant elevation in the ACCR was observed within 24 hours of starting intravenous Cimetidine therapy. The ACCR returned to normal within 72 hours and was not found to be elevated during chronic administration of the drug. These findings support evidence in the literature that Cimetidine therapy may be associated with pancreatic inflammation (99,100) and demonstrate how the ACCR might be used to screen for sub-clinical pancreatic inflammation in situations where a small but significant incidence of acute pancreatitis has been observed.

The presence of significant endotoxin in the blood of 40% of patients with acute pancreatitis and the increased activity of the complement system in all patients with acute pancreatitis were new and exciting observations which require to be followed up and hopefully put to clinical use. The finding of a highly significant correlation between the ACCR, estimated within 48 hours of admission to hospital with an attack of acute pancreatitis, and complement activation specifically via the classical pathway was both unexpected and stimulating. It seems clear that whatever activated the classical pathway also caused significant changes in the ACCR. In patients with established acute pancreatitis Trypsin seems by far the most likely candidate for this role, although other proteolytic enzymes released from the pancreas during an attack of acute pancreatitis cannot be excluded. This observation may be the best evidence to date that a substance released from the pancreas during an attack of acute pancreatitis is directly related to the changes in amylase clearance observed at that time. Further studies are required to confirm this finding and to relate the ACCR changes in acute pancreatitis to measured levels of Trypsin and its associated compounds. This finding is also entirely in keeping with the effect of Trasylol, an antitrypsin

agent, on the ACCR observed in Chapter 4 and supports the hypothesis put forward in that chapter.

4. CONCLUSIONS

In conclusion, therefore, the studies presented in this thesis have demonstrated that the ACCR is a simple test to perform and has a definite place in the diagnosis of acute pancreatitis. In these studies the ACCR achieved an overall 94% accuracy in the diagnosis of acute pancreatitis, including marked success in making the diagnosis in patients who for various reasons did not have significant hyperamylasaemia at the time of testing. The ACCR should be especially useful as a screening test for acute pancreatitis in patients admitted to hospital with abdominal pain of over 48 hours duration who have an amylase value above the normal range but below the level considered diagnostic for acute pancreatitis. There is good evidence that the ACCR is a sensitive test for pancreatic inflammation and can detect sub-clinical states. There is serious doubt however regarding the specificity of a significantly elevated ACCR for pancreatic inflammation alone and this makes the interpretation of a significant ACCR elevation in the absence of hyperamylasaemia and clinical findings suggestive of acute pancreatitis extremely difficult.

Despite this, the ACCR could be used to screen for pancreatic inflammation in situations where it might be expected to exist. The elevation of the ACCR in acute pancreatitis appears to be secondary to the release of Trypsin from the inflamed pancreas which results in reduced absorption of amylase from the renal tubules (38,39,40,41,42,135). This, together with an increase in amylase in the serum, leads to a rise in amylase clearance.

The ACCR is certainly not the ultimate diagnostic test for pancreatic inflammation and it is to be hoped that superior tests will be forthcoming in the near future. At the present time, however, the ACCR is as simple and at least as informative as any other diagnostic test in acute pancreatitis. The ACCR should be interpreted in conjunction with the serum amylase value by a clinician who understands the limitations of both tests. If these criteria are fulfilled then the ACCR is undoubtedly superior to any other single readily available diagnostic test for acute pancreatitis.

STATEMENT OF COLLABORATION AND PUBLICATION OF WORK CONTAINED IN THESIS

My interest in acute pancreatitis and its diagnosis stemmed from my appointment as Hospital Liaison Officer in the Western Infirmary, Glasgow, for the MRC Multicentre Study of Trasylol and Glucagon in acute pancreatitis. The work described in Chapter 3 was started on my own initiative and I continued to be responsible for the planning and co-ordination of the studies reported in this thesis. Dr L.B. Roberts of the Department of Biochemistry at the Western Infirmary and Gartnavel General Hospital has advised on the biochemical aspects of the projects, while Mr K.M. Taylor of the Department of Cardiothoracic Surgery at Glasgow Royal Infirmary collaborated in the study presented in Chapter 5. Mr J. Murie, then of the Department of Surgery at the Western Infirmary, collaborated on the study of the effect of chronic administration of Cimetidine on the ACCR presented in Chapter 6, while Drs A. Foulis, K. Whaley, C. McCartney and D. Galloway of the Departments of Pathology, Immunopathology, Bacteriology and Surgery at the Western Infirmary respectively, collaborated in the work presented in Chapter 7. All aspects of this work involving the ACCR were, however, fully my responsibility.

The work described in Chapter 3 was presented at the European Society for Surgical Research in April 1976 and the Scottish Society of Experimental Medicine in November 1976. The work described in Chapters 4, 5 and 7 has been presented at the Surgical Research Society in July 1978, January 1980 and January 1981 respectively.

The following abstracts and papers dealing with work presented in this thesis have been published or accepted for publication:

1. The amylase creatinine clearance ratio in acute pancreatitis. Murray WR, MacKay C.
   a. Eur Surg Res 1976; 8: 71
   b. Scot Med J 1976; 21: 95
   c. Br J Surg 1977; 64: 189-91.

4. The amylase creatinine clearance ratio following cholecystectomy. Murray WR, Roberts LB, MacKay C.
   Br J Surg 1978; 65: 813.

5. The amylase creatinine clearance ratio following cardiopulmonary bypass. Murray WR, Mitra S, Mitra D, Roberts LB, Taylor KM.
   a. Br J Surg 1980; 67: 386
   b. J Thorac Cardiovasc Surg 1981; (In Press).

7. Cimetidine and the amylase creatinine clearance ratio. Murie JA, Murray WR, Roberts JB.

Br J Clin Pharm 1980; 9: 279.

8. Is the amylase creatinine clearance ratio of prognostic value in acute pancreatitis? Murray WR, Foulis AK, Galloway DG, McCartney C, Whaley K.

Br J Surg 1981; (In Press).

A P P E N D I X

STATISTICAL ANALYSIS

The statistical analysis employed in the studies presented in this thesis was carried out using a programmable Hewlett Packard 9815A desk calculator. The following statistical tests were used where appropriate and reference was made to a text on medical statistics (136):

1. Student's unpaired t test
2. Student's paired t test
3. Fisher's exact test
4. Linear regression analysis.

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