Endoscopic electrolytic ablation – feasibility and safety in the treatment of pancreatic pathologies.

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by

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Abstract: Pancreatic cancer continues to have a dismal prognosis. It is a condition that tends to present late meaning that the majority of patients are only suitable for palliative therapy. Surgical, endoscopic nor medical palliation has proven to be completely successful, with many patients requiring a combination of therapies. This research aims to assess the feasibility and safety of using electrolysis, a locally ablative technique, as a potential tool in the treatment of pancreatic cancer and other pancreatic mass lesions either independently or as an adjunct to other modalities. Experimental work investigated the use of pancreatic electrolytic ablation, both open and endoscopic, in a porcine model.

Phase 1a: short term follow up open pancreatic electrolysis.

Twelve female white domestic pigs under went laparotomy, duodenotomy and open pancreatic duct cannulation. Six received pancreatic electrolysis, six were used as controls. All animals were followed up for 72 hours and then killed.

Phase 1b: medium and long term follow up open pancreatic electrolysis

Eight female white domestic pigs underwent open pancreatic electrolytic ablation. Four animals were killed at two weeks the remaining at eight weeks.

Phase 2: medium and long term follow up endoscopic pancreatic electrolysis

Fifteen female white domestic pigs all had endoscopic pancreatic duct cannulation. Ten pigs underwent endoscopic pancreatic electrolytic ablation and the other five pigs were used as controls. Five treatment animals and the five control animals were killed at two weeks and the remaining five treatment animals were killed at eight weeks.

All animals tolerated the procedure and had returned to normal diet by day three. In all phases there were no significant ($p>0.05$) differences between the treatment and control groups with respect to biochemical parameters or cytokine concentrations. Histological assessment of pancreata demonstrated significantly ($p<0.05$) increased inflammatory changes in all the treatment groups when compared with controls.

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Abbreviations

VICC	Vascular interstitial closed circuit
BCEC	Biologically closed electrical circuit
CCK	Cholecystokinin
PPPD	Pylorus preserving pancreatoduodenectomy
GOO	Gastric outlet obstruction
ERCP	Endoscopic retrograde cholangiopancreatography
CBR	Clinical benefit response
QOL	Quality of Life
5-FU	5-Fluorouracil
CRT	Chemoradiotherapy
EBRT	External beam radiotherapy
GITSG	Gastrointestinal tumor study group
ECOG	Eastern cooperative oncology group
EORTC	European organisation for research and treatment of cancer
RTOG	Radiation therapy oncology group
MMPI	Matrix metalloproteinase inhibitor
VEGF	Vascular endothelial growth factor
EGFR	Epidermal growth factor receptors
SPF	Specific pathogen free
DC	Direct current
mA	Milliamp
CRP	C-reactive protein
SCM	Sternocleidomastoid
C	Coulombs
ANOVA	Analysis of variance
ELISA	Enzyme linked immuno-sorbent assay
TNFα	Tumour necrosis factor alpha
IL-1β	Interleukin 1 beta
SIRS	Systemic inflammatory response syndrome
ARDS	Adult respiratory distress syndrome

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Publications arising solely from this work

1. Morrison CP, Court FG, Teague BD, Wemyss-Holden SA, Texler M, Metcalfe MS, Dennison AR, Maddern GJ.
   Endoscopic perductal electrolytic ablation of the pancreas: experimental studies of morbidity and mortality.
   Dig Dis. 2005; 23 (1):83-91.

2. Morrison CP, Court FG, Wemyss-Holden SA, Teague BD, Burrell A, Texler M, Metcalfe MS, Dennison AR, Maddern GJ.
   Perductal electrolytic ablation of the porcine pancreas: studies of morbidity and mortality.
   Surgical Endoscopy Ultrasound and Interventional Techniques. 2004 Oct; 18 (10);1435-41.

3. Morrison CP, Teague BD, Court FG, Wemyss-Holden SA, Metcalfe MS, Dennison AR, Maddern GJ.
   Experimental studies of serum cytokine concentration following pancreatic electrolytic ablation.
   Medical Science Monitor 2003; 9 (1): 43-46.

4. Teague BD, Morrison CP, Court FG, Nguyen T, Wemyss-Holden SA, Dennison AR, Maddern GJ.
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Surgical Endoscopy Ultrasound and Interventional Techniques 2003; 17 (2): 207-11.

Publications arising partly from this work

6. Court FG, Wemyss-Holden SA, Morrison CP, Teague BD, Dennison AR, Maddern GJ.
Post-operative deaths in sows as a result of gastric mucosal de-gloving at the pars oesophagea.
Lab Anim. 2005 Jul; 39 (3):308-13.

Objectives and Hypotheses

The objective of this experimental work was to assess the feasibility of delivering an ablative electrolytic dose to the porcine pancreas; using either open or endoscopic techniques with a long-term aim of investigating pancreatic electrolytic ablation as a potential treatment for pancreatic pathologies. While pancreatic cancer, especially of the pancreatic head, would be particularly amenable to debulking resulting from electrolysis this technique may also have a role in other pancreatic pathologies such as pancreatic masses associated with chronic pancreatitis.

This study aimed to investigate:

- Feasibility of porcine pancreatic duct cannulation, both open and endoscopic.
- Feasibility of delivering electrolytic ablation to the porcine pancreas, both as an open and an endoscopic procedure.
- Clinical assessment of short, medium and long-term morbidity and mortality post pancreatic electrolytic ablation.
- Effect of pancreatic electrolytic ablation on biochemical parameters.
- Effect of pancreatic electrolytic ablation on cytokine concentrations.
- Histological and radiological assessment of electrolysis and macroscopic and microscopic pancreatic morphology.

Chapter 1:

Introduction

Chapter 1: Introduction

1.1 Electrolysis

1.1.1 History

Much of the recent investigative work into the role and nature of electrolysis and its ablative effects, both at a theoretical and experimental level, can be attributed to the efforts of one man, Professor Bjorn Nordenstrom, a Swedish radiologist. His involvement in the field of electrolysis and more generally the relationship between electricity, tissue (both healthy and diseased) and the resulting electrical circuits, can be traced back to his investigation of 'corona' phenomena that surround necrotic lung malignancies as seen on plain radiographs. The most striking of the radiological signs that Nordenstrom identified and described were the 'A' and 'B' zones that surround each of these lung cancers. The radiolucent 'A' zone is directly adjacent to the lesion and is caused by the movement of water out of this area of tissue. The 'B' zone is radio-opaque and lies peripheral to the 'A' zone. This radio-opacity, which is greater than seen in normal lung tissue and is only adjacent to the radiolucent 'A' zone, is again the result of the movement of water and its accumulation within this region. Further investigations led him to conclude that the driving force behind this movement of tissue water was electrical potentials that occurred in the vicinity of the injured tissues. Furthermore he postulated that these electro-osmotic forces were the energy source for transport systems, tissue repair, growth and taxis of cells. He argued that electrical systems existed within the body, one of which he termed the 'vascular interstitial closed circuit (VICC)' the 'cables' of which were the blood vessels with their highly electrically resistant walls containing low resistance plasma (1-5).

Nordenstrom also demonstrated that newly injured tissue was electropositive in relation to its surrounding tissue and so an electrical potential was created; neutrophils, being electronegatively charged, are attracted to the site of injury and the healing and repair process can begin. However, as the healing process progresses the 'charge' of the injury site with regards to the normal tissue fluctuates from electropositivity to electronegativity and back again; as the healing continues the magnitude of the electropotential diminishes until equilibrium is reached. Nordenstrom claimed that this allowed for 'the simultaneous removal of destroyed material and supply of new material during both anodic and cathodic phases' (5). In an effort to use these findings therapeutically Nordenstrom conducted a series of treatments in which the inherent local response to a tumour was augmented by the application of a small positive charge. To this end an anode was placed within the lesion and a cathode was placed at a distance from the tumour within normal tissue. This theoretically would attract both neutrophils and negatively charged tumour cells to the anode and prevent spreading of cancerous cells during the treatment. The local environment around the anode became progressively acidic due to the movement of protons and led to localized necrosis of the tumour. In this series of treatments the growth of 12 out of 26 tumours was arrested (6). Professor Nordenstrom also hypothesised that the cytotoxic effect of this electrochemical treatment was also due in part to the distant effects of the artificially generated electrical field (2, 5). These poorly understood distant effects include activation of immune cells and regional microthomboses.

Despite Nordenstrom's continuing efforts to promote electrochemical treatment as a useful therapeutic tool there was only limited interest in Europe and North America. During the 1980's there was a second Swedish research group that investigated the use

of these techniques in the treatment of lung tumours in a number of experimental studies. Samuelsson and Jonsson investigated the chemical changes at the electrodes and within the electrolysis field that were responsible for tissue necrosis as well as the role of electrolysis in pre-sensitizing tumours prior to radiotherapy (7-16). However, this line of investigation has not been continued by these authors. The use of electrolysis in combination with chemotherapeutics has also been reported. Electrochemotherapy, or electroporation, as it is sometimes termed, is the use of pulsed direct current to potentiate the action of chemotherapeutic agents. This process is thought to act by temporarily increasing the permeability of the cell membrane to DNA-intercalating agents such as bleomycin (17-20).

The greatest body of clinical data of the use of electrolysis in the treatment of tumours has been from China. The concepts of electrolytic treatment were introduced into the Peoples' Republic of China in the late 1980's when Nordenstrom was invited to give a series of lectures on his research and findings. Only a few years later in 1994 Yu-Ling published the data from 4081 patients whom had been treated with electrolysis (21). There have also been a number of other published reports of the clinical use of electrolysis in China (22-24). However, this clinical data is not readily compared with the published experimental work from Europe and North America due to the eclectic nature of the data reported in these studies. The studies drew their data from 66 Chinese institutions and over 30 different tumours, both benign and malignant, and at various stages of progression. One paper reported a good short-term response in 78.1% of cases and also showed improved results when treating T1 and T2 tumours when compared to T3 and T4 tumours (22). However, the broad spectrum of pathologies and the lack of any comparative control groups made meaningful statistical analysis of the

data impossible. Electrolytic ablation continues to be used in China where it is regarded as a safe and cheap adjunct to conventional surgical management (21); to date over 15000 cases have been reported. However, these again are mainly published series rather than formally constructed trials of electrolytic ablation.

In the mid 1990's Maddern and Dennison, from Adelaide and Leicester respectively, commenced a structured investigation of electrolysis as a locally ablative technique in the treatment of colorectal liver metastases. This focused approach on a single disease process was hoped to provide a firm conclusion regarding the efficacy and safety of electrolysis as a locally ablative surgical tool. By the end of the 1990's small and large animal studies had shown electrolysis to be both safe and effective in the ablation of normal rodent and porcine liver tissue and hepatic tumours in rats (25-30). In a subsequent clinical pilot study colorectal liver metastases were ablated, in 5 human cases, using electrolysis immediately prior to resection. This study demonstrated significant necrosis of the tumour in all cases (31). This same group has also used electrolytic ablation in conjunction with surgical resection (32). Although the study was too small to generate statistically significant survival benefits, the authors state that electrolysis was delivered safely with no peri-procedural morbidity. To date 27 patients have undergone electrolytic ablation of hepatic colorectal metastases with no perioperative mortality (personal communication).

The use of electrolysis in clinical practise has been limited by the difficulty of real-time monitoring of lesion creation and the relatively slow nature of the ablation process. This lack of real-time monitoring has meant that all treatment doses have been extrapolated from dose-volume curves created from the ablation of normal, disease-

free porcine liver. The absence of large animal tumour models has necessitated this approach to dosage calculation; despite its obvious limitations regarding the extrapolation that tumour tissue and normal disease-free parenchyma will respond in the same manner and extent to the electrolytic ablative dose.

1.1.2 Mechanism of Tissue Destruction

There are a number of potential processes by which electrolysis results in tissue necrosis. Most authors are convinced that the main ablative pathways are that of pH change and release of radical species. What all authors agree on is that heat has no role in the ablative process (7, 30). This is where electrolysis differs importantly from other commonly used locally ablative techniques, in which extremes of temperature are used to create the ablative lesion; heat in the case of radiofrequency ablation and microwave ablation and cold in cryotherapy (33-35). With electrolytic tissue destruction, changes in tissue pH, release of cytotoxic chemicals and movement of tissue water are all thought to have an influence on local necrosis. In addition to this there are other factors that have been identified as effecting the subtler 'field effects' of electrolysis (1, 9, 11, 36-38).

1.1.2.1 Electrode Reactions

The agents responsible for tissue destruction in electrolysis are the product of interactions between the electrodes (platinum), the electrolyte (water and sodium chloride) and the current. When a direct current is passed between two or more electrodes inserted into vital tissue a number of reactions occur. The common use of

chemically inert metals such as platinum for electrode manufacture means that the main reactions involve the decomposition of water and substances dissolved in it (36). At the anode, formation of oxygen and chlorine is accompanied by the acidification and proton production as elucidated by the following reactions:

$$2\ H_2O = O_2 + 4\ H^+ + 4\ e^-$$

$$2\ Cl^- = Cl_2 + 2\ e^-$$

In addition the chlorine produced may itself react with water to cause further proton formation:

$$Cl_2 + H_2O = HClO + H^+ + Cl^-$$

Chlorine yield is relatively greater than oxygen production. The reduction in local pH to pH 1 at the electrode tissue interface, is mainly due to the decomposition of water rather than the formation of chlorine (3, 4). This acidification of the tissue leads to haematin formation with a resulting brown discolouration of the electrolytic lesion. The relative contributions to total necrosis of both chlorine liberation and acidification are determined by the ability of chlorine and hydrogen ions respectively to spread through the surrounding tissue. Chlorine diffusion is limited by its reaction with these that leads to bleaching of the tissue. The spread of protons is limited by the buffering capacity of these tissues, which itself is largely determined by the circulation. Indeed, the greater diameter of the ring of methaemoglobin stained acidified tissue around the anode when compared to the ring of chlorine bleached tissue is a visual indicator of the superior spread of protons over chlorine (3, 4, 36).

The cathodic reactions of electrolysis are also centred on the decomposition of water; resulting in the evolution of hydrogen and the formation of hydroxyl ions:

$$2\ H_2O + 2\ e^- = H_2 + 2\ OH^-$$

An additional reaction may also be the reduction of oxygen itself by the addition of hydrogen ions:

$$O_2 + 2\ H_2O + 4\ e^- = 4\ OH^-$$

This production of hydroxyl ions results in an alkalisation of the local environment and subsequent tissue destruction. The transformation of haemoglobin to haemochromogens by sodium hydroxide again results in a black colouration of the cathodic lesion (9).

The movement of tissue water from the anode to the cathode is also driven by this electrical circuit resulting in turgor at the cathode and desiccation at the anode; again these conditions may have a small role in the creation of the ablative lesion (1, 6), (37).

The role of ischaemia in the ablative process of electrolysis has been reported and postulated upon by various authors (25). Although the thrombosis of vessels is a common finding in electrolytic lesions these vessels are invariably of a narrow calibre and predominantly around the anode (1, 6). Thrombosis within large diameter vessels is rare; however the calibre at which vessels are preserved has not been determined. Nordenstrom in his original description of the BCEC compared vessels with electrical

cables, the vessel walls acting as an insulator (200 times the resistance of plasma) whilst plasma acting as the conductor (1, 5, 6). Experimental attempts to produce intraluminal thrombosis in large calibre vessels by placing the electrode within the lumen have been unsuccessful (39). This feature of electrolysis where the lesion extends right up to the vessel but does not disrupt it is something that separates electrolysis from the thermal ablative techniques that all display features of 'heat-sink' around vessels of a significant calibre. In reality determination of the calibre of vessel protected is likely to be multifactorial with current, coulomb dose, electrode proximity and velocity of blood flow all playing a part. Although wedge infarcts have been report following electrolysis in rodent livers (40) these have never been routinely produced in larger animal models. The assumption that an associated infarct could increase the ablative capacity of a given coulomb dose is unsafe and should not be relied upon.

1.1.3 Field effects of Electrolysis

Nordenstrom postulated that in addition to the local 'primary' ablative process caused by the electrode-electrolyte reactions that there were also other effects of the electrical field on tissue both normal and diseased generated by the electrolysis process. He termed these 'distant field effects' of electrolysis in order to differentiate them from the 'primary' ablative process (1-3). These effects are certainly less obvious and considerably more complex than the primary electrode-electrolyte reaction. In an early series of experiments Nordenstrom noted that, following a course of electrolytic ablation to a number of large pulmonary metastases, smaller metastases, which had not been treated with electrolysis, also regressed either completely or partially. In order to

try and explain these findings Nordenstrom proposed certain mechanisms by which the afore mentioned 'distant field effects' could result in these findings.

1.1.3.1 Microthrombosis

The presence of intravascular microthromboses in the primary electrolytic lesion has been reported by several authors (1, 5, 6, 40). Nordenstrom noted diapedetic bleeding and microthromboses scattered in the wider electrical field (1). He theorised that these microthromboses would suspend capillary blood flow and lead to regression or extinction of malignant cells in the area.

1.1.3.2 Tissue Water

During electrolysis tissue water always moves from the anode to the cathode. Nordenstrom postulated that this increase in tissue turgor around the cathode would cause suspension of capillary flow, whilst there would be microthromboses around the anode (1). This effective vascular insult, if of sufficient duration, Nordenstrom felt would seriously interfere with tissue viability, especially that of potentially sensitive tumour cells.

1.1.3.3 Immunological

Leukocytes being electronegatively charged will be attracted to the anode during electrolysis (1, 6). Leukocytes have also been noted at the cathode site(41). However, given that leukocyte infiltration is a normal component of the healing process and it has been shown that electrolytic lesions heal in a similar time frame to other traumatic lesions, it is difficult to determine whether the leukocyte presence is due to electromagnetic attraction or simply a normal inflammatory response. Interestingly,

electrolysis produced greater volumes of necrosis per coulomb dose in immunocompetent mice when compared to immunocompromised nude mice (42).

The exact role, if any, of immune system recruitment or up-regulation in electrolysis remains unresolved. However, in this study the focus is on using electrolysis as a surgical tool to create an area of ablation rather than trying to modify the host's immune response.

1.1.3.4 Membranes

As mentioned previously pulsed direct current termed electroporation has been used to increase the effectiveness of chemotherapeutic agents such as bleomycin and cisplatin (42-47). The pulsed direct current temporarily and reversibly destabilises the lipid bi-layer and either by creating pores or opening transport channels allows large molecules (e.g. bleomycin and platinum salts) into the cell. Although the technical parameters of electrolysis and electroporation are different, the potential exists that direct current in a continuous rather than pulsed form may also disrupt the lipid bi-layer leading to cell instability and death.

All of these field effects are somewhat esoteric in nature and a great deal subtler and more complex that the primary ablative process of electrolysis. Although they pose interesting questions regarding all the potential therapeutic uses of electrolysis they are not directly pertinent to this research and will not be discussed further.

1.1.4 Delivering an electrolytic 'dose'

As has been previously stated electrolysis is essentially a simple process. All that is required is a direct current generator, electrodes and an electrolyte solution. However, as with most apparently simple concepts the devil is in the detail. There are various parameters that can affect these three constituent parts and thereby affect the final electrolytic process. A few of these variables will be considered here.

1.1.4.1 Current Generation

In these series of experiments, as in most experimental reports, a direct current generator was specifically built for the experimental work (25, 38, 48). Electrolytic doses are typically measured in Coulombs. These generators either keep the current (amperage) constant and allow the voltage to vary between preset limits, dependent on tissue resistance, or vice-versa.

1.1.4.2 Electrodes

This is the area with the potential for most variation. Important areas where variation can have a dramatic impact on the electrolytic ablation process are electrode material, electrode shape and electrode placement. All these will now been dealt with in greater detail.

1.1.4.3 Electrode Material

Numerous metals have been used for the construction of electrolytic electrodes. Corrosion of metals such as steel and copper has limited their practical usability. Similarly formation of toxic compounds has restricted the use of metals such as silver

and brass. Platinum is now the most common electrode material in biological systems (37, 49). Its main advantages are that it is chemically inert for reactions at both the anode and cathode and also it dissolutes slowly (1, 16). Interestingly some authors have postulated that the formation of these platinum salts in some way aids the electrolytic process; at present there is no evidence to support this theory. On the down side platinum is expensive and also brittle making it difficult work with. However, platinum-iridium alloys, with 10% iridium, retain the important electrochemical properties of platinum whilst resolving the physical brittleness (1).

1.1.4.4 Electrode Shape and Size

Electrode shape and placement are the two main determinants for lesion shape. The lesions at the anode and cathode are initially elliptical or spherical in nature (16, 30). However if the electrodes are sufficiently close together to allow 'overlap' of these lesion more complex 'dumb-bell' shapes are created. The volume of ablation is greater at the anode than the cathode, approximately by an additional 50%, as previously mentioned.

The size of the electrode surface area has important bearings on the speed of the ablation process and current density. Larger surface area electrodes have the potential for greater rates of ablation but would require increased amperage to maintain current density. Also, the larger the electrode the greater the trauma associated with its placement. Smaller surface area electrodes, although safer to place, especially percutaneously, have slower ablation rates.

1.1.4.5 Electrode Placement

In studies where electrolysis has been performed in organs with fibrous septae the placement of electrodes on either side of a septum has resulted in disruption of the flow of current between these two electrodes and therefore a failure of the ablative process. However, given the lack of significant fibrous septae within the pancreas this phenomenon is unlikely to be an issue during this series of experiments.

1.1.4.6 The Electrolyte

For there to be a complete electrical circuit the current must be able to flow through the tissue in which the electrodes have been placed. This tissue and the availability of the raw materials of electrolysis will affect the extent and duration of the electrolytic ablation. The level of resistance at the electrode-electrolyte interface will determine the voltage required to deliver the electrolytic dose: high resistance interface, high voltage required and vice-versa. This can effect the duration of ablation, especially as most generators have preset voltage limits that can not be exceeded. Also high voltages lead to heat generation. Although heat generation plays no part in the ablative process of electrolysis, it is an inevitable product of both the electrical circuit and the electrochemical reactions. However, temperature increases of only 5 degrees centigrade have been noted with currents of less than 100mAmps.

1.1.4.7 The electrolytic 'dose'

Electrolysis doses are expressed in coulombs, being the SI units for charge(10, 36):

1 Coulomb = 1 Ampere x 1 Second

The coulomb is described as 'the quantity of electric charge which passes some point when a current of one ampere flows for a period of one second.'

The relationship between electrolytic dose/coulombs and volume of necrosis has been reported by several groups. Although there is variation between the figures quoted, the range seems to be between 12.5mm^3 / Coulomb and 50mm^3 / Coulomb(50, 51). These differences are attributable to variation in equipment, experimental set-up and the tissue being ablated. There have been a few reports of ablation volumes an order of magnitude greater than those noted above however these seem to be 'one-off' results and may reflect other factors in the necrosis of the tumours rather than just the electrolysis.

1.1.5 Practical considerations for the clinical use of electrolytic ablation

Although the vast majority of experimental studies have performed electrolytic ablation under general anaesthesia, in the clinical setting local anaesthetics have been used successfully. Obviously this requires a cooperative patient and good communication between patient and operator but this is the case with any procedure performed under local or regional anaesthesia. As previously discussed, electrode size not only effects the time taken to deliver the electrolytic dose but also the trauma associated with their placement; this being of particular import when considering the use of local anaesthesia.

1.2 Pancreatic Cancer

1.2.1 Background

In most published literature pertaining to pancreatic cancer the first thing that is mentioned is the dismal prognosis associated with the disease. This poor outcome has not significantly altered over the years (52-54) despite extensive research into, and increased understanding of, the mechanisms, natural history and management of the disease. Pancreatic cancer accounts for 5% of the cancer deaths in the USA and is the fourth and fifth leading cause of cancer death, in men and women respectively (55-57). Despite an increase in incidence in the middle of the last century, the incidence of pancreatic cancer has now plateaued at 9-10 cases per 100 000 people (57-62). Reported five-year survival varies considerably between amongst reports of individual studies (63-66); however, collated data suggests actual, not actuarial, five-year survival rates of between 0.5% and 3% (53, 54). One of the factors contributing to the poor prognosis of pancreatic cancer is the often advanced nature of the disease at the time of presentation, with only approximately 15% of patients being suitable for surgical resection (67, 68), which is still considered to be the only potentially 'curative' treatment available (69, 70). Indeed pancreatic cancer has one of the lowest 5-year survival rates of any cancer, with the yearly incidence and mortality rates being almost identical (71, 72).

There are several well-documented geographical and demographical differences in the incidence of pancreatic cancer. In the USA, African-Americans have a higher incidence of pancreatic cancer with a ratio of 1.5 to 1 when compared with Americans of European extraction (73, 74). Japanese-Americans have an even greater risk of the

disease, despite the low incidence of pancreatic cancer in the native Japanese (75). Within Europe there are marked differences between pancreatic cancer incidence in the northern countries (Denmark, Holland, Germany and Ireland) having the highest incidence that is twice that of the southern countries (France, Italy, Portugal and Greece) with the lowest rates (57, 76, 77).

1.2.2 Aetiology

The single strongest risk factor for the development of pancreatic cancer is age. The risks of pancreatic cancer increase exponentially with age, with patients older than 50 years having 20 times the chance of developing the disease than younger patients (73, 74). The diagnosis of pancreatic cancer is usually made in the seventh decade; however, in patients with chronic pancreatitis this is often brought forward by ten to twenty years (78).

1.2.3 Risks associated with other medical conditions

1.2.3.1 Chronic Pancreatitis

As previously mentioned chronic pancreatitis patients are at increased risk of developing pancreatic cancer (78-80). This risk increases with the duration of the disease process, with a cumulative risk of approximately 2% per decade (78). The same study demonstrated a relative risk of 16. However, the causal relationship between chronic pancreatitic and pancreatic cancer has not been confirmed by all authors (81).

1.2.3.2 Diabetes Mellitus

In the past diabetes mellitus has been cited as a risk for pancreatic cancer (54, 82, 83). However, more recently this association has been questioned. The crucial point of debate being whether diabetes mellitus is an aetiological factor in is its own right or merely an early manifestation and symptom of pancreatic cancer. There is certainly a considerably quantity of data showing that there is a marked increase in the incidence of pancreatic cancer in those patients who have been diagnosed with type-2 diabetes in the previous 6-24 months (84); although some of the same studies show that when diabetes of duration greater then 3 years is considered that there is no longer a significant association between diabetes and pancreatic cancer (84). However, a number of studies (prospective, case-control and meta-analysis) with large numbers of participants have shown that diabetes mellitus, either of short or long-term duration, probably is a true risk factor for pancreatic cancer, albeit a modest one (85-88).

Even if the causal relationship between diabetes and pancreatic cancer is accepted, the biological mechanism behind this association remains unresolved. Previous hypotheses that destruction of the endocrine pancreas by local tumour advancement has been widely discounted. This is based on data from pancreatic resection series in which partial pancreatic resection (albeit a greater tissue volume than typically involved in pancreatic cancer) had not significantly affected glucose metabolism and glucose metabolism had improved post-resection (89). Other studies have shown that fasting levels of insulin are actually elevated in patients with pancreatic cancer and abnormal glucose metabolism (90). This would imply that the diabetes associated with pancreatic cancer is a result of peripheral insulin resistance rather than decrease insulin production, a situation that is analogous with 'classical' type 2 diabetes.

1.2.3.3 Hereditary Pancreatitis

There is positive association between hereditary pancreatitis and pancreatic cancer. It has been shown that 1% of pancreatitis cases are due to hereditary pancreatitis, an autosomal dominant condition with variable penetrance (91). Disease specific mutations have been identified on chromosome 7q35 (91, 92). The first two were identified in 1996 and 1997 respectively: R117H (type I hereditary pancreatitis) (91) and N21I (type II hereditary pancreatitis) (93). Over the past few years these have been renamed R122H and N29I. However, recently two new mutations N29T and R122C (within the same cationic trypsinogen PRSS1) have been identified (94). All these mutations are thought to have an effect on trypsin metabolism within the pancreas. The R122H mutation has been demonstrated to eliminate the cleavage point in the "hinge" section of the trypsin molecule (91) thereby removing the self-dampening mechanism of cleavage of trypsin by trypsin or trypsin-like enzymes when the concentration becomes too high with in the pancreatic tissue. Although pancreatic specific trypsin inhibitor (PSTI) remains the first line of defence, if the levels of R122H trypsin were to exceed the inhibitory capacity of PSTI, the cascade of trypsin release and autodigestion would continue unchecked (95). Although the mechanism of action of the N29I mutation is not as clear cut as for R122H there is evidence that this mutation may prevent the R122 region from being folded in such a way as to make it accessible to cleavage by other trypsin or trypsin-like enzymes (96). It has been observed that cleavage at the R122 site requires that the side-chain containing R122 be moved to a less favourable thermodynamic position before it is susceptible to cleavage by trypsin or trypsin-like enzymes (97).

Lowenfels et al proposed that the risk of pancreatic cancer was significantly increased in chronic pancreatitics, regardless of the country or sex of the patient and the aetiology of the pancreatitis (78). Indeed this study showed that there was a 15-fold increase in the risk of developing pancreatic cancer in patients with documented pancreatitis when compared with a normal population.

1.2.3.4 Gastrectomy and Cholecystectomy

The majority of the published literature has shown that there is a positive association between cholecystectomy and pancreatic cancer (98-101). However the temporal relationship between these events has not been so thoroughly demonstrated. Silverman et al published a case-control study, in 1999, which examined this aspect of the relationship; they demonstrated that the odds risk of developing pancreatic cancer within twelve months of cholecystectomy was 57.9 (95% confidence interval: 27.3-123.0) (102). For time periods greater than twelve months post-cholecystectomy and up to 20 years post-cholecystectomy there was an increased odds risk of pancreatic cancer, but at a greatly reduced rate (odds ratio range: 1.4-2.7). Other studies have shown that when only cases of pancreatic cancer occurring more than one (103) or two (104) years after cholecystectomy are considered that the odds ratio returned to unity. Some authors have stated that due to the aggressive nature of pancreatic cancer that the increased risks of the disease, seen in some studies (101, 102), many years after cholecystectomy cannot be due to prodromal neoplastic disease (105). Biological bases for this causal relationship are the experimental studies showing cholecystokinin (CCK), present in increased levels following cholecystectomy, to be a promoter of pancreatic adenocarcinoma in rodents (106, 107).

The role of gastrectomy as an independent risk factor for pancreatic cancer is more tenuous than that of cholecystectomy, although there have been consistent reports of the association between gastric surgery and pancreatic cancer over the years (108). Despite this, several large case-control studies have shown that there is no significantly increased risk of pancreatic cancer with either peptic ulcer disease or previous partial or total gastrectomy (102, 103, 109). The presence and significance of such an association is still under debate. However, the decreasing incidence of gastrectomy for non-malignant disease is likely to make this area of discussion of less importance in the future.

1.2.4 Environmental and Lifestyle risk factors

1.2.4.1 Smoking

Smoking is the most common risk factor associated with pancreatic cancer. The International Agency for Research on Cancer reported in 1985 that 'cigarette smoking is an important cause of pancreatic cancer' (110). This statement is supported by many studies, both prospective and retrospective, that have shown that smoking causes a 1.5-3 fold increased risk of developing pancreatic cancer; and that the risk was proportional to the duration of the habit and the quantity smoked (111-116). Further more it has been estimated that 25% of pancreatic cancers in the USA and Europe could be due to smoking(111). A recent study has shown that there is no association between cigar smoking and pancreatic cancer, although the risk of other cancers is increased. However, in those cigar smokers who inhale, the smoke the relative risk of pancreatic cancer is 2.7 (117). A markedly increased risk of the disease has also been

shown in chewers of tobacco (115). It has been proposed that nicotine is the causative agent in cigarette smoke responsible for the development of pancreatic cancer (118).

1.2.4.2 Alcohol

The role of alcohol as an independent risk factor for pancreatic cancer has been in dispute since the 1960's. Many studies have failed to demonstrate a positive association between alcohol consumption and an increased risk of pancreatic cancer (119-122). Other than the role of alcohol in the aetiology of chronic pancreatitis there appears to be no increased risk, in terms of pancreatic malignancy, with modest alcohol consumption.

1.2.4.3 Diet

As discussed above with alcohol, coffee has been regarded as a potential independent aetiological factor in pancreatic cancer for many years. Despite some studies suggesting that there is a positive association between coffee consumption and an increased risk of pancreatic cancer, the majority of the published literature has failed to support such a contention. There is considerable risk that the results of epidemiological studies were significantly confounded by the smoking of tobacco. Indeed there is a strong association between smoking and high coffee consumption. Furthermore, some studies have shown that the 'coffee' association disappears when cigarette smoking is taken into account (123).

Although studies have reported that K-*ras* gene mutations, in pancreatic cancer, are more common in patients with heavy coffee consumption; Jacobsen and Heuch demonstrated that coffee consumption was positively associated with mutatious K-*ras*

harbouring pancreatic cancer, albeit weakly, but were more strongly negatively associated with pancreatic cancer with the wild-type gene resulting in an overall relative risk of approximately 1 (124).

High-carbohydrate and high-fat diets have also been linked with an increased risk of pancreatic cancer. In addition to this, in 1992 Howe et al published a multinational case-control study that demonstrated a positive association between 'total energy intake' and pancreatic cancer (80, 125, 126). The beneficial role of high vegetable and fruit intake has yet to be proven despite the emergence of epidemiological evidence to that effect.

1.2.4.4 Occupational exposure

The pesticide DDT has been shown to have a strong positive association with pancreatic cancer (127). Garabrant et al showed that the risk ratio of developing pancreatic cancer between those 'ever-exposed' and those 'never-exposed' was 4.8, in those subjects with prolonged exposure (mean 47 months) that risk increased to 7.4 (128).

1.2.5 Management

1.2.5.1 Surgical

Surgical resection is still considered to be the only curative treatment available for pancreatic cancer. Historically curative surgery has only been applicable to a small (10-15%) proportion of patients with pancreatic cancer (52, 67, 68). However, there

remains considerable debate regarding which surgical procedure should be considered the gold standard.

Although the first successful pancreatoduodenectomy was reported in 1912, by Kausch (129), it was not until 1935 that the original paper by Whipple et al reported three successful two-stage pancreatoduodenectomies (130). Following the discovery of vitamin K, in 1940 by a Danish physiologist, Whipple performed a modified one-stage version of this original technique which has become the eponymous procedure which is the mainstay of pancreatic head cancer surgery (131) accounting for two thirds of pancreatic head resections in the USA (132). In the time since the initial description of the pancreaticoduodenectomy, the procedure has been refined and modified as further generations of surgeons have attempted to improve peri-operative mortality and morbidity and long-term survival. The 'classical' Whipple procedure included resection of the pylorus and distal stomach in an attempt to maximise tumour clearance. However, the consequent alterations in upper gastrointestinal anatomy and physiology not infrequently result in long-term post-operative complications. In 1944, Watson described the first pylorus preserving pancreatoduodenectomy (PPPD), for the resection of a carcinoma of the ampulla of Vater (133). His rationale being, that not only was it a less time consuming operation but also, the preservation of the pylorus and formation of a duodenojejunostomy would lead to a reduction in anastamotic ulceration and that retaining the stomach would result in an improvement in digestion. However, it was not until 1978, when Traverso and Longmire (134) re-proposed the concept of a pylorus preserving pancreatoduodenotomy (although initially for chronic pancreatitis surgery), that the surgical community took note. These authors stated the theoretical advantages of PPPD as eliminating the complications of a reduced gastric

reservoir, improving gastrointestinal function and reducing jejunal exposure to excessive amounts of acid. Since the re-introduction of the PPPD in the management of pancreatic cancer there has been an ongoing debate regarding its suitability and adequacy as an oncological procedure. The concerns regarding PPPD as a cancer operation are mainly to do with tumour and lymph node clearance, which some authors feel is inadequate if the distal stomach is retained. Concerns regarding prolonged post-operative gastric paresis have also been expressed. Retrospective studies have, in the main, shown there to be no significant difference between classical Whipple procedure and PPPD in terms of mortality, morbidity, complication rate, survival and recurrence site (135-142). Furthermore, some of these same studies show improvement in post-operative weight gain and nutritional status (136, 141). However, some reports state improved long-term survival in patients who have undergone a classical Whipple resection rather than a PPPD (143).

Cancers of the body and tail of the pancreas are not amenable to resection using the Whipple procedure and are therefore usually treated with a distal pancreatectomy. A combination of factors leads to distal pancreatectomy being rarely used with curative intent in patients with pancreatic carcinoma. Firstly, the incidence of pancreatic cancer originating in the body or tail of the gland is low (144, 145) and secondly the absence of biliary obstruction as an early symptom results in delayed presentation of the patient with symptoms of pain or weight loss (146, 147). Unfortunately, by this point the disease is often either locally advanced or distant metastases are present thereby precluding an attempt at a curative resection. Some authors advocate total pancreatectomy in these patients, but this remains a controversial issue.

The role of surgery in the palliation of pancreatic cancer and its symptoms has returned as an area of contention in recent years. In the past the peri-operative morbidity and mortality associated with the Whipples procedure meant that its use for non-potentially curative purposes was considered inappropriate. Indeed there were some authors that felt that the associated morbidity and mortality meant that pancreaticoduodenectomy had no place in the management of pancreatic cancer, either as a palliative procedure or where used with curative intent. Therefore surgical intervention for palliation in pancreatic surgery was almost exclusively restricted to bypass surgery for either gastric outlet obstruction or biliary obstruction or both. However, in the past two decades, as morbidity and mortality rates of 30-50% and 0-5% respectively have been increasingly reported from tertiary centres with a specific interest in pancreatic surgery, the role of the Whipple's procedure in palliation of pancreatic surgery has had to be reassessed. Unfortunately this improvement in peri-operative outcome has not been seen universally. A significant difference in peri-operative mortality, for the Whipple procedure, has been shown when high-volume centres (>24 cases/year) are compared with low-volume centres (<5 cases/year); respective mortality rates were 1.5% and 16.4% (148). Data from the USA and the United Kingdom supports this relationship between mortality rate and unit case-volume (149, 150).

1.2.5.2 Bypass Surgery and Endoscopic management

As mentioned previously, bypass surgery, both biliary and gastrointestinal, was the mainstay of interventional palliation for the symptoms of pancreatic cancer for many years.

The rationale behind operative biliary bypass is to decompress the biliary system that has become obstructed, invariably by an unresectable malignancy usually pancreatic or bile duct in origin. The exact technique, timing and the actual indications for biliary bypass remains an area of contention. One of the main factors around which these differences revolve is the decision to stage pancreatic cancer progression either by laparotomy or laparoscopy.

The majority of authors advocate the formation of a Roux-en-Y choledochojejunostomy as the preferred technique for surgical biliary bypass. This technique has been shown to produce good symptom control whilst having a low re-obstruction rate. Drainage of the obstructed biliary tree via a choledochoduodenostomy has been discouraged by many authors due to concerns regarding anastamotic leaks and late obstruction due to tumour progression. Although the formation of a cholecystojejunostomy has been promoted by some authors, mainly due to the relative technical ease of the procedure, this technique has been widely criticised because the function of the biliary bypass is dependent on the patency of the cystic duct, which is not uncommonly involved in locally advanced disease.

The relevance of gastric outlet obstruction (GOO) in the management of unresectable pancreatic cancer is still under debate. As a result the role of gastrojejunostomy in these patients remains controversial. Prevalence of GOO remains to be well defined. Advocates of prophylactic gastrojejunostomy, at the time of surgical biliary bypass, quote data from retrospective laparotomy-based studies that suggest that 25% of patients eventually develop GOO. They argue that if the disease is found to be unresectable and a surgical biliary bypass is performed that the addition of a

prophylactic gastrojejunostomy is justified on the grounds that it will spare the patient the possibility of a second laparotomy and can be performed with similar morbidity and mortality to a biliary bypass alone. In 1999 Lillemoe et al published a prospective randomised trial that lent weight to this point of view (151). They found that 8 out of 43 patients who did not have a prophylactic gastrojejunostomy formed at the time of surgical biliary bypass subsequently developed GOO, with 7 of these patients requiring a second laparotomy and bypass. In addition to this their data showed there to be no significant difference, with regards to length of hospital stay and post-operative complications, between the group of patients that underwent biliary bypass alone and the cohort that had a gastrojejunostomy in addition to the biliary bypass. Based on this data the authors have proposed the routine use of gastrojejunostomy at the time of biliary bypass.

The use of laparoscopy to stage pancreatic cancer and the treatment of biliary obstruction by endoscopic or radiological stent placement complicate the issue of gastrojejunostomy even further. In a prospective study published by Espat et al in 1999, 155 patients, who were deemed to have unresectable pancreatic cancer at laparoscopy but did not undergo open enteric or biliary bypass at the time of laparoscopy, were followed up until death (152). This study showed that only 3 patients (2%) required a subsequent open procedure to treat either GOO or biliary obstruction. From these findings the authors propose that 'surgical biliary bypass can be advocated only for those patients with obstructive jaundice who fail endoscopic stent placement, and gastroenterostomy should be reserved for patients with confirmed gastric outlet obstruction'. Part of the difficulty in rationalising these variations in

reported incidence of GOO, and therefore the proposed role of gastrojejunostomy, is the lack of a consistent understanding of the term 'gastric outlet obstruction'.

1.2.5.3 Invasive Palliation - Stenting

Biliary Obstruction: The introduction of stents in the treatment of biliary obstruction has had a significant impact on the management strategies available for the palliation of pancreatic cancer. The placement of these stents can either be performed under radiological guidance via a trans-hepatic approach or at endoscopy during an endoscopic retrograde cholangiopancreatography (ERCP). The first reported case of endoscopic biliary stent placement was described in 1980 by Soehendra and Reynders-Frederix. In the past twenty years the use of biliary stents during ERCP has become routine (153).

Prior to ERCP any clotting abnormalities resulting from often prolonged obstructive jaundice are corrected with either vitamin K or fresh frozen plasma. It is imperative that both the biliary and pancreatic duct systems are evaluated to allow accurate definition of the malignant stricture within the biliary tree. At the time of ERCP wire-guided brush cytology, needle aspiration or forceps biopsy can be performed in order to derive a cytological diagnosis. Although all these techniques have been shown to be highly specific, with a positive predictive value approaching 100%, they are appreciably less sensitive, with a false negative rate of 40%. As a result there are some limitations to the usefulness of these procedures. Stent placement is achieved by passing a guide wire across the stricture to the level of the right or left hepatic ducts. Over this wire the stent is 'railroaded' until it is accurately positioned. The most commonly used stents for this procedure are solid polyethylene stents of either straight

or curved configuration, with flaps at either end to minimise stent migration. This technique allows the placement of stents with a diameter of up to 10-14 French into the biliary tree, although many authors use 10F stents as the obstruction rates for these is no different from that for greater calibre stents and the immediate complications are less. Although endoscopic placement of polyethylene stents can normally be successfully performed without a sphincterotomy these are occasionally required (154). In situations where it is impossible to gain deep cannulation of the bile duct via a solely endoscopic approach a combined 'percutaneous transhepatic-endoscopic' approach can be used. In this procedure a percutaneous approach is undertaken (as for percutaneous transhepatic stent placement as described earlier) this allows the placement of a guide wire (4m in length) across the bile duct stricture and into the duodenum. Once this guide wire has been secured in position, at ERCP the intra-duodenal guide wire is snared and brought out through the endoscope. This results in the guide wire lying across the stricture to be stented whilst give the endoscopist control of both proximal and distal ends of the guide wire; this techniques usually allows internal drainage of an obstructed biliary system to be achieved in most cases. A further advantage of the combined procedure is that it does not require the passage of large calibre instruments (12-14 French) through the liver, a situation that is necessary for percutaneous transhepatic stenting (155).

Complications of biliary stent placement can be divided into early complications, primarily related to ERCP, such as cholangitis, pancreatitis and perforation of either the bile duct or the duodenum; and late complications that are related to stent placement. These include problems such as migration, fracture and obstruction of the stent itself, acute cholangitis and duodenal perforation (156-159). Large reported series

of endoscopic biliary stent placement give morbidity rates of between 0-35%. However, significant procedure related morbidity is below 10%. Despite operative mortality remaining low, there is still a significant 30-day mortality of 10-20% (160-163). This is largely dependent on the progression of the pancreatic cancer, a biologically aggressive disease, rather than as a result of endoscopic intervention.

Of the early complications the most common and clinically most important is cholangitis. The role of antibiotic prophylaxis in ERCP remains controversial. The use of pre-ERCP antibiotics, in all cases of biliary obstruction, has been proposed by some centres (164). However, other studies have shown that cholangitis occurs in only 2-3% of cases and primarily in those patients in which adequate biliary drainage has not been achieved (165). These centres advocate the selective use of antibiotic cover in patients following failed biliary stenting.

Stent migration has been shown to occur in 5% of cases (166). Migration has also been shown to be associated with large stent diameter, proximal placement and sphincterotomy.

Stent occlusion and the associated clinical sequelae of jaundice, abnormal liver enzymes and cholangitis are the major late complication of palliation of biliary obstruction by the placement of plastic stents (167). Occlusion of plastic stents may occur at any time after insertion; however the mean duration of patency is 4.5-8 months. These two studies found that 20-33% of patients who had undergone stenting required stent replacement. Data from other studies have concurred with these findings. This has led many authors to recommend prophylactic stent replacement at

between 3 and 6 months. The cause of obstruction for most biliary stents is not tumour ingress but rather deposition of biliary sludge and bacterial 'biofilm' (168). Various attempts have been made to prevent stent occlusion. Although encouraging results have been seen in-vitro, using silver-coated or ultra-smooth stents or antibacterial and mucus suppressing agents, there has been no translation into improvements in terms of clinical outcomes (169-171). Stent design has also been shown to play a key role in stent occlusion. It was demonstrated in 1985 by Leung et al that stents with pigtail or tapered ends had substantially decreased flow rates when compared with straight or moderately curved stents (172), these findings have been subsequently supported by other investigators (173). From basic physics, in the form of Poiseuille's law, we know that flow through a tube is proportional to the radius^4 of that tube (174). Therefore, it has been correctly assumed that greater stent diameter leads to improved flow rates. A number of studies comparing 10 Fr stents with 7 and 8 Fr stents have shown that this theoretical advantage translates into clinical practice with significantly improved patency rates (175-179). However, a retrospective study performed by Kadakia et al comparing 10 and 11.5 Fr stents found no significant difference between the two diameters of stents in various measurable parameters such as: successful insertion rates, resolution of jaundice, complications or stent patency (180).

1.2.5.4 Medical Management

As was stated at the start of this section with only a small percentage of people with pancreatic cancer being suitable for resection the vast majority require medical management as the main stay of their treatment. Indeed, with 5-year survival rates of between 8-11% following definitive surgery alone, it is apparent that additional

adjuvant therapies are required in order to maximise potential survival even in those patients who are suitable for potentially curative surgery (181-183).

In addition to the potential survival benefit to be achieved with chemotherapy, with or without radiation therapy, there is increasing recognition of worthwhile palliation of symptoms and improved quality of life following chemotherapy even in the absence of a survival benefit. This has led some investigators to move away from survival as the end point for their investigations, as has been the tradition in phase III trials of chemotherapeutic agents. One of the alternate end points is Clinical Benefit Response (CBR) which is a composite of pain scores, performance status and weight gain. CBR has been used as the primary end point in a number of studies (184, 185). The development of a pancreatic cancer specific quality of life (QOL) module (QLQ PAN 26) will hopefully further help standardize the QOL assessment in this disease process (186).

1.2.5.5 Chemotherapy

Many chemotherapeutic agents have been studied for their efficacy in the management of pancreatic cancer, both individually and in combination.

1.2.5.6 Chemotherapy – Advanced Pancreatic Cancer

Gemcitabine, a nucleoside analogue, remains the standard of care for advanced pancreatic cancer. Phase II studies in the mid 1990's investigated gemcitabine using a weekly regimen administered by short infusion (187, 188). These studies reported a response rate of only 10-15% on radiological assessment. However, 27% of patients reported some clinical benefit in terms of performance status, weight gain and pain. A

subsequent Phase III multicentre randomised trial compared Gemcitabine and 5-fluorouracil (5-FU). Again although conventional radiological response rates were disappointing (0% for 5-FU and 5.4% for gemcitabine) improvements in CBR and 1 year survival were noted with Gemcitabine (185).

Investigations in the optimisation of gemcitabine administration suggest that this is an area of potential improvement. Gemcitabine requires intracellular phosphorylation to convert it to its active metabolite; this is a saturable process. Preclinical and clinical studies suggest that the drug may be more effective if administered using a fixed dose rate of 10mg/m^2/min (189). Phase II trials of this regimen are ongoing (190, 191).

5-FU is a pyrimidine analogue; it acts by inhibiting thymidylate synthase and also directly inhibiting DNA and RNA synthesis. As has been previously alluded to, bolus administration of 5-FU has little activity in the treatment of pancreatic cancer (185). Investigation of whether infusional or chronomodulated administration of 5-FU improved its efficacy have demonstrated no clear evidence of clinical benefit (192-195).

In addition to the use of single chemotherapeutic agents combination chemotherapies have also been studied. A number of Phase II have been undertaken using gemcitabine in combination with up to three other agents, each of which have typically demonstrated some single agent activity in pancreatic cancer (196-200). These Phase II studies have generally reported higher response rates; 26% with gemcitabine and cisplatin (196) and 51% with gemcitabine, cisplatin, epirubicin and 5-FU (197); than is typically seen with single agent studies. However, Phase III trials have not shown any

firm evidence of improved outcomes with combination therapy. An Italian randomised study comparing gemcitabine alone or with cisplatin showed a greater response rate with the combination therapy 26.4% opposed to 9.2% but no significant difference in CBR (52.6% to 49%) or median survival (30 weeks to 20 weeks) (201). Two further trials comparing single agent gemcitabine with combinations of either gemcitabine and 5-FU or gemcitabine and capecitabine respectively have been reported (202, 203). In neither of these trials was there any evidence of significant difference between single agent and combination therapy when assessing CBR, response rate or median survival. To date no single or combination therapy has been proven to be of greater efficacy than single agent gemcitabine.

1.2.5.7 Chemotherapy – Adjuvant Therapy

There is a limited volume of data pertaining to the use of adjuvant chemotherapy in the management of pancreatic cancer (181, 204, 205). Most published studies are of chemoradiotherapy (CRT) as adjuvant therapy. To date there has only been one randomised controlled trial of adjuvant chemotherapy in the management of pancreatic adenocarcinoma, the ESPAC-1 trial (206-208). This was a 2x2 factorial study of chemotherapy versus no chemotherapy and radiotherapy versus no radiotherapy. 285 patients were recruited to the full randomisation with another 256 recruited into single option limbs of the study. Chemotherapy was bolus 5-FU with folinic acid. This study showed there to be significantly improved median survival with chemotherapy (19.7 months versus 14 months) when all patients were considered. However, when analysis was limited to the 285 patients in the 2x2 trial statistical significance was not reached. A further trial ESPAC-3 is currently ongoing with three study limbs of observation, 5-FU/Folinic acid and gemcitabine.

1.2.5.8 Radiotherapy and Chemoradiotherapy

In 1969 Moertel et al reported improved median survival in patients with advanced pancreatic adenocarcinoma when treated with a combination of externalbeam radiotherapy (EBRT) and 5-FU when compared with EBRT alone (10.4 months versus 6.3 months) (209). The Gastrointestinal Tumor Study Group (GITSG) randomised unresectable patients into one of three study limbs 60 Gy EBRT, 40 Gy EBRT with 5-FU and 60 Gy EBRT with 5-FU. In this study both CRT limbs had superior survival when compared with the EBRT alone limb (10 months CRT versus 5.5 months EBRT) (210). In 1988 the same group compared CRT against chemotherapy alone and found superior 1-year survival in the CRT group (41% versus 19%) (211). However, a larger study published 3 years earlier in 1985 by Klaassen et al, reporting for the Eastern Cooperative Oncology Group (ECOG) found no survival benefit when comparing 5-FU with EBRT plus concurrent and maintenance 5-FU (212). Other published studies in this field have shown similar median survival for patients being treated with a variety of chemoradiotherapeutic regimens (45-60 Gy continuously, split-course or hyperfractionated with either 5-FU with or without folinic acid or cisplatin) (213-216). Studies into the use of gemcitabine as a radiosensitizer are ongoing (217, 218).

Although studies have shown there to be a reasonable survival benefit with CRT with or without maintenance chemotherapy in patients with unresectable pancreatic cancer when compared with observation there is little strong evidence to suggest improved survival when compared with chemotherapy alone.

As noted previously with reported 5-year post-operative survival between 8-11% adjuvant therapies must have a role to play (181-183). Local failure rates following surgery, with curative intent, have been as high as 50-86% in some series (219-222). To ensure better local disease control radiation therapy is routinely given to the pancreatic bed and locally draining lymph nodes. In the United States, standard adjuvant therapy is a combination of EBRT and CRT in an attempt to both improve local control and prevent early metastases.

There have been several published non-randomised studies on the use of CRT as an adjuvant treatment in pancreatic cancer (223-227). However, none of these have shown any clear survival benefit over either observation or chemotherapy alone. In 1999 a multicentre randomised phase III trial conducted by the European Organisation for Research and Treatment of Cancer (EORTC) was published (182). This study compared surgery alone with CRT following potentially curative surgery for pancreatic and ampullary cancers. Although there was an apparent survival benefit in the pancreatic cancer treatment group (17.1 versus 12.6 months for treatment and observation respectively) this did not reach statistical significance. It was concluded that although CRT was well tolerated there was no survival benefit. In the United States the regimen originally adopted by GITSG (210) was used for a randomised study that was published in 1985 and later added to in 1987. Here 43 patients with clear resection margins were randomised to either surgery alone or 40 Gy radiotherapy with 5-FU radiosensitisation followed by weekly 5-FU for 2 years. Median survival was significantly higher in the treatment group (20 months versus 11 months) with also a superior 2-year survival (42% versus 15%) (228). A further 30 patients were added to the treatment arm the combined data of which showed median survival of 18

months and 2-year survival of 46% (229). There have been concerns regarding these studies on grounds of poor compliance, small numbers, early termination and quality assurance. However it appears that the standard adjuvant regimen, especially in the States, is based on the findings (230-237). It is hoped that the recently closed Phase III Radiation Therapy Oncology Group (RTOG) study comparing gemcitabine and CRT with 5-FU and CRT will provide some clear indications as to what the 'standard' adjuvant therapy in pancreatic cancer should be.

1.2.5.9 Neoadjuvant Therapy

The role of neo-adjuvant therapy in the management of pancreatic cancer remains unclear. In 1998 ECOG published a Phase II study investigating the role of pre-operative CRT in patients with potentially respectable cancers. Of the 53 patients treated pre-operatively 24 went on to have resection. This group had a median survival of 15.7 months compared with 9.7 months for the whole group (238). Other studies of the role of neoadjuvant CRT have had similar results (239-244). Although feasible, there is no evidence that neoadjuvant CRT conveys any long-term advantage in outcome and should be regarded as an area for further study rather than being standard care (245, 246).

1.2.5.10 Matrix Metalloproteinase Inhibition

Matrix metalloproteinases (MMPs) are zinc- dependent endopeptidases involved in tissue re-modelling. Overexpression of MMPs has been demonstrated in pancreatic cancer (247). Although there is some pre-clinical data that inhibitors of these enzymes (MMPIs) slow tumour growth and diminish metastases, this has not been supported by clinical trials (248). Two published studies have investigated the utility of Marimastat,

a peptidomimetic MMPI, in pancreatic cancer. The first paper looked at a group of patients with advanced pancreatic cancer whom had no response to conventional therapies. These patients were divided into three groups, each one given different doses of marimastat. Although there was no evidence of survival benefit there was an improvement or stabilisation in pain, immobility and analgesic consumption in 50% of these patients. There was also a stabilisation or reduction in CA 19-9 levels in 30% of patients (249). A subsequent randomised study using gemcitabine with three dose levels of marimastat showed gemcitabine to have a superior response rate, median survival and clinical response compared to marimastat (250). At present MMPIs have not been conclusively demonstrated to have significant activity in pancreatic cancer. However, a recent study of inoperable gastric cancer has demonstrated improved 1-year survival with marimastat compared with placebo (251).

1.2.5.11 Angiogenesis Inhibition

Angiogenesis is essential for tumour growth. By the time a tumour has a volume of 1-2mm^3 it requires new blood vessels to continue growing (252). Vascular Endothelial Growth Factor (VEGF) has been demonstrated to be a potent regulator of both normal and pathological angiogenesis (253). Bevacizumab, a recombinant monoclonal antibody to VEGF, has been the most studied anti-angiogenesis agent to date. A Phase III trial of metastatic colon cancer showed significantly improved median survival with Bevacizumab, 5-FU and irinotecan when compared to 5-FU and irinotecan alone (20.3 months versus 15.6 months respectively) (254). A Phase I study of Bevacizumab, capecitabine and EBRT in locally advanced pancreatic cancer showed median survival, in a 48 patient study group, of 15.7 months and an actuarial 1-year survival of

65%. These encouraging data have prompted the RTOG to further investigate Bevacizumab in combination with capecitabine and EBRT.

1.2.5.12 Epidermal Growth Factor Inhibition

The importance of epidermal growth factor receptors (EGFR) in cancer is suggested by a number of observations. Overexpression of EGFR with its ligand has been shown to transform non-malignant cells in-vitro and EGFR is the most commonly overexpressed receptor in solid tumours. Pre-clinical data suggest that EGFR inhibition inhibits tumour cell proliferation and viability (255).

One of the approaches to target EGFR has been the development of specific antibodies. Two such antibodies have demonstrated clinical activity: Trastuzumab, Herceptin®, an anti-Her-2 antibody has been shown to be active in Her-2 overexpressing breast cancer (256) and cetuximab, an antibody to EGFR (Her-1), has activity in colon cancer (257). This is of relevance in pancreatic cancer as Her-1 is overexpressed in 30-50% of pancreatic cancers and Her-2 is overexpressed in 20% of pancreatic tumours (258, 259). Given that there is preclinical data that these agents work synergistically with chemotherapy, a number of ongoing trials are investigating combinations of these agents with conventional chemotherapeutics. Early results from phase II trials show encouraging evidence of activity with trastuzumab plus gemcitabine and cetuximab plus gemcitabine, respectively (260, 261).

1.2.5.13 Immunotherapy

The aim of immunotherapy is to stimulate the immune system to act specifically against tumour cells whilst limiting damage to normal tissue. Gastrin has been shown

to be a growth factor in gastrointestinal tumours, including pancreatic cancer. An anti-gastrin immunogen G17DT has been developed, it works by raising antibodies that inhibit gastrin stimulated growth (262, 263). Gilliam et al presented, in abstract form, a randomised study in 2004 of 154 patients with advanced pancreatic cancer who were either unable or unwilling to take conventional chemotherapy. These patients were randomly assigned to receive either G17DT or a placebo. Overall survival was significantly improved in those patients taking G17DT (151 days versus 82 days with placebo). Unfortunately a more recent Phase III trial has shown no survival benefit when comparing G17DT plus gemcitabine with gemcitabine alone.

1.3 Experimental Porcine Pancreatitis

This study's methodological validity is dependent on the premise that acute pancreatitis in pigs is a clinically significant condition. Obviously, if porcine pancreatitis were a trivial condition, then it would be difficult to determine significant differences between treatment groups if one of the potential outcomes, acute pancreatitis, is a non-significant physiological insult.

There have been numerous studies investigating animal models of acute pancreatitis. Although the porcine model is not the most commonly used (canine and rodent models being the most popular), there are many reports of porcine pancreatitis being used as an animal model to evaluate the effects of various interventions.

Thorpe described the induction of experimental pancreatitis in a porcine model by the injection of bile and bile salts into the pancreatic duct in 1971 (264). This methodology

had been used to produce animal models of significant acute pancreatitis in other species (265, 266).

Waterworth et al first described their model for producing experimental pancreatitis in pigs, again by injecting bile into the pancreatic duct in 1974 (267). In 1976, the same group published a paper on the use of glucagon in the management of experimental pancreatitis. Again, they produced a severe pancreatitis in a porcine animal model by injecting bile into the pancreatic duct. Of the 15 control animals who did not receive glucagon, 10 died within the first week (268).

There have been further published studies in which a porcine model of acute pancreatitis has been used to assess the efficacy of various treatments. The majority of these studies have induced pancreatitis by the injection of either bile or Na-taurocholate trypsin into the pancreatic duct. Although there are a wide range of interventions being assessed, all these papers have similar findings with regards to the outcome of the untreated control animals; invariably, the vast majority of the study pigs dying within a few days.

Schroder et al in 1978 published a controlled trial investigating the use of xylocaine infusion in the management of severe pancreatitis. Experimental haemorrhagic pancreatitis was induced in 12 piglets by infusing Na-taurocholate trypsin into the pancreatic duct with simultaneous intravenous secretin stimulation. All animals developed severe pancreatitis accompanied by the production of bloody ascites. All the pigs in the control limb of the study died within 24 h. Of the animals treated with xylocaine infusion (50 microgram/kg/min for 24 h), one died within 24 h, one during

the second day, and four lived for over a week, at which time they were killed(269). Further research from the same group in 1981, this time looking at the role of chlorpromazine in the treatment of pancreatitis, showed similar mortality in the untreated control group (n=10)(270).

In 1982 Zalaudek et al looked at hyperbaric oxygen therapy in acute pancreatitis; all the animals who did not receive hyperbaric oxygen therapy died from fulminate pancreatitis within 24 hours of its induction (271).

Rudd published a paper, in 1986, that reported a greater than 60% (5 of 8) mortality within 6 hours of Na-taurocholate induced experimental porcine pancreatitis (272).

Another group reported a mortality of 83% (10 of 12) within 24 hours again using the Na-taurocholate experimental porcine pancreatitis model (273). Similar results have been published by other authors(274).

Other studies have used histological assessment of pancreatic necrosis as an end point rather than mortality. Clemens in 1980 showed that the acute pancreatitis induced by injection of contrast medium into the porcine pancreatic duct lead to necrosis of 50% of the gland (275).

Although these papers have used a variety of methods and end points, they have universally found that porcine pancreatitis is a clinically significant condition that in its severest form conveys high mortality levels (60-100%) in a short time frame (6 hours to 7 days). As a result of this, we reasoned that if electrolytic ablation of the porcine

pancreas were to produce a significant acute pancreatitis, it was likely to be a clinically, biochemically and histologically detectable condition.

1.4 This study aims to investigate:

- Feasibility of porcine pancreatic duct cannulation, both open and endoscopic.
- Feasibility of delivering electrolytic ablation to the porcine pancreas, both as an open and an endoscopic procedure.
- Clinical assessment of short, medium and long-term morbidity and mortality post pancreatic electrolytic ablation.
- Effect of pancreatic electrolytic ablation on biochemical parameters.
- Effect of pancreatic electrolytic ablation on cytokine concentrations.
- Histological and radiological assessment of electrolysis and macroscopic and microscopic pancreatic morphology.

With a view to electrolysis ablation being an additional therapy in the management of pancreatic pathologies, particularly head of pancreas mass lesions caused either by adenocarcinoma or chronic pancreatitis.

Chapter 2:

Methods

Chapter 2: Methods

2.1 Ethics Declaration

The Animal Ethic Committees of The Queen Elizabeth Hospital, Woodville, South Australia and Adelaide University, North Terrace, Adelaide, South Australia approved all animal studies performed in this study. All studies were performed in accordance with The South Australian Prevention of Cruelty to Animals Act 1985 and the Australian Code of Practice for the Care and use of Animals for Scientific Purposes, 6th Edition 1997.

All animals used in this study were obtained from the Pig and Poultry Production Institute, Roseworthy Campus, Roseworthy, SA 5371, Australia. All pigs were raised from a closed herd and kept under strict quarantine conditions. The pigs were housed in individual pens maintained at 23±1˚C at ambient humidity. Lighting was artificial with a 12-hour on/off cycle. Pigs were fed and watered ad libitum (other than 12-hours pre-operatively and immediately post-operatively). Water was suitable for human consumption. These conditions complied with the Australian Code of Practice for the Care and use of Animals for Scientific Purposes.

2.2 Summary of Methodology

Study phase	Number of pigs	Treatment/ Electrolytic dose	Time of sacrifice	Investigations
Pilot	3			
1a	6	50 coulombs @ 50mA open	3 days	Bloods Cytokines Post-mortem Histology
1a	6	Control open	3 days	Bloods Cytokines Post-mortem Histology
1b	4	50 coulombs @ 50mA open	2 weeks	Bloods Cytokines Post-mortem Histology
1b	4	50 coulombs @ 50mA open	8 weeks	Bloods Cytokines Post-mortem Histology
2	5	25 coulombs @ 25mA endoscopic	2 weeks	Bloods Post-mortem Histology
2	5	Control endoscopic	2 weeks	Bloods Post-mortem Histology
2	5	25 coulombs @ 25mA endoscopic	8 weeks	Bloods Post-mortem Histology

2.3 Pilot Study

Three specific pathogen free (SPF) female domestic white pigs were used in a 'pilot study' to assess the porcine anatomy and the initial feasibility of pancreatic duct cannulation and pancreatic electrolytic ablation via the pancreatic duct. An ex-vivo pancreatogram was obtained by injecting the pancreatic duct with 50% dilute iodixanol 320 contrast (Visipaque, Nycomed, Chatswood, New South Wales, Australia) (Fig. 2.1). This demonstrated that 4-5cm of the proximal pancreatic duct ran immediately adjacent to the duodenum before the duct turned sharply (through about 90°) into the splenic lobe of the pancreas. The initial attempt at insertion of a 6 French electrolysis catheter was limited by this sharp angle in the pancreatic duct. Delivery of an electrolysis dose at this site resulted in direct damage to the duodenum. However, the mobilisation of the fascial attachments between the proximal pancreas and the duodenum, whilst maintaining the blood supply, allowed not only the distance between the pancreas and the duodenum to be maximised but also for the electrolysis catheter to be advanced to a depth of 60-80mm as a result of the reduction in angulation of the pancreatic duct. Delivery of electrolytic dose at this position resulted in no damage to the duodenum.

The introduction of finer calibre (4 and 3.7 French) electrolysis catheters in the subsequent phases (1b + 2) of this study meant that cannulation depth was no longer a limiting issue and the mobilisation of the proximal pancreas was discontinued.

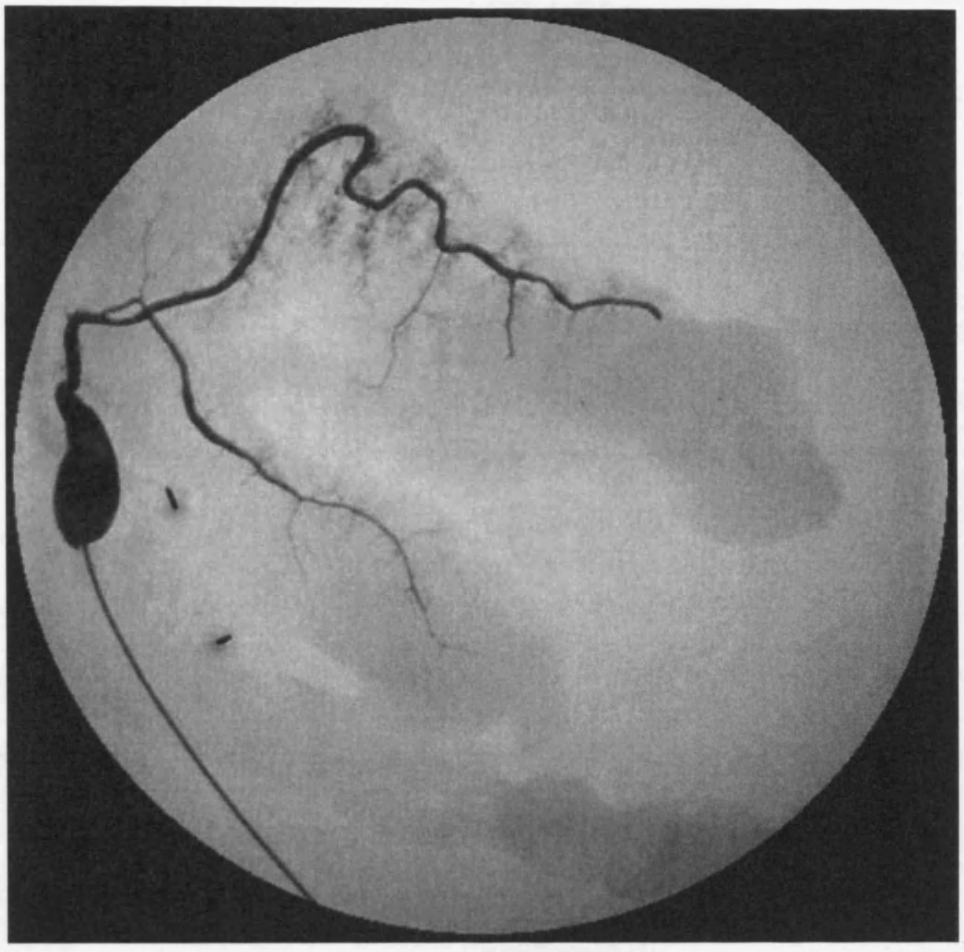

Figure 2.1: Porcine ex-vivo pancreatogram.

2.4 Operative techniques/procedures

2.4.1 Phase 1a: Open approach short-term.

Twelve specific pathogen free (SPF) female domestic white pigs were used for this phase of the study. Mean weight was 31kg (range 28-33.5kg). The study consisted of two treatment groups: 'control' group (n=6) and 'treatment' group (n=6).

All pigs were anaesthetised in the same manner. Sedation was achieved with an intra-muscular injection of Ketamine (20mg/kg) and Xylazine (1.5mg/kg). Each animal was cleaned with a solution of chlorhexidine and taken into the operating theatre. A laryngeal mask airway was inserted and anaesthesia maintained with 1.5% halothane in oxygen. Oxygen saturation and heart rate were monitored continuously throughout the procedure.

2.4.1.1 Intravenous femoral line insertion

A 10cm incision in the right groin crease was made and the right femoral vein was exposed. After the distal femoral vein has been tied off (2/0 silk) a venotomy was created. Following this a fine (1mm diameter lumen) catheter was inserted into the vein and introduced into the inferior vena cava, and then secured in position (2/0 silk). The catheter was routed subcutaneously along the right flank to between the scapulae. The groin wound was closed in layers with continuous 3/0 vicryl. This venous line allowed regular blood sampling as well as intravenous fluid administration.

2.4.1.2 Pancreatic electrolysis

A midline laparotomy incision was made. The pancreas was identified through the lesser sac, which was opened to allow access to the gland. The transverse colon was cleared from the ventral surface of the gland. The bowel was packed away from the pancreas and the duodenum. The fascial attachments of the proximal pancreas to the duodenum were divided, whilst maintain the vascular supply (as described previously). This manoeuvre allowed maximalisation of the distance between the pancreas and the duodenum, in order to avoid damage to the duodenum, from the electrolysis procedure, that was noted in the pilot study.

A 5cm duodenotomy was performed over the site of pancreatic duct insertion, approximately 20cm distal to the pylorus. The pancreatic ampulla was identified and a 6 French (2mm) electrolysis catheter was inserted to a depth of 60-80mm and secured in position (Fig. 2.2: Cordis Webster Inc, 4750 Littlejohn Street, Baldwin Park, CA 91706). Each catheter had four electrodes, which were 4mm long and separated by 3mm. For the electrolytic treatment the second most distal and third most distal electrodes were used. The "unused" electrodes were electrically isolated. The electrolysis catheter was then connected to a direct current (DC) generator (Fig. 2.3) (ECU 100, Söring GmbH, Justus-von-Liebig-Ring 10, D-25451, Quickborn, Germany).

Following this the animals were randomly allocated to either the 'control' (n=6) or 'treatment' (n=6) groups. An electrolysis dose of 50 coulombs, at 50 milliamps (mA), was then delivered to the pancreas in the treatment group animals. The mean duration for electrolysis delivery was 23 minutes (range: 19 to 25 minutes). In the control group

Figure 2.2: Electrolysis electrode.

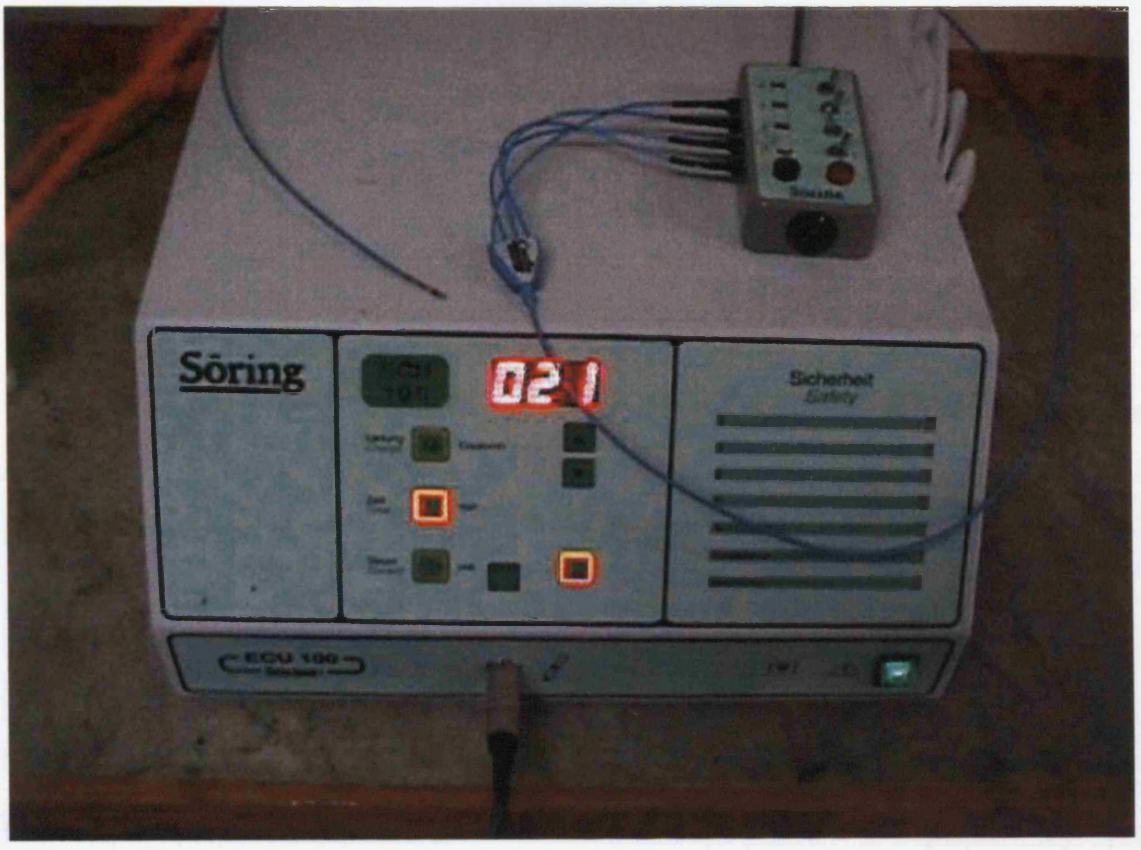

Figure 2.3: Direct current (DC) generator.

the electrolysis catheter was left connected to the generator but no electrolysis dose was delivered, the catheter was left in position for 21 minutes. The duration that the electrolysis catheter was left in position in the control animals was calculated as 110% of the minimal time required to deliver the coulomb dose to the treatment animals. Experience has shown that the actual dose delivery time is approximately 10% longer than the predicted time due to small variations in amperage during the procedure.

At the end of the treatment, the electrode catheter was removed from the pancreatic duct and the duodenotomy was closed with continuous 3/0 PDS. The midline laparotomy was closed in the following manner: the mass closure was used for the deep layers with continuous 1/0 or 0 nylon and the skin was closed with continuous subcuticular 2/0 vicryl. In the perioperative period each animal was given 1 litre of 0.9% saline by intravenous infusion. In addition to this intramuscular injection of Buprenorphine (0.01mg/kg) was given before the animal was recovered. A further litre of 0.9% saline was given on a daily basis to each animal for the first three post-operative days.

Blood samples were taken pre-operatively, immediately post-operatively, 1 hour post-operatively, 4 hours post-operatively and then daily for three post-operative days. These samples were analysed for serum amylase, glucose, C-reactive protein (CRP), calcium, urea & electrolytes. Further samples were centrifuged at 3500 rpm for 5 minutes at 4°C; the serum was then collected and stored at -80°C for subsequent evaluation of cytokine content.

After 72 hours, post-operatively, the animals were killed. This was performed by lethal injection of barbiturate via the femoral line. A post-mortem examination was performed. In particular, the pancreas was examined to determine the extent of the induced necrosis, the patency of the pancreatic duct, the presence of any peri-pancreatic fluid collections and any involvement of adjacent organs. The pancreata were excised along with a section of duodenum. In addition to this sections of both lungs and kidneys were removed. All the specimens were preserved in 10% formalin.

Each pancreas was examined histologically to determine: (1) the extent of the electrolytic necrosis, and (2) any associated pancreatitis in the surrounding parenchyma. Samples of lung and kidney were examined histologically for signs of a systemic inflammatory response.

Results were analysed statistically using the Student T-test procedure (SPSS Version 11.0).

2.4.2 Phase 1b: Open approach medium and long-term.

11 specific pathogen free (SPF) female domestic white pigs were used for this phase of the study. Mean weight was 31.5 kg (range 28-35kg). The study consisted of two treatment groups: two-week survivors (n=4) and eight-week survivors (n=4). (There were three premature deaths in this phase of the study, none of which were directly due to pancreatic electrolysis, which will be discussed at greater length in the phase 1b results section.)

All pigs were anaesthetised in the same manner. Sedation was achieved with an intra-muscular injection of Ketamine (20mg/kg) and Xylazine (1.5mg/kg). Each animal was cleaned with a solution of chlorhexidine and taken into the operating theatre. A laryngeal mask airway was inserted and anaesthesia maintained with 1.5% halothane in oxygen. Oxygen saturation and heart rate were monitored continuously throughout the procedure.

2.4.2.1 Intravenous external jugular line insertion

In this phase of the study venous access was switched from the right femoral vein catheter approach that was used in phase 1a to a left external jugular vein line. Following discussion with veterinary staff from the University of Adelaide, Institute of Veterinary and Medical Services, it was decided that the jugular line was technically simpler and quicker than the femoral line and as a result was adopted in this phase of the study.

A 7-8cm incision was made over the lateral aspect of the left sternocleidomastoid (SCM), and extended through the platysma. The left external jugular vein was identified lateral to SCM and cleared of surrounding adventitia. The vein was ligated distally with 2/0 silk, and following a venotomy a catheter was introduced in to the left external jugular vein, advanced into the superior vena cava and then sutured in place (2/0 silk). The line was then tunnelled subcutaneously to between the scapulae, to allow access for post-operative blood sampling and fluid administration.
Platysma was closed with continuous 3/0 vicryl and the skin with subcuticular 3/0 vicryl.

2.4.2.2 Pancreatic electrolysis

A midline laparotomy was performed and the pancreas identified. Any bowel loops attached to the pancreas were dissected free, to mobilize the gland. A 5cm duodenotomy was performed 20cm distal to the pylorus. The opening of the pancreatic duct was identified and cannulated with a 4 French (1.33mm) electrode catheter to the depth of 120mm. Each catheter had six electrodes, which were 2mm long and separated by 3mm. For the electrolytic treatment the most distal and third most distal electrodes were used. The "unused" electrodes were electrically isolated. The use of a narrower diameter electrolysis catheter in this phase (cf. 6 French (2mm) catheter in phase 1a) facilitated catheterization of the pancreatic duct and also allowed a greater depth of catheter insertion (as determined by markings on the catheter). Therefore the proximal pancreas was not mobilized from the duodenum. At this point the animals were randomly allocated to either the '2-week' or the '8-week' survival groups.

Each animal had an electrolytic "dose" of 50 coulombs (C), delivered to the pancreas at 50 milliamps (mA) using the direct current generator. Mean treatment time was 21 minutes (range: 18 to 22 minutes.)

At the end of the treatment, the electrode catheter was removed from the pancreatic duct and the duodenotomy was closed as previously described. The midline laparotomy was also closed in a standard fashion. An intramuscular injection of Buprenorphine (0.01mg/kg) was given before the animal was recovered. During the peri-operative period each animal was given one litre of 0.9% saline by intravenous infusion.

Post-operatively each animal was given one litre of 0.9% saline daily for the first three days. Blood samples were taken from the jugular catheter immediately pre- and post-operatively, daily for seven days with further samples at 10 days and 2 weeks post-operatively. These samples were analysed for serum amylase, glucose, C-reactive protein (CRP), calcium, urea & electrolytes. Further samples were centrifuged at 3500 rpm for 5 minutes at 4°C; the serum was then collected and stored at -80°C for subsequent evaluation of cytokine content. In the eight-week survival group the jugular catheters were removed at 2 weeks to prevent line-related sepsis.

After the allocated survival period the animals were killed. This was either done by lethal injection of barbiturate via the jugular line, if it was still in place; however if the line was absent, the injection was given via an intravenous cannula inserted into an ear vein, following sedation of the animal with a deep intramuscular injection of ketamine. A post-mortem examination was performed. In particular, the pancreas was examined to determine the extent of the induced necrosis, the patency of the pancreatic duct, the presence of any peri-pancreatic fluid collections and any involvement of adjacent organs. The pancreata were excised along with a section of duodenum and portal vein. In addition to this sections of both lungs and kidneys were removed. All the specimens were preserved in 10% formalin.

Each pancreas was examined histologically to determine: (1) the extent of the electrolytic necrosis, and (2) any associated pancreatitis in the surrounding parenchyma. Samples of lung and kidney were examined histologically for signs of a systemic inflammatory response.

Results were analysed statistically using the Student t-test procedure (SPSS Version 11.0).

2.4.3 Phase 2: Endoscopic approach medium and long-term.

16 specific pathogen free (SPF) female domestic white pigs were used for this phase of the study. Mean weight was 31.2 kg (range 29-32kg). The study consisted of three groups: 'control' group (n=5), 'two-week survivor' treatment group (n=5) and 'eight-week survivor' treatment group (n=5). (There was one premature death, not directly related to electrolysis, in this phase of the study that will be discussed at greater length in the results section.)

All pigs were anaesthetised in the same manner. Sedation was achieved with an intra-muscular injection of Ketamine (20mg/kg) and Xylazine (1.5mg/kg). Each animal was cleaned with a solution of chlorhexidine and taken into the operating theatre. A laryngeal mask airway was inserted and anaesthesia maintained with 1.5% halothane in oxygen. Oxygen saturation and heart rate were monitored continuously throughout the procedure.

2.4.3.1 Oesophagostomy technique

As will be discussed in greater length in the results section for Phase 1b, the three premature animal deaths were due to a combination of acute gastric dilatation and catastrophic gastrointestinal haemorrhage. Post-mortem examination of these animals revealed grossly distended stomachs in all three cases. In the case of the animal that died from upper GI haemorrhage a circumferential mucosal defect, exposing the underlying muscle, was also noted at the pars oesophagea. This phenomenon has been

widely reported in the literature, both in surgical (276-278) and veterinary (279, 280) journals. Previous investigators have shown that the incidence of these 'ulcers' is not affected by administration of either H2 antagonists or proton pump inhibitors(281). However, decompression of the stomach either by gastrostomy or gastrojejunostomy has been shown to reduce the incidence of gastric dilatation and ulceration at the pars oesophagea (282). Therefore a technique was required to allow decompression of the stomach in the early post-operative period, whilst keeping intra-abdominal intervention to a minimum in this the 'minimally invasive' phase of the project. In order to address both these requirements gastric decompression was achieved via an oesophagostomy tube inserted into the left side of the neck.

Following an incision over the medial border of the left SCM the oesophagus was mobilized from behind the trachea. A 14 French nasogastric tube was inserted into the oesophagus, as an oesophagostomy, and secured in position with a 3/0 Dexon purse string. The platysma was closed with continuous 3/0 vicryl and the skin with subcuticular 3/0 vicryl.

It was decided that insertion of an external jugular line, as well as an oesophagostomy tube, would be inappropriate. Therefore, venous access was achieved via a right femoral vein line, as was described in phase 1a.

2.4.3.2 Endoscopic pancreatic electrolysis

The anatomy of the porcine upper gastrointestinal tract, although similar to human anatomy, differs in a number of important ways. The insertion of the main pancreatic duct in humans is virtually perpendicular to the second part of the duodenum. This

angle allows cannulation of the ampulla via an endoscopic approach, as is widely practiced as endoscopic retrograde cholangio-pancreatography (ERCP). However in the pig, the pancreatic duct runs adjacent to the duodenum for approximately 4cm before opening into the lumen of the gut (FIG 2.1). This acute angle would make endoscopic cannulation of the ampulla of Vater, via a per-oral approach, extremely difficult even with a side viewing endoscope. To overcome this problem a 'retrograde' endoscopic approach was undertaken in this phase.

The aim of this phase of the study was not to show that endoscopic cannulation of the pancreatic duct was feasible, as this is well established with ERCP being a widely practiced procedure around the world; but rather to demonstrate whether the endoscopic delivery of an electrolytic dose was feasible and safe. Therefore, it was felt that this 'modification' of the normal endoscopic approach did not affect the validity of the data produced by this phase of the study.

A 10cm incision midline was made just superior to the umbilicus. A loop of distal duodenum/proximal jejunum was then located and delivered through the wound. This technique required neither division of the peritoneum nor mobilization of the bowel. A 15mm enterotomy was then formed, into which an end-viewing endoscope was inserted in a cephalic direction (EVIS Gastrointestinal Videoscope GIF 130, Olympus, Adelaide, Australia). The endoscope was connected to a standard endoscopic stack (Olympus CLV-U20 (light source), Olympus CV-100 (video processor)). In this phase a 3.7 French electrolysis catheter, that was 150cm in length, was used (Fig. 2.4: Revelation Tx Microcatheter, Ref No. 01-082012. Cardima, Fremont, California,

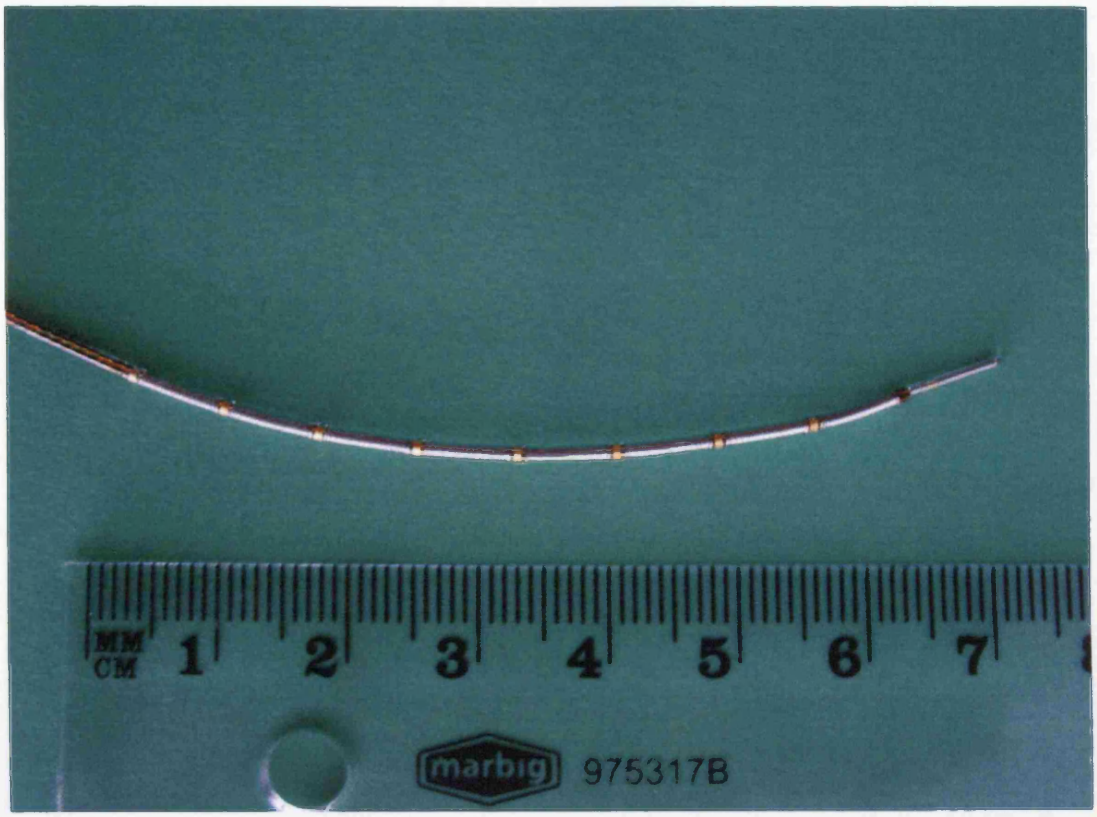

Figure 2.4: Endoscopic electrolysis electrode.

USA). This catheter had six electrodes, each 6mm in length and separated by 2mm. The catheter was inserted via the instrumentation channel of the endoscope.

Following visualisation of the pancreatic ampulla the fine electrolysis catheter was inserted to a depth of 10-12 cm, as assessed by markings on the catheter itself (VIDEO 2.1). At this point the animal was randomised to one of the three groups: 'controls', 'treatment: 2-week survivors' or 'treatment: 8-week survivors'. In order to minimise the effect of any 'learning curve' with regards to the technical aspects of endoscopic pancreatic duct cannulation, randomisation was performed in the following manner; five groups of three pigs were formed. In each of these five groups one animal was randomly allocated to each of the three experimental groups (i.e. 'controls', 'treatment: 2-week survivors' or 'treatment: 8-week survivors').

Following randomisation an electrolytic dose of 25 coulombs, at 25 mA, was delivered to the pancreata of the animals in the treatment groups, using the direct current generator. The most distal and second-most distal electrodes were used; all the other electrodes were electronically isolated. Mean treatment time was 10 minutes (range: 9 to 11 minutes). In the control group the electrolysis catheter was left in place for 10 minutes but no electrolysis dose was administered; again at 110% of the predicted delivery time.

Once the electrolysis dose had been delivered the catheter was removed from the pancreatic duct and the endoscope was withdrawn. The enterotomy was closed as previously described. The midline laparotomy was again closed in the previously

Video 2.1: Endoscopic cannulation of pancreatic duct.

described manner. An intramuscular injection of Buprenorphine (0.01mg/kg) was given before the animal was recovered. During the peri-operative period each animal was given one litre of 0.9% saline by intravenous infusion.

Post-operatively each animal was given one litre of 0.9% saline daily for the first two days. Blood samples were taken from the femoral venous catheter immediately pre- and post-operatively, daily for seven days with further samples at 10 days and 2 weeks post-operatively. These samples were analysed for serum amylase, glucose, C-reactive protein (CRP), calcium, urea & electrolytes. Further samples were centrifuged at 3500 rpm for 5 minutes at 4°C; the serum was then collected and stored at -80°C for subsequent evaluation of cytokine content. In the eight-week survival group the femoral lines were removed at 2 weeks to prevent line-related sepsis. The oesophagostomy tubes were also removed, at 2-weeks, if still present.

After the allocated survival period the animals were killed. This was either done by lethal injection of barbiturate via the femoral line, if it was still in place; however if the line was absent, the injection was given via an intravenous cannula inserted into an ear vein, following sedation with a deep intramuscular injection of ketamine. A post-mortem examination was performed and the pancreas was examined as previously described. The pancreata were excised, again with a section of duodenum and portal vein. In addition to this sections of both lungs and kidneys were removed. All the specimens were preserved in 10% formalin.

In eight of the fifteen resected pancreata (3 'controls', 2 'treatment: 2-week survivors' and 3 'treatment: 8-week survivors') a pancreatogram was performed by the injection

of 50% dilute iodixanol 320 contrast (Visipaque, Nycomed, Chatswood, New South Wales, Australia) into the pancreatic duct. These studies were then assessed for evidence of duct stenosis, dilatation and patency.

Data from this phase of the study was statistically analysed with analysis of variance (ANOVA) (SPSS Version 11.0).

2.5 Enzyme linked immuno-sorbent assays: serum cytokine concentrations

Enzyme linked immuno-sorbent assays (ELISAs) are well established, semi-quantative, techniques used for the determination of unknown concentrations of a given substance. In these studies the substances of interest were tumour necrosis factor alpha (TNFα) and interleukin 1 beta (IL-1β). Both these cytokines have pivotal roles in the cytokine cascade of the inflammatory response, over-activation of which leads to a SIRS reaction.

Both TNFα and IL-1β concentrations were assessed using commercially available porcine specific solid phase sandwich ELISA kits (Biosource International, Camarillo, USA). Each ELISA plate consisted of 96 wells, onto which monoclonal antibodies for either TNFα or IL-1β had been coated. The assay method will now been described in detail for IL-1β, variations in method specific for TNFα will be addressed later.

2.5.1 Interleukin 1 beta

All serum samples and solutions required for performance of the ELISA were at room temperature prior to commencing the following protocol. All the water used in this experiment for the reconstitution of solutions was distilled using a MilliQ water

sterilizer. All standards and samples were run in duplicate to minimise error. A standard curve was run on each plate and used to quantify samples from just that plate.

1. The standard 2500pg/ml swine IL-1β solution was reconstituted. From this samples of known concentrations of swine IL-1β, as listed below, were prepared for the generation of the standard curve:
    i. 2500pg/ml
    ii. 1250pg/ml
    iii. 625pg/ml
    iv. 312pg/ml
    v. 156pg/ml
    vi. 78.1pg/ml
    vii. 39.0pg/ml
    viii. 0pg/ml
2. 100 μL of standard buffer solution was added to 'zero' wells of the standard curve. For the standard curve 100 μL of the standards was added to the appropriate wells. For all samples 50 μL of standard buffer solution was added to each well followed by 50 μL of sample. The well reserved for the chromogen blank was left empty. The plate was tapped gently to mix the solutions.
3. The plate was then covered and incubated at 37°C for 2 hours.
4. Following incubation the plate was decanted and washed, with provided wash buffer, 4 times.

5. 100 μL of biotinylated anti-IL-1β (biotin conjugate) was added to each well, other than the chromogen blank. Again the plate was tapped gently to mix.
6. The plate was then covered and incubated at room temperature for 1 hour.
7. Following incubation the plate was decanted and washed, with provided wash buffer, 4 times.
8. 100 μL of streptavidin-HRP working solution was added to each well (other than the chromogen blank).
9. The plate was then covered and incubated at room temperature for 30 minutes.
10. Following incubation the plate was decanted and washed, with provided wash buffer, 4 times.
11. 100 μL of stabilized chromogen was added to each well, including the chromogen blank well (wells begin to turn blue).
12. The plate was then covered and incubated, in the dark, at room temperature for 20 minutes.
13. Following incubation 100 μL of stop solution was added to each well. The solution in the wells changes from blue to yellow.
14. The plates were read on an ELISA plate reader having blanked the reader against a 'chromogen blank' containing 100 μL of each stabilized chromogen and stop solution. The plates were read at 450nm and 30-45 minutes after the addition of the stop solution.
15. Means of the optical density of the standards were plotted against the known standard concentrations. The line of best-fit was then constructed

from these points and the equation of the graph and the correlation co-efficient (R^2) were determined (Excel 2000, Microsoft Corporation, USA).

16. The optical density of the samples were converted in to concentrations (pg/ml) and doubled to account for the dilution factor from step 2. Means of the duplicate assays were obtained and these data were used in the subsequent analysis.

2.5.2Tumour necrosis factor alpha

The ELISA assessment of TNFα concentrations was performed in essentially an identical manner to that described above for IL-1β, with the exception of the following points.

1. The standard curve for TNFα was derived from standard concentrations between 0pg/ml and 1000pg/ml.
2. In step 3 the TNFα ELISA plate was incubated at room temperature, rather than at 37°C.

2.6 Histological assessment of specimens

2.6.1 Specimen preparation

All specimens (pancreata, kidney and lung) from all phases (1a, 1b and 2) were preserved in ample quantities of buffered 10% formalin for duration of 3 weeks in all cases.

Sections of pancreas, including the ablation lesion and proximal and distal unablated pancreas, as well as sections of lung and kidney were taken and mounted in paraffin

blocks. Histological slides were prepared from these sections and stained with haematoxylin and eosin using a standard protocol.

2.6.2 Histological scoring system for experimental pancreatitis

All histological samples were examined by a pathologist, who was unaware to the experimental group from which the samples came. In the case of pancreatic specimens the pathologist was also blinded as to whether the sample was from the ablation lesion or from unablated proximal or distal pancreas.

A histological scoring system was used to assess all the pancreatic specimens. This system was devised by Spormann et al (283) in 1989 as an objective measure of the histological changes seen in experimental models of acute pancreatitis. The scoring system has five sections, which are scored individually, with the sum of these scores giving the final histological score, within the range of 11-27, for that pathological section (Table 2.1). Two histological preparations from each specimen were assessed and scored, the final 'score' for that specimen being the mean of these two figures.

Table 2.1: Scoring system for experimental pancreatitis

Oedema

1 = Mild
2 = Moderate
3 = Severe

Inflammatory Infiltrate

1 = Mild
2 = Moderate
3 = Severe

Fat Necrosis

3 = Mild
5 = Moderate
7 = Severe

Parenchymal Necrosis

3 = Singular
5 = Sub-Lobular (<1/3)
7 = Lobular (>1/3)

Haemorrhage

3 = Mild
5 = Moderate
7 = Severe

Lung and kidney specimens were also assessed for each experimental animal. A scoring system was not used in assessing these histological preparations. Histological examination of these specimens was primarily targeted at the gross changes associated with SIRS/ARDS. However, in the absence of these findings subtler, less specific, indications of inflammation such as fluid sequestration and neutrophil infiltration were described.

The mean histological scores for the pancreatic sections, from the various experimental groups, were compared and the data was statistically analysed using the Student T-Test and ANOVA (SPSS Version 11.0).

Chapter 3:

Results

Chapter 3: Results

3.1 Phase 1a

3.1.1 General

All the animals tolerated the laparotomy and electrolytic ablation well. There was one per-operative death due to an anaesthetic complication. This animal had been randomised to the control group. A post-mortem examination did not reveal the cause of death and the pig was subsequently replaced. At the time of euthanasia the mean weight in the control group was 30.5kg (28-31.5kg) and in the treatment group was 31kg (29.5-33kg); statistical analysis of this data showed no significant difference in pre- and post-operative weights either in or between the two experimental groups ($p>0.05$).

In both the control and treatment groups 4 of the 6 pigs were 'clinically' back to normal 48 hours after surgery. These animals were all active and mobile; in addition to this appetite, thirst, urine production and defecation were all normal by the second post-operative day. The remaining 4 animals, however, all experienced a more protracted recovery, although by the time of sacrifice on the third post-operative day, these 4 pigs were eating and drinking normally. Comparison of the biochemical parameters of the 4 animals with moderately delayed recovery with biochemical data from the 'normal' pigs failed to show any significant difference, either within the randomised groups or when comparing the data as a whole.

3.1.2 Macroscopic appearance of the pancreata

3.1.2.1 Control Group

At post-mortem examination all pancreata in the control group had an essentially normal macroscopic appearance, one of the glands was slightly oedematous in nature. However, there was no evidence of pancreatic erythema in any of the animals. In addition to this there were no peri-pancreatic collections, pancreatic fistulae or damage to adjacent viscera, in particular the duodenum and portal vein. The duodenotomy was healing well and the pancreatic ducts all appeared to be patent at their insertion in to the duodenum.

3.1.2.2 Treatment Group

The electrolytic lesion was clearly visible within the splenic lobe of all the pancreata at post-mortem examination. The lesion was spherical in nature with a mean diameter of 14.5mm (12.0 to 18.5 mm). The ablation lesion was well demarcated from the surrounding unablated pancreatic tissue (Fig. 3.1.1). In 4 of the 6 glands the electrolytic ablation lesion had breached the pancreatic capsule and was associated with small (10-45mls) fluid collections. However, there was no evidence of pancreatic fistulae or damage to adjacent organs, again particular note was taken of the duodenum and portal vein. The splenic lobe of one of the glands was mildly erythematous, but all the other pancreata were normal in appearance aside from the ablation lesion. Again the duodenotomy was healing well and the pancreatic ducts all appeared to be patent at the duodenal insertion.

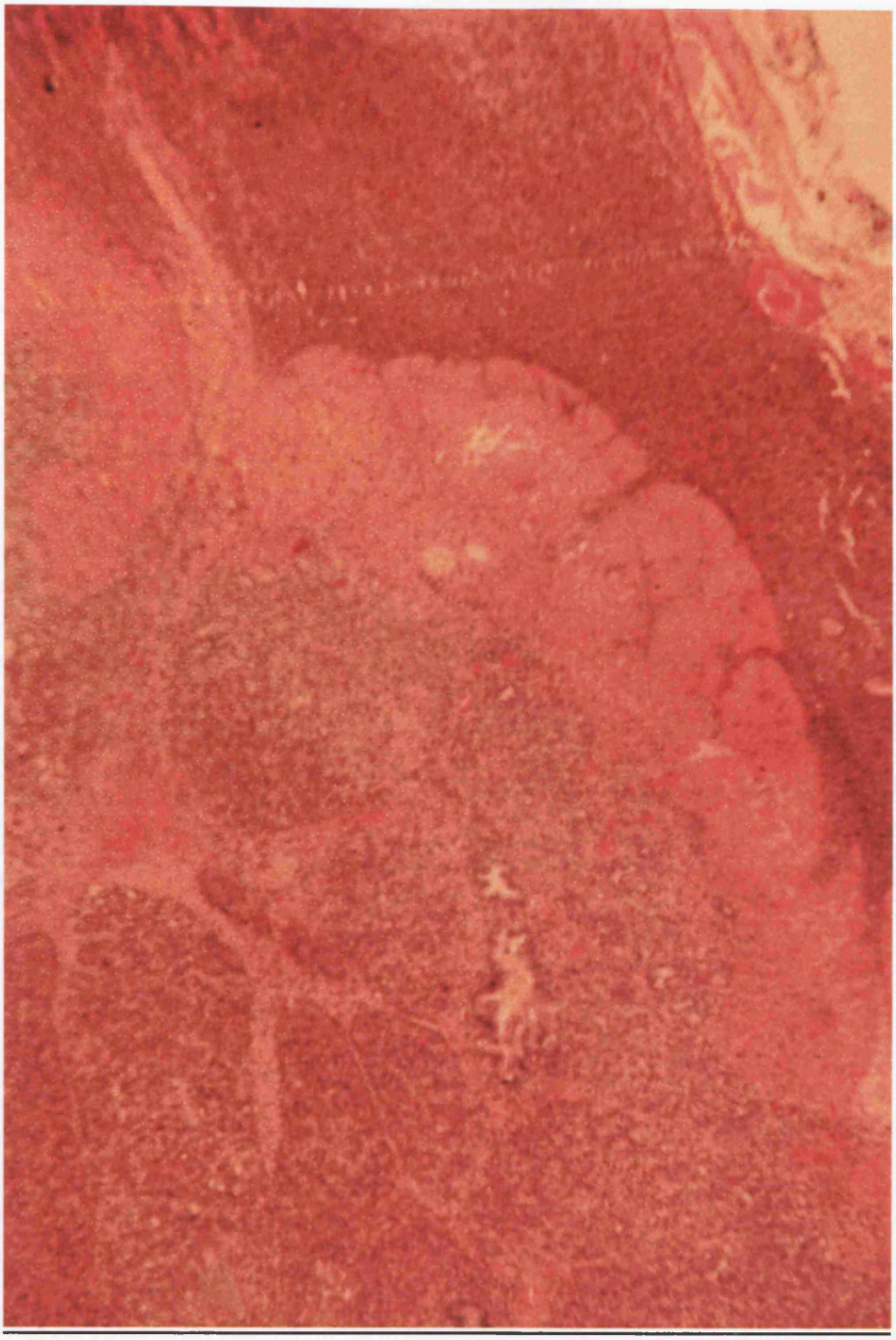

Figure 3.1.1: Electrolytic ablation lesion, day 3 post-operation.

3.1.3 Histological appearance

Sections of the pancreas were preserved in formalin, paraffin mounted and stained with haematoxylin and eosin. These sections were graded by a 'blinded' pathologist using an established scoring system for experimental pancreatitis, as described in the experimental methods.

3.1.3.1 Control Group

The mean histological score at the approximated site of the electrolysis electrodes was 15.1 ± 1.4; the mean score of the proximal unablated pancreas was 13.7 ± 2.3 (Fig. 3.1.2). In all the control pancreata there was no evidence of parenchymal necrosis, either at the 'ablation' site or in the unablated tissue (Figs. 3.1.3 & 3.1.4). Although in one animal there was minimal peri-ductal inflammation (Fig. 3.1.5) this was not widespread and did not extent more than 1mm radius from the duct. In all sections of pancreas examined the pancreatic duct remained patent.

3.1.3.2 Treatment Group

At the site of the electrolytic ablation lesion the mean histological score was 22.8 ± 3.1 and the mean score for the unablated proximal pancreas was 14.3 ± 2.8 (Fig. 3.1.2). The histological appearance of the ablation lesions was similar (Fig. 3.1.6). There was a central area of coagulative necrosis, in which there was no residual viable tissue. Surrounding this central area of necrosis there was a 'transition zone'; which was characterised by an inflammatory infiltrate of neutrophils and lymphocytes. This zone was less than 1mm in thickness; outside this zone the pancreatic parenchyma was

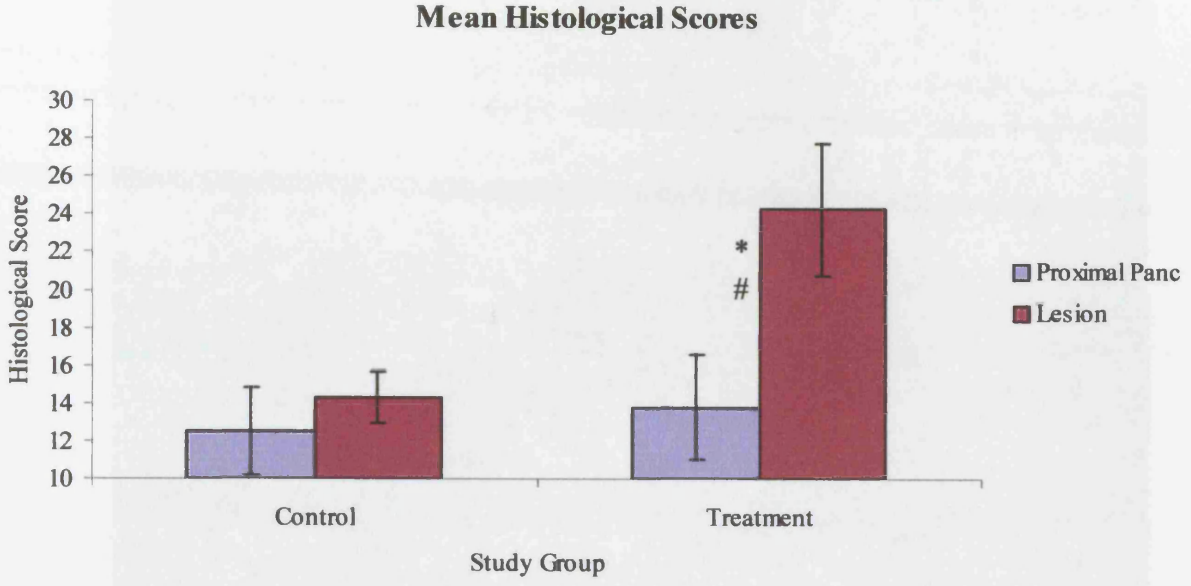

Figure 3.1.2: Mean histological scores, with standard deviations, for each experimental group. (* denotes significant [$p<0.05$] difference between scores within a group, # denotes significant [$p<0.05$] difference between scores for a region of pancreas between groups.)

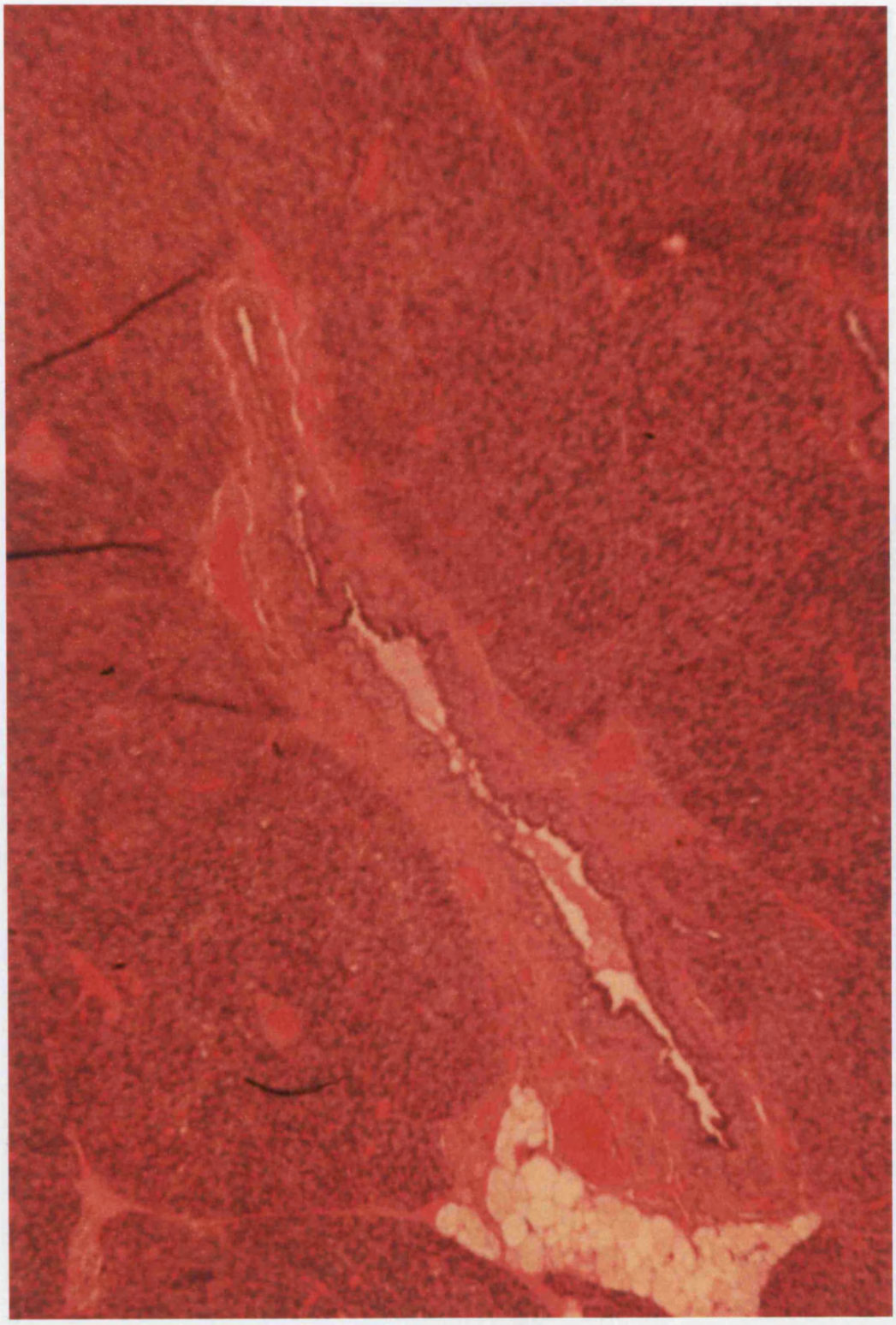

Figure 3.1.3: Control group: proximal pancreas at day 3. (H&E, x4)

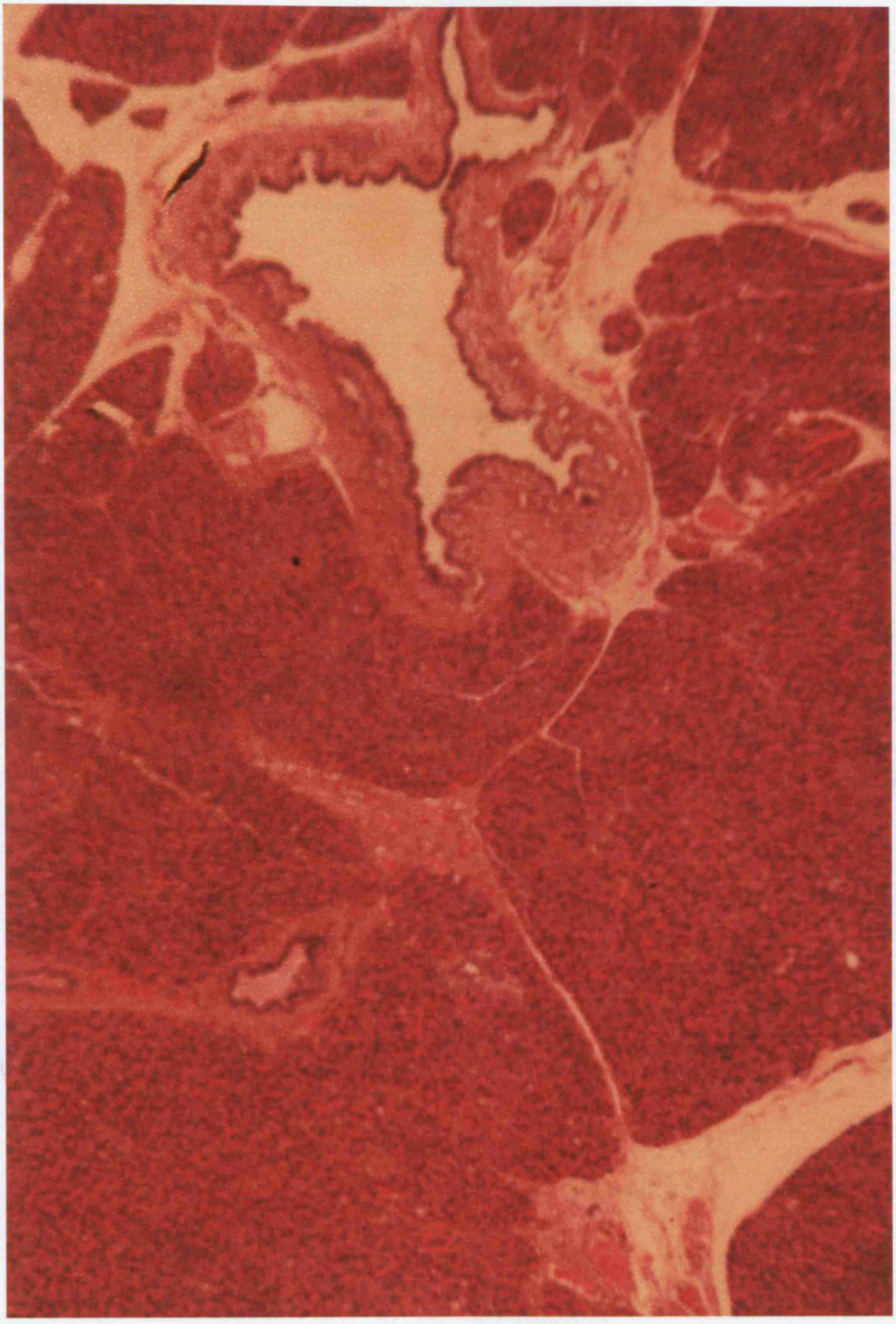

Figure 3.1.4: Control group: 'ablation site' at day 3. (H&E, x4)

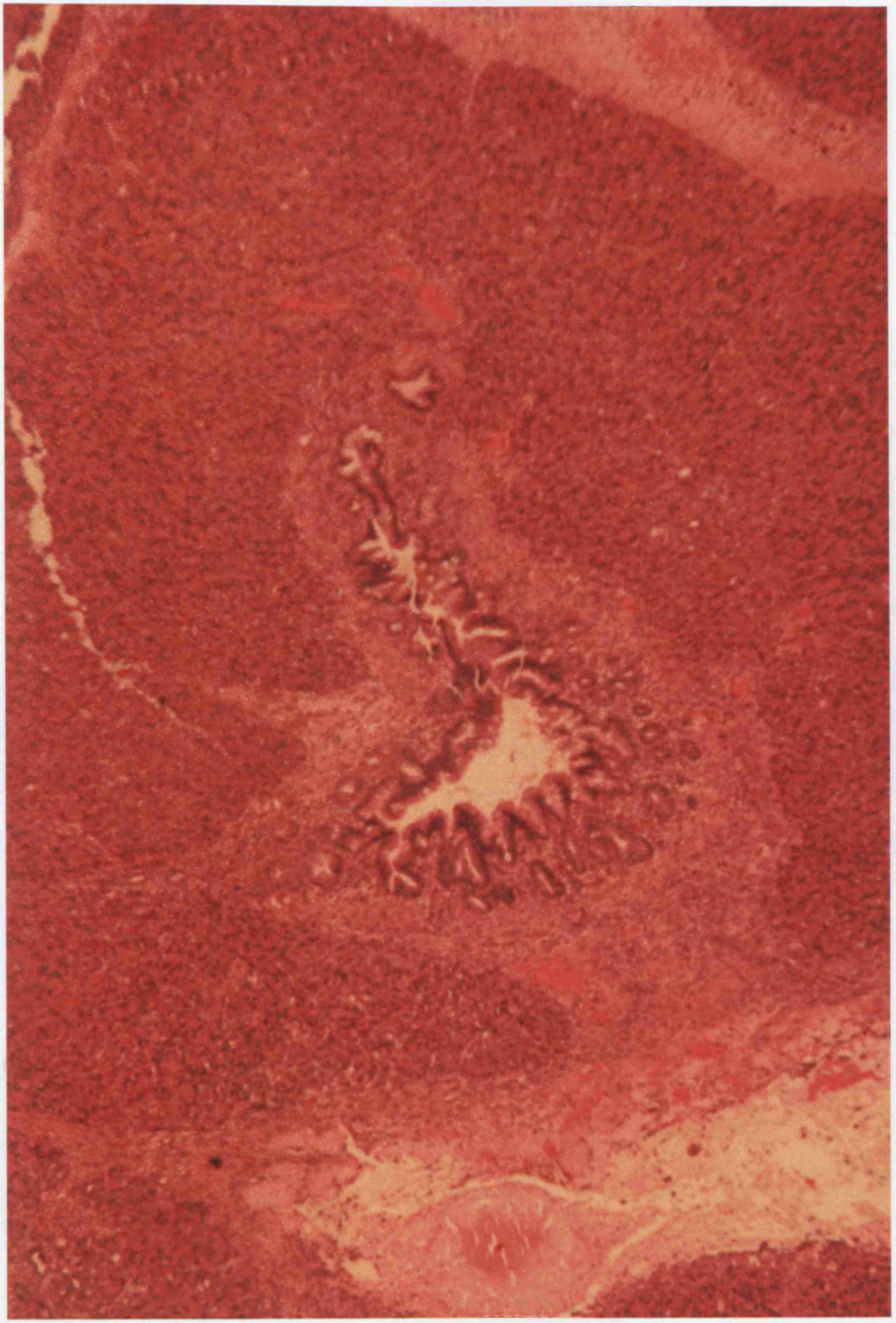

Figure 3.1.5: Control group: peri-ductal inflammation at day 3. (H&E, x4)

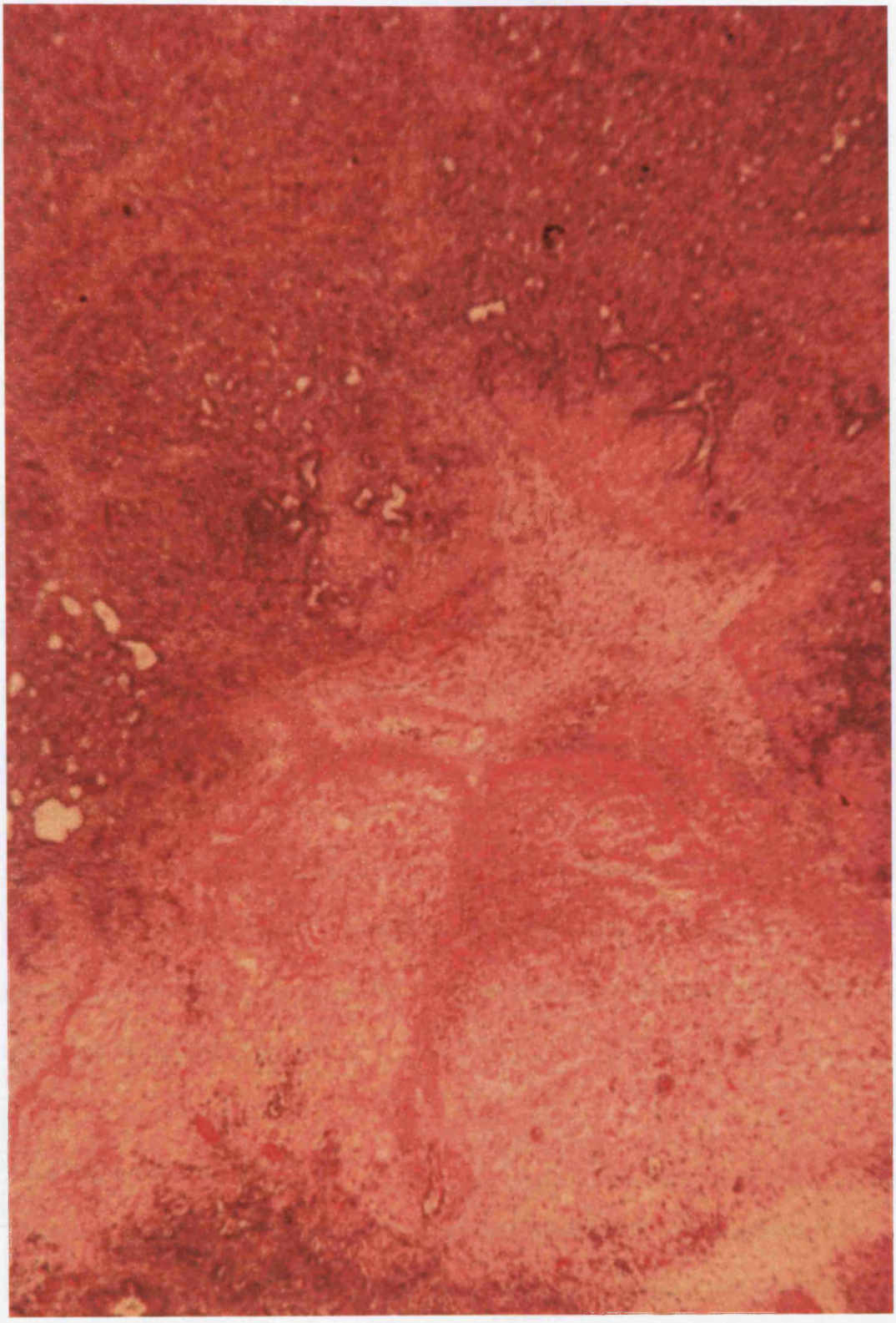

Figure 3.1.6: Treatment group: ablation lesion at day 3. (H&E, x4)

essentially normal. However, mild interlobar inflammation was noted to radiate, for a variable distance, from the electrolytic lesion in to the surrounding pancreatic tissue. There were no abscesses, either within the pancreatic parenchyma or adjacent to the electrolysis lesions. The sections of unablated pancreas showed little evidence of generalised inflammation or acute pancreatitis. However, some interlobar inflammation was evident (Fig. 3.1.7). Again the pancreatic duct remained patent in both sections from the ablated and unablated pancreas.

The data from the histological scoring of the pancreatic sections were statistically analysed. This demonstrated a significant difference between the score for the ablation lesion in the treatment group and the score for the unablated pancreas from the same group ($p<0.05$). The histological score for the electrolytic lesion, in the treatment group, was also significantly greater than the scores obtained, for both the 'ablation site' and the proximal unablated pancreas, in the control group ($p<0.05$). However, no significant differences were detected when the histological scores for the 'ablation site' and the proximal pancreas in the control group were compared or when the score for the proximal unablated pancreas in the treatment group was compared with either of the scores in the control group.

3.1.4 Biochemistry

The biochemical data from all the animals in the treatment and control groups were reviewed, with the exception of the animal that died per-operatively and was replaced. The data for the various biochemical parameters was statistically analysed, both comparing the treatment and control groups and comparing post-operative data with pre-operative level within each experimental group.

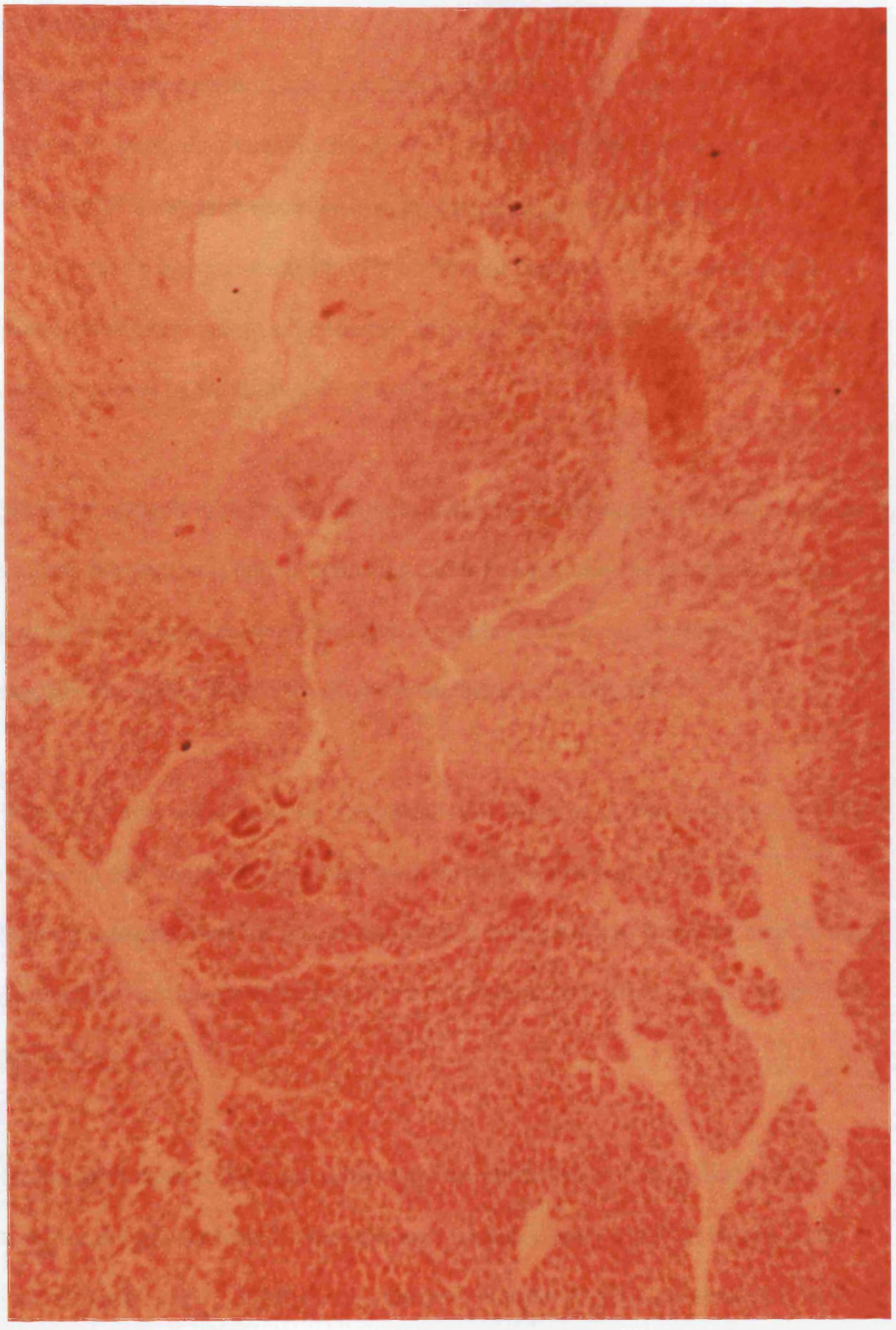

Figure 3.1.7: Treatment group: proximal pancreas at day 3. (H&E, x4)

3.1.4.1 Amylase

Transient hyperamylasaemia was evident in all animals, both treatments and controls, in the early postoperative period (Fig. 3.1.8). The mean serum amylase concentration peaked at 24 hours post-electrolysis in the treatment group, 6837 units/L ± 3241 units/L, and 48 hours post-operatively in the control group, 14195 units/L ± 8215 units/L. However, in both experimental groups mean serum amylase was returning towards normal by the third post-operative day.

Statistical analysis of the serum amylase data from the treatment and control groups showed that there was only a significant difference between the groups at 48-hours post surgery, where the serum amylase concentration in the control group was significantly greater than it was in the treatment group ($p<0.05$).

When the mean serum amylase concentrations within the experimental groups were compared and statistically analysed the following results were obtained:
In the treatment group, the only mean amylase concentrations that were significantly greater than the pre-operative level were at 24 hours ($p<0.05$) and 48 hours ($p<0.01$) post-electrolysis.

In the control group, the mean serum amylase concentrations were found to be significantly greater than the pre-operative level at 4 hours ($p<0.05$) and 24 hours ($p<0.05$) post surgery. Interestingly, the peak in mean amylase concentration at 48 hours post procedure was found to be not significantly different ($p=0.504$) from the pre-operative mean amylase concentration.

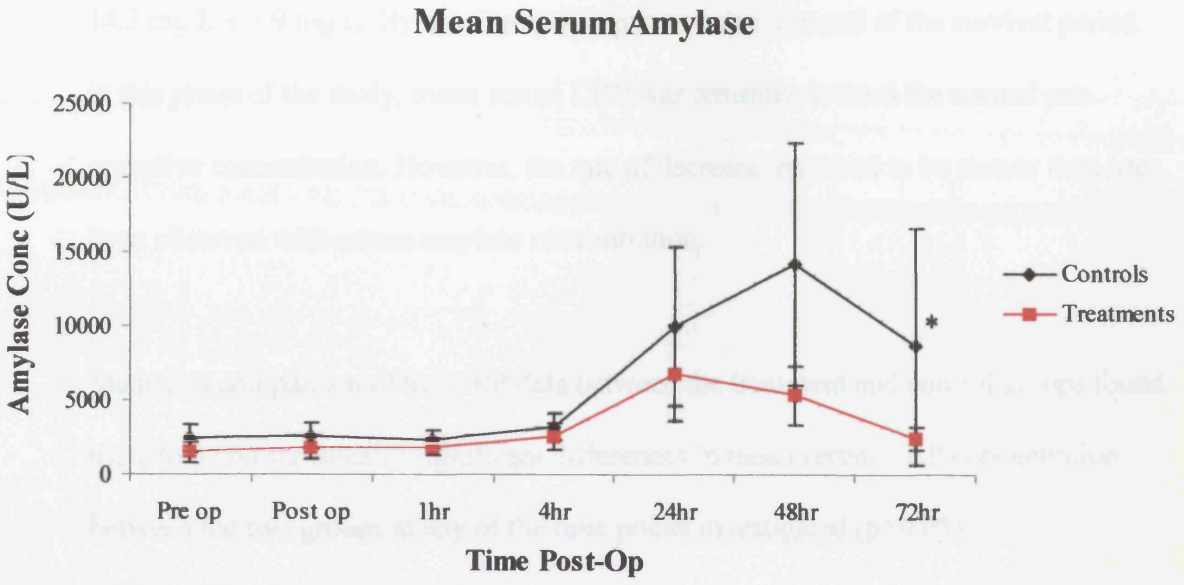

Figure 3.1.8: Mean post-operative Amylase concentration for each experimental group.
(* denotes significant [$p<0.05$] difference from control group.)

3.1.4.2 C-Reactive Protein (CRP)

The CRP was elevated in all the treatment and control animals after surgery (Fig. 3.1.9). The mean serum CRP reached a peak concentration in the treatment group 48 hours after electrolysis, 18.9 mg/L ± 3.9 mg/L, and after 24 hours in the control group, 14.5 mg/L ± 3.9 mg/L. By the third post-operative day, the end of the survival period in this phase of the study, mean serum CRP was returning toward the normal pre-operative concentration. However, the rate of decrease appeared to be slower than had been observed with serum amylase concentration.

Statistical comparison of the CRP data between the treatment and control groups found there to be no statistically significant differences in mean serum CRP concentration between the two groups at any of the time points investigated ($p>0.05$).

When the mean CRP concentrations were analysed within each experimental group the following results were obtained:

In the treatment group the serum CRP levels rose to significantly greater concentrations, when compared with the pre-operative levels, 24 hours post-electrolysis ($p<0.05$). By the end of the experimental period the mean CRP continued to be significantly greater than the pre-operative level ($p<0.05$).

In the control group mean CRP concentration was significantly increased, compared to pre-operative levels, by 4 hours post surgery ($p<0.05$). The CRP concentration remained at a significantly increased level until the end of the study period ($p<0.05$, 72 hours post-procedure.)

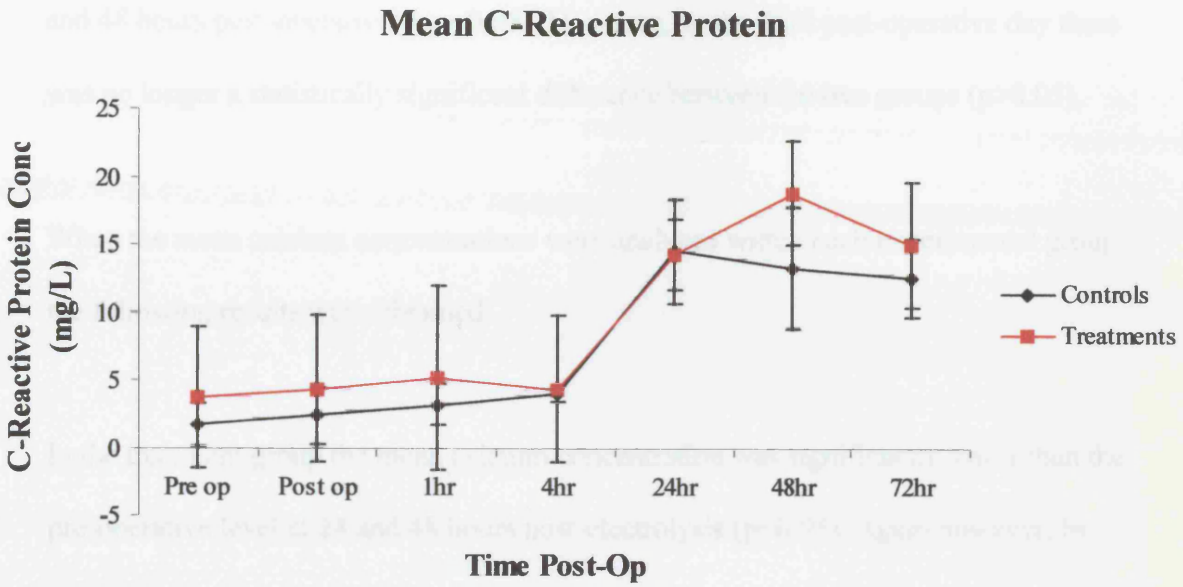

Figure 3.1.9: Mean post-operative C-Reactive Protein concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

3.1.4.3 Calcium

Mean serum calcium concentrations were statistically compared between treatment and control groups over the course of the study period (Fig. 3.1.10). This demonstrated that serum calcium concentration was significantly lower in the treatment group at 24 and 48 hours post-operatively ($p<0.05$). However, by the third post-operative day there was no longer a statistically significant difference between the two groups ($p>0.05$).

When the mean calcium concentrations were analysed within each experimental group the following results were obtained:

In the treatment group the mean calcium concentration was significantly lower than the pre-operative level at 24 and 48 hours post-electrolysis ($p<0.05$). Again however, by the third post-operative day this difference no longer existed ($p>0.05$).

In the control group, post-operative calcium concentrations were significantly lower than pre-operation levels at 4, 24 and 72 hours post-procedure ($p<0.05$).

However, throughout the course of the study period mean serum calcium never dropped below 2.33 mmol/L, which is within the normal range 2.10-2.55 mmol/L.

3.1.4.4 Glucose

There were no statistically significant differences in the mean glucose concentrations, between the two study groups, in the post-operative period ($p>0.05$, Fig. 3.1.11). However, when glucose concentration was analysed intra-group, it was found that in the treatment group the serum glucose level was significantly greater than the pre-

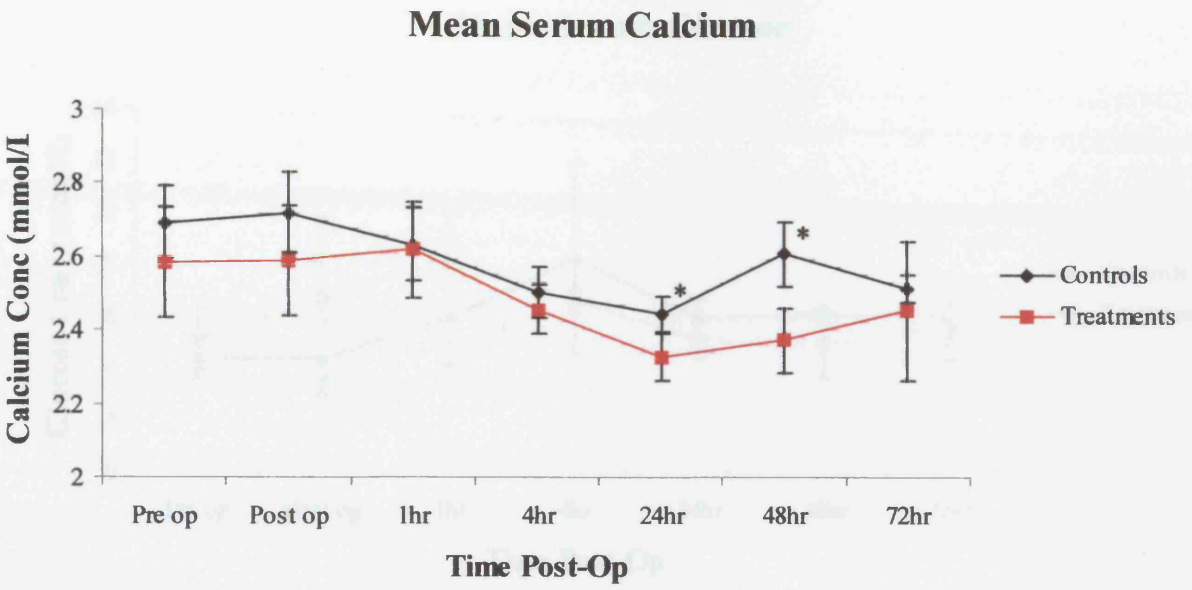

Figure 3.1.10: Mean post-operative calcium concentration for each experimental group.
(* denotes significant [$p<0.05$] difference from control group.)

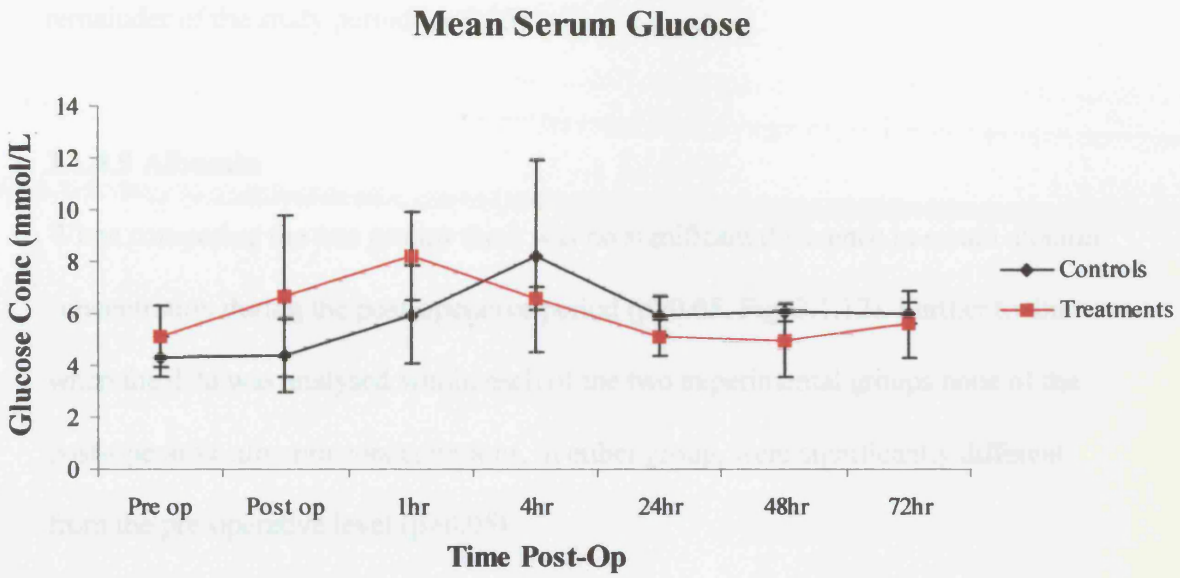

Figure 3.1.11: Mean post-operative glucose concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

operative level at 1 and 4 hours post-electrolysis ($p<0.05$) but that by the first post-operative day there was no significant difference ($p>0.05$). In the control group a significant rise in mean serum glucose was noted at 4 hours post-surgery, serum glucose remained at a level significantly higher than the pre-operative figure for the remainder of the study period ($p<0.05$).

3.1.4.5 Albumin

When comparing the two groups there was no significant difference in serum albumin concentration during the post-operative period ($p>0.05$, Fig. 3.1.12). Further to this when the data was analysed within each of the two experimental groups none of the post-operative albumin concentrations, in either group, were significantly different from the pre-operative level ($p>0.05$).

3.1.4.6 Urea

There was an increase in mean serum urea in both groups in the early post-operative period, although by the third day these levels were returning towards the pre-operative level (Fig. 3.1.13). Statistical analysis showed there to be no difference between serum urea concentrations during the course of the experiment between the treatment and control groups ($p>0.05$).

When the serum urea data pertaining to each group was analysed separately it was found that in the control group all mean urea concentrations were significantly higher than the pre-operative level at all post-operative time points other than 72 hours ($p<0.05$). In the treatment group all post-operative urea concentrations were significantly greater than the pre-electrolysis concentration ($p<0.05$).

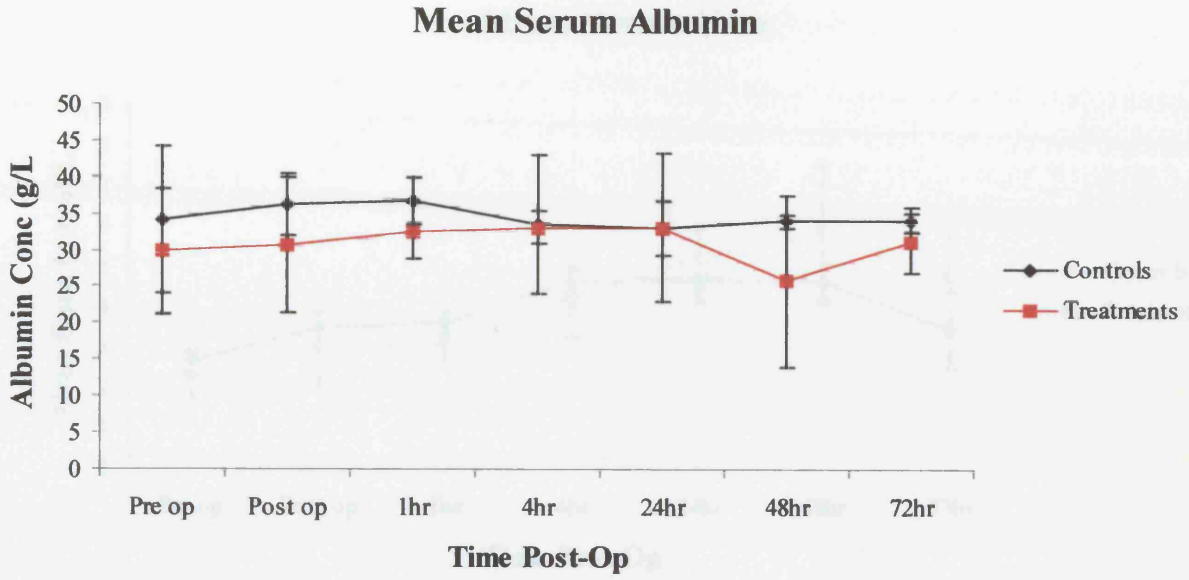

Figure 3.1.12: Mean post-operative albumin concentration for each experimental group.
(* denotes significant [$p<0.05$] difference from control group.)

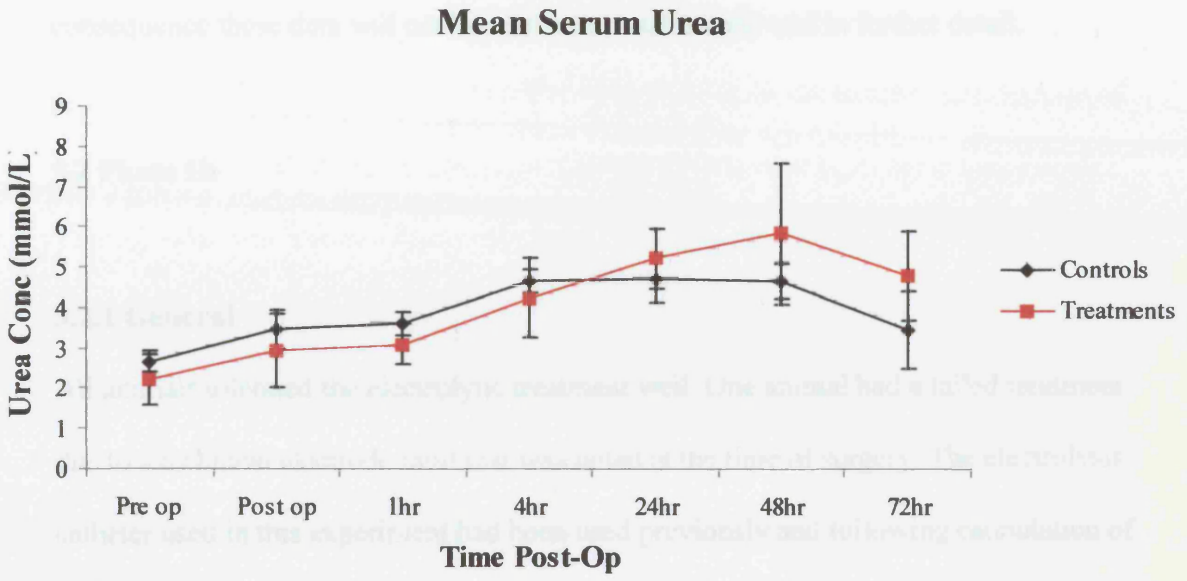

Figure 3.1.13: Mean post-operative urea concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

Other biochemical parameters, such as sodium, potassium, creatinine and bicarbonate concentrations, were also determined and statistically analysed for both the treatment and control groups. There were no significant changes in the mean serum levels of any of these substances during of the study, either between or within groups ($p>0.05$). As a consequence these data will not be addressed individually and in further detail.

3.2 Phase 1b

3.2.1 General

All animals tolerated the electrolytic treatment well. One animal had a failed treatment due to a technical electrode fault that was noted at the time of surgery. The electrolysis catheter used in this experiment had been used previously and following cannulation of the pancreatic duct it was noted that it was not possible to pass a direct current between the required electrodes. Upon removal of the catheter significant erosion of the catheter casing was noted around the previously used electrodes and the catheter was discarded. It was felt that re-cannulation of the pancreatic duct with a new catheter would be inappropriate as the re-instrumentation may well cause additional inflammation to the pancreas that would bias any resulting data from that animal. Therefore this pig was euthanased whilst still under general anaesthetic, with a lethal intravenous injection of barbiturate, and was subsequently replaced. The post-operative recovery of the animals was variable. The majority of the animals (n=8) were eating, drinking, passing urine and defaecating normally 48 hours after the procedure. All these animals started to gain weight between 7 and 10 days post-operatively. At the end of the study period the mean weight in the two-week group was 36kg (range 34-38kg); in the eight-week group the mean weight was 65kg (range 45-77kg).

3.2.2 Premature deaths

Three pigs died prematurely. Two pigs in the two-week survival group died prematurely at 7 and 8 days post-operatively. Post mortem examination showed marked gastric dilatation in both animals. One animal was also found to have a fluid collection, in the tail of the pancreas, at the site of the electrolytic treatment. Neither of these animals showed any evidence, clinically or biochemically, of acute pancreatitis. These deaths were attributed to acute gastric dilatation due to duodenal oedema, post-operative ileus and overeating. These two animals were subsequently replaced and post-operative management was modified to restrict feeding in the early post-operative period. There was a further premature death in the eight-week survival group. This animal suffered a catastrophic upper gastrointestinal bleed and was euthanased on the eighth post-operative day. Post-mortem examination demonstrated marked gastric dilatation associated with a large circumferential 'ulcer' at the gastro-oesophageal junction (Fig. 3.2.1). Histological examination suggested this to be a traction injury to the mucosa rather than classical gastric ulceration (Fig. 3.2.2). We proposed that this 'degloving' type injury to the stomach was most likely a result of the acute gastric dilatation that had been seen post-operatively in a number of the pigs, from both this project and other studies using a porcine model. This phenomenon will be addressed in greater detail in the discussion for this phase of the study.

Figure 3.2.1: Circumferential ‘ulcer’ at the pars-oesophagea.

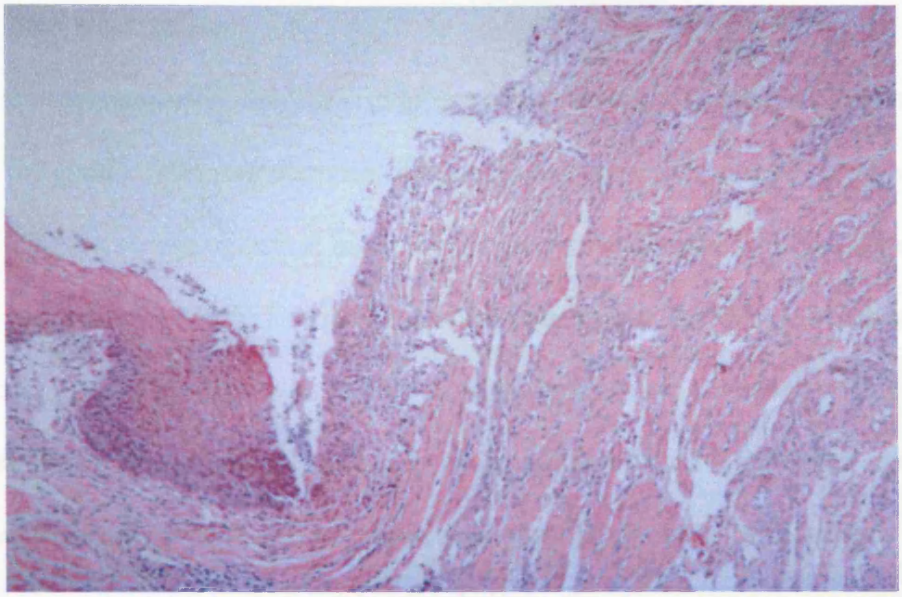

Figure 3.2.2: Histological section showing exposed smooth muscle fibres. (H&E x4)

3.2.3 Macroscopic appearances of pancreata

3.2.3.1 Two-week group

In all the pancreata (n=4), there was evidence of an electrolytic lesion in the splenic lobe of the gland. The mean diameter of these lesions was 2.4cm (range 1.8cm to 3.0cm). The lesion was spherical in nature and clearly demarcated from the adjacent normal pancreatic tissue. In 3 of the 4 glands the electrolytic lesion had breached the pancreatic capsule and there was an associated peri-pancreatic collection of fluid (30-120mls). There was no evidence of injury to any adjacent viscera; particular attention was paid to the portal vein with no evidence of thrombosis noted. In all animals the duodenotomy was well healed and remained patent. The lungs and kidneys were all macroscopically normal.

3.2.3.2 Eight-week group

All the animals in this group (n=4) were found to have dense intraperitoneal adhesions at post mortem examination. The scarring from the electrolytic lesions was still visible in the splenic lobe of the pancreas. There were no associated fluid collections, pseudocysts or fistulae. There was no evidence of damage to adjacent viscera in any of the animals; again the portal vein in all the animals was free of thrombosis. The duodenum remained well healed and patent at the site of the duodenotomy. The lungs and kidneys were all macroscopically normal.

3.2.4 Histological appearance

As previously described sections of the pancreas were preserved in formalin, paraffin mounted and stained with haematoxylin and eosin. These sections were graded by a 'blinded' pathologist using an established scoring system for experimental pancreatitis.

3.2.4.1 Two-week group

The mean histological score at the site of electrolysis was 23.6 ± 3.2; the mean score of the proximal unablated pancreas was 14.3 ± 2.9 (Fig. 3.2.3). In all four pancreata, abscess formation at the site of electrolytic ablation was noted. In addition to this, at the electrolysis site, there was marked inflammatory infiltration, extensive fat necrosis, predominately sub-lobular parenchymal necrosis and moderate intra-pancreatic haemorrhage (Fig. 3.2.4). There were only minimal signs of inflammation in the proximal pancreas (Fig. 3.2.5). In both sets of histological sections the pancreatic duct remained patent. In areas where the electrolytic lesion was adjacent to the portal vein there was no evidence of damage to the vessel wall (Fig. 3.2.6). There was no evidence of inflammatory changes in either the lung or kidney. There was however mild pulmonary oedema in one of the lung specimens, whether these were agonal changes or not it was not possible to say. A paired t-test was used to compare the histological grading scores from the site of the electrolytic ablation and the proximal unablated pancreas. There was a significant ($p<0.05$) difference between the scores for the two regions of pancreas examined.

3.2.4.2 Eight-week group

The mean histological score at the site of electrolytic ablation was 14.1 ± 3.4 the score

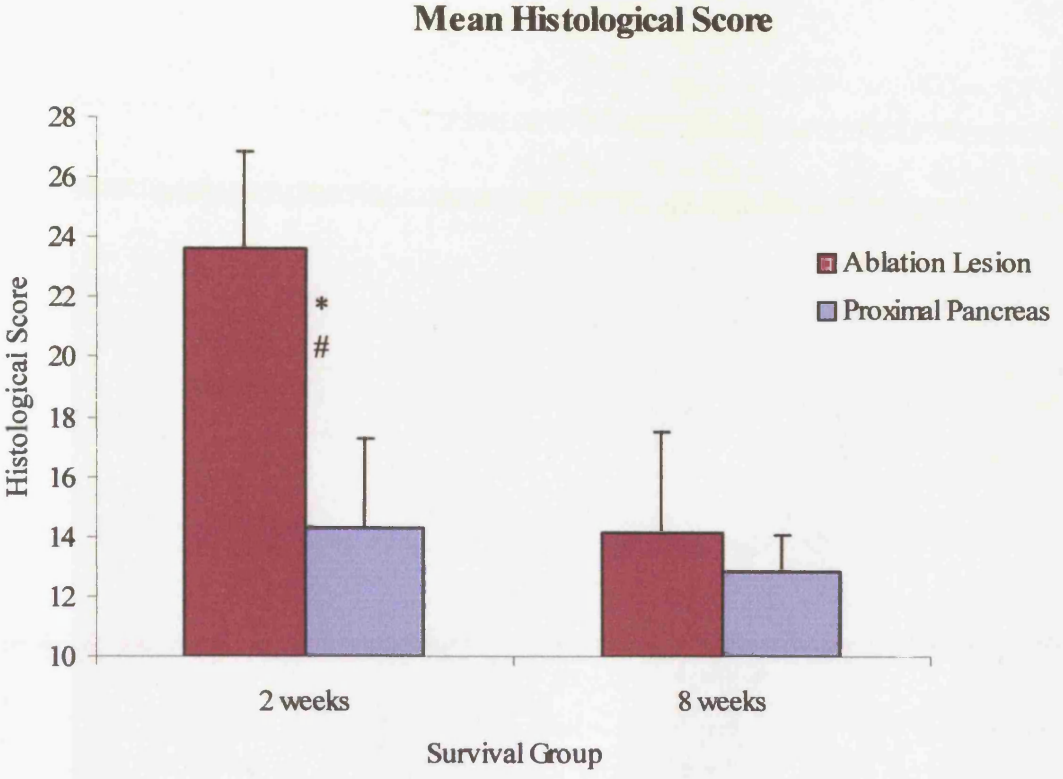

Figure 3.2.3: Mean histological scores, with standard deviations, for each experimental group. (* denotes significant [$p<0.05$] difference between scores within a group, # denotes significant [$p<0.05$] difference between scores for a region of pancreas between groups.)

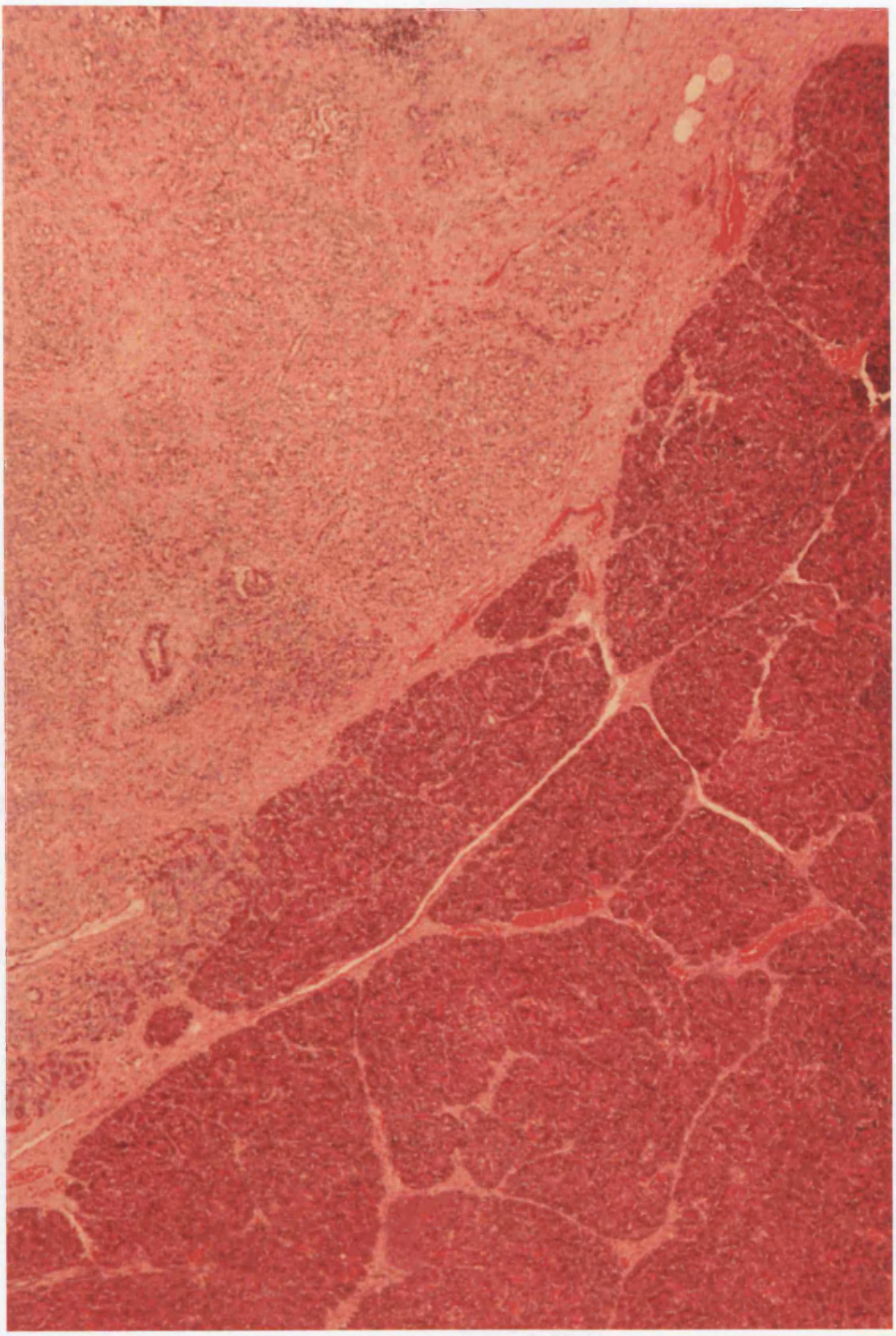

Figure 3.2.4: Two-week group: ablation lesion. (H&E x4)

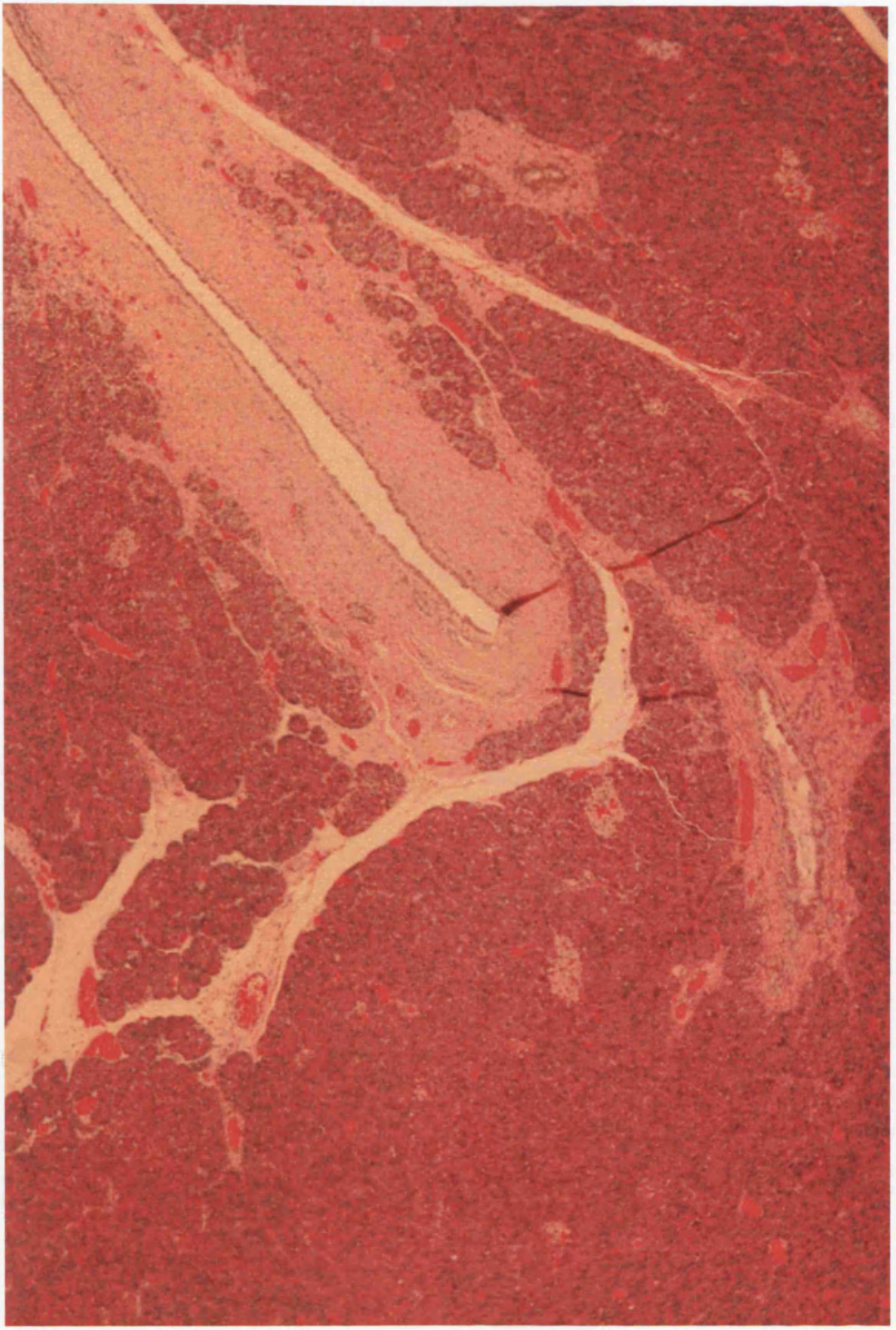

Figure 3.2.5: Two-week group: proximal pancreas. (H&E x4)

Figure 3.2.6: Two-week group: ablation lesion abutting directly onto portal vein.

(H&E x4)

of the proximal pancreas was 12.8 ± 1.3 (Fig. 3.2.3). There was no evidence of abscess formation in any of the examined pancreata. In this group, although there was again sub-lobular parenchymal necrosis at the site electrolysis, there were only minimal inflammatory changes, fat necrosis and haemorrhage (Fig. 3.2.7). In both samples the pancreatic duct remained patent. There was no evidence of inflammatory changes in either the lung or kidney. A paired t-test was used to compare the histological grading scores from the site of the electrolytic ablation and the proximal unablated pancreas. There was no significant difference between these two regions of pancreas ($p>0.05$). Further to this the histological scores from each of the survival groups at 2 and 8 weeks were compared (Fig 3.2.3). These data were statistically analysed using an unpaired Student t-test. This demonstrated that there was a statistically significant difference in the histological scores obtained at the electrolysis site between the 2-week and the 8-week survival groups ($p<0.05$). However, no statistically significant difference was found between the two histological scores obtained, at two and eight-weeks, from the unablated proximal pancreas ($p>0.05$).

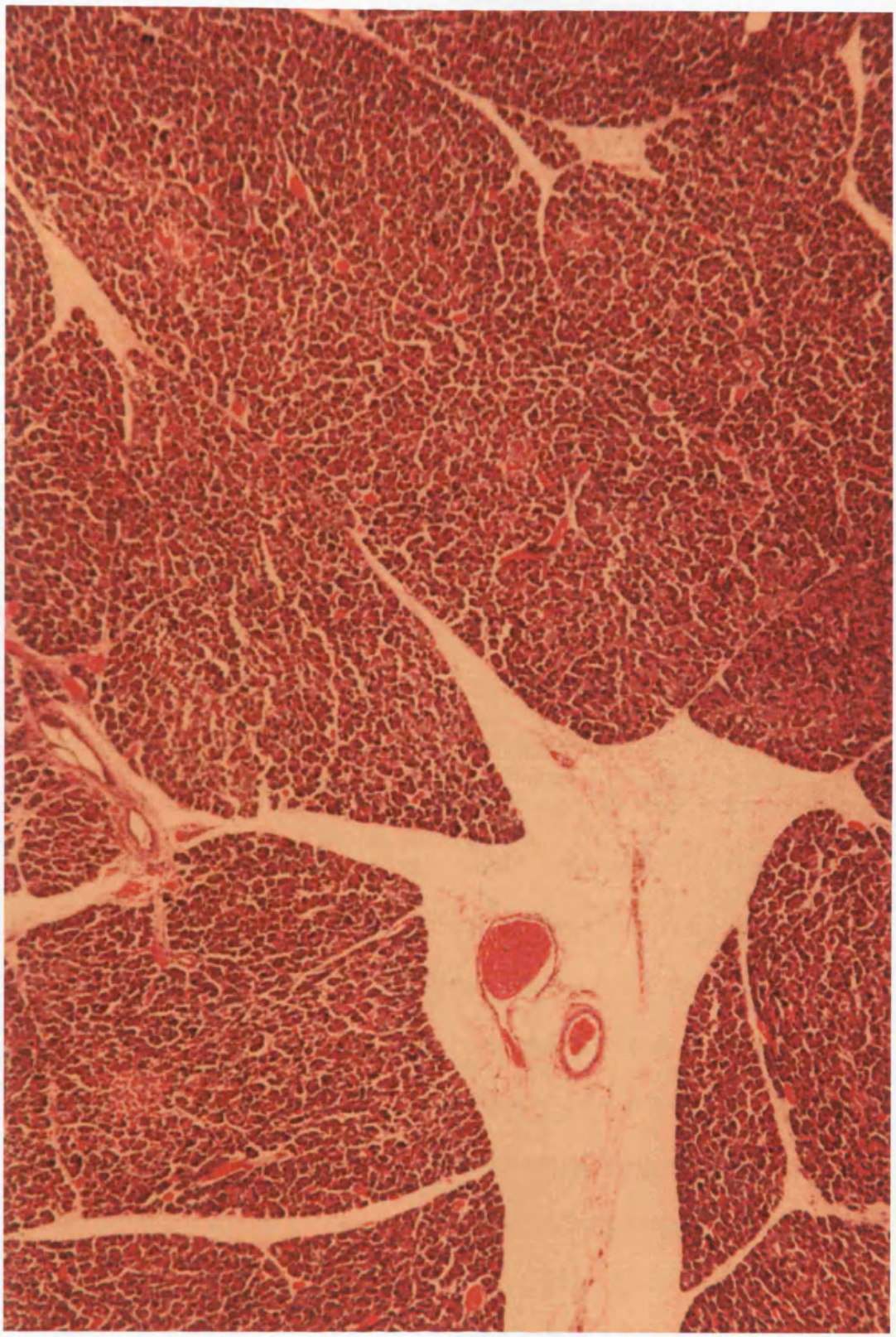

Figure 3.2.7: Eight-week group: ablation lesion. (H&E x4)

3.2.5 Biochemistry

The biochemical data from all animals (pre-mature deaths and exclusions due to technical difficulties not included) was reviewed. The amylase and CRP data were analysed both comparing the 2-week and 8-week groups and as a whole (i.e. combining the 2 and 8 week data.) Analysis of data pertaining to the other biochemical parameters demonstrated no significant differences ($p>0.05$) between the 2 and 8-week groups and therefore only the analysis of the combined data are addressed.

3.2.5.1 Amylase

All animals developed transient post-operative hyperamylasaemia. The mean serum amylase in both the 2 and the 8-week groups peaked at 24 hours post-operatively, 8219 units/L ± 3783 units/L and 11917 units/L ± 2272 units/L respectively (Fig. 3.2.8). Statistical analysis showed that these peaks were significantly ($p<0.05$) higher than the corresponding mean pre-operative values in both groups. However, in 2-week group the mean serum amylase had returned to 'normal' (i.e. not statistically significantly different from the pre-operative level) by the fourth post-operative day and in the 8-week group the mean serum amylase had returned to 'normal' by the third post-operative day ($p>0.05$). The mean amylase concentration for both the groups was compared at each post-operative time point. The only statistically significant difference was immediately post electrolysis where the mean serum amylase in the 8-week survivor group was significantly greater ($p<0.05$) than the means serum amylase in the 2-week survivor group.

When the data from the two survival groups was combined (Fig. 3.2.9), the post-operative peak occurred at 24 hours, again this was statistically significantly greater

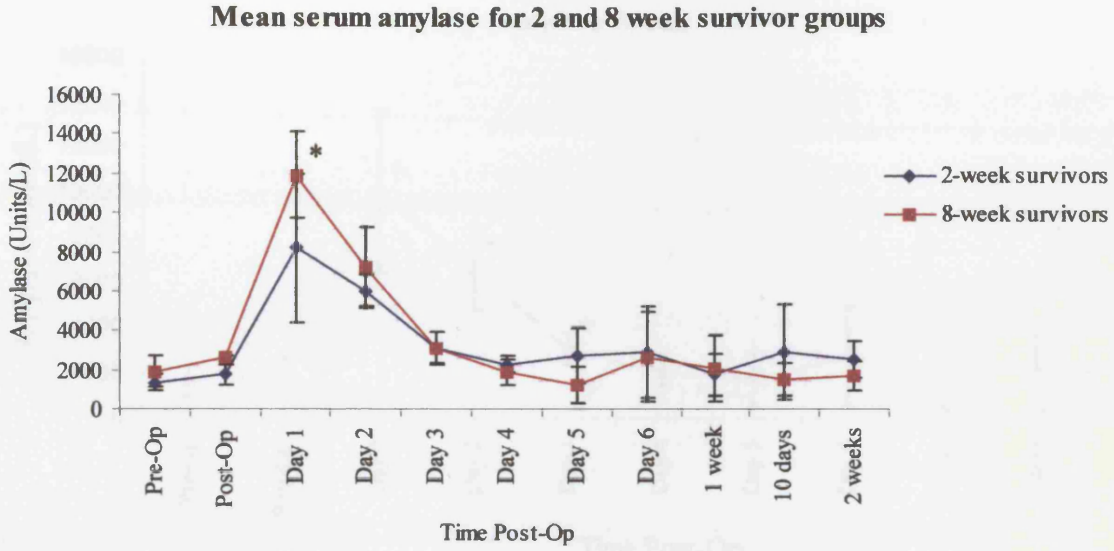

Figure 3.2.8: Mean post-operative Amylase concentration for each experimental group. (* denotes significant [$p<0.05$] difference between experimental groups.)

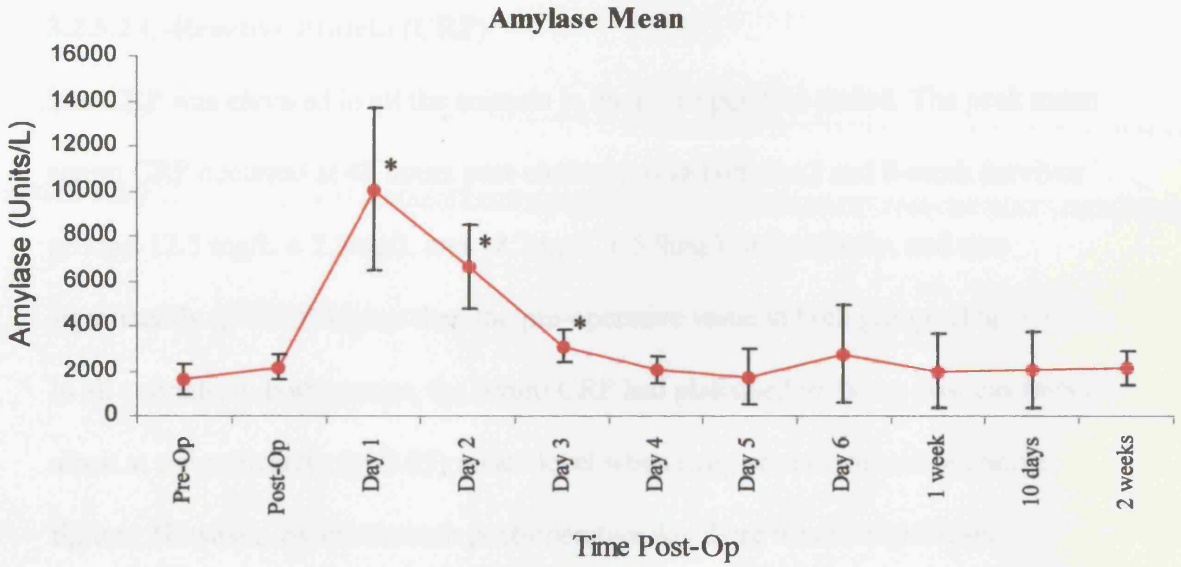

Figure 3.2.9: Combined mean post-operative Amylase concentration for both experimental groups.
(* denotes significant [$p<0.05$] difference from pre-operative concentration.)

than the pre-operative value ($p<0.05$). In the combined data the mean serum amylase concentration was not significantly different from the pre-operative level by the fourth post-operative day.

3.2.5.2 C-Reactive Protein (CRP)

The CRP was elevated in all the animals in the post-operative period. The peak mean serum CRP occurred at 48 hours post-electrolysis in both the 2 and 8-week survivor groups, 12.5 mg/L ± 2.5mg/L and 18.3mg/L ± 5.9mg/L respectively, and was significantly ($p<0.05$) higher than the pre-operative value in both groups (Fig. 3.2.10). In all animals, in both groups, the serum CRP had plateaued 96 hours post electrolysis, albeit at a significantly ($p<0.05$) raised level when compared to the pre-operative figures. However, by the seventh post-operative day there was no significant difference, in either the 2 or the 8-week group, between the mean serum CRP and the pre-operative concentration. When CRP data was statistically compared between the two and the eight-week groups a significant difference was only detected on the first post-operative day, where the mean CRP for the eight-week group was significantly ($p<0.05$) greater than the mean CRP for the two-week group.

When the data from both the groups was combined and analysed as a whole similar results were obtained (Fig. 3.2.11). The post-electrolysis peak occurred at 48 hours and again this was statistically significantly greater than the pre-operative value ($p<0.05$). Interestingly, the mean CRP concentration, from the combined data, remained significantly greater than the pre-operative figure until the tenth post-operative day.

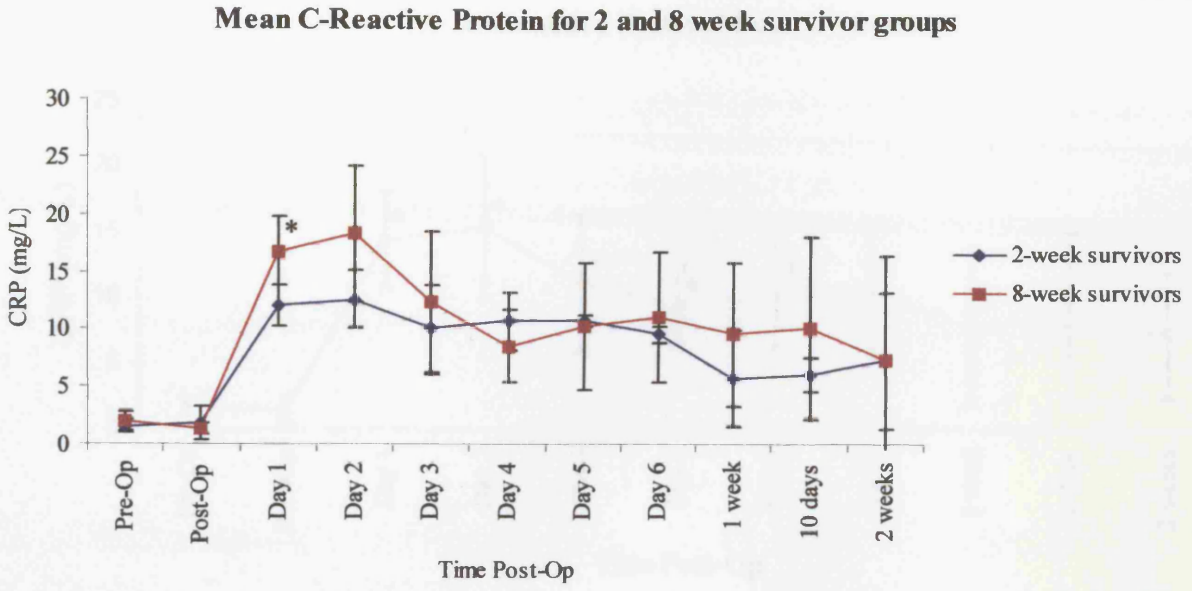

Figure 3.2.10: Mean post-operative C-Reactive Protein concentration for each experimental group.
(* denotes significant [$p<0.05$] difference between experimental groups.)

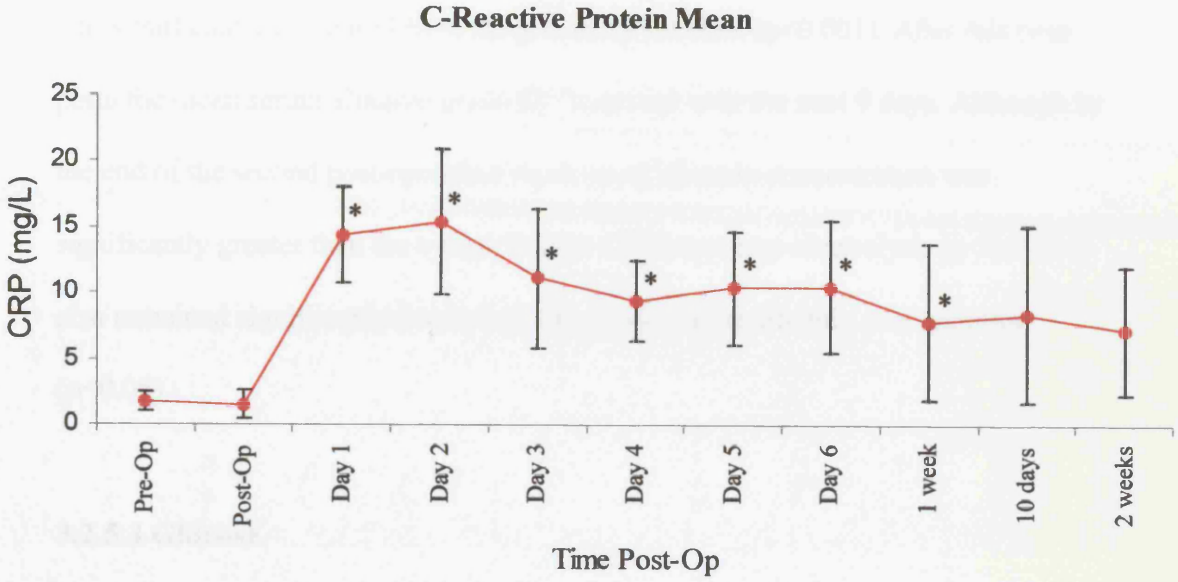

Figure 3.2.11: Combined mean post-operative C-Reactive Protein concentration for both experimental groups. (* denotes significant [$p<0.05$] difference from pre-operative concentration.)

3.2.5.3 Albumin

Mean serum albumin concentration was determined for all animals. The mean albumin concentration decreased post-operatively after the first 24 hours, reaching a low at the fifth post-electrolysis day (Fig. 3.2.12). This trough in mean albumin concentration was significantly decreased from the pre-operative level ($p<0.001$). After this time point the mean serum albumin gradually increased over the next 9 days. Although by the end of the second post-operative week mean albumin concentration was significantly greater than the trough level at 120 hours post-electrolysis ($p<0.005$) it also remained significantly lower than the pre-operative albumin concentration ($p<0.05$).

3.2.5.4 Glucose

The mean glucose concentration for all animals, in both experimental groups, was determined (Fig. 3.2.13). There was an immediate post-operative increase in serum glucose concentration, however this failed to reach statistical significance ($p=0.101$) when compared with the pre-operative mean concentration. Statistical analysis of the remaining data, using a Student t-test, showed there to be no significant differences between any of the other post-operative mean glucose levels and the pre-operative glucose concentration ($p>0.05$).

3.2.5.5 Calcium

Mean calcium concentration was calculated for all the pigs in both groups of animals (Fig. 3.2.14). There was a decrease in mean serum calcium from the pre-operative level of 2.73 mmol/L ± 0.10 mmol/L to a trough at 24 hours post-electrolysis (mean calcium concentration 2.37 mmol/L ± 1.8 mmol/L. Statistical analysis of these data

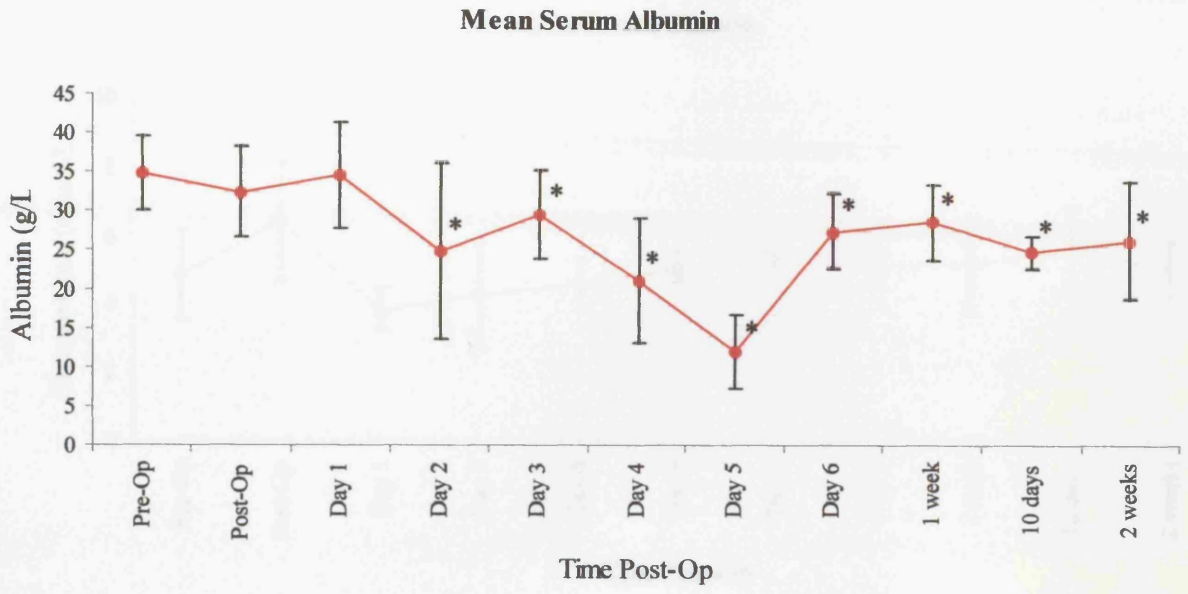

Figure 3.2.12: Mean post-operative albumin concentration for each experimental group. (* denotes significant [$p<0.05$] difference pre-operative concentration.)

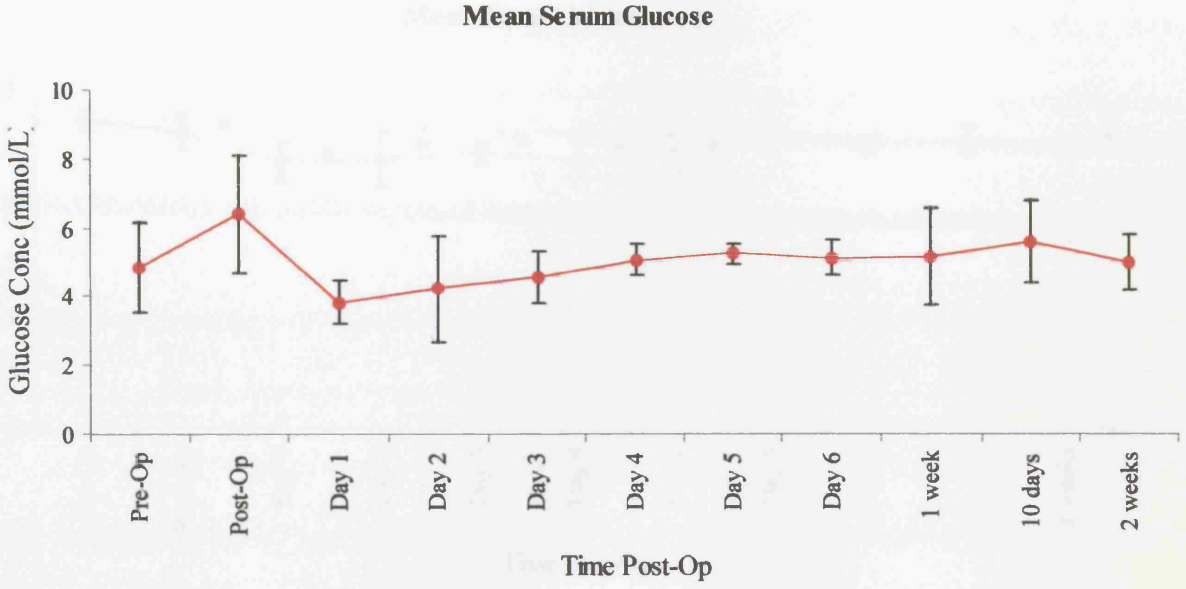

Figure 3.2.13: Mean post-operative glucose concentration for each experimental group.

(* denotes significant [$p<0.05$] difference pre-operative concentration.)

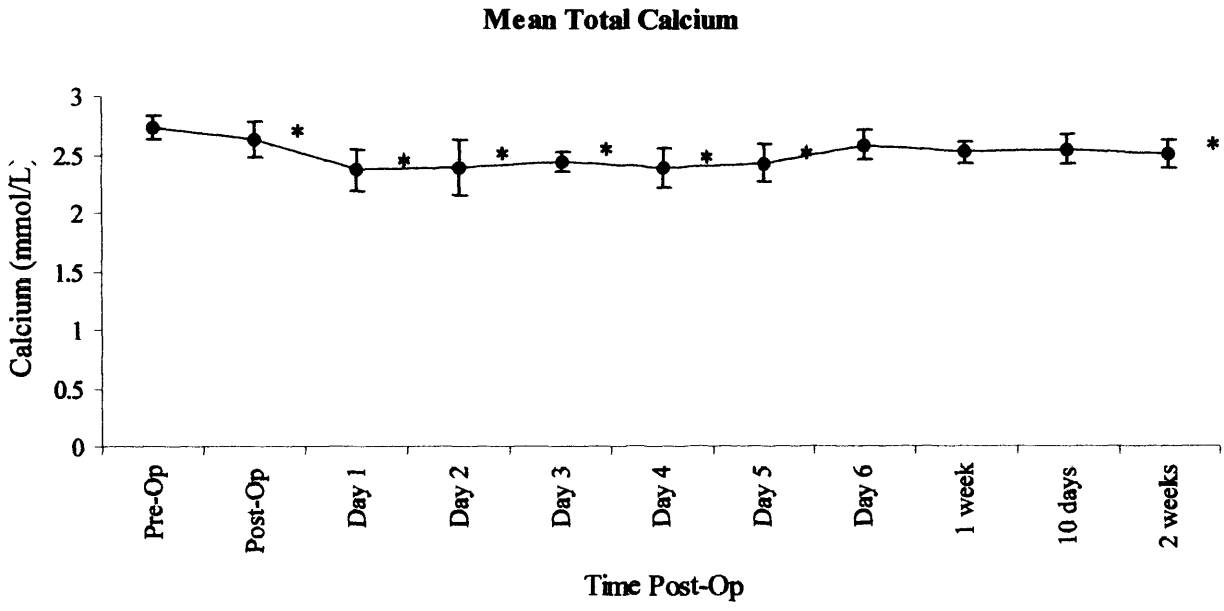

Figure 3.2.14: Mean post-operative calcium concentration for each experimental group. (* denotes significant [$p<0.05$] difference pre-operative concentration.)

showed that mean serum calcium concentration was significantly lower than the pre-operative level at all time points other than 6, 7 and 10 days post-electrolysis ($p<0.05$). Despite the statistically significant relative decrease in serum calcium concentration compared with the pre-operative levels, the mean serum calcium concentration never dropped below 2.37 mmol/L and remained within the normal range through out the study period.

3.2.5.6 Urea & Electrolytes

The mean urea concentration was also determined for the whole animal group for the initial 2 weeks of the experiment (Fig. 3.2.15). Mean serum urea increased in the early post-operative period from a pre-electrolysis level of 2.21 mmol/L ± 0.72 mmol/L to 8.54 mmol/L ± 7.75 mmol/L and 8.8 mmol/L ± 9.67 mmol/L at 48 hours and 72 hours post-electrolysis respectively. At all other time points mean serum urea concentration was less than 5.5 mmol/L and therefore within the 'normal' range. This marked increase in post-operative mean serum urea is almost solely due to increased urea concentration in the first two animals used in this phase of the study. In these two pigs, one from each of the 2 and 8-week survivor groups, inadequate peri-operative intravenous fluid administration resulted in a marked dehydration and a subsequent increase in serum urea at 48 hours (19.8 mmol/L and 19.6 mmol/L) and 72 hours (15.1 mmol/L and 27.7 mmol/L). In both these animals the dehydration was readily corrected with intravenous fluid administration. All the other animals used in this phase of the study were given 1 litre of 0.9% saline, intravenously, on a daily basis for the first 72 hours of the experiment (as previously described in the experimental method). If only the pigs in which the modified intravenous fluid regimen was used are

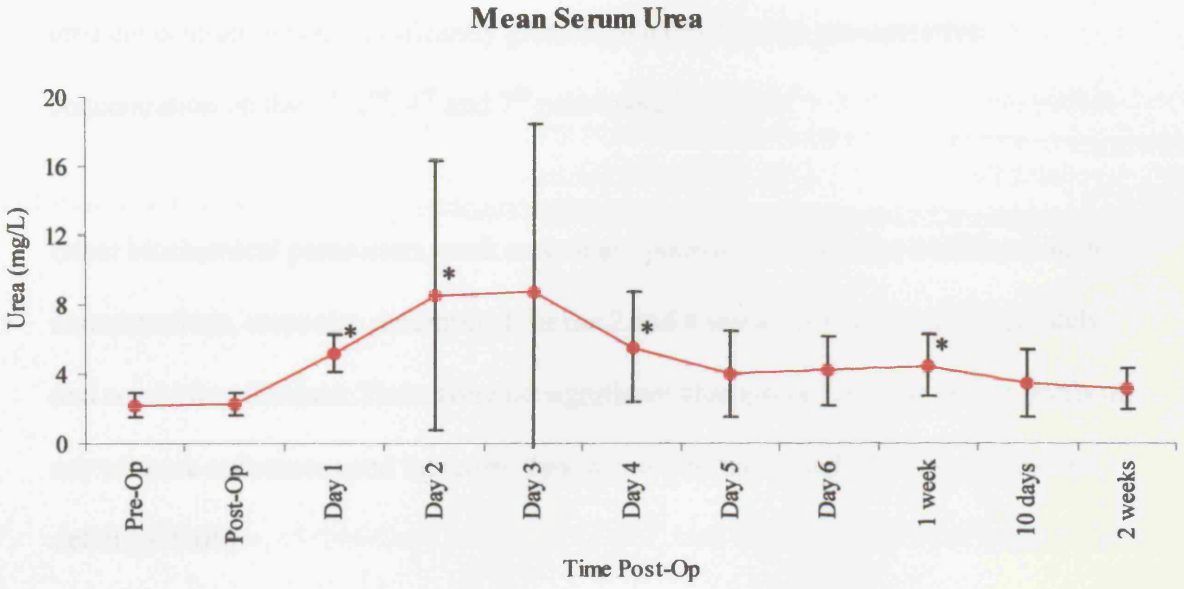

Figure 3.2.15: Mean post-operative urea concentration for each experimental group.

(* denotes significant [$p<0.05$] difference pre-operative concentration.)

considered then the mean serum urea is never greater than a 5.25 mmol/L ± 1.14 mmol/L peak at 24 hours post-electrolysis.

Statistical analysis of the urea data, from all eight animals, showed that mean serum urea concentration was significantly greater ($p<0.05$) than the pre-operative concentration on the 1st, 2nd, 4th and 7th post-operative days.

Other biochemical parameters, such as sodium, potassium, creatinine and bicarbonate concentrations, were also determined for the 2 and 8 week survival groups separately and combining the data. There were no significant changes in the mean serum levels of any of these substances and therefore they will not be addressed individually and in detail ($p>0.05$).

3.3 Phase 1 a & b Cytokines.

Serum concentrations of TNF-α and IL-1β were determined by commercially available, porcine specific sandwich ELISA's, as has been described in the methodology. Serum from animals in phases 1a and 1b were analysed for cytokine levels. These data were statistically analysed using the Student T-test in both cases; an unpaired test was used in phase 1a and a paired test was used in phase 1b.

3.3.1 Phase 1a

The mean serum cytokine concentrations were determined for both the treatment and control groups.

Detectable levels of serum TNF-α were found in most of the experimental animals, from both study limbs, at all time points of this experiment. Mean concentration of TNF-α peaked immediately post-operatively in both treatment and control groups, 49.2 pg/ml ± 41.0 pg/ml and 44.0 pg/ml ± 28.9 pg/ml respectively (Fig. 3.3.1). From this peak concentration, mean serum levels returned towards pre-operative levels and remained essentially constant in both groups during the course of the study. Statistical analysis of the mean serum TNF-α concentrations within each of the experimental groups showed there to be no statistically significant ($p>0.05$) difference in any of the post-operative concentrations when compared to the pre-operative TNF-α levels from the respective study group. Statistical comparison of mean serum TNF-α concentrations between the treatment and the control group at each of the experimental time-points failed to detect any statistically significant difference between the two groups ($p>0.05$).

Detectable levels of serum IL-1β were only found in a few serum samples in both groups of animals. Indeed at each time point at least four of the six samples, in either group, had no detectable IL-1β. The resulting mean concentrations are therefore derived from only one or two samples, as is indicated by the wide standard deviations (Fig. 3.3.2). Statistical analysis of mean IL-1β concentrations showed there to be no significant difference ($p>0.05$) when either treatment group and control group data were compared or when post-operative mean IL-1β concentrations were compared to pre-operative levels within each experimental group.

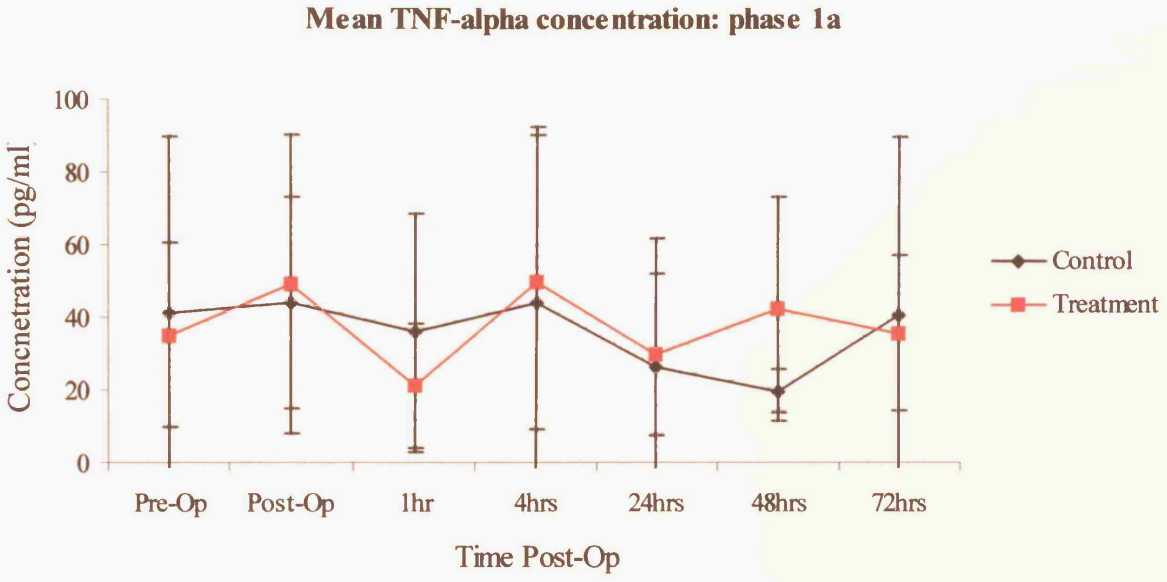

Figure 3.3.1: Phase 1a: Mean post-operative TNF-alpha concentration for each experimental group.
(* denotes significant [$p<0.05$] difference from control group.)

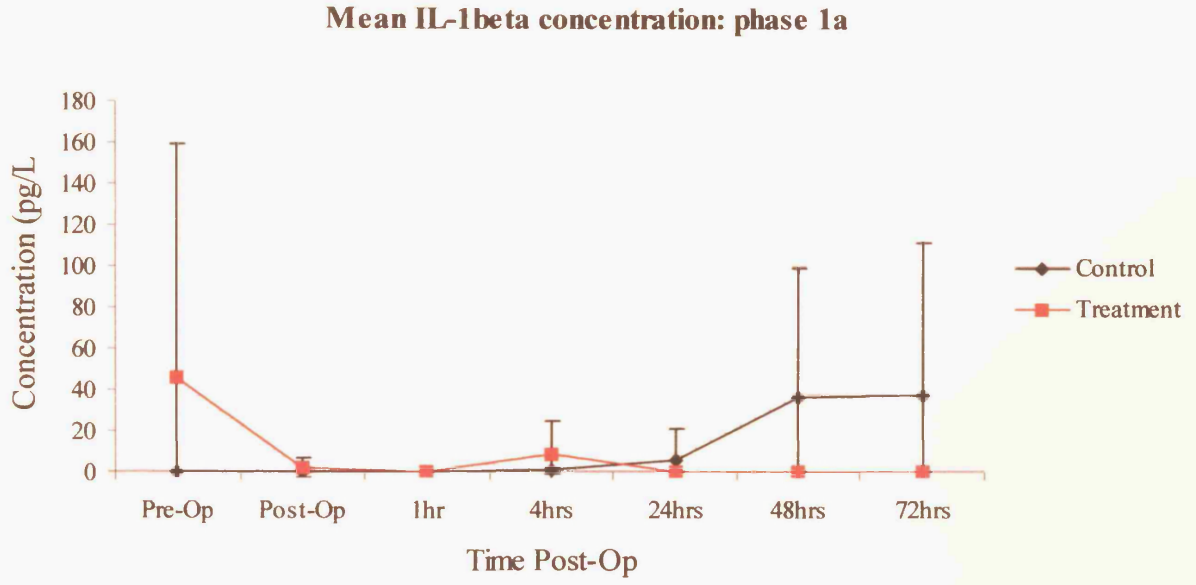

Figure 3.3.2: Phase 1a: Mean post-operative IL-1beta concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

3.3.2 Phase 1b

In this phase of the study all cytokine data has been analysed together rather than dividing it into 2 and 8-week survivor groups. All animals underwent identical treatment and management in the initial two weeks of the experiment. Therefore combining the data from the two survival groups will allow greater statistical power in the analysis of the resulting information.

Serum TNF-α was detectable at all stages, both pre and post-operatively, through the duration of the experiment (Fig. 3.3.3). The peak TNF-α level was detected immediately post electrolysis. The mean concentration of TNF-α, at this point, was 84.0 pg/ml with a standard deviation of 12.7 pg/ml. Although this is greater than the pre-operative concentration (mean 76.7 pg/ml ± 8.7 pg/ml), when the data was statistically analysed using a paired t-test no significant difference was detected ($p=0.078$). Further statistical analysis of post-operative mean TNF-α concentrations during the remainder of the study period showed that, when compared to the pre-operative data, there was no significant difference in any of the experimental time-points ($p>0.05$). As observed in the previous phase of this study only minimal serum IL-1β was detectable at any stage of this experiment (Fig. 3.3.4). Once again mean concentrations have been derived from only a few detectable IL-1β levels at each time-point, resulting in the wide standard deviations. Although the IL-1β concentrations decreased post-operatively, from the pre-operative mean serum concentration of 57.1 pg/ml, to almost undetectable concentrations (<15pg/ml), these differences were not statistically significant ($p>0.05$). Indeed statistical analysis of the all the post-operative IL-1β concentrations failed to detect any significant differences from the pre-operative figure.

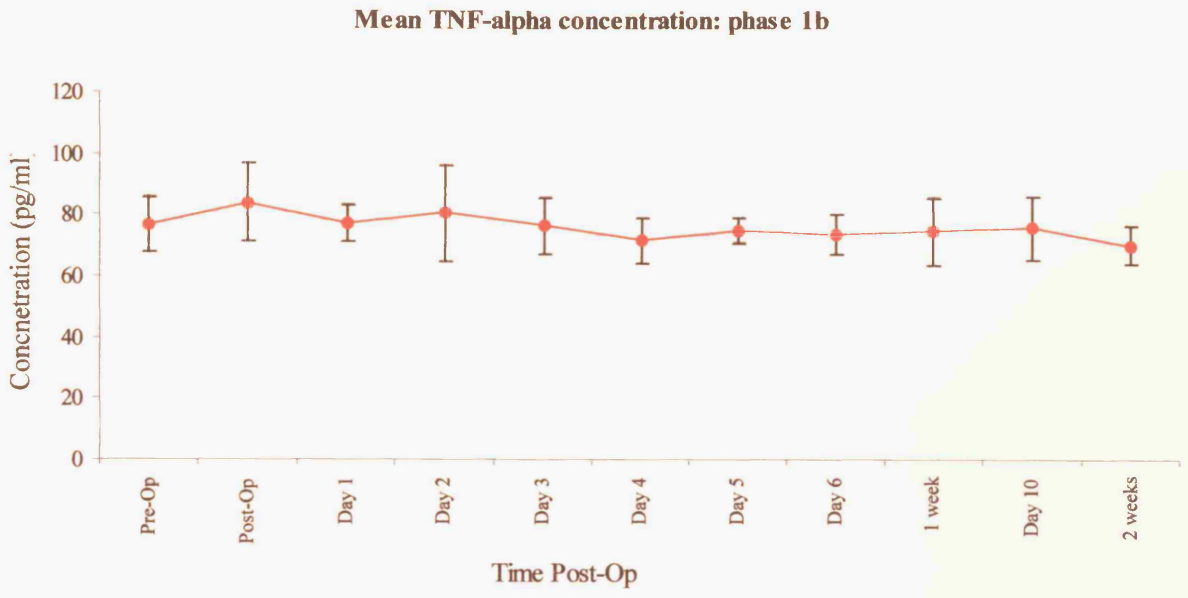

Figure 3.3.3: Phase 1b: Mean post-operative TNF-alpha concentration.
(* denotes significant [$p<0.05$] difference from pre-operative concentration.)

Figure 3.3.4: Phase 1b: Mean post-operative IL-1beta concentration.

(* denotes significant [$p<0.05$] difference from pre-operative concentration.)

3.4 Phase 2

3.4.1 General

All the animals, in both the treatment groups and control group, used in this phase of the study tolerated the operative procedure without event.

However, one of the animals in the 2-week treatment group clinically deteriorated dramatically on the fifth post-operative day. Despite aggressive fluid resuscitation the animal's condition continued to deteriorate and therefore it was euthanased prematurely on the fifth post-operative day. Post-mortem examination revealed a perforated ceacum with resulting faecal peritonitis. There was no evidence of colonic or small bowel distension or obstruction. In addition to this the duodenotomy was intact and healing well, there were no of peri-pancreatic collections and there was no evidence of damage to surrounding from the electrolytic process. The site of enteric perforation was distant to the site of electrolysis and therefore it was felt that it was unlikely that the electrolytic process itself was directly related to the ceacal perforation, even though it was impossible to deduce the exact cause of the perforation. The data from this animal was excluded from the study and the animal itself was replaced.

The post-operative recovery of the other animals in all the experimental groups was unremarkable. All animals were mobile and eating and drinking normally by the third post-operative day. At the end of the two-week survival period the mean weight in the treatment group was 36.5kg (range 33-38kg) and in the control group 36kg (range 32.5-37.5kg), statistical comparison of these sets of data using an unpaired Student t-

test showed there to be no significant difference ($p>0.05$) between the two groups. In the eight-week treatment group mean weigh was 74kg (range 68-81k).

3.4.2 Macroscopic appearances of pancreata

3.4.2.1 Two-week treatment group

There was evidence of intraperitoneal adhesion formation in all animals. Two of the five animals had peripancreatic fluid collections adjacent to the ablation lesion (Fig. 3.4.1). However, there was no evidence of damage to the adjacent viscera. The enterotomies were well healed in all animals and the lungs and kidneys were macroscopically normal.

Pancreatic ductogram, in three of the resected glands, confirmed that the pancreatic ducts remained patent both proximal and distal to the site of ablation (Fig 3.4.2).

3.4.2.2 Two-week control group

All the pancreata in this group had a macroscopically normal appearance (Fig. 3.4.3). There was no evidence of pancreatic erythema, peri-pancreatic collections or pancreatic fistulae. The enterotomy was well healed in all animals and there was no damage to any surrounding viscera. The lungs and kidneys were macroscopically normal.

Pancreatic ductogram was performed in three of the resected pancreata. In all cases these demonstrated that the pancreatic ducts remained patent, both proximal and distal to the site of the electrolysis catheter electrodes (Fig. 3.4.4).

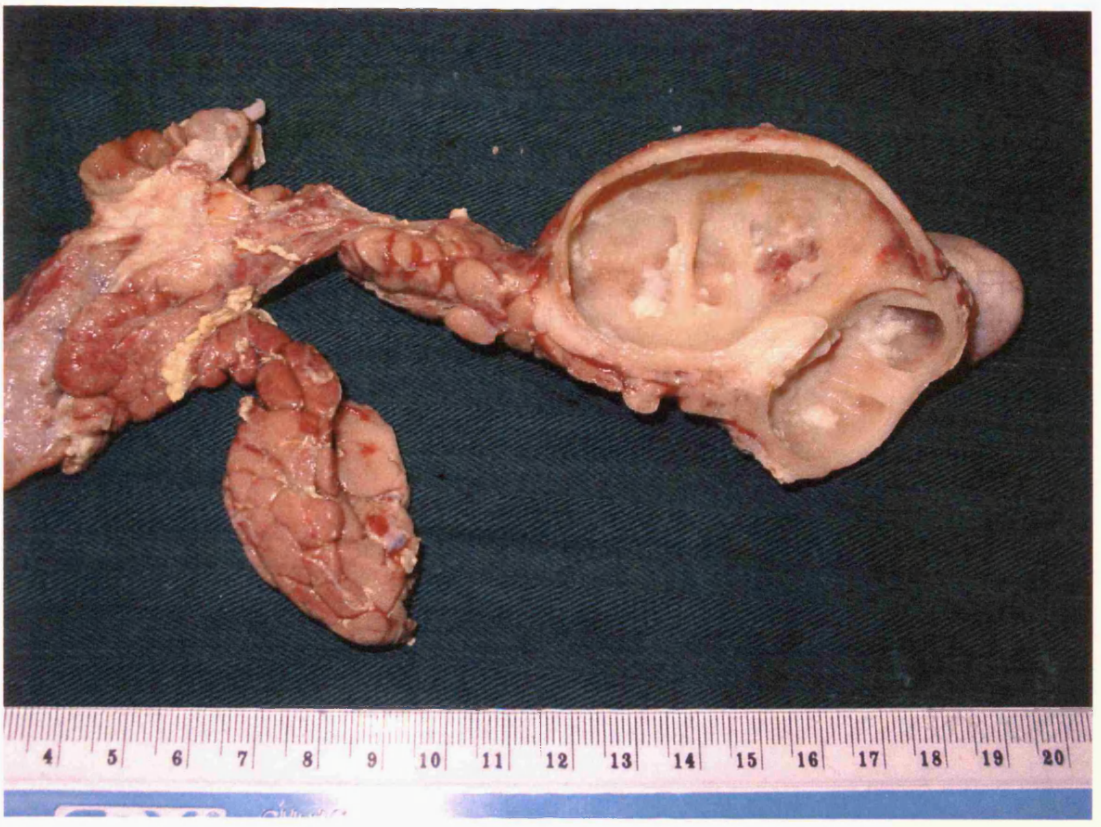

Figure 3.4.1: Two-week treatment group: pancreas with adjacent cyst.

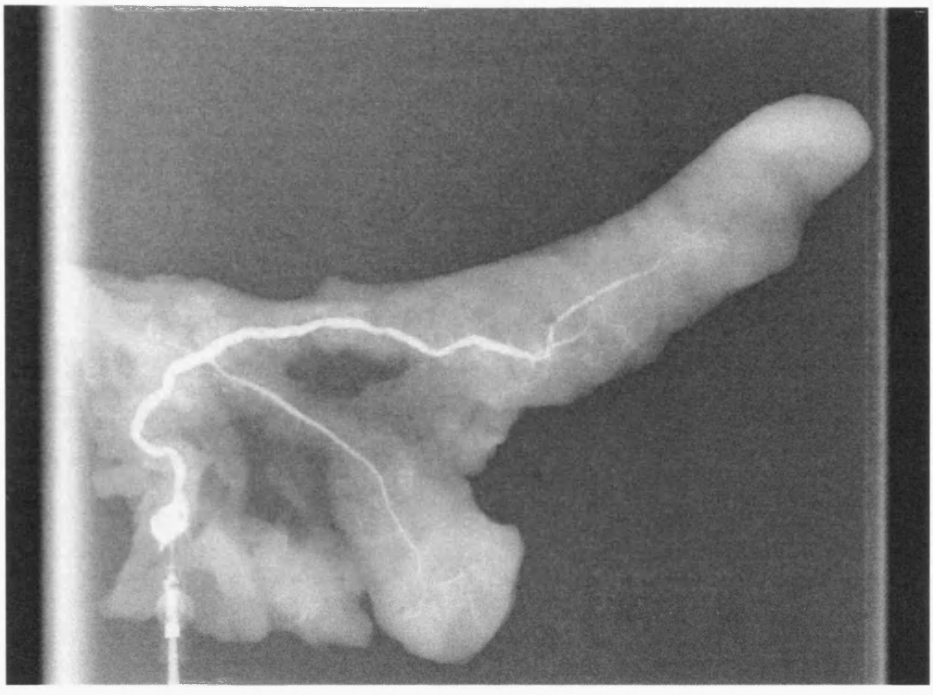

Figure 3.4.2: Two-week treatment group: pancreatic ductogram showing patent duct proximal and distal to ablation lesion.

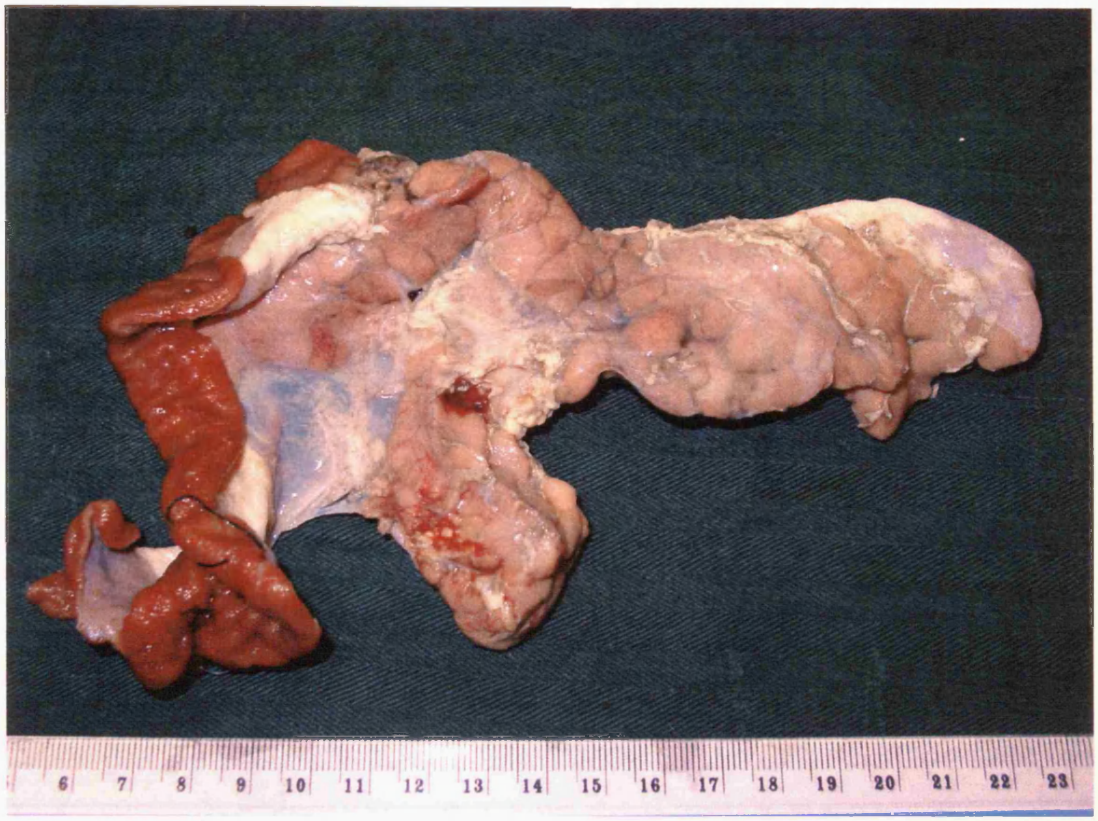

Figure 3.4.3: Two-week control group: excised pancreas

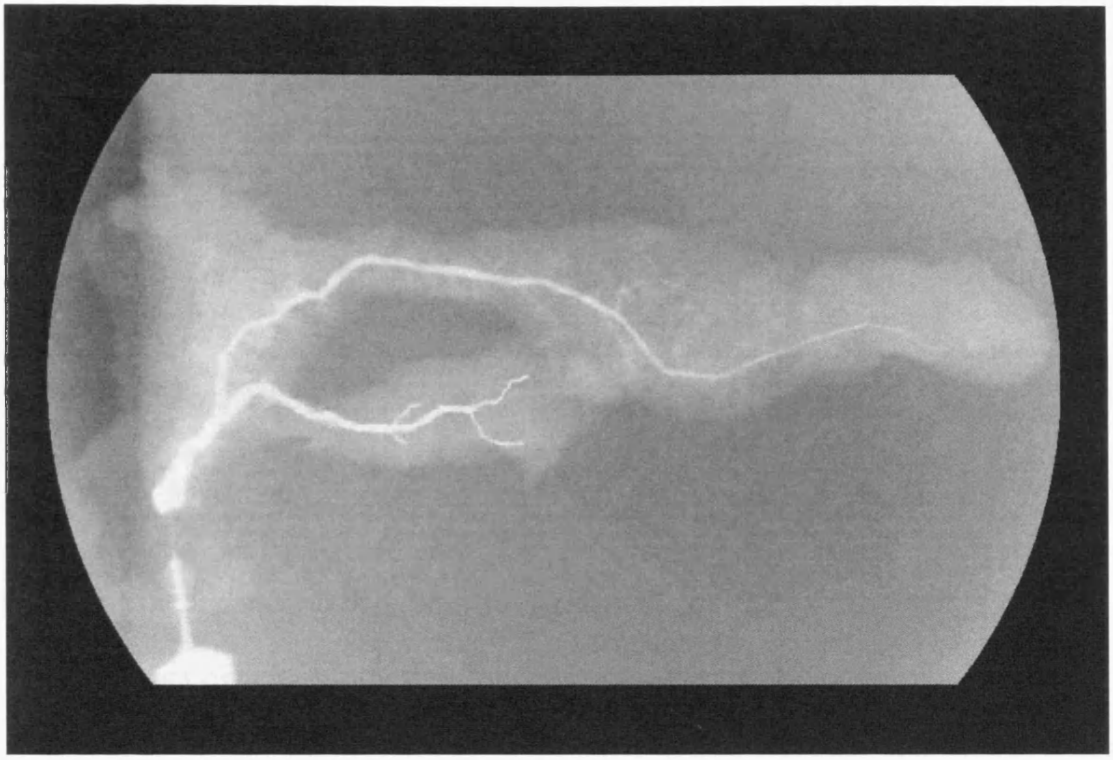

Figure 3.4.4: Two-week control group: pancreatic ductogram showing patent duct proximal and distal to 'ablation lesion'

3.4.2.3 Eight-week treatment group

In all animals in this group there were dense intra-peritoneal adhesions at post-mortem examination. The site of electrolytic ablation was evident in all the pancreata due to the localised scarring (Fig. 3.4.5). In one of the glands, the tail of the splenic lobe appeared to be particularly scarred through out. The enterotomy was well healed in all animals. None of the animals had evidence of peri-pancreatic collections, pancreatic fistulae or damage to surrounding viscera, particularly the portal vein and duodenum. The portal vein was free of thrombosis. Macroscopically, all lungs and kidneys were again normal.

Pancreatic ductograms were performed in three of the resected pancreata from this group. In all cases these ductograms showed that the ducts remained patent in all animals, both proximal and distal to the site of the electrolytic ablation lesions (Fig 3.4.6).

3.4.3 Histological appearance

Paraffin mounted and haematoxylin and eosin stained histological sections were examined by a pathologist, blinded to the experimental groups, again using an established histological scoring system (range 11-27) for experimental pancreatitis. Three sections of each pancreas were examined: the ablation lesion, pancreas proximal to the lesion and pancreas distal to the lesion.

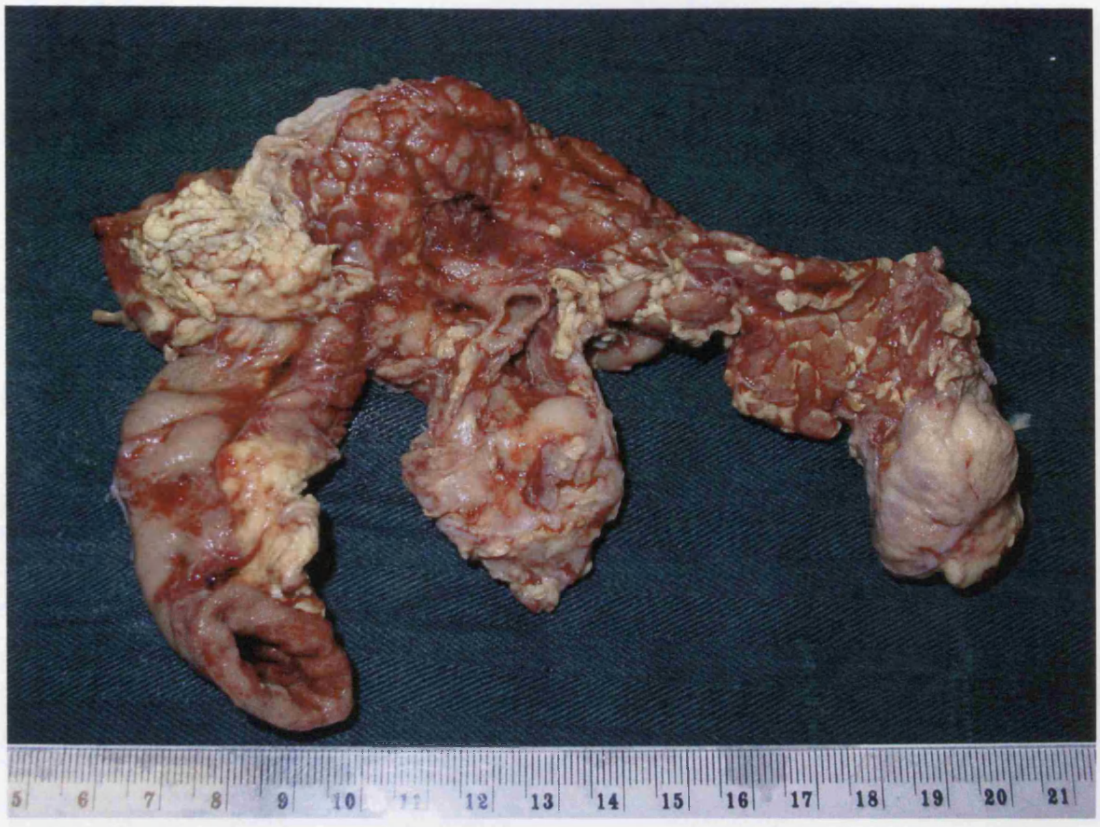

Figure 3.4.5: Eight-week treatment group: scarred pancreas in region of ablation.

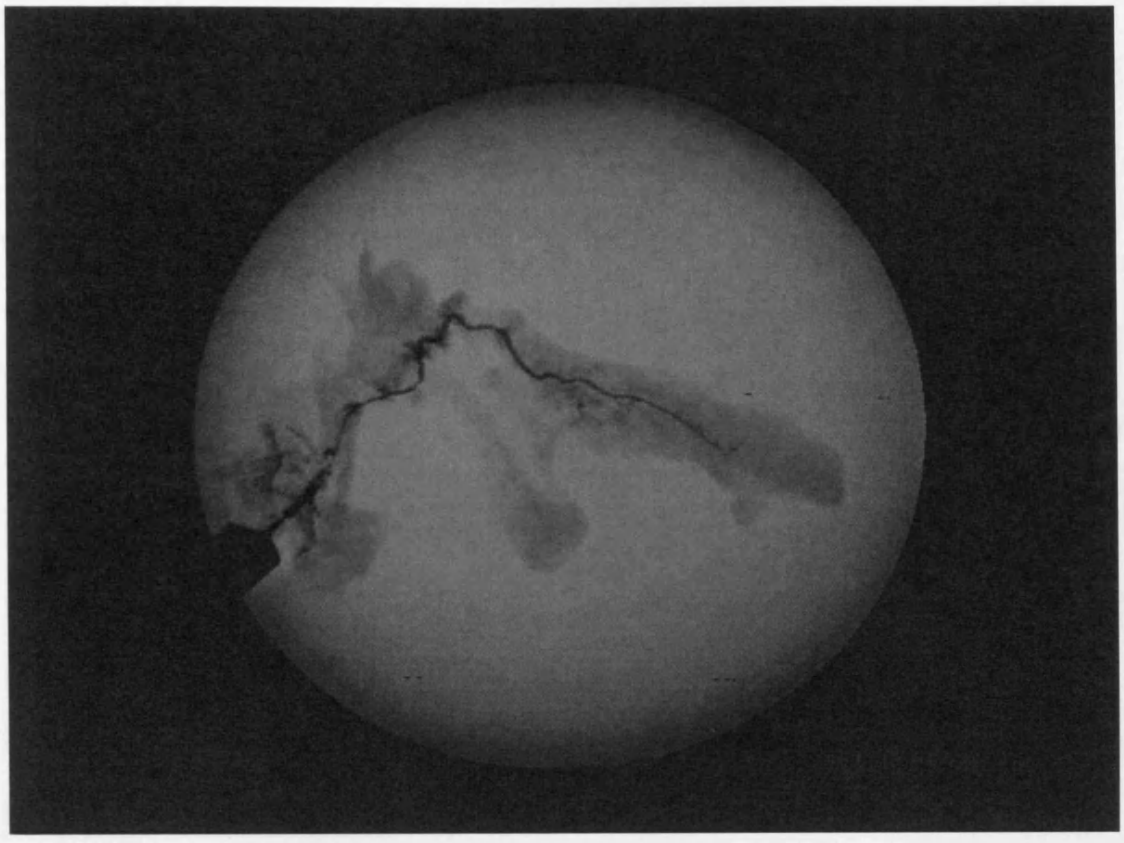

Figure 3.4.6: Eight-week treatment group: pancreatic ductogram showing patent duct proximal and distal to ablation lesion.

3.4.3.1Two-week treatment group

The mean histological score at the site of electrolysis was 17.8 ± 3.27; the mean score of the proximal unablated pancreas was 11.5 ± 0.5 and of the distal unablated pancreas was 16.3 ± 2.05 (Fig. 3.4.7). All specimens in this group showed evidence of localised tissue necrosis and acute inflammatory change, with infiltration of polymorphs and monocytes, at the site of electrolytic ablation (Fig. 3.4.8). Histological examination of the pancreas distal to the ablation site also demonstrated evidence of parenchymal necrosis and inflammatory infiltration. The portion of the pancreas proximal to the site of electrolysis was essentially normal. Statistical analysis of the histological scores for the three sections of pancreas examined from the five animals in this group was performed using analysis of variance. This showed that the histological scores for the proximal unablated pancreas were significantly lower than the scores for both the ablation lesion and the distal pancreas ($p<0.05$). There was no significant difference between scores for the ablation lesion and the distal pancreas ($p>0.05$).

3.4.3.2 Two-week control group

The mean histological score at the site of electrolysis was 12.6 ± 3.03; the mean score of the proximal unablated pancreas was 12.9 ± 2.88 and of the distal unablated pancreas was 11.1 ± 0.22 (Fig. 3.4.7). In four of the five animals in the control group there was no evidence of necrosis, of either fat or parenchyma, in any of the three areas of the pancreata that were examined. In the remaining animal however moderate fat necrosis and sub-lobular parenchymal necrosis was noted at the site of the electrode tip and in the proximal portion of the gland (Fig. 3.4.9). In the distal sections of the pancreata no inflammatory infiltrate or necrosis was detected, although one specimen showed moderate oedema. All the other sections of distal pancreas in this group were

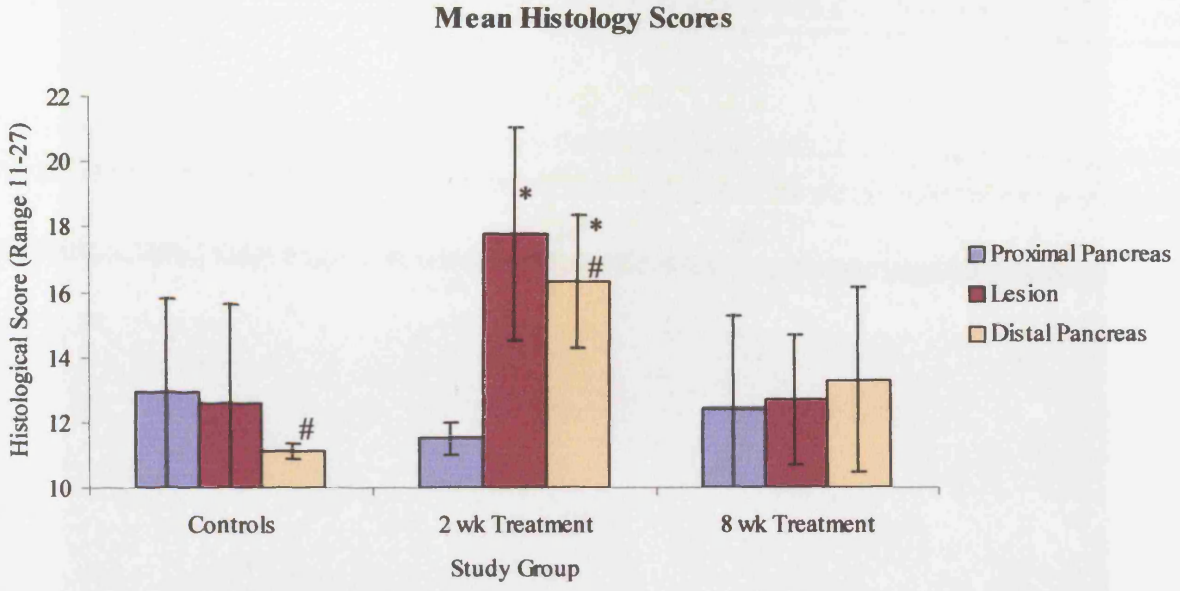

Figure 3.4.7: Mean histological scores, with standard deviations, for each experimental group. (* denotes significant [$p<0.05$] difference between scores within a group, # denotes significant [$p<0.05$] difference between scores for a region of pancreas between groups.)

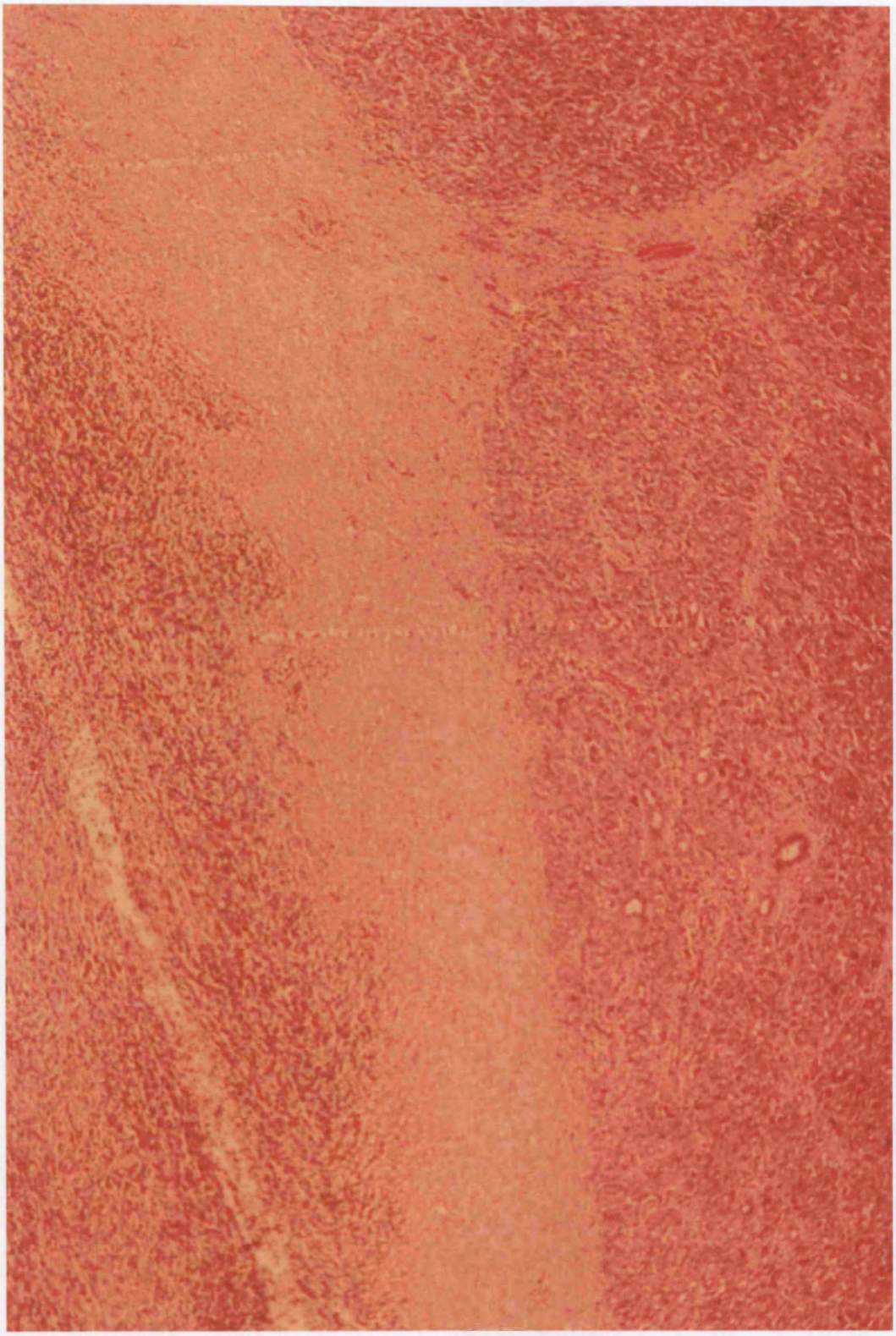

Figure 3.4.8: Two-week treatment group: histological section of ablation lesion.

(H&E x4)

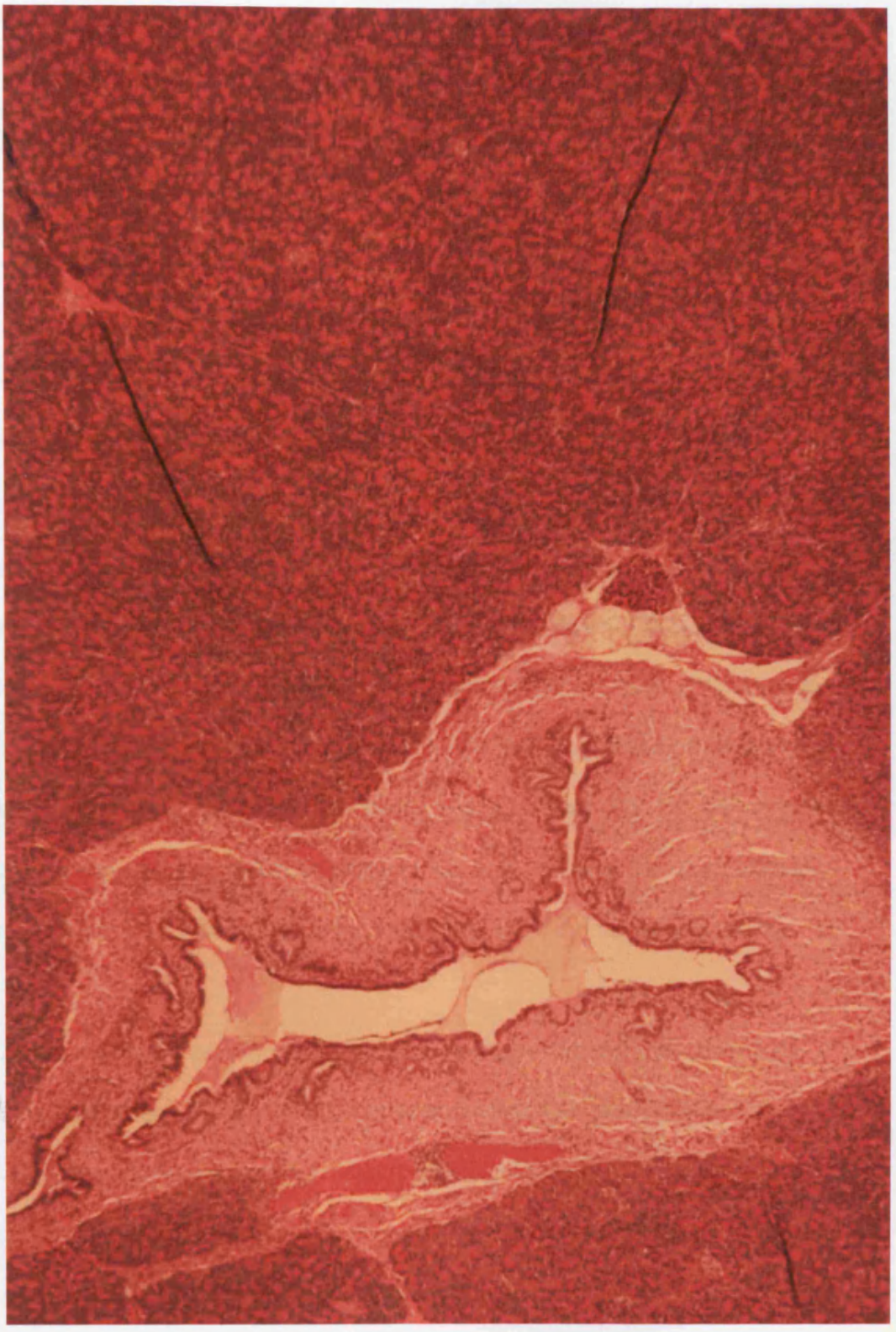

Figure 3.4.9: Two-week control group: histological section of proximal pancreas.

(H&E x4)

scored as '11', the lowest score available using this system. Statistical analysis of these data found there to be no significant difference in histological score between any of the three areas of pancreas examined ($p>0.05$).

3.4.3.3 Eight-week treatment group

The mean histological score at the site of electrolysis was 12.7 ± 1.99; the mean score of the proximal unablated pancreas was 12.4 ± 2.86 and of the distal unablated pancreas was 13.3 ± 2.86 (Fig. 3.4.7). Oedema and inflammatory infiltrate where both scored as 'minimal' the lowest score available, in all sections from this experimental group. However, there was evidence of parenchymal necrosis both at the site of ablation and in the distal pancreas. In the histological sections through the ablation lesion there was predominately scarring of the parenchyma. In the sections of distal pancreas there was evidence of ductal proliferation in conjunction with parenchymal scarring that resembled chronic pancreatitis in appearance (Fig. 3.4.10). As with the control group no statistically significant differences between the three pancreatic regions was found on analysing the data using analysis of variance ($p>0.05$).

The histological scores for each region of pancreas examined were statistically compared between the control group, the two-week treatment group and the eight-week treatment group using analysis of variance. There was no significant difference between scores for the proximal pancreas or the ablation site between the three experimental groups ($p>0.05$). However the histological scores for the distal section of pancreas in the control group was significantly less ($p<0.005$) than the scores for the distal pancreas in the two-week treatment group. There were no other significant

Figure 3.4.10: Eight-week treatment group: histological section of scarred pancreatic parenchyma. (H&E x4)

differences between the experimental group scores for the distal region of the pancreas ($p>0.05$).

3.4.4 Biochemistry

The biochemical data from all animals, other than the pig with the ceacal perforation, was reviewed. The data, for each experimental group, was analysed and compared between groups using analysis of variance.

3.4.4.1 Amylase

A post-operative increase in mean serum amylase concentration was noted in all three experimental groups, peaking on the second post-operative day in all groups (Fig. 3.4.11 & Table 3.4.1). Although these peak concentrations were significantly greater ($p<0.05$) than the pre-operative amylase concentration no statistically significant difference was demonstrated between the three peak concentrations themselves ($p>0.05$). Statistical analysis of the data at the other time-points also failed to show any significant differences between either of the treatment groups and the control group ($p>0.05$). The mean amylase concentrations returned towards normal after the 48-hour peak in all the groups. The eight-week survivor group mean amylase concentration returned to levels that were not significantly different from the pre-operative figure by the third post-operative day ($p>0.05$). In the other two groups there was a more fluctuating decrease in mean serum amylase. However, by the end of the initial two-week post-operative period there was no significant difference in the mean amylase concentration, in any of the three experimental groups, and the corresponding pre-operative level ($p>0.05$).

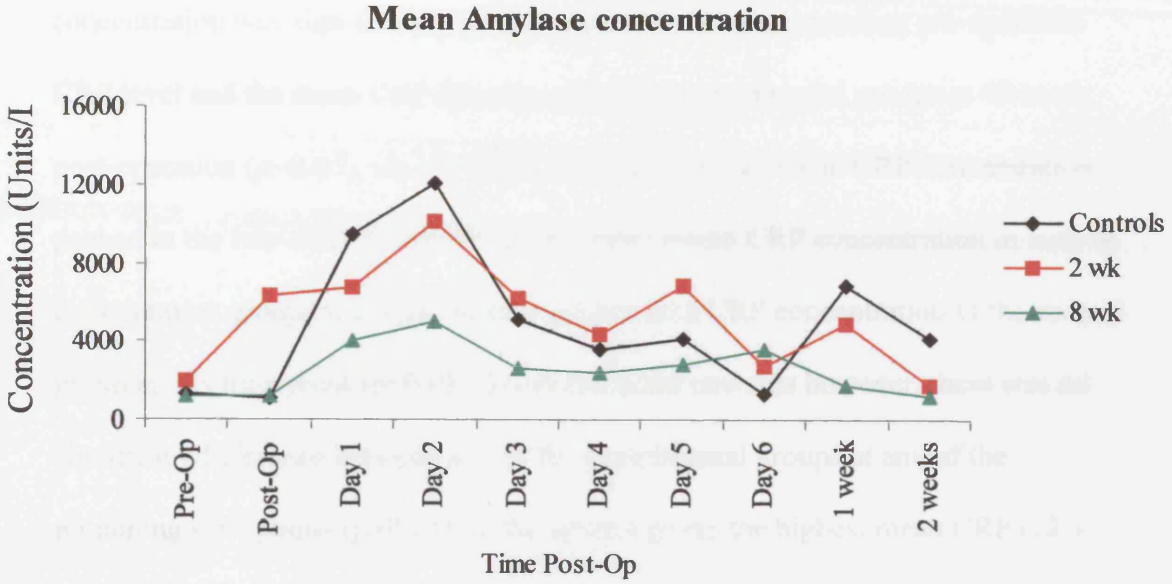

Figure 3.4.11: Mean post-operative serum amylase for each experimental group.

3.4.4.2 C-Reactive Protein (CRP)

There was a post-operative increase in serum CRP in all animals in the early post-operative period (Fig. 3.4.12 & Table 3.4.2). In the eight-week survival group this peaked at 48-hours post-electrolysis. Statistical analysis showed this peak concentration was significantly greater than both the corresponding pre-operative CRP level and the mean CRP from the other two experimental groups at 48-hours post-operation ($p<0.05$). On the third post-operative day mean CRP concentration peaked in the two-week treatment group; mean serum CRP concentration in both of the treatment groups was significantly greater than CRP concentration in the control group at this time point ($p<0.05$). From this point onwards however, there was no significant difference between any of the experimental groups at any of the remaining time points ($p>0.05$). In the control group the highest mean CRP (12.3 mg/L) was detected on the seventh post-operative day, however this high mean concentration was mainly due to a single CRP level of 26 in one animal, as is demonstrated by the wide standard deviation of 12.3, although this was show to be significantly greater ($p<0.05$) than the pre-operative mean CRP concentration in the control group it was not significantly different from either of the two treatment groups ($p>0.05$).

3.4.4.3 Leukocyte count

In both treatment groups the white cell count peaked on the second post-operative day (Fig. 3.4.13 & Table 3.4.3). Statistical analysis showed that these peaks at 48 hours were significantly greater than the pre-operative concentrations and also the mean white cell count in the control group at 48-hours post surgery ($p<0.05$). The only other time point at which the mean white cell count in either of the treatment

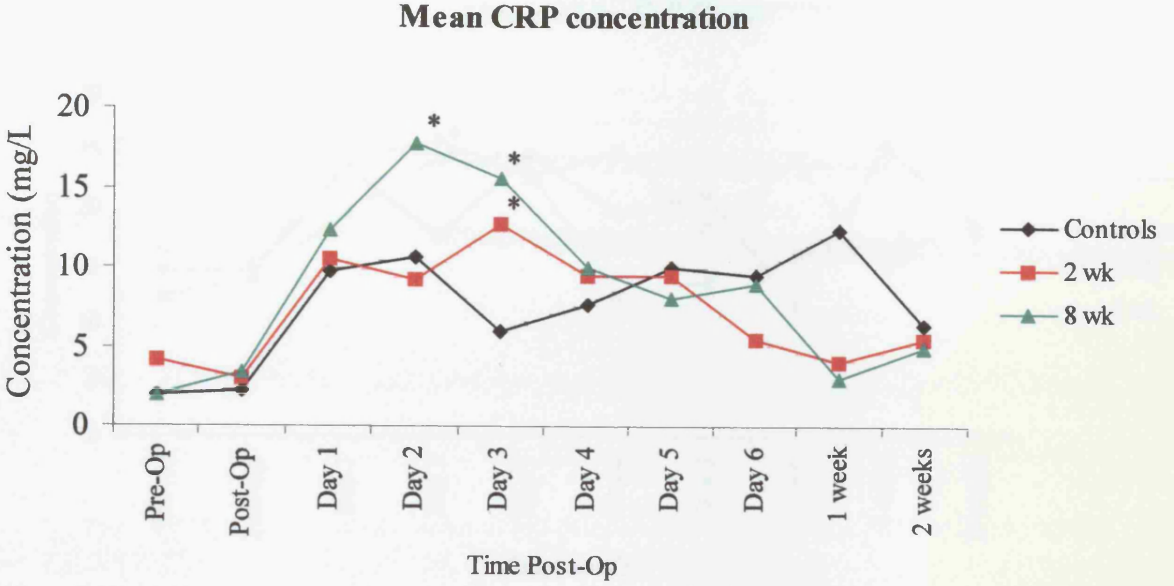

Figure 3.4.12: Mean post-operative C-Reactive Protein for each experimental group.
(* denotes significant [$p<0.05$] difference from control group.)

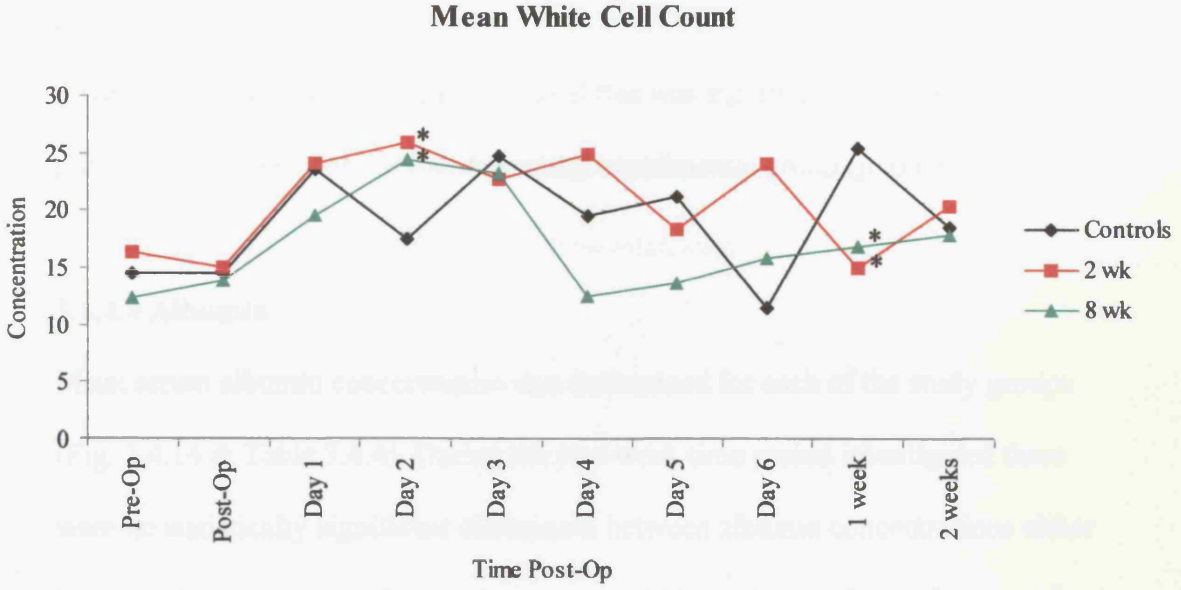

Figure 3.4.13: Mean post-operative leukocyte count for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

groups was significantly different from the control group was at 1-week post-operatively. At this time point the mean white cell count was significantly greater in the control group (mean 24.2 ± 10.1) than the mean counts in either of the treatment groups ($p<0.05$). By the end of the second post-operative week the mean white cell count in only the

8-week survival group remained at a level that was significantly greater than the pre-operative figure for the corresponding experimental group ($p<0.05$).

3.4.4.4 Albumin

Mean serum albumin concentration was determined for each of the study groups (Fig. 3.4.14 & Table 3.4.4). During the two-week time period investigated there were no statistically significant differences between albumin concentrations either between the treatment and control groups or within each experimental group when comparing pre and post-operative albumin concentrations ($p>0.05$).

3.4.4.5 Glucose

Serum glucose concentration data were examined and the mean concentrations in each group were calculated (Fig. 3.4.15 & Table 3.4.5). Statistical analysis of this data showed there to be no difference between glucose concentration in the control group and the 8-week treatment group at any of the investigated time points ($p>0.05$). However the glucose concentration in the 2-week treatment group on the sixth post-operative day was found to be significantly lower than the glucose concentration in both the control group and the 8-week treatment group ($p<0.05$).

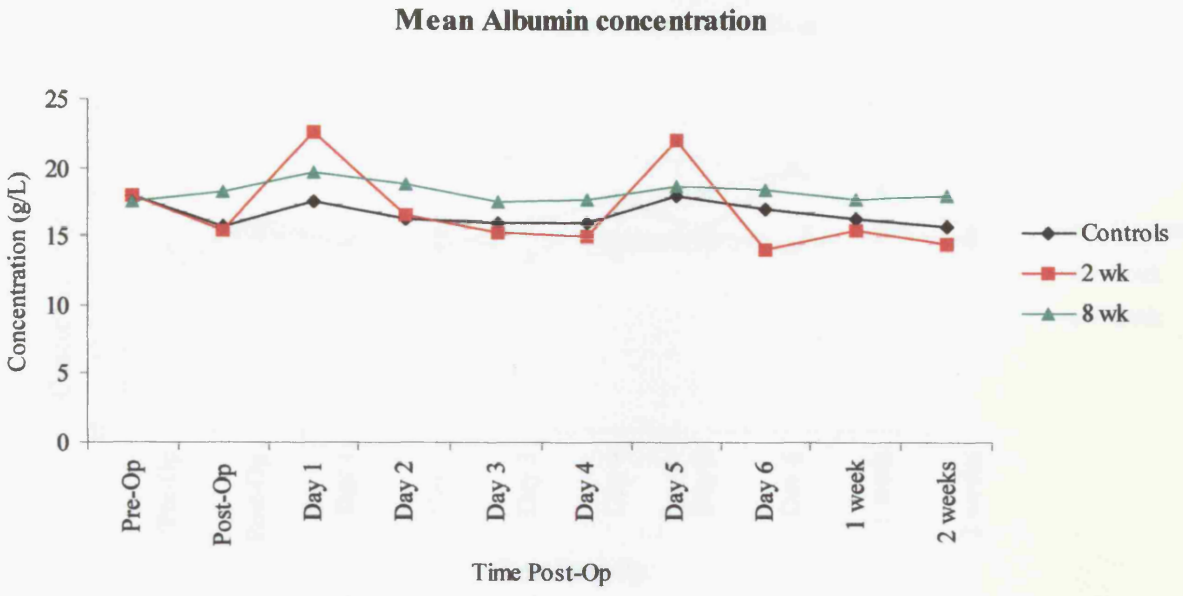

Figure 3.4.14: Mean post-operative albumin concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

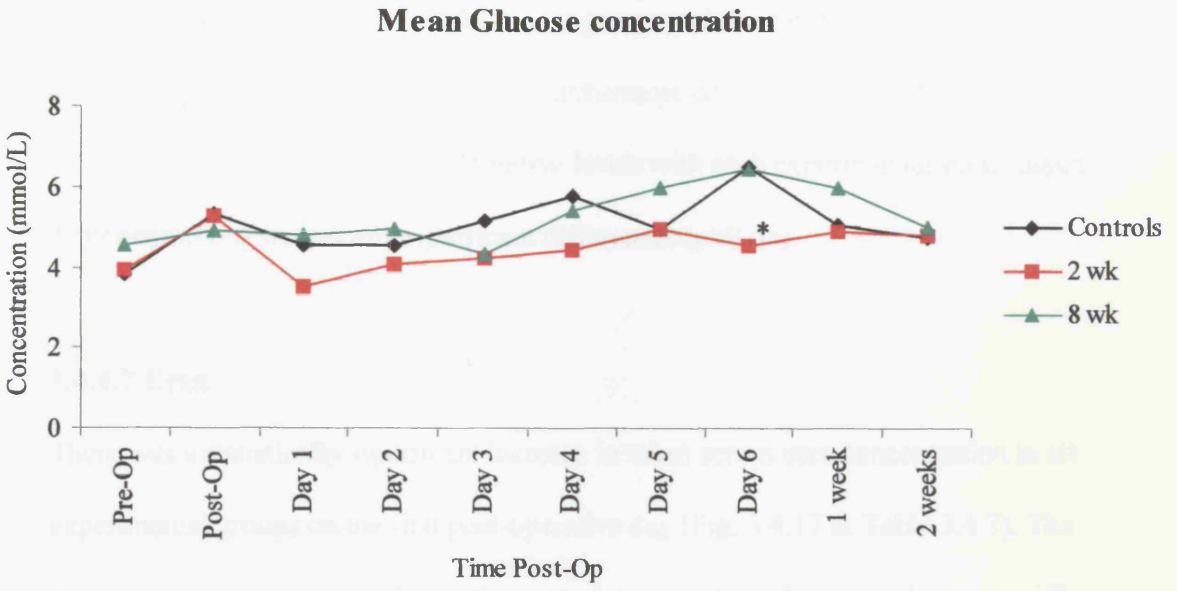

Figure 3.4.15: Mean post-operative glucose concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

3.4.4.6 Calcium

The post-operative mean calcium concentration fluctuated during the post-operative period in all three experimental groups between 2.2 and 2.55 mmol/L (Fig. 3.4.16 & Table 3.4.6). Statistical analysis of the data showed there to be no significant differences between either of the treatment groups and the control group at any time point during the study period ($p>0.05$). Furthermore comparison of post-operative calcium concentrations with pre-operative levels with each experimental group again demonstrated no statistically significant differences ($p>0.05$).

3.4.4.7 Urea

There was a statistically significant increase in mean serum urea concentration in all experimental groups on the first post-operative day (Fig. 3.4.17 & Table 3.4.7). The mean serum urea concentration in the control group returned to normal more rapidly than the treatment groups; however, there was no significant difference between any of the three groups during the study period ($p>0.05$). The greatest mean serum urea concentration detected during the experiment was in the 8-week treatment group on the fourth post-operative day. This peak of 8.3 mmol/L was predominately due to a urea reading of 18.6 mmol/L in one of the animals, the standard deviation at this point is 8.6 mmol/L.

Other biochemical parameters, such as sodium, potassium, creatinine and bicarbonate concentrations, were also determined and statistically analysed for both the treatment and control groups. There were no significant changes in the mean serum levels of any of these substances during of the study, either between or within groups ($p>0.05$). As a consequence these data will not be addressed individually and in further detail.

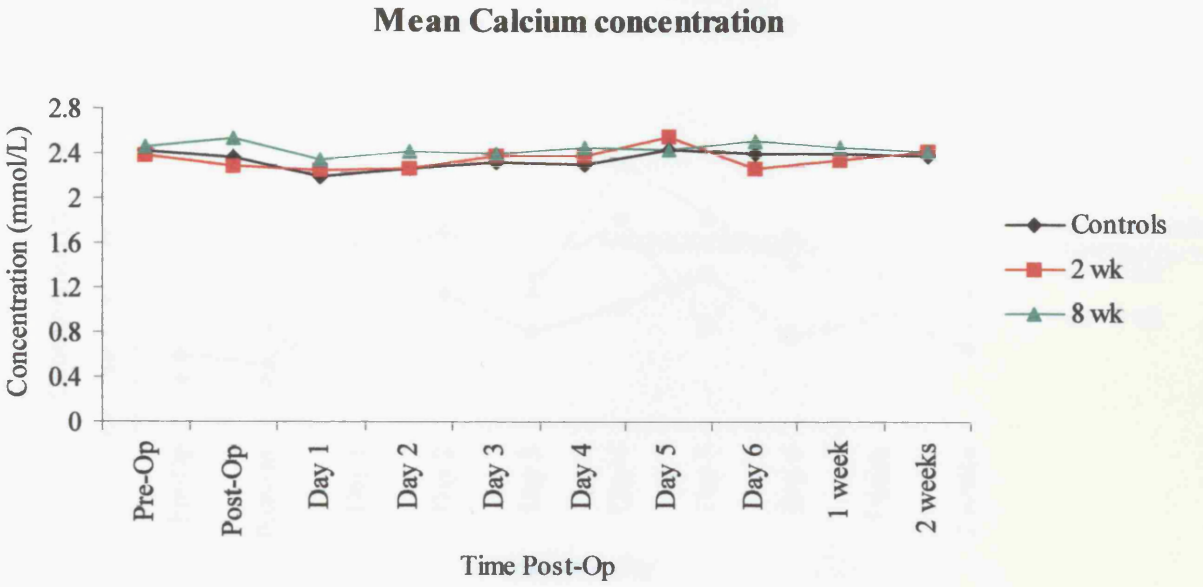

Figure 3.4.16: Mean post-operative calcium concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

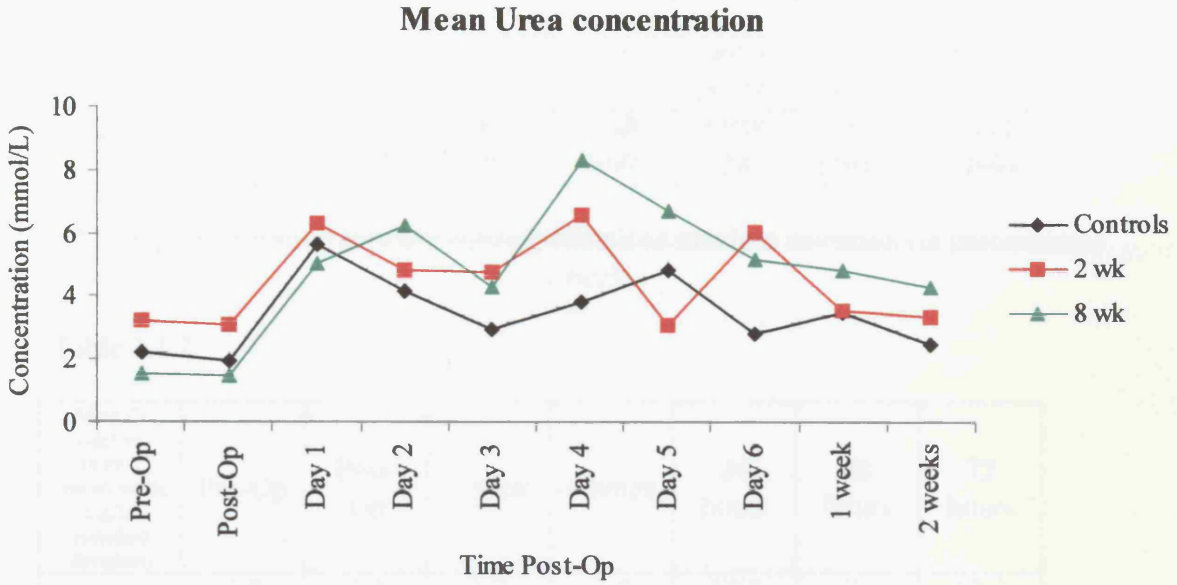

Figure 3.4.17: Mean post-operative urea concentration for each experimental group. (* denotes significant [$p<0.05$] difference from control group.)

Tables (3.1) Biochemical Data Phase 1a

Table 3.1.1

Mean amylase concentration, units/L. (standard deviation)	Pre-Op	Post-Op	1 hour	4 hours	24 hours	48 hours	72 hours
Controls	2418 (978)	2579 (940)	2362 (633)	3245 (918)	9954 (5378)	14195 (8215)	8663 (8052)
2-week Treatment	1481 (687)	1767 (767)	1826 (593)	2546 (906)	6838 (3242)	5336 (2012)	2434 (799)

Mean post-operative amylase concentration and standard deviation (in parentheses) units/L.

Table 3.1.2

Mean C-reactive protein concentration, mg/L. (standard deviation)	Pre-Op	Post-Op	1 hour	4 hours	24 hours	48 hours	72 hours
Controls	1.7 (1.6)	2.3 (2.1)	3.2 (1.5)	4.0 (0.6)	14.5 (3.9)	13.3 (4.5)	12.5 (2.9)
2-week Treatment	3.7 (5.2)	4.3 (5.4)	5.2 (6.8)	4.3 (5.4)	14.3 (2.6)	18.8 (3.9)	15 (4.7)

Mean post-operative C-reactive protein concentration and standard deviation (in parentheses) mg/L.

Table 3.1.3

Mean albumin concentration, g/L. (standard deviation)	Pre-Op	Post-Op	1 hour	4 hours	24 hours	48 hours	72 hours
Controls	34.2 (10.0)	36.3 (4.2)	36.8 (3.2)	33.5 (9.5)	33.2 (10.3)	34 (1.0)	34.2 (1.7)
2-week Treatment	29.8 (8.6)	30.7 (9.2)	32.7 (3.8)	33.2 (2.3)	33.2 (3.7)	25.8 (11.8)	31.2 (4.1)

Mean post-operative albumin concentration and standard deviation (in parentheses) g/L.

Table 3.1.4

Mean glucose concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	1 hour	4 hours	24 hours	48 hours	72 hours
Controls	4.3 (0.8)	4.4 (1.4)	6.0 (1.9)	8.3 (3.7)	5.9 (0.7)	6.1 (0.3)	6.0 (0.4)
2-week Treatment	5.1 (1.2)	6.7 (3.1)	8.2 (1.7)	6.6 (0.4)	5.1 (0.7)	5.0 (1.4)	5.6 (1.3)

Mean post-operative glucose concentration and standard deviation (in parentheses) mmol/L.

Table 3.1.5

Mean calcium concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	1 hour	4 hours	24 hours	48 hours	72 hours
Controls	2.7 (0.1)	2.7 (0.1)	2.6 (0.1)	2.5 (0.1)	2.4 (0.1)	2.6 (0.1)	2.5 (0.0)
2-week Treatment	2.6 (0.1)	2.6 (0.2)	2.6 (0.1)	2.5 (0.1)	2.3 (0.1)	2.4 (0.1)	2.5 (0.2)

Mean post-operative total calcium concentration and standard deviation (in parentheses) mmol/L.

Table 3.1.6

Mean urea concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	1 hour	4 hours	24 hours	48 hours	72 hours
Controls	2.7 (0.3)	3.5 (0.5)	3.6 (0.4)	4.7 (0.3)	4.7 (0.6)	4.7 (0.4)	3.5 (1.0)
2-week Treatment	2.2 (0.6)	2.9 (0.9)	3.1 (0.5)	4.3 (1.0)	5.2 (0.7)	5.9 (1.8)	4.8 (1.1)

Mean post-operative urea concentration and standard deviation (in parentheses) mmol/L.

Tables (3.2) Biochemical Data Phase 1b

Table 3.2.1

Mean amylase concentration, units/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
2-week Treatment	1343 (442)	1733 (548)	8219 (3783)	6000 (856)	3130 (776)	2261 (408)	2713 (1424)	2913 (2333)	2888 (2427)	2558 (945)
8-week Treatment	1910 (777)	2574 (180)	11917 (2222)	7243 (2041)	3072 (838)	1849 (642)	1203 (930)	2663 (2283)	2071 (1661)	1693 (742)
Combined Groups	1626 (707)	2153 (528)	10068 (3594)	6621 (1854)	3101 (728)	2025 (610)	1707 (1238)	2746 (2152)	1969 (1661)	2125 (769)

Mean post-operative amylase concentration and standard deviation (in parentheses) units/L.

Table 3.2.2

Mean C-reactive protein concentration, mg/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
2-week Treatment	1.5 (0.6)	1.8 (1.5)	12.0 (1.8)	12.5 (2.5)	10.0 (3.8)	10.7 (2.5)	10.7 (2.5)	9.5 (0.7)	5.7 (4.2)	7.3 (9.2)
8-week Treatment	2.0 (0.8)	1.3 (0.5)	16.8 (3.0)	18.3 (5.9)	12.3 (6.2)	8.5 (3.1)	10.3 (5.6)	11.0 (5.7)	9.5 (6.2)	7.3 (5.9)
Combined Groups	1.8 (0.7)	1.5 (1.1)	14.4 (3.6)	15.4 (5.5)	11.1 (5.3)	9.4 (3.1)	10.4 (4.3)	10.5 (5.0)	7.9 (5.9)	7.3 (4.9)

Mean post-operative C-reactive protein concentration and standard deviation (in parentheses) mg/L.

Table 3.2.3

Mean albumin concentration, g/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
2-week Treatment	34.5 (6.0)	33.3 (7.1)	36.3 (8.4)	29.5 (12.2)	26.8 (6.8)	16.3 (5.3)	11.5 (5.4)	29.3 (6.2)	28.3 (4.9)	24.8 (3.3)
8-week Treatment	35.3 (3.4)	31.5 (2.1)	33.0 (1.6)	18.7 (8.1)	32.5 (4.4)	27.7 (13.3)	14.0 (0.8)	25.0 (9.6)	29.5 (7.8)	31.7 (1.5)
Combined Groups	34.9 (4.8)	32.4 (5.8)	34.6 (6.8)	24.9 (11.3)	29.6 (5.7)	21.1 (8.0)	12.8 (4.8)	27.4 (4.9)	28.7 (4.9)	27.7 (4.2)

Mean post-operative albumin concentration and standard deviation (in parentheses) g/L.

Table 3.2.4

Mean glucose concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
2-week Treatment	6.9 (1.0)	4.1 (0.7)	5.1 (1.4)	4.8 (0.6)	5.2 (0.4)	5.3 (0.3)	5.1 (0.5)	5.2 (1.4)	5.6 (1.7)	4.9 (1.0)
8-week Treatment	5.9 (3.6)	3.5 (0.3)	3.3 (0.6)	4.3 (0.8)	5.0 (0.4)	5.3 (0.5)	5.2 (0.8)	5.1 (0.4)	5.6 (0.4)	5.2 (0.3)
Combined Groups	4.9 (1.3)	6.4 (1.7)	3.8 (0.6)	4.2 (1.6)	4.6 (0.7)	5.1 (0.5)	5.3 (0.3)	5.1 (0.5)	5.2 (1.4)	5.0 (0.8)

Mean post-operative glucose concentration and standard deviation (in parentheses) mmol/L.

Table 3.2.5

Mean calcium concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
2-week Treatment	2.7 (0.12)	2.6 (0.18)	2.5 (0.22)	2.5 (0.28)	2.4 (0.10)	2.4 (0.19)	2.4 (0.18)	2.5 (0.13)	2.5 (0.09)	2.5 (0.11)
8-week Treatment	2.8 (0.07)	2.7 (0.08)	2.3 (0.05)	2.3 (0.08)	2.5 (0.13)	2.4 (0.14)	2.5 (0.03)	2.7 (0.28)	2.6 (0.30)	2.6 (0.07)
Combined Groups	2.7 (0.10)	2.6 (0.15)	2.4 (0.18)	2.4 (0.24)	2.4 (0.09)	2.4 (0.17)	2.4 (0.14)	2.6 (0.13)	2.5 (0.09)	2.5 (0.12)

Mean post-operative total calcium concentration and standard deviation (in parentheses) mmol/L.

Table 3.2.6

Mean urea concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
2-week Treatment	2.4 (0.8)	2.4 (0.8)	4.9 (1.2)	12.5 (8.4)	13.0 (11.0)	7.4 (3.1)	4.5 (2.7)	4.5 (2.4)	4.8 (1.8)	3.4 (1.3)
8-week Treatment	2.0 (0.4)	2.1 (0.5)	5.5 (0.8)	4.6 (0.8)	4.6 (1.9)	3.7 (1.4)	2.2 (0.3)	3.8 (1.3)	3.8 (2.1)	2.8 (1.3)
Combined Groups	2.0 (0.7)	2.0 (0.6)	5.3 (1.1)	8.5 (7.7)	8.8 (9.7)	5.6 (3.2)	3.5 (2.3)	4.2 (2.1)	4.5 (1.8)	3.1 (1.2)

Mean post-operative urea concentration and standard deviation (in parentheses) mmol/L.

Tables (3.4) Biochemical Data Phase 2:

Table 3.4.1

Mean amylase concentration, units/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
Controls	1254 (457)	1057 (326)	9462 (4818)	12090 (6421)	5133 (2085)	3555 (3197)	4140 (4743)	1253 (495)	6894 (7419)	4139 (4622)
2-week Treatment	1978 (1594)	6286 (11649)	6793 (5292)	10089 (5495)	6223 (4789)	4351 (2943)	6840 (4025)	2668 (1191)	4871 (2254)	1693 (1095)
8-week Treatment	1200 (572)	1210 (410)	4072 (2494)	5008 (2486)	2603 (1579)	2345 (2043)	2871 (2564)	3597 (3510)	1786 (1614)	1208 (144)

Mean post-operative amylase concentration and standard deviation (in parentheses) units/L.

Table 3.4.2

Mean C-reactive protein concentration, mg/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
Controls	2.0 (0.0)	2.2 (0.4)	9.8 (1.3)	10.7 (3.2)	6.0 (4.0)	7.7 (4.9)	10.0 (9.8)	9.5 (2.1)	12.3 (12.3)	6.4 (6.1)
2-week Treatment	4.2 (3.8)	3.0 (1.7)	10.5 (4.4)	9.2 (5.9)	12.8 (4.5)	9.5 (6.4)	9.5 (6.4)	5.5 (4.9)	4.0 (0.0)	5.4 (4.5)
8-week Treatment	2.0 (0.0)	3.4 (1.9)	12.4 (3.2)	17.8 (1.9)	15.6 (2.7)	10.0 (1.7)	8.0 (2.2)	9.0 (6.9)	3.0 (1.4)	5.0 (1.0)

Mean post-operative C-reactive protein concentration and standard deviation (in parentheses) mg/L.

Table 3.4.3

Mean leukocyte concentration, 10^9/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
Controls	14.5 (2.8)	14.4 (3.0)	23.5 (6.4)	17.6 (2.5)	24.7 (9.8)	19.6 (3.5)	21.3 (3.5)	11.5 (0.0)	25.4 (5.2)	18.5 (8.3)
2-week Treatment	16.4 (1.9)	15.0 (1.0)	24.1 (2.5)	26.0 (1.8)	22.7	25.0	18.3 (10.1)	24.2 (0.9)	15.0	20.4 (2.8)
8-week Treatment	12.3 (1.9)	13.8 (5.4)	19.6 (1.8)	24.5 (5.3)	23.3 (1.2)	12.4	13.7 (3.5)	15.9 (3.7)	16.9 (2.3)	17.9 (0.7)

Mean post-operative leukocyte concentration and standard deviation (in parentheses) 10^9/L.

Table 3.4.4

Mean albumin concentration, g/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
Controls	18.0 (1.9)	15.8 (2.1)	17.6 (2.1)	16.3 (2.2)	16.0 (1.8)	16.0 (1.7)	18.0 (3.0)	17.0 (1.4)	16.3 (0.6)	15.8 (1.9)
2-week Treatment	18.0 (1.9)	15.5 (2.1)	22.6 (11.6)	16.6 (2.1)	15.3 (3.0)	15.0 (2.8)	22.0 (14.1)	14.0 (1.4)	15.5 (3.5)	14.4 (1.9)
8-week Treatment	17.6 (2.8)	18.2 (1.3)	19.6 (2.1)	18.8 (1.9)	17.5 (1.3)	17.7 (2.1)	18.8 (2.2)	18.3 (0.6)	17.7 (1.2)	18.0 (1.6)

Mean post-operative albumin concentration and standard deviation (in parentheses) g/L.

Table 3.4.5

Mean glucose concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
Controls	3.8 (1.5)	5.3 (3.0)	4.5 (0.4)	4.6 (1.1)	5.2 (0.8)	5.8 (0.5)	5.0 (0.6)	6.5 (1.0)	5.1 (1.0)	4.8 (1.0)
2-week Treatment	3.9 (1.6)	5.2 (4.2)	3.5 (0.2)	4.1 (0.8)	4.2 (0.3)	4.5 (0.2)	5.0 (0.8)	4.6 (0.5)	4.9	4.8 (0.5)
8-week Treatment	4.5 (1.2)	4.9 (0.3)	4.8 (0.4)	5.0 (2.0)	4.4 (0.8)	5.4 (1.2)	6.0 (1.3)	6.4 (1.4)	6.0 (1.6)	5.0 (0.9)

Mean post-operative glucose concentration and standard deviation (in parentheses) mmol/L.

Table 3.4.6

Mean calcium concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
Controls	2.4 (0.13)	2.4 (0.22)	2.2 (0.09)	2.3 (0.14)	2.3 (0.11)	2.3 (0.21)	2.4 (0.33)	2.4 (0.29)	2.4 (0.13)	2.4 (0.13)
2-week Treatment	2.4 (0.09)	2.3 (0.15)	2.3 (0.14)	2.3 (0.10)	2.4 (0.06)	2.4 (0.02)	2.5 (0.09)	2.3 (0.19)	2.3	2.4 (0.08)
8-week Treatment	2.5 (0.12)	2.5 (0.09)	2.3 (0.09)	2.4 (0.09)	2.4 (0.09)	2.5 (0.06)	2.4 (0.07)	2.5 (0.09)	2.5 (0.14)	2.4

Mean post-operative total calcium concentration and standard deviation (in parentheses) mmol/L.

Table 3.4.7

Mean urea concentration, mmol/L. (standard deviation)	Pre-Op	Post-Op	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	1 week	2 weeks
Controls	2.2 (0.8)	2.0 (0.3)	5.6 (0.6)	4.2 (0.6)	3.0 (0.5)	3.8 (0.9)	4.8 (0.3)	2.9 (0.8)	3.5 (0.7)	2.5 (1.1)
2-week Treatment	3.2 (1.4)	3.1 (1.6)	6.3 (0.4)	4.8 (0.7)	4.8 (1.6)	6.6 (4.6)	3.1 (0.40	6.1 (4.3)	3.6 (0.6)	3.4 (0.7)
8-week Treatment	1.5 (0.5)	1.5 (0.4)	5.0 (0.7)	6.2 (4.7)	4.3 (2.9)	8.3 (8.6)	6.7 (4.0)	5.2 (1.4)	4.8 (2.7)	4.3 (1.0)

Mean post-operative urea concentration and standard deviation (in parentheses) mmol/L.

Chapter 4: Discussion and Conclusion

4.1 Summary of Results

The feasibility of delivering an electrolytic ablation 'dose' to the porcine pancreas, via the pancreatic duct, has been shown both at an 'open' approach and at a 'minimally invasive endoscopic' approach. In both phases of this study pancreatic electrolysis was performed safely and without serious adverse effects on the outcome of the animal.

Five pigs died prematurely in the course of this study. In phase 1a one pig, randomised to the control group, died whilst under general anaesthetic and was subsequently replaced and the associated data excluded. In phase 1b, two animals died at 7 and 8 days respectively with gross gastric dilatation and a further animal was prematurely killed at 8 days following a catastrophic upper gastro-intestinal haemorrhage. This phenomenon of gastric dilatation and subsequent haemorrhage from a mucosal lesion at the pars oesophagea has been widely reported in both surgical and veterinarian literature (276-278, 284). In many experimental series using pig models there is a documented attrition rate amongst the animals due to massive gastro-intestinal haemorrhage, although in a considerable proportion of these studies detailed post-mortem examination of the animals has not been performed. Interestingly there are reports of this phenomenon in pigs who have not undergone surgical procedures, especially where the animals are intensively reared or subjected to unusual levels of excitation and stimulation (284, 285). These lesions, although almost certainly multifactorial in nature, are predominately a result of traction at the mucosal junction at the pars oesophagea leading to a 'de-gloving' type mucosal injury. The combination

of post-operative ileus, possible over indulgence by the pigs and the oblique nature of the gastro-oesophageal junction, with a flap-like mucosal valve, are the likely causative factors in the development and progression of this complication. A number of experiments performed in conjunction with colleagues working on other research projects using porcine model produced some interesting results. In an effort to replicate the 'traction and de-gloving' model in producing these mucosal defects the following experiment was performed. At the end of the appropriate survival period the animals were re-anaesthetised and the stomach was isolated on its vascular supply. The oesophagus and duodenum were divided, into these a tube attached to a manometer and a rigid laparoscope was secured respectively. Insufflation of air resulted in distension of the stomachs and at a pressure of 80-100 mmHg mucosal tearing at the pars oesophagea was noted. The animals were then killed and at post-mortem examination their stomachs were removed in addition to the pancreas and other specimens. Examination of the stomachs confirmed the presence of mucosal lesions in the region of the pars oesophagea that were similar in distribution to the 'ulcers' noted in the animals which had experienced the gastric haemorrhage. This replication of the mucosal injury suggested that physical decompression of the stomach in the post-operative period might well be beneficial in combating this invariably fatal complication. As outlined in the previous sections of this thesis a policy of routine insertion of a nasogastric tube into the stomach via an oesophagostomy was adopted at the time of initial operation. This gastric decompression was found to considerably reduce the incidence of this complication and further supports the underlying hypothesis of aetiology. The three animals in which this phenomenon was identified were replaced and the related data excluded from further analysis. In the second phase of the study another animal was prematurely sacrificed on the fifth post-operative day.

Post-mortem examination of this animal revealed a ceacal perforation, as this lesion was distant from the site of electrolytic ablation, it was concluded that this complication was unlikely to be a direct result of the ablative process. As in the first phase of the study this animal was replaced and associated data excluded.

The thirty-five pigs (11 controls and 24 treatments) that successfully underwent electrolytic ablation of the pancreas were all mobile, alert and eating and drinking normally by the third post-operative day.

A transient hyperamylasaemia was evident in all animals, both treatments and controls, in all phases of this study. However, as has been shown in phases 1a and 2 there is no statistically significant difference between mean amylase concentrations between treatment and control groups. This finding suggests that this is most likely caused by cannulation of the pancreatic duct, either open or endoscopically, rather than as a result of electrolytic ablation of pancreatic tissue. Similarly, the increase in post-operative C-reactive protein concentration and leukocyte count, evident in both control and treatment groups, suggests that these are in part a result of invasive surgery rather than being solely attributable to pancreatic ablation. Although there was no significant differences between controls and treatment groups in phase 1a, in phase 2 of this project, post-operative mean CRP levels and mean leukocyte counts were significantly greater in the treatment groups than in the control group on the 2^{nd} and 3^{rd} day and 2^{nd} day respectively. Interestingly also in phase 2 of the study mean leukocyte concentration was significantly greater, at 7 days post-operation, in the control group than it was in either of the treatment groups. The lack of significant differences in post-operative cytokine concentrations between treatment and control groups and with

respect to pre-operative levels in phases 1a and 1b add further evidence to suggest that the process of electrolytic ablation of pancreatic tissue is safe and not inherently associated with risks of generalised acute pancreatitis or the systemic inflammatory response.

Localised complications such as peri-pancreatic collections are most likely due to a combination of factors. Firstly the electrolytic coulomb doses used in this study were extrapolated from dose-volume data generated by previous experimental studies into the use of electrolysis in ablation of the porcine liver (286). Interestingly the ablation volume per coulomb appears to be greater in the pancreas than it is in the liver. The mechanism resulting in this difference in ablation volume may be the higher concentration of electrolytes and ions within the pancreas as compared to the liver. These may contribute to an 'electrolyte soup' which allows the current from electrolysis to flow more readily and thereby leads to a larger ablation lesion. Another possibility is that the electrolytic destruction of pancreatic acinar tissue results in the release of pancreatic proteolytic enzymes into the electrolysis ablation lesion and that it is the combination of proteolytic action of these enzymes in addition to the cytotoxic action of the electrolytic process that produces the increase ablation volume per coulomb dose that has been noted in this study. The result of this was that the electrolytic ablation lesion breached the pancreatic capsule in a proportion of the animals, thereby increasing the likelihood of peripancreatic collection formation. In addition to this the anatomical morphology of the pig pancreas is such that there is no region of the porcine gland similar to the head of pancreas as found in humans. Therefore, a greater cannulation depth of about 10cm was required to enable the electrolytic dose to be delivered to an area of pancreas of equivalent proportions to the

human pancreatic head. Consequently drainage of necrotic products of ablation may have been compromised by the distance between the ablation lesion and the duodenum. In the clinical setting however, the use of electrolysis as an ablative technique would be envisaged most commonly in the pancreatic head as this is the region of the gland in which most tumours arise (59). The greater proximity of such ablation lesions to the duodenum would allow ready drainage of any necrotic products following electrolytic ablation. In addition to this the placement of stent across the ablation site would also be anticipated to facilitate the rapid drainage of necrotic material.

Histological analysis of the ablation lesion and sections of unablated pancreas, both proximal and distal to the site of ablation, using an established scoring system for experimental pancreatitis enabled a semi-quantative assessment to be made of the impact of pancreatic electrolysis on the histological architecture of the pancreas. The most consistent finding in all phases of this study was the presence of marked acute inflammation and necrosis at the site of ablation in the early post-operative period (3-14 days.) Importantly this acute inflammatory response appears to be localised to the site of electrolysis as there were no incidences of generalised acute pancreatitis and also self-limiting in nature as the early acute inflammatory changes were replaced by scarring in the eight-week survival groups. The absence of marked inflammatory change in the proximal sections of unablated pancreas further supports this. In the second phase of this study, the mean histological score for distal sections of pancreas at two-weeks post-electrolysis were significantly greater than the score for the proximal unablated pancreas in the same group and the histological scores for the distal pancreas in the control group. The histological findings of parenchymal and fat

necrosis suggest that the ablative process affects pancreatic tissue distal to the electrolytic lesion. As has been addressed above, the drainage of necrotic tissue via the pancreatic duct is imperative to prevent duct occlusion and ductal distension. The scarring of the pancreas distal to the electrolytic lesion may be a consequence of compromised ductal drainage, which itself lead to an obstructed duct system, the ablative process itself or more likely a combination of these two factors. The demonstrable patency of the ducts as seen on the ductograms performed in phase 2 would suggest that complete obstruction of the ducts had not occurred and therefore would be an unlikely sole cause of the scarring of the distal regions of the pancreas. It is anticipated that in clinical practise stent placement across the ablation lesion and the likely greater proximity of the lesion to the duodenum would facilitate drainage of the products of electrolytic ablation. However, at this stage it is impossible to say whether these factors would resolve the issue of scarring of the distal pancreas. Indeed, given that many patients with pancreatic cancer have an element of pancreatic duct obstruction, it may be that the impact of electrolytic ablation on the distal gland is limited as a result of this already partially obstructed duct system and possibly pre-existing scarring of the pancreatic parenchyma.

When considered as a whole the results of this study have shown that is it feasible to deliver electrolytic ablation to the pancreas via the pancreatic duct, both at open and endoscopic approaches. In addition to this it has been shown that despite transient hyperamylasaemia, a short-term increase in CRP and leukocyte concentration and an incidence of self-limiting peripancreatic collection formation that this technique is not associated with significant morbidity and mortality.

4.2 Strengths and Limitations of the Study

The most critical limitation of this study is the use of normal pancreatic tissue for electrolytic ablation rather than pancreatic adenocarcinoma. Unfortunately at this time there are no large animals models of pancreatic adenocarcinoma and therefore this has necessitated the use of non-tumour models, commonly swine (287-289), for the investigation of the potential role of locally ablative techniques in the management of pancreatic cancer. The primary concern is that the extrapolation of findings and data from experimental ablation of non-tumourous pancreatic tissue to the clinical scenario of ablating a pancreatic malignancy is not a straightforward undertaking. Firstly, there is no guarantee that tumour tissue will behave in exactly the same manner as non-tumourous tissue when subjected to electrolysis, or other ablation modalities. Indeed, pancreatic adenocarcinomas are typically fibrous in natural and as a result it would not be unexpected for there to be differences in response to electrolysis. Furthermore this alteration in tissue reaction would also effect any dose-volume calculations and relationships that were based on data resulting from ablation of non-tumour pancreatic tissue. The currently available animals models of pancreatic adenocarcinoma are primarily confined to small animals: mice, rats, hamsters and guinea pigs (290-293). Attempts have been made to produce large animal models of the disease with limited success. Ductal, epithelial and acinar hyperplasia has been produced in dogs by local administration of benzopyrene (294) and deoxycholate (295) and in rabbits by administration of dimethylhydrazine into the pancreatic duct (296). Japanese researchers Kamano et al and Sato have succeeded in producing ductal adenocarcinoma in a few dogs following the administration of N-ethyl-N'-

nitrosoguanidine (297-299). However the clinical usefulness of the majority of these models remains unproven (300).

In previous work from this institution investigating electrolysis as a locally ablative technique in the management of liver tumours, this problem was over-come by electrolytically ablating liver lesion in patients who immediately proceeded to hepatic resection, including the area of ablation. This approach enabled not only the observation of the efficacy of electrolysis in tumour tissue it also enabled the generation of a dose-volume relationship specifically for tumour ablation. This strategy is not readily applicable to the investigation of electrolytic ablation of pancreatic cancer. The relative paucity of patients undergoing resection for pancreatic cancer, due to the oft-advanced nature of the disease at the time of diagnosis, significantly limits the potential candidates in whom electrolytic ablation could be trialled prior to pancreaticoduodenotomy.

A further limitation of the experimental model used in this study is the difference between porcine biliary anatomy and human. In pigs the common bile duct drains into the first part of the duodenum approximately 5cm distal to the pylorus. This complete separation of the biliary and pancreatic systems is obviously different from human anatomy. As a result of this it is not possible to determine the effect of pancreatic electrolytic ablation, and the resulting lesion and scarring, on biliary drainage in a porcine model. Although this situation is not ideal, the placement of stents, either plastic or SEMS, in clinical practise within the biliary system is commonplace and therefore stent placement across the electrolytic lesion would be anticipated to ensure adequate drainage of the biliary tract following electrolysis.

This study has had a number of logistical limitations. Given that the premise of the study is essentially a 'proof of concept' the number of control groups and animals has been kept to an absolute minimum. This has been for two main reasons: Firstly, there were ethical concerns regarding large numbers of animals undergoing 'sham' operations as controls. Secondly, there are financial considerations again regarding the expense of having large control groups at each time point in each experimental phase. This would have required an additional 13 pigs and would have increased the total animal number from 35 to 48. Future studies into pancreatic electrolysis would ideally be constructed with control groups for each study end point. Hopefully the findings and data from this thesis will support their inclusion.

Although 35 animals have been used in this study the numbers in each group are relatively small (n= 4 to 6). As a result of this, the absence of statistically significant differences between groups for some parameters in various phases of this study may be in part due to a type II error. Furthermore, the relatively small number of animals (n=24) who have undergone perductal pancreatic electrolysis means that infrequent complications of the procedure may have occurred within this study; as any complication with a incidence of less than 4% would not necessarily be expected to be noted in a study population of this size. Despite this, however, significant differences have been consistently detected within study groups over the time course of experiments for amylase, C-reactive protein and leukocyte count. Also, significant differences for specific parameters have been detected between treatment and control groups at individual time points (CRP and leukocyte count in phase 2). The combination of these two factors suggests that given the reproducibility of the results

in this study that any differences missed due to a type II error are likely to be small in nature.

Another limitation of the study was the assessment of the presence or absence of the systemic inflammatory response syndrome by the determination of systemic concentrations of the inflammatory cytokines TNF-α and IL-1β. Although both these cytokines have been identified as playing a central role in the inflammatory process their presence in detectable quantities in the circulation is often of a limited duration (301-304), as was the case with the IL-1β assay in both phases 1a & 1b. As a result of this any conclusions made from these data need to be assessed in conjunction with findings from other areas of investigation. In this study however, the absence of a significant increase in serum cytokine levels is well supported by the clinical and biochemical data that indicates that there was no significant or prolonged inflammatory reaction following pancreatic electrolysis. Ideally local TNF-α and IL-1β concentrations within the ablation lesion and surrounding tissue would be evaluated following electrolytic ablation. It has been shown that local concentrations of these cytokines can increase to physiologically significant levels without corresponding increases in serum concentration. However, this approach has a number of logistical concerns. Firstly multiple biopsies would be required from the pancreatic ablation lesion and surrounding unablated pancreas, this would necessitate either repeated laparotomy in individual animals and ethically unacceptable levels of suffering or dramatically increasing animal numbers and sacrificing a proportion of the study group at the time of each biopsy. Furthermore biopsying the ablation lesion and unablated pancreas may effect assessment of histological changes in these regions of the glands, as a result of increased inflammation and possibly haemorrhage at the site of biopsy.

One of the main strengths of this study is the novelty of the concept that has been investigated. Prior to this study there have been reports of perductal pancreatic electrolytic ablation in the world literature. Indeed the limited reports of locally ablative techniques, including electrolysis, being used in the pancreas have required the external penetration of the pancreatic parenchyma in order to gain access to the desired area for ablation, with the inherent risk of haemorrhage and damage to adjacent viscera.

Although as previously stated the porcine model used is by no means perfect, it does provide a large animal model with anatomy and physiology that are sufficiently similar to that of humans to allow reasonable confidence in the interpretation of data with respect to possible clinical usage. Furthermore the use of porcine models in surgical research is commonplace and as a result there is already a body of knowledge and expertise that facilitates the smooth progression of a research project, more so than may have been the case if another animal model had been used. Also the long-term follow-up of a number of these experimental animals helps support the safety of this technique. With mean survival durations of only 4-6 months the 8-week follow-up in this study represents approximately one third to a half of the anticipated life expectancy of patients undergoing palliation for pancreatic cancer. The absence of significant complications within this timeframe is encouraging for similarly low complication rates within the expected survival period of this cohort of patients.

A vitally important aspect of this study was that the animal model used was susceptible to acute pancreatitis. An aspect of the reasoning behind the selection of a porcine

model, other than the advantages previously discussed, was the documented ability to produce acute pancreatitis in pigs. Experimental pancreatitis has been produced in the porcine pancreas by various methods (305, 306), most commonly by the injection of an irritant (for example bile salt-trypsin solution, contrast medium or oleic acid) into the pancreatic duct (307-309). Although there have been no reported cases of acute pancreatitis in experimental studies following local ablation of the pancreas, using various ablative techniques, the number of glands ablated remains relatively small. It is therefore reassuring and important to know that the ability to produce acute pancreatitis in a porcine model has previously been successfully demonstrated.

Another strength of the study is the minimal difference between the nature of the 'endoscopic' approach used in the second phase of the project and the envisaged delivery system for clinical practice, ERCP. Other than the method of gaining access to the gastrointestinal tract the experimental model has been kept as 'minimally invasive' as possible. This in turn makes the resulting data more applicable to the clinical setting and reduces the number of new variables that will have to be considered when and if clinical trials of pancreatic electrolytic ablation are established.

4.3 Relation to the Literature

The discussion of the results of this study with regards to its relation to the literature is difficult. There is a paucity of published experimental studies that have addressed similar areas of investigation. The published work on the local ablation of the pancreas has either used other ablative techniques such as radiofrequency ablation (287, 288) or has been performed in a small animal model (310). Furthermore in these three studies

animal numbers have been comparatively small being: six, thirteen and ten respectively. In addition to this, although all the authors stated the perceived safety of the locally ablative technique for use in pancreatic tissue ablation, whether it be RFA or electrolysis, there have been no further published studies from these investigators on the use of these locally ablative techniques in the pancreas. Another important difference between the cited studies and this research is the anatomical approach to the pancreas that has been adopted. This project is the first attempt at investigating the feasibility and safety of delivering a locally ablative technique via the pancreatic duct whereas an external, transperitoneal approach has been used previously. The result of these factors is the absence of a comparable body of work using a similar model, experimental technique and duration of follow-up with which the results of this thesis can be compared. However, in accordance with the limited available data relating to pancreatic ablation the results of this series of experiments suggests that perductal pancreatic electrolytic ablation, in addition to being feasible, is also safe and not associated with either acute generalised pancreatitis or significant local or systemic complications.

It is possible however to compare the results of this study with both the published data on electrolytic ablation of other tissues, mainly liver, and the results obtained from other locally ablative techniques, primarily radiofrequency ablation and cryotherapy, also mainly in the area of hepatic ablation.

Electrolysis has been and is being widely used in China for the local ablation of tumours of various types. The limited published data that is starting to appear from the Chinese electrolytic experience is predominately in the form of case studies rather than

well-planned research, either clinical or experimental. However in addition to this, as was addressed in the introduction, there has also been rigorous scientific investigation of electrolysis and its locally ablative effects (25, 27, 28, 31, 32, 40, 48, 310-312). Radiofrequency ablation and cryotherapy, on the other hand, are more widely used in the western world and as such there is a greater quantity of published material regarding these two modalities (33, 313-321). Although there continues to be debate as to what should be the 'gold-standard' approach to the local ablation of tumours, with the various involved factions strongly advocating their chosen technique, the overwhelming conclusion of the literature is that all these techniques can be delivered safely with minimal morbidity and mortality. The arguments for and against each modality are based around more esoteric concerns, for example the proximity of vessels, the time required to ablate a given tumour volume and the ability to deliver the technique percutaneously. Although there is a documented association between cryotherapy and the 'cryoshock' phenomenon this complication is limited to less than 1% of hepatic ablations and its incidence is directly related to the volume of tissue ablated, often greater than 30% of the liver (34, 318, 322). The findings from this thesis provide no evidence to suggest that the safety aspects of perductal electrolytic ablation of the pancreas differ markedly from those that have been reported for electrolysis and both radiofrequency ablation and cryotherapy.

Obviously categorical statements regarding the absolute safety of any given procedure or technique are impossible to make. This is no less true for pancreatic electrolysis than it is for anything else, especially given the relatively small number of animals and the somewhat 'artificial' experimental model, as has been discussed at greater length in the previous section. Therefore further studies are necessary, preferably from a number

of institutions to both corroborate the results of this thesis and to further the investigation of this potentially useful application of electrolytic ablation.

4.4 Implications of This Study

The management of pancreatic tumours continues to present a difficult challenge to the medical community. As has been discussed in the introduction palliation remains the primary therapeutic option for over 80% of patients with this disease. Debate continues as to what is the optimal approach to palliation of these patients. There are advocates for both operative bypass, biliary and gastrointestinal, and ERCP with stenting. Furthermore there is much published literature to support both sides of the argument. The one fact that is not in dispute is that there is currently no therapeutic option available that is universally applicable to all patients requiring palliation for the symptoms of pancreatic cancer.

This study has shown that perductal electrolytic ablation of the pancreas, both at open and endoscopic approaches, is feasible and safe. Although this technique is still in the experimental phases of investigation the results reported in this thesis suggest that it warrants further and more detailed study as to its application in the palliation of pancreatic cancer. Potentially, endoscopic delivery of an electrolytic dose would combine the best aspects of the currently available palliative modalities; the efficacy of symptom control associated with surgery with the minimal morbidity and mortality of ERCP and stenting and the shortened hospital stay associated with a minimally invasive procedure. Minimisation of the amount of time spent in hospital is of particular importance when considering patients with incurable pancreatic cancer as

these people have a mean survival of only 4-6 months from the time of diagnosis (323). Therefore palliative interventions that result in prolonged hospitalisation are especially unacceptable in this cohort of patients.

When the concept for this research study was conceived the original anticipated application of perductal pancreatic electrolytic ablation was in the palliative management of unresectable pancreatic cancer. However, there is no reason why this technique could not be used in chronic pancreatitis for the ablation of head of pancreas masses. This study has demonstrated that electrolytic ablation of pancreatic tissue results in localised necrosis and inflammation without progressing to generalised inflammation of the entire gland. One of the sequelae noted was the presence of scarring in the pancreas distal to the site of ablation, resulting in an appearance not dissimilar to that of chronic pancreatitis. As discussed above, whether this scarring is due to inadequate drainage of necrotic material via the pancreatic duct or is a direct consequence of the electrolytic process remains uncertain. However, this outcome would obviously be of less import in an already scarred and diseased pancreas as would be expected in patients with chronic pancreatitis. The use of locally ablative techniques, of any sort, in the management of chronic pancreatitis related pancreatic head masses would be dependent on the ability to exclude neoplasms as a possible cause of such lesions.

Although endoscopic perductal electrolysis pancreatic tissue will not be a panacea for all cases of pancreatic cancer, it may be that its use in conjunction with biliary and pancreatic stent placement, and possibly enteric wall stenting, will offer improved and prolonged palliation in a proportion of patients.

4.5 Areas for Future Research

A number of areas for further investigation have been identified from this study and the discussion of the resulting data.

The determination of an accurate dose-volume relationship is essential if this technique is to have a clinical application. Although the problems of not having an available tumour model have already been discussed it would be important to establish the relationship between volume of necrosis and coulomb dose delivered, even though it would be within normal pancreatic tissue. As part of such a study the reasoning as to why greater volumes of necrosis per coulomb are produced in the pancreas as compared to the liver could also be investigated. Determination of the relationship between lesion pH, the principle cytotoxic factor in electrolysis, and volume would also help to clarify whether the release of pancreatic proteolytic enzymes into the ablation lesion contributes to tissue necrosis. Furthermore the quantification of enzyme concentration within the ablation lesion would also provide useful data.

Another approach to establishing the dose-volume relationship would be to perform electrolytic ablation, using low doses of coulombs, within pancreatic tumours in the clinical setting in conjunction with ERCP with stenting in the presence of biliary obstruction. Magnetic resonance imaging (MRI) could then be used to assess the size and volume of the resulting electrolytic lesion, indeed due to the non-interventional and non-irradiating nature of MRI, sequential scanning of the lesions would be possible and enable the progression of the electrolytic lesions to be monitored.

Previous unpublished work from The Queen Elizabeth Hospital, Adelaide has demonstrated a close correlation between the MRI appearance of electrolytic lesions and histological findings. The use of MRI is perceived to offer a degree of accurate non-invasive assessment of electrolytic lesions and their morphology that has previously been impossible without histological assessment of a resected lesion. Magnetic resonance imaging would facilitate the assessment of electrolytic ablation lesion progression and allow dose-volume formulas to be created within a clinical study by dramatically increasing the number of patients in whom the technique could be trailed by removing the dependence on histological assessment of the ablation lesions. As previously discussed the proportion of patients with pancreatic cancer who undergo resection is limited by the advanced nature of the disease at presentation.

Another avenue of further study would be the investigation of the impact of stent placement across the ablation lesion. As stated previously it is not unreasonable to predict that stent placement would facilitate the drainage of necrotic material from the ablation lesion. However the impact of stenting on the development of the electrolytic lesion, the extent of scarring of the distal pancreas, long-term duct patency and the incidence of local complications would be of significant interest in the development of this technique for use in the clinical environment. The per-operative use of an image intensifier would allow accurate stent placement across the electrolytic lesion, in addition to this in-situ ductograms could be performed to access both stent and ductal patency at the time of electrolysis and at post-mortem examination prior to the resection of the pancreata.

Ultimately the usefulness, in terms of palliation, of perductal electrolytic ablation of pancreatic tumours can only be established in a clinical trial comparing this novel technique against currently accepted best practice, which in this case would most likely be ERCP with stenting. Until such a trial has been established and is in progress it would be unethical to undertake electrolytic ablation of pancreatic tumours in an ad-hoc manner. However, the work presented in this thesis supports the further investigation of this technique as a potentially beneficial intervention in the management of pancreatic tumours.

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SURGICAL ENDOSCOPY
and Other Interventional Techniques

Palliation of pancreatic cancer using electrolytic ablation

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Abstract
Background: Inoperable pancreatic cancer has a dismal prognosis. Palliation involves either stenting or surgical bypass. Stenting does not relieve gastric outlet obstruction, and surgical bypass is a major procedure. A minimally invasive procedure is needed that relieves both gastric outlet and biliary obstruction, with the potential for relieving pain.
Methods: In an experimental model, pancreatic electrolysis was investigated. The pancreatic duct was cannulated via a transduodenal approach with an electrode catheter. In 6 animals an electrolytic "lesion" was created using a direct current generator. 6 animals were controls. The local and systemic effects of electrolysis were assessed using histological and biochemical parameters.
Results: The pancreatic duct was cannulated in all animals and treatment was uneventful. Electrolytic lesions comprised a central area of necrosis with a sharp demarcation between necrotic and viable pancreas. All animals developed transient hyperamylasemia after electrolysis. There was no significant difference between treatment and controls. Importantly, no animal had clinical, biochemical, or histological evidence of pancreatitis.
Conclusions: This experimental study suggested that electrolytic palliation of inoperable pancreatic cancer via the gastrointestinal tract is potentially safe. In patients, this treatment could be performed during endoscopic retrograde cholangiopancreatography and may have therapeutic advantages when compared to stenting or biliary bypass.

Pancreatic cancer is the fifth most common cause of cancer death in the United States, with an incidence of approximately 10 per 10,000 population [14]. The prognosis is dismal. Median untreated survival from the time of diagnosis is between 4 and 7 months, and the overall 5-year survival is between 1 and 3% [29]. Radical surgery offers the only chance of cure [15, 20], but is possible in only 15% of patients [16, 21]. Additionally, of those who undergo potentially curative surgery, the median survival is 10–18 months [4, 5, 30], and only approximately 10% survive to 5 years [7, 12]. Therefore, for the majority of patients, palliation is the only therapeutic option.

Effective palliation should aim to relieve the distressing symptoms of locally advancing disease, namely jaundice (often with its intolerable pruritis), gastric outlet obstruction, and pain. Managing these patients is a therapeutic conundrum. The treatment options currently available are limited and are at the extremes of a spectrum of intervention. Therapeutic endoscopic retrograde cholangiopancreatography (ERCP) with endoscopic stenting of the malignant stricture is the least invasive technique available [10]. This procedure has the advantage of rapidly relieving the symptoms of obstructive jaundice without the need for a prolonged hospital admission in a patient with a very limited survival. However, the symptoms of gastric outlet obstruction and pain remain unpalliated, and readmission rates for stent occlusion and cholangitis are high [2].

Conversely, surgical bypass achieves extremely effective palliation of the symptoms of both biliary and gastric outlet obstruction, and recurrent jaundice or cholangitis occurs in less than 10% of patients [24]. However, surgical bypass is associated with a higher 30-day mortality than stent insertion [11, 24], and patients often experience prolonged hospital admission. Much controversy exists regarding how these patients should be managed. The implication is that neither is ideal.

After the recent introduction of new chemotherapeutic agents including gemcitabine, it was hoped that survival would improve [3, 19]. This may have led clinicians to consider the more permanent palliation associated with biliary bypass worthwhile. Unfortunately, following early optimism [3], results have been disap-

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pointing, although large multicenter trials continue [23]. The benefit of adjuvant and neoadjuvant chemotherapy remains unresolved.

The need clearly exists for a palliative treatment that combines the highly effective palliation of surgical bypass with the minimally invasive nature of an endoscopic procedure. Potentially, this could be achieved by "debulking" the tumor using an ablative technique, thereby relieving the locally compressive effects of the tumor. Locally ablative techniques, including radiofrequency ablation [13], cryotherapy [22], and recently, electrolysis [6], have been used in an experimental setting. However, with each technique the approach to the pancreas was transperitoneal, with the potential attendant risk of pancreatic fistulae, bleeding, and enteric perforation. If a locally ablative technique is to become accepted as a palliative option, it must be associated with the minimal morbidity of an endoscopic procedure. It is therefore proposed that ablation should be performed via the pancreatic duct, such that the necrotic products of tumor ablation can drain into the gastrointestinal (GI) tract rather than into the peritoneal cavity. However, it is accepted that cannulation of the biliary tree at ERCP is associated with a significant risk of acute pancreatitis and death [9].

Using the pancreas of a large animal model, this study aimed to determine (1) the feasibility of performing pancreatic electrolytic ablation using an electrode catheter introduced into the pancreatic duct via the duodenum, (2) the nature of pancreatic necrosis caused by electrolytic ablation, and (3) whether pancreatic electrolysis caused acute pancreatitis or other significant local or systemic effects.

Materials and methods

Specific pathogen-free (SPF) female domestic white pigs were used for the study. Mean weight was 31 kg.

All pigs were anesthetized in the same way. Sedation was achieved with an intramuscular injection of ketamine (20 mg/kg) and xylazine (1.5 mg/kg). Each animal was cleaned with a solution of chlorhexidine and taken into the operating theater. A laryngeal mask airway was inserted and anesthesia maintained with 1.5% halothane in oxygen. Oxygen saturation and heart rate were monitored continuously throughout the procedure.

In a preliminary pilot study, three animals were used to determine the pancreatic anatomy, feasibility, and technical requirements for pancreatic duct cannulation and ablation. A pancreatogram was obtained by injecting the pancreatic duct (PD) with contrast medium (Fig. 1). This study showed that the proximal 6 cm of the PD ran parallel and in close approximation to the second part of the duodenum (Fig. 1, P) before turning approximately 90°. Therefore, it was evident that the pancreas should be mobilized from the medial wall of the duodenum before cannulation with the electrode catheter to avoid duodenal perforations. Additionally, it was shown that the electrode catheter should be inserted to a minimum depth of 80 mm, such that the tip of the catheter would be in the main "splenic" lobe of the gland.

Following the pilot study, the main study was performed using 12 SPF pigs. A tunneled catheter was inserted into the right femoral vein for postoperative blood sampling. A midline laparotomy was performed, and a 5-cm longitudinal duodenotomy was performed 20 cm distal to the pylorus. The ampulla was identified and a 6-Fr (2-mm) electrode catheter (Fig. 2; Part No 1086-547-S, Cordis Webster, Baldwin Park, CA, USA) was inserted into the PD of the splenic lobe to a depth of 80 mm and secured with a suture (Fig. 3). Each catheter had four electrodes, which were 4 mm long. Each electrode was separated by 3 mm. Electrolytic treatment was given using the tip and middle electrodes. The "unused" electrodes were electrically isolated.

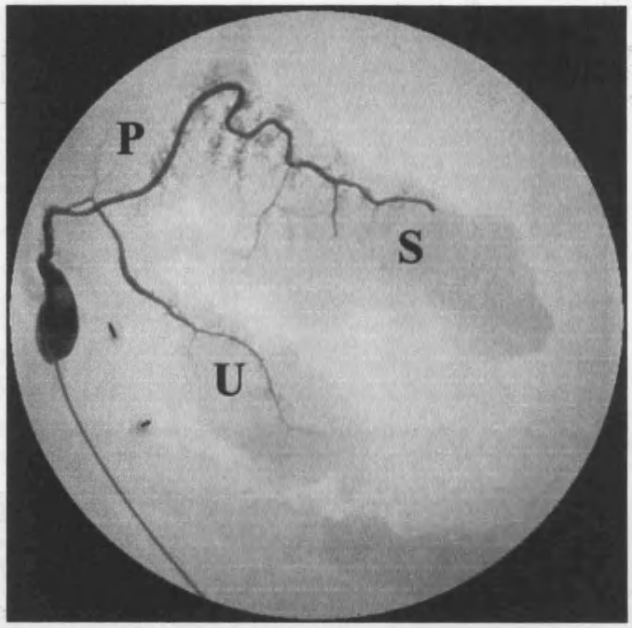

Fig. 1. Pancreatogram showing the unique anatomy of the porcine pancreas. S, splenic lobe; U, uncinate; P, Paraduodenal segment.

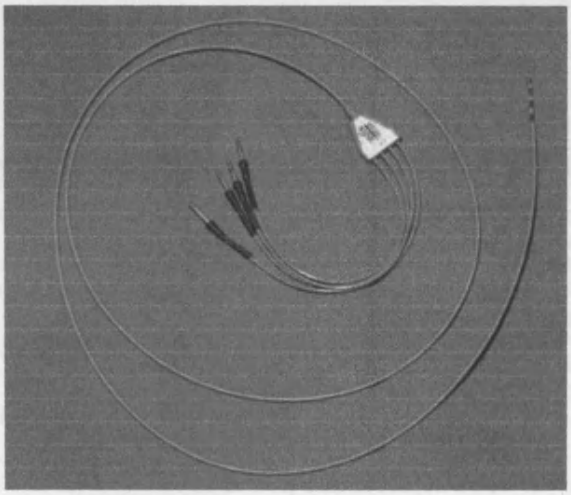

Fig. 2. A 6-Fr (2-mm) electrode catheter. The distal and third electrodes were connected to the DC generator. The "unused" electrodes were electrically isolated.

Each animal was then randomized to either the treatment or control arm of the trial. In the treatment group (n = 6), the electrode catheter was connected to a direct current (DC) generator (ECU 100, Söring, Quickborn, Germany), and an electrolytic "dose" of 50 C was delivered to the gland at 50 mA. Median treatment time was 23 min. (range, 19–25 min). In the control group (n = 6), the electrode catheter was connected to the DC generator, but no current was delivered.

At the end of treatment, the electrode catheter was removed from the PD and the duodenotomy was closed. An intramuscular injection of buprenorphine (0.01 mg/kg) was given before the animal was woken.

Blood samples were taken using the femoral catheter immediately before and after electrolysis and at 1, 4, 24, 48, and 72 h postoperatively for the measurement of serum amylase, glucose, C-reactive protein (CRP), calcium, urea, and electrolytes.

After 72 h, the animals were euthanized. A postmortem examination was performed. In particular, the pancreas was examined to

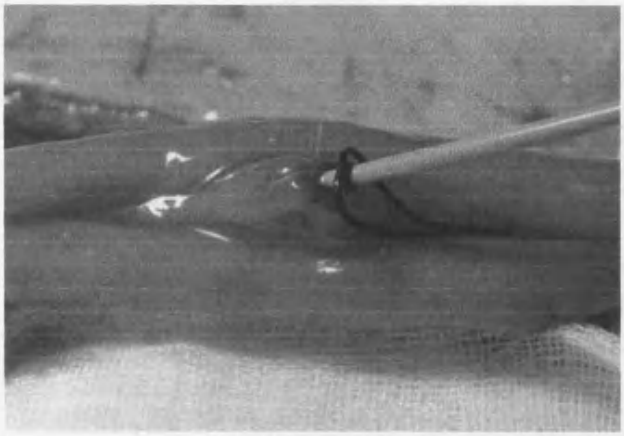

Fig. 3. Cannulation of the pancreatic duct using a 6-Fr (2-mm) electrode catheter. The catheter was inserted to a depth of 80 mm and secured with a suture.

determine the extent of the induced necrosis, the patency of the PD, the presence of any peripancreatic fluid collections, and any involvement of adjacent organs. The pancreas was examined histologically to determine the extent of the electrolytic necrosis and whether there was any associated pancreatitis in the surrounding parenchyma. Results were analyzed statistically using the unpaired *t*-test.

Results

All animals tolerated the electrolytic treatment well. One animal died during surgery from an anesthetic-related complication. This animal was a control and was replaced. Recovery was variable. The majority of the animals ($n = 8$) were eating, drinking, passing urine, and defecating normally 48 h after the procedure. However, four animals experienced a more prolonged return to normal activities. This prolonged recovery was equal in both the treatment ($n = 2$) and control ($n = 2$) groups and was unrelated to the increase in serum amylase or other biochemical parameters. No animal was euthanized prematurely.

Macroscopic appearances

Control group

One of the pancreata was slightly edematous. Otherwise, the glands of the control animals were of normal macroscopic appearance. Specifically, there was no saponification, fluid collections, or pancreatic fistulae. The pancreatic duct was patent in all specimens. There was no damage to adjacent organs.

Treatment group

In all the pancreata, there was evidence of an electrolytic "lesion" in the splenic lobe of the pancreas with a mean diameter of 1.45 cm (range, 1.2–1.85 cm). This lesion was spherical in nature and clearly demarcated from the adjacent normal pancreas. In four of the six glands, the electrolytic lesion had breached the pancreatic capsule, and there were associated small fluid collections. However, the proximal and distal pancreatic duct remained patent in all specimens, and there was no damage to adjacent organs. One of the glands was mildly erythematous, but there was no other evidence of acute pancreatitis in any of the pancreata.

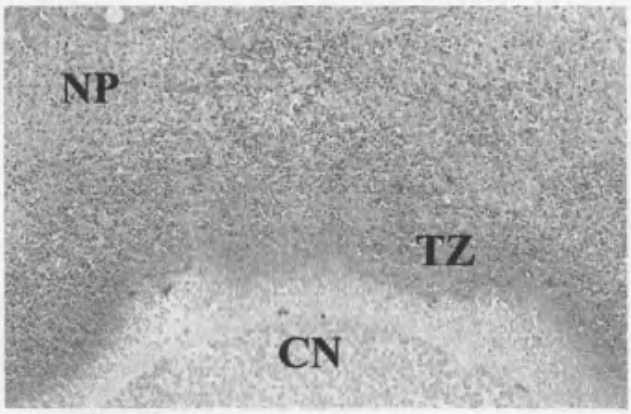

Fig. 4. Typical histological appearances (pancreatic ablation). C, coagulative necrosis; NP, normal pancreas; TZ, transition zone.

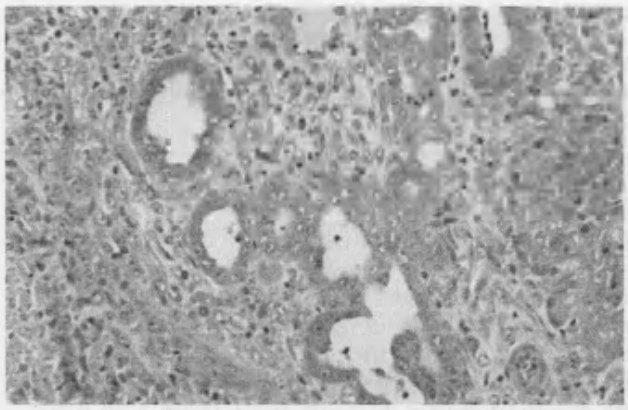

Fig. 5. Typical histological appearances adjacent to the PD (control).

Histological appearances

Treatment group

All of the electrolytic lesions were of similar appearance (Fig. 4). There was a central zone of coagulative necrosis in which there was no residual viable tissue. There was a transition zone surrounding this area of coagulative necrosis. This area was less than 1 mm wide and was infiltrated by neutrophils and lymphocytes. Cell viability in this area was variable.

Outside this transition zone, the pancreatic parenchyma was normal, apart from mild interlobar inflammation that radiated out a variable distance from the lesion. Bubbles of gas were also seen in these fibrous septa. There were no abscesses.

Control group

There was no evidence of parenchymal necrosis or inflammation in any of the control specimens (Fig. 5). However, in one pancreas there was a necrotic area surrounding the pancreatic duct (2.5-mm diameter) with associated local inflammatory changes.

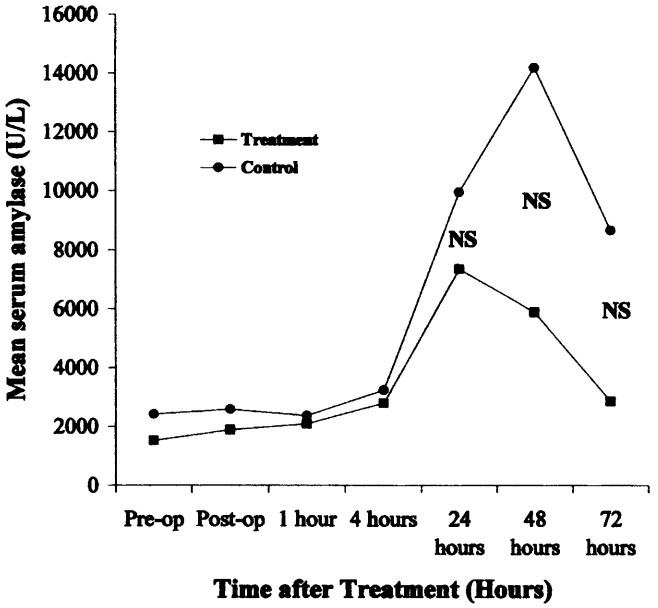

Fig. 6. Mean change in serum amylase after electrolysis. NS, not significant.

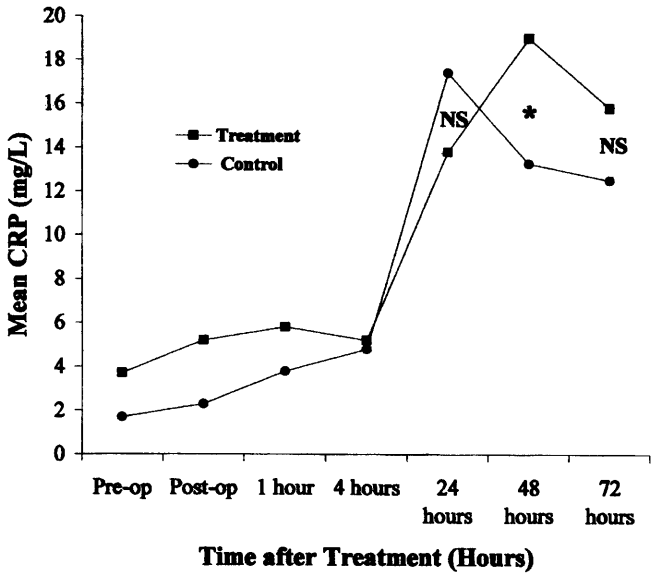

Fig. 7. Mean change in CRP after electrolysis. NS, not significant, $^{*}p < 0.05$.

Biochemistry

Amylase

All of the control and treated animals developed postoperative hyperamylasemia (Fig. 6). The mean serum amylase reached a peak concentration after 48 h in the control group and after 24 h in the treatment group. However, in both groups the serum amylase was returning toward normal at 72 h. There was no significant difference between the two groups with regard to the elevation in serum amylase after operation.

C-reactive protein

The CRP was elevated in all the animals after operation (Fig. 7). The mean serum CRP reached a peak concentration after 24 h in the control group and after 48 h in the treatment group. The mean serum CRP was significantly ($p < 0.05$) higher after 48 h in the treatment group. However, in both groups the serum CRP was returning toward normal at 72 h.

Glucose, calcium, urea, and electrolytes

There was no significant change in serum levels of glucose, calcium, urea, and electrolytes after operation in either group.

Discussion

The optimal management of patients with inoperable pancreatic cancer remains controversial. Despite their relative merits, the current therapeutic options of either endoscopic stenting or surgical bypass are suboptimal. Debulking the tumor from within the gland using the novel technique of electrolytic ablation is an attractive option. This method potentially offers the excellent palliation associated with surgical bypass without the need for a major surgical procedure or the stent-related complications of ERCP [2].

Electrolytic ablation has been extensively investigated for use in patients with unresectable liver tumors [18, 26–28]. Unlike other forms of local ablation, electrolysis is a nonthermal technique [1] that causes cell death by a more subtle chemical action. Platinum electrodes are polarized using a small direct electrical current. As a result, the intracellular pH changes and cytotoxic gases are released into the local environment, causing cell necrosis.

It has been shown that electrolytic ablation can be safely used in close proximity to large intrahepatic veins, with the vein wall remaining intact [25]. Conversely, thermal methods of ablation may cause a direct burn to large vessels. Indeed, cryotherapy has been shown to damage large veins due to cracking of the "ice-ball" during the freezing phase of the process [8, 17]. It is proposed that the ability of electrolysis to treat tumors directly adjacent to large veins may confer a benefit when compared to other forms of ablation for debulking tumors of the pancreas. This is of relevance because of tumors of the pancreas are usually situated in the head of the gland, intimately associated with the portal vein. Damage to the portal vein would be rapidly fatal.

The electrolytic "dose" chosen for this study was based on dose–response data from work in the liver [26]. However, in four of the six animals treated with electrolysis, the pancreatic capsule was breached by the lesion, which was larger than predicted. It is likely that this is due to relatively high concentrations of electrically active ions in the pancreatic parenchyma compared to the liver. Consequently, in future studies a lower dose should be used to avoid fistula formation. In the clinical setting, breaching of the pancreatic capsule would be far less likely because the electrode catheter would be inserted into the center of a bulky tumor rather than into a normal gland.

If electrolytic ablation is to become accepted as a palliative treatment, it is important that complications are minimal. In this study, the pancreatic duct both proximal and distal to the electrolytic lesion remained patent after treatment. This is vital, because cellular debris can drain effectively into the GI tract, thereby preventing fistula formation. It is proposed that a stent could also be inserted at the time of treatment to facilitate drainage.

Although all the animals developed transient hyperamylasemia after operation, none developed clinically, biochemically, or histologically significant pancreatitis, and all were well at the time of sacrifice. Interestingly, although not statistically significant, the three animals with the highest peak amylase level were all controls. This is likely a result of the fact that these animals were the first three to be operated on due to the randomization process. This was early in the operator's learning curve, and the time taken to cannulate the duct was prolonged in these animals.

The serum CRP was elevated in all animals after operation. This finding is consistent with the trauma associated with laparotomy. However, there was a significantly higher mean serum concentration in the treated animals 48 h after operation. It is proposed that this resulted from the additional effect of electrolytic necrosis. This effect was transient.

This experimental study confirms previous findings that pancreatic ablation using electrolysis is technically feasible and potentially safe for debulking inoperable pancreatic tumors. Importantly, there was no evidence of pancreatitis after treatment. This is the first study in which pancreatic ablation was achieved by accessing the pancreas from the GI tract. This not only has implications for minimizing complications, such as fistula formation, but also the treatment could potentially be given during ERCP, thereby avoiding a prolonged hospital stay.

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The Lack of a Systemic Inflammatory Response Syndrome Supports the Safety of Pancreatic Electrolysis: Experimental Studies[1]

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Background. **Per-ductal pancreatic electrolysis is a new minimally invasive ablation treatment. Possible applications include tumor debulking and treatment of chronic pancreatitis. Both solid organ ablation and pancreatitis are associated with the risk of an overwhelming systemic inflammatory response syndrome (SIRS) and multiorgan failure. TNF-α and IL1-β are important cytokine mediators of this response. The aim of this study was to measure the circulating levels of IL1-β and TNF-α following pancreatic electrolytic ablation as a marker of the risk of SIRS complicating per-ductal pancreatic electrolysis.**

Methods. **Serum TNF-α and IL1-β were measured in six treatment and six control pigs before and after laparotomy and pancreatic electrolytic ablation via a per-ductal approach.**

Results. **There was no significant rise in serum TNF-α and IL1-β in association with per-ductal pancreatic electrolysis.**

Conclusion. **This study supports the evidence that per-ductal electrolysis is a safe procedure with potential for palliative treatment of pancreatic cancers.**

Key Words: **electrolysis; ablation; pancreas; SIRS; cytokine.**

INTRODUCTION

Pancreatic cancer and chronic pancreatitis continue to pose management problems for the surgeon due to inadequate symptom control with conventional surgical treatments. Ablative techniques such as cryotherapy [1], radio-frequency ablation [2], and electrolysis [3] have been proposed as potential treatments for pancreatic disease. The potential benefit of such treatments is effective tissue destruction, via a minimally invasive approach.

Electrolytic ablation is a nonthermal, local ablation modality that uses a low-level direct current passed between two or more electrodes. Localized tissue necrosis results from the decomposition of interstitial fluid and changes in tissue pH around anode and cathode [4]. We have developed a swine model for per-ductal electrolytic pancreatic ablation, which would allow for an electrolysis catheter to be inserted into the pancreas via a minimally invasive approach [5]. The aim of this model is to develop a palliative treatment for pancreatic cancer allowing tumor debulking alone, or in combination with endoscopic biliary stent placement, to relieve symptoms of biliary obstruction, gastric outlet obstruction, and intractable pain.

Both solid organ ablation and endoscopic pancreatic duct cannulation are associated with a small but clinically significant risk of an overwhelming systemic inflammatory response leading to multiorgan failure. Cryoshock, a variant of the systemic inflammatory response syndrome (SIRS), occurs in 1% of hepatic cryoablations and has a significant associated mortality of 35% [6]. Endoscopic pancreatic or biliary duct instrumentation alone can be complicated by acute pancreatitis, SIRS, and death [7]. The proinflammatory cytokine mediators of the SIRS seen with cryoshock or severe acute pancreatitis include interleukin (IL)1, IL6, and tissue necrosis factor (TNF)-α [8, 9].

The aim of this study was to measure the circulating levels of IL-1β (the extracellular circulating form of

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0022-4804/04 $30.00

IL1) and TNF-α following per-ductal pancreatic electrolytic ablation to assess the risk of SIRS, secondary to pancreatitis or a cryoshock-like phenomenon, complicating per-ductal pancreatic electrolysis.

METHODS

The study was approved by the Animal Ethics Committees of the University of Adelaide and the North Western Adelaide Health Service. Twelve 30 kg female specific, pathogen-free white pigs underwent laparotomy and duodenotomy under general anesthetic. The pancreatic duct opening into the duodenum was identified and cannulated with a 6-French electrolysis catheter (Cordis Webster Inc., CA, USA) with a 4-mm platinum anode and cathode 10 mm apart. The electrolysis catheter was connected to a direct current generator (ECU 100, Söring GmbH, Quickborn, Germany) capable of delivering a charge of 50 C at 50 mA over 17 min. Following duct cannulation, pigs were randomized to receive electrolytic ablation or 17 min of no treatment such that there were six treatment pigs and six controls. At the end of the treatment period, the catheter was removed and the duodenotomy and laparotomy were closed in a standard fashion.

Blood samples were taken via an intravenous catheter prior to the laparotomy, immediately following closure of the laparotomy and then at 1, 4, 24, 48, and 72 h postoperatively. Samples were centrifuged and serum was stored at −80°C. At 72 h postoperatively the pigs were euthanized.

Serum IL1-β and TNF-α concentrations were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using kits specific for swine IL1-β and TNF-α (BioSource International, CA, USA) with minimum detectable levels of 6.0 pg/ml for IL1-β and 15 pg/ml for TNF-α. Standard solutions of each cytokine were assayed to obtain a standard curve of optical densities from which the unknown cytokine concentrations of the sera could be extrapolated. ELISA was performed twice for each sample and the mean of the two results was taken as the final level.

Statistical analysis was performed using an unpaired t test to compare cytokine levels between treatments and controls at the each time period. Two-tailed P values of <0.05 were considered significant.

RESULTS

Laparotomy and duodenotomy with cannulation of pancreatic duct was successfully performed in all pigs. Postoperatively the pigs recovered uneventfully with no clinical evidence of organ failure. By the third postoperative day, all pigs were clinically well with normal feeding habits.

Serum TNF-α levels showed no rise postoperatively in treatment or control pigs (Fig. 1). There was no significant difference in serum TNF-α levels at any of the time points examined. Serum IL1-β levels were below the minimum detectable level in at least five of six pigs in each group of treatment and controls at each time period. For this reason, no meaningful statistical analysis was possible for IL-1β. No pig in either group showed a consistent change in serum IL-1β over the time period studied.

Histological and biochemical data for these pigs has been previously reported [5]. Per-ductal electrolysis produced spherical zones of coagulative necrosis of a mean diameter of 14.5 mm were surrounded by a transition zone of less than 1 mm of neutrophil and lymphocytic infiltrate and only mild interlobar inflammation outside the lesion. There was a transient rise in mean serum amylase in both groups peaking at 24 to 48 h and returning to normal at 72 h. Similarly a transient rise in mean CRP was noted which was significantly higher at 48 h in the treatment group than in controls, returning toward normal at 72 h. Renal function was normal in both treatment and control pigs.

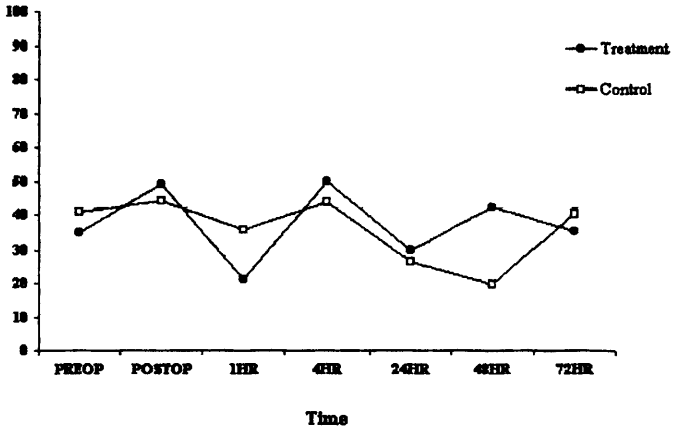

FIG. 1. Mean serum TNF-α concentrations (pg/L) over time for treatment and control animals. PREOP = prior to laparotomy; POSTOP = immediately postoperatively; HR = hours.

DISCUSSION

Although tissue necrosis is the end result of all forms of ablation, a specific shock syndrome has only consistently been reported for cryoablation. Studies of hepatic ablation in rat livers compared RFA with cryotherapy and found that with equivalent volumes of necrosis, only cryotherapy was associated with systemic proinflammatory cytokine activation and lung inflammation [10].

Systemic inflammatory activation following cryosurgery results from the systemic release of necrotic products. In pigs after per-ductal electrolysis, the pancreatic duct was found to be patent and at 3 days [5] and at 2 and 8 weeks follow-up [11]. This is important as a patent duct allows drainage of necrotic material, as well as reducing the risk of fistula formation or "obstructive" pancreatitis. Subsequent studies have also demonstrated that even at 1 to 2 weeks there is no rise in serum TNF-α or IL1-β levels [11].

This study has been undertaken in pigs with normal pancreata as there is no suitable large animal pancreatic tumor model for validation of the technique. As this was a nontumor model, the dose of electrolytic ablation was limited due to the risk of damage to adjacent organs. Larger doses or repeated treatments may be needed to achieve significant tumor debulking in a tumor setting. It is not clear whether larger doses

of ablation with resultant larger volumes of tissue necrosis may produce systemically a cytokine response.

In this study pancreatic duct cannulation was performed at laparotomy, following duodenotomy, to overcome pig pancreatic duct anatomy. In the pig the pancreatic duct enters the duodenum after running parallel with the duodenum for approximately 4 cm. This makes a conventional per-oral endoscopic approach impossible. As endoscopic biliary and pancreatic duct cannulation is an established technique in patients, we do not anticipate any technical difficulties in performing this procedure in patients with pancreatic disease.

The findings of the current study add to the current evidence [5] that per-ductal electrolysis is a safe procedure in the short term. This is of paramount importance for a technique that is proposed as a palliative procedure. Such a procedure should be minimally invasive, have a low level of morbidity and mortality, and be effective in relieving both biliary and gastrointestinal obstruction. Further studies are needed to confirm the safety of this procedure before clinical trials of its effectiveness.

SUMMARY

Per-ductal electrolysis is a new minimally invasive technique of pancreatic ablation. Solid organ ablation and pancreatic duct cannulation are associated with a small but significant risk of systemic inflammatory response syndrome. This study measured systemic proinflammatory cytokine levels to assess the risk of this syndrome complicating pancreatic electrolysis. There was no rise in IL1 or TNF-α following pancreatic electrolysis.

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SURGICAL ENDOSCOPY
and Other Interventional Techniques

Perductal electrolytic ablation of the porcine pancreas

A minimally invasive option-studies of morbidity and mortality

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Abstract
Background: Pancreatic cancer has a dismal prognosis. Few patients are suitable for surgical resection, leaving the majority requiring symptom palliation. Current palliative techniques such as surgical bypass and endoscopic retrograde cholangiopancreatography (ERCP) are imperfect. A novel palliative therapy combining the symptom control of surgical bypass with the minimally invasive nature of ERCP is required.
Methods: Perductal electrolytic ablation of pancreatic tissue, in a porcine model, was performed. There were two survival groups of 2 weeks (n = 4) and 8 weeks (n = 4). Postoperatively, serum biochemistry, amylase and C-reactive protein (CRP) were assessed. Histological examination of the pancreas, lungs, and kidneys was performed to determine the presence of acute pancreatitis or systemic inflammatory response.
Results: An immediate transient increase in both amylase and CRP was seen. Although pancreatic histology demonstrated localised necrosis at the electrolytic site at 2 weeks, there was no evidence of generalized pancreatitis or a systemic inflammatory response at either 2 or 8 weeks.
Conclusions: This study suggests that, although there is localized pancreatic necrosis and transient hyperamylasemia, perductal pancreatic electrolytic ablation is safe, with neither generalized pancreatitis nor a systemic inflammatory response, in the medium and long term. Although performed in normal porcine pancreas, because of the absence of a large-animal model of pancreatic cancer, this study suggests that electrolytic pancreatic ablation is safe. This technique may have a role in the palliation of pancreatic cancer, especially if delivered via a minimally, invasive approach, and warrants further investigation.

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Key words: Pancreas — Electrolysis — Local ablation — Palliation — Experimental studies

Pancreatic carcinoma accounts for 15% of cancer deaths in the United States [14]. Despite extensive and continuing research the overall 5-year survival remains at ~3% [29]. Radical surgery remains the only potentially curative treatment option [13, 26]. Untreated the median survival of these patients is 4 to 7 months [36]. Unfortunately only 15% of patients are suitable for surgical intervention [15, 27]. Therefore, for the vast majority of patients, palliation is the only therapeutic option for their symptoms of gastric outlet obstruction, pain, and jaundice, with its associated pruritis.

Neither surgical bypass nor endoscopic stenting has proven to be ideal in the management of these patients. Bypass surgery, although associated with good symptom control and low reintervention rates, continues to have a high perioperative mortality and morbidity [11, 30]. Therapeutic endoscopic retrograde cholangiopancreatography (ERCP) with stenting is a far less invasive procedure with minimal morbidity and mortality. Although ERCP relieves the symptoms of jaundice, gastric outlet obstruction and pain remain unaffected. In addition to this, the rates of readmission for stent occlusion and cholangitis are high [2].

There is, therefore, a need for a treatment that produces the good palliative results of surgical bypass in combination with the minimally invasive nature of an endoscopic procedure. Theoretically, "debulking" the tumor using an ablative technique could achieve this. Indeed several locally ablative techniques, including radiofrequency ablation [12], cryotherapy [28], and electrolysis [6], have been investigated, albeit to a limited extent, in experimental studies. However, if local ablation is to become a feasible palliative option it must be associated with the minimal invasiveness and compara-

tive risks of an endoscopic procedure. It is accepted that ERCP and biliary cannulation are not without risk and that there is a significant associated risk of complications, such as acute pancreatitis, and even death [10].

Previous work from this group has shown that it is feasible and safe to deliver an electrolytic dose to the pancreas via the pancreatic duct [35]. A large animal study has shown that although there is a transient rise in both serum amylase and C-reactive protein there is no evidence of acute pancreatitis on clinical, biochemical, or histological parameters. However, if this treatment modality is to be acceptable as a potential palliative treatment in the management of pancreatic carcinoma, the determination of any long-term sequelae or complications associated with it, such as fistulas, pseudocysts, or abscesses, is essential. Therefore the aim of this study was to determine, in a porcine model, the medium- and long-term mortality and morbidity associated with pancreatic electrolytic ablation. To achieve this, an ablative "dose" was delivered to the pancreas via the pancreatic duct. The intent was not to completely destroy the gland but rather to replicate the degree of ablation that theoretically would be used to "debulk" a tumor within the pancreas.

Electrolytic ablation works by the flow of a direct current between two electrodes within an ionic medium. The flow of ions that this generates results in a pH change at the electrodes [16, 17]. There is also a release of cytotoxic chemicals at the anode and cathode as is expressed by the following equations.

Anodic reactions:

$$2H_2O = O_2 + 4H^+ + 4e^-$$

$$2Cl^- = Cl_2 + 2e^-$$

Cathodic reactions:

$$2H_2O + 2e^- = H_2 + 2OH^-$$

The pH change in combination with the released chlorine gas and hydroxyl free radicals results in a locally cytotoxic environment that leads to parenchymal necrosis.

Electrolysis differs from other ablative techniques as it is nonthermal in nature [1, 21]. Other ablative techniques such as radiofrequency ablation, microwave coagulation, and cryotherapy all use extremes of temperature to create local tissue necrosis. Because of this chemical, nonthermal mechanism electrolysis is not adversely influenced when performed in the vicinity of large blood vessels [31], unlike the thermally based ablation techniques, which are all limited by the "heat-sink" phenomenon in which the ablative stimulus, heat or freezing, is dissipated by the flow of blood through adjacent blood vessels [23]. This is of particular importance when considering pancreatic ablation given the proximity of the pancreas to a number of major arterial and venous structures.

Materials and methods

Eight specific pathogen–free (SPF) female domestic white pigs were used for the study. Mean weight was 31.5 kg (range 28–35 kg). The study consisted of two treatment groups: 2-week survivors and 8-week survivors (n = 4).

All pigs were anesthetized in the same manner. Sedation was achieved with an intramuscular injection of ketamine (20 mg/kg) and xylazine (1.5 mg/kg). Each animal was cleaned with a solution of chlorhexidine and taken into the operating room. A laryngeal mask airway was inserted and anesthesia maintained with 1.5% halothane in oxygen. Oxygen saturation and heart rate were monitored continuously throughout the procedure.

A catheter was inserted in to the left external jugular and tunnelled subcutaneously to between the scapulae for postoperative blood sampling. A midline laparotomy was performed and the pancreas identified. Any bowel loops attached to the pancreas were dissected free, to mobilize the gland. A 5-cm duodenotomy was performed 20 cm distal to the pylorus. The opening of the pancreatic duct (PD) was identified and cannulated with a 4 French (1.33-mm) electrode catheter (Cordis Webster, Baldwin Park, CA) to a depth of 120 mm. Each catheter had six electrodes, which were 2 mm long and separated by 3 mm. For the electrolytic treatment the most distal and third most distal electrodes were used. The "unused" electrodes were electrically isolated.

Each animal had an electrolytic "dose" of 50 C, delivered to the pancreas at 50 mA using a direct current generator (ECU 100, Sõring, Quickborn, Germany). Mean treatment time was 21 min (range: 18–22 min). The dose of 50 C was decided after analysis of both the data from a pilot study investigating the feasibility and safety of pancreatic electrolytic ablation and preexisting dose/volume data generated from previous studies into hepatic electrolytic ablation [32, 34, 35].

At the end of the treatment, the electrode catheter was removed from the pancreatic duct and the duodenotomy was closed. The midline laparotomy was closed in a standard fashion and intramuscular injection of buprenorphine (0.01 mg/kg) was given before the animal was allowed to recover. During the perioperative period each animal was given 1 L of 0.9% saline by intravenous infusion.

Postoperatively each animal was given 1 L of 0.9% saline daily for the first 3 days. Blood samples were taken from the jugular catheter immediately pre- and postoperatively, daily for 7 days with further samples at 10 days and 2 weeks postoperatively. These samples were analyzed for serum amylase, glucose, C-reactive protein (CRP), calcium, urea, and electrolytes. In the 8-week survival group the jugular catheters were removed at 2 weeks to prevent line-related sepsis.

After the allocated time period the animals were euthanized. A postmortem examination was performed. In particular, the pancreas was examined to determine the extent of the induced necrosis, the patency of the pancreatic duct, the presence of any peripancreatic fluid collections, and any involvement of adjacent organs. Each pancreas was examined histologically to determine (1) the extent of the electrolytic necrosis, and (2) any associated pancreatitis in the surrounding parenchyma. Samples of lung and kidney were examined histologically for signs of a systemic inflammatory response.

Results were analysed statistically using a paired Student t-test (SPSS).

The ethics committees of the North-West Adelaide Health Service and University of Adelaide provided ethical approval for this study. The study was conducted under the guidelines of and conformed with the "Code of Practice for the Care and Use of Animals for Scientific Purposes" (NHMRS/CSIRO/AAC 1997) and the South Australian Prevention of Cruelty to Animals Act 1985.

Results

Premature deaths

Three pigs died prematurely. All of these animals died within 3 days of operation. At postmortem examination gross gastric dilatation was discovered; in addition to this there was a circumferential "ulcer" at the pars

esophagea in all animals. Acute gastric distension and catastrophic gastrointestinal hemorrhage has been well documented in pigs, in both surgical and veterinary literature [3–5, 7, 25]. Gastric decompression via a nasogastric tube inserted into the stomach through an oesophagostomy has successfully eliminated this phenomenon (unpublished data). The replacements for these animals and all animals subsequently used in this study underwent esophagostomy and nasogastric tube placement.

General

All other animals tolerated the electrolytic treatment well. The postoperative recovery of the animals was variable. However, all animals were eating, drinking, passing urine, and defecating normally 72 h after the procedure. All animals started to gain weight between 7 and 10 days postoperatively. At the end of the study period the mean weight in the 2-week group was 36 kg (range 34–38 kg), in the 8-week group the mean weight was 65 kg (range 45–77 kg).

Macroscopic appearances

Two-week group

In all the pancreata ($n = 4$), there was evidence of an electrolytic lesion in the splenic lobe of the gland. The mean diameter of these lesions was 2.4 cm (range 1.8–3.0 cm). The lesion was spherical and clearly demarcated from the adjacent normal pancreatic tissue. In three of the four glands the electrolytic lesion had breached the pancreatic capsule and there was an associated collection of pancreatic fluid (30–120 ml). There was no evidence of injury to any adjacent viscera. In all animals the duodenotomy was well healed. The lungs and kidneys were all macroscopically normal.

Eight-week group

All the animals in this group ($n = 4$) were found to have dense intraperitoneal adhesions at postmortem examination. The scarring from the electrolytic lesions was still visible in the splenic lobe of the pancreas. There were no associated fluid collections, pseudocysts, or fistulas. There was no evidence of damage to adjacent viscera in any of the animals. The lungs and kidneys were all macroscopically normal.

Histological appearance

A histological grading system for experimental acute pancreatitis [24] was used to score each of the pancreatic specimens (scoring range: 11 to 27). The grading pathologist was blinded as to whether the specimen was from the 2- or the 8-week group. From each animal samples from both the electrolytic lesion and the proximal pancreas were examined.

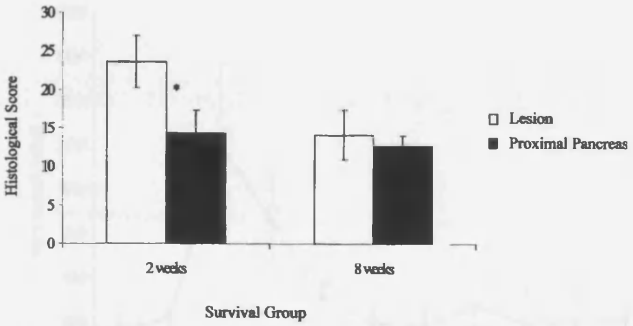

Fig. 1. Mean histological scores for pancreatitis at 2 and 8 weeks. *, Significant ($p < 0.05$) difference between values.

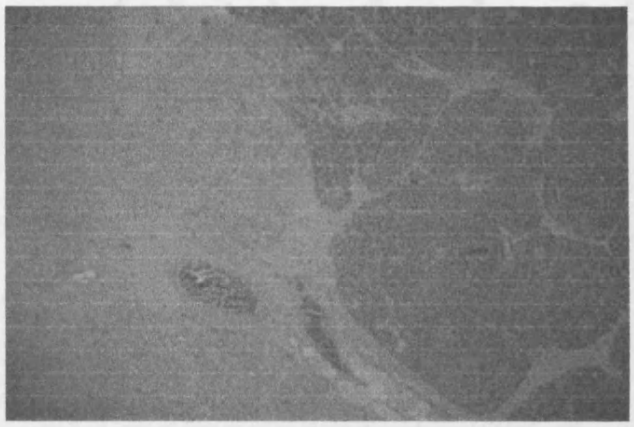

Fig. 2. Histological section through the electrolytic lesion at 2 weeks. (Stain H + E, ×4 original magnification.)

Two-week group

The mean histological score at the site of electrolysis was 23.6 (standard deviation SD 3.2); the mean score of the proximal pancreas was 14.3 (SD 2.9) (Fig. 1). In all four pancreata, abscess formation at the site of electrolytic ablation was noted. In addition to this, at the electrolysis site, there was marked inflammatory infiltration, extensive fat necrosis, predominately sublobular parenchymal necrosis, and moderate intrapancreatic hemorrhage (Fig. 2). There were only minimal signs of inflammation in the proximal pancreas (Fig. 3). In both samples the pancreatic duct remained patent. In areas where the electrolytic lesion was adjacent to the portal vein there was no evidence of damage to the vessel wall (Fig. 4). There was no evidence of inflammatory changes in either the lung or kidney. There was, however, mild pulmonary edema in one of the lung specimens; whether these were agonal changes or not it was not possible to say. A paired t-test was used to compare the histological grading scores from the site of the electrolytic ablation and the proximal pancreas. There was a significant ($p < 0.05$) difference between these two scores.

Eight-week group

The mean histological score at the site of electrolytic ablation was 14.1 (SD 3.4) score of the proximal

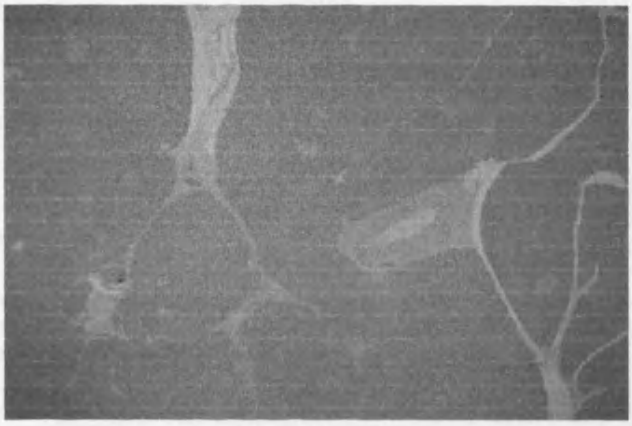

Fig. 3. Histological section through the pancreas, proximal to the electrolysis lesion, at 2 weeks. (Stain H + E, ×4 original magnification.)

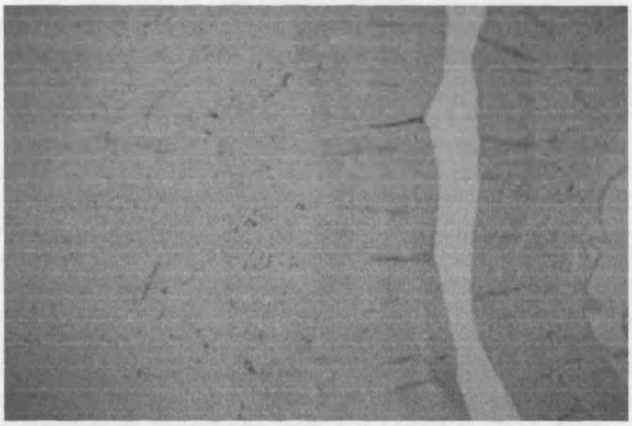

Fig. 4. Intact portal vein directly adjacent to the electrolysis ablation lesion. (Stain H + E, ×4 original magnification.)

pancreas was 12.8 (SD 1.3) (Fig. 1). There was no evidence of abscess formation in any of the examined pancreata. In this group, although there was again sublobular parenchymal necrosis at the site electrolysis, there were only minimal inflammatory changes, fat necrosis, and hemorrhage. In both samples the pancreatic duct remained patent. There was no evidence of inflammatory changes in either the lung or kidney. A paired *t*-test was used to compare the histological grading scores from the site of the electrolytic ablation and the proximal pancreas. There was no significant difference between these two groups.

Biochemistry

The biochemistry from all animals has been reviewed together, as there were no significant differences between the 2- and 8-week survival groups.

Amylase

All animals developed transient postoperative hyperamylasemia (Fig. 5). The mean serum amylase peaked at

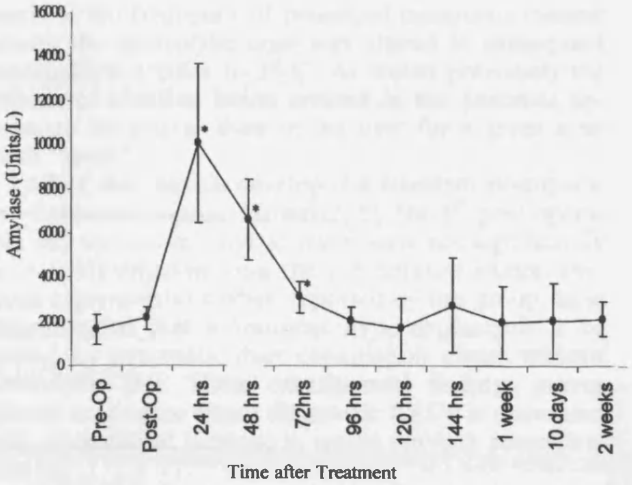

Fig. 5. Mean postoperative serum amylase. *, Significant ($p < 0.05$) difference from preoperative value.

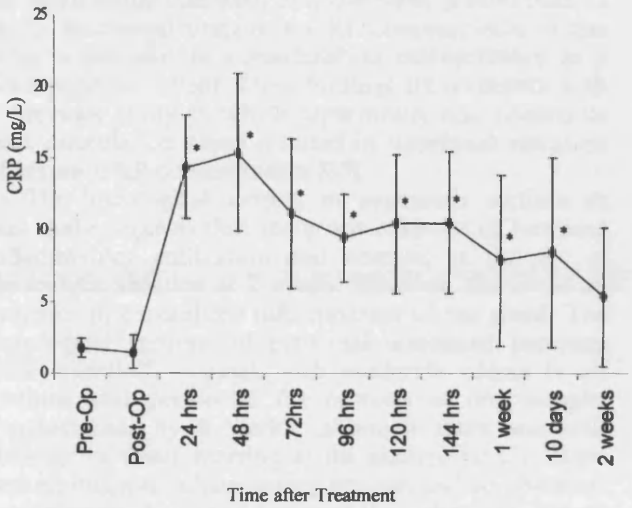

Fig. 6. Mean postoperative C-reactive protein. *, Significant ($p < 0.05$) difference from preoperative value.

24 h postoperatively and was significantly ($p < 0.05$) higher than the preoperative value. However, in all animals the serum amylase had returned to normal by the 4th postoperative day.

C-Reactive Protein (CRP)

The CRP was elevated in all the animals in the postoperative period (Fig. 6). The peak in mean serum CRP occurred at 48 h postoperation and was significantly ($p < 0.05$) higher than the preoperative value. In all animals the serum CRP had plateaued 96 h after the operation, albeit at a significantly ($p < 0.05$) raised level when compared to the preoperative figures. However, by 2-weeks postoperation there was no significant difference between the mean serum CRP and the preoperative value.

Glucose, Calcium, Urea, and Electrolytes

There was no significant change in serum levels of glucose, calcium, urea, or electrolytes after operation in any of the animals.

Discussion

Palliation continues to be the main therapeutic option for most patients with pancreatic cancer. Neither of the two most commonly used palliative techniques, bypass surgery or ERCP with stenting, have been shown to be without fault. Local ablation of pancreatic lesions using a minimally invasive approach has the potential to combine the positives of these techniques while, it is hoped, avoiding the more negative aspects.

The findings of this study suggest that electrolytic ablation of the porcine pancreas via the pancreatic duct is not associated with significant long-term complications. The premature deaths were due to gastric dilatation and ulceration with subsequent hemorrhage, a well-recognized phenomenon in postoperative pigs as previously mentioned [3–5, 7]. Indeed, female pigs are susceptible to gastric dilatation and resultant death when intensively farmed, even without the added stress of surgery [22].

The presence of fluid collections in three of the animals in the 2-week survival group is probably due to a combination of two factors: breaching of the pancreatic capsule and failure of the pancreatic duct to adequately drain the necrotic tissue. However, as peripancreatic collections were not found in the 8-week survival group, it would appear that these collections are self-limiting. The electrolytic dose used in this study has been extrapolated from dose-response data from previous work in the liver [32]; this finding suggests that electrolysis produces lesions of greater volume per coulomb dose in the pancreas than in the liver. We hypothesize that this may be due to the large quantities of electrically active ions in the pancreatic acinar cells and secretions, although this remains unproven. Failure of the pancreatic duct to adequately drain the products of electrolytic ablation postoperatively is a significant potential problem as this could result in both pseudocyst and fistula formation. In this experimental model the drainage of the porcine duct is more likely to be compromised than one would expect in the clinical situation. First, because of the anatomical morphology of the porcine anatomy there is no "pancreatic head" as such; therefore the site chosen for electrolytic ablation was within the splenic lobe some 12 cm away from duodenum. With the vast majority of human pancreatic tumors occurring in the head of the pancreas [20] the drainage distance would be significantly reduced in the clinical situation. Furthermore, in clinical practice the placement of a PD stent across the ablation lesion after electrolytic ablation in combination with sphincterotomy formation would be a logical step in maintaining PD patency and facilitating drainage of necrotic tissue. It should be noted that as a result of the frequency of presumed pancreatic capsule breach the electrolytic dose was altered in subsequent experimental studies to 25 C. As stated previously the volume of ablation lesion created in the pancreas appears to be greater than in the liver for a given coulomb "dose."

All of the animals developed a transient postoperative hyperamylasemia. However, by the 4th post-operative day the serum amylase levels were not significantly ($p > 0.05$) different from the preoperative values. Previous experimental studies, reported by this group, have demonstrated that a transient hyperamylasemia is be caused by pancreatic duct cannulation alone, without electrolysis [35]. These experimental findings mirror clinical experience where diagnostic ERCP is associated with a significant increase in serum amylase concentration [9].

The CRP values were also significantly elevated in the early postoperative period, peaking at 48 h. Although the CRP concentration remained significantly greater than the preoperative level after this time point, the mean serum concentration was never greater than 12 mg/L. In clinical practice a CRP concentration of this order would not be considered as extraordinary in a postoperative patient. These findings are consistent with a previous study in which laparotomy and pancreatic duct cannulation alone resulted in significant elevation of serum CRP concentration [35].

The histological scoring of pancreatic sections in this study suggests that there was evidence of localized inflammatory infiltration and necrosis at the site of electrolytic ablation at 2 weeks. However, there was no evidence of generalized inflammation of the gland. The histological sections of proximal unablated pancreas were essentially normal, with moderate edema in all sections and periductal fat necrosis in one sample. Furthermore, by 8 weeks, although there was still evidence of tissue scarring at the electrolytic site, there was no ongoing inflammatory process and no abscesses were present. This would suggest that, although present at 2 weeks postoperation, the pancreatic inflammation is both localized to the ablation area and self-limiting in nature. The presence of abscesses in the acute phase may be as a result of poor drainage of necrotic pancreatic tissue rather than a direct result of acute pancreatitis. As previously discussed, this complication may well be resolved by stent placement in clinical practice.

Electrolysis has been extensively investigated in the treatment of patients with unresectable liver tumors [19, 32–34]. Unlike other local ablative techniques electrolysis does not rely on extremes of temperature to create an area of tissue ablation. Rather, the flow of a direct current between two electrodes produces a flux of ions that results in a localized pH change [1]. This subtle chemical action has been shown to be safe when used adjacent to vascular structures [35], which is not the case with either radiofrequency ablation or cryotherapy [8, 18]. Histological findings from this study support this, as there was no damage to the portal vein even when directly adjacent to the electrolytic lesion. This is of particular note when considering electrolytic ablation of

pancreatic lesions, given the proximity of the portal vein to the head of the gland.

The data from this medium-to-long term follow-up study suggests that perductal electrolytic ablation is not associated with significant morbidity and mortality. Although there is evidence of localized pancreatic inflammation and necrosis at 2 week, there is no evidence of generalized pancreatitis. The transient increase in serum amylase concentration is most likely due to a combination of pancreatic duct cannulation and localized tissue ablation. The moderate CRP rise is consistent with recent laparotomy. The absence of peripancreatic collections in the 8-week group suggests that early complications are self-limiting in nature. Further investigation into the electrolytic dose–volume relationship in pancreatic tissue, in conjunction with post-tablation stent placement, likely to address the issue of collection formation due to capsule breach and inadequate drainage of ablation products.

The lack of a large animal model for pancreatic adenocarcinoma necessitates the use of normal pancreatic tissue in experimental studies such as this. Given that the response of cancerous tissue to any ablative technique may not exactly replicate the experimental findings in normal pancreas, data interpretation can be difficult, especially with regard to clinical applications of novel techniques. However, the results of this study suggest that perductal electrolytic ablation of the pancreas is safe and that it is not associated with significant morbidity or mortality in the medium and long term. Although electrolytic pancreatic ablation will not be a panacea for all patients with unresectable pancreatic cancer requiring palliation, it may well have a role in a proportion of such patients. The authors believe that further investigation of this technique is warranted. Future studies could usefully investigate minimally invasive approaches to the delivery of pancreatic electrolysis; such an approach would be essential for this technique to be acceptable in the clinical setting.

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Experimental studies of serum cytokine concentration following pancreatic electrolytic ablation

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
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Summary

Background: Pancreatic cancer has a poor prognosis, with symptomatic palliation being the priority in the majority of cases. Alternative palliative techniques, such as local ablation, are under investigation. Palliative per-ductal electrolytic ablation of the pancreas has been reported, in an experimental series. It is important to establish that this technique is associated with low morbidity and mortality if it is to be acceptable. The 'systemic inflammatory response syndrome' (SIRS) is a recognised and serious complication of both acute pancreatitis, and certain locally ablative techniques. This study aimed to determine whether pancreatic electrolytic ablation is associated with an increase in serum IL-1β and TNF-α concentrations, these cytokines playing integral roles in the inflammatory pathway of SIRS.

Material/Methods: Eight pigs underwent per-ductal electrolytic ablation of the pancreas. Serum samples, taken pre-operatively and post-operatively for two weeks, were analysed for IL-1β and TNF-α concentrations. Variations in cytokine levels were statistically analysed.

Results: Post-operative serum IL-1β and TNF-α concentrations did not significantly increase on pre-operative figures. There were no other clinical, biochemical or histological indicators of a SIRS-like phenomenon.

Conclusion: The results from this study suggest that electrolytic ablation of normal pancreas is not associated with either an increase in serum concentrations of IL-1β and TNF-α or a SIRS phenomenon. Although this study has only investigated the serum levels of two cytokines, IL-1β and TNF-α have both been shown to have a central role in the inflammatory cascade that leads to SIRS.

key words: pancreatic cancer • electrolytic ablation • SIRS • cytokines

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BACKGROUND

Pancreatic cancer is the fifth most common cause of cancer death in the USA [1,2]. Surgical resection is the only curative treatment available. However, the vast majority of patients are not suitable for surgery because of either locally advanced disease or distant metastases [3]. This leaves symptom control and palliation as the principle management option for most patients. Surgical biliary bypass and endoscopic retrograde cholangiopancreatography (ERCP) with stenting are the most commonly used palliative techniques. Untreated median survival is between 3 and 9 months, depending on the stage of the disease at diagnosis. This short life expectancy makes the morbidity and duration of hospitalisation associated with any palliative intervention of particular importance. Therefore ideally, any novel palliative treatment should combine effective palliation, of jaundice, pain and gastroduodenal obstruction, with the low morbidity associated with a minimally invasive technique.

The use of locally ablative techniques as potential treatments for the palliation of pancreatic cancer has been investigated [4,8]. Unfortunately the lack of a large animal model of pancreatic adenocarcinoma necessitates the use of normal pancreatic tissue for experimental studies such as these. To date, most of these techniques have utilised extremes of temperature to produce localised cell necrosis, for example cryotherapy [4], radiofrequency ablation [5] and laser-induced thermotherapy [6]. More recently this institution has reported successful ablation of the pancreas, in an experimental setting, using electrolysis [7,8]. Electrolysis may be more suitable for pancreatic ablation than other modalities because of: (1) its chemical rather than thermal mechanism (2) its demonstrable safety in the vicinity of vascular structures and (3) the relatively slow, controlled and predictable progression of ablation lesions produced [9].

Any procedure involving handling or manipulation of the pancreas has the potential to result in acute pancreatitis; even diagnostic ERCP is known to cause pancreatitis in up to 3% of patients [10]. Therefore it is possible that any form of localised pancreatic tissue ablation will lead to acute pancreatitis. The significant morbidity and mortality associated with acute pancreatitis is often associated with the systemic inflammatory response syndrome (SIRS) and subsequent organ failure. Furthermore, another locally ablative technique, cryotherapy, is known to produce a SIRS-type phenomenon in 1% of cases of liver ablation. This has been termed 'cryoshock' and has an associated mortality of 30–35% [11]. The determination of the presence of SIRS, or an increase in the serum levels of cytokines, as surrogate markers of SIRS, is therefore an important component in the investigation of the safety of new palliative treatments for pancreatic cancer.

The systemic inflammatory response syndrome is mediated by various cytokines. The interleukins 1 and 6 (IL-1 & IL-6) and tissue necrosis factor alpha (TNF-α) have been shown to have central roles in the pathogenesis of both SIRS and 'cryoshock', and serum concentrations of these cytokines have also been shown to be associated with outcome [12,14].

The aim of this study was to determine whether perductal electrolytic ablation of the normal pancreas results in either an increase in serum cytokine concentrations or a SIRS-type response, either as a result of acute pancreatitis or an ablation related 'cryoshock'-type phenomenon, by measuring circulating levels of IL-1β and TNF-α during the initial post-electrolysis period.

MATERIAL AND METHODS

Eight specific pathogen free (SPF) female domestic white pigs were used for the study. Mean weight was 31.5 kg (range 28–35 kg). The study consisted of two treatment groups: two-week survivors (n=4) and eight-week survivors (n=4).

All pigs were anaesthetised in the same manner. Sedation was achieved with an intra-muscular injection of Ketamine (20 mg/kg) and Xylazine (1.5 mg/kg). Each animal was cleaned with a solution of chlorhexidine prior to surgery. Anaesthesia was maintained with 1.5% halothane in oxygen. Oxygen saturation and heart rate were monitored continuously throughout the procedure.

A catheter was inserted in to the left external jugular vein and tunneled subcutaneously to between the scapulae for post-operative blood sampling. A midline laparotomy was performed and the pancreas identified. Any bowel loops attached to the pancreas were dissected free, to allow visualisation of the gland. A 5 cm duodenotomy was performed 20 cm distal to the pylorus. The opening of the pancreatic duct was identified and cannulated with a 4 French (1.33 mm) electrode catheter to a depth of 120 mm. Each catheter had six electrodes, which were 2 mm long and separated by 3 mm. For the electrolytic treatment the most distal and third most distal electrodes were used. The 'unused' electrodes were electrically isolated.

Each animal had an electrolytic 'dose' of 50 coulombs (C), delivered to the pancreas at 50 milliamps (mA) using a direct current generator (ECU 100, Söring GmbH, Justus-von-Liebig-Ring 10, D-25451 Quickborn, Germany.) Mean treatment time was 21 minutes (range: 18 to 22 minutes.) This coulomb dosage has been previously shown to produce spherical electrolytic lesions with a mean diameter of 15mm in porcine pancreatic tissue [7].

At the end of the treatment, the electrode catheter was removed from the pancreatic duct and the duodenotomy was closed. The midline laparotomy was closed in a standard fashion. An intramuscular injection of Buprenorphine (0.01 mg/kg) was given before the animal was recovered. During the peri-operative period each animal was given one litre of 0.9% saline by intravenous infusion.

Post-operatively each animal was given one litre of 0.9% saline daily for the first three days. Blood samples were taken from the jugular catheter immediately pre- and post-operatively, daily for seven days with further samples at 10 days and 2 weeks post-operatively. Post-operative blood samples were taken between 9 am and 10 am, this time period was selected as it allowed samples to be consistently taken at multiples of 24 hours post-electrolysis. These samples were centrifuged and serum was stored at –80°C.

After the allocated survival period the animals were euthanased and a post-mortem examination was performed.

Serum IL-1β and TNF-α concentrations were assessed using solid phase sandwich ELISAs specific for swine (Biosource International, California, USA.) Assays for each sample were performed twice and mean figures were taken as the final result. Results were analysed statistically, using a two-tailed paired t-test.

Results

All the animals tolerated the pancreatic electrolysis without event. At the end of the survival periods all animals had a normal diet and were gaining weight.

Histological examination of the pancreata demonstrated localised necrosis at the site of ablation, but no evidence of generalized pancreatitis was found. Serum C-reactive protein levels peaked at 15 mg/L on the second post-operative day.

Serum TNF-α was detectable at all stages, both pre and post-operatively, through the course of the experiment (Figure 1). The peak TNF-α level was detected immediately post electrolysis. The mean concentration of TNF-α, at this point, was 84.0 pg/ml with a standard deviation of 12.7. Although this is greater than the pre-operative concentration (mean 76.7 pg/ml, standard deviation 8.7), when the data was analysed using the paired t-test no significant difference was detected ($p=0.078$). Furthermore, when compared to the pre-operative data, there was no statistically significant difference in any of the other post-operative TNF-α concentrations ($p>0.05$).

Only minimal IL-1β was detectable at any stage of the experiment (Figure 2). Although the IL-1α concentrations decreased post-operatively, from the pre-operative level of 57.1 pg/ml, to almost undetectable concentrations (<15 pg/ml), these differences were not statistically significant ($p>0.05$).

Discussion

The morbidity and mortality associated with any procedure, especially a palliative one, is important in determining its suitability and acceptability both to medical staff and more importantly to patients. A significant incidence of a serious complication such as SIRS would obviously negate any potential benefits gained from the treatment.

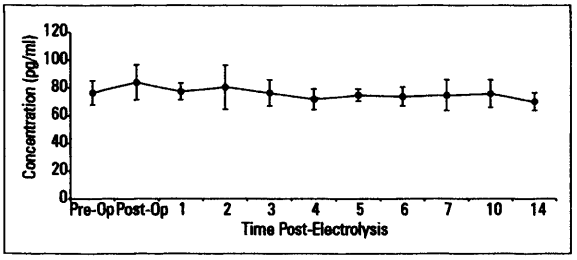

Figure 1. Mean TNF-α concentrations during the course of the experiment, with 1 standard deviation.

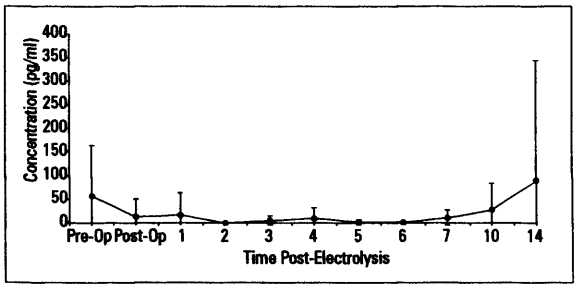

Figure 2. Mean IL-1β concentrations during the course of the experiment, with 1 standard deviation.

The results from this study suggest that perductal electrolytic ablation of the pancreas does not result in either a rise in systemic levels of known cytokine mediators (IL-1β and TNF-α) or a SIRS or 'cryoshock'-type phenomenon. This finding is supported by clinical and biochemical data that also demonstrated no evidence of significant or prolonged systemic inflammation. Although only two cytokines have been investigated both IL-1β and TNF-α have been shown to play central roles in the inflammatory pathway [13,15]. This data supports previous evidence that the technique of electrolytic ablation of the pancreas is safe [7,8]. Given that the temporal association between an initiating 'insult' and the associated increase in serum cytokine levels or the onset of SIRS has been demonstrated to be in the order of hours or days rather than weeks [16,18]; it would be unlikely that a SIRS-type picture would develop after the second post-operative week. Indeed, there were no clinical indicators of SIRS in the animals in the 8-week survivor group beyond the second post-operative week.

The association between cryoablation and SIRS is, in part, related to the extent of hepatic ablation and has been more frequently reported with lesions greater than 35-50% of the liver volume [16,19]. The absence of SIRS in this study may be in part due to the discrete nature of the electrolytic ablation lesion created. As the intention of this technique is palliation and tumour debulking, even in a clinical situation there would be no need to ablate large volumes of pancreatic tumour.

Histological analysis of the ablation lesions demonstrated localised areas of necrosis, at the ablation site, at two

weeks post-operatively being replaced with scarring by the eighth post-operative week. In both the 2 and 8-week groups the pancreatic duct remained patent proximal and distal to the ablation site. In the clinical setting the placement of a stent cross the ablation lesion would facilitate the drainage of any necrotic tissue. Examination of kidney and lung samples did not demonstrate any evidence of an acute inflammatory response. (unpublished data)

CONCLUSION

This study suggests that electrolytic ablation of the normal pancreas is not associated with either an increase in the systemic concentrations of inter-leukin 1β and tissue necrosis factor-α or the systemic inflammatory response syndrome.

The lack of a large animal model for pancreatic cancer hampers the investigation of the potential suitability of all locally ablative techniques, such as electrolysis, in the palliative management of pancreatic cancer; for it is difficult to predict how tumour tissue will respond to the ablative treatment when compared to normal pancreatic tissue. However this data, in conjunction with that from previous experimental studies [7,8], suggests that electrolytic ablation of pancreatic tissue is safe. The authors feel that this technique warrants further investigation as a potentially useful treatment option in the palliation of pancreatic cancer.

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Original Paper

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Endoscopic Perductal Electrolytic Ablation of the Pancreas: Experimental Studies of Morbidity and Mortality

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Key Words
Palliation · Pancreatic carcinoma · Electrolysis · Ablation · Stent · Animal model

Abstract
Background: Palliation of pancreatic cancer remains the only option for the majority of patients. Palliative techniques such as surgical bypass and endoscopic retrograde cholangiopancreatography (ERCP) with stenting are not ideal. The 'ideal' palliative technique would combine the efficacy of surgery with the minimal complications of an endoscopic procedure. Endoscopically delivered perductal electrolytic ablation of pancreatic lesions has the potential to meet these criteria. ***Methods:*** Fifteen pigs were used. The pancreatic duct was cannulated with an electrolysis catheter. Animals were randomised to either: controls, treatment 2-week survivor or treatment 8-week survivor. An electrolytic dose was administered to the treatment animals. Post-operatively, serum amylase and leucocyte count were assessed. Pancreata were histologically examined to detect evidence of acute pancreatitis. ***Results:*** Electrolysis was well tolerated. There was no difference in post-operative hyperamylasaemia and leucocyte count between the groups. Histological examination showed inflammation at the ablation site at 2 weeks, by 8 weeks this was replaced by scarring. ***Conclusion:*** The results of this study suggest that endoscopic perductal electrolytic ablation of the pancreas is feasible and safe. Biochemical and histological findings indicate self-limiting localised inflammation of the pancreas. This technique may have a role in the palliation of pancreatic cancer and warrants further investigation.

Introduction

Despite having a relatively low incidence of 8–10 per 100,000 population, pancreatic cancer is the fourth or fifth most common cause of cancer death in the western world [1]. Surgical resection remains the only potentially curative treatment available [2, 3]. However, due to the often-advanced nature of the disease process at the time of presentation, only 15% of patients are suitable for surgery [4]. This leaves palliation of symptoms as the main therapeutic option for the management of pancreatic carcinoma in the vast majority of patients.

Currently, the most commonly used palliative techniques are bypass surgery and endoscopic retrograde cholangiopancreatography (ERCP) with stenting. Although each of these established techniques has their own

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inherent advantages and disadvantages, neither has been shown to be ideal. The ideal palliative intervention would combine the good symptom control and low re-intervention rate of bypass surgery [5, 6] with the minimal morbidity, mortality [7] and reduced inpatient stay associated with endoscopic procedures.

Various locally ablative techniques have been investigated as to their potential role in the management of pancreatic cancer [8–10]. The authors of this study feel that electrolysis may be more suitable for use in ablation of pancreatic tissue than other local ablative techniques such as cryotherapy and radiofrequency ablation (RFA). Unlike many other locally ablative techniques, electrolysis utilises changes in tissue pH to generate regions of necrosis rather than extremes of temperature. In addition to this, electrolysis has been shown to be safe in close proximity to vascular structures, which is not the case with other ablative techniques [11]. Previous experimental studies have shown that the delivery of an electrolytic dose to pancreatic tissue via a catheter inserted into the pancreatic duct is both feasible and safe in the short to long term [12]. In these studies, cannulation of the pancreatic duct was performed as an open procedure. However, if the technique of electrolytic ablation of pancreatic lesions is to be of use in the clinical setting, it is necessary that the electrolytic dose can be successfully and safely delivered in a minimally invasive fashion.

The aim of this study, therefore, was to investigate the feasibility and safety of endoscopically delivered electrolytic ablation to the pancreas in a porcine model. Assessment of the theoretical morbidity and mortality potentially associated with endoscopic electrolytic ablation of the pancreas is important in determining whether this technique may have a role in the management of pancreatic cancer.

Material and Methods

Fifteen specific pathogen-free (SPF) female domestic white pigs were used for this phase of the study. Mean weight was 31.2 kg (range 29–32 kg). The study consisted of three groups: 'control: 2-week survivor' group (n = 5), 'treatment: 2-week survivor' group (n = 5) and 'treatment: 8-week survivor' group (n = 5).

All pigs were anaesthetised in the same manner. Sedation was achieved with an intra-muscular injection of ketamine (20 mg/kg) and xylazine (1.5 mg/kg). Each animal was cleaned with a solution of chlorhexidine and taken into the operating theatre. A laryngeal mask airway was inserted and anaesthesia maintained with 1.5% halothane in oxygen. Oxygen saturation and heart rate were monitored continuously throughout the procedure.

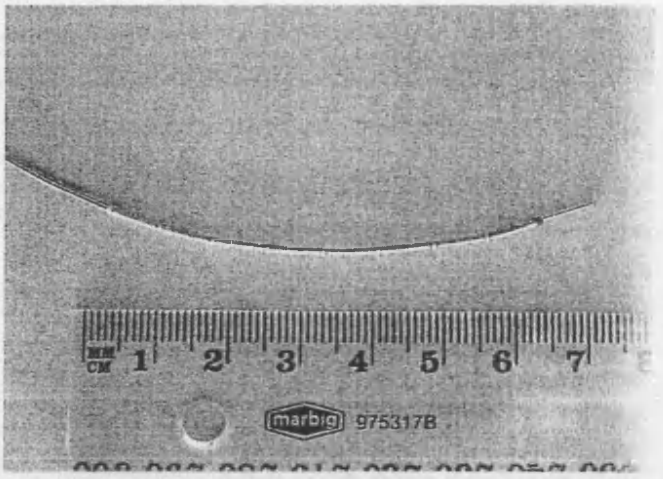

Fig. 1. Electrolysis catheter 3.7 french.

In previous experimental studies of pancreatic electrolysis a number of cases of upper GI haemorrhage secondary to gastric dilatation and ulceration at the pars oesophagea were observed. This phenomenon is a well-recognised complication in pigs, both post-operatively and in intensive farming conditions [13–16]. Mechanical decompression of the stomach by a nasogastric tube (NGT) inserted into the stomach via an oesophagostomy has been successful in eliminating this complication (unpublished data). Therefore all animals in this study underwent oesophagostomy formation and NGT placement.

Long-term intravenous access was achieved via a right femoral vein line tunnelled subcutaneously to between the scapulae on the back of each animal.

The anatomy of the porcine upper gastrointestinal tract, although similar to human anatomy, differs in a number of important ways. In the pig, the pancreatic duct runs directly parallel to the duodenum for approximately 4 cm before opening into the lumen of the gut. This acute angle would make endoscopic cannulation of the ampulla of Vater, via a per-oral approach, extremely difficult even with a side-viewing endoscope. To overcome this problem, a 'retrograde' endoscopic approach was undertaken in this study.

The purpose of this study was to demonstrate whether the endoscopic delivery of an electrolytic dose was feasible and safe. Therefore, it was felt that this 'modification' of the normal endoscopic approach did not affect the validity of the data produced by this phase of the study.

A 10-cm midline incision was made just superior to the umbilicus. A loop of distal duodenum/proximal jejunum was then located and brought out through the wound. No further mobilisation of the bowel was required. A 15-mm enterotomy was formed, into which an end-viewing endoscope was inserted in a cephalic direction (EVIS Gastrointestinal Videoscope GIF 130; Olympus, Adelaide, Australia). The endoscope was connected to a standard endoscopic stack (Olympus CLV-U20 (light source), Olympus CV-100 (video processor)). A 3.7-french electrolysis catheter, 150 cm in length, was used to deliver the electrolytic dose (fig. 1) (Revelation Tx Microcatheter, Ref. No. 01-082012; Cardima, Fremont,

Calif., USA). The catheter is comprised of six electrodes, each 6 mm in length and separated by 2 mm. The catheter was inserted via the instrumentation channel of the endoscope.

Following visualisation of the pancreatic ampulla, the pancreatic duct was cannulated with the electrolysis catheter to a depth of 10–12 cm. At this point, the animal was randomised to one of the three groups: 'controls', 'treatment: 2-week survivors' or 'treatment: 8-week survivors'. In order to minimise the effect of any 'learning curve' with regard to the technical aspects of endoscopic pancreatic duct cannulation, randomisation was performed in the following manner. Five groups of 3 pigs were formed. In each of these five groups, 1 animal was randomly allocated to each of the three experimental groups (i.e. 'controls', 'treatment: 2-week survivors' or 'treatment: 8-week survivors').

In the treatment group, an electrolytic dose of 25 C, at 25 mA, was delivered to the pancreata of the animals in the treatment groups, using a direct current generator (ECU 100; Söring GmbH, Quickborn, Germany.) The most distal and second-most distal electrodes were used; all the other electrodes were electrically isolated. Mean treatment time was 10 min (range: 9–11 min). In the control group, the electrolysis catheter was left in place for 10 min, but no electrolysis dose was administered.

Once the electrolytic dose had been delivered, the catheter was removed from the pancreatic duct and the endoscope was withdrawn. The enterotomy was closed with 3/0 PDS. The midline laparotomy was closed in a standard fashion. An intramuscular injection of buprenorphine (0.01 mg/kg) was given before the animal was recovered. During the peri-operative period each animal was given 1 litre of 0.9% saline by intravenous infusion.

Post-operatively, each animal was given 1 litre of 0.9% saline daily for the first 2 days. Blood samples were taken from the femoral venous catheter immediately pre- and post-operatively, daily for 7 days with further samples at 2 weeks post-operatively. These samples were analysed for serum amylase, glucose, C-reactive protein (CRP), calcium, urea and electrolytes and leucocyte count. In the 8-week survival group, the femoral lines were removed at 2 weeks to prevent line-related sepsis. The oesophagostomy tubes were also removed, at 2 weeks, if still present.

After the allocated survival period, the animals were killed by lethal injection. A post-mortem examination was performed and the macroscopic appearance of the pancreas was examined. The pancreata were excised with a section of duodenum and portal vein. In addition to this, sections of both lungs and kidneys were removed. All the specimens were preserved in 10% buffered formalin.

Pancreatograms were performed by the injection of contrast medium into the pancreatic duct. These studies were then assessed for evidence of duct stenosis, dilatation and patency.

Data from this study were statistically analysed using analysis of variance and Student's t test.

The ethics committees of the North-West Adelaide Health Service and Adelaide University provided ethical approval for this study. The study was conducted under the guidelines of and conformed with the 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (NHMRS/CSIRO/AAC 1997) and the South Australian Prevention of Cruelty to Animals Act 1985.

Results

General

All the animals tolerated the operative procedure without event.

The post-operative recovery of the animals in all the experimental groups was unremarkable. All animals were mobile and eating and drinking normally by the third post-operative day. At the end of the 2-week survival period, the mean weight in the treatment group was 36.5 kg (range 33–38 kg) and in the control group 36 kg (range 32.5–37.5 kg); statistical comparison of these sets of data using an unpaired Student t test showed there to be no significant difference ($p > 0.05$) between the two groups. In the 8-week treatment group, mean weight was 74 kg (range 68–81 kg).

Macroscopic Appearances of Pancreata

Two-Week Treatment Group. There was evidence of intraperitoneal adhesion formation in all animals. Two of the five animals had peripancreatic fluid collections adjacent to the ablation lesion. However, there was no evidence of damage to the adjacent viscera. The enterotomies were well healed in all animals, and the lungs and kidneys were macroscopically normal.

Pancreatic ductogram confirmed that the pancreatic ducts remained patent both proximal and distal to the site of ablation.

Two-Week Control Group. All the pancreata in this group had a macroscopically normal appearance. There was no evidence of pancreatic erythema, peri-pancreatic collections or pancreatic fistulae. The enterotomy was well healed in all animals and there was no damage to any surrounding viscera. The lungs and kidneys were macroscopically normal.

Pancreatic ductogram demonstrated that the pancreatic ducts remained patent, both proximal and distal to the site of the electrolysis catheter electrodes.

Eight-Week Treatment Group. In all animals in this group ($n = 5$), there were dense intra-peritoneal adhesions at post-mortem examination. The site of electrolytic ablation was evident in all the pancreata due to the localised scarring. In one of the glands, the tail of the splenic lobe appeared to be particularly scarred throughout. The enterotomy was well healed in all animals. None of the animals had evidence of peri-pancreatic collections, pancreatic fistulae or damage to surrounding viscera, particularly the portal vein and duodenum. The portal vein was free of thrombosis. Macroscopically, all lungs and kidneys were again normal.

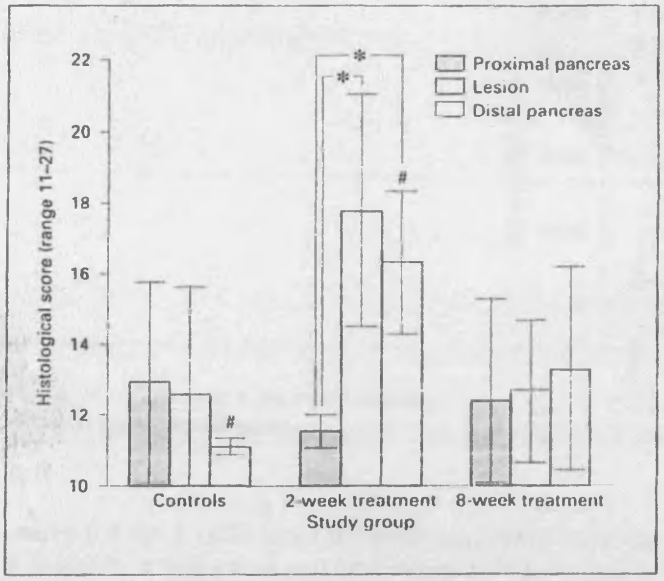

Fig. 2. Mean histological scores, with standard deviations, for each experimental group. * Denotes significant ($p < 0.05$) difference between scores within a group; # denotes significant ($p < 0.05$) difference between scores for a region of pancreas between groups.

Pancreatic ductograms showed that the ducts remained patent both proximal and distal to the site of the electrolytic ablation lesions.

Histological Appearance

Paraffin-mounted and haematoxylin-and-eosin-stained histological sections were examined by a pathologist, blinded to the experimental groups, using an established histological scoring system (range 11–27) for experimental pancreatitis [17]. Three sections of each pancreas were examined: the ablation lesion, pancreas proximal to the lesion and pancreas distal to the lesion.

Two-Week Treatment Group. The mean histological score at the site of electrolysis was 17.8 (standard deviation SD 3.27); the mean score of the proximal unablated pancreas was 11.5 (SD 0.5) and that of the distal unablated pancreas was 16.3 (SD 2.05) (fig. 2). All specimens in this group showed evidence of localised tissue necrosis and acute inflammatory change, with infiltration of polymorphs and monocytes, at the site of electrolytic ablation (fig. 3). Histological examination of the pancreas distal to the ablation site also demonstrated evidence of parenchymal necrosis and inflammatory infiltration. The portion of the pancreas proximal to the site of electrolysis was essentially normal. Statistical analysis of the histological

Fig. 3. Electrolytic lesion at 2 weeks after ablation. HE. × 3.

Fig. 4. Pancreatic duct at 2 weeks in control group. HE. × 3.

Fig. 5. Distal pancreas, 8 weeks after ablation. HE. × 3.

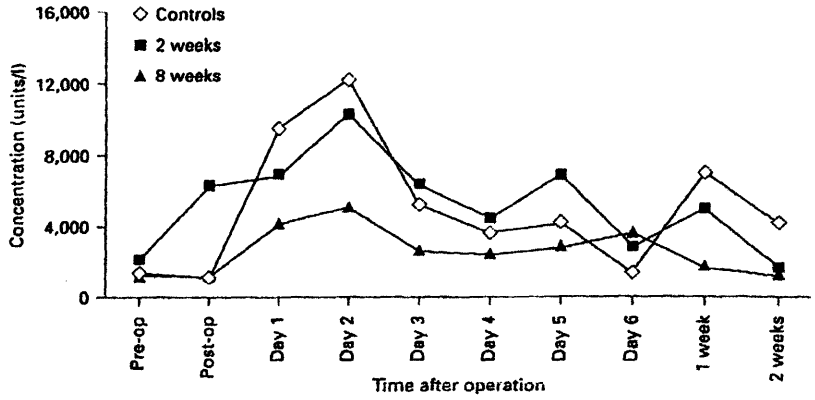

Fig. 6. Mean post-operative serum amylase for each experimental group.

scores for the 3 sections of pancreas examined from the 5 animals in this group was performed using analysis of variance. This showed that the histological scores for the proximal unablated pancreas were significantly lower than the scores for both the ablation lesion and the distal pancreas ($p < 0.05$). There was no significant difference between scores for the ablation lesion and the distal pancreas.

Two-Week Control Group. The mean histological score at the site of electrolysis was 12.6 (SD 3.03); the mean score of the proximal unablated pancreas was 12.9 (SD 2.88) and that of the distal unablated pancreas was 11.1 (SD 0.22) (fig. 2). In 4 of the 5 animals in the control group there was no evidence of necrosis, of either fat or parenchyma, in any of the three areas of the pancreata that were examined. In the remaining animal, however, moderate fat necrosis and sublobular parenchymal necrosis was noted at the site of the electrode tip and in the proximal portion of the gland (fig. 4). In the distal sections of the pancreata no inflammatory infiltrate or necrosis was detected; although 1 specimen showed moderate oedema, all other specimens in the group received the lowest score available. Statistical analysis of these data found there to be no significant difference in histological score between any of the three areas of pancreas examined.

Eight-Week Treatment Group. The mean histological score at the site of electrolysis was 12.7 (SD 1.99); the mean score of the proximal unablated pancreas was 12.4 (SD 2.86) and that of the distal unablated pancreas was 13.3 (SD 2.86) (fig. 2). All specimens were given the lowest score available for both oedema and inflammatory infiltrate. However, there was evidence of parenchymal necrosis both at the site of ablation and in the distal pancreas. In the histological sections through the ablation lesion there was predominately scarring of the parenchyma. In the sections of distal pancreas there was evidence of ductal proliferation in conjunction with parenchymal scarring that resembled chronic pancreatitis in appearance (fig. 5). As with the control group, no statistically significant differences between the three pancreatic regions was found on analysing the data.

The histological scores for each region of pancreas examined were statistically compared between the control group, the 2-week treatment group and the 8-week treatment group using analysis of variance. There was no significant difference between scores for the proximal pancreas or the ablation site between the three experimental groups. However, the histological scores for the distal section of pancreas in the control group was significantly less ($p < 0.005$) than the scores for the distal pancreas in the 2-week treatment group. There were no other significant differences between the experimental group scores for the distal region of the pancreas.

Biochemistry

The biochemical data from all animals were reviewed. The data, for each experimental group, were analysed and compared using analysis of variance.

Amylase

A post-operative increase in mean serum amylase concentration was noted in all three experimental groups, peaking on the second post-operative day in all groups (fig. 6). Although these peak concentrations were significantly greater than the pre-operative amylase concentration, no statistically significant difference was demon-

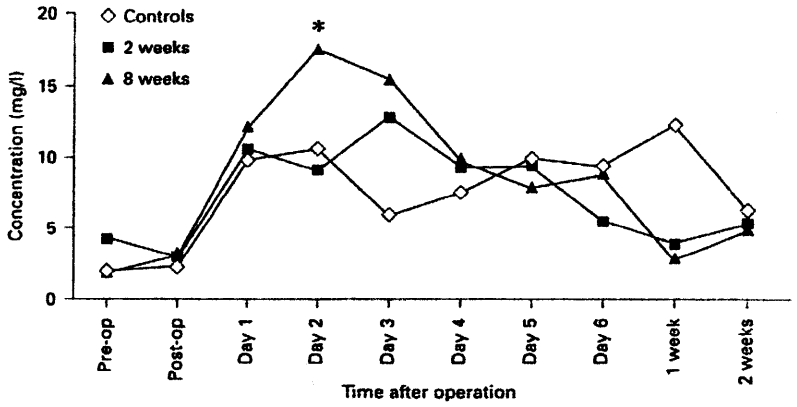

Fig. 7. Mean post-operative CRP for each experimental group. * Denotes significant ($p < 0.05$) difference from control group.

strated between the three peak concentrations themselves. The mean amylase concentrations returned towards normal after the 48-hour peak in all the groups. The 8-week survivor group mean amylase concentration returned to levels that were not significantly different from the pre-operative figure by the third post-operative day. In the other two groups there was a more fluctuating decrease in mean serum amylase. However, by the end of the initial 2-week post-operative period there was no significant difference in the mean amylase concentration, in any of the three experimental groups, and the corresponding pre-operative level.

C-Reactive Protein

There was a post-operative increase in serum CRP in all animals in the early post-operative period (fig. 7). In the 8-week survival group this peaked at 48 h following electrolysis. Statistical analysis showed this peak concentration was significantly greater than both the corresponding pre-operative CRP level and the mean CRP from the other two experimental groups at 48 h following the operation. On the third post-operative day, mean CRP concentration peaked in the 2-week treatment group; mean serum CRP concentration in both of the treatment groups was significantly greater than CRP concentration in the control group at this time point. From this point onwards, however, there was no significant difference between any of the experimental groups at any of the remaining time points. In the control group, the highest mean CRP (12.3 mg/l) was detected on the 7th post-operative day; however, this high mean concentration was mainly due to a single CRP level of 26 in 1 animal, as is demonstrated by the wide standard deviation of 12.3.

Leucocyte Count

In both treatment groups the white cell count peaked on the second post-operative day (fig. 8). Statistical analysis showed that these peaks at 48 h were significantly greater than the pre-operative concentrations and also the mean white cell count in the control group at 48-hour after surgery. The only other time point at which the mean white cell count in either of the treatment groups was different from the control group was at 1-week post-operatively. At this time point, the mean white cell count was significantly greater in the control group than the mean counts in either of the treatment groups (fig. 8). By the end of the second post-operative week, the mean white cell count in only the 8-week survival group remained at a level that was significantly greater than the pre-operative figure for the corresponding experimental group.

Glucose, Calcium, Albumin, Urea and Electrolytes

There was no significant change in serum levels of glucose, calcium, albumin, urea or electrolytes after operation in any of the animals.

Discussion

Palliation remains the mainstay of therapy for patients with pancreatic cancer. However, controversy remains as to what is the optimal treatment modality for this group of patients. Neither surgery nor ERCP with stenting have proven to be ideal palliative interventions. Furthermore the treatment options available to clinicians have recently increased with the introduction of endoscopically placed enteric metal stents (e.g. Wallstent) for the man-

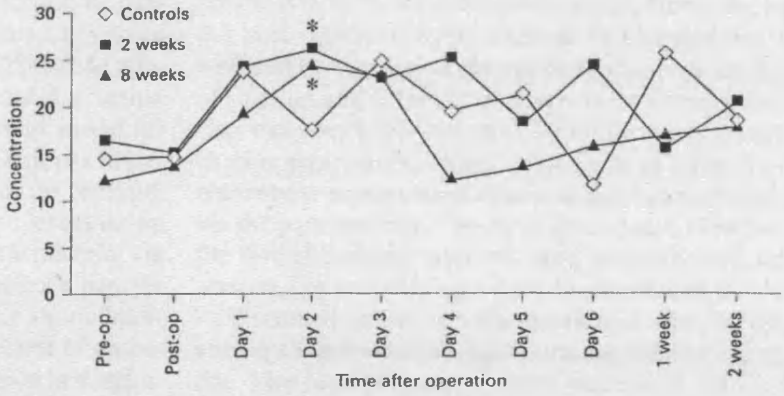

Fig. 8. Mean post-operative leucocyte count for each experimental group. * Denotes significant ($p < 0.05$) difference from control group.

agement of malignant gastric outlet obstruction [18, 19]. De-bulking of pancreatic tumours as part of patient palliation has a number of potential advantages. Biliary decompression may be facilitated and stent patency prolonged by ablation of the central regions of pancreatic tumours. In addition to this de-bulking of pancreatic head, lesions may improve symptoms of gastric outlet obstruction in its own right or in conjunction with enteric stenting. Electrolysis may be particularly suitable for pancreatic ablation because of its relatively slow, non-thermal action. Passage of a small direct current between platinum electrodes results in local tissue pH change and also the release of cytotoxic free radicals and gases [20], leading to an area of tissue necrosis. Another advantage of electrolysis over other locally ablative techniques is the demonstrable safety of electrolytic ablation in the vicinity of large vessels. Commonly used thermal ablative techniques such as RFA, microwave coagulation therapy and cryotherapy have been associated with significant haemorrhage [21–23]. This safety aspect is of particular relevance when considering pancreatic ablation given the close proximity of the portal vein to the pancreatic head, the site of most pancreatic cancers.

Previous studies have shown that per-ductal electrolytic ablation of the pancreas is both feasible and safe in the porcine model [12]. The results of this study not only support these previous findings but also show that it is possible to endoscopically deliver an electrolytic ablative dose to the pancreas via the pancreatic duct, safely and with minimal short- or long-term morbidity.

The electrolytic dose delivered to the pancreas in this and previous studies has been extrapolated from dose-volume data generated from work on electrolytic ablation in the liver [24]. In this study there was evidence that the pancreatic capsule had been breached in 2 of the 5 animals in the 2-week treatment group as peripancreatic fluid collections were found at post-mortem examination in 3 animals. However, it appears that these collections are self-limiting in nature as in the 8-week survival group no such collections were found at post-mortem. Another potential cause of collection formation is pancreatic duct obstruction, however this seems to be unlikely given that the pancreatic ducts were shown to be patent proximal and distal to the lesion on post-mortem ductogram (fig. 9).

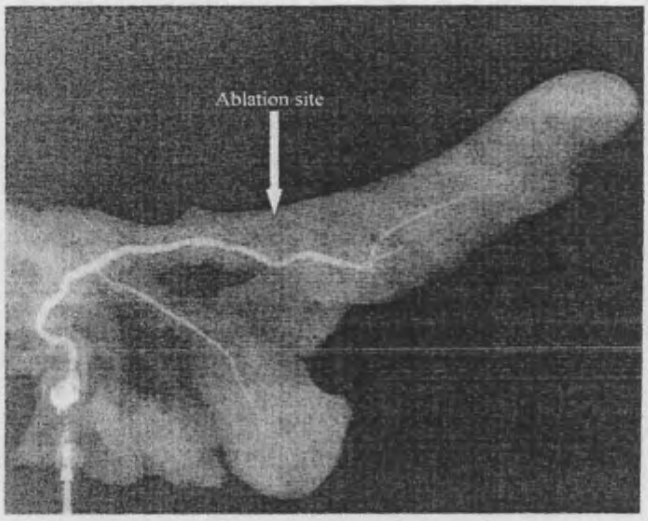

Fig. 9. Pancreatic ductogram showing patent duct proximal and distal to ablation lesion. Arrow indicates ablation site.

Inadequate drainage of necrotic tissue from the ablation lesion is a potentially serious complication of pancreatic electrolytic ablation, although in this study there was no evidence of ductal obstruction or stenosis. In clinical practice it could be envisaged that ductal obstruction would be less likely to occur than in a porcine model for the following reasons. Firstly, as the vast majority of human pancreatic cancers occur in the head of the pancreas [25], the physical proximity of the ablation lesion to the duodenum would aid in drainage of necrotic material via the pancreatic duct. In contrast, the absence of a pancreatic head in the pig necessitates a greater cannulation depth (100 mm). Furthermore, the placement of an expandable metal stent across the ablation lesion in the clinical setting would further aid rapid drainage of necrotic tissue into the duodenum.

Transient post-operative hyperamylasaemia was evident in all three experimental groups. Therefore it is likely to be largely due to pancreatic duct cannulation, although electrolytic ablation of pancreatic tissue may well contribute. Despite this temporary increase in serum amylase there was no clinical, biochemical or histological evidence to suggest acute generalized pancreatitis. Indeed, increases in serum amylase concentration are recognised sequelae to any manipulation of the pancreas, diagnostic ERCP for example [26]. Interestingly, in this study the peak mean amylase concentration was greatest in the control group rather than in either of the treatment groups, although this difference did not reach statistical significance.

There was a significant increase in mean serum CRP concentration in the early post-operative period in all three experimental groups. However, this increase was significantly greater in the treatment groups than it was in the control group, which is in keeping with the additional impact of pancreatic electrolysis over and above the trauma that is inherently associated with any surgical procedure. Despite the statistical significance of the post-operative increase in CRP, even the highest mean concentration of 17.8 mg/l in the 8-week treatment group on the second post-operative day would not be considered as a greatly elevated CRP in clinical practice.

Again the moderate increase in white blood cell count in all experimental groups would suggest a generalized response to laparotomy and surgery rather than to the electrolytic process itself.

The quantification of the histological changes seen in the pancreata in the three experimental groups shows that inflammation at the ablation site occurred by the second post-operative week. In addition to this, moderate parenchymal necrosis was evident in the distal regions of the pancreas in the 2-week treatment group. However, by the 8th post-operative week, these acute changes had been replaced by scarring of the parenchyma, both at the site of ablation and in the distal pancreas. In places, this scarring was associated with ductal proliferation resembling chronic pancreatitis. Whether this was as a result of the electrolytic process itself or due to sub-optimal drainage via the pancreatic duct is impossible to say. However, all the 8-week survivor animals were clinically well with a normal diet and gaining weight by the end of the study. As discussed earlier, the placement of a stent across the ablation lesion would facilitate drainage of necrotic material. The incorporation of stent placement into subsequent studies would help to determine the exact aetiology of the scarring of the distal pancreatic segment.

This is the first study in which local pancreatic ablation has been achieved via the pancreatic duct at endoscopy. The data suggest that perductal electrolytic ablation of pancreatic tissue via a minimally invasive, endoscopic technique is feasible and can be performed without significant morbidity or mortality in the short and long term. These findings are of considerable importance as minimally invasive delivery of electrolysis, presumably at ERCP, would be essential if this ablative technique is to have a clinical application.

Interpretation of the data from this study with regard to its implications for clinical practice is hampered by the necessity of using non-tumour models. The lack of a large animal model of pancreatic adenocarcinoma means that all experimental studies investigating localised ablation of pancreata are performed in normal, non-tumorous tissue. As a result, the behaviour of neoplastic tissue, in a clinical setting, to any given locally ablative technique cannot be guaranteed to replicate exactly what was found at experimental investigation. Despite this, the results of this study suggest that the endoscopic perductal electrolytic ablation of the pancreas is not only technically possible but also associated with minimal significant complications. This technique will certainly not be suitable for all patients nor in all circumstances; however, it may have a role in palliation of pancreatic cancer symptoms in a proportion of patients and so warrants further investigation.

Acknowledgements

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Postoperative death in sows as a result of gastric mucosal de-gloving at the pars oesophagea

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Summary

It is well documented that pigs frequently die from postoperative acute gastric dilatation, and proximal gastric 'stress' ulceration. Three cases of gastric mucosal 'de-gloving' are reported. This was secondary to acute gastric dilatation and resulted in death from acute haemorrhage. All animals had undergone major abdominal surgery. Histology confirmed that the proximal gastric mucosa had been 'de-gloved', or torn from the gastro-oesophageal junction, leaving exposed muscle fibres.

This syndrome has not been reported previously. The postmortem appearances of this mechanical injury could easily be mistaken for extensive oesophago-gastric peptic ulceration. This has major implications for prevention.

Keywords Pig; major surgery; gastric mucosa; de-gloving; gastric ulceration

The pig is a commonly used species in the development of experimental surgical techniques. Experimental studies using this species are often thwarted by unexpected postoperative deaths, complicating the analysis of data. During the development of novel surgical techniques at this institution, we have observed postoperative mortality in a number of cases, all of which showed unusual but similar features despite the animals having undergone different procedures. Analysis of the literature suggests that the postmortem findings in these animals may be related to what has previously been described as *peptic* ulceration and, furthermore, that this problem may be widespread in the porcine species, both within experimental studies and animal husbandry. Despite a number of potential possible explanations for the cause of these lesions being proposed previously, prevention has not been achieved. The aetiology of these lesions is of paramount importance in their prevention, and thereafter a reduction in the morbidity and high mortality seen with these lesions. The authors of this paper suggest a novel hypothesis for the cause of this phenomenon.

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Materials and methods

Nineteen female specific pathogen-free (SPF) domestic white pigs (weight range 27–35 kg) were obtained from the Pig and Poultry Production Institute, Roseworthy Campus, Roseworthy, SA, Australia. The pigs were raised from a closed herd and kept under strict quarantine protocol. There was a period of conditioning prior to operation varying from 4–6 days. The study conformed with the 'Code of Practice for the Care and

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Use of Animals for Scientific Purposes' (NHMRC/CSIRO/AAC 1990) and the SA Prevention of Cruelty to Animals Act 1985.

The pigs were housed in individual pens maintained at 23 ± 1°C at ambient humidity. Lighting was artificial, with a 12 h on/off cycle. The air exchange rate and airflow speed complied with the Australian code of practice for the care and use of experimental animals. The pigs were fed and watered *ad libitum* (standard grower diet of 0.7 g of available lysine 1 MJ digestible energy, with a digestible energy content of 14 MJ/kg). Water quality was suitable for human consumption. Preoperatively the pigs were fasted from food for 12 h; however, water was available *ad libitum*.

The pigs were part of two large surgical projects (Court *et al.* 2004, Morrison *et al.* 2004) involving laparotomy and abdominal surgery of variable duration (2–4 h). The operations ($n = 19$) consisted of midline laparotomy comprising either resection of 80–90% of liver volume ($n = 4$), or duodenotomy and intraductal electrolytic ablation of the pancreas ($n = 15$).

All animals were initially sedated with a deep intramuscular injection (forequarter paraspinal muscle) of ketamine (20 mg/kg) and xylazine (1.5 mg/kg). The pigs were then washed with a solution of aqueous iodine solution and taken into the operating theatre. A size 4 laryngeal mask airway was inserted and anaesthesia maintained using 1.5% halothane in oxygen titrated to a depth of anaesthesia. All pigs were allowed to self-ventilate throughout the operation. Oxygen saturation and heart rate were monitored throughout the operation. Central venous access was established with a tunnelled catheter from the right femoral vein. This remained *in situ* until death to allow blood sampling and intravenous fluid administration. At the end of the operation, the abdomen was closed using a mass closure technique and the skin was closed with absorbable sutures. Halothane administration was discontinued and an intramuscular injection of buprenorphine (0.005 mg/kg) was given before waking and as required, intravenously, in the postoperative period. Each pig was allowed access to food and water *ad libitum* in the postoperative phase. The animals were kept for a maximum of three weeks postoperatively in the liver resection group and two weeks in the pancreatic ablation group prior to euthanasia, unless death occurred earlier.

Results

General

Sixteen of the animals made an uneventful postoperative recovery. Unfortunately, three animals died from upper gastrointestinal haemorrhage between eight and 13 days after surgery as detailed below. In all animals, the clotting studies and platelets were within normal ranges at the time of death.

Case 1

This animal was noted to be retching repeatedly with small volumes of vomit in the first 2–3 days after liver resection. The animal then recovered, and was eating normally by the seventh postoperative day. However, there were signs of progressive anaemia with increasing pallor and decreasing serial haemoglobin levels with no obvious cause. Prothrombin ratio was mildly elevated 24 h postoperatively at 1.5, but returned to normal within 48 h and remained within normal limits until death. The animal was euthanized on the 12th postoperative day, due to symptomatic anaemia.

At autopsy, the liver was found to have regenerated back to full volume; however, the stomach was dilated and contained a large amount of blood and feed. There was a 5 cm circumferential area of exposed muscular wall, extending from the gastro-oesophageal junction, with a sharply demarcated distal edge, consisting of gastric mucosa (Figure 1).

Case 2

This animal died 13 days after liver resection following an acute upper gastrointestinal bleed. The animal was found dead unexpectedly overnight in its pen, having showed signs of normal postoperative

Figure 1 Case 1: Grossly dilated stomach showing extensive circumferential 'de-gloving' at pars oesophagea

recovery. Haemoglobin levels had been stable up until the fourth postoperative day, when the central line was lost. At autopsy it was determined that the bleeding had originated from a denuded area of muscular wall at the gastro-oesophageal junction. The lesion was macroscopically identical to the previous case (Figure 2). Again the liver was found to have regenerated macroscopically back to normal volume.

Case 3

The third animal had undergone pancreatic surgery and showed early postoperative signs of retching, with small volumes of vomit, associated with watery diarrhoea. This

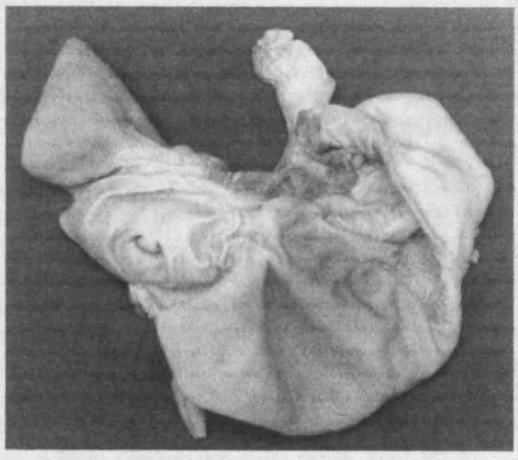

Figure 2 Case 2: Grossly dilated stomach showing extensive circumferential 'de-gloving' at pars oesophagea

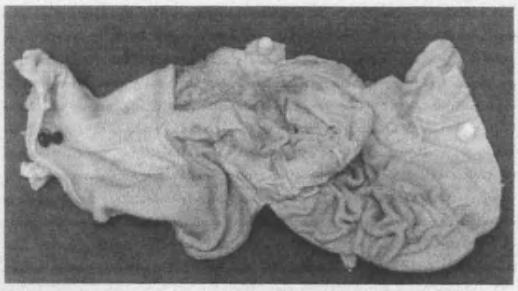

Figure 3 Case 3: Grossly dilated stomach showing extensive circumferential 'de-gloving' at pars oesophagea

animal never regained full appetite and was euthanized on the eighth postoperative day due to pallor and lethargy, following an acute bleed. Haemoglobin and prothrombin levels were not measured routinely in this study. At autopsy, an identical lesion was seen at the gastro-oesophageal junction, associated with gross dilatation of the stomach (Figure 3).

Histopathology

The histology of each of the lesions was identical and showed an absence of mucosa, with denuded muscle fibres in the base of each lesion. The proximal margin of each lesion consisted of stratified squamous cells (Figures 4 and 5), and the distal margin of glandular mucosa (Figures 6 and 7). There was no evidence of scarring or fibrinous exudate in the base of any of the lesions, which would be expected if the lesions were peptic in origin. However, an intense inflammatory reaction was seen.

Discussion

'Gastric ulceration' in the proximal stomach is well documented in pigs, and is often found to be haemorrhagic in nature. Most reported cases have described extensive ulceration at the pars oesophagea. This is an area of stratified squamous epithelium extending just below the gastro-oesophageal junction, which has no equivalent in the human stomach (Lee 2000). This area is an unusual site for peptic ulceration, given that it contains no glandular epithelium.

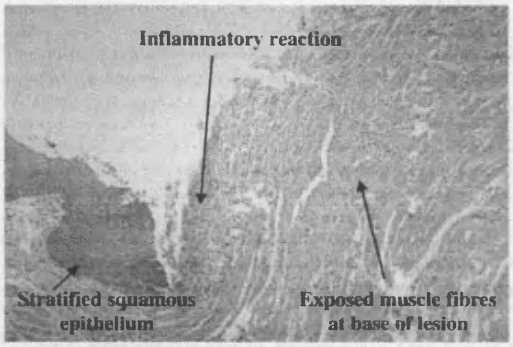

Figure 4 Proximal margin of lesion showing normal stratified squamous epithelium and denuded muscle fibres in base of lesion. Haematoxylin and eosin stain (× 100)

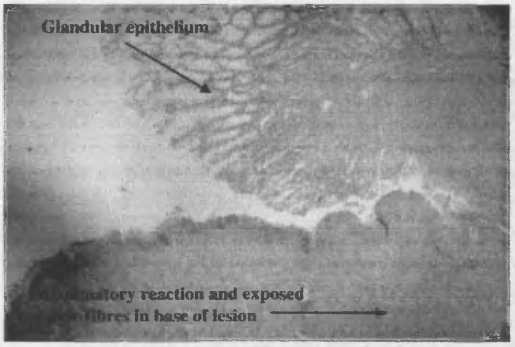

Figure 6 Distal margin of lesion showing normal glandular epithelium and denuded muscle fibres in base of lesion. Haematoxylin and eosin stain (× 40)

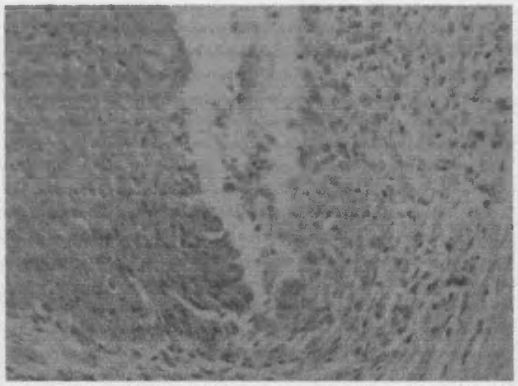

Figure 5 High-power view of Figure 4 indicating normal stratified squamous mucosa to the left and base of lesion on the right. Haematoxylin and eosin stain (× 200)

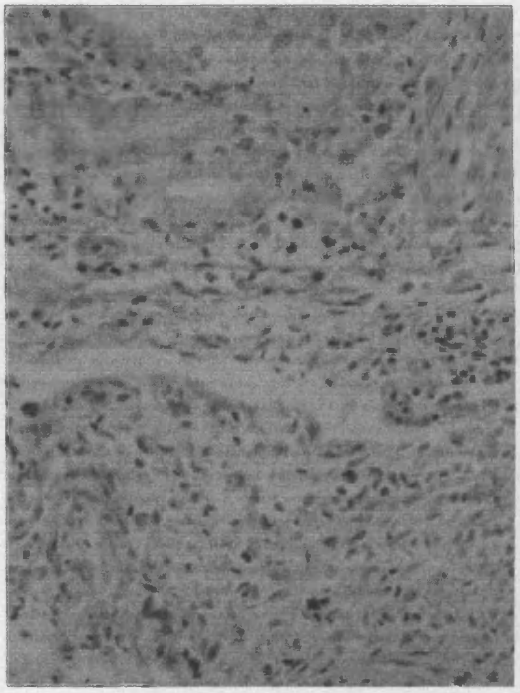

Figure 7 High-power view of Figure 6 indicating normal glandular mucosa superiorly and base of lesion inferiorly. Haematoxylin and eosin stain (× 200)

Such lesions were first reported in veterinary literature in the 1960s, in relation to the increase in intensive farming of pigs, and the increasing incidence of such lesions was thought to be related to the stress of overcrowding and overfeeding (Kowalczyk *et al.* 1960, O'Brien 1969). Intensively farmed pigs, that have not been subjected to surgery, have a 14% incidence of mortality associated with gastric ulceration resulting in major haemorrhage or perforation (Blackshaw *et al.* 1980, Gibson 1980, Straw *et al.* 1983), indicating that there may be a natural predisposition in this species. Subsequently, cases have been intermittently reported in experimental studies using pigs. These animals are often found to die 5–10 days after major surgery, such as liver or renal transplantation, due to massive haemorrhage from the lesion (Dent *et al.* 1971, Golby *et al.* 1971). The incidence of ulcerative lesions does not appear to be operation-specific; however, bile duct ligation is quoted as having a 100% incidence

of ulceration with 60% mortality, and a relationship with biliary obstruction has been proposed (Dent *et al.* 1971). Although all the animals in this study had surgical intervention in the biliary/pancreatic region, none of the animals showed evidence of biliary obstruction at postmortem.

Techniques to reduce acid secretion would be expected to have some impact on the incidence of such lesions if they were due to peptic ulceration; however, neither omeprazole nor H2 receptor antagonists have been successful in reducing the incidence, or mortality from this type of ulceration (Hedde *et al.* 1985, Friendship *et al.* 2000). Similarly, highly selective vagotomy did not alter the incidence in a small study comparing vagotomy with gastroenterostomy (van Hoorne-Hickman *et al.* 1978).

Pigs are known to be susceptible to acute severe gastric dilatation, which has been seen to be a cause of sudden death, particularly in sows (Straw *et al.* 1983). The cause for this is not known, as it occurs even without the animals being subjected to surgery; however, it is hypothesized by several authors that it may be due to a combination of rapid intake of large volumes of food and water and excess excitement (Bilkei 1987, Wendt 1987).

This study seems to suggest that pigs are particularly prone to the development of gastric dilatation after major abdominal surgery, with subsequent 'de-gloving' of the mucosa at the pars oesophagea. It is proposed that the competence of the lower oesophageal sphincter prevents these animals from adequately decompressing their stomach when it becomes distended. With increasing gastric distension over a period of hours to days, the mucosal junction splits at the point of transition between stratified squamous and glandular mucosa, whereas the muscular wall of the stomach is more elastic and has the potential for continued distension. The muscular wall of the stomach is thus denuded and left exposed to the erosive effects of gastric acid over succeeding days, leading to blood loss and eventual death.

Acute haemorrhage from gastric mucosal de-gloving, resulting from acute gastric dilatation, has not previously been reported. This purely mechanical phenomenon may previously have been mistaken for proximal *peptic* ulceration. We suggest that all such cases are fully investigated by postmortem examination and histology in order to more fully define the syndrome.

It is likely therefore that prevention of postoperative death from upper gastrointestinal haemorrhage may be achieved using effective gastric decompression rather than anti-secretory drugs.

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