INTRAPERITONEAL NUTRITION IN DOGS: A POSSIBLE ALTERNATIVE ROUTE FOR NUTRITIONAL SUPPORT

by

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The author would like to dedicate this thesis to the faithful and loving dogs that contributed to the many important findings of this study.

ABSTRACT

Prolonged protein-energy malnutrition in dogs and other species has serious and wide-ranging adverse effects on organ systems. Separating the effects of poor nutritional status from those of the underlying disease mechanism is difficult and has made documenting a significant improvement in the long-term survival of patients frustrating. Despite these problems, nutritional support continues to be an important component of the treatment in critically ill or injured patients. In Chapter 1 the consequences of prolonged malnutrition throughout the body are discussed and the methods of providing nutritional support assessed. For patients who have a functioning gastrointestinal tract (GIT), nutritional support should be provided so that as much of the GIT as possible is used. In companion animals, enteral nutrition can be provided in various ways, ranging from forced oral feeding to an indwelling jejunostomy tube. Enteral nutritional support is a more physiological route for nutrient absorption, is less likely to result in serious adverse effects and is cheaper than parenteral nutrition. However, there are circumstances under which the GIT cannot be utilised as the primary route for nutritional support. This prompted the development of intravenous parenteral nutrition. Administration of total daily caloric requirements necessitates the use of a central venous line and a continuous 18 to 24 hour infusion. Unfamiliarity with central venous catheters, expensive and the inability to provide 24 hour monitoring for critically ill patients has precluded the use of intravenous parenteral nutrition in many veterinary hospitals. For these reasons alternative routes of nutritional support have been examined.

Chapter 2 examines the work achieved over the past 20 years by workers who have investigated the peritoneal cavity as an alternative route for parenteral nutritional support. The peritoneum is capable of absorbing electrolytes, dextrose, complex carbohydrates, amino acids, intact plasma proteins, lipids and particulate matter. Previous studies using experimental animal models have demonstrated that intraperitoneal nutritional support is feasible.

The pilot study (Chapter 3) in this experimental series examined the physical, haematological, biochemical and peritoneal cytological response in dogs receiving a total nutrient admixture (TNA) comprised of dextrose, amino acids and a lipid emulsion administered into the peritoneal cavity by a repeat puncture technique. This study identified a number of significant adverse effects associated with intraperitoneal nutrition (IPN) when using a TNA given in sufficient quantities to meet 100% of daily energy requirements (RER). Acute non-septic peritonitis, hypoalbuminaemia, mild anaemia, electrolyte and glucose derangements, and sudden fluid shifts from the vascular space into the peritoneal cavity were the problems recognised.

The study reported in Chapter 4 was undertaken to investigate the cause of the marked peritoneal inflammatory response and to pursue possible explanations for the clinicopathological changes that occurred in the pilot study. This was achieved by administering the components of the TNA as individual nutrients and comparing the peritoneal response over a 5 day period. This study demonstrated that the lipid component of the TNA was responsible for the majority of the peritoneal inflammation seen in the pilot study, causing a 13 fold greater increase in peritoneal total white cell count (TWCC) compared to a 5% amino acid solution and a 10% dextrose solution. Although there was a significant increase in peritoneal TWCC in dogs receiving the lipid emulsion, there were no signs supportive of a clinically significant peritonitis at the dose administered. The mild anaemia, hypoproteinaemia (particularly hypoalbuminaemia) and electrolyte disturbances noted in the pilot study were again seen in the study described in this chapter.

Although well tolerated by the peritoneal cavity, 10% dextrose in the volume administered in Chapter 4 failed to supply enough calories on a daily basis to make this route of nutritional support feasible. It was decided to try and increase the percentage of daily caloric requirements supplied by using a dextrose polymer. This nutrient solution allowed more calories to be provided for a given osmolality without the risk of fluid shifts into the peritoneal cavity because of it's isotonic nature. The study presented in Chapter 5 identified that a 21.5% dextrose polymer solution caused an initial significant increase

in peritoneal TWCC, which then declined to near baseline concentrations by the end of the study.

It was concluded that twenty percent of resting energy requirements can be safely given to clinically healthy dogs in the form of a 10% dextrose solution, 5% amino acid solution, 10% lipid emulsion and a 21.5% dextrose polymer solution via a repeat abdominal puncture technique. Further studies are required before this form of nutritional support can be widely recommended to the veterinary profession.

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INTRODUCTION

Nutritional support for critically ill or injured dogs has become commonplace in veterinary medicine.[1,2,3,4,5,6,7] Infectious, inflammatory, neoplastic and functional diseases can have profound effects on the ability of a patient to consume enough energy, protein and micronutrients to maintain adequate organ function. Poor appetite, the inability to ingest food, reduced digestive and absorptive function of the gastrointestinal tract and metabolic derangements are but a few of the potential factors that can lead to malnutrition in these patients.

Nutritional support is beneficial in a number of clinical situations in people and dogs including trauma,[8] severe burns,[9] increasing the tolerance and reducing the side-effects associated with chemotherapy,[10,11] severe pancreatitis,[12,13,14] reducing the infection rate associated with bone-marrow transplants,[15] reducing mortality in severely malnourished children,[16] decreasing mortality associated with HIV infection,[10] short bowel syndrome,[14] chronic intestinal obstruction,[8] severe neurological impairment,[8] Crohn's disease,[11] enterocutaneous fistulas,[8] inflammatory bowel disease,[11] decreasing the length of hospital stay and decreasing the numbers of infections in the perioperative period.[10,17,18,19,14,9] Furthermore, nutritional support has been demonstrated to reverse a number of the adverse consequences of malnutrition by increasing serum protein concentrations,[8] improving nitrogen balance,[8] increasing bodyweight,[8] improving immune-function,[9,43] reducing bacterial translocation,[9,39] reducing the incidence of septic and non-septic complications post-operatively[8] and improving wound strength after surgery.[9,40]

It has been difficult to consistently demonstrate the effect of nutritional support on survival in these patients.[20] This may occur for two reasons. First, critical illness and severe injuries are themselves associated with significant morbidity and mortality. To separate the adverse effects of malnutrition from disease-related effects can be extremely difficult. Secondly, a difference may be hard to document, as the beneficial effects may be small. As a result, large patient numbers are required to identify statistically significant differences. To date, the majority of studies have utilised small patient numbers. There is a paucity of published material on the adverse effects of malnutrition in dogs. However, it

is generally agreed that the basic principles of human critical care nutrition can be applied safely to critically ill or injured dogs.

Techniques for administration of nutrients to dogs have developed rapidly over the last 30 years. Many of the newer techniques, such as intravenous parenteral nutrition, have been adopted following experience gained in the treatment of critically ill or injured people. Despite the paucity of well controlled prospective clinical trials determining the efficacy of nutritional support in dogs it remains an important part of caring for our debilitated patients.[7]

Before discussing the ways in which nutritional support can be provided to patients and the associated advantages and disadvantages of each technique, it is important to have a thorough understanding of the role different nutrients play in the day to day metabolism of dogs and of the adverse effects of prolonged malnutrition.

CONSEQUENCES OF MALNUTRITION

PROTEIN-ENERGY MALNUTRITION

The adverse effects of long-term protein-energy malnutrition on organ systems have been well documented in people.[20,21,22,23,24] However, the long-term consequences of malnutrition and its role in the outcome of dogs suffering from critical illness or injury have yet to be determined.

The consequences of malnutrition are dependent upon a number of variables. The severity of the underlying illness, the nutritional status of the patient prior to illness, the duration of malnutrition, and the magnitude of the malnutrition are considered important.[1,2,3,4,5,22,25] It should be highlighted that the changes in intermediary metabolism induced by "simple" starvation versus those induced by starvation in association with a hypermetabolic state: such as sepsis, severe inflammatory disease, or cancer ("stress starvation") are vastly different.

As an adaptation to "simple" starvation, the body switches from utilising glucose as the major energy substrate to fatty acids and ketone bodies. By reducing glucose requirements, the need for protein catabolism to fuel gluconeogenesis (valine predominantly) is significantly reduced, thus sparing essential body proteins.[1] In contrast, the elevated concentrations of counter-regulatory hormones such as glucocorticoids, catecholamines, growth hormone, and glucagon that are commonly present during "stress starvation" inhibit the action of insulin and lipoprotein lipase. As a consequence, decreased lipolysis and lipoprotein metabolism reduces the availability of fatty acids and ketones as an energy fuel. Glucose intolerance, through insulin resistance, places further demands on protein catabolism to supply amino acids for gluconeogenesis. As a result, patients undergoing "stress" starvation have a rapid loss of lean body mass.[1,23,25,26,27,28,29,30,31,32,33]

Studies in people have identified that almost every organ system in the body is affected by malnutrition to some degree. The following is a brief summary of those findings.[20,21,22,23,26]

Cardiovascular system

In the early stages of "stress" starvation the cardiovascular system is minimally affected by protein-energy malnutrition. As weight loss becomes more severe bradycardia, hypotension, reduced stroke volume and cardiac output ensue. These changes can be associated with ECG alterations such as a prolonged QT interval, decreased voltage deflections and right axis shift. This predominantly results from a reduction in cardiac muscle mass as a result of protein catabolism to supply amino acids for gluconeogenesis.[26]

Respiratory system

Histological changes in the lungs are rare in protein-energy malnutrition but significant abnormalities in respiratory function tests have been identified. Reduced minute ventilation, vital capacity, and tidal volume associated with decreased host defences have been the major reasons cited for the high incidence of pneumonia in malnourished humans. Death due to pneumonia is the most common complication of protein-energy malnutrition identified in people.[26]

Gastrointestinal

There appears to be tremendous species variability in the susceptibility of the gastrointestinal tract to protein-energy malnutrition.[34,35,36,37] Laboratory animals such as guinea pigs, rats and mice seem to be the most susceptible to mucosal atrophy and the development of bacterial translocation. There is very little published work on mucosal atrophy in malnourished dogs. One study in healthy dogs identified a significant number of bacteria in the mesenteric lymph nodes indicating that bacterial translocation in this species may be a normal event.[38] Decreased gastric acidity, reduced gastrointestinal motility, and reduced concentrations of disaccharidase in the mucosal brush border have been consistent findings in humans with protein-energy

malnutrition.[26] The concomitant reduction in serum protein concentration leads to mucosal oedema and congestion.[105] This results in a significant reduction in the absorptive capacity of the gut, exacerbating protein-energy malnutrition and promoting diarrhoea.[105] Villous atrophy and bacterial translocation can be reduced by nutritional repletion.[39,9] A study in dogs identified that nutritional support improved intestinal wound collagen content and bursting pressure.[40]

Hepatobiliary system

During protein-energy malnutrition the liver becomes smaller as a result of a marked reduction in glycogen[25] and fat stores[26] and hepatic protein is utilised to maintain glucose production for the vital organs. Prolonged sulfobromophthalein (BSP) retention has been identified during long-term starvation in people. However, other liver function tests are not affected consistently.[26]

Immune function

Decreased cell-mediated immunity is the most consistent change in immune function associated with malnutrition.[3,26,41,42] Reduced lymphocyte responsiveness to the mitogens pokeweed mitogen, concanavalin A and phytohaemoglutanin have been documented in people with prolonged "stress" starvation.[26] Reduced peripheral lymphocyte counts and reduced thymic and lymphoid tissue mass have been observed in malnourished human neonates.[26] These factors can be improved by nutritional support.[43,9] Humoral immunity is also affected by starvation, but to a lesser degree. The absolute number of antibodies is reduced, but their responsiveness to antigenic stimulation remains within normal limits.[26] Plasma fibronectin and cell-associated fibronectin concentrations (necessary for opsonisation, normal macrophage activity and cellular barrier integrity) begin to decline within 24 to 48 hours of protein-energy malnutrition.[3] Serum complement, fibrinogen and globulin synthesis is significantly reduced with 72 hours of malnutrition.[3] Patients who receive nutritional support in the perioperative period have a reduced rate of infectious complications compared with patients who do not.[10,17,18,19,9] There is also a reduced rate of infection in bone-marrow transplant recipients receiving nutritional support.[15]

Wound Healing

Studies have identified that wound healing has a biological priority.[25,44] Amino acids required for fibroblast proliferation and orderly wound healing are derived from lean muscle mass in the face of negative nitrogen balance. However, selected aspects of wound healing are significantly delayed by malnutrition. These include neovascularisation, collagen synthesis, and wound remodelling.[25] Concurrent severe hypoproteinaemia causes wound oedema, further delaying the healing process. Bowel oedema and hypoproteinaemia has deleterious effects on gastrointestinal anastomoses in people[26] but not in dogs.[45] However, nutritional support was shown to increase the collagen content of intestinal anastomoses, decrease the time required to reach the healing end-point and increase the bursting strength of the anastomoses in a group of research dogs.[40]

MICRONUTRIENT DEFICIENCIES

Essential fatty acids

The only essential fatty acid of both dogs and humans is linoleic acid. "Essential" means that these species cannot synthesise linoleic acid from other fatty acids in the body. Dogs require a minimum of 2% of the daily caloric intake as linoleic acid to prevent essential fatty acid deficiency.[24] People and dogs can convert linoleic acid to arachidonic acid, and as a result, do not require a source of arachidonic acid in their diet, unlike cats.[24] Fatty acids are involved in many bodily functions such as constituents of cell membranes, the synthesis of prostaglandins and the regulation of cholesterol synthesis.[46] Deficiency of essential fatty acids can be seen in dogs fed for prolonged periods diets low in fat, poorly formulated dry diets and diets utilising beef tallow as the sole source of fat in the diet. Parenteral nutrition may be associated with essential fatty acid deficiency if lipids are not a component of the nutritional solution, or if only a small percentage of calories are supplied using lipid. Fat stores in healthy dogs can supply linoleic acid but due to the anti-lipolytic action of insulin (which is continuously released by the stimulus of glucose infusions) endogenous linoleic acid often cannot be utilised in patients receiving parenteral nutrition.[26]

The classical manifestation of essential fatty acid deficiency in dogs and people is desquamative dermatitis preceded by a dry lustreless coat, alopecia, and oedema.[24,26] Other abnormalities associated with essential fatty acid deficiency include delayed wound healing, anaemia, thrombocytopenia and hepatic dysfunction.[47]

Trace elements

Trace elements (elemental metals found in very small quantities in normal human and animal tissues) are involved in a wide range of body functions. Zinc, copper, manganese, chromium, selenium, and molybdenum deficiency have all been reported in people receiving long-term parenteral nutrition. There are no case reports of confirmed trace element deficiencies in dogs treated with total parenteral nutrition, probably because of the relatively small number of veterinary patients that have received total parenteral nutrition (TPN) for prolonged periods.

Zinc is the best known of the trace elements. It is required for the normal function of several metalloenzymes such as alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase. It is thought to play an important immunological role, with zinc deficiency resulting in T-cell dysfunction. Acrodermatitis enterohepatica-like syndrome is the most common manifestation of zinc deficiency. Zinc deficiency may occur in people during TPN if adequate amounts are not supplemented (100 μg/kg/day)[26] or if excessive losses of zinc are occurring. Accelerated zinc loss may be seen in severe diarrhoea, especially in people with small-bowel fistulae.[26]

The other trace elements listed above function as co-factors, activate enzyme-metal-substrate complexes, participate as structural constituents of metalloenzymes, facilitate oxidation-reduction reactions, or influence carbohydrate and lipid metabolism.[47] The varied role of trace elements in cellular function means that deficiencies can have a wide impact throughout the body. Clinical signs associated with trace element deficiencies include anorexia, diarrhoea, hypothermia, muscle pain and tenderness, cardiomyopathy, peripheral neuropathy and glucose intolerance.

Most trace elements are intracellular in nature. Serum concentrations are thus an inaccurate measure of total body stores, making documentation of trace elements deficiencies difficult and their reported association with disease tenuous.

METHODS OF PROVIDING NUTRITIONAL SUPPORT

ENTERAL NUTRITION

Nutritional support via the gastrointestinal tract has long been considered the ideal route of feeding because of the trophic effect of nutrients on mucosal, hepatic and pancreatic cells.[9] In addition, enteral nutrition avoids the high serum concentrations of glucose, amino acids and triglycerides that commonly occur during parenteral nutrition.[12,48,49] There are many methods for supplying nutrients through the gastrointestinal tract ranging from forced oral feeding to jejunostomy tubes. The method utilised for each critically ill or injured patient depends on a number of factors. These include the presence or absence of vomiting, the length of time nutritional support is required, the safety of the method chosen, the facilities and equipment available, the experience of the clinician, and the cost of each method. The patient's neurological status and type of injury or disease process are additional factors that need consideration. For example, cranio-facial trauma or severe stomatitis would preclude the use of forced oral feeding, oro-gastric intubation and possibly a pharyngostomy tube. Generally speaking, as much of the gastrointestinal tract should be used as possible to avoid maldigestion or malabsorption of enteral feeding solutions. However, the region of the gastrointestinal tract into which the feeding solution is administered will depend on the site of gastrointestinal dysfunction.[1]

Forced Oral Feeding

This method of nutritional support should be restricted to co-operative patients that require short-term support, e.g. two-three days maximum. It should not be utilised for patients with traumatic injury of the head and neck.[1] Forced oral feeding can be accomplished in several ways. Moist solid foods such as canned food can be placed as boluses into the pharynx and the dog's mouth closed. Massaging the glottis can

stimulate swallowing. An alternative method is to use liquid gruel prepared by adding water to a variety a commercial dog foods. The gruel can then be syringed into the back of the mouth. This method is best suited for dogs with moderate to long noses as large quantities can be placed in the mouth, reducing the amount of time needed to feed the dog.

The main limitation of forced oral feeding is the willingness of the patient to co-operate. Another disadvantage of this technique is the caloric density of the food (1100 to 1800 kcal/kg).[1] This necessitates the use of large volumes of food that can result in gastric distension and vomiting.[1]

Nasogastric tube feeding

The advantages of nasogastric or naso-oesophageal tube feeding are ease of placement of the feeding tube and cost-effectiveness. The major limitation is that only small diameter feeding tubes can be placed through the nasal cavity, which in turn makes the use of gruel difficult. Commercially prepared veterinary enteral diets e.g. Clinicare®[a] or human products such as Jevity®[b] can be used instead of gruels. These are well tolerated by most patients but in some animals have been associated with abdominal cramping and diarrhoea.[1] Mechanical problems associated with nasogastric tubes include vomiting the tube, aspiration of the tube after vomiting and regurgitation of meals. Placing the dog in the sitting position during feeding can reduce this latter complication.[1] Naso-oesophageal tubes are preferred by some authors and are considered to be safer than nasogastric tubes because they do not traverse the lower oesophageal sphincter.[1,2,5] Oesophagitis associated with the passage of gastric contents along the outside of the nasogastric tube into the distal oesophagus has been reported.[2] Other complications of tube placement include rhinitis and dacrocystitis.[5] Patients with gastroparesis or a reduced level of consciousness are poor candidates for this method of nutritional support as the risk of aspiration pneumonia is high.

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[a] Jevity. Ross Laboratories. Ohio, USA.
[b] Clinicare. PetAg, Inc. IL, USA.

Pharyngostomy tubes

Placement of a pharyngostomy tube has been well described elsewhere.[4,50,51,52] Pharyngostomy tubes can be used when there is nasal or facial trauma or when there is difficulty in placing or maintaining a nasogastric tube.[5] Complications associated with using a pharyngostomy tube include vomiting the tube, aspiration pneumonia, oesophageal excoriation, infection at the ostomy site and interference with the normal movement of the glottis.[4] This latter complication is more commonly associated with larger diameter feeding tubes. Soft feeding tubes are more likely to curl around the larynx compared with rigid tubes and are associated with a higher risk of aspiration. Meticulous care of the ostomy site is needed to prevent serious wound infection that necessitates removal of the feeding tube. When using larger diameter feeding tubes (16-20 French); gruels or slurries made by blending high-energy canned food (e.g. Hills'® a/d)[c] with water or enteral solutions can be given. This can significantly reduce the cost of feeding. The relatively high incidence of complications[2] and the development of alternative enteral feeding methods such as the gastrostomy tube have meant that pharyngostomy tubes are less frequently used in specialist facilities.[52,53]

Oesophagostomy tubes

Oesophagostomy tubes are a relatively new method for supplying nutritional support to dogs and cats.[6] The method was developed to overcome the complications of pharyngostomy tubes, particularly in cats that have a higher incidence of complications than dogs.[4,52] Experience with this technique is limited, although one report identified that the technique was well tolerated and associated with minimal complications.[6] The greatest concern with oesophagostomy tubes is the development of oesophageal strictures.[54,55] Indications for using an oesophagostomy tube include cranio-facial trauma, pharyngeal trauma and poor tolerance of other methods. No specialised equipment is required to place the tube. Two techniques for introducing the tube into the oesophagus can be used: fine-needle percutaneous tube placement and a surgical cut-down technique. Both techniques require general anaesthesia or heavy sedation for tube placement.[6]

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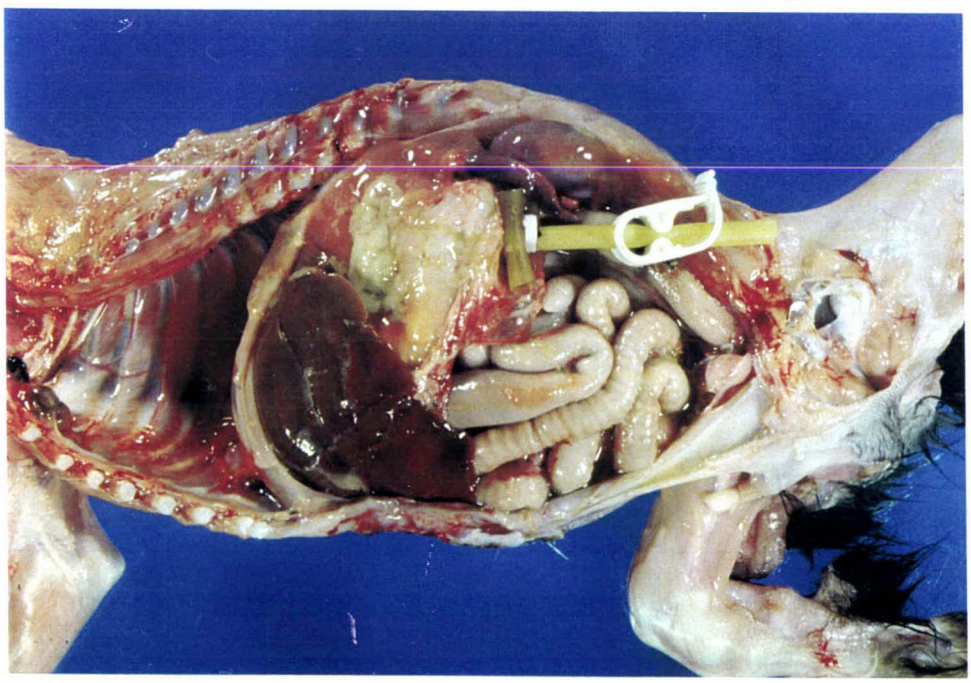
[c] Hills' Pet Nutrition, Kansas, USA.

Gastrostomy tubes

Gastrostomy tubes have now become the most widely used method for long-term nutritional support of veterinary patients throughout the world. With the development of the percutaneous endoscopic gastrostomy (PEG) technique the procedure can be performed in less than 10-15 minutes. This has made gastrostomy tubes cost effective and resulted in their use in a wide range of situations rather than reserving them exclusively for patients with disease of the nose, pharynx and oesophagus. Gastrostomy tubes have been associated with minimal complications in cats and dogs and have been used successfully for as long as a year.[4] Minor excoriation at the ostomy site, peritonitis (Figure 1-1.), and migration of the tube distally in the intestinal tract has been recorded in a number of animals.[5]

Figure 1-1. Percutaneous endoscopic gastrotomy (PEG) tube associated with septic peritonitis in a dog.



The incidence of peritonitis can be minimised by ensuring the tube is left in place a minimum of 5 to 7 days following placement, to allow secure stoma formation.[1,4,5] The use of PEG tubes has largely superseded the need for surgical placement. Recently, a gastrostomy tube-placing device has been developed that overcomes the necessity for an endoscope to place a gastrostomy tube.[d] Large diameter gastrostomy tubes (16-20 French) can be used in medium to large breed dogs.

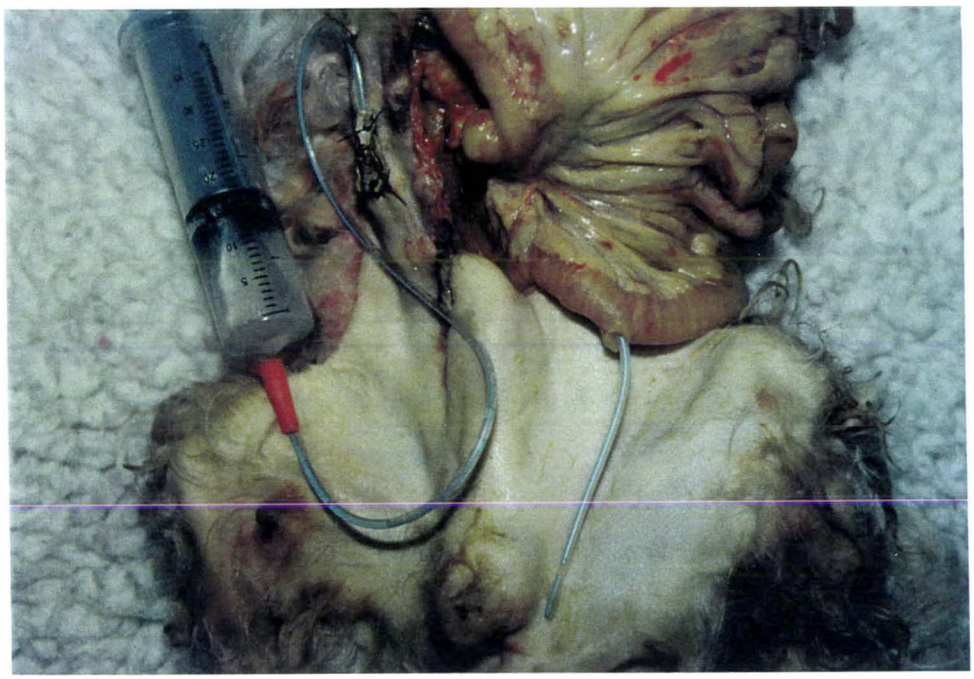
Jejunostomy tubes

The placement of a jejunostomy tube has been reviewed in detail elsewhere.[2,3,4,5] Patients with a poor gag reflex, disease of the pharynx, oesophagus or stomach, gastric hypomotility or frequent vomiting are good candidates for the placement of a jejunostomy tube.[4] Jejunostomy tubes require a functional but not necessarily *fully* functional small intestine for the successful use of this feeding technique.[56] The placement of a jejunostomy tube should be considered when an exploratory laparotomy is performed in any critically ill or injured animal that cannot be fed by a more proximal route. If a full celiotomy is not desired, a mini-celiotomy can be performed to place a jejunostomy tube. Jejunostomy tubes utilise fine diameter (2.5-5 French) feeding tubes that necessitate the need for liquid enteral diets. If absorptive function is poor, the use of "monomeric" diets containing amino acids, monosaccharides and disaccharides that do not require digestion has been advised.[4] However, the high osmolality of these formulae have been associated with nausea, vomiting, abdominal cramping and diarrhoea.[2,3,4,5] Furthermore, it is now known that digested protein is absorbed from the intestinal tract in the form of di- or tri-peptides calling into question the need for elemental amino acid diets. Polymeric, isotonic formulas can be used when small intestinal function is adequate. This significantly reduces the incidence of adverse effects.[4] Other complications associated with jejunostomy tubes include perforation of the small intestine and peritonitis (Figure 1-2.).[57] The incidence of peritonitis is thought to be related to the position of the distal end of the jejunostomy tube. At least 20 to 30 cm of jejunostomy tube should be within the lumen of the jejunum.[4] Infusion of nutrients into the jejunum is best given via constant rate infusion (CRI) starting at 0.5 ml/kg/hr and increasing

[d] Cook® Veterinary Products. Qld, Australia.

intermittent bolus feeding as large osmotic loads result in diarrhoea. There is conflicting data regarding whether to start with dilute nutritional solutions, gradually increasing to full strength over a 48 to 72 hour period, or to start with full strength solutions. Several studies have identified that as long as the full strength solutions are started slowly, adverse effects are minimal.[52,58,59] Reducing the time taken to begin feeding full-strength solutions shortens the period of under-feeding, improving the efficacy of nutritional support.

Figure 1-2. Perforation of the jejunum by migration of a jejunostomy tube in a dog.



INTRAVENOUS PARENTERAL NUTRITION

Parenteral nutrition involves the infusion of nutrients into a peripheral or central vein to supply either partial or total daily caloric requirements. There are many circumstances in which enteral feeding is not a suitable method for nutritional support. Examples include patients with severe vomiting or diarrhoea, maldigestion, malabsorption and intestinal obstruction. Bowel-rest for severe pancreatitis, and in people, enterocutaneous fistulas or ulcerative colitis are further indications for an alternative route for nutritional support, although the preference for parenteral nutrition over enteral nutrition in pancreatitis has recently been challenged in humans.[10]

The use of parenteral nutrition should be restricted to those cases that cannot be managed by the use of the more physiological enteral route. There are large numbers of studies in humans, dogs, cats and laboratory animals comparing the efficacy, safety, complications, and outcome of patients treated either with enteral nutrition, parenteral nutrition, or a combination of both.[47,50,51,52,58,59,60,61,62,63,64,65,66,67] Results have been variable and often conflicting, thus no firm conclusions or recommendations can be made at the present time. It is clear that serious metabolic derangements are less common when using enteral nutrition and the cost of parenteral nutrition far exceeds that of enteral nutritional support. However, as described above there are a number of patients that can only be supported by parenteral nutrition.

Partial parenteral nutrition (PPN) typically utilises a combination of glucose, amino acids and a lipid emulsion via a peripheral vein. Small diameter peripheral veins (e.g. cephalic, medial or lateral saphenous) are very susceptible to damage (thrombophlebitis) from hypertonic nutritional solutions. Because of this, the osmolality of the nutritional solution should be no more than 600 mOsmol/l.[68] This maximum osmolality can be increased to 800 to1000 mOsmol/l if parenteral heparin or parenteral hydrocortisone is used to help prevent thrombophlebitis.[68] This information is extrapolated from studies in people, as data from veterinary studies are lacking.

Restricting the osmolality of the solution directly affects the total number of calories that can be supplied in a 24 hour period. In people PPN is usually provided using 10% dextrose, 8.5% amino acids, and 10% or 20% lipid emulsion. Lipid emulsions are iso-osmolar allowing a greater proportion of non-protein calories to be supplied without inducing thrombophlebitis. PPN has been successfully used in dogs and people for up to 14 days.[69,70,71] The incidence of thrombophlebitis in humans in one study was reported to be 64%, which was reduced to 27% by decreasing the osmolality from 930 mOsmol/l to 735 mOsmol/l.[72] Whether osmolality directly affected the incidence of thrombophlebitis could not be determined, as the nutritional solutions used were different.

Total parenteral nutrition (TPN) can only be achieved using a central vein. Large diameter veins are less susceptible to damage from hypertonic solutions. This allows osmolalities as high as 1600 to 1800 mOsmol/l to be used. The types of nutrients used in TPN are the same as those used in PPN but the concentrations of the nutrients are higher. A combination of 50% dextrose, 10% amino acids and 20% lipid emulsion is often used to supply total daily caloric requirements.[73,76,53] The nutrients can be infused from separate bottles or can be given as a complete nutritional admixture. When individual nutrients are mixed it is important to add the dextrose and amino acids first, before adding the lipid. If the lipid comes into direct contact with the dextrose a precipitate will form, making the solution unusable.[5,31,73,74] Most pharmaceutical companies now prepare nutrient admixtures in a single bag that is partitioned with the dextrose and amino acids in one section and the lipid in the other. The solution is prepared for use by simply removing the partition and allowing the lipid and dextrose /amino acids to mix.

Parenteral nutrition is best given via a constant rate infusion aided by the use of a fluid-pump. Gravity-dependent infusions have also been successful[30,31,33] but suffer from frequent interruptions in administration that can lead to serious metabolic complications (see below). If this method is to be used, continuous monitoring becomes essential. Patients receiving constant rate infusions are often given a 4 to 6 hour period "off" TPN to allow lipaemia or other metabolic derangements to resolve.

Monitoring patients receiving parenteral nutrition often necessitates 24-hour supervision. This is generally not feasible in private practice and is one of the reasons for the limited use of this form of nutritional support by veterinary practitioners. Common complications of parenteral nutrition in dogs include phlebitis, venous thrombosis, hyperglycaemia, sepsis, hyperlipidaemia, and mechanical faults. These will be discussed in detail in the next section. Regular measurement of bodyweight and hydration status will allow the success of parenteral nutritional support to be assessed. Rapid fluctuations in bodyweight indicate changes in body fluid content rather than lean body weight. Daily assessment of triglycerides, glucose and acid-base status is ideal, and changes in the nutrient formulation, or the rate at which it is given should be based on these results. Most dogs will require a 48 to 72-hour period to adjust metabolically to the sudden infusion of large quantities of glucose.[7]

Electrolyte imbalances are common in parenteral nutrition, necessitating the measurement of sodium, potassium, chloride, phosphate, magnesium and calcium to be measured at least once daily during the initial stages of parenteral nutrition.[73,76,53] If serious complications arise, monitoring electrolytes every 12 hours may be required.

Intermittent analysis of the complete blood count and serum chemistry parameters such as blood urea nitrogen, creatinine, liver enzymes, and cholesterol are required to identify any septic or metabolic complications. Early recognition and aggressive medical management of these problems are essential to ensure the success of parenteral nutritional support.

Strict aseptic technique in placing the peripheral or central venous catheter and maintaining the nutritional line is mandatory if septic complications are to be avoided. Dedicating the line to nutritional support has been shown to minimise septic complications.[7,31,74,75,76]

COMPONENTS USED IN PARENTERAL NUTRITION

CARBOHYDRATES

Glucose

Glucose is the most commonly used carbohydrate source for parenteral nutrition.[21,77,78] Reasons for this include its ease of manufacture, cost effectiveness and minimal adverse effects. Furthermore, glucose does not require metabolism prior to utilisation by cells and is the energy substrate of choice for the central nervous system (insulin independent), peripheral nerves, red blood cells, white blood cells and certain phagocytes.[78]

Glucose is a dextrogyrate hexose that is essential for the synthesis of glycogen and glucuronic acid. The utilisation of glucose by most cells in the body is insulin-dependent.[77] It is not uncommon for severely ill or injured patients to have some degree of insulin resistance.[79] This can result in under-utilisation of the glucose supplied with significant adverse effects (see later).

Glucose is available in many formulations, ranging from 5% to 70%. Each gram of glucose supplies approximately 3.4 kcal. Table 1-1. illustrates the variation in osmolality and caloric density of the available preparations. Ten-percent glucose is the maximum concentration that can be safely infused via a peripheral vein for prolonged periods in dogs.[71] Concentrations greater than this necessitate the use of a central vein.

Non-protein calories must be given in an optimal ratio with protein. For people, cats and dogs this ratio is approximately 100 to 200 kcal/gram of nitrogen.[29] If non-protein calories are less than this requirement, endogenous protein catabolism occurs to provide glucose via gluconeogenesis.

Fructose

Fructose is a levogyrate hexose that is initially transported and phosphorylated independently of insulin. This may provide an advantage over glucose in situations of

insulin resistance.[77] However, data from people suggest that only the initial steps of fructose metabolism are insulin-independent.[79] The majority of human adult tissues utilise fructose only after conversion to glucose in the liver. Nagel[78] states that fructose has more "protein sparing" effects than glucose and that if given equally as fast as glucose, significantly less fructose will be eliminated in the urine, reducing wasted calories. However, fructose has not found favour as a source of carbohydrate in people because it has been associated with lactic acidosis, hypophosphataemia, elevated bilirubin and uric acid.[78]

Table 1-1. Commercially available glucose preparations.[80]

Glucose Concentration (%)	Caloric Content (kcal/l)	Osmolality (mOsmol/l)
5	170	252
10	340	505
20	680	1010
40	1360	2020
50	1700	2525
60	2040	3030
70	2380	3535

Sorbitol and Xylitol

Sorbitol and xylitol are sugar alcohols that have been assessed as potential carbohydrate substitutes in people because of their partial insulin-independence. Sorbitol is metabolised to fructose by sorbitol-dehydrogenase and further metabolised in the liver. Again, only the initial steps of sorbitol metabolism are independent of insulin. Both substrates have been associated with adverse metabolic effects similar to those seen with fructose. Data has been presented suggesting that the side effects were dose-dependent and if sorbitol is given in combination with glucose at a rate no more than 0.5 g/kg/hour, few adverse effects are seen.[81]

Alcohol

Alcohol appeared an attractive alternative carbohydrate because of its increased energy density (7.1 kcal/gram), but due to its extensive list of adverse effects has not found favour for parenteral nutrition. Ethanol has been shown to be toxic to muscle, brain and liver; inhibits gluconeogenesis and impairs leukocyte migration and phagocytosis. Acetaldehyde, a product of alcohol metabolism that is exhaled, is known to be irritating to pulmonary alveoli.[77]

Protein

Hydrolysates

When total parenteral nutrition was first developed, protein supplementation was given in the form of casein or fibrin hydrolysates. The hydrolysates were the result of acid hydrolysis of fibrin or pancreatic proteolysis of casein. The end product was a combination of free amino acids, di- and tripeptides, and larger polypeptides.

Hydrolysates are effective in supporting normal growth and maintaining positive nitrogen balance in people[78] and dogs.[33] The bioavailability of nitrogen contained within a hydrolysate has been questioned as studies indicate that between 30 to 53% of the nitrogen is in the form of dextro-isomers[30,78] These isomers form within the hydrolysate solution as part of the manufacturing process, cannot be utilised by the body and are excreted in the urine.[78] The significance of this finding for dogs receiving protein hydrolysates intravenously remains unresolved as several studies have successfully maintained dogs for prolonged periods of time using hydrolysates as the protein source.[31,33]

Protein hydrolysates have been assessed as to their suitability for dogs and cats. Whilst they contain the essential amino acids for dogs they lack taurine, an essential amino acid for cats.[30] Protein hydrolysates vary considerably in their specified composition depending on which company manufactures the product. Between-batch variation has also been identified.[78] Clinically significant concentrations of ammonia are present in the hydrolysate products. This can cause metabolic problems, especially in patients with

hepatic, renal and neurological disease. The fact that a small proportion of the hydrolysate contains large polypeptide fragments increases the risk of anaphylaxis. This has been reported with their use in people,[26] but not in dogs. The product variability, potentially adverse metabolic and immunological affects of the hydrolysates and the development of high quality L-amino acid solutions have resulted in a decline in the use of hydrolysates and today this form of protein supplementation is rarely used.

L-Amino acids

Crystalline L-amino acids have revolutionised the ability to supply high quality protein via the parenteral route. These solutions are manufactured by numerous companies[e,f,g,h,i,j] which illustrates the current market size. The solutions have been specifically formulated to contain the correct ratio of essential to non-essential amino acids. In addition, some solutions have been formulated for specific diseases e.g. hepatic and renal disease. Because not all amino acids require catabolism before they are utilised, only 0.4 g/kg/day of amino acids are required to maintain nitrogen equilibrium compared with 0.8 g/kg/day for a hydrolysate in people.[78] The branch-chain amino acids alanine, leucine, and isoleucine provided in the amino acid solutions can be utilised directly by skeletal muscle as an energy source. They can also be converted to alanine and glutamine in extra-hepatic sites thereby providing a protein-sparing effect in the face of hepatic dysfunction.[30]

Glutamine, one of the most abundant amino acids in circulation, is considered a "conditionally essential" amino acid, meaning that during times of severe illness or injury it becomes essential for maintaining health.[82] Glutamine plays a vital role in nitrogen transport between tissues, is essential for renal ammoniagenesis and is a key energy source for rapidly dividing cells, such as enterocytes, lymphocytes, fibroblasts

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[e] Baxter HealthCare Corporation. IL, USA.
[f] Cutter Laboratories. CA, USA
[g] Travenol Laboratories Inc. IL, USA
[h] B.Braun Medical Inc. CA, USA.
[i] McGaw Inc. CA, USA.
j Abbott Laboratories. USA.

and endothelial cells.[82] Although not a standard component of parenteral nutrition amino acid formulas due to its poor stability,[13,83,14] inclusion of glutamine in TPN solutions has been documented to be beneficial in a number of areas. It reduces the severity of mucosal atrophy associated with TPN,[84,82] increases the villous height and mucosal DNA content in patients being fed via TPN,[13,82] enhances gut immune function,[82,84] and decreases bacterial translocation associated with TPN.[13,82,83]

Lipids

Total parenteral nutrition can be based on one of two systems. The "glucose system", in which all the non-protein calories are supplied in the form of glucose, or the "lipid system", where between 50 to 70% of the calories are supplied in the form of a lipid emulsion. Currently, three types of lipid emulsion are commercially available for clinical use.[85] They are emulsions based on safflower oil or soybean oil (long-chain triglycerides) and emulsions containing a mixture of medium-chain triglycerides (MCT) and long-chain triglycerides (LCT). MCT are thought to be advantageous in parenteral nutrition because they are cleared quickly from circulation and oxidised rapidly.[78,86] This helps prevent the hepatic reticuloendothelial system from becoming congested with lipid, which has been implicated as a possible cause of immunosuppression in patients receiving parenteral nutrition.[73,78]

None of the lipid emulsions contain appreciable quantities of arachidonic acid. This is not of concern for dogs and people. However, as mentioned previously, cats cannot synthesise arachidonic acid from linoleic acid and have an obligatory requirement of approximately 0.17% arachidonic acid on a dry matter basis in their diet.[24] Given the short-term nature of TPN support in cats, a deficiency of this essential fatty acid is unlikely to be clinically significant.

Lipid emulsions are supplied as either a 10% or 20% formulation and have an energy density of 1.1 or 2.0 kcal/ml, respectively. The high-energy density of the emulsion allows small volumes of nutritional solution to be given to meet total daily caloric requirements. Lipid emulsions are isotonic (280 to 300 mOsmol/l) which allows them

to be given by either a peripheral or central vein without risks of thrombophlebitis. Unlike glucose infusions, lipid emulsions can be given at full strength and at maximal rates immediately without the need for an adjustment period. If the infusion is accidentally stopped, no adverse metabolic effects occur, which is not true of the "glucose" system. By supplying calories in the form of lipid, the total amount of glucose can be reduced, minimising the adverse effects sometimes seen with glucose-based solutions. Lipid emulsions also prevent deficiency of linoleic acid from occurring.[5,31,73,74]

Lipid emulsions can result in some complications. Lipid emulsions have been associated with tachycardia, pyrexia and hypotension.[31] Hypertriglyceridaemia and lipaemia also occur commonly in dogs given TPN and has been previously considered a relative contraindication for the use of lipid-containing TPN in dogs with pancreatitis.[12] However, this has not been shown to be the case in people with pancreatitis being treated with lipid containing TPN[66] and was not identified as a complication in a recent review of total parenteral nutrition in dogs.[7] The rapid infusion of large quantities of lipid can alter the function of the reticuloendothelial and immune systems and will be discussed later in this chapter. Lipid emulsions are expensive compared with glucose and this fact may influence the frequency of its use in dogs.

Vitamins, Trace elements and Electrolytes

As previously mentioned, vitamins and trace elements are involved in vital roles throughout the body. Vitamins and minerals are not contained in standard intravenous nutritional solutions and must be specifically added to the nutritional formulation if considered necessary, or administered separately. Because it can be very difficult to document micronutrient deficiencies, and little is known about the minimum daily requirements for seriously ill or injured patients receiving parenteral nutrition, most patients are supplemented with a standard amount of multi-vitamins and trace elements. In veterinary medicine most dogs and cats receive an empirical dose every 5 to 7 days. Until further research identifies minimum daily requirements, this empirical form of vitamin and mineral supplementation remains the most practical approach.

Patient fluid and electrolyte balance has to be carefully monitored throughout the period of parenteral nutritional support. Most nutritional solutions meet or exceed total daily fluid requirements. However, if hyperglycaemia results in an osmotic diuresis, or patients suffer from intractable vomiting or diarrhoea, additional fluid support may be necessary. Amino acid solutions provide lactate, acetate, phosphate, and chloride in significant quantities, but in an unbalanced manner. To address electrolyte balance, many companies now include a balanced electrolyte product as part of the amino acid solution e.g. Freamine III with electrolytes®.[k]

Insulin

Dogs receiving large doses of glucose may require parenteral insulin to ensure adequate glycaemic control and efficient utilisation of the glucose in the early stages of TPN.[12] This is usually a short-term phenomenon, although insulin may be required for the duration of parenteral nutritional support in some patients. The ideal method of insulin administration is to add a set quantity (usually 1 IU/5 grams of glucose) to the nutritional solution at the time of formulation. This results in a concomitant change in insulin dose as the rate of TPN infusion is adjusted. A drawback of this approach is that insulin binds to plastic in the administration set and fluid bag, necessitating an increase in the amount of insulin initially added to the nutritional solution.[26] However, insulin binding to polyvinyl chloride surfaces is a saturable phenomenon.[87] Therefore, running insulin through the giving set prior to use will saturate the binding sites and the additional insulin delivered to the patient in the nutrient solution will have the same concentration as that originally added to the nutrient bag. An alternative and more practical approach is to give the insulin at set times during the day, via the subcutaneous route. However, a recent review of TPN in 209 dogs revealed that with careful patient monitoring and adjustments in the rate of TPN administration exogenous insulin administration was not necessary in any of the non-diabetic dogs that experienced episodes of hyperglycaemia.[7]

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[k] McGaw Inc. CA, USA.

COMPLICATIONS OF PARENTERAL NUTRITION

Unfortunately, parenteral nutrition is associated with a significant number of side effects, which compromise the value of this technique and have continued to stimulate interest in other methods of nutritional support.

Thrombophlebitis and Catheter-Related Sepsis

The terms thrombophlebitis and sepsis, when associated with parenteral nutrition are not synonymous. Thrombophlebitis implies inflammation of a peripheral or central vein with thrombus formation, whereas catheter sepsis results from the colonisation of the intravenous catheter by bacteria or fungal organisms. There is a direct correlation between the quantitative bacterial count from intravenous catheters and the risk of sepsis.[88,89] Systemic infection quickly occurs once the quantitative bacterial count reaches a critical threshold.[89]

The definition of catheter-related sepsis varies according to the study reviewed. The gold standard diagnostic procedure is the acquisition of duplicate, quantitative blood cultures with one sample taken through the offending catheter and a second sample taken from a peripheral vein, at a distant site. Higher quantitative bacterial counts from the catheter site in comparison to the peripheral venous site are highly suggestive of catheter-related infection.[89] Culture of the catheter-tip may yield additional information but requires the removal of the catheter, which is not always desirable.

The reported prevalence of phlebitis and catheter sepsis associated with parenteral nutrition varies considerably[31,60,65,68,72,88,89,90] ranging from 2 to 65%. This wide variation is a result of different patient populations, experimental protocols, definitions of phlebitis and sepsis, and the experience of the personnel providing the nutritional support.

Thrombophlebitis occurs as a result of trauma to the endothelial lining of a vein, which exposes sub-endothelial collagen. This trauma stimulates aggregation of platelets, fibrin, red blood cells, and polymorph-neutrophils, resulting in the release of vasoactive peptides and the production of inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumour necrosis factor (TNF).[65] The local inflammatory response causes intense vasoconstriction, limiting the blood flow through and around the catheter. This exacerbates the endothelial trauma by reducing the dilutional effect of blood flow on the hypertonic solutions and increasing the exposure of the vessel wall to irritating chemicals.[65] If the phlebitis is severe, fibrin formation can completely occlude the vessel and result in peripheral or central vein thrombosis. This is a serious complication, especially if other veins have already been thrombosed and can result in "caval syndrome" or the development of thromboemboli.

Many factors are involved in the pathogenesis of phlebitis. Excessive trauma to the vein at the time of catheter insertion increases the risk of phlebitis and sepsis.[68,88,89] The composition of the catheter material influences the frequency of phlebitis. Some catheter materials produce less trauma to the vein and decrease the ability of micro-organisms to colonise the catheter. For example, Teflon and polyurethane allow less colonisation compared with silicone and polyvinyl chloride catheters.[88,89] However, silicone is the least "reactive" catheter material and also the "softest". Teflon is intermediate in reactivity and is the stiffest material.[91] (Table 1-2.)

Table 1-2. Properties of catheters for intravenous use.[91]

Material	Stiffness	Thrombo-genicity	Reactivity
Silicone	1+	1+	1+
Polypropylene	3+	3+	3+
Teflon	2+	4+	2+
Polyethylene	3+	3+	3+
Polyvinyl chloride	4+	3+	3+
Polyether -based Polyurethane	1+	2+	1+
Polyester-based Polyurethane	2+	3+	2+

Relative values: 1+ = minimal, 2+ = mild, 3+ =moderate, 4+ = marked (Modified from DiBartola: Fluid therapy in small animal practice. W. B. Saunders, Philadelphia, 1992.)

The adherence to strict aseptic technique when catheters are inserted also influences the incidence of catheter-related phlebitis and sepsis. A six-fold reduction in the incidence of catheter sepsis can be achieved when an operator using sterile gloves, a mask and gown, and a large drape, compared with sterile gloves and a small drape while inserting the catheter.[89] There is disagreement in the literature as to the influence of specialised staff (nutrition support teams) on the prevalence of catheter-related sepsis, thrombophlebitis and other complications associated with nutritional support.[60,68,89] However, there is widespread opinion that patients treated by personnel experienced in administering TPN suffer fewer complications compared to those treated by inexperienced medical staff.[67]

Single-lumen catheters are associated with a lower incidence of catheter sepsis compared with multiple-lumen catheters.[88] This is a direct consequence of reduced handling and manipulation of the nutritional line, resulting in a smaller chance of a break in strict asepsis. Physical characteristics of the nutritional solution such as osmolality, pH, nutrient type and particulate matter also play a role in the pathogenesis of catheter-related phlebitis and sepsis. While the author was unable to find any studies that have primarily addressed the issue of osmolality in dogs, it is believed that an

osmolality exceeding 600 mOsmol/l in people and 750 mOsmol/l in dogs is likely to result in phlebitis.[71] Central veins, because of their larger diameter, can withstand osmolalities around 1200 to 1600 mOsmol/l. Buffering the pH of the nutritional solution to that of plasma (7.2 to 7.4) may reduce the incidence of phlebitis.[68,72] A study comparing the incidence of phlebitis using a nutritional solution based on 10% glucose to one based on glycerol identified a reduction from 68% to 27%, respectively. The authors proposed that glucose solutions promote phlebitis when compared to glycerol and lipids.[72] Whether this was related to osmolality or the chemical irritation of glucose itself remains unanswered. Adding heparin (1 IU/ml) and/or hydrocortisone (1 to 10 mg/l) to nutritional formulations based on glucose has been reported to reduce the incidence of phlebitis in people.[31,68]

Immune Function

Metabolic disturbances associated with over-supplementation of nutrients are potential causes for septic episodes in patients receiving parenteral nutritional support. Severe hyperglycaemia has been identified as a risk factor for the development of sepsis in people.[65] Whether hyperglycaemia is a cause or an effect of sepsis remains unclear. Diabetic people with poor glycaemic control are more likely to suffer a septic episode compared with well-controlled diabetics as a consequence of reduced adherence, chemotaxis, and phagocytic function of polymorph-neutrophils.[65] It is tempting to extrapolate these findings to patients suffering periods of severe hyperglycaemia (> 12.3 mmol/l) associated with excessive administration of glucose, although no studies have specifically addressed this issue.

Infusion of large quantities of lipid into the systemic circulation may potentially predispose to sepsis by causing changes in the immune system.[65,73] Linoleic acid is the predominant fatty acid present in commercially available lipid emulsions. Lipid emulsions based on soybean oil also contain significant quantities of linolenic acid. Linoleic acid is subsequently desaturated and elongated within the body to form arachidonic acid. This fatty acid is the precursor for the eicosanoids – leukotrienes and prostaglandins. Many of these inflammatory mediators can modify the immune

response and have been associated with reduced or enhanced immune function.[65]

Rapid infusion of lipids can alter the functional capacity of the reticuloendothelial system. When Kuppfer cells in the liver become saturated with lipid globules their ability to phagocytose and clear bacteria from the blood stream is impaired.[65] This did not occur when an equivalent dose of lipid (as that given over 12 hours) was infused over a longer (24 hour) period. Nor did it occur when the same amount of lipid was given in the form of MCT as opposed to LCT.[65] MCT are oxidised more rapidly and completely compared with LCT. It is proposed that the rapid metabolism of MCT does not allow significant quantities of lipid to be stored intracellularly, thereby minimising any disturbance in the ability of the Kuppfer cells to phagocytose bacteria or other micro-organisms.

Structured lipids are a new class of lipid emulsion composed of different length fatty acids esterified to the same glycerol backbone. These emulsions are not yet commercially available. Emulsions based on *structured* lipids may have significantly beneficial effects for patients by incorporating omega-3 fatty acids that potentially reduce the production of the pro-inflammatory eicosanoids.[85]

The most common micro-organisms isolated from dogs with catheter-related sepsis are gram-negative.[31] Recent reviews in human patients have identified coagulase-negative *Staphylococci, Staphylococcus aureus*, and *Candida* species.[88,89] Migration of skin organisms along the outside of the catheter and migration of bacteria from the hub of the catheter along the inside surface are the two most frequent routes of entry of bacteria and fungi into the body in people receiving TPN.[89] The application of broad-spectrum antibiotic and anti-fungal ointment at the catheter insertion site may prove effective in preventing sepsis. Mupirocin[1], an antibacterial agent effective against gram-positive bacteria, and chlorhexidine are considered the most effective agent's.[89] Newer strategies to prevent catheter-related sepsis include the use of catheters impregnated with rifampicin-minocycline and negatively charging the catheter material to inhibit

[1] Bactroban®. SmithKline Beecham (NZ) Ltd. Auckland, NZ.

bacterial colonisation and the development of the bio-film layer that occurs on all intravenous catheters.[89] Further research is needed in this area before the use of such strategies become commonplace.

Metabolic Complications

Most dogs receiving parenteral nutrition undergo a period of adaptation to the dramatically increased glucose load administered during TPN.[7] During adaptation, pancreatic beta cells respond to neuro-endocrine signals by increasing the rate of insulin production and secretion. A study in rats indicates this phenomenon may be mediated through branches of the hepatic vagus nerve, which act as sensory afferent pathways to the brain stem, with efferent signals travelling to the pancreas via the celiac branch of the vagus nerve.[92]

Adaptation does not occur in all individuals, with some exhibiting varying degrees of insulin resistance and alterations in carbohydrate metabolism.[30,79] The exact mechanism for insulin resistance in seriously ill or injured patients is not fully understood. However, it is postulated to involve a combination of reduced glucose-receptor binding and post-receptor defects leading to abnormal intracellular glucose metabolism.[79] This occurs due to the release of IL-1, IL-6, and TNF, which in turn stimulate the release of the counter-regulatory hormones:- catecholamines, glucagon and cortisol.[65,79]

Altered glucose metabolism can result in several serious adverse effects such as severe hyperglycaemia, glucosuria, and non-ketotic hyperosmolar syndrome.[30] Severe hyperglycaemia, defined in people as a blood glucose concentration greater than 12.3 mmol/l (220 gm/dl), has previously been discussed in terms of its potential role in the pathogenesis of sepsis and immunosuppression.[65] Blood glucose concentrations exceeding the renal threshold in dogs (> 10.0 mmol/l) result in glucosuria. Glucosuria has two potential adverse effects. Firstly, glucose excreted in the urine results in lost calories that cannot be utilised for anabolic purposes. Secondly, persistent glucosuria is a predisposing factor for the development of urinary tract infections. This may have serious consequences for patients already immuno-compromised. Non-ketotic

hyperosmolar syndrome is an uncommon clinical entity reported in diabetic dogs[93] and people, as well as people receiving total parenteral nutrition.[67] If left untreated, it is a life-threatening disorder and has been associated with mortality rates in people of between 40% to 50%.[30]

Careful monitoring of blood and urinary glucose concentrations during the initial 24 to 48 hours of TPN administration is essential if prompt recognition and successful management of this metabolic derangement is to occur. Maintaining euglycaemia can be achieved through altering the concentration and/or rate of glucose administration, by substituting lipid calories for glucose calories and by the use of insulin administration. A gradual reduction in glucose administration when TPN is no longer required will help prevent hypoglycaemia from insulin over-secretion.

Hyperchloraemic metabolic acidosis, hyperammonaemia, and pre-renal azotaemia have been associated with the administration of TPN.[30] Earlier formulations of crystalline amino acid solutions were based on chloride salts, and their metabolism in the tricarboxylic acid cycle resulted in the liberation of a chloride cation and free hydrogen anion. As a consequence, newer amino acid solutions contain lactate and phosphate salts, and have a much lower concentration of chloride. Protein hydrolysates have a pH of approximately 2.5 to 3.5, which adds to the net acid load if they are used as the source of protein in TPN formulations.[30]

Metabolism of amino acids with the production of ammonia is normally accompanied by a concurrent increase in the conversion of ammonia to urea (within the liver) and its subsequent excretion in the urine. Patients suffering from renal or hepatic disease may have abnormalities in these metabolic pathways, resulting in the development of hyperammonaemia. This typically manifests as alterations in mental demeanour, coma, seizures or limb rigidity.[30] Pre-renal azotaemia can be a direct consequence of the increased protein load and/or changes in hydration status. This may be exacerbated by osmotic diuresis associated with overzealous glucose administration. Usually the azotaemia is short-lived and not clinically significant.

Derangement of phosphate balance occurs frequently in people receiving TPN[26] but is not a common complication recognised in dogs.[7,94] Phosphate is distributed in a similar manner to potassium with the highest concentration located intracellularly. Seriously ill or injured patients may already be phosphate depleted despite normal to low-normal serum phosphate concentrations. Phosphate concentrations can decline rapidly with the introduction of TPN.[26] This occurs because phosphate is incorporated into tissue protein or undergoes transcellular shifts mediated by insulin administration. A study of seriously ill people undergoing TPN identified 42% of patients as hypophosphataemic during the initial stages of therapy.[95] The clinical signs of hypophosphataemia include weakness, tremours, mental depression, increased red blood cell fragility, shifts in the oxygen-haemoglobin dissociation curve and reduced white cell phagocytosis and chemotaxis.[26,31,96,97] Studies in people suggest that supplementation of between 7.0 mmol/l and 14.0 mmol/l,[31,95] or 0.4 to 0.5 mmol/kg[98] should be provided to patients receiving TPN. In dogs, an initial starting dose of 0.01 to 0.03 mmol/kg/hr has been recommended for intravenous phosphate supplementation.[96] For this reason it has been recommended that potassium supplementation is provided in the form of potassium phosphate, rather than potassium chloride to patients receiving TPN.[30]

Magnesium, an often forgotten cation in critically ill patients, is important for many body functions e.g. oxidative phosphorylation, high-energy phosphate transfer, and myocardial integrity.[26,31,99] Due to its intracellular location, total body magnesium can be difficult to assess and serum concentrations often do not accurately reflect total body stores.[99] Clinical signs of hypomagnesaemia are closely related to other electrolyte imbalances such as calcium and phosphorus and therefore require laboratory documentation to confirm a diagnosis. Most TPN formulations have adequate concentrations of magnesium to help prevent the development of clinically significant hypomagnesaemia.

Complications of lipid infusions include tachycardia, pyrexia, hypotension, and tachypnoea.[30] These adverse reactions are less common with the newer formulations, but still occur. The sodium oleate content of the emulsion and the purification process

for lecithin may be involved in the pathogenesis of these reactions.[100]

Hepatobiliary Complications

Hepatobiliary complications in people receiving TPN are common, ranging from benign elevations in liver enzyme concentrations to severe steatohepatitis and cirrhosis.[100] Differentiating complications of TPN support from the underlying disease process can be difficult. If hepatobiliary complications are recognised, a thorough search for other possible explanations should be performed before the blame is attributed to the administration of TPN.

Abnormalities in people, including steatosis, steatohepatitis, intrahepatic cholestasis, and biliary tract disease have all been recognised in association with TPN.[100] Cholestasis and biliary tract disease occur less frequently than steatosis or steatohepatitis.[100] A functional defect of the reticuloendothelial system associated with lipid infusion has previously been discussed.[65]

Benign elevations in liver enzyme concentrations are very common in people receiving TPN,[100] with increases in AST and ALT the earliest recognised. Increased ALP and total bilirubin occur later, usually around three weeks after initiating TPN.[100] Enzyme increases are mild and usually do not exceed two to three-times the upper limit of the reference interval. Articles reviewing TPN in dogs and cats do not state the prevalence of hepatobiliary complications. The reported prevalence in people varies from 20 to 100%. This variation is probably due to different patient populations and TPN formulations. Notably, the incidence has declined since lipids have become routinely added to the TPN formulation.[100]

Steatosis is the most common histological finding in people with elevated liver enzymes, although there is no correlation between the severity of the lesions and the degree of increase in serum activity of the liver enzymes.[100] Lesions tend to be periportal in location, but in severe cases may extend to become centrilobular to panlobular. The fat vesicles seen histologically are predominantly triglycerides and are thought to be caused

by excessive glucose administration and persistently high insulin concentrations.[100] Insulin promotes lipogenesis and reduces hepatic triglyceride secretion[100,101,102] Reduced lipoprotein synthesis from deficient protein intake may alter lipoprotein metabolism, leading to triglyceride accumulation in hepatocytes. Steatohepatitis probably reflects a natural progression from severe steatosis.[100]

Cholestasis is usually not seen until around three weeks after the onset of TPN. Increases in ALP are commonly seen, but only occasionally will there be elevations in total bilirubin concentrations. Small intestinal bacterial overgrowth (SIBO) and alterations in gut-barrier function have been postulated as possible factors in the pathogenesis of the TPN-induced cholestasis.[100] SIBO may result in increased conversion of chenodeoxycholic acid to lithocholic acid. Lithocholic acid is considered toxic to hepatocytes and other cells within the biliary system.[85] The neuro-endocrine stimulation of mucosal cells induced by enteral feeding, that may be absent in TPN patients, could play a vital role in the pathogenesis of altered gut-barrier function. Mucosal atrophy, alterations in cellular tight junctions, a reduced mucus layer and increased bacterial translocation across the gut wall may result in gram-negative septicaemia,[35,36,59] potentially causing cholestasis. The majority of studies investigating altered gut-barrier function have used laboratory animal species as their model. It is clear that significant species variation exists[34,35,36,38,59] and that extrapolation from one species to another may result in incorrect conclusions.

Prolonged anorexia also causes reduced bile flow. People receiving TPN have reduced gallbladder contractions compared with enterally fed patients.[100] This promotes bile stasis and reduces the release of cholecystokinin that is known to have trophic effects on the intestinal mucosa.[103]

Gastrointestinal Complications

The adverse effects of prolonged parenteral nutrition on the gastrointestinal tract have attracted an enormous amount of research. Fundamental questions repeatedly asked include:- does total parenteral nutrition alter gut-barrier function? And if so, does it

adversely affect patient outcome?

Many studies have investigated the site,[35] route,[35,38] and species[35,36] associated with bacterial translocation, mucosal atrophy,[34] and bowel permeability[36,59] in relation to enteral versus parenteral nutrition.[64] The majority of the studies demonstrating mucosal atrophy, increased bowel permeability and greater numbers of bacteria translocating across the bowel wall have been performed in laboratory animal species such as rats, mice and guinea pigs. A recent review of this topic[59] was critical of most studies addressing these issues and stated that significant species variation exists in the susceptibility to mucosal atrophy and inherent bowel permeability. Extrapolation of results from rats to human beings, and perhaps dogs, has only led to erroneous conclusions. This review stressed that it is extremely difficult to demonstrate clinical benefit for enteral nutrition over parenteral nutrition because of many confounding variables and the difficulty in separating the effects of treatment from those of the underlying disease. The increased prevalence of infectious complications seen in parenteral nutrition may simply reflect the increased risk of sepsis in TPN patients, rather than being caused by greater numbers of translocating bacteria.

There is conflicting opinion as to whether the organisms associated with translocation across the bowel wall are the cause of sepsis in critically ill or injured patients.[35,38] There is little published work in the veterinary literature regarding bacterial translocation and its role in the pathogenesis of sepsis in critically ill patients. A recent paper identified that in clinically normal dogs undergoing an elective ovariohysterectomy, 52% of 50 dogs had bacteria isolated from their mesenteric lymph nodes.[38] Bacterial numbers ranged from 50 to $> 10^5$ organisms/g of tissue. This finding highlights the fact that the prevalence of bacterial translocation may vary significantly between species. Further work is required to compare the quantitative bacterial counts from critically ill patients receiving TPN to patients who are being fed by the enteral route. If a significant difference exists, the next appropriate step would be to determine if the offending organisms are the cause of sepsis in these patients.

CHAPTER 2.

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- Peritoneal Physiology.
- History of Intraperitoneal Nutrition.
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- Summary of Intraperitoneal Nutrition.

INTRODUCTION

Nutritional support has been shown to be important in the care of critically ill or injured animals.[1,5,7,29,33,76,104] Consequences of under nutrition develop rapidly in these animals, with serious and wide-ranging effects.[105] Various methods of providing daily protein-energy requirements have been developed, ranging from forced oral feeding to indwelling jejunostomy tubes.[1,5] However, there are many circumstances in which the gastrointestinal tract (GIT) cannot be utilised as the primary route of nutritional support, e.g. severe GIT infections, GIT neoplasia and profound motility disorders.[1,28,29,30] This prompted the development of intravenous parenteral nutrition, which has been successful in supplying daily energy-protein requirements to dogs in both short and long-term situations.[32,33,74,76]

Unfortunately, intravenous (IV) parenteral nutrition is not without complications and hazards.[5,31,33] As discussed in Chapter 1, the most well recognised of these problems in dogs and people are sepsis, phlebitis and thrombosis. Metabolic complications associated with IV nutrition in people include hyperammonaemia, hyperglycaemia, hypertriglyceridaemia, hyperchloraemic metabolic acidosis, liver dysfunction and azotaemia. To limit these complications, total caloric requirements can only be achieved using continuous (18 to 24 hour) infusion by way of a central vein. Unfamiliarity with central venous catheters and the lack of intensive monitoring capabilities has precluded the use of this form of nutrition in many veterinary hospitals.

The development of an alternative route for the administration of parenteral nutrition is needed to fill a niche in small animal clinical nutrition. The ideal route would be one that is easy to use, available to all veterinarians regardless of expertise, cost effective and allows complete nutritional support with a minimum of adverse effects and complications. There is evidence that the intraperitoneal route for nutritional support may fulfil some of these requirements.

PERITONEAL ANATOMY

The peritoneal cavity is completely enclosed in the male but remains potentially open in the female through the ovarian tubes. The peritoneum consists of three parts: the parietal peritoneum, which lines the abdominal wall; the visceral peritoneum, which covers the organs within the abdominal cavity; and the mesenteries, which consist of a series of double folds connecting the parietal and visceral parts. Approximately 90% of the surface area of the peritoneal membrane is visceral in origin.[106]

The peritoneal cavity of the healthy animal consists of a number of clefts, most of which are capillary in dimension. The collective space contains only a few millilitres of fluid that is high in phospholipids and provides lubricant properties, allowing the abdominal organs to slide freely over one another. The peritoneum has a large surface area that has been estimated in several species, including man (1.7-2.0 m^2), rats (595 cm^2), and rabbits (820 cm^2).[107] However, the surface area of the peritoneum is but a fraction of that of the small intestine. For example, man has a small intestinal surface area of approximately 175m^2.[108]

The peritoneal membrane is composed of four distinct layers (Figure 2-1.). An outer sheet of mesothelial cells that rest on a prominent basement membrane, the interstitial or connective tissue layer[109] and the peritoneal elastic lamina, consisting of an irregularly organised layer of collagen fibres, which, in parts, becomes continuous with fibres from visceral organ capsules.[110] Peritoneal mesothelial cells are flattened to cuboidal in shape, and vary in appearance according to their anatomical location. The apical borders of the cells have an abundant covering of microvilli (Figure 2-2.). The lateral borders of neighbouring cells form tight junctions, except in areas of peritoneal stomata (see later). Peritoneal mesothelial cells contain numerous cytoplasmic organelles including rough endoplasmic reticulum and Golgi apparatus. The cells also contain numerous, small electron dense vesicles that contain phospholipids, which can be extruded into the peritoneal cavity. The secreted phospholipids are thought to possess lubricant and surfactant properties, and repel water.[107] The secretory properties of peritoneal mesothelial cells are becoming better understood. The cells have been

shown to secrete a number of substances including prostaglandins, interleukins 1, 6, and 8, granulocyte colony-stimulating factor, granulocyte-monocyte colony-stimulating factor and colony-stimulating-factor 1. The secretion of these molecules increases in response to contact with dialysis solutions,[107] although the mechanism is poorly understood.

Figure 2-1.[109] Human parietal mesothelium showing typical flattened cells (m). The cells have a prominent basement membrane (bm), which rests on a dense fibrous connective tissue (ct) that becomes continuous with the peritoneal elastic lamina (not shown). (From Slatter N J et al. The ultrastructure of human abdominal mesothelium. J Anat 167: 47-56, 1989. With permission).

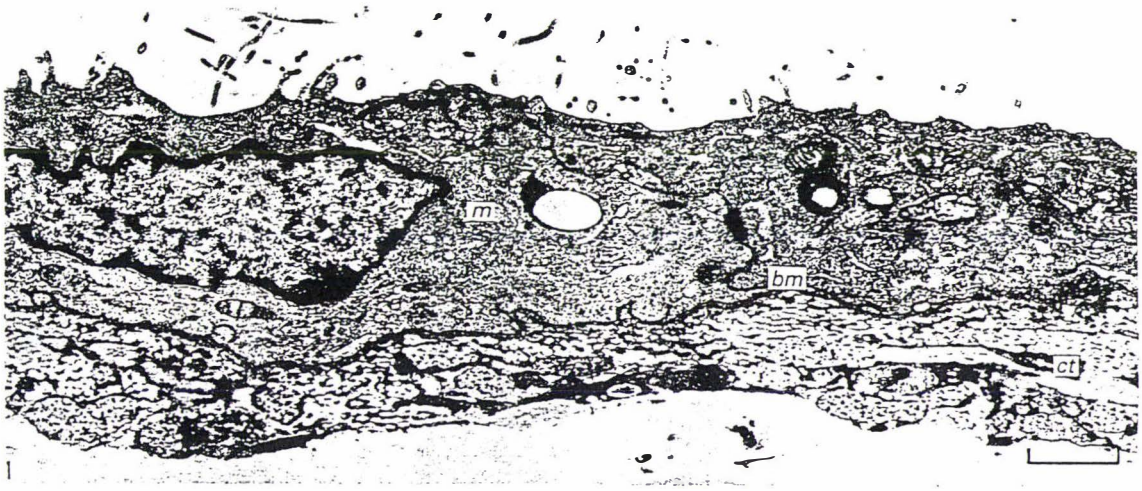


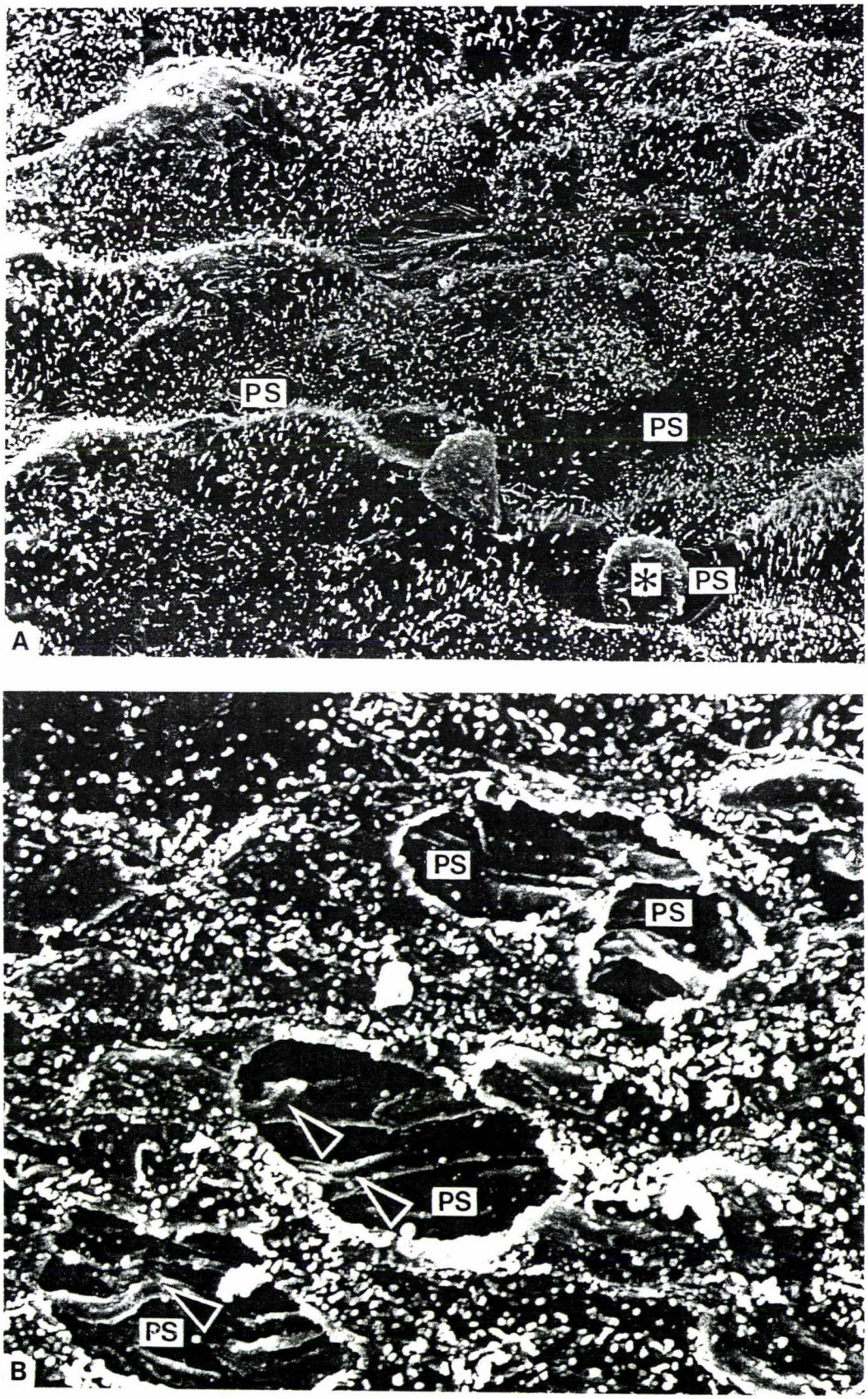
Figure 2-2.[109] Human mesothelial cell and microvillus. Microvilli significantly increase the surface area of the peritoneal membrane. (From Slatter N J et al. The ultrastructure of human abdominal mesothelium. J Anat 167: 47-56, 1989. With permission).

Peritoneal blood vessels and lymphatics course throughout the interstitial layer of the peritoneal membrane. The location of vessels within the interstitium (generally within 500 to 800 μm of the luminal surface of the peritoneal cavity) has specific effects on solute and water transport across the peritoneal membrane. This effect is due to regional pressure influences on the capillary beds.[111] The interstitial tissue also acts as a barrier to transcapillary transport of solutes and water. The ability of substances to diffuse through the interstitium is governed by several factors, including molecular size and nett charge. Larger molecules are excluded from up to 90% of the peritoneal

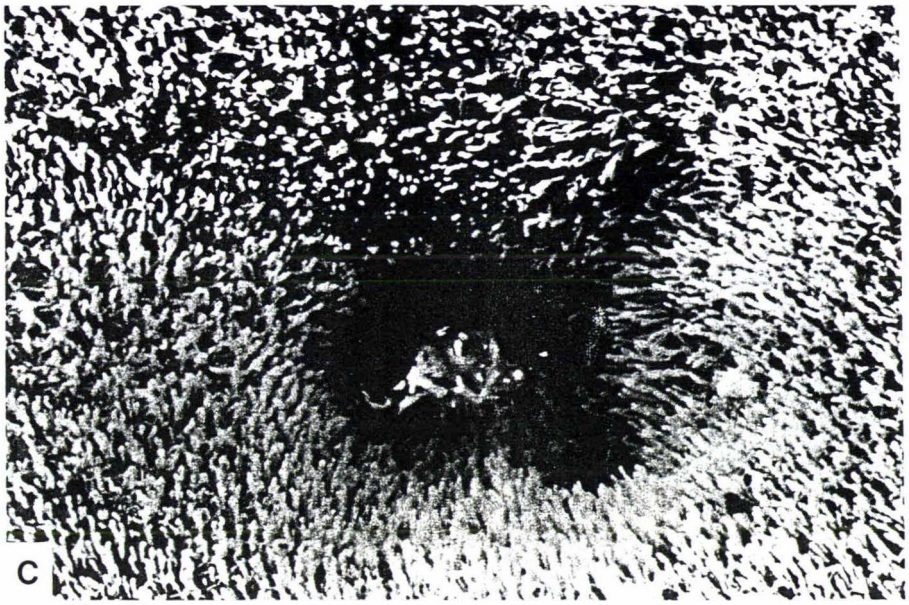
interstitial extravascular space because of the "solid structures" (i.e. connective tissue and structural supportive tissue for the blood vessels and lymphatics) located within the interstitium.[111] Peritoneal mesothelial cells and capillary endothelial cells have a nett negative charge that provides a permeability barrier to negatively charged molecules. This selective permeability is effective down to a molecular size of 2 nm and a molecular weight of 2 kD.[112] Peritoneal capillaries have numerous fenestrae that facilitate the absorption of substances across the peritoneum. The degree to which these fenestrae occur is dependent on species and anatomical site within the peritoneal membrane. For example, 2% of human parietal peritoneal capillaries, 29% of rabbit diaphragmatic capillaries and 27% of mouse mesenteric capillaries contain fenestrae.[107]

Peritoneal stomata (openings) remain the most controversial anatomic features of the peritoneal membrane. Whilst some anatomic studies have failed to identify these structures, a recent study in rats[113] conclusively identified their existence. The stomata were seen in many different areas of the peritoneal membrane, including the parietal, hepatic, diaphragmatic, pelvic, ovarian, omental and mesenteric regions.[113] Peritoneal stomata are channel-like structures connecting the surface of the peritoneal membrane to the lumen of the underlying interstitial lymphatic vessels. The stomata appear as "pocket-like" slits or "valve-shaped" openings, which penetrate the interstitial tissue and are associated with a loss of the normal mesothelial cell and lymphatic endothelial basement membrane. The lymphatic lumens are generally greater than 20 μm in diameter at these sites (Figures 2-3 a, b, c.). Stomata are thought to be involved in metastatic spread of abdominal tumours and ascitic fluid resorption. They may also be important sites of transperitoneal flux during peritoneal dialysis, intraperitoneal nutrition and intracavitary chemotherapy.

Figure 2-3.[113] Appearance of peritoneal stomata at different anatomic locations in the rat. (a) Diaphragm: the peritoneal stomata (ps) appear as valve-shaped openings. Free cells (asterisk) partly occlude the lumen. (b) Abdominal wall: Peritoneal stomata (ps) arranged in clusters. The openings expose underlying structures of the submesothelial connective tissue (arrows).



(c) Liver: A channel-like invagination appears as a stomata-like structure. (From Wassilev W, Wedel T, Michailova K, et al. A scanning electron microscopy study of peritoneal stomata in different peritoneal regions. Ann Anat 180: 137-143, 1998. With permission.)



PERITONEAL PHYSIOLOGY

Extensive studies have investigated the site, rate, and the factors affecting absorption of solutions and particles from the peritoneal cavity.[114,115,116,117,118,119] Absorption is facilitated by spread of the fluid throughout the abdomen. The specific gravity and viscosity of the fluid, as well as the size of particles within the fluid affect the rate of spread within the peritoneum. Gravitational forces heavily influence these variables.[116] However, body position has no effect on the net absorption from the peritoneal cavity of dogs.[116] As viscosity increases, the rate of spread decreases. Particle size and specific gravity are also inversely related to the speed of particle movement and fluid movement, respectively. The presence of fibrinogen or thromboplastin within the peritoneal cavity markedly impairs the spread of fluids. However, less severe peritoneal inflammation, which is not associated with fibrin formation within the peritoneal cavity, does not affect absorption. In fact, the resulting inflammatory fluid enhances the spread of particulate matter within the peritoneal cavity.[116] Flow of water and solutes across the peritoneum may occur either: down a concentration gradient; by solvent drag, through the effect of bulk-flow of water; or by all of these mechanisms.[120] The volume of peritoneal fluid also influences equilibration of water and solutes across the peritoneal membrane. Equilibration becomes progressively slower as the volume of the peritoneal fluid increases. This is thought to result from the accumulating fluid attaining a more spherical shape, which is less favourable for solute equilibration.[115] This observation has clinical importance, as large intraperitoneal fluid volumes have been associated with pulmonary dysfunction (due to the impairment of diaphragmatic movement against the fluid bolus) and reduced cardiac output in people.[115]

Lymphatic drainage from the peritoneal cavity occurs via a dynamic process in which the caudal (inspiratory) movement of the diaphragm stretches the lymphatic channels creating negative pressure within the lymphatic lumen, allowing fluid to be sucked into the lymphatic vessel.[119] A study in cats has identified that as the respiratory rate increases, the rate of lymphatic absorption increases[118] (Table 2-1.). The vast majority of

the lymphatic fluid passes through the sternal lymphatics, right thoracic duct and eventually into the venous systemic circulation.

Table 2-1. Factors known to influence the spread and absorption of particulate matter and solutes within and from the peritoneal cavity of dogs.

Factor	Spread	Absorption
↓ Specific gravity	↑	↑
↑ Viscosity	↓	↓
↑ Particle size	↓	↓
Fibrinogen	↓	↓
Thromboplastin	↓	↓
↑ Fluid bolus	↑	↓
↑ Respiration	↑	↑

↓ = decreasing, ↑ = increasing, ↓ = decreases, ↑ = increases

The permeability of the peritoneal membrane is reduced in certain disease states. A study in people undergoing chronic ambulatory peritoneal dialysis identified reduced clearance of urea, creatinine and urates from the systemic circulation into the peritoneal cavity when chronic renal failure was complicated by systemic vasculitis.[121] The vasculitis was postulated to alter the size, shape, or charge of the fenestrae that occur between capillary endothelial cells and mesothelial cells.

HISTORY OF INTRAPERITONEAL NUTRITION

The occurrence of hypertriglyceridaemia and obesity in people undergoing chronic ambulatory peritoneal dialysis (CAPD) with hypertonic glucose solutions and the relatively high prevalence of serious complications associated with intravenous nutrition, led to the detailed investigation of glucose transport across the peritoneal membrane as a possible alternative route for nutritional support. Studies identified that significant quantities of glucose are absorbed from the dialysis solutions and contribute to the daily caloric load of CAPD patients.[122,123] This finding led to the theory that

patients undergoing CAPD could have their daily caloric intake adjusted (depending on their nutritional status) by manipulating the glucose concentration of the dialysate.
The increasing success and popularity of CAPD for treating people with chronic renal failure resulted in the discovery that patients develop abnormal plasma aminograms, negative nitrogen balance and protein malnutrition.[124] These complications have been attributed to protein loss in the dialysate, but are difficult to differentiate from those of underlying renal disease. Identification of these abnormalities stimulated research into the use of dialysis solutions that either combined glucose and amino acids,[125] or used amino acid solutions alone.[124] Studies identified that using amino acid dialysis solutions resulted in an improved nitrogen balance, maintained serum protein concentrations better than glucose-based dialysis solutions and that amino acid solutions performed as well as standard dialysis solutions in their ability to maintain serum urea and creatinine concentrations.[124,125]

Use of lipid emulsions for intraperitoneal nutrition has been investigated for several reasons. First, lipid emulsions are energy dense and provide an ideal non-protein energy source for critically ill patients. Second, lipid emulsions are isotonic and would not be expected to draw fluid into the peritoneal cavity, as occurs with hypertonic glucose and amino acid solutions. Finally, lipid emulsions may reduce the metabolic complications that have been observed in people given dialysis solutions consisting entirely of glucose i.e. hyperglycaemia, hypertriglyceridaemia and hepatic steatosis.[123]

ABSORPTION OF CARBOHYDRATE FROM THE PERITONEAL CAVITY

There are many factors that affect the absorption of carbohydrate from the peritoneal cavity. These include: the type and size of the carbohydrate molecule;[126,127] the concentration of the carbohydrate solution infused into the peritoneal cavity;[27,122] the total volume of carbohydrate infused;[37,116] the rapidity of carbohydrate metabolism within the systemic circulation[106] and the surface area available for carbohydrate absorption.[128,129]

Glucose has a relatively small molecular weight (180 Daltons) and diffuses across the peritoneum through intercellular spaces between the mesothelial cells.[126] The glucose then enters the submesothelial capillary or lymphatic network. Because of the rapidity of glucose absorption the majority of glucose probably enters the capillaries and flows into the portal and systemic circulation. The ability of the peritoneum to absorb larger and more complex carbohydrates has been examined. The advantages of using larger carbohydrate molecules are that they provide a greater number of calories (5.5 to 8.0 times) for a given osmolality, and that the lower osmolality solution prevents significant fluid shifts from occurring. Glucose, sucrose and a dextrose polymer[m] are capable of diffusing across isolated porcine peritoneal membranes.[127] Although the net molecular flux decreases as the molecular weight increases, the total number of calories transferred across the membrane is greater for the complex carbohydrates (assessed *in-vitro* by enzymatic digestion using aminoglucosidase).[127] Absorption of a dextrose polymer solution and the subsequent *in-vivo* conversion to glucose has been confirmed in rats.[127] Increased serum glucose concentrations were identified starting at 15 minutes after the dextrose polymer infusion into the peritoneal cavity. Compared with an isocaloric intraperitoneal glucose infusion, the serum glucose concentrations after the glucose polymer remained lower, but stayed elevated for a more prolonged time period.[127] Quantities of glucose absorbed from the peritoneal cavity are directly correlated to the concentration of glucose in the peritoneal infusate and the total amount to glucose infused.[3]

The maximal absorption rate of glucose occurs at around 30 minutes after intraperitoneal administration, with the majority of the infused glucose being absorbed within the first two hours.[27,106,128,129] Absorption of glucose from the peritoneal cavity increases as the concentration of the glucose solution increases.[27,122] A similar increase in absorption rate is seen when the volume of infused glucose increases. However, as mentioned previously, there is a maximal volume that can be administered, after which, further increases in volume lead to a decrease in absorption rate.[37] Although increasing the total volume of glucose administered into the peritoneal cavity can increase the

[m] Polycose®. Ross laboratories. Ohio, USA.

amount of glucose absorbed, it also produces hyperglycaemia.[116] Rapid metabolism of the intraperitonealy administered glucose not only helps reduce the hyperglycaemia, but also maintains a glucose concentration gradient between the peritoneal cavity and systemic circulation, therefore promoting ongoing peritoneal glucose absorption.[106]

The large surface area of the peritoneum contributes to the rapid absorption of carbohydrate from the peritoneal cavity.[129] D-xylose absorption from the peritoneal cavity of dogs has been documented to be faster than absorption from the jejunum when given as a bolus.[129] Given the very large surface area of the jejunum of dogs, a possible explanation for this finding is the time required for peristaltic contractions to spread the D-xylose bolus. Spread would occur more rapidly within the peritoneal cavity. The effect of reducing the available surface area within the peritoneal cavity on absorption has been examined. Omental and 40% small bowel resection (SBR) result in a 6.5% reduction in glucose absorption, whilst 60% SBR causes a 14% decrease.[128]

POTENTIAL COMPLICATIONS OF RAPID GLUCOSE ABSORPTION

The higher the glucose concentration and the larger the volume of administration, the greater the chance of hyperglycaemia occurring.[128] People do not tolerate large intraperitoneal fluid volumes[116] and experience discomfort and pain when the peritoneal infusate exceeds 500 mOsmol/l.[27] Systemic hyperglycaemia and hyperosmolar comas are encountered when using more concentrated glucose solutions in people.[122] Systemic hyperglycaemia can also influence the rate of peritoneal glucose absorption by reducing the concentration gradient that drives the movement of glucose from the peritoneal cavity into the systemic circulation. The systemic concentration of glucose is also influenced by the rate at which it is metabolised. There does not appear to be a correlation between peak serum glucose concentrations and peak serum insulin concentrations following peritoneal administration of glucose.[122]

ABSORPTION OF PROTEIN FROM THE PERITONEAL CAVITY

The site of protein absorption from the peritoneal cavity is dependent on the molecular size of the substrate being absorbed. Intact proteins, such as albumin and globulins are absorbed from different peritoneal locations when compared to individual amino acids. Courtice and Steinbeck[118] carefully elucidated the drainage of heparinized feline plasma from the peritoneal cavity of the cat in the early 1950's. They identified that the majority of plasma proteins are absorbed via the lymphatic channels beneath the mesothelium of the diaphragm. The absorbed proteins pass through the sternal lymphatic system to the right thoracic duct and eventually into the systemic circulation.[118] The remaining protein is absorbed across the mesothelium of the mesentery, omental, parietal and visceral peritoneum into the left thoracic duct and systemic circulation. The later route is slower than the diaphragmatic one.[118] No appreciable difference in the rate of absorption of albumin versus globulins can be detected.[117] Szabo and Magyar[114] state that absorption of radiolabelled albumin from the peritoneal cavity of dogs is four times faster across the peritoneal membrane covering the liver compared to that across the diaphragmatic peritoneum.[114] The methodology of this experiment was vastly different from previous studies making it difficult to compare the two.

The site of absorption of individual amino acids from the peritoneal cavity is less controversial. It is widely accepted that absorption occurs across mesothelial cells into the venous capillary network into the mesenteric vessels, which lead to the portal circulation.[106,124,125,129] Much smaller quantities also pass through the diaphragmatic route.

Absorption of a 2% amino acid solution from the peritoneal cavity in people is 90% complete within 6 hours, with 50% of the total absorption occurring within the first hour.[124] Amino acid dialysis solutions have been successful in reducing protein malnutrition in people and are well tolerated by patients.[125] Intraperitoneal infusion of amino acids significantly improves the plasma aminogram and concentrations of retinol binding protein, alpha-1 glycoprotein and transferrin concentrations.[125] A study in rabbits demonstrated that structure or molecular weight does not influence the rate or

completeness of peritoneal absorption of essential, non-essential and branch-chain amino acids.[27]

Compared with glucose, amino acids are absorbed more rapidly and to a greater extent from the peritoneal cavity.[27] There does not appear to be a significant difference between the rate of absorption of amino acids from the peritoneal cavity and the jejunum.[129] Like glucose, the metabolism of the absorbed amino acids influences the rate at which they are absorbed from the peritoneal cavity. Amino acids enter the mesenteric blood supply and hence the portal circulation. The liver has an enormous capacity for metabolism of amino acids, which facilitates their absorption from the peritoneal cavity.[106] Using a two-compartment pharmacokinetic model it has been estimated that the absolute bioavailability of peritoneal amino acids is around 98%.[106]

ABSORPTION OF LIPID FROM THE PERITONEAL CAVITY

The absorption of lipid emulsions from the peritoneal cavity has been extensively investigated.[130,131,132,133,134] The size of fat particles in lipid emulsions are similar to chylomicrons (0.4 μm) and their absorption occurs predominantly through the lymphatic channels that cover the diaphragm. A small proportion of the lipid is phagocytosed by peritoneal mesothelial cells and large mononuclear cells present in the peritoneal cavity.[133] As previously mentioned, fluid transport through the lymphatic network is much slower than that through blood capillaries. In one study the maximal absorption rate of intraperitoneal lipid occurred between 60 to 120 minutes after infusion, with 78% of the lipid having been absorbed within four hours.[130]

Utilisation of lipids administered by the intraperitoneal route has been demonstrated by a concomitant increase in serum free fatty acids and serum triglyceride concentrations[130,131] due to activation of serum lipoprotein lipase.[130] Changes in serum and hepatocellular lipoprotein profiles after intraperitoneal (IP), intravenous (IV) and intragastric (IG) administration of a 10% lipid emulsion in rabbits have been investigated.[131] An increase in triglycerides (TG), phospholipids (PL) and total

cholesterol (TC) concentrations, as well as alterations in the lipoprotein profile of serum and hepatocytes occurred in the IP and IV rabbits, but not in the IG group. Absolute values tended to be greater in the IP animals. Diffuse microvesicular changes in the hepatocytes, consistent with hepatic steatosis were identified only in the IV group.

Absorption of lipid particles from the peritoneal cavity of dogs is not influenced by simultaneous absorption of glucose or amino acids.[130] Studies in people using lipid emulsions as a component of peritoneal dialysis solutions identified that the absorption of lipid increases as the concentration of the lipid infusate increases. No significant increases in serum triglyceride concentrations occurred following the use of a 0.6 to 0.8% solution,[134] but up to 32 g of fat per day was absorbed when concentrations greater than 10% was used.[132] However, failure to document an increase in serum triglyceride concentration following the administration of less concentrated emulsions does not disprove significant lipid absorption occurred as systemic metabolism may exceed the rate of absorption.

ABSORPTION OF WATER AND ELECTROLYTES FROM THE PERITONEAL CAVITY

The absorption of electrolytes from the peritoneal cavity is largely dependent on a solute concentration gradient. The greater the concentration gradient, the more rapid the rate of absorption, obeying the law of bulk solute-solvent flow, with water being dragged along with the electrolytes. Therefore, water and solute movements are inextricably linked. The rate of absorption of water and electrolytes from the peritoneal cavity of well hydrated dogs has been estimated at approximately 21 ml/hr.[135] More recent work using radio-labelled sodium sulphate estimates fluid absorption from the peritoneal cavity of dogs at 52 ml/hr. The majority of fluid absorption from the peritoneal cavity occurs via the capillary-portal venous route with systemic venous and lymphatic absorption accounting for 33% and 17%, respectively.[136] Absorption of small molecules across the peritoneum is not only concentration and total-volume dependent, but for some molecules is also dependent on the rate of administration. For

example, although not an electrolyte, insulin's absorption from the peritoneal cavity of the dog and subsequent effectiveness at reducing serum glucose concentrations is influenced significantly by the rate of infusion of the solution.[137]

ABSORPTION OF TOTAL NUTRIENT ADMIXTURES FROM THE PERITONEAL CAVITY

Total nutrient admixtures (TNA) are nutritional solutions containing a combination of carbohydrates (usually dextrose), protein (amino acids) and a lipid emulsion. Studies assessing the absorption and utilisation of TNA from the peritoneal cavity are comprehensive.[138,139,140,141,142,143,144] Drawing comparisons between the studies, however, is difficult, due to the tremendous variation in experimental design.

Investigations of intraperitoneal nutrition using TNA have concentrated on a number of key issues including the absorption of the individual nutrient components from a TNA; the effectiveness of intraperitoneal nutrition; and the effect of reducing the surface area available for absorption by performing a partial small bowel resection.

Four hours after a bolus infusion of 40 ml/kg of a TNA in rats, the glucose, amino acid and lipid absorption was 84%, 90% and 60%, respectively.[139] These values compare favourably to a study in dogs that reported 91.7%, 91.9% and 65.1%, respectively, five hours after a 50 ml/kg bolus of a similar TNA.[142] When compared to the absorption rate of single nutrients from the peritoneal cavity, nutrient absorption from a TNA is slower.[142] Although the studies used for the comparison of absorption rates utilised different animal models, different nutrient concentrations and different volumes of infusion,[143] another study supports this statement.[142] Total nutrient absorption from the peritoneal cavity of dogs is greater when infusion of glucose and amino acids (combined) is alternated with a lipid emulsion, compared to dogs that receive all three nutrients at once.[141] In addition to this finding, total nutrient absorption of glucose and lipids in dogs that receive amino acids is less than in dogs that do not receive amino acids.[141] This implies that amino acids somehow decrease the absorption of glucose and lipids from the peritoneal cavity, however, this is not a consistent finding.[130]

The effectiveness of intraperitoneal nutrition has been assessed predominantly by monitoring changes in bodyweight and serum protein concentrations and by determining changes in nitrogen balance. The ability to provide total daily protein-energy requirements appears to be the most important factor governing the efficacy of intraperitoneal nutrition (IPN). It has been estimated that two to three boluses of 50 ml/kg of a TNA could supply resting energy requirements to dogs.[142] A comparison between rats fed intraperitonealy (IPN) and rats fed orally using an identical nutritional solution identified that nitrogen balance was poorer in IPN rats.[140] Both groups of rats were in negative nitrogen balance at the completion of the study as a consequence of the diet supplying only 80% of daily resting energy requirements. The authors did not give reasons for why the IPN rats had a poorer nitrogen balance. Long-term survival of rabbits fed a TNA by repeated abdominal injections has been reported.[138] The rabbits lost an average of 22% of their starting bodyweight and serum albumin concentrations declined to between 20% to 25% of baseline values. The details as to the percentage of daily protein-energy requirements supplied by the diet were not provided. The significance of the marked weight loss is therefore hard to determine. A significant reduction in bodyweight has also been reported in dogs being fed a TNA.[145] At the completion of a 30 day IPN feeding trial using indwelling peritoneal catheters and a constant rate infusion (CRI), the dogs had lost an average of 14% of their initial bodyweight. There were many interruptions to the CRI and the dogs did not receive their total nutritional requirements.[145] In contrast, weight gain has been observed in rabbits being given a TNA by way of an indwelling peritoneal catheter,[138] although serum albumin concentrations declined from 36 g/l to 30 g/l. Two further studies in dogs have documented that bodyweight can be maintained by IPN over a prolonged time period (up to 28 days).[143,144] No significant change in bodyweight occurred in these studies. However, the dogs had a reduction in serum albumin concentration[144] and were in negative nitrogen balance by the end of the study.[143] Both studies provided 0.5 g protein/kg/day, which is below the absolute minimum protein requirement for maintenance in dogs of 1.3 to 1.5 g/kg/day.[146]

The surface area available for nutrient absorption can influence the rate of absorption of nutritional solutions from the peritoneal cavity,[138,143] although the magnitude of this effect varies. Total nutrient absorption from the peritoneal cavity of rabbits declined from 95.7% ± 1.2% to 93.6% ± 2.5% following 50% small bowel resection.[138] The minor reduction in absorptive capacity is in contrast to dogs that were receiving IPN, in which 80% of the small bowel was resected. The dogs suffered significant decreases in bodyweight, serum protein concentrations and negative nitrogen balance.[143] Species variation and the greater magnitude of SBR in the dogs could explain the difference in these two studies.

HISTOPATHOLOGY OF ABDOMINAL ORGANS AND THE PERITONEUM FOLLOWING INTRAPERITONEAL NUTRITION

Histological assessment of the abdominal organs and, visceral, parietal and mesenteric peritoneum following intraperitoneal nutrition is extensive.[130,131,133,138,141,143,144,145] Comparison between studies and quantifying the changes associated with specific nutrient components is difficult because of variation with respect to species, types of nutritional solutions, length of treatment and the volumes of nutritional solution given is marked.

A weak association exists between the amount of nutrients provided, the frequency of dosing and the duration of nutritional support and, the severity of the gross and histological changes seen within the peritoneal cavity. Gross observations include erythema of the peritoneal membrane,[140,141,143,145] peritoneal thickening [133,143,144,145] and fat accumulation within intestinal folds and on the ventral surface of the diaphragm.[140,143,144] Lymphatic engorgement with a milky-white lipid material was commonly reported.[133,143,144] In one study in rats, these changes were severe, with haemorrhagic ascitic fluid and inspissated lipid adherent along intestinal folds.[140] More than 50% of the rats in this study died during the experimental period and several positive peritoneal cultures were obtained. Histological changes in abdominal organs

such as kidney, spleen, intestines and pancreas are generally unremarkable.[130,133,138,141,143,144]

Studies have investigated the occurrence of TPN-induced cholestasis and hepatopathy in animals receiving IPN.[131,138,141,143] This is a common complication of IV nutritional support in dogs and people.[7] Light microscopy identified vacuolation of hepatocytes but no evidence of portal inflammation, fibrosis, or cholestasis.[138,141,143] Vacuolation is not a consistent feature, as other studies failed to show any significant changes using either light, or electron microscopy.[130,131,133,138,144]

Histopathology of the peritoneal membranes typically reveals varying degrees of acute and chronic inflammation. Mixed inflammatory cells such as neutrophils, plasma cells, lymphocytes and macrophages are often present.[133,138,143,145] In general, the peritoneal inflammation was mild to moderate in nature. The peritoneal mesothelial cells become hyperplastic, increasing the thickness of the peritoneal membrane.[133,138,143,145] Fat particles can be seen both intracellularly and within intercellular spaces.[133,138,143,144,145] A prominent feature in one study was marked capillary proliferation, producing a pseudovillus formation on both the parietal and visceral peritoneal surfaces.[145] Neovascularisation was noted in another study.[144] These changes had completely resolved 30 days after resuming oral nutrition.

COMPLICATIONS ASSOCIATED WITH INTRAPERITONEAL NUTRITION

A variety of complications of intraperitoneal nutrition have been identified. They can be classified as acute versus delayed complications or related to mechanical, septic, metabolic and nutritional complications.

Acute Complications

Acute complications that have been identified include abdominal pain, during or after infusion;[132] rapid fluid shifts from the vascular space into the peritoneal cavity;[140,145] nausea; and vomiting.[143] Many of these acute reactions are related to the osmolality of the nutritional solution used. Pain associated with peritoneal dialysis in people has

been documented when the osmolality exceeds 500 mOsmol/l.[27] The greater the osmolality of the nutritional solution, the greater the amount of water drawn from the vascular space into the peritoneal cavity and the more likelihood of hypovolaemia and shock.[138,140,145] Due to the deleterious effects of high osmolality, intraperitoneal nutritional solutions have to be maintained between 500 to 900 mOsmol/l. This restricts the energy density of the solutions requiring the infusion of large volumes to satisfy daily caloric requirements. Administration of very large volumes produces abdominal distension and may contribute to nausea and vomiting seen in some animals.[143] Stimulation of the chemoreceptor trigger zone by high serum concentrations of glucose, triglycerides and free fatty acids, and stimulation of the vomiting centre by high serum osmolality are other possible causes of these clinical signs. An additional potential complication of large-volume infusions is restriction of diaphragmatic movements and impairment of respiratory function. This has been reported in people undergoing peritoneal dialysis.[27]

Mechanical Complications

Several IPN studies utilise indwelling peritoneal catheters to facilitate nutrient infusion. Some were commercially available peritoneal dialysis catheters used in people,[130,141,142,143,144] whilst others were custom-made from various sizes of silastic tubing.[138,145] Catheter blockage occurred in a number of cases, limiting the amount of nutritional solution provided and may have contributed to the weight loss that occurred.[141,145] In one study in dogs, infusion rates were increased to compensate for lost time, resulting in serious complications including death.[145] Surgical resection of the omentum was performed in rabbits to try and minimise catheter blockage and adhesion formation, with mixed success.[140] Additional complications associated with indwelling catheters include twisting and kinking of fluid lines and removal and chewing of lines by fractious animals.[141,145] The advantage of using indwelling catheters rests with the ability to give constant rate infusions, which minimises bolus administration that often results in rapid fluid shifts.

Septic Complications

With any form of indwelling catheter the risk of sepsis is high. Infectious peritonitis occurred infrequently in dogs receiving IPN. One study reported an overall infection rate of 7.7%,[144] another reporting an incidence of 16.7%.[143] The latter infection rate was reduced to 10.5% with the use of cephalosporin antibiotics added to the nutritional solution. The incidence of sepsis associated with intraperitoneal nutrition compares favourably to sepsis associated with intravenous nutrition.[88,90] When encountered, septic peritonitis can be treated by halting intraperitoneal nutrition, performing peritoneal lavage and administering systemic antibiotics for two to three days.[143] Septic peritonitis, both clinically and experimentally, is considered easier to manage than sepsis associated with intravenous nutrition.[143,144] Contamination of the intraperitoneal nutrition solution has been identified as the most likely cause of sepsis in one study.[143] Experimental protocols are sufficiently different to make it difficult to compare the incidence of sepsis in animals with indwelling catheters and animals receiving nutritional solutions via repeat abdominal puncture.

Metabolic Complications

Hyperglycaemia is a common complication of IPN,[130,131,132,138,141,143,144,145] although it is not a consistent finding.[106,144] The severity of hyperglycaemia depends upon the total amount of glucose infused, the concentration gradient between the peritoneal cavity and serum and patient factors governing carbohydrate metabolism. The use of insulin administration to facilitate glycaemic control in patients receiving IPN has not been specifically studied. However, improved glycaemic control in dogs given insulin by the intraperitoneal route has been reported.[143] Insulin absorption from the peritoneal cavity is volume, concentration and time dependent.[138] Whether intraperitoneal insulin administration offers definite advantages over the more traditional routes requires further investigation.

Hypertriglyceridaemia often occurs during IPN when lipid is included in the nutritional admixture.[130,131,132,138,141,143,144,145] Although direct consequences of this metabolic derangement have not been reported, possible adverse effects include

pancreatitis, seizure activity, hyperviscosity syndrome and impairment of the immune system.[101,147] Elevated serum triglyceride concentrations occur as a direct result of absorption from the peritoneal cavity down a concentration gradient. The ability of the liver to clear triglycerides from the circulation is dependent upon the activity of lipoprotein lipase and hepatic lipid metabolism.[102]

Hypoproteinaemia is frequently encountered during IPN.[138,143,144,145] The aetiology has been poorly defined but is probably multi-factorial. Loss of albumin (intravascular and interstitial) into the peritoneal fluid due to movement along a concentration gradient would seem the most plausible explanation. Peritoneal inflammation, as a consequence of chemical irritation from the nutritional solutions or peritoneal sepsis, would be expected to exacerbate protein loss into the peritoneal cavity. It has been clearly shown that including amino acids in the nutritional admixture reduces the severity of hypoproteinaemia but does not prevent it from occurring.[130,138,148] Insufficient amounts of protein and calories supplied to some experimental animals would have played a role in the development of hypoproteinaemia.[143,144] Additional factors involved in the aetiology of hypoproteinaemia may include over-hydration leading to dilutional hypoproteinaemia and blood loss into the peritoneal cavity during catheter placement or repeated peritoneal puncture.

Anaemia has been reported less frequently in association with intraperitoneal nutrition.[130] Many of the above factors may also play a role in the aetiology of anaemia. Anaemia may also result in part from repeated blood sampling throughout the experimental period and from iron sequestration secondary to chronic peritoneal inflammation.

A single case report of severe hypophosphataemia has been described in a woman undergoing IPN in association with chronic ambulatory peritoneal dialysis.[149] The specific cause of the electrolyte disorder in this patient was not determined but was thought to occur as a result of intracellular phosphate shifts associated with a positive

nitrogen balance and protein synthesis. Hyperphosphataemia has also been reported in association with IPN in dogs.[145]

SUMMARY OF INTRAPERITONEAL NUTRITION

The peritoneal membrane of animals and people has a tremendous potential for nutrient and fluid absorption. Simple and complex carbohydrates, as well as amino acids, protein hydrolysates, intact proteins and lipid emulsions are all capable of being absorbed across the peritoneal membrane. The absorption of carbohydrates and amino acids from the peritoneal cavity occurs predominantly across the mesothelial cells into the peritoneal interstitial capillary network and flows to the portal circulation. This route of absorption is rapid, with the majority of absorption occurring within the first 60 to 120 minutes. Lipid absorption is slower and occurs primarily through the lymphatic channels on the ventral surface of the diaphragm.

Complications of intraperitoneal nutrition have been recognised. The most important include rapid fluid shifts from the vascular space into the peritoneal cavity; pain associated with the use of hypertonic intraperitoneal nutritional solutions; significant reductions in serum protein concentrations and bod weight and the inflammatory response by the peritoneal membrane to the infusion of nutritional solutions into the peritoneal cavity.

Histopathological changes to the peritoneal membrane typically reveal varying degrees of acute and chronic inflammation. The inflammatory response as a result of the intraperitoneal administration of nutritional solutions resolves quickly, and is completely resolved four weeks following the conclusion of intraperitoneal nutrition.

Several studies have identified that dogs can survive for prolonged periods when fed solely by this route, however, difficulties in providing total daily protein-energy requirements have resulted in a significant reduction in lean body mass in some studies.

Further research is required before IPN becomes widely accepted in veterinary medicine. Future work will need to concentrate on establishing data that can be used to monitor patients receiving IPN. These parameters should provide clinicians with the necessary information to ensure the safe and effective administration of nutritional support in their patients. The development of an alternative method for nutritional administration that is safe, effective and easy to perform may alleviate some of these complications. Repeat abdominal puncture and bolus administration of the solution is one such technique that warrants further investigation.

The overall purpose of this thesis is to expand knowledge on the administration, tolerance, complications and monitoring of intraperitoneal nutrition in dogs.

CHAPTER 3.

- Pilot Study of Intraperitoneal Nutrition in Dogs Using a Complete Nutritional Admixture via a Repeat Abdominal Puncture Technique.

  Introduction.

  Objectives.

  Materials and Methods.

  Results.

  Discussion.

  Summary and Conclusions.

PILOT STUDY OF INTRAPERITONEAL NUTRITION IN DOGS USING A COMPLETE NUTRITIONAL ADMIXTURE VIA A REPEAT ABDOMINAL PUNCTURE TECHNIQUE.

INTRODUCTION

Previous studies using experimental animal models have demonstrated that all three of the nutritional components commonly used for intravenous total parenteral nutrition (glucose, crystalline amino acids and lipid emulsions) can be absorbed from the peritoneal cavity. However, intraperitoneal nutrition has a number of concerning aspects. First, there is a paucity of data documenting the maximum allowable osmolality of nutritional solutions that can be safely infused into the peritoneal cavity of dogs. Secondly, there have been no studies assessing the short-term cytological response by the peritoneum to a total nutrient admixture (TNA). This information is required to provide clinicians with the ability to detect nascent infectious peritonitis. Thirdly, studies providing a detailed clinical assessment of dogs receiving intraperitoneal nutrition are lacking. In particular, there is insufficient information available on the changes in cardiovascular status resulting from intraperitoneal nutrition. Lastly, the use of a repeated abdominal puncture technique for the administration of nutritional solutions has not been adequately examined.

OBJECTIVES

The overall purpose of this pilot study was to critically examine the suitability of a total nutrient admixture similar to that previously reported to be successful for intraperitoneal nutrition in dogs.[143,144] Specific experimental objectives were to:

- develop a repeated abdominal puncture technique for the administration of intraperitoneal solutions.
- identify any clinical evidence of pain or clinically significant fluid shifts associated with the infusions of hypertonic nutritional solutions into the peritoneal cavity of dogs.
- define the peritoneal cytological changes that occur in dogs given a TNA into the peritoneal cavity via a repeated abdominal puncture technique.

- determine the prevalence of metabolic complications such as hyperglycaemia and hypercholesterolaemia in dogs receiving a TNA.
- initiate the development of reference ranges for diagnostic peritoneal lavage in dogs receiving an intraperitoneal TNA.

MATERIALS AND METHODS

Dogs

Two adult Border collie-cross dogs weighing 19.5 kg and 22.5 kg were used for this study. IPN was commenced after an initial 12 hour fast. Both dogs had access to water ad-libitum throughout the study.

Nutritional Admixture

On Day 1, each dog received maintenance energy requirements (MER = 132 x $BW^{0.75}$) in the form of a total nutrient admixture (TNA) that was comprised of 500 ml of 8.5% amino acids,[n] 500 ml of 50% dextrose[o] and 250 ml of a 20% lipid emulsion.[p] The volume of nutrient solution administered was subsequently modified depending on how well the solutions were tolerated (Table 3-1.). The calculated osmolality of the TNA was 835 mOsmol/l. The TNA admixture was commercially prepared under sterile conditions.[q] The lipid fraction of the TNA was separated from the glucose and amino acids in the nutritional bag until immediately prior to nutrient infusion (Figure 3-1.). The nutritional solution was planned to be administered daily for ten days in two divided boluses at 9.00am and 5.00pm by repeated abdominal puncture.

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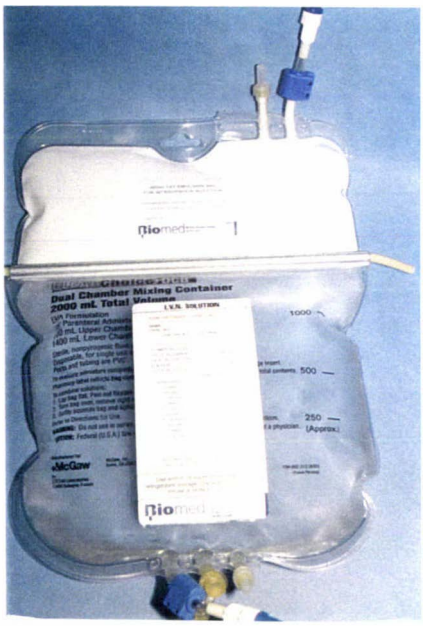
[n] FreAmine® III. McGaw, Inc. CA, USA.
[o] Hypertonic Dextrose Injections USP. McGaw, Inc. CA, USA.
[p] Lipofundin® MCT/LCT. B.Braun. Melsungen, Germany.
[q] McGaw BioMed Ltd. Auckland, NZ.

Table 3-1. Volume of nutritional solution administered to Dog 1 and Dog 2 on each day.

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Volume Infused	Dog 1	1000	500	1000	1500	2000	1000	0
(ml/day)	Dog 2	1000	500	1000	1500	1000	1000	0

Figure 3-1. Total nutrient admixture bag prior to mixing the lipid and dextrose / amino acid components.



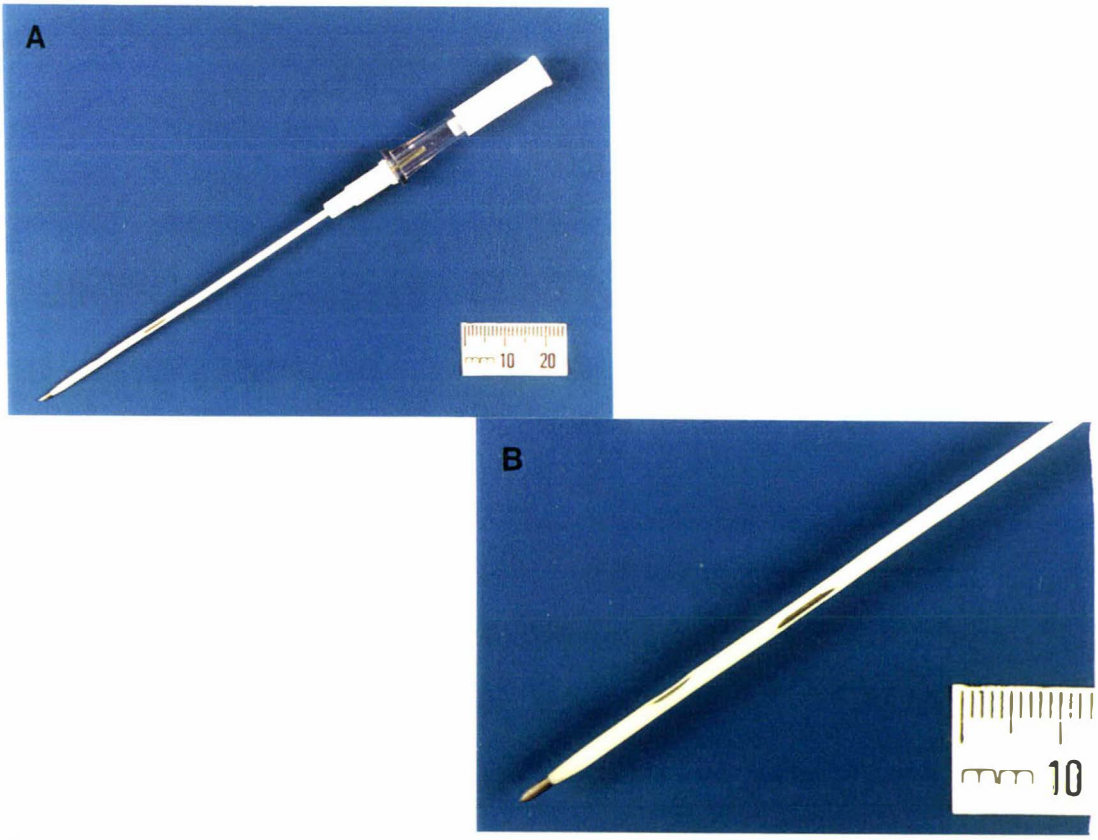
Diagnostic peritoneal lavage and nutrient infusion

The dogs were clipped along the ventral abdomen and surgically prepared using a standard technique (aqueous chlorhexidine, then chlorhexidine tincture, followed by alcohol) prior to each infusion. Before each infusion, 1 ml of local anaesthetic (2% lignocaine[r]) was infiltrated into the skin and abdominal wall at the site of the puncture using a 1 inch, 25 G needle. A 12 G over-the-needle catheter[s] with three to five side-holes

[r] Lopaine®. Ethical Agents Ltd. Auckland, NZ.
[s] Angiocath™. Becton Dickinson Infusion Therapy systems Inc. Utah, USA.

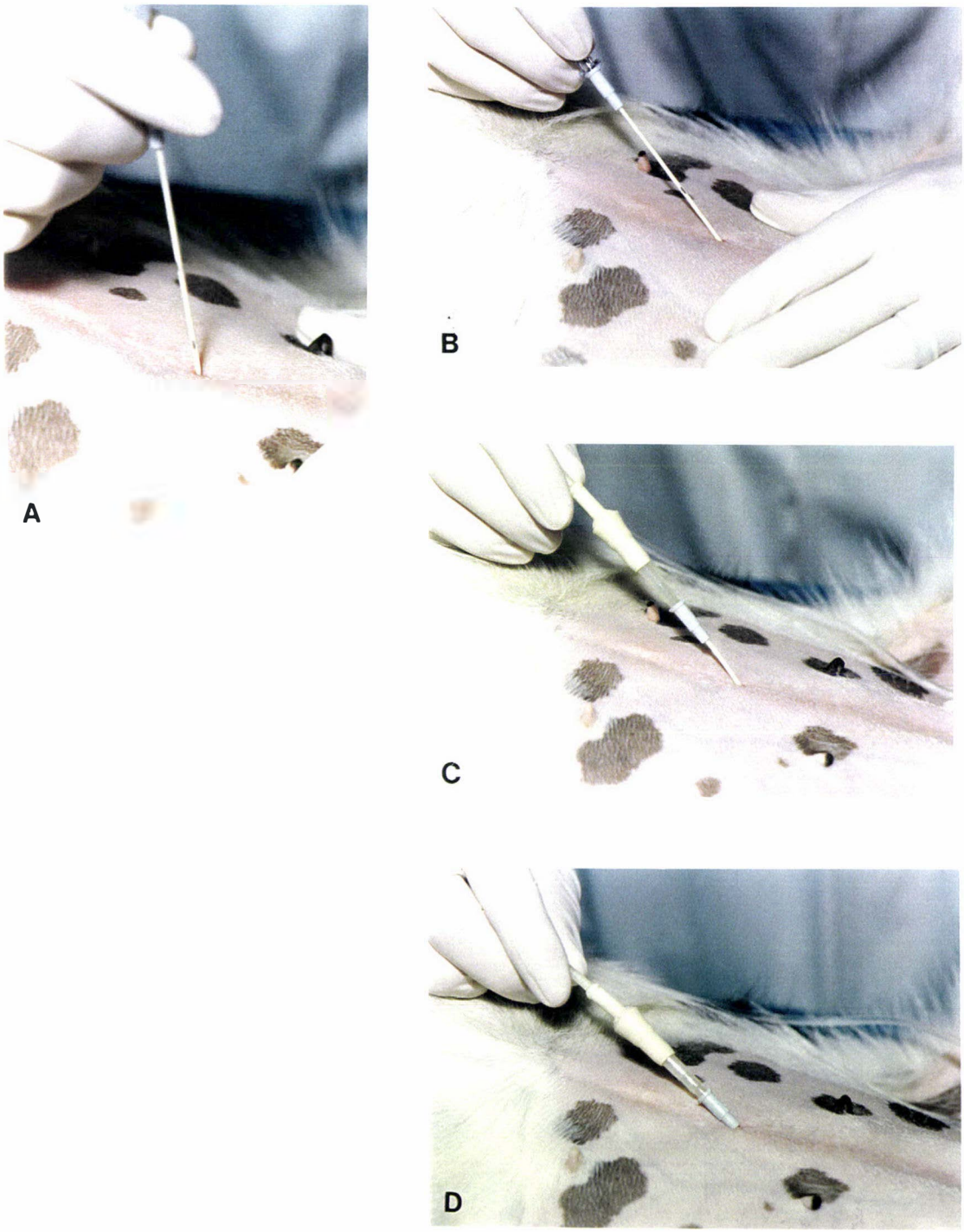
cut using a No.11 scalpel blade (Figure 3-2 a, b.) was then inserted into the peritoneal cavity on the ventral midline, approximately three centimetres caudal to the umbilicus. (Figures 3-3 a, b, c, d.) Prior to the first nutrient infusion on Day 1, a DPL was performed via the catheter using 25 ml/kg lactated Ringer's solution.[t] A 10 ml sample was obtained for cytological analysis (see below) and 10 ml for aerobic and anaerobic culture. Following the DPL, the catheter was connected to the parenteral nutrition bag by a 10 drop/ml infusion set.[u] The calculated volume of nutritional solution was infused by gravity-flow over a 15 to 20 minute period. At the completion of the infusion the catheter was withdrawn from the peritoneal cavity and light pressure applied with a gauze swab for several minutes to prevent leakage of fluid into the subcutaneous tissues. The dogs were restrained in dorsal recumbency for the duration of the DPL and nutrient administration.

Figure 3-2. 12 G Over-the-needle catheter used for the DPL and nutrient infusion (a) an over-the-needle design is very cost effective (b) additional side holes were cut using a No. 11 scalpel blade.



[t] Hartmans Solution. Baxter Healthcare PTY Ltd. NSW, Australia.
[u] Solution Set, Flashball Device®. Baxter Healthcare Corporation. IL, USA.

Figure 3-3. Sequential insertion of the over-the-needle catheter for DPL and nutrient administration. (a) catheter inserted on midline (b) once through the abdominal wall the catheter is angled caudally and to the right to avoid the spleen (c) the giving set is attached for the final advancement of the catheter (d) final position of the catheter.



Experimental time course and monitoring

The DPL was planned to be repeated on Days 3, 5, 8 and 10, prior to the first nutrient infusion for the day and the same assessments made as on Day 1. On Days 1, 4, 7 and 10, a cephalic venous blood sample was collected and a complete blood count (CBC) and serum chemistry panel with electrolytes were performed. A voided urine sample was collected for urinalysis on the same days as the blood samples. A schematic outline of the planned experimental protocol can be seen in Table 3-2. However, due to the unfavourable response by the dogs in the early stages of the study the experiment was stopped on Day 7. On the days when the blood and urine samples coincided with the DPL and nutrient administration the former samples were collected first. Each day at 10.00am, 2.00pm and 6.00pm, the dogs' temperature, respiration rate, heart rate, mucous membrane colour, capillary refill time, pulse pressure and general demeanour were assessed. In addition, the presence of abdominal pain was assessed by examining for behavioural and postural changes, and by thorough abdominal palpation. The dogs were weighed once daily in the morning prior to the DPL and nutrient infusion.

Table 3-2. Schematic representation of the planned experimental protocol.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
DPL	✓		✓		✓			✓		✓
IPN	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Cult	✓		✓		✓			✓		✓
Haem	✓			✓			✓			✓
Urin	✓			✓			✓			✓

DPL = diagnostic peritoneal lavage, IPN = intraperitoneal nutrition, Cult = aerobic and anaerobic culture
Heam = complete blood count and serum chemistry panel with electrolytes, Urin = complete urinalysis

Analysis of samples

The peripheral CBC and the total white cell count of the DPL fluid was performed using an automated haematology machine.[v] The differential peripheral and DPL differential white cell counts were calculated after counting 500 white cells (using a rapid staining technique).[w] The differential white cell count and cell morphology of the DPL fluid were assessed following cyto-centrifugation[x] and staining by the author. The serum chemistry analysis was performed using an automatic analyser[y] and the urine chemistry analysis performed using commercially available urinalysis strips.[z] As a consequence of systemic hypoglycaemia, a serum insulin concentration was measured in Dog 2 on Day 5 using a radioimmunoassay technique.

Statistics

Given the small number of dogs used in this pilot study descriptive statistics only were used for the assessment of the data collected.

RESULTS

The bodyweights of Dog 1 and Dog 2 on Day 1 were 19.5 kg and 22.5 kg, respectively. Although there was a moderate variation on a day to day basis, the bodyweights changed only marginally to 20.5 kg and 20.5 kg respectively, by Day 7 (Table 3-3.).

Table 3-3. Changes in bodyweight throughout the study.

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
BW (kg)	Dog 1	19.5	23.0	22.5	20.5	20.5	22.5	20.5
	Dog 2	22.5	22.0	20.0	22.5	23.0	24.0	20.5

BW = bodyweight

[v] Cobas Minos-Vet. ABX International. Levallois, France.
[w] Diff-Quik®. Dade Diagnostics of PR. Inc. Aguada, USA.
[x] Shandon Cytospin SCA 0300. Shandon Southern Instruments Inc. PA, USA
[y] Hitachi Boerhinger Mannheim 704. Boerhinger Mannheim Diagnostics. IN, USA.
[z] Combur[9] Test. Boerhinger Mannheim (Diagnostics and Biochemicals) Ltd. East Sussex, England.

Both dogs appeared lethargic and vomited within 10 minutes of the first intraperitoneal nutrient infusion. By 60 minutes, the dogs had evidence of abdominal pain, a stilted hindlimb gait, ataxia and congested mucous membranes. The clinical signs gradually deteriorated, until by 6 hours after the administration of the intraperitoneal nutrients the dogs were displaying signs of hypovolaemic shock, as indicated by tachycardia, weak femoral pulse, prolonged capillary refill time and weakness. A short-term increase in peripheral packed cell volume (PCV) and total protein of 72% and 110 g/l, and 66% and 100 g/l in Dog 1 and Dog 2, respectively occurred. Both dogs became hypoglycaemic, with a serum glucose concentration of 2.35 mmol/l in Dog 1 and 2.70 mmol/l in Dog 2. The skin on the ventral abdomen appeared erythematous and warm to touch, and was associated with abdominal discomfort. As soon as the clinical signs of hypovolaemic shock and abdominal discomfort occurred the dogs were given intravenous lactated Ringer's solution at 90 ml/kg/hr to improve cardiac output and buprenorphine[aa] (0.01 mg/kg subcutaneously every eight hours) for pain relief. The dogs made an uneventful recovery and no further nutritional solution was given on Day 1.

Given the complications on Day 1, the volume of infusion on Day 2 was reduced to 25% of the calculated maintenance calories (equal to 2000 ml). This was split into two divided doses of 250 ml at 9.00am and 5.00pm. There were no complications seen on Day 2 and the dogs remained bright and alert with no evidence of abdominal discomfort. On Days 3 and 4, the volume of nutritional solution administered increased to 50% and 75% of the calculated maintenance calories, respectively. On Day 3, the dogs tolerated the increase in nutritional administration without complication. No change in cardinal signs or general demeanour was noted. On Day 4, Dog 1 developed a fever (39.8°C) that was associated with moderate abdominal discomfort on deep palpation but otherwise remained bright and alert. The dog was observed overnight and reassessed the next morning and a DPL performed.

On Day 5, the fever and abdominal discomfort in Dog 1 were still present. The DPL analysis revealed a markedly increased TWCC of 36.5 x 10^9/l and consisted

[aa] Temgesic®. Reckett and Colman. 289 Lincoln Road, Auckland, NZ.

predominantly of mature, non-degenerate neutrophils. No bacteria were seen and culture of the DPL fluid was negative. The fever was treated with subcutaneous ketoprofen[bb] (2 mg/kg) and quickly resolved. Dog 1 received a total of 2000 ml (maintenance caloric requirements) on Day 5. Dog 2 developed systemic hypoglycaemia (2.63 mmol/l) after the first infusion of 1000 ml on Day 5. Consequently, no additional nutritional solution was given that day to prevent further metabolic complications.

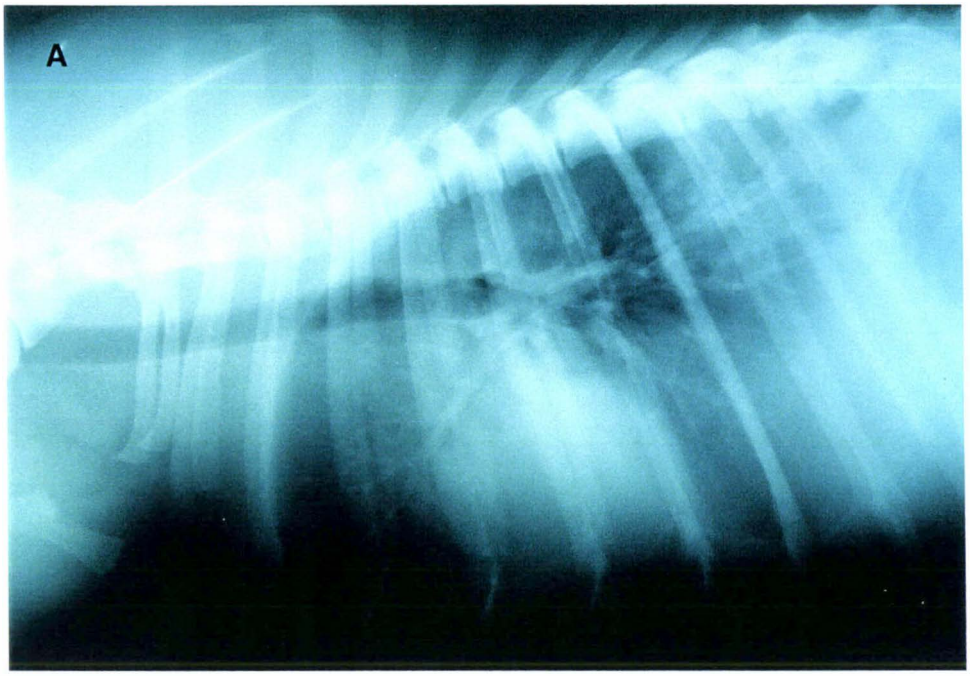
On Day 6 both dogs were given their morning infusions of 1000 ml without incident. However, immediately after the infusion of the second 1000 ml to Dog 1 that evening, the dog vomited and became depressed, dyspnoeic, tachycardic and had congested mucous membranes. The capillary refill time was prolonged in association with a weak femoral pulse. The 1000 ml bolus was withdrawn from the peritoneal cavity and the dog started on intravenous fluid therapy. The vomiting persisted and required the use of intravenous metoclopramide[cc] to control the clinical signs. As a precaution, Dog 2 did not receive any further nutritional solution on Day 6. As a result of the second major episode of hypovolaemic shock and the unacceptable adverse clinical signs associated with the infusion of total daily caloric requirements, it was decided to stop the study. The dyspnoea in Dog 1 was still present the next morning and chest radiographs demonstrated a mild pleural effusion (Figure 3-4 a, b.). This resolved over the following five days without the need for treatment. Both dogs made a complete recovery within seven days.

On Day 3 of the study there was a mild increase in peripheral TWCC in Dog 1 and an increase in immature neutrophils on Days 5 and 7, and Day 7, in Dog 1 and Dog 2, respectively (Table 3-4.). The dogs were desexed as an elective surgery approximately four weeks after the conclusion of the study. At this time the peritoneal cavity was grossly normal.

[bb] Ketofen.™ Rhone Merieux. Lyon, France.

[cc] Maxalon.® SmithKline Beecham (NZ) Ltd, Auckland, NZ.

Figures 3-4. Chest radiographs of Dog 1 on Day 7 (a) left lateral (b) ventro-dorsal. Note the loss of the diaphragmatic line and cardiac silhouette.



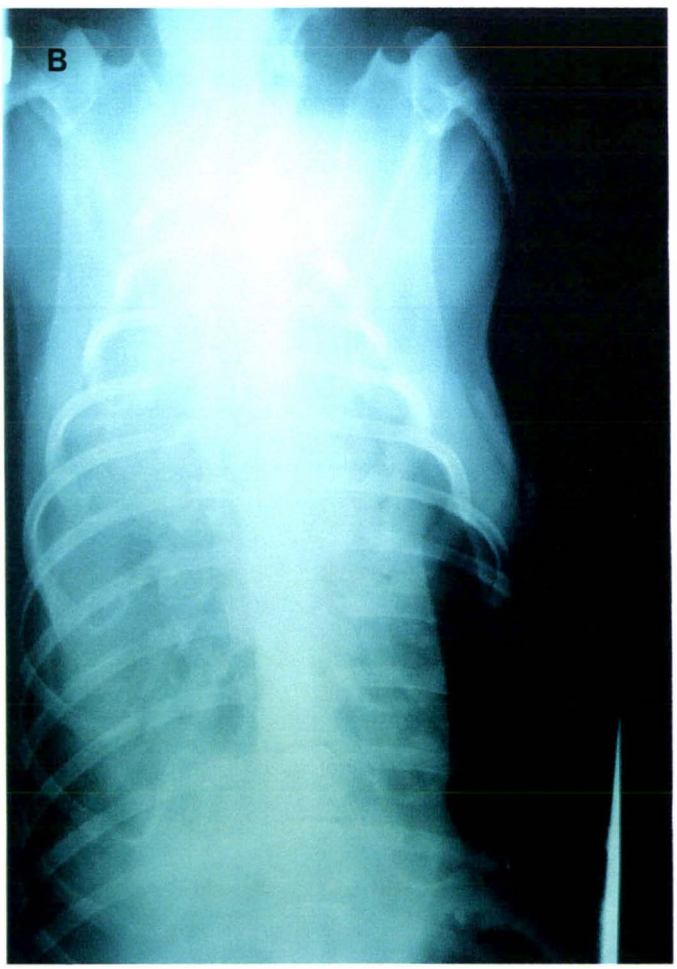


Table 3-4. Comparison of the Day 1, 3, 5 and 7 complete blood counts in Dog 1 and Dog 2.

		Day 1	Day 3	Day 5	Day 7	Reference Range
HB (g/l)	Dog 1	178	172	154	176	120-180
	Dog 2	162	175	194	198	
PCV (l/l)	Dog 1	0.48	0.44	0.43	0.46	0.37-0.55
	Dog 2	0.43	0.45	0.52	0.51	
RBC (x 10^{12}/l)	Dog 1	7.27	6.69	6.48	6.93	5.8 - 8.5
	Dog 2	6.62	6.72	7.93	7.79	
MCV (fl)	Dog 1	67	67	68	67	60 - 77
	Dog 2	66	68	66	66	
MCH (g/l)	Dog 1	24.4	25.7	23.7	25.3	None
	Dog 2	24.4	26.0	24.4	25.4	
MCHC (g/l)	Dog 1	367	383	350	376	310 - 340
	Dog 2	369	383	371	386	
Retics (x 10^9/l)	Dog 1	0	0	0	0	None
	Dog 2	0	0	0	0	
PLT (x 10^9/l)	Dog 1	340	314	236	112	200 - 900
	Dog 2	477	392	400	312	
WBC (x 10^9/l)	Dog 1	11.5	21.1	9.6	11.3	6.0 - 15.0
	Dog 2	12.6	19.1	9.6	14.8	
Neuts (x 10^9/l)	Dog 1	6.9	18	6.1	7.7	3.6 - 11.5
	Dog 2	7.6	15	3.7	9.9	
Bands (x 10^9/l)	Dog 1	0	0	0.9	0.6	0.00 - 0.54
	Dog 2	0	0	0	0.9	
Lymph (x 10^9/l)	Dog 1	3.2	1.3	2.1	2.4	1.0 - 4.8
	Dog 2	2.3	1.5	2.6	2.1	
Mono (x 10^9/l)	Dog 1	0.2	1.1	0.6	0.7	0.18 - 1.5
	Dog 2	1.5	1.9	1.7	1.8	
Eosin (x 10^9/l)	Dog 1	1.2	0.6	0	0	0.12 - 1.5
	Dog 2	1.3	0.2	1.6	0.1	
Baso (x 10^9/l)	Dog 1	0	0	0	0	0
	Dog 2	0	0	0	0	

HB = haemoglobin, PCV = packed cell volume, RBC = red blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, Retics = reticulocytes, PLT = platelets, WBC = white blood cells, Neuts = mature neutrophils, Bands = immature neutrophils, Lymph = lymphocytes, Mono = monocytes, Eosin = eosinophils, Baso = basophils.

On Days 3 and 5, serum urea nitrogen (SUN) concentration decreased in Dog 1, whilst on Day 7, SUN in both dogs increased and was associated with a concomitant increase in serum creatinine on Day 7 in Dog 1. Serum ALP increased modestly in both dogs on Days 3, 5 and 7 and was associated with an increase in serum bilirubin concentration on Day 7 in both dogs.

Hypoglycaemia was documented on Day 1 in both dogs and Day 5 in Dog 2. The second episode of hypoglycaemia in Dog 2 was associated with a markedly increased serum insulin concentration of 278.22 μU/l (reference range: 5 to 20 μU/l) The serum cholesterol concentration increased mildly on Day 7 in Dog 1 and Dog 2. The most striking change in the chemistry panel occurred in the total protein and serum albumin concentrations. Total protein declined from 67.8 g/l to 53.9 g/l and from 63.3 g/l to 61.8 g/l by Day 7, in Dog 1 and Dog 2, respectively. The albumin concentration reduced to a greater extent than globulins, declining from 35.8 g/l to 19.1 g/l in Dog 1 and 32.5 g/l to 21.3 g/l in Dog 2, by Day 7.

Significant electrolyte abnormalities were restricted to sodium and phosphate. The serum sodium concentration in Dog 1 and Dog 2 underwent a marked decline on Day 7, and Days 5 and 7, respectively. The serum phosphate concentration increased on Days 5 and 7 in both dogs. A summary of the serum chemistry and electrolyte data can be seen in Table 3-5.

The only significant change to occur in the urinalysis was a decline in the urine specific gravity to 1.007 and 1.006 for Dog 1 and Dog 2, respectively on Day 3, and 1.013 in Dog 1, on Day 5 (Table 3-6.).

Table 3-5. Comparison of the Day 1, 3, 5 and 7 serum chemistry and electrolyte data in Dog 1 and Dog 2.

		Day 1	Day 3	Day 5	Day 7	Reference Range
Urea (mmol/l)	Dog 1	3.87	3.1	2.25	24.04	2.5 - 8.4
	Dog 2	5.16	3.38	5.64	19.36	
Creatinine (µmol/l)	Dog 1	80	64	63	174	75 - 138
	Dog 2	74	64	71	101	
Lipase (IU/l)	Dog 1	73	57	95	274	< 500
	Dog 2	62	33	87	589	
ALP (IU/l)	Dog 1	65	99	151	277	< 185
	Dog 2	136	148	251	394	
ALT (IU/l)	Dog 1	29	28	34	32	< 58
	Dog 2	50	36	25	26	
CK (IU/l)	Dog 1	153	188	1145	798	< 800
	Dog 2	115	79	127	179	
Glucose (mmol/l)	Dog 1	3.5	4.7	4.5	5.7	3.8 - 5.8
	Dog 2	4.6	6.2	4.2	5.7	
Cholesterol (mmol/l)	Dog 1	6.88	4.7	9.1	10.31	3.2 - 9.3
	Dog 2	5.73	6.61	9.27	10.07	
TP (g/l)	Dog 1	67.8	68.8	65.4	53.9	62 - 85
	Dog 2	63.3	64.5	71	61.8	
Albumin (g/l)	Dog 1	35.8	33.7	27.9	19.1	28 - 35
	Dog 2	32.5	31.5	29.3	21.3	
Globulins (g/l)	Dog 1	32	35.1	37.5	34.8	27 - 57
	Dog 2	30.8	33	41.7	40.5	
A/G	Dog 1	1.12	0.96	0.74	0.55	None
	Dog 2	1.06	0.95	0.7	0.53	
T Bilirubin (µmol/l)	Dog 1	2.6	2.6	3.4	30.3	< 6.0
	Dog 2	1.9	1.9	5.7	7.2	
Sodium (mmol/l)	Dog 1	150.7	144.9	144.9	125.2	141 - 155
	Dog 2	149.7	146.9	138.9	131.6	
Potassium (mmol/l)	Dog 1	4.54	4.34	4.74	5.02	3.6 - 5.6
	Dog 2	5.33	4.2	5.55	5.51	
Calcium (mmol/l)	Dog 1	2.67	2.57	2.48	2.45	2.05 - 3.00
	Dog 2	2.49	2.47	2.51	2.38	
Phosphate (mmol/l)	Dog 1	1.1	1.6	1.75	2.59	0.8 - 1.6
	Dog 2	1.78	1.6	2.65	3.17	

ALP = Alkaline phosphatase, ALT = Alanine aminotransferase, CK = Creatine kinase, TP = Total protein, A/G = albumin to globulin ratio, T Bilirubin = Total bilirubin.

Table 3-6. Comparison of the Day 1, 3, 5 and 7 urinalysis results in Dog 1 and Dog 2.

		Day 1	Day 3	Day 5	Day 7
S.G.	Dog 1	1.030	1.007	1.013	1.044
	Dog 2	1.034	1.006	1.031	1.026
pH	Dog 1	9	7	8	6
	Dog 2	8	7	8	6
Protein	Dog 1	1+	No	Trace	2+
	Dog 2	Trace	Trace	Trace	1+
Glucose	Dog 1	no	no	4+	No
	Dog 2	no	no	no	No
Ketones	Dog 1	no	no	no	No
	Dog 2	no	no	no	No
Bilirubin	Dog 1	no	no	no	No
	Dog 2	no	no	no	No
Urobilinogen	Dog 1	no	no	no	No
	Dog 2	no	no	2+	No
Blood	Dog 1	no	no	2+	3+
	Dog 2	no	no	2+	2+
Nitrate	Dog 1	no	no	no	No
	Dog 2	no	no	no	No

S.G. = specific gravity.

The TWCC of the DPL fluid on Day 1 was 0.9 x 10^9/l and 0.6 x 10^9/l in Dog 1 and Dog 2, respectively. The TWCC had increased markedly to 26.4 x 10^9/l and 56 x 10^9/l in Dogs 1 and 2, respectively by Day 7 (Table 3-7.). The differential white cell counts of the DPL fluid on Days 1 and 7 followed the same trend as the TWCC. The inflammatory response was predominantly neutrophilic with mature neutrophils accounting for 98% and 99% of the TWCC on Day 3 in Dog 1 and Dog 2, respectively (Figure 3-5 a, b.). The peritoneal inflammation resolved rapidly after discontinuing the infusion of intraperitoneal nutrition, with the TWCC declining to 0.8 x 10^9/l in Dog 1 and 1.6 x

10^9/l in Dog 2, four days after stopping the study. The cytoplasm of both peritoneal neutrophils and large mononuclear cells became highly vacuolated by Day 3 and persisted throughout the study (Figure 3-6 a, b.). Using a methylene blue-Sudan red stain, the cells were identified as being heavily laden with lipid vacuoles. All direct culture and enrichment broth cultures from both dogs were negative. Figures 3-7. and 3-8. summarises the DPL data from Dog 1 and Dog 2, respectively.

Table 3-7. Total white cell count and differential of the diagnostic peritoneal lavage fluid in Dog 1 and Dog 2.

		Day 1	Day 3	Day 5	Day 7
TWCC (x 10^9/l)	Dog 1	0.90	8.60	36.50	26.40
	Dog 2	0.60	19.60	21.40	56.00
Neuts (x 10^9/l)	Dog 1	0.42	8.47	33.40	24.63
	Dog 2	0.22	19.4	20.59	44.13
Large Mono (x 10^9/l)	Dog 1	0.32	0.13	3.10	1.72
	Dog 2	0.19	0.30	0.81	11.87
Lymph (x 10^9/l)	Dog 1	0.08	0	0	0.05
	Dog 2	0.11	0	0	0
Eosin (x 10^9/l)	Dog 1	0.07	0	0	0
	Dog 2	0.08	0	0	0

TWCC = total white cell count, Neuts = neutrophils, Large Mono = large mononuclear cells, Lymph = lymphocytes, Eosin = eosinophils.

Figure 3-5. Cytological appearance of the DPL fluid on Day 3 of the study (a) Dog 1 (b) Dog 2. Note the predominantly neutrophilic appearance.

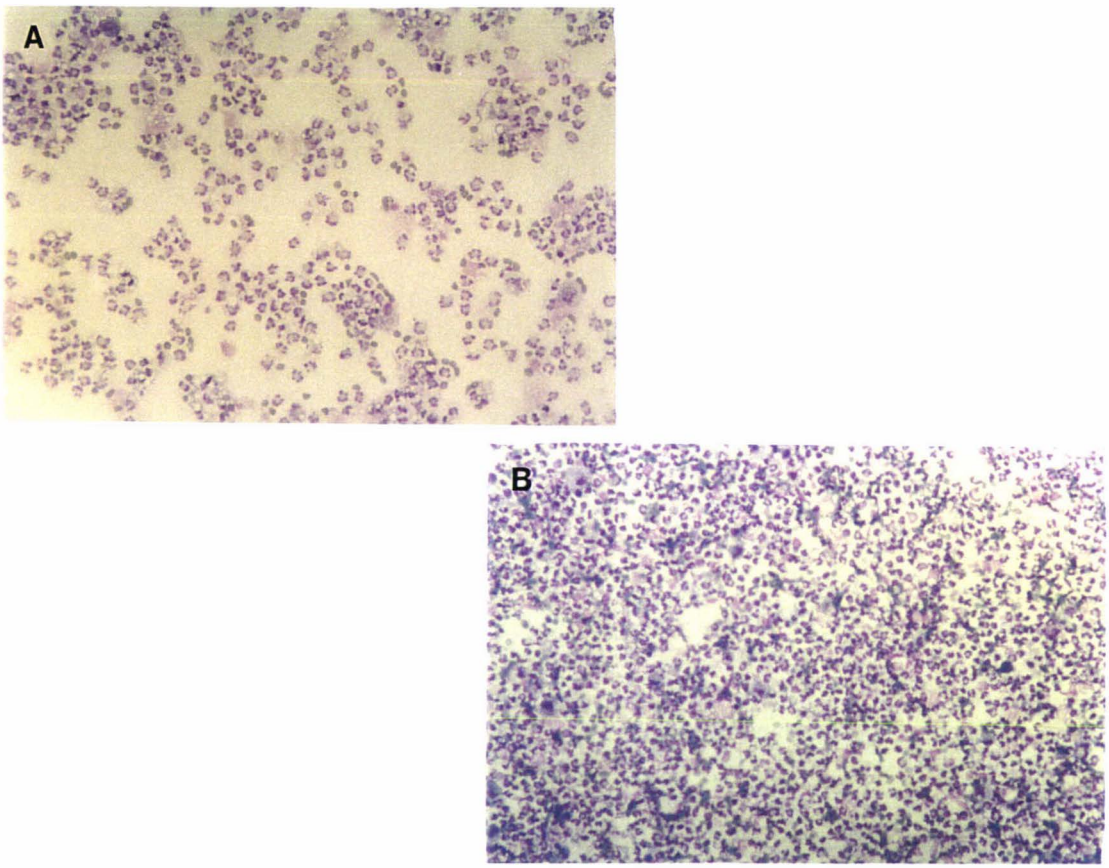


Figure 3-6. Lipid-laden mononuclear cells (open arrows) and neutrophils (small arrows) seen in the DPL fluid on Day 7 (a) Dog 1 (b) Dog 2.

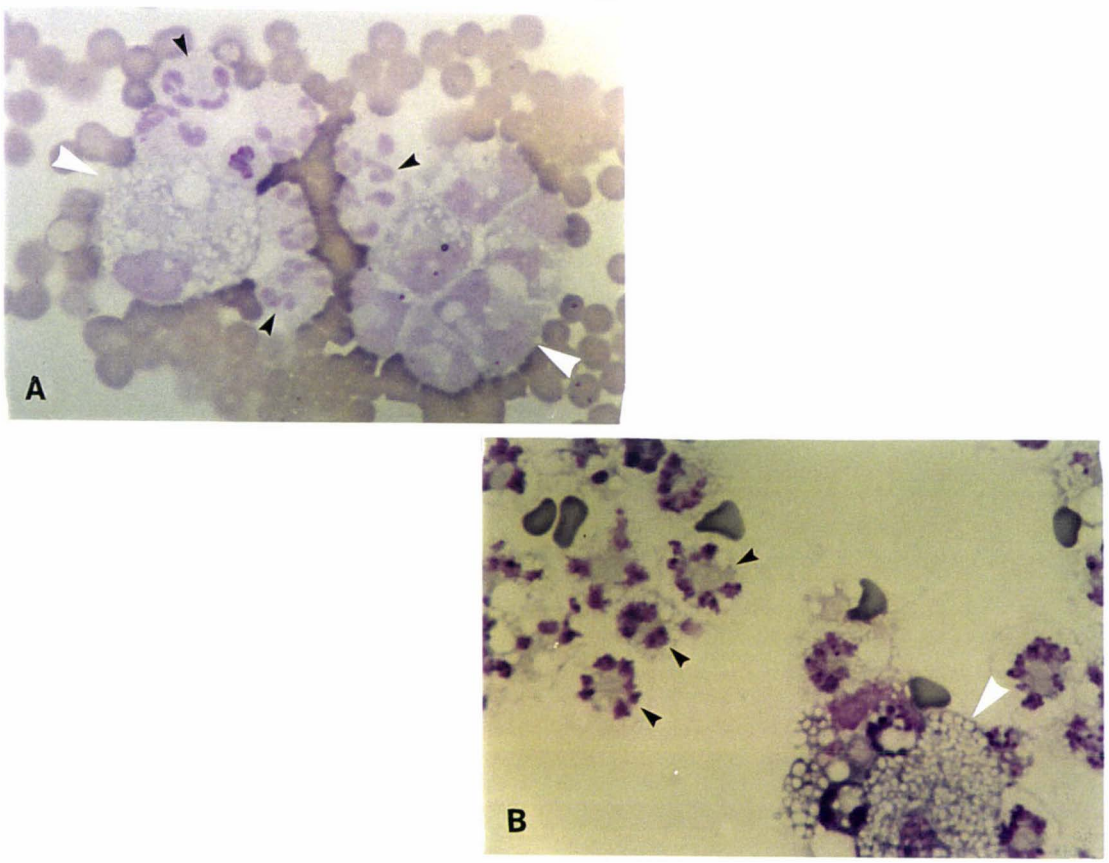
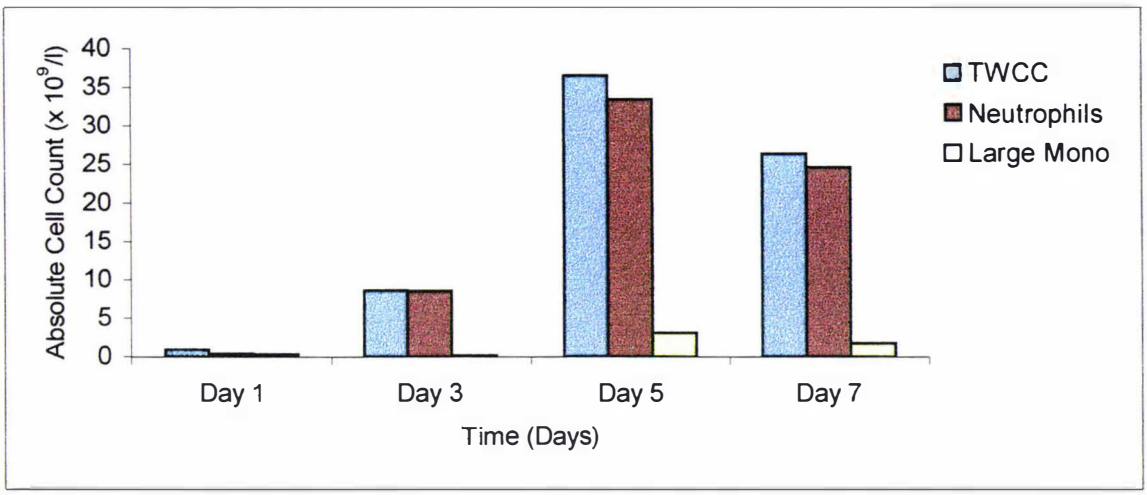
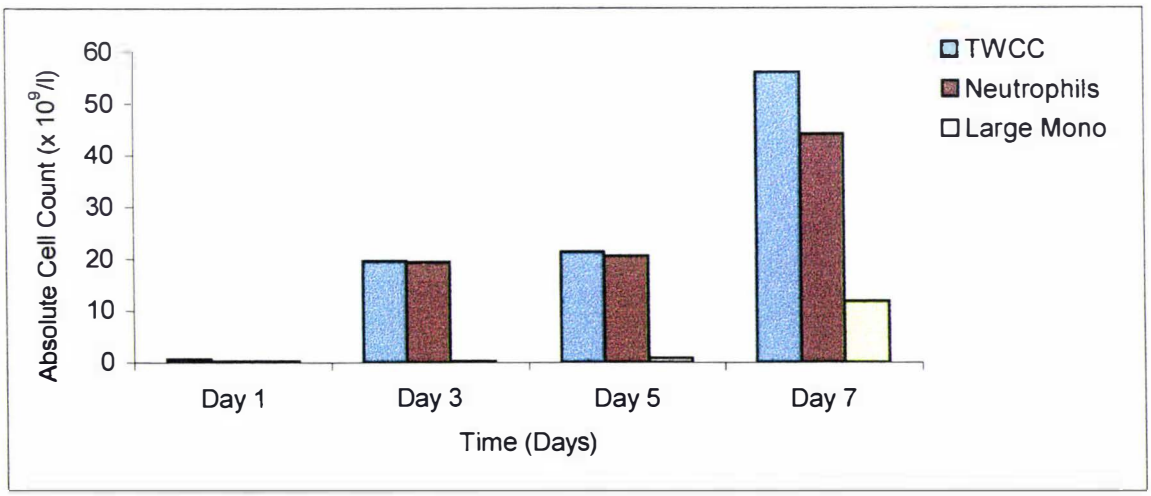


Figure 3-7. Comparison of the total white cell count, neutrophil count and large mononuclear cell counts on Days 1, 3, 5 and 7 in Dog 1.



TWCC = total white cell count, Large Mono = large mononuclear cells.

Figure 3-8. Comparison of the total white cell count, neutrophil count and large mononuclear cell counts on Days 1, 3, 5 and 7 in Dog 2.



TWCC = total white cell count, Large Mono = large mononuclear cells.

DISCUSSION

Two previous studies of intraperitoneal nutrition in dogs did not report significant adverse reactions using a TNA of similar composition to that used in this study.[143,144] In contrast, the dogs in this pilot study experienced marked complications. Although not identical, the TNA in these studies were of very similar composition with both containing a lipid emulsion.

The aetiology of the vomiting after the administration of intraperitoneal nutrients is probably multifactorial. Stimulation of the chemoreceptor trigger zone or vomiting centre by high serum concentrations of glucose, triglycerides and amino acids; pain; stimulation of stretch receptors in the peritoneal membrane as a consequence of a large intraperitoneal (IP) bolus; and rapid changes in serum osmolality may all have potential roles.

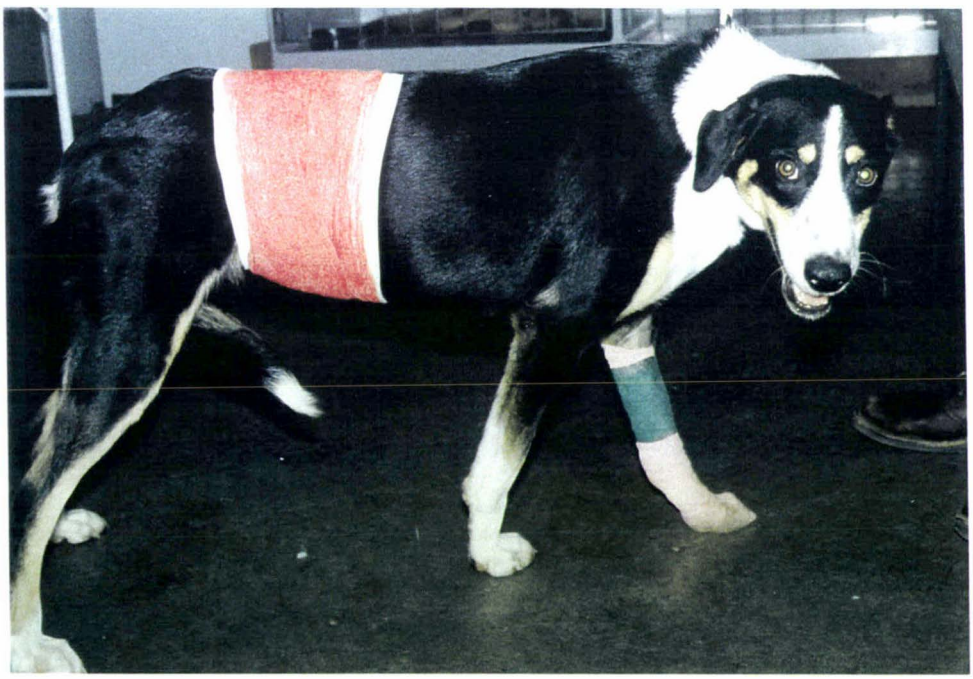
The rapid development of abdominal discomfort after nutrient infusion most likely occurred as a consequence of the hypertonicity of the TNA infused into the peritoneal cavity. Studies in people with chronic renal failure who are being treated with peritoneal dialysis have identified that abdominal discomfort is associated with dialysis solutions with an osmolality greater than 500 mOsmol/l.[27] The maximum tolerable osmolality in dogs was unknown prior to this pilot study. However, previous intraperitoneal nutrition studies in dogs have not reported adverse effects when using nutritional solutions with osmolalities up to 1200 mOsmol/l.[143,144] It is clear from the results of this study that dogs do not tolerate intraperitoneal infusion of a nutritional admixture with an osmolality of 835 mOsmol/l. The discordance in results when comparing these studies is difficult to explain. Both studies used bolus administration of large volumes of hypertonic nutritional solutions, although the previous studies divided the total volume into three boluses, as opposed to two in this study.

The speed with which the TNA was administered into the peritoneal cavity, the high osmolality of the nutritional solution and the use of bolus administration could have contributed to the rapid fluid shifts from the vascular space into the peritoneal cavity and the development of hypovolaemic shock. The acute peritoneal inflammatory response

induced by the nutritional solution may have exacerbated the fluid shifts by increasing the "permeability" of the peritoneal membrane.

Two further complications of using large bolus infusions into the peritoneal cavity that were encountered included dyspnoea and leakage of fluid into the subcutaneous tissue through the site of peritoneal puncture. Dyspnoea occurred in Dog 1 on Day 6 after the second 1000 ml bolus had been given that evening. The dyspnoea improved dramatically after the TNA was withdrawn from the peritoneal cavity, indicating that physical obstruction to the movement of the diaphragm was the most likely cause of the dyspnoea. Leakage of fluid into the surrounding subcutaneous tissue occurred despite application of pressure over the peritoneal puncture site for several minutes following the end of each infusion. There were no direct consequences of the leakage and the problem was prevented after subsequent infusions by applying an abdominal pressure bandage at the completion of the nutrient administration, which remained in place for approximately 60 minutes (Figure 3-9.).

Figure 3-9. Abdominal wrap bandage to minimise subcutaneous leakage of nutrient infusion from the peritoneal cavity.



The aetiology of the mild pleural effusion identified in Dog 1 on Day 6 was not specifically identified. It is probable; however, that this occurred for similar reasons to the pleural effusion that develops in some dogs with severe acute pancreatitis.[150] That is, inflammatory mediators (absorbed from the peritoneal cavity through the lymphatics of the diaphragm, para-sternal lymphatics and sternal lymph nodes) cause lymphangitis. The nodes may act as a barrier to markedly increased lymphatic flow,[118] and the combination of increased lymphatic flow and lymphangitis results in a modified transudate accumulating in the pleural space.

The elevated SUN and creatinine concentrations seen on Day 7 were most likely attributable to the fluid shifts from the vascular space into the peritoneal cavity and the development of pre-renal azotaemia. This explanation is supported by the values returning to normal following aggressive fluid therapy. Both dogs experienced a modest increase in serum ALP, total bilirubin and cholesterol concentration. These findings suggest a degree of cholestasis, the cause of which remains obscure. The changes in the serum chemistry profile are common in people being treated with intravenous total parenteral nutrition[100] but have not been documented in animals undergoing IP nutrition. Hepatocyte swelling as a consequence of fatty microvacuolation may be a possible explanation.

Hypoglycaemia, documented in Dog 1 and Dog 2 on two occasions, has not been previously reported in association with IP nutrition. In fact, systemic hyperglycaemia is more commonly recognised.[27,122,127,128,129,138,139,142,143] The markedly increased serum insulin concentration in Dog 2 of 278.22 μU/l present concomitantly with a serum glucose concentration of 2.63 mmol/l indicates that there may have been a prior overwhelming stimulus for insulin secretion due to the rapid absorption of IP glucose, or lack of negative feedback on the secretory activity of the endocrine pancreas. Work in rats has identified that the afferent branch of the hepatic vagus nerve transmits information to the lateral hypothalamus that can result in increases in pancreatic insulin secretion.[92] Stimulation of glucose receptors in the liver (in response to high portal glucose concentrations) decreases the afferent input to the hypothalamus. This results in dis-inhibition of efferent fibres travelling to the pancreatic beta cells by way of the vagus

nerve (including the celiac branch) and increases the serum insulin concentration. The portal glucose concentration is inversely proportional to the afferent hepatic vagal activity. Interestingly, IP injection of glucose in rats causes a much greater increase in serum insulin concentration compared to an intravenous glucose injection.[92] These findings may help explain why hypoglycaemia occurred in these dogs.

Hypoproteinaemia, in particular, hypoalbuminaemia was prominent by Day 7 in both dogs. This has been a consistent finding in animals being fed via the peritoneal cavity.[138,143,144,145] The aetiology has been poorly defined but is probably multifactorial. Loss of albumin (both intravascular and interstitial) into the peritoneal cavity due to movement along a concentration gradient would seem the most plausible explanation for the reduced serum protein concentrations. Peritoneal inflammation, as a consequence of chemical irritation from the nutritional solutions or peritoneal sepsis, would be expected to exacerbate protein loss into the peritoneal cavity. It has been clearly shown that including amino acids in the nutritional admixture reduces the severity of hypoproteinaemia but does not prevent it from occurring.[130,138,148] However, insufficient amounts of protein supplied to some experimental animals would have played a role in the development of hypoproteinaemia in those studies.[143,144] In the present study, protein was given at 1.5 g/kg/day, which is considered the absolute minimum daily protein requirement for adult dogs.[146] The rapid decline in serum albumin concentration, as opposed to the long serum half-life of albumin in dogs (approximately 8 days), as well as the greater percentage decline in albumin versus globulins, supports the hypothesis that loss of albumin in the peritoneal fluid ("third space") plays a significant role in the hypoproteinaemia. Additional factors involved in the aetiology may include over-hydration: leading to dilutional hypoproteinaemia and protein loss into the peritoneal cavity concomitantly with blood during catheter placement and repeated peritoneal puncture.

Low serum sodium concentrations on Day 7 in Dog 1, and Days 5 and 7 in Dog 2 may have been due to the rapid and large volume fluid shifts from the vascular space into the peritoneal cavity. The rapid movement of water across the peritoneum drags sodium along with it, a phenomenon known as "sodium-sieving".[120] Two possible explanations

exist for the hyperphosphataemia documented on Day 5 and 7 in both dogs. A phosphate-containing lipid emulsion was used as part of the TNA as well as phosphate salts in the amino acid solution. Rapid absorption from the peritoneal cavity may have led to an increase in serum concentrations. The pre-renal azotaemia would also have contributed to the problem.

The short-term reduction in urine specific gravity on Days 3 and 5 in both dogs most likely represents a normal physiological adaptation to volume overload, cause by the infusion of a large volume of intraperitoneal fluid.

The most striking feature of the DPL fluid assessment was the marked inflammatory response seen as early as Day 3. The aetiology of the inflammation cannot be definitively attributed to one of the nutritional components of the total nutrient admixture in particular, although suspicion must fall on a lipid-induced peritonitis. One study in rats has documented an intense inflammatory response by the peritoneal membrane to lipid.[140]

Another feature of the DPL cytology was the presence of large mononuclear cells and neutrophils heavily laden with lipid vacuoles. The presence of lipid vacuoles in neutrophils was surprising and probably reflects the overwhelming amount of lipid that the peritoneal mononuclear cells had to contend with. Neutrophils would not normally be expected to phagocytose this type of material but are capable of doing so.[151] It appears that some component of the lipid emulsion attracts large numbers of neutrophils into the peritoneal cavity and stimulates a phagocytic reaction by both neutrophils and large mononuclear cells.

SUMMARY AND CONCLUSIONS

This pilot study has revealed that dogs are unable to tolerate intraperitoneal bolus feeding of a total nutrient admixture containing 500 ml of 50% dextrose, 500 ml of 8% amino acids and 250 ml of 20% lipid, with an osmolality of 835 mOsmol/l in quantities sufficient to meet their daily nutritional requirements.

The severe peritoneal inflammation, hypovolaemia, metabolic complications, pain and inability to provide total daily caloric requirements to dogs in this study has highlighted several key issues regarding intraperitoneal nutrition. First, more research is required to identify which of the individual nutrients (glucose, amino acids, lipid emulsion) in the TNA was associated with the inflammatory reaction and whether this inflammatory potential is a function of the osmolality or the physical characteristics of the nutrients. Secondly, fluid shifts from the vascular space into the peritoneal cavity cause severe complications when encountered, limiting the osmolality and, with current total nutrient admixtures, the caloric density of the nutritional solutions used. Finally, the two-way movement of solutes (including intact proteins) is problematic in maintaining serum protein concentrations.

Several positive and encouraging findings came from this study. The repeat abdominal puncture technique was well tolerated by the dogs and was considered easy to perform by the author. The use of over-the-needle catheters with three to four additional side holes cut into the catheter using a No. 11 scalpel blade provided adequate drainage when performing DPL's and administering the nutritional solution. With careful skin preparation and the use of clean examination gloves, there were no episodes of septic peritonitis encountered throughout the study period. A total of 28 abdominal punctures were performed during this experiment, indicating that, although the risk of sepsis associated with IPN will always be present, the incidence is low using this technique of administration.

Further studies are required to answer questions that have been raised by this study. These include:- ascertaining the peritoneal cytological response to the individual nutrients that composed the total nutrient admixture in the present study; determine the safety of using individual nutrient solutions with an osmolality significantly reduced from the 835 mOsmol/l used in the pilot study; and to assess how well dogs tolerate intraperitoneal nutrient solutions with this osmolality.

CHAPTER 4.

- A Comparison of Peritoneal Cytology in Dogs Given 10% Dextrose, 5% Amino acids or a 10% Lipid emulsion into the Peritoneal Cavity by Repeat Abdominal Puncture.

  Introduction.

  Objectives.

  Materials and Methods.

  Results.

  Discussion.

  Summary and Conclusions.

A COMPARISON OF PERITONEAL CYTOLOGY IN DOGS GIVEN 10% DEXTROSE, 5% AMINO ACIDS OR A 10% LIPID EMULSION INTO THE PERITONEAL CAVITY BY REPEAT ABDOMINAL PUNCTURE.

INTRODUCTION

Given the inflammatory reaction by the peritoneal membrane to the total nutrient admixture (TNA) used in the pilot study (Chapter 3), we elected to identify which nutrient solution caused this response. By identifying which of the nutrient component(s) is responsible for the peritonitis, we may be able to modify the nutritional composition to avoid this happening in the future. To avoid the significant fluid shifts from the vascular space into the peritoneal cavity noted in the pilot study, we decided to keep the osmolality of the nutrient solutions to less than 600 mOsmol/l and to reduce the total number of osmols administered by restricting the calories provided to 20% of resting energy requirements. Because the peritonitis occurred within a 48 hour period after starting IPN in the pilot study, we elected to administer intraperitoneal nutrients for a total of 4 days in the present study to allow adequate time to identify an inflammatory response. The inflammatory effects of the nutrients were compared on a kcal for kcal basis in this study rather than on an osmol for osmol basis. This decision was made because we were primarily interested in determining the nutrients of most practical value for intraperitoneal nutrition.

OBJECTIVES

The objectives of this study were to:

- quantify the cytological response by the peritoneal membrane to infusion of 10% dextrose, 5% amino acids or a 10% lipid emulsion via a repeat-puncture technique given in sufficient quantities to meet 20% of daily caloric requirements.

- assess the safety of infusing nutrient solutions with an osmolality ranging from 345 mOsmol/l to 505 mOsmol/l given in sufficient quantities to meet 20% of daily caloric requirements with respect to excessive fluid shifts from the extravascular space into the peritoneal cavity.

- assess whether intraperitoneal infusion of nutrients with an osmolality ranging from 345 mOsmol/l to 505 mOsmol/l are associated with abdominal discomfort in dogs.

- identify any haematological or biochemical complications associated with infusion of 10% dextrose, 5% amino acids or a 10% lipid emulsion into the peritoneal cavity of dogs.

MATERIALS AND METHODS

Dogs

Twelve healthy adult Border collie-cross dogs were used for this study with a mean bodyweight (BW) of 23.9 kg (range 18.9 kg to 31.5 kg). The dogs were housed in individual cages and were allowed out twice daily to urinate and defecate. The dogs had access to water ad-libitum throughout the study.

Nutrient solutions

The dogs were randomly assigned to three groups. Group One (n = 4) received 20% of resting energy requirements (RER = [30 x BW (kg)] + 70) (kcal/day) in the form of a 10% dextrose solution.[dd] Group Two (n = 4) received 20% of RER in the form of a 5% amino acid solution[ee] and Group Three (n = 4) received 20% of RER from a 10% lipid emulsion.[ff] The volume of fluid administered to each group on Day 1 to 4 is shown in Table 4-1. All nutrients were commercially prepared under sterile conditions and packaged into individual single-dose flexi-packs or syringes (lipid). The osmolality of the dextrose, amino acids and lipid emulsion, were 505 mOsmol/l, 475 mOsmol/l and 345 mOsmol/l, respectively. The remaining daily caloric intake was provided orally in the form of dry dog food.[gg]

[dd] Hypertonic Dextrose Injections USP - McGaw Inc. CA, USA.
[ee] 10% Freamine® III. B.Braun Medical Inc. CA, USA.
[ff] Intralipid®. Kabi Pharmacia. Stockholm, Sweden.
[gg] Eukanuba®. The Original Premium. The IAMS Company. Ohio, USA.

Table 4-1. Volume of nutrient solution infused in each group on Day 1 - 4.

	Dextrose	Amino Acid	Lipid
ml/kg	19.5	30.4	6.8

Intraperitoneal administration and diagnostic peritoneal lavage (DPL)

The intraperitoneal nutrients were given once daily for a total of four days via a repeat abdominal puncture technique. The dogs were prepared for intraperitoneal infusions and the catheter inserted into the peritoneal cavity in an identical manner to the dogs used in Chapter 3. A 12 G over-the-needle catheter[hh] with three to four side holes (cut using a No. 11 scalpel blade) was used for the DPL and nutrient infusions. During the DPL and nutrient infusions the dogs were manually restrained in dorsal recumbency. Dogs that did not tolerate manual restraint were given light sedation using intravenous xylazine[ii] at a dose rate of between 0.1 to 0.3 mg/kg to facilitate the procedures. There was no bias as to the number of dogs in each group that received xylazine sedation. Xylazine was chosen because of its predictable level and duration of sedation and cost-effectiveness.

Experimental time course and monitoring

On Day 1 a baseline complete blood count (CBC), serum chemistry panel with electrolytes and DPL were performed. The DPL was performed using 25 ml/kg of warmed lactated Ringer's solution.[jj] A 10 ml sample of DPL fluid was collected and analysed for total white cell count (TWCC), differential white cell count and morphological assessment of cells. The remaining lavage fluid was left within the peritoneal cavity. Following the DPL, the dogs were given their appropriate nutrient solution using gravity-flow over a ten-minute period. At the completion of the infusion

[hh] Angiocath™. Becton Dickinson Infusion Therapy systems Inc. Utah, USA.
[ii] Xylazine 2%. Phoenix Pharm Distributors Ltd. Auckland, New Zealand.
jj Hartmans Solution. Baxter Healthcare PTY Ltd. NSW, Australia.

On Days 2, 3 and 4, the DPL and nutrient infusions were repeated. On Day 5, the DPL was repeated as well as the CBC and serum chemistry panel with electrolytes (Table 4-2.). Twice daily the dogs' temperature, respiration rate, heart rate, mucous membrane colour, capillary refill time, pulse pressure and general demeanour were assessed. In addition, behavioural and postural changes were assessed and the abdomen was palpated thoroughly to detect the presence of abdominal discomfort.

Analysis of samples

Analysis of the CBC, serum chemistry panel and DPL fluid were carried out in an identical manner to that described in Chapter 3.

Statistics

Repeated measures ANOVA was used to compare the mean values between the three treatment groups on Day 1 and Day 5; the mean change between Day 1 and Day 5 within each group; and the mean change between Day 1 and Day 5 for all 12 dogs for the following parameters: bodyweight; CBC (RBC count, packed cell volume, haemoglobin, white cell count, neutrophils and total protein; serum chemistry panel (urea, creatinine, lipase, alkaline phosphatase (ALP), alanine amino transferase (ALT), creatine kinase (CK), glucose, cholesterol, total protein, albumin, globulin, total bilirubin, sodium, potassium, calcium and phosphorus; DPL fluid: (TWCC, neutrophils and large mononuclear cells). For the purposes of the statistical analysis of the DPL fluid the data were log-transformed due to the wide variation within the treatment groups. All results are tabulated as the mean ± the standard deviation (s.d.). Statistical significance was set at $p \leq 0.05$.

Table 4-2. Schematic representation of experimental protocol.

	Day 1	Day 2	Day 3	Day 4	Day 5
IPN	✓	✓	✓	✓	
DPL	✓	✓	✓	✓	✓
CBC	✓				✓
Chem	✓				✓

IPN: intraperitoneal nutrients, DPL: diagnostic peritoneal lavage, CBC: complete blood count, Chem: serum chemistry panel with electrolytes.

RESULTS

All twelve dogs remained bright and alert throughout the study period and maintained a normal appetite. One dog in the lipid treatment group experienced a single episode of an elevated body temperature (39.8°C) on Day 4 that was associated with a DPL TWCC of 13.9 x 10^9/l, 91% of which, were non-degenerate neutrophils. The elevated body temperature spontaneously resolved the following morning and did not recur. There was no evidence of abdominal pain and the dog remained bright and alert during this episode. No other clinically significant changes were detected in the physical parameters assessed throughout the study. Although there were no clinically significant fluid shifts from the vascular space into the peritoneal cavity in any of the dogs, residual peritoneal fluid was palpable on the fifth morning of the study. The fluid wave was palpable in the dogs that received dextrose or amino acids but not in the dogs receiving the lipid emulsion.

Bodyweight

The dogs in the dextrose group (28.25 ± 2.66 kg) started the study significantly ($p = 0.005$) heavier than the dogs receiving the amino acid solution (23.65 ± 3.07 kg) and lipid emulsion (19.95 ± 2.03 kg). The changes in bodyweight throughout the study were not

significantly different between the groups but taken across the three treatment groups (all 12 dogs), there was a marginal (p= 0.08) tendency to gain weight by Day 5 (Table 4-3.).

Table 4-3. Day 1 versus Day 5 bodyweights in the dextrose, amino acid and lipid groups.

		Dextrose Mean	s.d.	Amino acid Mean	s.d.	Lipid Mean	s.d.
Bodyweight	Day 1	28.25^{λ}	2.66	23.65	3.07	19.95	2.03
(kg)	Day 5	28.75^{Φ}	4.44	28.88^{Φ}	3.28	20.25^{Φ}	1.89

s.d. = standard deviation, $^{\lambda}$ = significantly different (p= 0.005) on day 1, $^{\Phi}$ = marginally different (p= 0.08) from day 1.

Complete blood count (CBC)

The dogs in all groups had a significant (p= 0.008) reduction in RBC count by Day 5. Dogs in all the treatment groups had a significant (p = 0.01) reduction in haemoglobin and packed cell volume by Day 5. There were no significant differences between the groups on Day 1 and Day 5 with respect to the above parameters (Table 4-4.).

Serum chemistry panel and electrolytes

The dogs receiving the amino acid solution and lipid emulsion experienced a significant (p= 0.032) reduction in serum urea nitrogen concentration by Day 5. However, no significant differences between the groups could be detected on Day 1 and Day 5 for this analyte. There was a statistically (p= 0.04) but not clinically significant decrease in serum creatine kinase concentration from 137 ± 49.93 IU to 67.25 ± 39.18 IU by Day 5 in the lipid group. The serum glucose concentration increased marginally (p= 0.08) from 4.83 ± 0.61 mmol/l to 5.50 ± 0.14 mmol/l by Day 5 in the amino acid group, whereas no significant changes were seen in the dextrose or lipid groups. There was a significant reduction in total protein (p= 0.035) and albumin (p= 0.005) concentrations by Day 5 in all three treatment groups, although a significant difference between the groups could not be detected for this analyte. Dogs in the dextrose group started the study with a marginally (p= 0.055) higher globulin concentration (38.30 ± 3.54 g/l). However, during the course of the study, the globulin concentration did not change significantly within or

between the treatment groups. A marginal (p= 0.083) reduction in serum sodium concentration occurred in all three treatment groups by Day 5. The serum total calcium concentration declined significantly (p= 0.003) by Day 5 in the dextrose (2.54 ± 0.04 mmol/l), amino acid (2.65 ± 0.22 mmol/l) and lipid group (2.62 ± 0.18 mmol/l). No significant differences could be detected for the remaining parameters studied (Table 4-5.).

DPL fluid analysis

In the present study it was noted that if insertion of the DPL catheter was slightly off the midline, abdominal muscle wall trauma led to gross haemorrhagic discolouration of the DPL fluid that was retrieved (Figure 4-1.). As the author became more experienced with inserting the DPL catheter blood contamination was rarely encountered. The mean total white cell count (TWCC) for the dextrose group did not change significantly from baseline throughout the study period (Table 4-6.). The lipid group had a significantly ($p < 0.05$) higher TWCC than the dextrose group on Days 2, 3, 4 and 5, and was also significantly ($p < 0.05$) higher than the amino acid group on Days 2 and 4. On Day 5, all three TWCC group means were significantly ($p = 0.05$) different, with the amino acid group being greater than that of the dextrose group, and the lipid group being higher than the amino acid group (Figure 4-2. and 4-3.).

The mean neutrophil count in the dextrose group increased significantly ($p = 0.05$) above baseline on Days 2, 3 and 4 but had declined by Day 5. The mean neutrophil count in the amino acid group was significantly ($p = 0.05$) greater than baseline on Days 3, 4 and 5. On Days 2, 3, 4 and 5 the mean neutrophil count of the lipid group was significantly ($p = 0.05$) greater than baseline, and that of the dextrose group. On Days 2 and 4, the mean neutrophil count of the lipid group was higher than that of the amino acid group. The mean neutrophil counts of all three groups were significantly ($p = 0.05$) different on Day 5 (Figures 4-4. and 4-5.).

The mean large-mononuclear cell count (LMCC) of the three treatment groups underwent a different pattern of change throughout the study compared to the TWCC and neutrophil counts. On Day 2, the mean LMCC for the lipid group was significantly

($p = 0.05$) higher than baseline and was greater than both the dextrose and amino acid mean values ($p < 0.05$). On Day 3, there were no significant differences between the three treatment groups. On Day 4, the lipid group mean was significantly ($p = 0.05$) greater than the mean of the dextrose group but not greater than that of the amino acid group. Finally, on Day 5 the mean of the lipid and amino acid group was significantly greater ($p = 0.05$) than the dextrose mean. (Figures 4-6. and 4-7.)

Table 4-4. Day 1 versus Day 5 values for CBC in the dextrose, amino acid and lipid groups.

		Dextrose Mean	s.d.	Amino acid Mean	s.d.	Lipid Mean	s.d.	Reference Range
HB (g/l)	Day 1	157.00	3.74	171.00	16.15	173.00	11.52	120-180
	Day 5	147.50#	6.81	163.75#	17.76	148.75#	26.95	
PCV (l/l)	Day 1	0.45	0.01	0.49	0.04	0.48	0.04	0.37-0.55
	Day 5	0.41#	0.02	0.45#	0.06	0.43#	0.08	
RBC (x 10^{12}/l)	Day 1	6.37	0.25	6.74	0.47	6.55	0.39	5.8 - 8.5
	Day 5	5.76Ψ	0.35	6.22Ψ	0.73	5.82Ψ	0.90	
MCV (fl)	Day 1	70.25	0.96	72.50	3.59	73.50	1.91	60 - 77
	Day 5	72.25	0.96	72.25	3.70	73.50	3.11	
MCH (g/l)	Day 1	24.63	0.62	25.30	1.22	26.38	0.54	None
	Day 5	25.58	0.60	26.33	0.93	25.40	1.11	
MCHC (g/l)	Day 1	350.25	4.92	350.75	2.99	359.25	6.75	310 - 340
	Day 5	354.50	3.11	364.75	33.28	346.50	6.45	
PLT (x 10^9/l)	Day 1	284.25	35.50	275.50	45.27	247.25	63.03	200 - 900
	Day 5	291.00	29.68	264.75	20.90	217.25	43.12	
WBC (x 10^9/l)	Day 1	10.80	1.82	9.28	1.62	10.30	2.04	6.0 - 15.0
	Day 5	9.65	0.85	8.43	1.77	11.75	3.31	
Neuts (x 10^9/l)	Day 1	5.83	1.05	5.08	1.09	5.20	1.12	3.6 - 11.5
	Day 5	5.28	1.36	4.18	1.44	7.05	4.11	
Bands (x 10^9/l)	Day 1	0.00	0.00	0.03	0.05	0.00	0.00	0.00 - 0.54
	Day 5	0.25	0.50	0.02	0.05	0.00	0.00	
Lymph (x 10^9/l)	Day 1	2.85	0.95	2.63	0.34	3.05	1.19	1.0 - 4.8
	Day 5	2.40	1.12	2.55	0.39	2.70	0.73	
Mono (x 10^9/l)	Day 1	1.18	0.29	0.46	0.20	0.53	0.27	0.18 - 1.5
	Day 5	0.73	0.33	0.43	0.25	0.28	0.22	
Eosin (x 10^9/l)	Day 1	0.98	0.61	1.14	0.68	1.55	1.19	0.12 - 1.5
	Day 5	1.19	0.57	1.25	0.75	1.74	1.50	
Baso (x 10^9/l)	Day 1	0.02	0.05	0.03	0.05	0.03	0.05	0
	Day 5	0.10	0.14	0.00	0.00	0.03	0.06	

s.d. = Standard deviation, HB = haemoglobin, PCV = packed cell volume, RBC = red blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, Retics = reticulocytes, PLT = platelets, WBC = white blood cells, Neuts = mature neutrophils, Bands = immature neutrophils, Lymph = lymphocytes, Mono = monocytes, Eosin = eosinophils, baso = basophils. Ψ = significantly different (p= 0.008) from day 1. # = significantly different (p = 0.01) from Day 1.

Table 4-5. Day 1 versus Day 5 values for the serum chemistry panel and electrolytes values in the dextrose, amino acid and lipid groups.

		Dextrose Mean	s.d.	Amino acid Mean	s.d.	Lipid Mean	s.d.	Reference Range
Urea (mmol/l)	Day 1	5.93	1.27	8.21	2.30	6.60	1.53	2.5 - 8.4
	Day 5	6.30	1.22	5.86$^{\Psi}$	1.73	3.22$^{\Psi}$	0.70	
Creatinine (µmol/l)	Day 1	90.00	3.37	83.75	6.99	84.00	12.96	75 - 138
	Day 5	97.50	6.86	89.00	7.75	79.50	11.45	
Lipase (IU/l)	Day 1	113.00	53.65	140.00	178.67	51.50	48.34	< 500
	Day 5	134.25	66.35	56.50	20.17	54.25	44.33	
AP (IU/l)	Day 1	47.75	12.34	50.75	14.48	48.75	18.89	< 185
	Day 5	43.50	7.51	38.00	6.22	37.00	22.29	
ALT (IU/l)	Day 1	28.25	10.50	44.50	9.85	52.75	34.34	< 58
	Day 5	33.25	8.26	41.00	10.89	46.25	17.08	
CK (IU/l)	Day 1	94.50	24.28	102.00	26.50	137.00	49.93	< 800
	Day 5	74.75	12.53	110.75	37.85	67.25$^{\Psi}$	39.18	
Glucose (mmol/l)	Day 1	5.78	0.17	4.83	0.61	5.45	0.86	3.8 - 5.8
	Day 5	5.48	0.21	5.50$^{\Phi}$	0.14	5.43	0.45	
Cholesterol (mmol/l)	Day 1	5.76	1.42	6.68	0.95	6.32	1.07	3.2 - 9.3
	Day 5	5.29	1.20	5.97	1.59	6.09	0.65	
TP (g/l)	Day 1	71.93	3.55	68.08	5.06	70.05	8.57	62 - 85
	Day 5	66.18$^{\Psi}$	4.04	65.58$^{\Psi}$	10.90	65.33$^{\Psi}$	5.92	
Albumin (g/l)	Day 1	33.63	3.25	34.95	4.50	37.80	6.94	28 - 35
	Day 5	31.00$^{\Psi}$	1.89	33.25$^{\Psi}$	5.93	32.13$^{\Psi}$	3.49	
Globulins (g/l)	Day 1	38.30$^{\Omega}$	3.54	33.10	0.65	32.25	4.31	27 - 57
	Day 5	35.18	4.53	32.33	5.44	33.20	4.21	
A/G	Day 1	0.89	0.14	1.05	0.12	1.19	0.25	None
	Day 5	0.90	0.14	1.03	0.11	0.98	0.14	
T Bilirubin (µmol/l)	Day 1	1.63	0.75	2.55	1.37	1.55	0.74	< 6.0
	Day 5	1.28	0.48	2.18	1.12	1.88	0.85	
Sodium (mmol/l)	Day 1	153.13	2.48	155.58	4.02	158.10	7.07	141 - 155
	Day 5	153.93	2.93	150.70$^{\Phi}$	3.96	150.80$^{\Phi}$	3.23	
Potassium (mmol/l)	Day 1	4.52	0.15	4.17	0.18	4.22	0.38	3.6 - 5.6
	Day 5	4.76	0.68	4.50	0.48	4.21	0.29	
Calcium (mmol/l)	Day 1	2.85	0.22	2.79	0.21	2.76	0.27	2.05 - 3.00
	Day 5	2.54$^{\Psi}$	0.04	2.65$^{\Psi}$	0.22	2.62$^{\Psi}$	0.18	
Phosphate (mmol/l)	Day 1	1.39	0.26	1.38	0.37	1.35	0.24	0.8 - 1.6
	Day 5	1.57	0.24	1.43	0.05	1.56	0.14	

s.d. = standard deviation, ALP = alkaline phosphatase, ALT = alanine aminotransferase, CK = creatine kinase, TP = to tal protein, A/G = albumin to globulin ratio, T Bilirubin = total bilirubin. $^{\Phi}$ = marginally different (p = 0.1) from day 1, $^{\Psi}$ = significantly different (p = 0.05) from day 1, $^{\Omega}$= marginally different (p = 0.1) on day 1.

Cell Morphology

The baseline DPL's consisted of approximately 60 to 70% mature, non-degenerate neutrophils and 30 to 40% large mononuclear cells, with the occasional lymphocyte, eosinophil and peritoneal mesothelial cell seen (Figure 4-8 a, b.). As the study progressed, the percentage of mature neutrophils increased significantly, and by Day 5, constituted approximately 90% of the TWCC. In all three treatment groups the neutrophils remained non-degenerate and developed hyper-segmented, pyknotic nuclei (Figure 4-9.). The large mononuclear cells (LMC) became activated as the study progressed in all treatment groups, especially in the lipid group where the cells contained abundant intracytoplasmic vacuoles (Figure 4-10.).

Table 4-6. Day 1 versus Day 5 values for the DPL fluid total white cell count and differential in the dextrose, amino acid and lipid groups.

		Dextrose Mean	s.d.	Amino acid Mean	s.d.	Lipid Mean	s.d.
TWCC (x 10^9/l)	Day 1	0.10	0.14	0.11	0.13	0.23	0.29
	Day 5	0.34	0.58	1.13^{Σ}	0.57	13.25^{Σ}	19.99
Neuts (x 10^9/l)	Day 1	0.08	0.10	0.08	0.09	0.07	0.13
	Day 5	0.28	0.50	0.82^{Σ}	0.59	12.08^{Σ}	18.45
Large Mono (x 10^9/l)	Day 1	0.03	0.04	0.03	0.04	0.15	0.28
	Day 5	0.04^{Σ}	0.06	0.21^{Σ}	0.20	1.12^{Σ}	1.59
Lymph (x 10^9/l)	Day 1	0.00	0.00	0.00	0.01	0.01	0.02
	Day 5	0.01	0.01	0.09	0.12	0.04	0.05
Eosin (x 10^9/l)	Day 1	0.00	0.00	0.00	0.01	0.01	0.02
	Day 5	0.01	0.01	0.01	0.01	0.01	0.01

s.d. = standard deviation, TWCC = total white cell count, Neuts = neutrophils, Large Mono = large mononuclear cells, Lymph = lymphocytes, Eosin = eosinophils. $^{\Sigma}$ = significantly greater (p = 0.05) than day 1.

Figure 4-1. Diagnostic peritoneal lavage fluid. Note the varying degrees of iatrogenic blood contamination secondary to abdominal wall trauma.

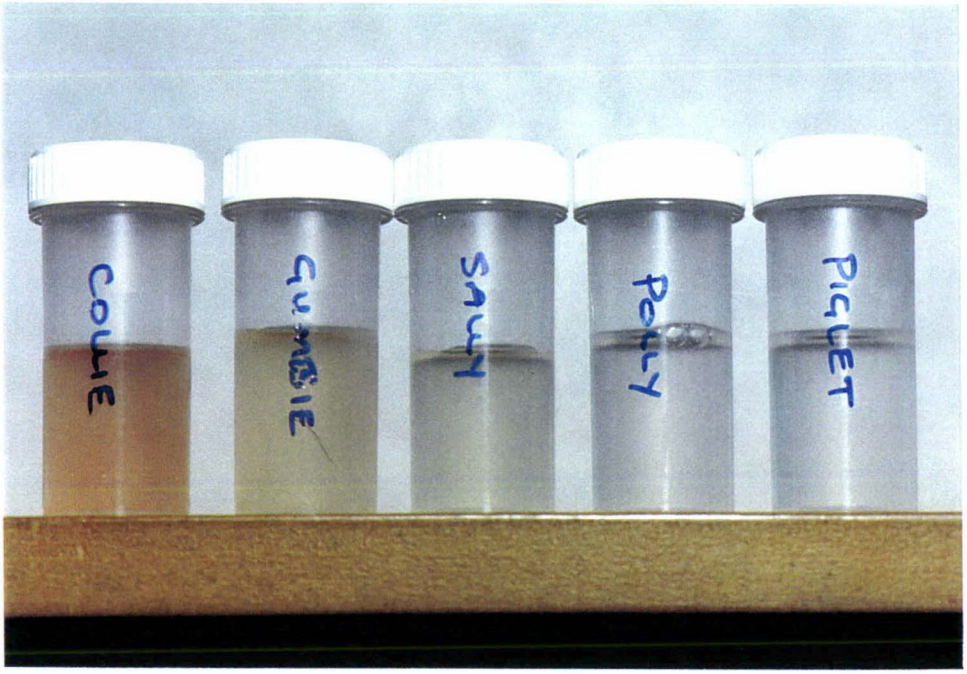
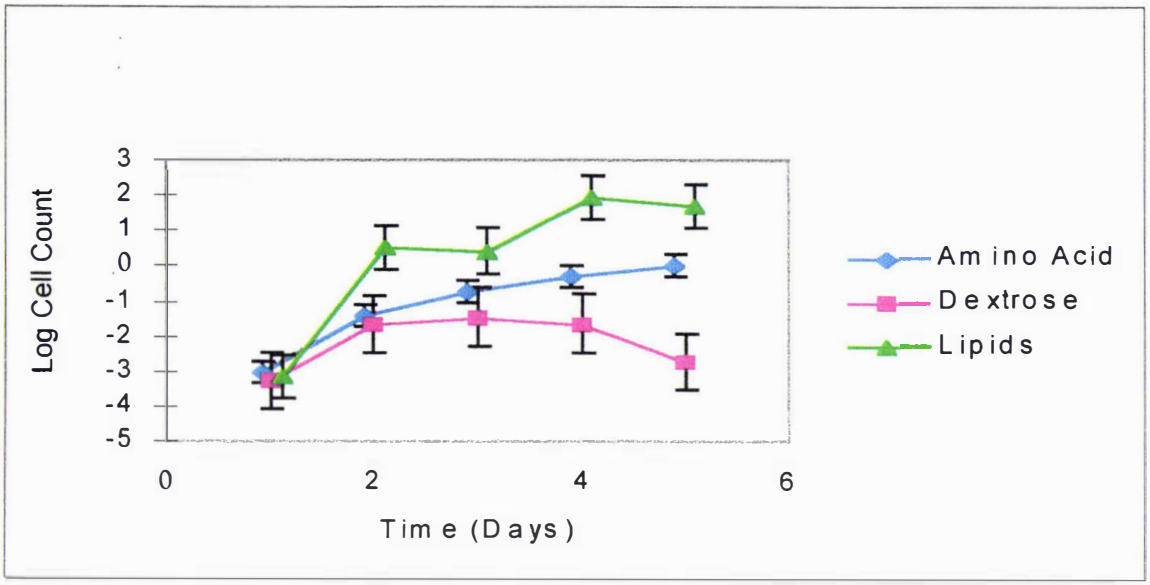
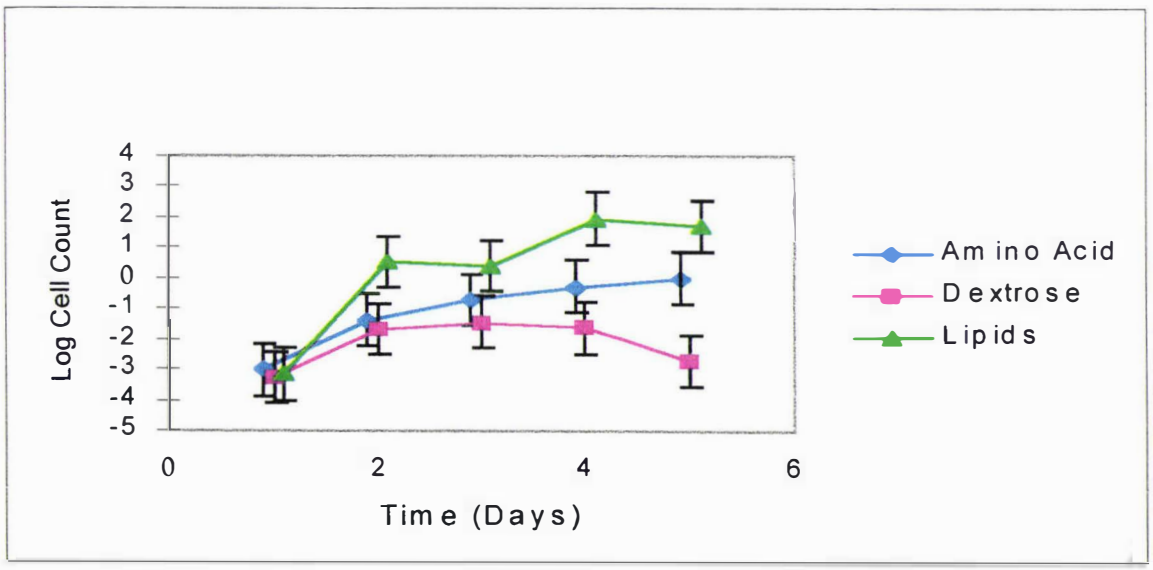


Figure 4-2. Mean peritoneal total white cell count ± SEM vs. time graph in the dextrose, amino acid and lipid groups.



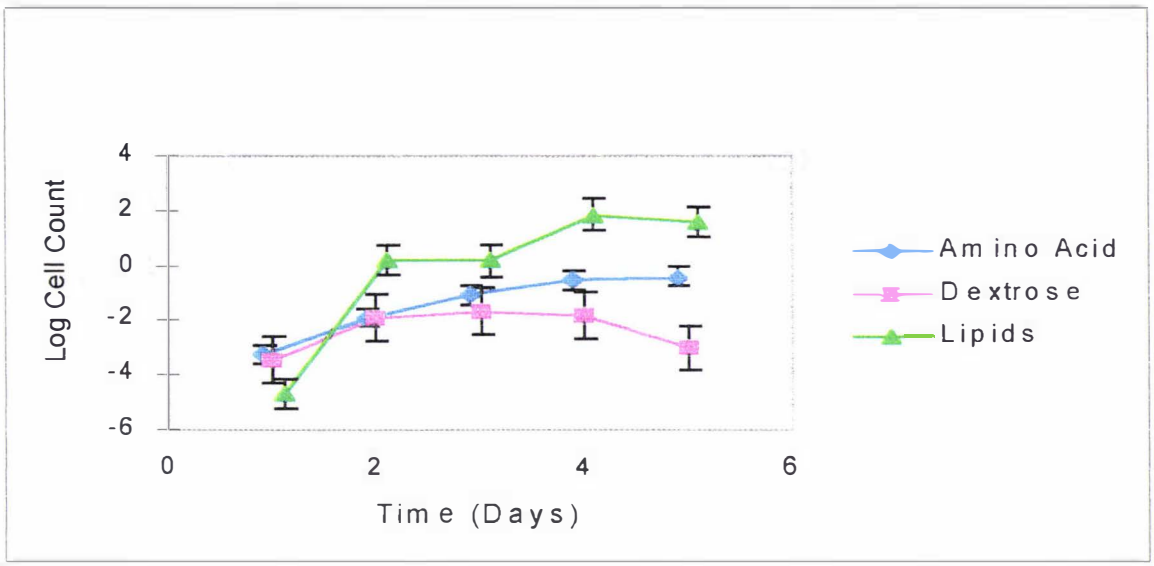
SEM = standard error of the mean.

Figure 4-3. Mean peritoneal total white cell count ± ½ LSD bars vs. time graph in the dextrose, amino acid and lipid groups.



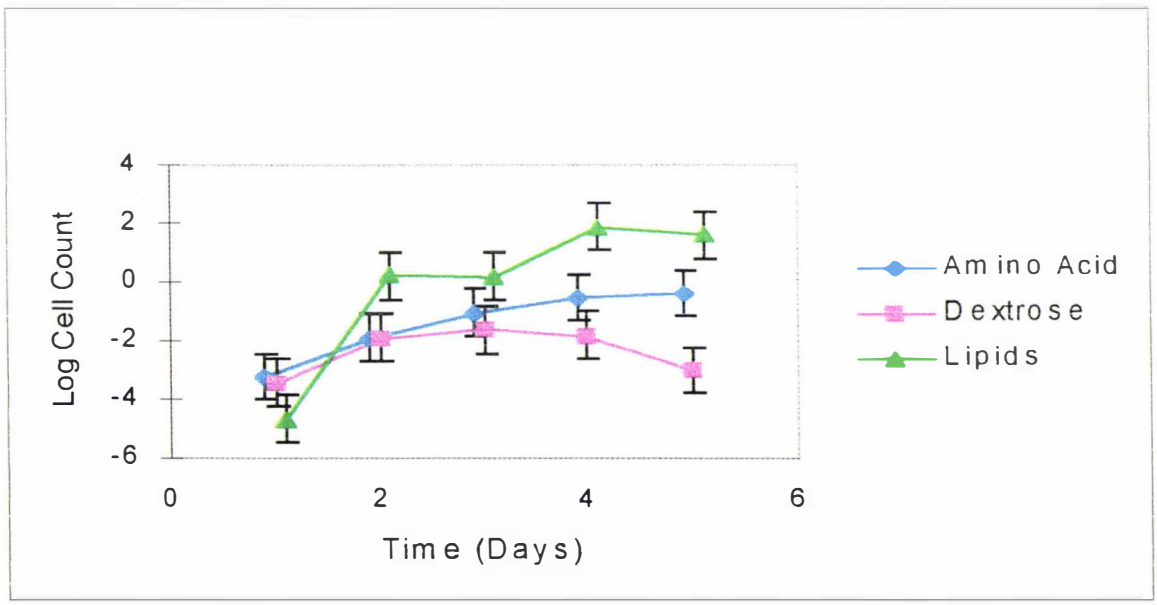
½ LSD = Half the least significant difference. If the ½ LSD bars do not overlap the means are significantly different (p = 0.05).

Figure 4-4. Mean peritoneal neutrophil count ± SEM vs. time graph in the dextrose, amino acid and lipid groups.



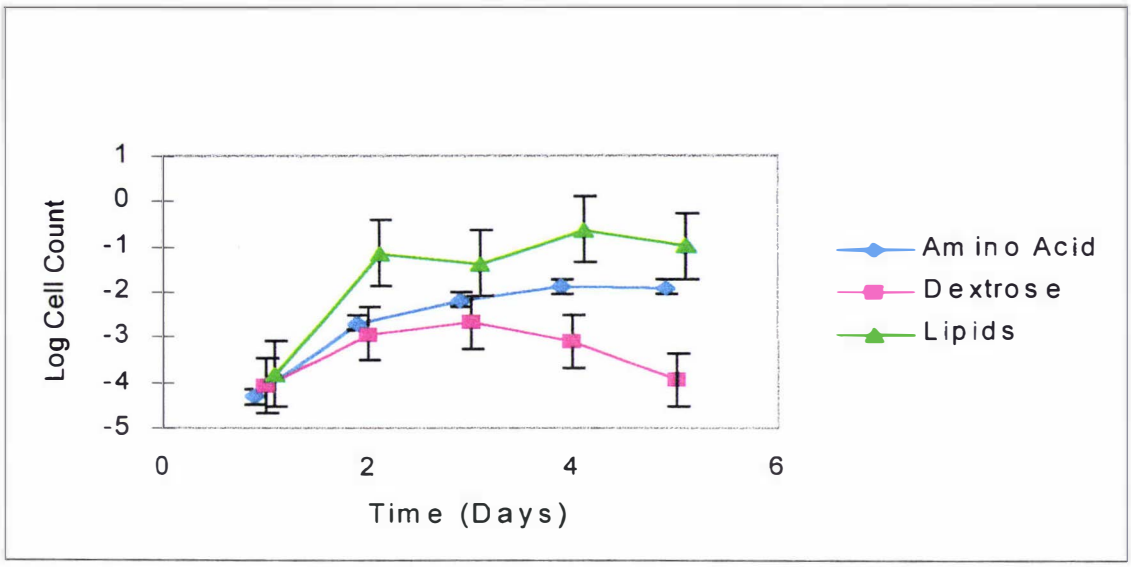
SEM = standard error of the mean.

Figure 4-5. Mean peritoneal neutrophil count ± ½ LSD bars vs. time graph in the dextrose, amino acid and lipid groups.



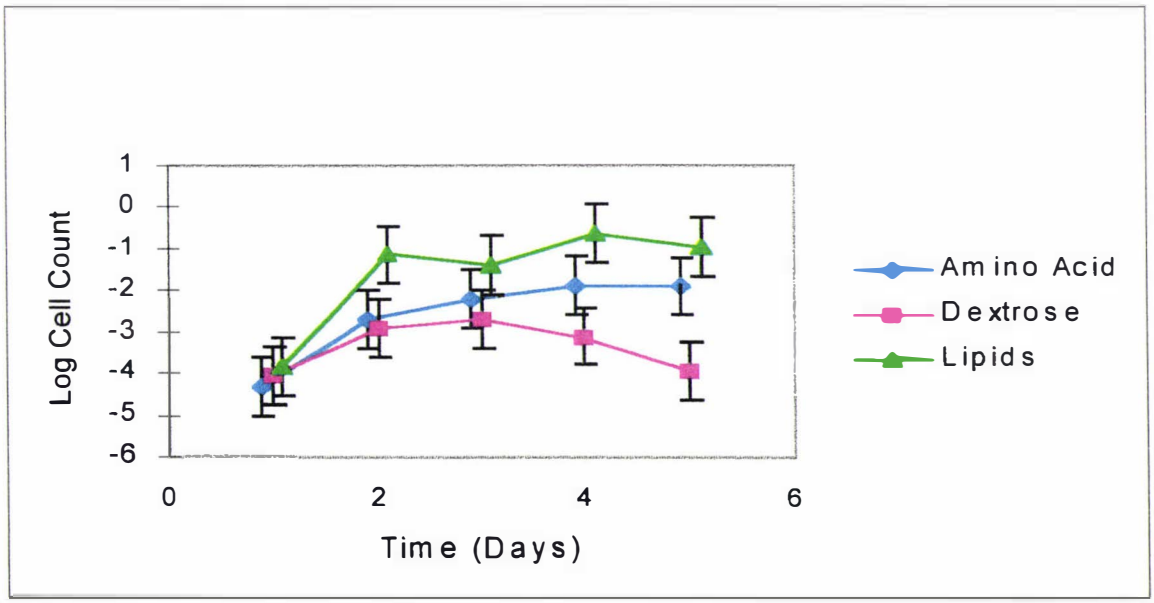
½ LSD = Half the least significant difference. If the ½ LSD bars do not overlap the means are significantly different (p = 0.05).

Figure 4-6. Mean peritoneal large mononuclear cell count ± SEM vs. time graph in the dextrose, amino acid and lipid groups.



SEM = standard error of the mean.

Figure 4-7. Mean peritoneal large mononuclear cell count ± ½ LSD bars vs. time graph in the dextrose, amino acid and lipid groups.



½ LSD = Half the least significant difference. If the ½ LSD bars do not overlap the means are significantly different (p = 0.05).

Figure 4-8. Baseline (Day 1) DPL cytology (a) two large mononuclear cells (open arrows), one non-degenerate polymorph-neutrophil (large arrow) and an eosinophil (small arrow) (b) three peritoneal mesothelial cells.

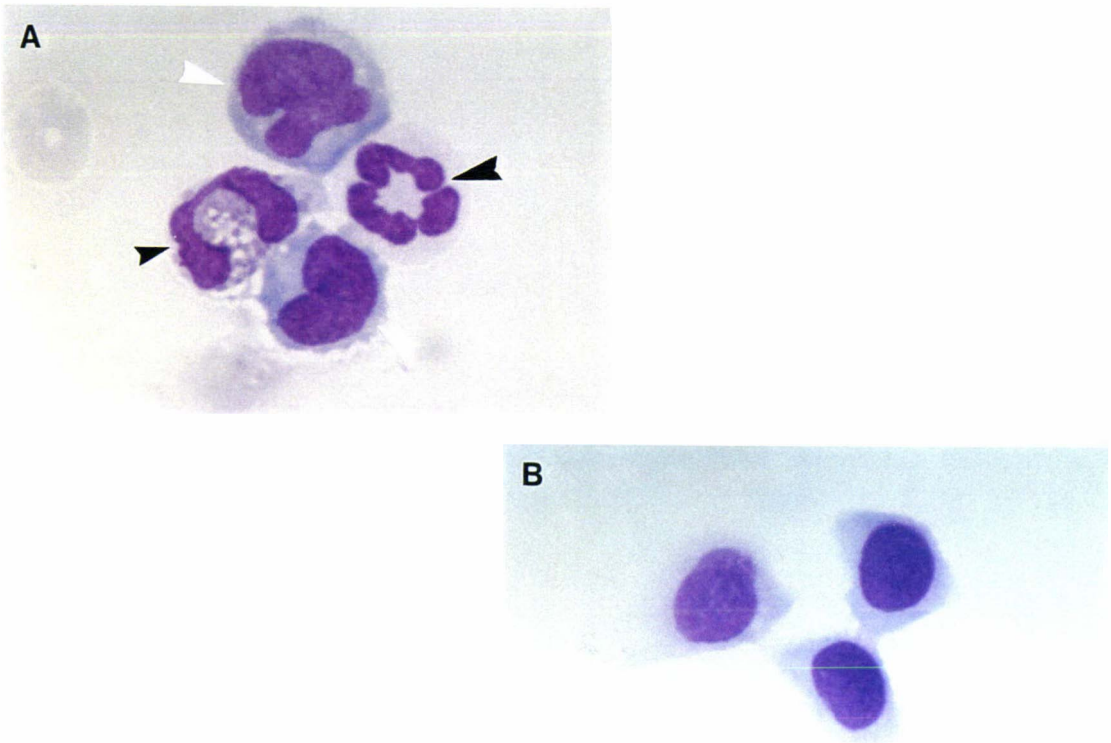


Figure 4-9. Mature, hypersegmented polymorph-neutrophils. Note the pyknotic nucleus (arrow) in the upper most cell.

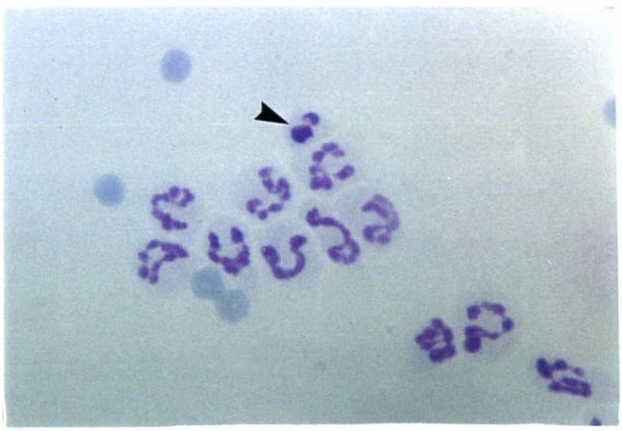
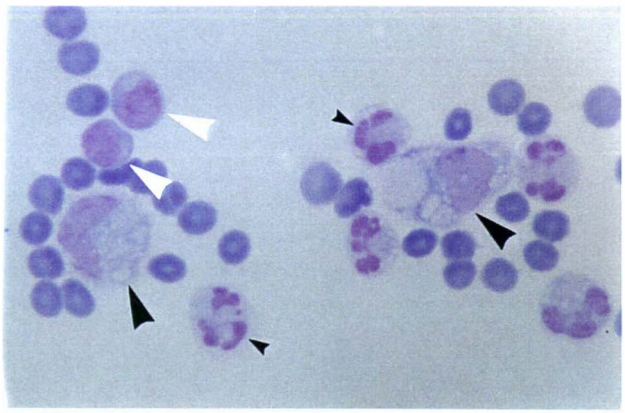


Figure 4-10. Vacuolated large mononuclear cells (large arrows), lymphocytes (open arrows) and neutrophils (small arrows) in the DPL from the lipid group.



DISCUSSION

The repeated abdominal puncture technique for the administration of peritoneal nutrients was easy to perform and very well tolerated by the dogs in this study. The use of a 12 G over-the-needle catheter allowed a relatively large bolus of DPL fluid or nutrient solution to be administered rapidly without discomfort to the dogs. Insertion of the catheter on the ventral midline is consistent with a number of reports previously describing DPL techniques in dogs[152,153,154,155,156] and has been shown to minimise iatrogenic haemorrhage that may make interpretation of the retrieved DPL fluid difficult.

The residual peritoneal fluid that was present in the dogs receiving the dextrose and amino acid solutions may have contributed to the marginal gain in weight that occurred in these dogs. Movement of extracellular fluid into the peritoneal cavity was expected in these dogs as both the solutions are hypertonic (505 mOsmol/l and 475 mOsmol/l, respectively) and will create a small degree of osmotic draw. The amount of caloric support provided by the intraperitoneal nutrients and the short duration of the study

precludes a discussion as to the efficacy of intraperitoneal nutrition and it's feasibility to maintain critically ill or injured dogs. However, it is very encouraging that 20% of RER can be safely administered to dogs in the form of 10% dextrose, 5% amino acids and a 10% lipid emulsion for short periods of time. It is also of note that there were some observations collectively supporting the inclusion of amino acids in an intraperitoneal nutrition protocol. For example, there was a greater weight gain in the dogs receiving the amino acid solution, along with a smaller decline in serum albumin concentration, although, these changes were individually not significantly different.

Mild anaemia, hypoproteinaemia, hypoalbuminaemia, decreased serum urea nitrogen concentration and decreased serum total calcium concentration were identified in the present study. These changes were also observed in the pilot study and have been previously reported by other workers.[130,138,143,144,145] The proposed pathogenesis of the haematological and biochemistry changes were discussed previously in Chapter 3. A difference from the pilot study was the decline in the serum creatine kinase concentration in the lipid group by Day 5. Whilst the difference was considered statistically significant, the pathogenesis remains obscure and was clinically insignificant.

The baseline (Day 1) mean DPL TWCC of 0.15 ± 0.19 x 10^9/l for all 12 dogs in this study is consistent with previously reported values in dogs.[152,153,154,155,156,157,158,159] The significant increase in TWCC by Day 5 in the lipid treatment group has confirmed that the lipid component of the total nutrient admixture used in the pilot study was the major cause of peritonitis in the dogs. This study has identified that intraperitoneal amino acids also causes a mild chemical peritonitis, whilst intraperitoneal dextrose is not associated with a significant increase in peritoneal white cell count in dogs when administered as a 10% solution at 20% of resting energy requirements. Despite the marked increase in peritoneal white cell count in the lipid group, it was not associated with a change in peripheral TWCC or clinical signs supportive of peritonitis. In addition, serum total protein and albumin concentrations in the lipid group were not significantly different from the dextrose or amino acid groups. This indicates that the peritonitis was not of sufficient magnitude to increase peritoneal membrane permeability to the point whereby albumin loss into the peritoneal cavity occurred at a sufficiently rapid rate to produce a

significantly lower serum albumin concentration than the dogs in the other groups. In fact, the remaining peritoneal fluid volume on Day 5 in the lipid group appeared to be much smaller than in either the dextrose and amino acid groups. This suggests that a "cytological" peritonitis may not necessarily equate to a "histological" peritonitis and the associated adverse clinical, haematological and biochemical effects of a "clinical" peritonitis.

It is worthy of note that the nutrients in the present study were compared on a kcal for kcal basis. As a result they were administered in different volumes and at different osmolalities. It is possible that these variables may have affected the quantitative peritoneal cytological response. However, it is unlikely that the conclusions of the study would have differed had the osmolality or volume been held constant and the amount of kcal allowed to vary because the lipid solution had the lowest osmolality and was administered in the lowest volume (approximately half of the other nutrients) but resulted in a peritoneal TWCC that was 13 fold higher than that caused by the other solutions.

The aetiology of the "cytological" peritonitis in the lipid group is unknown. Both neutrophils and large-mononuclear cells were observed to phagocytose lipid particles. The lipid emulsion used in this study consisted of glycerol, triglycerides, cholesterol, sodium oleate and phospholipids. One or all of these lipids may be involved in the initiation of the inflammatory response. It is feasible that as peritoneal mesothelial cells absorb lipid particles, intracellular signalling occurs with the production and release of inflammatory cytokines into the peritoneal cavity. Peritoneal mesothelial cells have been reported to be capable of cytokine production and release into the peritoneal cavity[107] and an increase in peritoneal cytokine concentration is considered an early indicator of impending peritoneal sepsis in people.[160] Once attracted into the peritoneal cavity, neutrophils would be capable of phagocytosis and further release of cytokines and other inflammatory mediators. The length of time lipid remains in the peritoneal cavity (a function of molecular size) may also influence the peritoneal membrane response to this nutrient.

Additional factors that may play a role in the inflammatory and / or irritant nature of lipids, and to a lesser extent amino acids, are the pH of the nutrient solution and the osmolality. The closer the nutrient pH to 7.4, theoretically, the less irritating it should be to the peritoneal membrane.[161] However, if we look at the pH of the nutrient solutions used in this study we find that 10% dextrose has an approximate pH of 3.2 and causes much less inflammation than does a 5% amino acid solution (pH = 6.0 to 7.0) or a 10% lipid emulsion (pH = 6.5 to 8.5). This implies that pH, as an individual factor, plays an insignificant role in the aetiology of the peritoneal inflammation. Finally, the osmolality of the nutrient solution would be expected to influence the peritoneal membrane's response. This was not shown to be the case in this study, as the dextrose solution with the greatest osmolality (505 mOsmol/l) caused the least inflammation, whilst the lipid emulsion (345 mOsmol/l) stimulated the greatest inflammatory response. Osmolality per se, does not seem to play a vital role in the pathogenesis of the "cytological" peritonitis identified in the lipid treatment group.

SUMMARY AND CONCLUSIONS

This study has reinforced the observations in the pilot study that a repeat abdominal puncture technique for the administration of nutrient solutions into the peritoneal cavity is easy to perform. In contrast to the pilot study, the smaller volumes and lower osmolality of the solutions used in the present study were not associated with significant adverse effects and were well tolerated by the dogs. A further explanation for the differences between these two studies is that whilst individual nutrients may be tolerated by the peritoneum, a total nutrient admixture with greater osmolality, the potential for chemical interactions and the complex nature of it's absorption, may overwhelm the peritoneal membrane creating the inflammatory reaction.

The results of this study have identified that 20% of resting energy requirements in the form of a 10% dextrose solution is well tolerated by the peritoneal membrane, whereas a 5% crystalline amino acid solution causes a mild "cytological" peritonitis and a 10% lipid emulsion is associated with a marked non-infectious suppurative peritonitis. However, the cytologic peritonitis in the dogs receiving intraperitoneal lipid was not associated with

greater haematological or biochemical alterations that are commonly seen with the use of intraperitoneal nutrition, nor were signs supportive of a clinically significant peritonitis detected.

Infusion of a 5% amino acid solution in sufficient quantities to meet 20% of RER causes mild peritoneal inflammation but can be safely administered to dogs via the peritoneal cavity. Twenty percent of RER in the form of amino acids equates to approximately 1.0 g of protein/kg/day, which although below the minimum daily requirements of adult dogs, would provide significant protein-sparing effects. To give additional protein to dogs via the peritoneal cavity would necessitate a greater amino acid concentration or a larger volume. If the volume of nutrient administration were to increase significantly above that used in the present study, the author would anticipate reduced respiratory function due to impairment of diaphragmatic movements. Leakage of peritoneal fluid (a consequence of increased intra-abdominal pressures) at the site of catheter insertion would also require cumbersome abdominal bandaging. Increasing the osmolality of the nutrient solution above 600 mOsmol would simply increase the risk of life-threatening fluid shifts into the peritoneal cavity and outweigh the advantage of increasing the energy density of the solution. An alternative protein source that can meet the recommended daily allowance of protein when administered in small volumes and without excessive hypertonicity would be therefore be advantageous.

The aetiology of the marked peritoneal inflammation associated with the infusion of a lipid emulsion into the peritoneal cavity of dogs' remains poorly understood and is probably multifactorial. Despite the lack of clinical signs supportive of acute suppurative peritonitis, it would be prudent to investigate the use of an alternative non-protein energy-dense nutrient to avoid the potential complications that may occur as a consequence of using greater quantities of lipid than that used in the present study in order to provide adequate caloric support. One such nutrient solution is Polycose®[kk], a dextrose polymer solution that can be formulated to suit the required osmolality and energy density considered by the author to be safe for intraperitoneal administration in dogs. A 21.5% Polycose® solution has an energy density of 1.0 kcal/ml and a calculated

[kk] Ross Laboratories, USA.

osmolality of 315 mOsmol/l. Determining the peritoneal cytological response to this nutrient solution, along with haematological and biochemical complications warrants further investigation.

CHAPTER 5.

- A Comparison of Peritoneal Cytology in Dogs Given 0.9% Sodium Chloride, 10% Dextrose or a 21.5% Dextrose Polymer Solution into the Peritoneal Cavity by Repeat Abdominal Puncture.

  Introduction.

  Objectives.

  Materials and Methods.

  Results.

  Discussion.

  Summary and Conclusions.

A COMPARISON OF PERITONEAL CYTOLOGY IN DOGS GIVEN 0.9% SODIUM CHLORIDE, 10% DEXTROSE OR A 21.5% DEXTROSE POLYMER SOLUTION INTO THE PERITONEAL CAVITY BY REPEAT ABDOMINAL PUNCTURE.

INTRODUCTION

The study reported in Chapter 4 identified that twenty percent of resting energy requirements (RER) in the form of 10% dextrose, 5% amino acids or a 10% lipid emulsion can be given safely to dogs via the peritoneal cavity but that the lipid emulsion was associated with a cytological peritonitis. These results suggest that a clinically significant peritonitis may occur if the volume of lipid infused into the peritoneum is increased, which would be necessary to meet a larger percentage of daily caloric needs. The study in Chapter 4 also identified that fluid shifts into the peritoneal cavity from the extracellular fluid compartment occurred as a consequence of the dextrose and amino acid infusions due to the hypertonic nature of the nutrients. Although the fluid shifts were not clinically significant, they emphasise the potential hazards of using solutions with osmolalities significantly greater than that of plasma.

For these reasons an alternative non-protein energy source to monomeric dextrose was investigated. Polycose®[ii], a dextrose polymer was selected because it can be formulated to suit the required energy density and osmolality established to be safe by our previous studies for intraperitoneal administration in dogs. By using a dextrose polymer as the caloric source the energy density of the nutritional solution can be increased concomitantly with a reduced osmolality.

In this study we elected to include a control group to determine the changes in peritoneal white cell count that occur as a consequence of the repeated abdominal puncture technique for nutrient infusion. In the previous experiments the dogs acted as their own controls by performing a baseline DPL prior to nutrient infusion, with comparisons being made back to the baseline value. One could argue that it is clinically irrelevant to separate the effects of repeat abdominal puncture from the effects of the intraperitoneal

[ii] Ross laboratories, USA.

nutrients, as the two cannot be mutually exclusive. However, if we are to confidently make statements regarding the inflammatory potential of intraperitoneal nutrients a study incorporating a control group receiving a non-irritant, non-nutrient intraperitoneal solution is required. We thus compared the peritoneal cytological response of dogs given 0.9% saline (control), 10% dextrose or a 21.5% dextrose polymer solution via a once-daily, repeat puncture technique.

OBJECTIVES

The objectives of this study were to:

- quantify the cytological response by the peritoneal membrane to intraperitoneal infusion of 0.9% sodium chloride, or a 10% dextrose or 21.5% dextrose polymer via a repeat-puncture technique given in sufficient quantities to meet 20% of daily caloric needs.

- determine if there is a significant difference in the peritoneal inflammation caused by the above electrolyte and nutrient solutions.

- identify any haematological or biochemical complications associated with infusion of the above electrolyte and nutrient solutions into the peritoneal cavity of dogs.

MATERIALS AND METHODS

Dogs

Eleven healthy adult border collie-cross dogs were used with a mean bodyweight (BW) of 25.3 kg (range 19.6 kg to 40.5 kg). The dogs were housed in individual cages measuring approximately 1.25 cubic metres and were allowed out twice daily to urinate and defecate. The dogs had access to water ad-libitum throughout the study.

Nutrient solutions

The dogs were randomly allocated into three groups. Group One (n = 3) received 12 ml/kg BW 0.9% sodium chloride,[mm] Group Two (n = 4) received 20% of RER (RER = [30 x BW (kg)] + 70) in the form of a 10% dextrose solution,[nn] and Group Three (n = 4)

[mm] Baxter Healthcare PTY Ltd. NSW, Australia.
[nn] Baxter Healthcare PTY Ltd. NSW, Australia.

received 20% of RER from a 21.5 % dextrose polymer solution.[oo] The volume of each nutrient solution administered each day is shown in Table 5-1. The dextrose and dextrose polymer solutions were commercially prepared under sterile conditions and packaged into individual single-dose flexi-packs.[pp] The measured osmolality of the sodium chloride, dextrose and dextrose polymer solutions were 310 mOsmol/l, 505 mOsmol/l, and 322 mOsmol/l, respectively. The osmolality of the dextrose polymer solution was measured using the freezing-point diffraction technique and compared favourably to the calculated value of 315 mOsmol/l. The remaining daily caloric intake was given to the dogs in the form of dry dog food.[qq]

Table 5-1. Volume of nutrients infused in each group on Days 1 - 4.

	0.9% NaCl	10% Dextrose	21.5% Polymer
ml/kg	12.0	19.4	6.6

Intraperitoneal administration and Diagnostic peritoneal lavage (DPL)

The intraperitoneal nutrients were given once daily for a total of four days by a repeat abdominal puncture technique. The dogs were prepared for intraperitoneal infusions and the catheter inserted into the peritoneal cavity in an identical manner to that used in the pilot and second study. A 16 G over-the-needle catheter[rr] was used for the DPL and nutrient infusions. The DPL was performed using 25 ml/kg of warmed lactated Ringer's solution.[ss] During the DPL and nutrient infusions the dogs were manually restrained in dorsal recumbency. Dogs that did not tolerate manual restraint were given light sedation using intravenous xylazine[tt] at a dose rate of between 0.1 to 0.3 mg/kg intravenously to facilitate the procedures. There was no bias between the groups as to the number of dogs that received xylazine sedation. Xylazine was chosen because of its repeatable sedation characteristics, ease of administration and cost-effectiveness.

[oo] Polycose®. Ross Laboratories, USA.
[pp] McGaw BioMed. Auckland, NZ.
[qq] Eukanuba® The Original Premium. The IAMS Company. Ohio, USA.
[rr] Angiocath™. Becton Dickinson Infusion Therapy systems Inc. Utah, USA.
[ss] Hartmans Solution. Baxter Healthcare PTY Ltd. NSW, Australia.
[tt] Xylazine 2%. Phoenix Pharm Distributors Ltd. Auckland, NZ.

Experimental time course and monitoring

On Day 1 a baseline complete blood count (CBC), serum chemistry panel with electrolytes and DPL were performed. A 10 ml sample of DPL fluid was collected and analysed for total white cell count (TWCC), differential white cell count and morphological assessment of the cells. The remaining DPL solution was left in the peritoneal cavity. Following the DPL, dogs were given the electrolyte or nutrient solution into the peritoneum using gravity-flow over a ten minute period. At the completion of the infusion the catheter was withdrawn from the peritoneal cavity and pressure applied using a gauze swab to prevent leakage of fluid and nutrients into the subcutaneous tissues. On Days 2, 3 and 4 the DPL and nutrient infusions were repeated. On Day 5 the DPL was repeated as well as the CBC and serum chemistry panel with electrolytes (Table 5-2.). Twice daily the dogs' temperature, respiration rate, heart rate, mucous membrane colour, capillary refill time, pulse pressure, general demeanour and abdominal pain were assessed. In addition, behavioural and postural changes were assessed and the abdomen was palpated thoroughly to detect the presence of abdominal discomfort.

Table 5-2. Schematic representation of experimental protocol.

	Day 1	Day 2	Day 3	Day 4	Day 5
IPN	✓	✓	✓	✓	
DPL	✓	✓	✓	✓	✓
CBC	✓				✓
Chem	✓				✓

IPN: intraperitoneal nutrients, DPL: diagnostic peritoneal lavage, CBC: complete blood count, Chem: serum chemistry panel with electrolytes.

Analysis of samples

Analysis of the CBC, serum chemistry panel and DPL fluid were carried out in an identical manner to that described in Chapters 3 and 4.

Statistics

Repeated measures ANOVA was used to compare the mean values between the three groups on Day 1 and Day 5; the mean change (between Day 1 and Day 5) within each group; and the mean change between Day 1 and Day 5 for all 11 dogs for the following parameters: bodyweight; resting heart rate and body temperature; CBC (haematocrit, packed cell volume, haemoglobin, RBC count, platelet count, total protein, white cell count and neutrophils); serum chemistry panel and electrolytes (urea, creatinine, lipase, alkaline phosphatase (ALP), alanine amino transferase (ALT), creatine kinase (CK), glucose, cholesterol, total protein, albumin, globulin, total bilirubin, sodium, potassium, calcium and phosphorus); and DPL fluid (TWCC, neutrophils and large mononuclear cells). For the purpose of the statistical analysis of the DPL fluid the data were log-transformed due to the wide variation within the treatment groups. All results are tabulated as the mean ± the standard deviation (s.d.). Statistical significance was set at $p \leq 0.05$.

RESULTS

Physical parameters

All eleven dogs remained bright and alert throughout the study period and maintained a normal appetite. There were no significant differences in the bodyweight of the dogs between the treatment groups at the start of the study. The dogs receiving the dextrose solution were significantly ($p = 0.05$) heavier by Day 5, whereas the dogs receiving the saline and dextrose polymer did not have a significant change in bodyweight by Day 5. There was a marginal ($p = 0.087$) difference between the treatment groups on Day 5 with the dextrose dogs being heavier (Table 5-3.). The dogs that received the dextrose monomer had a palpable fluid wave on the fifth morning of the study. This peritoneal fluid retention could not be palpated in the dogs that received saline or the dextrose polymer. The mean heart rate on Day 1 in the dogs that received the dextrose was significantly ($p = 0.05$) higher than the mean heart rate in the dogs receiving the dextrose polymer but not that of the dogs receiving the saline. The mean heart rate of the dogs receiving the dextrose polymer increased significantly ($p = 0.05$) above baseline on Days 2

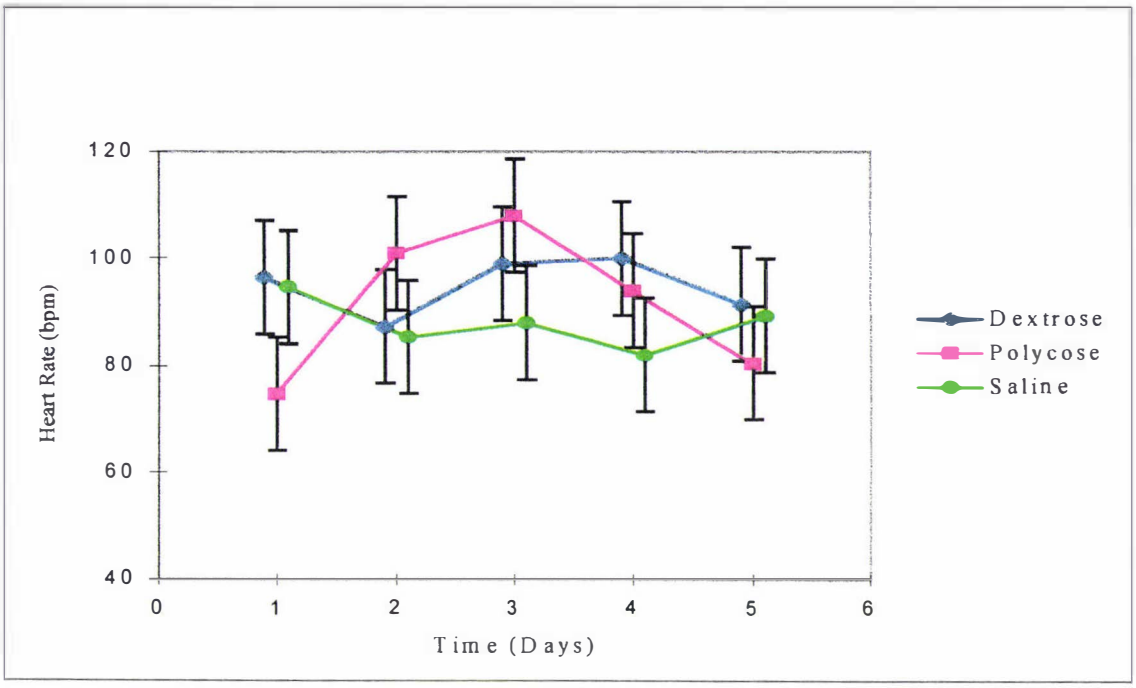
and 3 but subsequently declined on Days 4 and 5. There were no significant differences between the three treatment groups on Days 2 to 5 with respect to heart rate (Figure 5-1.). The mean body temperature in the dogs receiving the saline and dextrose did not change significantly from Day 1 throughout the study period, nor did the two groups differ significantly in terms of the changes in body temperature that occurred on Days 2 to 5. In contrast, the dogs receiving the dextrose polymer solution had a significantly ($p = 0.05$) increased body temperature above baseline on Days 2 to 5. The mean body temperature of the dogs in this group was also significantly ($p < 0.022$) higher than the dogs receiving the saline and dextrose on Days 2, 3 and 4 and Days 2 and 3, respectively (Figure 5-2.).

Table 5-3. Day 1 versus Day 5 bodyweights in the saline, dextrose and dextrose polymer groups.

		Saline		Dextrose		Polymer	
		Mean	s.d.	Mean	s.d.	Mean	s.d.
Bodyweight	Day 1	26.73	11.92	26.65	5.32	22.88	1.80
(kg)	Day 5	26.50	12.56	28.70$^{\Psi}$	6.47	22.75	0.29

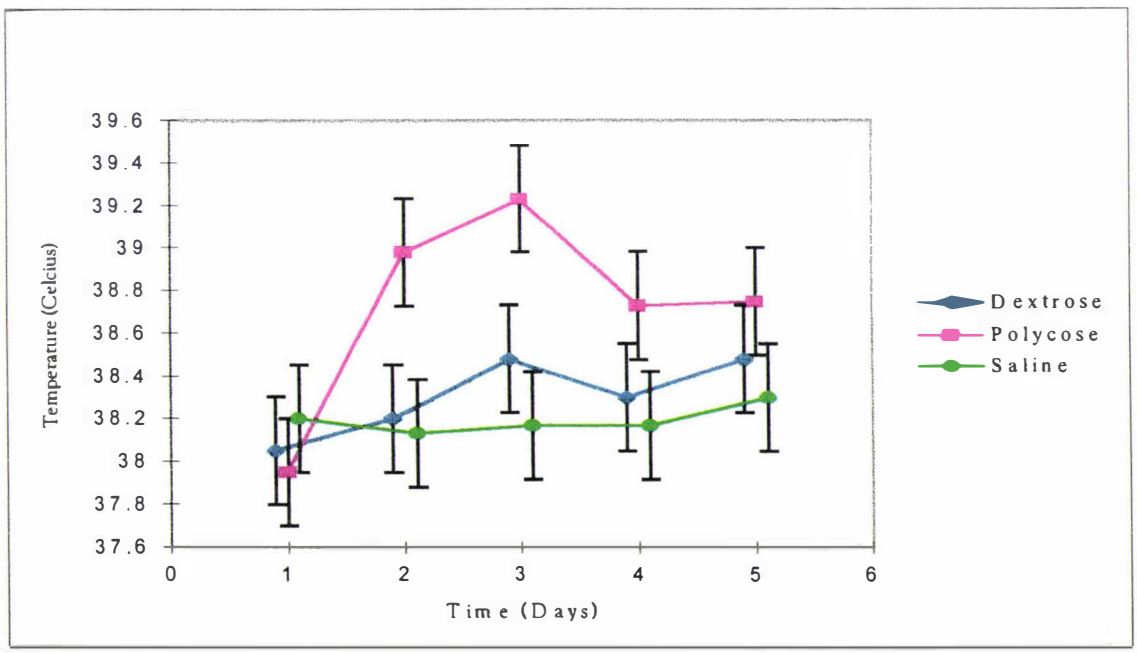
$^{\Psi}$ = significantly different from day 1($p = 0.05$).

Figure 5-1. Mean heart rate ± ½ LSD bars vs. time graph in the saline, dextrose and dextrose polymer groups.



½ LSD = Half the least significant difference. If the ½ LSD bars do not overlap the means are significantly different (p = 0.05).

Figure 5-2. Mean body temperature ± ½ LSD bars vs. time graph in the saline, dextrose and dextrose polymer groups.



½ LSD = Half the least significant difference. If the ½ LSD bars do not overlap the means are significantly different (p = 0.05).

Complete blood count

There were no significant differences in the packed cell volume (PCV) and red blood cell count (RBCC) of the dogs between the treatment groups at the start of the study, nor was there a significant difference between the treatment groups for the change in the PCV and red blood cell count (RBCC) that occurred throughout the study period. However, all three groups experiences a significant ($p = 0.002$) reduction in the PCV and RBCC by Day 5. The dogs receiving saline started the study with a marginally ($p = 0.099$) lower platelet count than the dogs receiving the dextrose or dextrose polymer. There were no significant differences between the treatment groups with respect to the changes that occurred in the platelet count throughout the study. Dogs receiving the dextrose polymer started the study with a significantly ($p = 0.007$) lower total protein concentration than dogs in the other two groups. There were no significant differences between the groups with respect to the changes that occurred in total protein throughout the study period. However, when the data for all eleven dogs were pooled "pooled data", there was a significant ($p = 0.02$) reduction in the total protein concentration by Day 5. There were no significant differences between the treatment groups on Day 1 with respect to the peripheral total white cell count (TWCC). However, there was a significant difference between the groups in the changes that occurred throughout the study for this parameter. Dogs receiving the dextrose polymer had a significant ($p = 0.05$) drop in the TWCC by Day 5, whilst the dogs receiving the saline and dextrose did not change significantly. Analysis of the neutrophil counts did not reveal any significant changes within or between treatment groups (Table 5-3.).

Serum chemistry panel and electrolytes

There was a statistically ($p = 0.002$) but not clinically significant difference in the serum urea nitrogen (SUN) concentration between the three treatment groups at the start of the study. There was a marginal ($p = 0.097$) difference between the treatment groups with respect to the decline in SUN that occurred over the study period. The higher the Day 1 SUN concentration, the greater the reduction in SUN concentration that was seen. The "pooled data" for this analyte underwent a significant ($p = 0.001$) decline in concentration by Day 5. There were no significant differences in the creatine

concentration between the groups at the start of the study, nor where there any differences between the groups in terms of the changes that occurred in the creatine concentration by Day 5. Once again, however, there was a significant ($p = 0.003$) drop in the creatine concentration by Day 5 for the "pooled data". The same pattern of change occurred with the serum alkaline phosphatase (ALP) concentration, which underwent a statistically ($p = 0.019$) but not clinically significant reduction by Day 5. For the "pooled data" there was a marginal ($p = 0.059$) decline in the serum creatinine kinase by Day 5. The between-group comparisons for creatinine kinase concentration on Day 1 and Day 5 did not reveal a significant difference. Dogs receiving the saline started the study with a significantly ($p = 0.017$) lower serum glucose concentration compared to the dogs receiving the dextrose or dextrose polymer. No significant differences were detected between the groups with respect to the decline in the glucose concentration that occurred throughout the study, but taken across all eleven dogs there was a statistically ($p = 0.004$) but not clinically significant reduction in the glucose concentration on Day 5. Analysis of the "pooled data" identified a statistically significant ($p = 0.004$) drop in cholesterol concentration on Day 5. Between-group comparisons on Day 1 and Day 5 for this analyte did not identify a significant difference. Dogs receiving the dextrose polymer started the study with a marginally ($p = 0.057$) lower total protein (TP) concentration compared to the dogs receiving the saline and dextrose. There were no significant differences between the groups with respect to the reduction that occurred in the TP concentration throughout the study, whilst the "pooled data" had a significant ($p = 0.02$) decline in the TP concentration by Day 5. There was a significant ($p = 0.003$) reduction in the serum albumin concentration in all groups by Day 5. Between-group comparisons on Day 1 and Day 5 did not show any significant differences for this parameter. There was a marginal ($p = 0.069$) difference in the serum globulin concentration at the start of the study. Dogs receiving saline had a significant ($p = 0.05$) decline in globulin concentration by Day 5, whereas the dogs receiving dextrose and dextrose polymer did not have a significant change. There was a marginal ($p = 0.059$) difference between the treatment groups with respect to their serum sodium concentrations at the start of the study. There was also a difference between the groups in the change that occurred for the sodium concentration. Dogs receiving the saline had a significant ($p = 0.05$) increase in sodium concentration on

Day 5, whereas dogs receiving dextrose and dextrose polymer did not have a significant change in sodium concentration. There was a significant decrease in the serum potassium ($p = 0.044$) and calcium ($p = 0.028$) concentrations on Day 5 when the "pooled data" were analysed. Between-group comparisons on Day 1 and Day 5 did not reveal any significant differences for these two electrolytes. For the remaining analytes studied: lipase, alanine aminotransferase (ALT), total bilirubin and phosphate, there were no within or between-group differences detected on Day 1 and Day 5 (Table 5-5.).

Diagnostic peritoneal lavage (DPL) fluid

The mean DPL total white cell counts (TWCC) in the three treatment groups were not significantly different on Day 1 (baseline). However, throughout the course of the study the changes in the TWCC that occurred within each group were significantly ($p = 0.05$) different (Table 5-6.). The mean TWCC in the dogs receiving the dextrose polymer were significantly ($p = 0.001$) higher than baseline on Days 2, 3, 4 and 5. However, by the conclusion of the experiment on Day 5 the mean TWCC in the dextrose polymer group had begun to decline and there was no longer a significant difference between the groups. The mean TWCC in the dogs receiving the dextrose polymer was significantly ($p < 0.05$) higher than the mean TWCC in the dogs receiving saline on Days 2 and 4, and higher than the dogs receiving dextrose on Days 2, 3 and 4. The mean TWCC in the dogs receiving the saline and dextrose did not increase significantly above baseline throughout the study period, nor where there any significant differences between these two treatment groups with respect to the changes in the TWCC on Days 2 to 5 (Figure 5-3. and 5-4.).

The mean DPL neutrophil counts in the three treatment groups were not significantly different on Day 1 (baseline). However, throughout the course of the study the changes in the neutrophil count that occurred within each group were significantly ($p = 0.05$) different (Table 5-6.). The mean neutrophil count in the dogs receiving dextrose polymer were significantly ($p = 0.001$) higher than baseline on Days 2, 3, 4 and 5. The mean neutrophil count in the dogs receiving the dextrose polymer were significantly ($p < 0.05$) higher than the mean neutrophil count in the dogs receiving the saline and dextrose on Days 2, 3 and 4. The mean neutrophil count in the dogs receiving the saline and dextrose did not increase significantly above baseline throughout the study period, nor where

there any significant differences between these two treatment groups with respect to the changes in the neutrophil count on Days 2 to 5 (Figure 5-5. and 5-6.).

The within and between-group comparisons of the mean large mononuclear cell counts (LMCC) at baseline and on Days 2 to 5 were the same as that described above for the neutrophil counts (Table 5-6; Figure 5-7. and 5-8.).

Table 5-4. Day 1 versus Day 5 values for the CBC in the saline, dextrose and dextrose polymer groups.

		Saline Mean	s.d.	Dextrose Mean	s.d.	Polymer Mean	s.d.	Reference Range
HB (g/l)	Day 1	147.33	17.50	145.75	15.41	148.50	9.33	120-180
	Day 5	135.00$^{\Psi}$	30.45	127.50$^{\Psi}$	17.41	121.50$^{\Psi}$	9.33	
PCV (l/l)	Day 1	0.47	0.06	0.47	0.04	0.50	0.03	0.37-0.55
	Day 5	0.45$^{\Psi}$	0.10	0.42$^{\Psi}$	0.05	0.41$^{\Psi}$	0.03	
RBC (x 10^{12}/l)	Day 1	6.39	0.45	6.32	0.59	6.67	0.41	5.8 - 8.5
	Day 5	6.14$^{\Psi}$	1.12	5.84$^{\Psi}$	0.73	5.48$^{\Psi}$	0.43	
MCV (fl)	Day 1	74.00	3.00	74.25	1.26	75.00	0.82	60 - 77
	Day 5	73.33	3.79	73.25	0.50	74.75	0.50	
PLT (x 10^{9}/l)	Day 1	353.33	75.08	243.75	67.19	261.50	37.64	200 - 900
	Day 5	311.33	29.87	282.50	23.22	248.25	15.31	
WBC (x 10^{9}/l)	Day 1	9.40	1.37	9.53	0.96	9.55	1.39	6.0 - 15.0
	Day 5	10.13	0.93	10.88	1.54	7.83$^{\Psi}$	0.94	
Neuts (x 10^{9}/l)	Day 1	5.10	0.52	5.85	1.39	4.70	1.26	3.6 - 11.5
	Day 5	5.23	1.34	7.00	1.24	3.90	0.61	
Bands (x 10^{9}/l)	Day 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00 - 0.54
	Day 5	0.00	0.00	0.00	0.00	0.02	0.05	
Lymph (x 10^{9}/l)	Day 1	2.23	0.55	2.55	0.72	2.93	0.47	1.0 - 4.8
	Day 5	2.73	0.81	2.65	0.70	2.63	0.38	
Mono (x 10^{9}/l)	Day 1	0.54	0.20	0.24	0.10	0.44	0.13	0.18 - 1.5
	Day 5	0.66	0.26	0.44	0.13	0.37	0.18	
Eosin (x 10^{9}/l)	Day 1	1.58	0.58	0.96	0.49	1.45	0.17	0.12 - 1.5
	Day 5	1.52	0.88	0.85	0.63	0.95	0.50	
Baso (x 10^{9}/l)	Day 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Day 5	0.00	0.00	0.00	0.00	0.00	0.00	

s.d. = standard deviation, HB = haemoglobin, HCT = haematocrit, RBC = red blood cells, MCV = mean corpuscular volume, Retics = reticulocytes, PLT = platelets, WBC = white blood cells, Neuts = mature neutrophils, Bands = immature neutrophils, Lymph = lymphocytes, Mono = monocytes, Eosin = eosinophils, baso = basophils. $^{\&}$ = significantly different ($p = <0.05$) on Day 1, $^{\Psi}$ = significantly different ($p = <0.05$) from Day 1.

Table 5-5. Day 1 versus Day 5 values for the serum chemistry panel and electrolytes in the saline, dextrose and dextrose polymer groups.

		Saline Mean	s.d.	Dextrose Mean	s.d.	Polymer Mean	s.d.	Reference Range
Urea (mmol/l)	Day 1	10.57$^{\Phi}$	1.42	7.57$^{\Phi}$	1.54	4.93$^{\Phi}$	1.09	2.5 - 8.4
	Day 5	4.82$^{\Psi}$	1.78	4.22$^{\Psi}$	0.45	3.23$^{\Psi}$	1.14	
Creatinine (µmol/l)	Day 1	82.33	7.23	82.75	5.56	86.75	4.65	75 - 138
	Day 5	90.00$^{\Psi}$	11.36	87.50$^{\Psi}$	7.05	89.25$^{\Psi}$	6.34	
Lipase (IU/l)	Day 1	141.67	201.23	109.25	105.35	79.00	28.25	< 500
	Day 5	55.33	64.38	111.25	74.55	105.50	97.52	
AP (IU/l)	Day 1	34.33	3.21	37.00	10.61	35.75	5.74	< 185
	Day 5	31.00$^{\Psi}$	4.00	35.25$^{\Psi}$	7.14	31.25$^{\Psi}$	4.72	
ALT (IU/l)	Day 1	43.67	12.90	50.50	12.07	78.75	60.32	< 58
	Day 5	44.67	8.74	99.75	101.21	52.50	20.93	
CK (IU/l)	Day 1	157.67	26.27	132.50	51.99	97.25	18.55	< 800
	Day 5	169.67$^{\Phi}$	44.07	92.00$^{\Phi}$	22.14	78.75$^{\Phi}$	4.35	
Glucose (mmol/l)	Day 1	4.30	0.53	5.73	0.71	5.63	0.30	3.8 - 5.8
	Day 5	3.73$^{\Psi}$	0.47	5.08$^{\Psi}$	0.28	5.30$^{\Psi}$	0.59	
Cholesterol (mmol/l)	Day 1	6.53	0.80	6.50	0.65	5.67	0.77	3.2 - 9.3
	Day 5	5.36$^{\Psi}$	1.70	6.13$^{\Psi}$	0.47	4.87$^{\Psi}$	0.67	
TP (g/l)	Day 1	70.37$^{\Omega}$	2.83	67.95$^{\Omega}$	3.73	63.35$^{\Omega}$	3.12	62 - 85
	Day 5	66.57$^{\Psi}$	2.14	66.03$^{\Psi}$	4.84	61.18$^{\Psi}$	2.97	
Albumin (g/l)	Day 1	34.97	2.87	35.38	1.66	35.23	1.07	28 - 35
	Day 5	33.63$^{\Psi}$	4.51	34.25$^{\Psi}$	2.46	31.75$^{\Psi}$	1.05	
Globulins (g/l)	Day 1	35.40$^{\Omega}$	4.39	32.58$^{\Omega}$	4.00	28.13$^{\Omega}$	2.10	27 - 57
	Day 5	32.93$^{\Psi}$	2.75	31.78	3.95	29.43	1.93	
A/G	Day 1	1.00	0.19	1.10	0.17	1.26	0.06	
	Day 5	1.03	0.21	1.09	0.15	1.08	0.04	
T Bilirubin (µmol/l)	Day 1	1.47	0.64	1.03	0.46	1.48	0.88	< 6.0
	Day 5	1.10	0.40	2.00	1.07	1.38	0.64	
Sodium (mmol/l)	Day 1	148.53	0.96	150.30$^{\Omega}$	1.41	148.20	0.61	141- 155
	Day 5	150.90$^{\Psi}$	1.30	149.90	3.13	147.63	0.22	
Potassium (mmol/l)	Day 1	4.63	0.65	4.53	0.47	4.40	0.18	3.6 - 5.6
	Day 5	4.74	0.65	4.26$^{\Psi}$	0.23	4.16$^{\Psi}$	0.19	
Calcium (mmol/l)	Day 1	2.68	0.05	2.66	0.09	2.57	0.06	2.05- 3.00
	Day 5	2.61$^{\Psi}$	0.08	2.62$^{\Psi}$	0.06	2.56$^{\Psi}$	0.09	
Phosphate (mmol/l)	Day 1	1.37	0.06	1.26	0.21	1.31	0.21	0.8 - 1.6
	Day 5	1.35	0.16	1.39	0.21	1.47	0.22	

s.d. = standard deviation, ALP = alkaline phosphatase, ALT = alanine aminotransferase, CK = creatine kinase, TP = total protein, A/G = albumin to globulin ratio, T Bilirubin = total bilirubin, $^{\Omega}$= marginally different on day 1, $^{\Phi}$= significantly different (p = <0.05) on Day 1, $^{\Psi}$= significantly different (p = <0.05) from Day 1.

DPL Cell Morphology

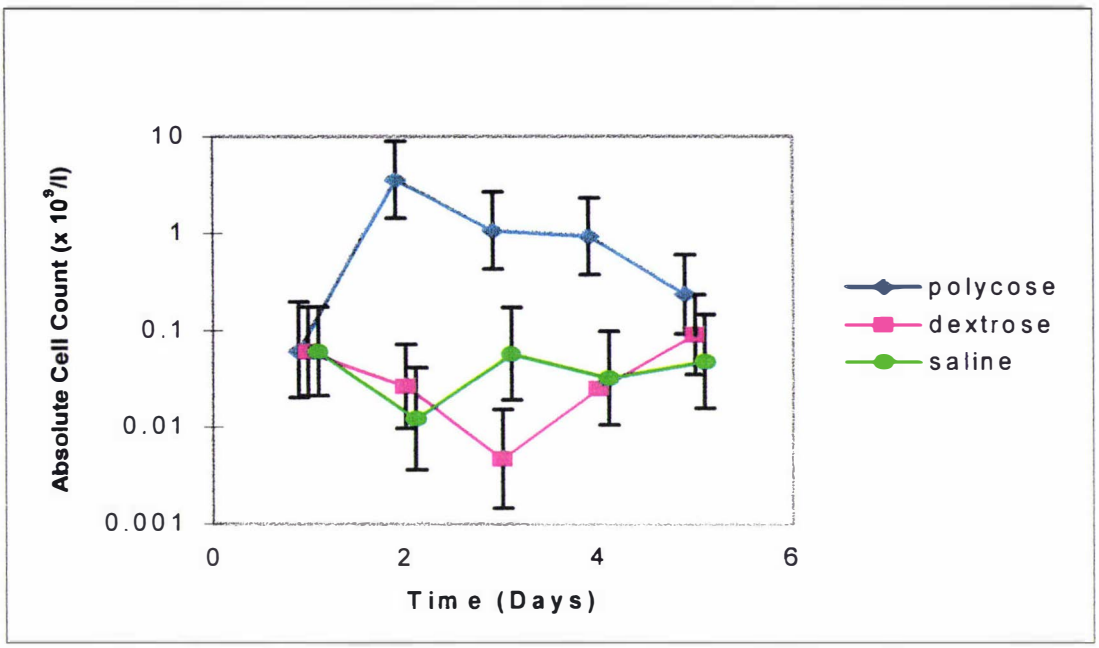
The baseline DPL's consisted of approximately 50 to 66% mature, non-degenerate neutrophils and 34 to 50% large-mononuclear cells, with the occasional lymphocyte, eosinophil and cluster of peritoneal mesothelial cells (Figure 5-9 a, b, c, d.). By Day 5 the percentage of mature neutrophils in the three groups increased markedly and ranged from approximately 76 to 86% of the TWCC. The neutrophils in all eleven dogs remained non-degenerate and developed hyper-segmented nuclei (Figure 5-10.). The large mononuclear cells in the dogs receiving the dextrose polymer became distended as a consequence of several to numerous intracytoplasmic vacuoles that contained small basophilic granules surrounded by a clear area (Figure 5-11 a, b.). The nucleus of these cells often became displaced to the periphery of the cell. The intracytoplasmic vacuoles in the large mononuclear cells in the dextrose polymer group most likely represent phagocytosed particulate matter present in the polymer solution, as the solution was constituted by dissolving a dextrose polymer powder in sterile water.

Table 5-6. Day 1 versus Day 5 cell counts for the DPL fluid in the saline, dextrose and dextrose polymer groups.

		Saline		Dextrose		Polymer	
		Mean	s.d.	Mean	s.d.	Mean	s.d.
TWCC (x 10^9/l)	Day 1	0.06	0.02	0.06	0.02	0.06	0.03
	Day 5	0.16	0.12	0.29	0.35	0.82$^{\Psi}$	0.73
Neuts (x 10^9/l)	Day 1	0.04	0.01	0.03	0.02	0.04	0.02
	Day 5	0.13	0.12	0.22	0.26	0.71$^{\Psi}$	0.68
Large Mono (x10^9/l)	Day 1	0.02	0.01	0.03	0.01	0.02	0.00
	Day 5	0.02	0.01	0.06	0.09	0.11$^{\Psi}$	0.07
Lymph (x 10^9/l)	Day 1	0.00	0.00	0.00	0.00	0.00	0.00
	Day 5	0.01	0.00	0.01	0.01	0.00	0.00
Eosin (x 10^9/l)	Day 1	0.00	0.00	0.00	0.00	0.00	0.00
	Day 5	0.00	0.00	0.00	0.00	0.00	0.00

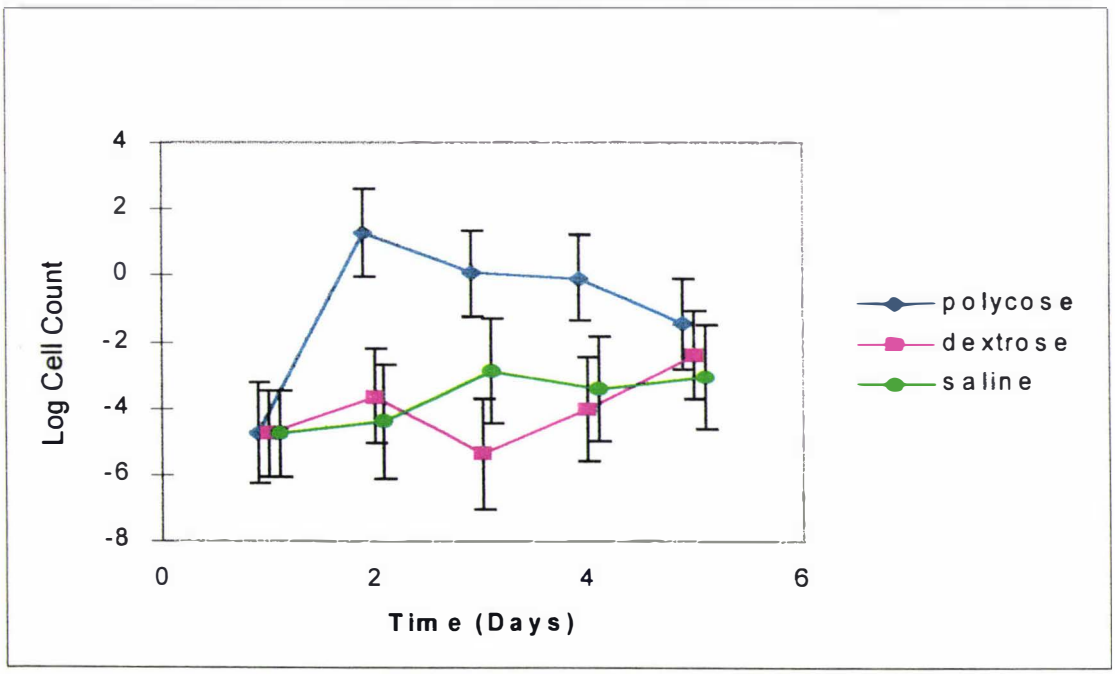
s.d. = standard deviation, TWCC = total white cell count, Neuts = neutrophils, Large Mono = large mononuclear cells, Lymph = lymphocytes, Eosin = eosinophils, $^{\Psi}$ = significantly different ($p = <0.05$) from Day 1.

Figure 5-3. Mean peritoneal total white cell count ± SEM vs. time graph in the saline, dextrose and dextrose polymer groups.



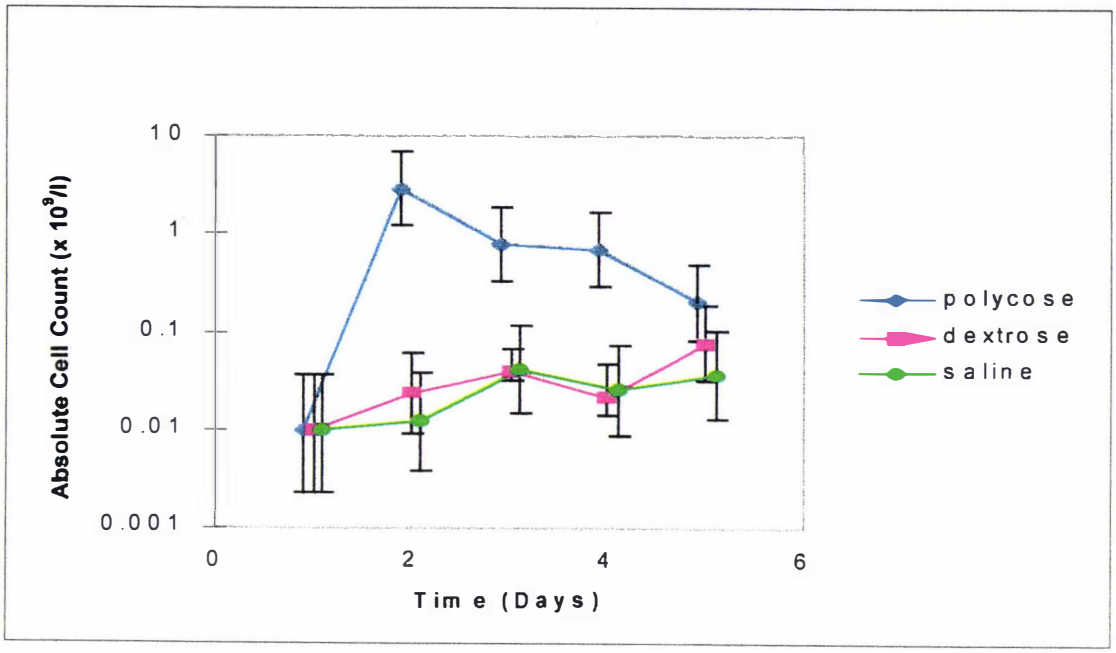
SEM = standard error of the mean.

Figure 5-4. Mean peritoneal total white cell count ± ½ LSD bars vs. time graph in the saline, dextrose and dextrose polymer groups.



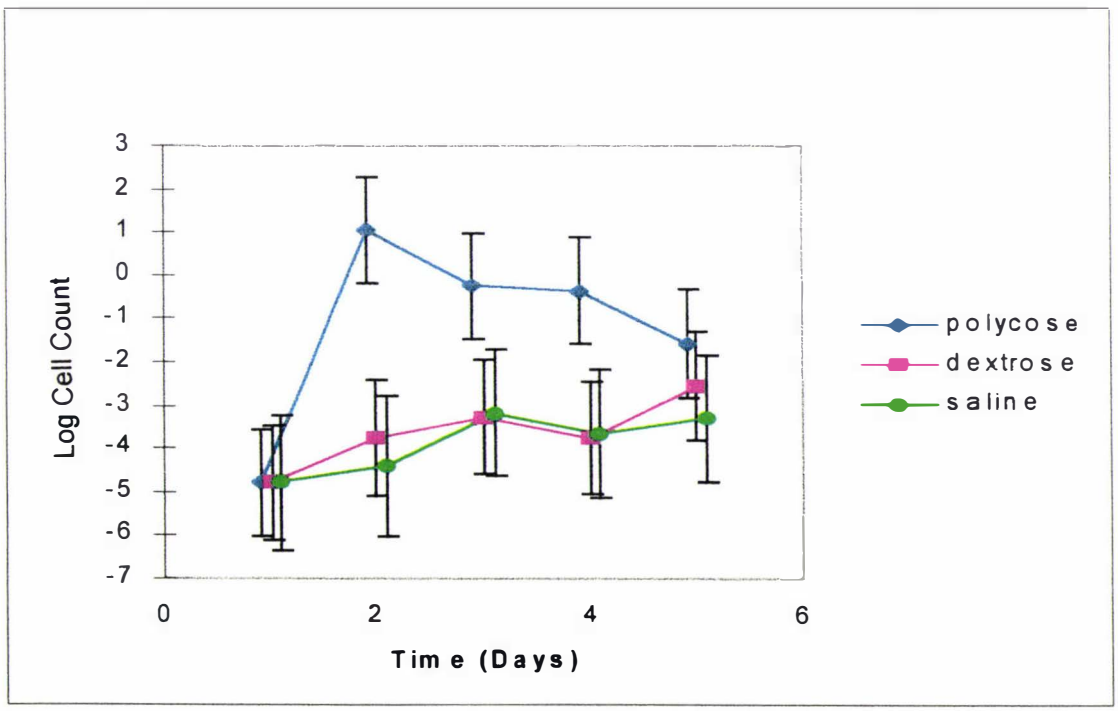
½ LSD = half the least significant difference. If the ½ LSD bars do not overlap then the means are significantly different. (p = 0.05).

Figure 5-5. Mean peritoneal neutrophil count ± SEM vs. time graph in the saline, dextrose and dextrose polymer groups.



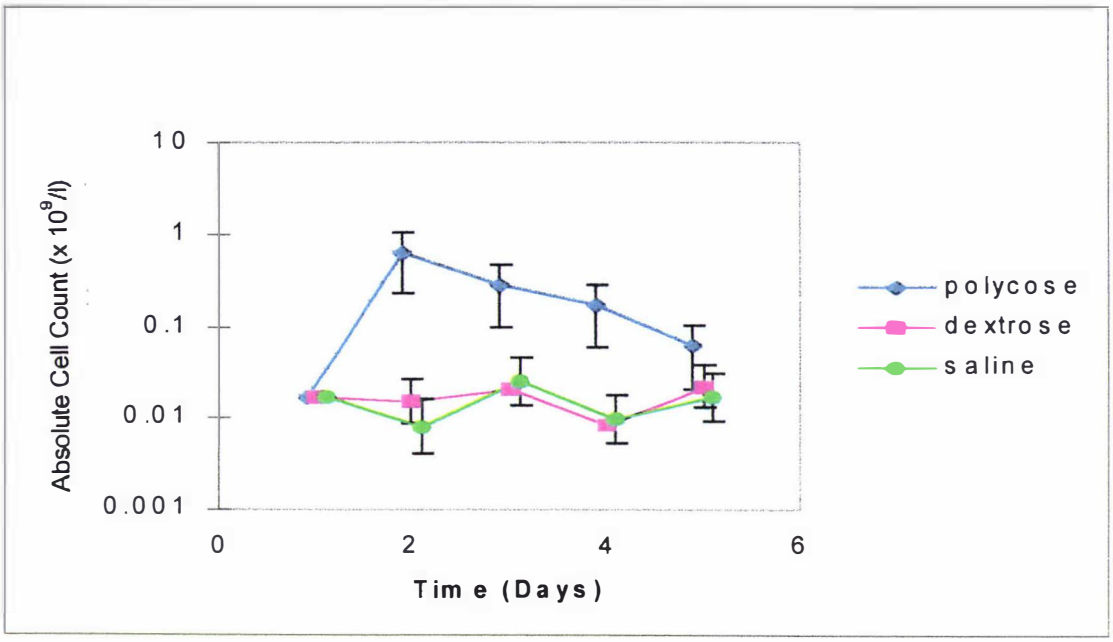
SEM = standard error of the mean.

Figure 5-6. Mean peritoneal neutrophil count ± LSD bars vs. time graph in the saline, dextrose and dextrose polymer groups.



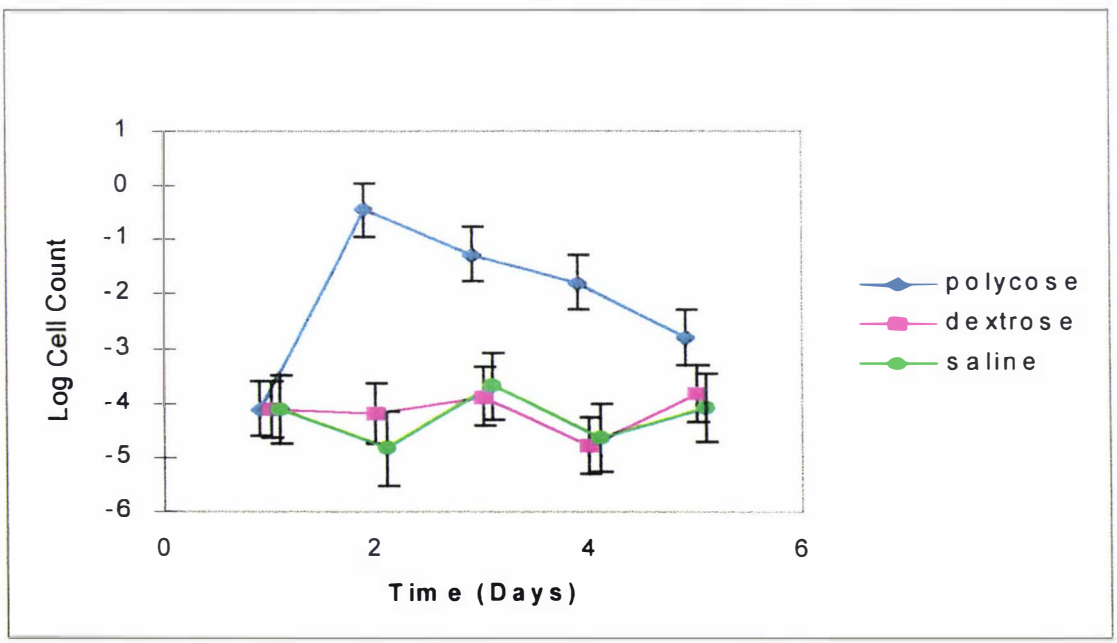
½ LSD = ½ the least significant difference. If the ½ LSD bars do not overlap the means are significantly different (p = 0.05).

Figure 5-7. Mean peritoneal large mononuclear cell count ± SEM vs. time graph in the saline, dextrose and dextrose polymer groups.



SEM = standard error of the mean.

Figure 5-8. Mean peritoneal large mononuclear cell count ± LSD bars vs. time graph in the saline, dextrose and dextrose polymer groups.



½ LSD = ½ the least significant difference. If the ½ LSD bars do not overlap the means are significantly different (p = 0.05).

Figure 5-9. Baseline (Day 1) diagnostic peritoneal lavage fluid cytology (a) mature non-degenerate polymorph neutrophils and single eosinophil (arrow) (b) cluster of large mononuclear cells: note the intracytoplasmic phagosome (arrow) (c) an eosinophil: note the classical red-orange intracytoplasmic granules (arrow) (d) a small cluster of peritoneal mesothelial cells.

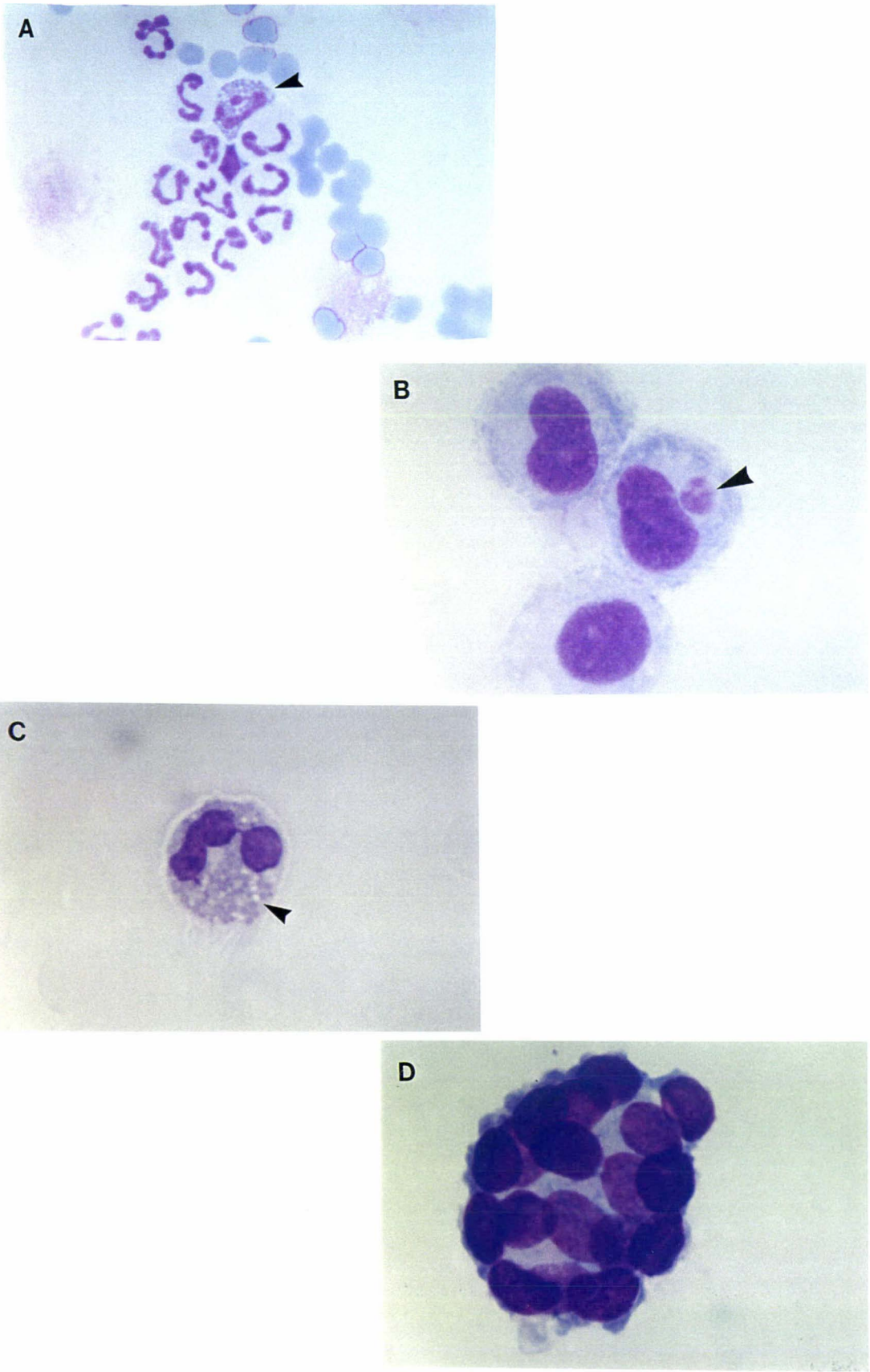


Figure 5-10. A group of large mononuclear cells (open arrow) surrounded by several mature, non-degenerate, hypersegmented polymorph neutrophils (small arrows).

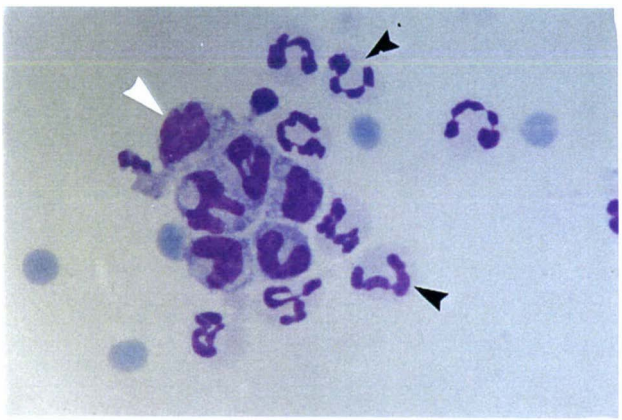
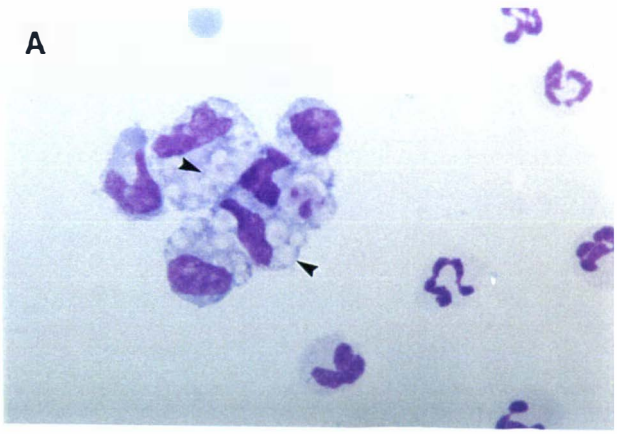
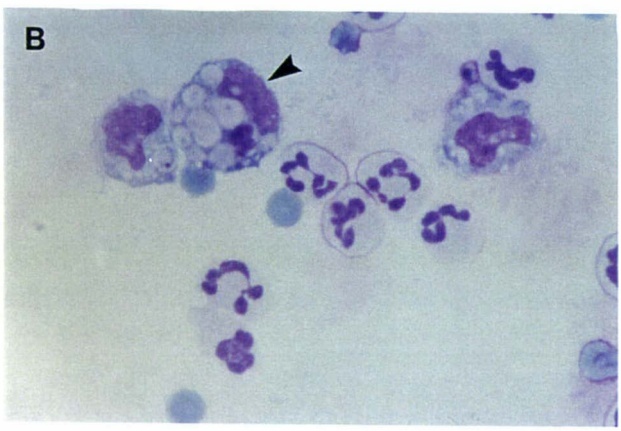


Figure 5-11. DPL fluid: large mononuclear cells in the dogs receiving the dextrose polymer (a) note the numerous intracytoplasmic vacuoles that contain a central basophilic body surrounded by a clear area (arrows) (b) note the nucleus is being displaced to the periphery of the cell (arrow).





DISCUSSION

A total of 55 peritoneal punctures were performed during this study and no major complications were encountered. The concomitant increase in resting heart rate and body temperature with the increased peritoneal TWCC and neutrophils on Days 2, 3 and 4 in the dogs receiving the dextrose polymer solution may indicate a systemic response to the mild "cytological" peritonitis. An alternative explanation for the elevated body temperature is "post-prandial thermogenesis" from the metabolism of the administered dextrose polymer.[162] Whilst other factors may have influenced these physical parameters, such as stress, fear, handling or excitement, these factors would have been expected to affect dogs in all treatment groups. Although the changes in the heart rate and body temperature were statistically significant, they were not of sufficient magnitude to be clinically significant, nor were there any additional signs of a clinically significant peritonitis detected during the physical examination in any of the dogs. It is also important to note that the magnitude of these parameters were on the decline by day 5, suggesting a developing peritoneal "tolerance" to the dextrose polymer solution.

The residual peritoneal fluid identified in the dogs receiving the dextrose solution may explain the significant increase in body weight that occurred by Day 5 in this group. The osmolality of the saline and dextrose polymer solutions is very similar to that of plasma and would have minimised any fluid flux from the extracellular fluid space into the peritoneal cavity in these dogs.

The reduction in the peripheral TWCC on Day 5 in the dogs receiving the dextrose polymer solution could indicate an acute demand for white blood cells and migration into the peritoneal cavity, as there was a significant increase in peritoneal TWCC that was identified on Days 2 to 5 in this group. However, the predominant cell type within the peritoneal cavity on these days in the dogs receiving the dextrose polymer solution was the neutrophil, which did not change significantly from Day 1 in the peripheral blood of the dogs in this group. Additionally, in dogs, an acute demand for white blood cells should have been well compensated for within five days of a pro-inflammatory stimulus[163] resulting in an increase in the number of mature neutrophils in circulation, or if the demand is great enough, an increase in band neutrophils in circulation. Neither of

these two phenomenon where seen. The decline in peripheral TWCC may have been an unrelated event, as there was a decline in the neutrophil, lymphocyte and eosinophil counts also. The cumulative decline in these cell counts would thus explain the decline in the TWCC. Therefore, the clinical significance of this finding remains unknown.

The data from this present study support the observations in our previous studies and those of others that a mild anaemia, hypoalbuminaemia, reduction in serum urea nitrogen concentration and total serum calcium concentration are consistent haematological and biochemical complications changes associated with short-term intraperitoneal administration of nutrient and electrolyte solutions in dogs. The proposed aetiology of these complications has been discussed previously in Chapter 3. An interesting feature of the dogs receiving the saline in the present study was the increased sodium concentration on Day 5. This probably reflects the degree of sodium loading the dogs underwent throughout the study, as the dogs received sodium from both the DPL solution (lactated Ringer's) and the control solution (0.9% sodium chloride). The decreased potassium concentration on Day 5 seen in the dogs receiving the dextrose and dextrose polymer may have resulted from an increase in insulin secretion (in response to the dextrose loads) and its subsequent effect on the intracellular movement of potassium into cells. Another notable finding in the dogs receiving the saline was the decline in total protein and albumin concentrations by Day 5. This observation supports the notion that the movement of protein from the extravascular space down a concentration gradient into the peritoneal cavity, or "third space", plays a role in the pathogenesis of the hypoproteinaemia that occurs commonly in dogs undergoing intraperitoneal nutrition.

The baseline (Day 1) mean peritoneal TWCC of $0.06 \pm 0.02 \times 10^9/l$ for all eleven dogs in the present study is lower than in the previous study by this author and values previously reported by other workers.[153,154,155,156,157,158,159] This may reflect differing dog populations or reduced blood contamination from iatrogenic haemorrhage as the author gained more experience using this technique.

This study has identified that intraperitoneal administration of 20% of RER in the form of a 21.5% dextrose polymer solution caused a mild, predominantly neutrophilic

"cytological peritonitis" that peaked on Day 2 after starting nutrient infusions, but then subsequently declined back to values approaching baseline on Day 5. Intraperitoneal infusion of 10% dextrose was not associated with a significant change in the peritoneal TWCC and the cytological response to this nutrient solution was very similar to that of the control (0.9% sodium chloride) solution. This data suggests that the procedure of repeat abdominal puncture itself, does not contribute significantly to the change in white cell count that occurred in the dogs that received the dextrose polymer solution.

Although not a specific objective of this study, it is the author's opinion that the 16 G over-the-needle catheter used in the present study was easier to insert and was subjectively associated with less blood contamination of the DPL fluid. Reducing the catheter diameter from 12 G to 16 G did not appreciably increase the time required to perform the DPL and infuse the nutrient solutions, nor did it affect the ability to obtain a satisfactory DPL fluid sample for analysis.

Whilst the increase in peritoneal TWCC in the dextrose polymer group was associated with a small but statistically significant increase in resting heart rate and body temperature, there were no signs of a clinically significant peritonitis in these dogs. In addition, the haematological and biochemical changes that were identified in the dogs receiving the dextrose polymer were not significantly different from the changes that occurred in the dogs receiving the saline and dextrose solutions. This indicates that any increase in peritoneal membrane permeability that may have occurred as a consequence of the inflammatory response was not of sufficient magnitude to change the rate of albumin loss into the peritoneal cavity compared to the dogs in the saline or dextrose groups.

The exact aetiology of the peritoneal inflammatory response to the dextrose polymer solution is unknown, but most likely involves the initial phagocytosis of dextrose polymer molecules by resident peritoneal mononuclear - macrophage cells and the subsequent release of pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-8 (IL-8) and tumour necrosis factor alpha (TNF-α). IL-8 is the archetypal chemoattractant for neutrophils, whilst IL-1 and TNF-α stimulate local production of the alpha-chemokines

that further enhance neutrophil migration to the affected area.[164] Cells capable of alpha-chemokine production include fibroblasts and epithelial cells. It is unknown whether peritoneal mesothelial cells are capable of this function. Once attracted into the peritoneal cavity, neutrophil activation would result in the release of inflammatory enzymes, free radicals and neutrophil-derived IL-8, leading to a self-perpetuating inflammatory cascade. The reason(s) why the inflammatory response to the dextrose polymer solution did not persist, or worsen, despite repeated administration on Days 2, 3 and 4 are unclear but may involve the development of a tolerance to this nutrient solution.

SUMMARY AND CONCLUSIONS

Data from the present study have shown that intraperitoneal infusion of a 21.5% dextrose polymer solution causes a transient and mild "cytological" peritonitis when given in sufficient quantities to meet 20% of RER, but is clinically very well tolerated by dogs. Intraperitoneal administration of either 0.9% sodium chloride or 10% dextrose causes minimal change to the peritoneal TWCC, whilst the repeat abdominal puncture technique has no apparent effect on the quantitative changes in peritoneal TWCC that occur during intraperitoneal nutrition.

Despite the mild peritonitis caused by the dextrose polymer, this nutrient solution has significant advantages when compared to a dextrose monomer solution. First, the osmolality of the 21.5% dextrose polymer solution is similar to that of plasma, but significantly lower than that of 10% glucose (322 mOsmol/l and 505 mOsmol/l, respectively). Although osmolality per se does not influence the inflammatory potential of the commonly used nutrient solutions, it is strongly correlated with fluid flux across the peritoneal membrane into the peritoneal cavity, which can have serious effects on the cardiovascular status of dogs. Secondly, the energy density of the dextrose polymer solution is much greater than that of 10% dextrose. Therefore, a greater proportion of daily caloric needs can be met when identical volumes of each solution are given. Whether increasing the amount of dextrose polymer solution given to dogs would result

in a greater and clinically significant peritonitis warrants further investigation, because this nutrient solution shows great potential for use in intraperitoneal nutrition in dogs.

CHAPTER 6.

- Experimental Summary and Conclusions.
- Future Developments.

SUMMARY AND CONCLUSIONS

The experiments described in this thesis have identified that a repeat abdominal puncture technique using a modified 16 G over-the-needle catheter for the administration of intraperitoneal nutrients is safe, easy to perform, does not require expensive or specialised equipment and, most importantly, is well tolerated by dogs.

The pilot study's objectives were to critically examine the suitability of a total nutrient admixture (TNA) similar to that previously reported to be successful for intraperitoneal nutrition in dogs.[143,144] However, a number of significant problems were identified in this study. The problems were hypovolaemia, hypoalbuminaemia, electrolyte imbalances and clinically significant signs of peritonitis. Additional complications reported in the pilot study were a mild non-regenerative anaemia, hypoglycaemia, leakage of the TNA from the peritoneal cavity into the subcutaneous tissues and a pleural effusion in one dog.

The study reported in Chapter 4 revealed that the lipid component of the TNA used in the pilot study was responsible for the peritoneal inflammatory response, and when compared to dextrose and amino acids (on a kcal for kcal basis), caused a 13 fold greater increase in peritoneal total white cell count (TWCC) when given in sufficient quantities to meet 20% of resting energy requirements (RER). The peritoneal inflammatory response seen in the dogs receiving the lipid emulsion in Chapter 4 was not associated with signs of clinically significant peritonitis. However, it would be prudent to avoid the use of a lipid emulsion of this type for IPN given the potential complications that may occur as a consequence of using the greater quantities of lipid that would be required to provide adequate caloric support to critically ill or injured dogs. The study reported in Chapter 4 revealed similar haematological and biochemical abnormalities to those encountered in the pilot study (mild anaemia, hypoproteinaemia, hypoalbuminaemia and hyponatraemia). These changes are a consistent finding in dogs undergoing intraperitoneal nutrition (IPN). They were

present in the third study of this series (Chapter 5) and have been previously reported by other workers.[138,143,144]

In Chapter 5, we compared 0.9% sodium chloride, 10% dextrose and a 21.5% dextrose polymer[uu] solution with respect to their inflammatory nature when administered into the peritoneal cavity. The use of a saline control group in this study allowed us to separate the effects of a repeat abdominal puncture from that of the irritant nature of the nutrient solutions on the change in peritoneal TWCC when performing IPN. The results of this study revealed the act of "repeat abdominal puncture" had little influence on the peritoneal TWCC during IPN and that a 10% dextrose solution (when given in sufficient quantities to meet 20% of RER) did not cause a significant increase in the peritoneal TWCC after four days of feeding.

Dextrose, as a monomer solution is also well tolerated by the peritoneal membrane when administered as a 10% solution. Whether the peritoneum of dogs would tolerate greater concentrations of dextrose monomer solutions, such as 15%, 20% or even 25% remains unknown. However, glucose solutions of this concentration or greater (as was used in the pilot study) have an osmolality that would be too great to safely administer into the peritoneal cavity without the risk of rapid fluid shifts from the extravascular space into the peritoneal cavity, resulting in hypovolaemia and shock. For sufficient energy to be supplied on a daily basis in the form of a 10% dextrose monomer solution, a large volume would be needed. This cannot be easily achieved as large boluses significantly limit diaphragmatic movement and would impair respiratory function. Leakage of the nutrient solution was encountered in the pilot study as a consequence of large volume boluses being given. It is for these reasons that we assessed the peritoneal response to a 21.5% dextrose polymer solution, which allows a greater proportion of daily energy requirements to be given whilst keeping the volume of administration and osmolality of the nutrient solution within acceptable limits. The 21.5% dextrose polymer was associated with a mild increase in the peritoneal TWCC, which subsequently declined to near baseline cell counts by Day 5 of the study.

[uu] Polycose®. Ross Laboratories, USA.

Infusion of a 5% amino acid solution in sufficient quantities to meet 20% of RER can be safely administered to dogs via the peritoneal cavity. Twenty percent of resting energy requirements in the form of amino acids equates to approximately 1.0 g of protein/kg/day, which although below the minimum daily requirements of adult dogs, would provide significant protein-sparing effects. To give additional protein to dogs via the peritoneal cavity would necessitate a greater amino acid concentration or a larger volume and would potentially result in the same complications described for the dextrose solution above. An alternative protein source that meets the recommended daily protein requirements when administered in small volumes and without excessive hypertonicity would be advantageous.

The changes in peritoneal TWCC (Day 1 vs. Day 5) induced by each of the nutrient and electrolyte solutions used in the three studies that comprised this thesis are shown in Table 6-1. Whilst the results of the different studies can not be directly compared, the experimental protocols were sufficiently similar to provide some insights.

Table 6-1. Comparison of Day 1 and Day 5 peritoneal total white cell counts of the nutrient solutions in the pilot study, second study and third study.

		TNA	Dextrose Study 2	Dextrose Study 3	Amino acids	Lipid	Dextrose polymer	Saline
Mean	Day 1	0.75	0.10	0.06	0.11	0.23	0.06	0.06
s.d.		n.c.	0.14	0.02	0.13	0.29	0.03	0.02
Mean	Day 5	28.95*	0.34	0.29	1.13$^{\Psi}$	13.25$^{\Psi}$	0.82$^{\Psi}$	0.16
s.d.		n.c.	0.58	0.35	0.57	19.99	0.73	0.12

TNA = total nutrient admixture, s.d. = standard deviation, n.c. = not calculated, $^{\Psi}$ = significantly different ($p = <0.05$) from Day 1. * = not statistically compared.

FUTURE DEVELOPMENTS

Further studies are required to determine the maximum amount of dextrose polymer that can be safely administered on a daily basis to dogs and thus the proportion of daily caloric requirements that can be given via the peritoneal cavity. The peritoneal response to a combination of dextrose polymer and crystalline amino acids could then be examined to assess the suitability of this nutrient admixture for IPN in dogs. The development of a protein hydrolysate that could be safely administered intraperitonealy so greater quantities of protein can be provided in a practical manner warrants further investigation. Finally, clinical trials in critically ill or injured dogs will need to be performed before this alternative route for nutritional support in dogs can be widely recommended to the veterinary profession.

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