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**INVESTIGATIONS INTO THE
FEASIBILITY OF THE
INTRAPERITONEAL ROUTE FOR
PROVISION OF NUTRITIONAL
SUPPORT TO COMPANION ANIMALS**

A thesis prepared in partial fulfilment of
the requirements for the degree of
Master of Veterinary Science
Massey University 2000
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ABSTRACT

Poor nutrition is associated with alterations in metabolism and intracellular enzyme activities, defects in the function of multiple body systems and impaired wound healing. Enteral nutrition is preferred over parenteral nutrition in most circumstances. A proportion of patients, however, are not suitable to receive nutrition via the enteral route and require parenteral nutritional support. Unfortunately, the use of intravenous parenteral nutrition is largely confined to referral institutions because of expense, potential complications and lack of 24-hour care facilities. A number of reports in the scientific literature suggest conventional parenteral nutrition solutions are well absorbed after delivery into the peritoneal cavity. This route of nutritional support may provide an alternative to currently available intravenous parenteral nutrition.

The peritoneum is composed of a single layer of mesothelial cells built upon a basal lamina and underlying connective tissue. Within the connective tissue layer is a complex network of lymphatics and blood vessels. Mesothelial cells are biologically active, playing roles in local immune response, host defence and locally regulating inflammation and vascular tone. The large surface area of the peritoneum has huge absorptive potential. Water and solutes are able to traverse the mesothelial cell layer, travel through the interstitium and into the semi-permeable capillary network or lymphatics. Macromolecules are absorbed through dilated mesothelial intercellular spaces or 'stomata' and enter underlying lymphatic channels. These transport properties of the peritoneum have been exploited for many years in the technique of peritoneal dialysis and are the basis of intraperitoneal nutrition.

Amino acids, glucose and lipid solutions have been demonstrated to be absorbed after intraperitoneal administration in rats, rabbits, dogs and people. Experimentally, intraperitoneally delivered nutrient solutions have been used to provide nutritional support for dogs and rabbits for periods up to 30 days. Side effects of intraperitoneal nutrition include fluid shifts into the peritoneal cavity and septic peritonitis. Careful control of

volume and osmolality of the fluid infused and implementation of sterile technique should limit the occurrence of these problems. Current literature would suggest intraperitoneal nutrition is a practical and effective means by which to provide nutritional support.

Originally used as a tool to detect intra-abdominal haemorrhage, diagnostic peritoneal lavage (DPL) is now used as an aid in the diagnosis of a wide variety of medical and surgical conditions of the abdomen. Diagnostic peritoneal lavage is reported to be highly sensitive in the detection of septic peritonitis. The technique of DPL would be easily combined with intraperitoneal nutrient instillation and may provide a useful means of monitoring the peritoneal cavity for potential sepsis during intraperitoneal nutrition.

Two original studies are reported in this work. The first describes the suitability of glucose, amino acid and lipid solutions for instillation into the peritoneal cavity of the cat. Two cats, one who received intraperitoneal amino acids and the other intraperitoneal glucose, developed clinical signs attributable to hypovolaemic shock. Another two cats received two doses of intraperitoneal lipids 24 hours apart and developed clinical signs consistent with peritonitis: abdominal pain, pyrexia and vomiting. Diagnostic peritoneal lavage performed two days after the second intraperitoneal lipid infusion in these cats returned fluid with elevated total nucleated cells counts, an increased proportion of neutrophils and no evidence of infectious organisms. Aerobic and anaerobic culture of DPL fluid grew no bacteria. These findings suggest that the lipid solution used in this study incited a clinically apparent, sterile peritonitis in these two cats.

The second study was undertaken to investigate the effects, in a different species, of intraperitoneal administration of the same lipid solution used in the feline pilot study. Five rats received three doses of intraperitoneal lipid 24 hours apart and another five rats intraperitoneal saline infusions. Diagnostic peritoneal lavage, post mortem and histological examination of peritoneum and selected intra-abdominal organs was performed. No clinical signs of peritonitis were detected in any rat. Lipid treated rats, however, displayed cytological and histological evidence of peritoneal inflammation.

ACKNOWLEDGEMENTS

Thanks to McGaw Biomed for their assistance in formulating and packaging the intraperitoneal nutrient solutions. I'd also like to thank the Animal Health Services Centre of Massey University and staff from the Rat Research Facility of the Crop and Food Division of AgResearch for their co-operation.

I would like to thank my supervisors, Dr Grant Guilford, Frazer Allan and Roz Machon for their advice and guidance. Particular thanks to Frazer for his support, assistance in preparation of this thesis and much appreciated enthusiasm.

I would also like to extend my gratitude to Phillip Clarke, Rebecca Allan, Roz Power and Sheryl Bayliss for their patience and assistance as I learnt to process clinical pathology samples. Wendi Roe provided me with a pathologists view-point of rat peritoneal histology for which I am also very appreciative. Additional thanks to Rebecca and Wendi for their photographic skills.

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CHAPTER 1

GENERAL INTRODUCTION

The provision of nutritional support to veterinary patients has received increasing attention in recent years. We are now becoming aware of the deleterious effects poor nutrition can have on the ill or recovering patient. Poor nutrition and weight loss are associated with alterations in protein and fat metabolism, as well as intracellular enzyme activities.^{1,2} In addition, they have also been associated with defects in the function of skeletal muscle, the respiratory system, the immune system and in wound healing.^{1,2} The benefits of nutritional support for critically ill or traumatised patients are clear. Nutritional support has been provided to veterinary patients in a number of ways, by both enteral and parenteral routes. For most patients the simpler and cheaper methods of enteral nutritional support are the best choice for providing nutritional support. A small proportion of patients, however, have non-functional gastrointestinal tracts and cannot be supplied with nutrition via the enteral route. Traditionally these patients have required parenteral nutritional support via the intravenous route. Intravenous total parenteral nutrition is considered by many veterinarians in general practice to be a complicated and costly procedure and is rarely undertaken outside of referral institutions. Current literature (reviewed in Chapter 3) suggests that the intraperitoneal delivery of nutrient solutions may provide an effective, simple and practical alternative to intravenous nutrition with fewer complications.

Many forms of enteral nutritional support have been used successfully in veterinary patients.² They include appetite stimulation, forced oral feeding and indwelling feeding tubes placed at various locations along the gastrointestinal tract. Each form of nutritional support has indications and relative contraindications, as well as a number of potential

complications. Only a brief discussion of the commonly used methods currently used to provide nutritional support to veterinary patients is presented here.

Many pharmacological agents have been recommended to stimulate voluntary food intake including benzodiazepines, serotonin antagonists and glucocorticoids.³ Controlled studies on the use of these agents as appetite stimulants in veterinary patients are lacking. Anecdotally, benzodiazepines and serotonin antagonists are reportedly effective for psychogenic, fear-induced anorexia seen in hospitalised patients but are unlikely to resolve disease-induced anorexia.³ Forced oral intake, either by force feeding or repeated orogastric intubation, can be achieved in a co-operative patient. Using commercial pet foods, relatively large quantities need to be given to provide sufficient calories.² Long-term use of this mode of nutritional support can become stressful and impractical for patient and operator.² Forced oral feeding may be easier in debilitated patients but care needs to be taken to ensure that swallowing is not impaired due to the increased risk of aspiration.

Naso-oesophageal or nasogastric feeding tubes can be placed in fully conscious or lightly sedated animals and are generally well tolerated for short term feeding.^{4,5} Clinical reports describe the use of such feeding tubes for periods up to two weeks with only minor complications, such as slight nasal irritation. Liquid diets need to be employed due to limitations on the size of tube that can be passed.^{2,5,6} Placement of nasal tubes can be difficult in brachycephalic dog breeds and is contraindicated in animals with nasal disease or facial trauma. Nasal feeding tubes are also not recommended for comatosed patients, those lacking a gag reflex, patients with oesophageal motility disorders or those that are frequently vomiting.⁵ Complications arising from the placement or use of nasal feeding tubes include epistaxis, nasal or laryngeal trauma, tube dislodgement or inadvertent tracheal placement.⁶

Other indwelling feeding tubes require general anaesthesia for placement, including pharyngostomy, oesophagostomy, gastrostomy and enterostomy tubes.^{3,4} If well maintained, these tubes can remain in place for weeks to months. Relatively large bore tubes can be placed for pharyngostomy, oesophagostomy and gastrostomy, conveniently allowing the feeding of blenderised canned food. In contrast, enterostomy tubes require specialised elemental diets, which are often expensive and not specifically designed for veterinary patients.⁷

Numerous complications have been reported with indwelling feeding tubes. Many of these can be avoided when appropriate placement and maintenance protocols are followed. With all feeding tubes, cellulitis, local infection, swelling, and pressure necrosis of the tube exit site can occur with improper or inadequate care.^{5,6} Tubes can become blocked, or damaged by patients necessitating their removal.^{5,6} Other serious complications include: iatrogenic trauma at time of tube placement; respiratory compromise from pharyngostomy tubes; oesophageal inflammation and stricture due to oesophagostomy tubes; peritonitis secondary to premature removal of gastrostomy or enterostomy tubes; and pressure necrosis of the stomach resulting from gastrostomy tubes.^{5,6,7,8}

Providing nutrition via the enteral route has many advantages over parenteral nutrition, some of which have only recently been recognised. Enteral nutrition appears to play a role in maintenance of the gut mucosal barrier and gut associated lymphoid tissue as well as supporting splanchnic perfusion.⁹ Much of the nourishment for the intestinal mucosa comes from the lumen itself; even short periods of starvation can lead to mucosal atrophy.¹⁰ Critically ill patients are susceptible to intestinal mucosal injury, subsequent alterations in gut permeability and failure of intestinal defence mechanisms.¹¹ Bacterial translocation across the gastrointestinal mucosa may play a role in systemic infections and multiple organ dysfunction experienced by some critically ill patients.¹⁰ Enteral nutrition appears to be important in restoring normal gut function and may prevent atrophic

changes in intestinal morphology.¹² Initiation of enteral nutrition early in the course of illness may have a significant beneficial effect for the patient by reduction of septic complications.¹³

Despite its advantages, there is a small proportion of patients requiring nutritional support for which enteral nutrition is unsuitable or inadvisable. Parenteral nutrition is required for patients whose gastrointestinal tracts are temporarily non-functional such as those with impaired intestinal motility, malabsorption, severe diarrhoea, intractable vomiting, massive small intestinal resection, or prolonged pancreatitis.^{6,14} Parenteral nutrition has also been provided to veterinary patients considered poor anaesthetic candidates for surgically placed ostomy tubes.¹⁴

Solutions used for total parenteral nutrition are usually a combination of glucose, amino acids and lipids.¹⁴ In order to provide sufficient protein and calories to meet metabolic requirements these solutions are hypertonic and need to be administered via a central vein in order to avoid thrombosis.¹⁴ Placement and maintenance of central venous catheters can be difficult and numerous complications have been reported. These include sepsis, central vein thrombosis and a variety of systemic metabolic disturbances.^{15,16} Air embolus or serious haemorrhage can occur if the catheter becomes accidentally detached or dislodged by the patient.¹⁵ Mechanical infusion equipment can also present problems such as equipment malfunction and line blockages.¹⁶ Patients receiving intravenous nutrition require regular supervision and frequent monitoring. The expense of providing intravenous nutrition, the required level of care, and the lack of 24-hour facilities in most veterinary hospitals, limit its use.

The transport properties of the peritoneum have been exploited in the management of renal failure patients by peritoneal dialysis for many years.¹⁷ Not only are solutes removed from the body during dialysis but substances present in the dialysis solutions are absorbed.¹⁸ Such observations initiated investigations into the use of the peritoneal cavity

for the delivery of nutrients. Glucose, amino acid, and lipid solutions, have been shown to be absorbed after intraperitoneal administration.^{19,20,21} Investigators have also attempted to provide total parenteral nutrition via this route. Several species have been maintained for periods of up to 30 days on intraperitoneal nutrition.^{22,23} In addition, a number of publications report the clinical use of intraperitoneal nutrition in human patients.^{24,25}

The infusion of a hypertonic solution into the peritoneal cavity has the potential to draw fluid into the cavity from the interstitium and intravascular space.²⁶ Fluid shifts have been of sufficient magnitude to result in hypovolaemic shock and death of animals in some experimental reports of intraperitoneal nutrition.^{22,26} Such complications should be avoidable if the volume and tonicity of the solutions infused intraperitoneally is limited. Unfortunately, at this time, there are no guidelines regarding what volume or osmolality of solution can safely be administered intraperitoneally.

A potentially serious complication of intraperitoneal nutrition is the development of septic peritonitis. Combining all reports of long-term intraperitoneal nutrition in dogs, a total of five days of peritonitis were detected in approximately 1,300 days of nutritional support.^{23,27} In all cases, peritonitis was quickly and easily resolved with systemic antibiotics and temporary cessation of intraperitoneal nutrient delivery.

Although apparently an uncommon complication, it would be desirable to have a surveillance system for the detection of septic peritonitis should it occur. Early detection of intraperitoneal sepsis would enable prompt treatment and reduce the potential for serious sequelae. Diagnostic peritoneal lavage has been associated with a high sensitivity in the detection of septic peritonitis.²⁸ In addition, diagnostic peritoneal lavage could easily be combined with procedure of intraperitoneal nutrient infusion and provides a means for the veterinarian to monitor cellular, and possibly septic, changes within the peritoneal cavity.

Intraperitoneal nutrition has previously been shown to induce inflammatory or hypertrophic changes to the peritoneum.^{22,23,26} Erythema and thickening of the peritoneum has been grossly noticeable at laparotomy or necropsy.^{22,23,26} This is thought to be due, in part, to compensatory changes of the peritoneum in response to increased absorptive demands. The hypertonicity of many nutrient solutions may be a possible contributing factor to the inflammatory changes. Histologically, hyperplasia and hypertrophy of the peritoneal mesothelium are reported.^{23,26,27} Diagnostic peritoneal lavage has not been used to monitor the peritoneal cavity in previous intraperitoneal nutrition studies. Some changes in the cellularity of the fluid collected from DPL are expected and new reference ranges for cytological data may need to be established. Cytological appearance of the DPL fluid sample, in addition to nucleated cell numbers, will be important in evaluation for sepsis.

Intraperitoneal nutrition appears to be a promising potential route for nutrient delivery in veterinary patients. Amino acids, glucose and lipid solutions have been shown to be absorbed, individually and in combination, from the peritoneal cavity of a number of species. Nutrient solutions have been delivered intraperitoneally in a bolus fashion giving it a practical advantage over intravenous nutrition.^{21,23} Providing sterile technique is maintained and precautions observed, complications encountered during intraperitoneal nutrition have reportedly been minor and infrequent. The technique of intraperitoneal nutrition appears to be safe, effective and practical. It deserves serious investigation as a potential route for provision of parenteral nutrition to veterinary patients.

REFERENCES

1. Thatcher CD. Nutritional needs of critically ill patients. *Compendium on Continuing Education for the Practicing Veterinarian*. 1996;**18**:1303-1313
2. Crowe DT. Enteral nutrition for the critically ill or injured patient – part I. *Compendium on Continuing Education for the Practicing Veterinarian*. 1986;**9**:603-612
3. Buffington CAT. Enteral nutritional support. *Proceedings, IV International Veterinary Emergency and Critical Care Society Meeting*, San Antonio, Texas. 1994 pp 327-330
4. Tennant B, Willoughby K. The use of enteral nutrition in small animal medicine. *Compendium on Continuing Education for the Practicing Veterinarian*. 1993;**15**:1054-1068
5. Armstrong PJ. Enteral feeding of critically ill pets: The choices and techniques. *Veterinary Medicine*. 1992;**87**:900-909
6. Armstrong PJ, Lippert AC. Selected aspects of enteral and parenteral nutritional support. *Seminars in Veterinary Medicine and Surgery*. 1988;**3**:216-226
7. Crowe DT. Enteral nutrition for critically ill or injured patients – Part III. *Compendium on Continuing Education for the Practicing Veterinarian*. 1986;**8**:826-838
8. Crowe DT. Enteral nutrition for critically ill or injured patients – Part II. *Compendium on Continuing Education for the Practicing Veterinarian*. 1986;**8**:719-732
9. Purcell PN, Davis K, Branson RD *et al*. Continuous duodenal feeding restores gut blood flow and increases gut oxygen utilisation during PEEP ventilation for lung injury. *American Journal of Surgery*. 1993;**165**:188-193
10. Deitch EA. Nutrition and the gut mucosal barrier. *Current Opinion in General Surgery*. 1993:85-91
11. Barber AE, Jones WG, Minei JP *et al*. Bacterial overgrowth and intestinal atrophy in the etiology of gut barrier failure in the rat. *American Journal of Surgery*. 1991;**161**:300-304
12. Buchman AL, Moukarzel AA, Bhuta S *et al*. Parenteral nutrition is associated with intestinal morphologic and functional changes in humans. *Journal of Parenteral and Enteral Nutrition*. 1995;**19**:453-460

13. Groos S, Hunefeld G, Luciano LJ. Early enteral feeding, compared with parenteral, reduces postoperative septic complications. The results of a meta-analysis. *Annals of Surgery*. 1992;**216**:172-183
14. Buffington CAT. Parenteral nutrition. *Proceedings, IV International Veterinary Emergency and Critical Care Society Meeting*, San Antonio, Texas. 1994 pp 339-341
15. Lippert AC, Fulton RB, Parr AM. A retrospective study of the use of total parenteral nutrition in dogs and cats. *Journal of Veterinary Internal Medicine*. 1993;**7**:52-64
16. Reuter JD, Marks SL, Rogers QR *et al*. Use of total parenteral nutrition in dogs: 209 cases (1988-1995). *Journal of Veterinary Emergency and Critical Care*. 1998;**8**:201-213
17. Carter LJ, Wingfield WE, Allen TA. Clinical experience with peritoneal dialysis in small animals. *Compendium on Continuing Education for the Practicing Veterinarian*. 1989;**11**:1335-1343
18. Hanning RM, Balfe JW, Zlotkin SH. The effect of amino acid containing dialysis solutions on plasma amino acid profiles in children with chronic renal failure. *Journal of Pediatric Gastroenterology and Nutrition*. 1987;**6**:942-947
19. Gilsdorf RB, Selby R, Tillach P. Systemic appearance of nutrients placed into the peritoneal cavity. *Journal of Parenteral and Enteral Nutrition*. 1985;**9**:148-152
20. Stabile BE, Borzatta M. Transperitoneal absorption of glucose and amino acids for nutritional support. *Archives of Surgery*. 1987;**122**:344-348
21. Klein MD, Coran AG, Drongowski RA *et al*. The quantitative transperitoneal absorption of a fat emulsion: Implications for intraperitoneal nutrition. *Journal of Pediatric Surgery*. 1983;**18**:724-731
22. Stone MM, Mulvihill SJ, Lewin KJ *et al*. Long-term total intraperitoneal nutrition in a rabbit model. *Journal of Pediatric Surgery*. 1986;**21**:267-270
23. Garcia-Gamito FJ, Moran JM, Mahedero G *et al*. Long-term peritoneal nutrition in dogs, both normal and after intestinal resection. *International Surgery*. 1991;**76**:235-240
24. Giordano C, Capodicasa G, De Santo NG. Artificial gut for total parenteral nutrition through the peritoneal cavity. *International Journal of Artificial Organs*. 1980;**3**:326-330

25. Merrit RJ, Atkinson JB, Whalen TV *et al.* Partial peritoneal alimentation in an infant. *Journal of Parenteral and Enteral Nutrition.* 1988;**12**:621-625
26. Gilsdorf RB, Selby RR, Schon DA *et al.* Total nutritional support through the peritoneal cavity. *Journal of the American College of Nutrition.* 1985;**4**:461-469
27. Moran JM, Garcia-Gamito FJ, Mahedero G *et al.* Peritoneal nutrition in dogs after intestinal resection: A comparative study. *Nutrition.* 1989;**5**:315-320
28. Crowe DT. Diagnostic abdominal paracentesis techniques: Clinical evaluation in 129 dogs and cats. *Journal of the American Animal Hospital Association.* 1984;**20**:223-230

CHAPTER 2

ANATOMY AND PHYSIOLOGY OF THE PERITONEAL CAVITY

2.1 ANATOMY OF THE PERITONEAL CAVITY

The peritoneal cavity is a potential space contained largely within the abdominal cavity but also extending into the pelvic cavity.¹ In males, it also extends into the scrotal cavity. It is formed by the peritoneum, a serous membrane which lines the cavity as well as covering and reflecting from organs within the peritoneal cavity.¹

There are two forms of peritoneum, parietal and visceral.¹ Parietal peritoneum covers most of the inner surface of the walls of the abdominal, pelvic and scrotal cavities. The visceral peritoneum covers, entirely or in part, the organs contained within those cavities. A double sheet of peritoneum extends between organs or connects organs to the parietal peritoneum. Mesenteries, omenta and ligaments within the peritoneal cavity are composed of these folds of peritoneum. Their nomenclature depends on the size and location of these folds, and the vessels which pass within them.¹

In the male, the peritoneal cavity is a closed space, whereas in the female, there is an opening at the cranial end of each uterine horn and thus a potential connection from the peritoneal cavity to the exterior.¹ No organs or tissues are normally contained within the peritoneal cavity, with the exception of oocyst/s at the time of ovulation in intact females. Organs or structures lying against the walls of the abdominal or pelvic cavity and only covered on one surface by peritoneum are said to be retroperitoneal.¹ Most of these organs are small and embedded in fat, including the kidneys and adrenal glands. Organs projecting freely into the abdominal, pelvic or scrotal cavities and receiving nearly a complete covering of peritoneum are termed intraperitoneal.¹

The 'peritoneal membrane' is the cellular and extracellular elements between the peritoneal cavity and the peritoneal vessels. It is comprised of a single layer of mesothelial cells overlying a basal lamina and connective tissue.^{2,3} Many investigators report the occurrence of three types of peritoneal mesothelial cells: flat cells, high cells, and transitional forms.² Other authors consider this an artifactual finding, and claim high cells are the result of temporary mesothelial cell swelling or a consequence of pathology. Differences in cellular size and configuration, intracellular organelles and specialisation, however, appear to distinguish these cells as separate populations. All mesothelial cells possess surface microvilli, their length and density varying with location within the peritoneal cavity and potentially with alterations in physiological conditions.^{2,3}

Mesothelial flat cells are the most numerous. Scanning electron microscopy demonstrates these cells to be irregularly polygonal or rounded.^{5,6} Borders between adjacent flat cells are indistinct due to the numerous microvilli in the intercellular zone. Transmission electron microscopy reveals these cells have an elongated nucleus with scant heterochromatin and thin cytoplasm.⁵ Mitochondria are limited in number but microvesicles are numerous.⁵

High cells are distributed more scantily, and are typically found in the mesothelium covering the spleen, bladder, uterus and rectum.² Large clusters of these cells have been described in the area of the diaphragmatic stomata.^{2,5} High cells are smaller in diameter than flat cells and their cellular borders more discrete.² Transmission electron microscopy reveals these cells have an indented nucleus, rich in heterochromatin, often with nucleoli.⁵ The golgi zone is larger and intracellular organelles are more prominent and numerous than those of flat cells.⁵ Numerous microvilli are found on the apical surface of mesothelial high cells.⁵

Several types of mesothelial cell junctions have been reported including tight junctions, anchoring junctions and gap junctions.^{4,5} Tight junctions function to seal the mesothelial cells together in sheets and are predominant in the apical zones of contact between mesothelial cells.⁴ Typically these junctions exclude the passage of water and solutes; however, their permeability varies between epithelia.⁴ There is evidence that the 'tightness' of these junctions can be modified to regulate flow of material through them but the exact function of mesothelial tight junctions is unclear.

Anchoring junctions mechanically attach cells to neighboring cells or to underlying basement membrane. Those found in the mesothelium are of two types: adherens junctions and desmosomes.^{4,5} Adherens junctions are located just below the tight junctions and connect cytoskeletal elements of adjacent mesothelial cells. Desmosomes are deeper points of intercellular contact and function as an anchoring site for intermediate filaments. Intermediate filaments are well developed in mesothelial cells and form part of the cellular cytoskeleton necessary to resist cellular detachment by frictional forces.⁴

The mesothelium is also reported to contain gap junctions.⁴ Gap junctions are dynamic membrane channels allowing molecules to pass directly from cell to cell. These junctions therefore enable electrical and metabolic coupling of cells.

Mesothelial intercellular spaces are generally described as narrow and tortuous but widened or rounded intercellular spaces also occur.^{4,6} Scanning electron microscopy has also revealed round or oval shaped orifices bordered by mesothelial cells which have been termed peritoneal stomata.⁷ The difference between these 'dilated' intercellular spaces and the controversial peritoneal stomata are unclear. Stomata are reported to often be covered by cellular processes or microvilli. Occasionally they are described as being in an open state, exposing components of the submesothelial connective tissue beneath them.⁷

Many studies suggest peritoneal stomata are restricted to the mesothelium lining the peritoneal surface of the diaphragm.^{8,9} There are, however, reports of stomata being detected at other sites including ovarian, pelvic, mesenteric, omental, hepatic and abdominal wall peritoneum.⁷ Some authors have not detected peritoneal stomata and question their existence.⁶ This discrepancy may be accounted for by variations in definition of true 'stomata'. Abu-Hijleh *et al*¹⁰ suggest that true stomata must have an inner lymphatic endothelial orifice rather than being just a dilated intercellular space.

A network of elastic fibres, the peritoneal elastic lamina, is found beneath the mesothelial basement membrane.^{2,11} This elastic layer is found throughout the visceral and parietal peritoneum but its thickness varies with location.¹¹ Within the submesothelial connective tissue, collagen fibres, predominantly type I and III, form a lattice within which cells can migrate and interact.⁴ Fibroblasts, macrophages, adipocytes and mast cells also reside within the connective tissue layer.^{2,3}

Lymph vessels are located near the surface of the connective tissue.³ These lymph vessels can be very large and give rise to lymphatic lacunae reaching 3-6 mm in diameter.³ The endothelium of these vessels is thin and the basement membrane incomplete.³ Deeper within the connective tissue lie peritoneal blood vessels, capillaries and post-capillary venules.³

The surface area of the peritoneum is large. Post-mortem findings in a number of species have proposed a variety of values for peritoneal surface area. In the rat it has been estimated as 595cm², man 7,792cm² and rabbits 820cm².³ In man and rat there appears to be no correlation between peritoneal surface area and body area, weight, or xyphoid to pubis distance.¹²

2.2 PERITONEAL PHYSIOLOGY

The peritoneum serves to reduce the friction between organs and tissues of the peritoneal cavity which would otherwise occur with peristalsis and respiration. By expressing microvilli and secreting various substances, the mesothelial layer of the peritoneum provides a protective and lubricated surface. In people and animals the mesothelium is known to secrete a surface active material consisting primarily of phospholipids.³ This hydrophilic, surfactant material is highly lubricant.³

The mesothelium is an active and responsive biological membrane. Its normal functions are only beginning to be uncovered. Cultured mesothelial cells have been shown to secrete a variety of substances including prostaglandins, interleukins and many unidentified proteins.³ Many of these substances are potent vasodilators or vasoconstrictors, suggesting a role for mesothelial cells in modulating peritoneal microcirculation.

Mesothelial cells play a role in the host defence mechanism by secreting a variety of cytokines which act as chemoattractants and influence cellular adhesion.⁴ Other roles played by mesothelial cells in host defence include ingestion of bacteria and antigen presentation.^{4,13} By secreting both pro-inflammatory and anti-inflammatory substances, the mesothelium may be pivotal in controlling the peritoneal response to injury or inflammation.⁴

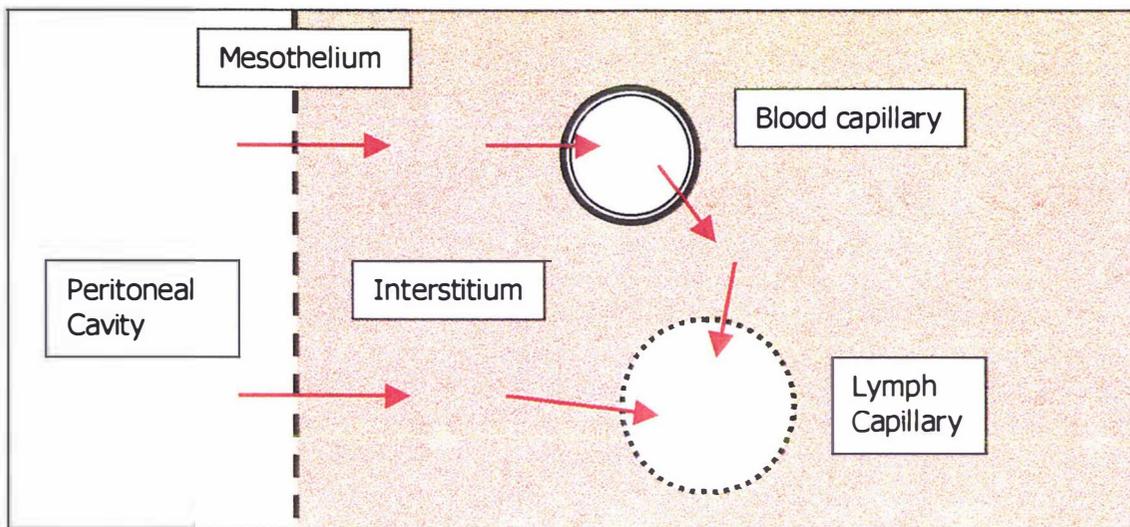
The mesothelium is normally non-thrombogenic.⁴ Cultured mesothelial cells have been demonstrated to secrete fibrinolytic substances, such as tissue plasminogen activator, in addition to fibrinolytic inhibitors. Mesothelial cells therefore may play a role in regulating fibrin deposition.⁴ Mesothelial cells may also contribute to repair and remodelling of the peritoneal membrane. Cultured mesothelial cells produce a variety of extracellular matrix constituents such as collagens, elastin and glycoproteins. They also synthesise and secrete enzymes responsible for degradation of matrix components.⁴

Below the mesothelium, collagen fibres of the connective tissue layer provide strength and absorb stress as well as acting as a frame work for the migration and interaction of other cells.⁴ The peritoneal elastic lamina contributes to the resilience of the peritoneal membrane.¹¹

2.3 PERITONEAL TRANSPORT

In the past, peritoneal transport models have focused on the idea that the peritoneum behaves as a single semi-permeable membrane but there is little support for this concept, either clinically or physiologically.¹⁴ Peritoneal blood vessels are not bathed directly in solutions placed within the peritoneal cavity. Discrete microvessels are located within the tissue spaces surrounding the peritoneal cavity. A varying amount of interstitial tissue therefore separates these vessels from the peritoneal cavity. For a substance to be absorbed from the peritoneal cavity into the systemic circulation it must pass through the mesothelium, into the interstitial space and then into blood vessels or lymphatics contained within the interstitium. This concept is demonstrated diagrammatically in Figure 2.1.

Most authors agree that the peritoneal mesothelium presents little barrier to solute passage.^{14,15} This is supported by *in vitro* studies. Conversely, large differences in peritoneal permeabilities have been noted when *in vivo* studies are compared to *in vitro* studies. Rapid degeneration of the peritoneal mesothelium has been demonstrated to occur *in vitro* and may explain these experimental findings.¹⁶ This membrane degradation casts uncertainty on the value of *in vitro* models of peritoneal permeability. *In vivo* studies and clinical experience of peritoneal dialysis, however, appear to confirm the hypothesis that the mesothelium does not present a significant osmotic barrier to solute absorption.^{16,17}

Figure 2.1 : Major elements in the peritoneal transport pathway

The exact mechanism by which substances are transported across the mesothelium is not certain. It is likely that multiple transport processes exist. Based on morphological studies, pinocytosis has been proposed as a mechanism of transmesothelial protein transport.¹⁸ Physiological studies however, indicate that transmesothelial protein transport occurs through intercellular junctions.¹⁹ These intercellular junctions may potentially be peritoneal stomata.

Peritoneal lymphatics play an important role in absorption from the peritoneal cavity. Fluid, solutes and macromolecules enter lymphatic lacunae via widened intercellular junctions or stomata. As previously mentioned, the existence of stomata is debated by some but accepted by most.^{6,7,8,9} Another point of controversy is the distribution of stomata throughout the peritoneal cavity. The diaphragm appears to be their most prominent location.¹⁰ Once entry has been gained into diaphragmatic stomata, the diaphragm is traversed via intrinsic lymphatics which empty into collecting ducts.¹⁰ Collecting ducts drain predominantly into parasternal lymphatic trunks from which entry is gained into the venous circulation.¹⁰

Substances absorbed from the peritoneal cavity via peritoneal capillaries and lymphatics traverse varying thicknesses of interstitial tissue. The peritoneal interstitium has been described as a two-phase model in which a colloid-rich, water-poor phase is equilibrated with a colloid-poor, water-rich phase.²⁰ Mucopolysaccharides comprise much of the colloid-rich phase, or ground substance. Water may travel freely throughout the interstitium. However, proteins and other solutes are, to a varying degree, excluded from the colloid-rich phase. In the normally hydrated individual, it has been estimated that from 10-30% of the interstitium is available for transport of small solutes, and 5-15% available for macromolecules.^{15,16} Random distribution of the two phases requires that solutes traverse a tortuous and potentially lengthy path to pass through the interstitium.^{15,16} Due to this volume exclusion, tortuosity and the interactions which occur between the colloid-rich phase and solutes, diffusion through the interstitium occurs at rates much slower than through an equivalent thickness of water. The interstitium is thus able to attenuate the magnitude of osmotic and hydrostatic pressure changes within the peritoneal cavity and provides some barrier to diffusion.¹⁶

Concentrations of solutes within the interstitium have been measured after intraperitoneal delivery. From these studies it has been concluded that transport across the visceral peritoneal interstitium is primarily due to diffusion.¹⁵ Transfer of substances across the peritoneum also occurs with bulk movement of water, a process termed convection.¹⁵ In solute transfer across the parietal peritoneum there is a significantly greater contribution from convection than diffusion.¹⁵ High concentrations of solutes are demonstrated in the diaphragm, likely due to the presence of stomata and specialised lymphatic channels. Macromolecular transport, in particular protein transport, has been shown to occur predominantly by convection through parietal peritoneum.¹⁵

Hydrostatic forces also have an effect on fluid transport across the peritoneum. Interstitial hydrostatic pressure has been demonstrated to fall with dehydration but remains constantly between 0 and 1 mmHg despite significant oedema of the peritoneal interstitium.¹⁵ The addition of a fluid volume into the peritoneal cavity increases intraperitoneal pressure which tends to drive fluid into interstitial tissues. The interstitium maintains its hydrostatic pressure at normal levels despite this increased fluid volume. Thus, the driving gradient for fluid absorption will only be dissipated by decreasing the peritoneal fluid volume. The placement of hypertonic solutions into the peritoneal cavity initially overpowers this hydrostatically driven fluid absorption. Fluid is drawn into the peritoneal cavity until the osmotic pressure decreases and the fluid flux reverses.¹⁵

Having entered the interstitium, substances must be transported across capillary membranes or into lymphatic channels to gain entry into the systemic circulation. Transport of substances into blood could be limited by its removal from the capillary, i.e. blood flow. Alternatively, absorption may be limited by passage of the substance across the endothelial membrane. Using the rate of disappearance of gases delivered into the peritoneal cavity, peritoneal blood flow has been estimated as being 4-7% of cardiac output.¹⁶ Diffusion impairment imposed by the interstitium was not considered, so this may be an under-estimate of peritoneal blood flow. Most authors agree that peritoneal blood flow is not a limiting factor to peritoneal transport; however, this has not been convincingly demonstrated.¹⁵

The capillary wall is considered a major barrier to transperitoneal solute transport.¹⁴ The 'three-pore theory' of peritoneal capillary transport is the most comprehensive and physiologically plausible explanation for the barrier properties of the 'peritoneal membrane' described so far.^{14,15} According to this model, the primary route for movement of water and water-soluble solutes is through ultra-small, small or large pores in the

capillary wall, while lipid-soluble substances are able to permeate endothelial cell membranes.

The endothelial cell wall is to some extent permeable to water. Water passage occurs through ultra-small channels in the endothelium which have a radius of less than 0.8 nanometers.^{14,15} Such pores comprise only a small amount of the transcellular pore area and are impermeable to solutes.^{14,15} It is likely that these pores are formed by specialised intramembrane proteins.¹⁴ As solutes are excluded, osmotic pressure difference is the predominant driving force for the transfer of water through these pores.¹⁵

A large proportion of transcapillary water and small solutes transfer occurs through pores with a radius of four to six microns. These 'small pores' comprise approximately 90-93% of the transcellular pore area and represent clefts between endothelial cells^{14,15}. Water and small solutes are allowed free passage through these pores but macromolecules are generally excluded. The bulk of fluid transport across the peritoneum occurs through these pores. Because the osmotic force of small solutes is rapidly minimised, the net osmotic force is due mainly to protein concentration. Both hydrostatic and osmotic forces operate to affect fluid transport through these pores.

Transcapillary passage of macromolecules also occurs and pores with a radius greater than 20 microns are described.¹⁵ It is likely that these pores represent widened or modified clefts between endothelial cells.¹⁴ Such pores offer little resistance to transport of any molecules; as such there is essentially no osmotic force present across them. These pores are few in number and contribute little to bulk fluid transport compared with other pores.^{14,15} Capillary hydrostatic pressure is always higher than that of interstitial tissue. Therefore, protein transport is primarily unidirectional, out of the capillary, as a result of convective forces (ie. proteins are 'dragged' out with the bulk flow of water). This means macromolecules cannot be transported from interstitium to capillary directly

but must traverse the lymphatic system to enter the circulation, emphasising the role of lymphatics in macromolecular absorption from the peritoneal cavity.¹⁵ This “three pore theory” of capillary membrane transport is depicted diagrammatically in Figure 2.2.

Figure 2.2: The three-pore theory for capillary membrane transport

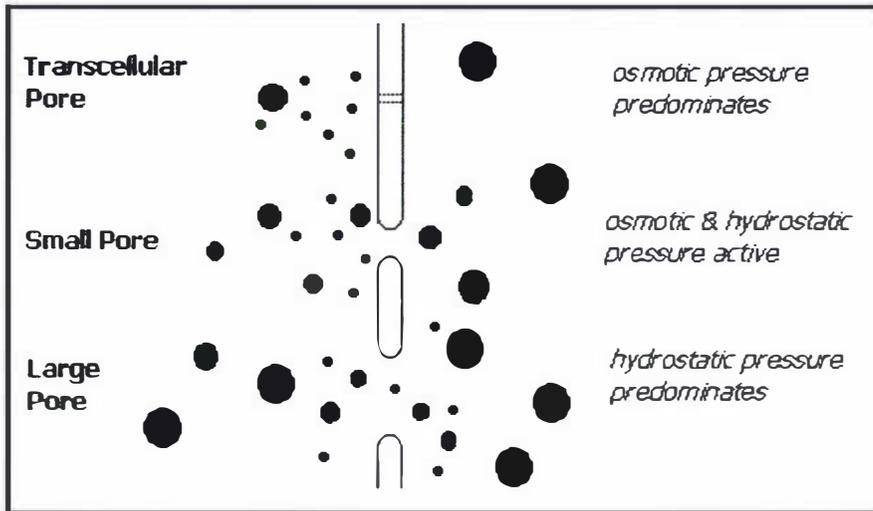


Figure 2.2: Small solid circles represent small solutes and large solid circles represent protein molecules.

Much remains to be understood regarding peritoneal transport physiology. The mechanisms and rate of transport of substances may be greatly influenced by physiological state, presence of local vasoactive substances and pathological processes. Intraperitoneal nutrition may influence peritoneal transport by osmotically-induced fluid shifts and changes induced in mesothelial cells. The exact impact of these factors on peritoneal absorption of nutrients is not known.

REFERENCES

1. Miller ME, Christensen GC, Evans HE. Chapter 13: Digestive system and abdomen. In: *Anatomy of the Dog*. WB Saunders Co. 1964 pp 672-679
2. Michailova KN. A combined electron microscopic investigation of the peritoneal mesothelium in the rat. *European Journal of Morphology*. 1995;**33**:265-277
3. Di Paolo N, Sacchi G. Anatomy and physiology of the peritoneal membrane. *Contributions in Nephrology*. 1990;**84**:10-26
4. Nagy JA. Peritoneal membrane morphology and function. *Kidney International*. 1996;**50**(suppl 56):S2-S11
5. Michailova KN. A combined electron microscopic investigation of the peritoneal mesothelium in the rat. *European Journal of Morphology*. 1995;**33**:265-277
6. Slater NJ, Raftery AT, Cope GH. The ultrastructure of human abdominal mesothelium. *Journal of Anatomy*. 1989;**167**:47-56
7. Wassilev W, Wedel T, Michailova K *et al*. A scanning electron microscopy study of peritoneal stomata in different peritoneal regions. *Annals of Anatomy*. 1998;**180**:137-143
8. Tsilibary EC, Wissig SL. Light and electron microscopic observation of the lymphatic drainage units of the peritoneal cavity of rodents. *American Journal of Anatomy*. 1987;**180**:195-207
9. Abu-Hijleh MF, Reid O, McGadey J *et al*. Distribution of mesothelial stomata in the rat. *Clinical Anatomy*. 1994;**7**:189-203
10. Abu-Hijleh MF, Habbal OA, Moqattash ST. The role of the diaphragm in lymphatic absorption from the peritoneal cavity. *Journal of Anatomy*. 1995;**186**:453-467
11. Knusden PJT. The peritoneal elastic lamina. *Journal of Anatomy*. 1991;**177**:41-46
12. Rubin BJ, Clawson BS, Planch A *et al*. Measurements of peritoneal surface area in man and rat. *American Journal of Medical Science*. 1988;**295**:453-458
13. Valle MT, Degl'Innocenti ML, Bertelli R *et al*. Antigen presenting function of human peritoneum mesothelial cells. *Clinical and Experimental Immunology*. 1995;**101**:172-176

14. Rippe B. A three-pore model of peritoneal transport. *Peritoneal Dialysis International*. 1992;**13**:S35-S38
15. Flessner MF. Peritoneal transport physiology: Insights from basic research. *Journal of the American Society of Nephrology*. 1991;**2**:122-135
16. Flessner MF. The importance of the interstitium in peritoneal transport. *Peritoneal Dialysis International*. 1996;**16**:S76-S79
17. Flessner MF. Osmotic barrier of the parietal peritoneum. *American Journal of Physiology*. 1994;**267**:F861-F870
18. Shostak A, Gotloib L, Jaichenko J *et al*. Transcytosis of intraperitoneally injected albumin in mice peritoneum (abstract). *Journal of the American Society of Nephrology*. 1990;**1**:392
19. Regoeczi E, Zaimi O, Chindemi PA *et al*. Absorption of plasma proteins from peritoneal cavity of normal rats. *American Journal of Physiology*. 1989;**256**:E447-E452
20. Weiderhielm CA. The interstitial space. In: *Biomechanics: Its Foundations and Objectives*. Ed: Fung YC, Perrone N, Andeker M. Prentice-Hall, N.J., USA. 1972:273-286

CHAPTER 3

**INTRAPERITONEAL NUTRITION:
A REVIEW OF THE LITERATURE**

3.1 INTRODUCTION

Recognition of the peritoneum's ability to function as a semi-permeable membrane resulted in the development of peritoneal dialysis for the management of chronic renal failure. During peritoneal dialysis, osmotically active substances, typically glucose and/or amino acid solutions, are infused intraperitoneally to draw fluid and blood solutes into the peritoneal cavity. The filtering function of the kidney is thus mimicked by the peritoneum and solute waste is excreted when the peritoneal fluid is drained. However, a bidirectional flow of solutes has been noted to occur during peritoneal dialysis.¹ Elevated serum levels of glucose and amino acids are detectable after infusion of peritoneal dialysis solutions. The concept arose that intraperitoneal administration of nutrient solutions may serve as an alternative route for the provision of nutritional support. A number of studies have examined individual nutrient absorption across the peritoneal cavity as well as attempts to provide total parenteral nutrition via this route.

3.2 ABSORPTION OF INTRAPERITONEALLY ADMINISTERED GLUCOSE

Many investigators have demonstrated that glucose is rapidly and almost totally absorbed from the peritoneal cavity.^{2,3,4} Serum glucose concentrations rise quickly after intraperitoneal glucose administration with peak levels occurring within 15 to 30 minutes.^{2,3,4} More than 80% of the total amount of glucose absorbed after intraperitoneal delivery occurs within the first two hours.⁵

Attempts have been made to quantify the maximum rate of peritoneal glucose absorption. This task is difficult because multiple factors influence the rate and method of glucose absorption. Both the volume infused and the concentration of glucose in the solution have been shown to influence peritoneal glucose absorption.³ In this study, the rate of glucose absorption rose as the concentration of glucose in the solution increased and as the volume of solution delivered into the peritoneal cavity was increased. The rises however, did not occur in a linear fashion. The exact nature of the complex effects of concentration, osmolality and volume on absorption from the peritoneal cavity is yet to be fully elucidated.

Glucose molecules are osmotically active and anything other than a dilute solution, for example 5% dextrose, is hypertonic with respect to plasma. Intraperitoneal instillation of a hypertonic solution draws fluid from the interstitial and vascular space into the peritoneal cavity. The magnitude of the fluid shift that results is variable. Death as a result of hypovolaemic shock secondary to instillation of large volumes of hypertonic solutions into the peritoneal cavity has been reported.^{6,7}

The bioavailability of glucose eight hours after intraperitoneal administration is 94% (+/- 4% SD).⁸ This observation demonstrates the great potential for the provision of nutrients via the intraperitoneal route but also points to the potential for excessively rapid administration, resulting in fluctuations in blood glucose levels. It has been demonstrated that glucose polymers are well absorbed after intraperitoneal administration, producing slightly lower but more sustained elevations in blood glucose concentrations.² An additional benefit of glucose polymers is that they are less osmotically active than glucose. A 20% glucose solution has an osmolality of approximately 1200 mOsmol/L compared to a 20% solution of a glucose polymer, *D*-xylose, which provides equivalent gm/L of glucose but has an osmolality of 380 mOsmol/L.² Thus the potential exists to

provide calories without hypertonicity. The pathways by which glucose polymers are metabolised once they have reached the systemic circulation are unknown.

3.3 ABSORPTION OF INTRAPERITONEALLY ADMINISTERED AMINO ACIDS

Amino acids used in addition to, or instead of, glucose in peritoneal dialysis solutions have established benefits for the patient. When the dialysate does not contain amino acids, patients become depleted of proteins and amino acids, developing a condition known as "depletion syndrome".^{2,9} A significant rise in total body nitrogen is noted in those patients in which amino acids are used in the dialysate.¹⁰ As a component of parenteral nutrition solutions, amino acids provide a source of nitrogen, essential amino acids and calories. Various mixtures of amino acids are commercially available for intravenous use. The ability of these amino acid solutions, and individual amino acids, to be absorbed from the peritoneal cavity has been investigated.^{2,4,5}

Amino acid solutions are rapidly absorbed from the peritoneal cavity. Peak plasma amino acid concentrations are reached within 15 to 20 minutes.^{2,4} One study reports this rise dissipating relatively quickly, with plasma amino acid values returning to baseline one hour after intraperitoneal infusion.² Over 90% of the amino acids were absorbed from the peritoneal cavity within the first two hours.⁵ Absorption of the amino acid alanine has also been described to fit this pattern.⁸

The ability of amino acids to be metabolised is likely to impact upon their absorption rates from the peritoneal cavity. Small molecular weight substances, such as amino acids, are absorbed through capillary pores and drain towards the portal circulation.¹¹ As such, these substances must pass through the liver prior to reaching the systemic circulation. A substance that is easily and rapidly metabolised would be cleared faster thus creating a greater concentration gradient across the peritoneum favouring its absorption. A study on

the absorption of the D-form of the amino acid alanine, which cannot be metabolised, compared to the metabolisable L-form gives support to this theory.⁸

As is the case with glucose, the bioavailability of intraperitoneally administered amino acids is high, being 90% (+/- 11% SD) at eight hours.⁸ Amino acid solutions are absorbed rapidly from the peritoneal cavity and the capacity for absorption is great. Commercially available amino acid solutions are significantly hypertonic with respect to plasma and carry similar concerns in that respect to intraperitoneal glucose administration.

3.4 ABSORPTION OF INTRAPERITONEALLY ADMINISTERED LIPIDS

Emulsified fats are a concentrated form of calories and exert little osmotic pressure.¹² Such properties are advantageous when compounding parenteral nutrient solutions. They allow reductions in volume and tonicity of the solutions compared to solutions based on glucose and amino acids alone. Additionally, lipids have been shown to be a necessary component of total parenteral nutrition formulas in people in order to avoid essential fatty acid deficiency.¹³

An early report describing intraperitoneal administration of lipid for nutritional purposes involved 34 critically ill human patients.¹⁴ Vascular access in these patients was limited and they were judged unsuitable for enteral nutritional support. In total, 53 doses of 10, 20 or 40% lipid emulsion^a, each of 200 to 500 ml volumes, were delivered intraperitoneally by percutaneous injection. Exact protocols for each patient were not specified. Lipid absorption was assessed by saline abdominal lavage and calculation of the triglyceride content of resultant fluid. On average, 32 grams of fat per day were absorbed (range 0 to 80 gm). It should be noted that this method of assessing lipid absorption is crude and subject to a high degree of error.

^a Intralipid®, source not specified.

Further research investigating the absorption of intraperitoneally administered lipid in a variety of animal species followed in the mid to late 1980s. The results of seven published studies investigating lipid absorption from the peritoneal cavity of dogs, rats and rabbits are summarised in Table 3.1. Multiple protocols were used with respect to lipid source, concentration and volume administered, as well as species of animal studied. These studies have shown that lipid absorption does occur after instillation into the peritoneal cavity of dogs, rats and rabbits but that it appears to occur at a slower rate than glucose and amino acid absorption.

Table 3.1: Intraperitoneal Lipid Absorption Studies in Animals: A Summary

Species	n.	Body wt (kg) (mean)	Solution	Conc. (%)	Volume (ml/kg)	Fat Dose (mg/kg)	Experimental Results	Ref.
Rabbits	9	2.3-3.0	Intralipid ^a	20	16.7	3.34	1.7g TG absorbed in first hour	¹⁵
Rabbits	8	2.3-3.0	Intralipid ^a	20	16.7	3.34	50% S.Int resection resulted in 30% reduction in TG absorption	¹⁵
Rats	n.s.	0.4	n.s.	5	14	0.7	Serum [TG] elevated at 90-120 mins and peaked at 150 mins	²
Rabbits	2	2.0	Lipofundin ^b	20	1	0.2	Serum TG peak at 2 hrs, returned to baseline by 7 hrs. Serum lipid peak at 2 hrs and baseline by 3 hrs.	⁴
Dogs	12	4	Liposyn ^c	10	25	2.5	Serum [TG] elevated at 4 hrs, still elevated at 48 hrs, normal by 7 days.	¹⁶
Rats	8	0.25	Intralipid ^d	20	20	4	Peak serum TG at 6 hrs. Bioavailability at 8 hrs 85%.	¹⁷
Rats	58	0.23	Intralipid ^e	20	20	4	Peak serum TG and FA at 6 hrs. TG and FA elevated from baseline from 3 to 9hrs.	¹⁸
Rats	4	0.3	Liposyn ^f	10	100	10	90% of administered TG absorbed in 24 hrs.	¹⁹

Key:

n. = number of animals in study

TG = Triglycerides

FA = Fatty acids

n.s. = not stated in article

□ = values assumed or extrapolated from text

^a Intralipid, Cutter Medical

^b Lipofundin, Braun, Melsungen, Germany

^c Liposyn, Abbott Laboratories, IL, United States

^d Intralipid, Kabi-Pfrimmer, Sweden

^e Intralipid, manufacturer not specified

^f Liposyn, Travenol Laboratories, IL, United States

Peak serum triglyceride levels after intraperitoneal lipid administration have reportedly been reached two to six hours post intraperitoneal administration.^{2,4,16,17} Elevated serum triglyceride levels were detected by one group of investigators 48 hours after a single dose of intraperitoneal lipid.¹⁶ Such persistent serum triglyceride elevations are not a consistent feature of other studies. One study showed a return to baseline serum triglyceride levels seven hours after administration.⁴ Another demonstrated no significant elevation in serum triglyceride levels nine hours after intraperitoneal lipid administration.¹⁸ Eight hours after intraperitoneal lipid administration, another group of investigators found bioavailability to be 84.5%.¹⁷ Based upon the results of these studies, it appears repeat doses of intraperitoneal lipid could safely be administered every six to eight hours.

No serious adverse effects of intraperitoneal lipid infusions were mentioned in the studies referred to in Table 3.1. Unexpected deaths of rats occurred in further studies conducted by one group of investigators.¹⁹ Previously, these investigators had administered 30mls of 10% lipid intraperitoneally to rats and found 90% of administered triglyceride was absorbed within 24 hours.¹⁹ The next study involved intraperitoneal administration of 60 mls of a 10% lipid emulsion to eight rats.¹⁹ No mention was made of the bodyweight of these rats nor the technique used for intraperitoneal delivery. If the weight of the rats is presumed to be similar to that of rats mentioned in their initial study (300 grams), these rats would have received an intraperitoneal volume of approximately 200 ml/kg. Experimental results state four of eight rats died within three hours of receiving intraperitoneal lipid and a further rat died within three hours of a second dose. No post mortem findings were mentioned, nor cause of death postulated. Three remaining rats were euthanased five days after receiving the initial lipid injection. At post mortem, large amounts of inspissated lipid were seen on the serosal surface of mucosal folds within the peritoneal cavity. The number of intraperitoneal lipid doses received by these rats was not stated.

Based upon this author's calculations, the rats would have received a large volume of fluid into their peritoneal cavities (approximately 200 mls/kg). This is twice the volume used in this group's initial study and up to 20 times the volume used in other studies (see Table 3.1). It is plausible that such a volume of fluid in the peritoneal cavity could impede venous return and impair respiration to such an extent that death may have resulted.

Lipid solutions provide excellent growth conditions for micro-organisms. Intraperitoneal injections of intralipid have been shown to increase mortality when administered prior to inoculating the peritoneal cavity of rats with a sub-lethal dose of low-virulence *Streptococcus* species.²⁰ The effect of the lipid solution may have been two-fold. Lipid may have provided a substrate for enhanced microbial growth. Alternatively, lipid emulsions have been thought to impair immune system function, possibly due to an oversupply of linoleic acid.²¹ It is possible the lipid solution used in the study of Rubin *et al*¹⁹ was contaminated prior to or during administration, and fulminant sepsis or peritonitis resulted in death of the rats.

Intraperitoneal lipid administration may be contraindicated if thoracic lymphatics are obstructed. Lymphatic absorption and transport has been shown to play an important role in movement of lipid emulsion from the peritoneal cavity into the circulation.^{15,18} Obstruction of lymphatic flow may prevent lipid clearance from the peritoneal cavity, predisposing to inspissation. The presence of lymphatic obstruction should be suspected in patients with central vein thrombosis, chylothorax or forms of cardiac disease associated with high venous pressures. Lymphatic obstruction was proposed as a major contributing factor in the development of diffuse lipogranulomatous lesions throughout the thoracic and abdominal cavities of an infant in whom partial peritoneal nutrition was used.²² This infant had been seriously ill since birth with diffuse, multisystemic disease. Prior to intraperitoneal nutrition, the child had been maintained on intravenous nutrition,

presumably containing lipid. Intravenous catheterisation had become impossible in this child due to numerous thrombosed vessels. Both the thrombosis and long-term use of lipid containing nutritional solutions may have contributed to formation of the lipogranulomatous lesions found at post-mortem. Experimental studies on the effects of lymphatic obstruction on lipid absorption from the peritoneal cavity are lacking. However, it would seem prudent to exercise caution in the use of intraperitoneal lipid in such cases until further information is gained.

3.5 EXPERIMENTAL ATTEMPTS AT NUTRITIONAL SUPPORT VIA THE PERITONEAL CAVITY IN ANIMALS

As well as conducting experiments investigating absorption of individual nutrients from the peritoneal cavity, a number of investigators have attempted to provide total nutritional support via this route. Dogs, rats and rabbits have been maintained for periods of one to four weeks by the intraperitoneal infusion of nutrient solutions.^{6,23,24} Issues considered by studies attempting to provide parenteral nutrition via this route have included whether adequate protein, calories and free water can be supplied, and the potential impact upon the patients' health and well being. Alterations in body weight, nitrogen balance, blood cell counts, serum biochemistry and changes in the demeanour and behaviour of animals have been monitored during these studies. Many studies have evaluated post mortem or laparotomy findings with some reporting histological assessment of the peritoneum and internal organs. Assessment techniques vary and not all studies mention or examine all of the above parameters making generalisations difficult.

A nutrient solution composed of glucose, amino acids and lipids, delivered intraperitoneally, was able to maintain the body weight of 12 dogs for 30 days.²³ Other investigators have achieved similar results in rabbits, rats and dogs.^{6,24,,25} In contrast,

some groups of investigators have reported weight loss in intraperitoneally fed animals.^{4,7,19} These groups all provided calories at below maintenance levels. Gilsdorf *et al*⁷, although intending to provide dogs with a caloric intake of 50kcal/kg/day, only achieved an average of 34.3 kcal/kg/day due to technical problems with infusion lines and pumps. An average decrease of 13.9% in body weight occurred over the study period. Interestingly, there appeared to be no correlation between an individual animal's caloric intake and its subsequent weight change. Similar observations have not been made in other studies.

For a number of reasons, investigators have found supplying sufficient quantities of amino acids via the peritoneal route difficult. Commercially available amino acid solutions for parenteral administration contain low concentrations of amino acids to limit their tonicity to clinically acceptable levels. To supply sufficient protein, either large volumes or markedly hypertonic solutions must be given.^{23,25} Another factor may further complicate delivery of adequate protein. It has been proposed that amino acids may be absorbed so rapidly from the peritoneal cavity that there is insufficient time for their utilisation by the animal. Amino acids thus enter gluconeogenic pathways rather than being utilised for protein synthesis.^{8,23}

The protein content of intraperitoneal nutrient solutions administered experimentally to dogs has varied from 0.5g/kg/day to 1.5g/kg/day.^{7,23,25,26,27} Protein requirements for various species in disease states are not well known. The adequacy of protein supply is not easily assessed, unless the deficiency is extreme. Nitrogen balance of dogs in long-term intraperitoneal nutrition studies has not been examined. However, many long-term intraperitoneal nutrition studies report decreases in serum concentrations of albumin, urea nitrogen or both. This may be indicative of insufficient supply of nitrogen or amino acids. Other factors such as overhydration may also play a role.

Intraperitoneal nutritional support originated with observations made during peritoneal dialysis.^{1,10} A distinction can be made between those investigators who attempt to improve the nutritional status of peritoneal dialysis patients by manipulation of the dialysate and those who aim at providing total nutritional support.^{6,7,19,24} Attempts need to be made to maintain fluid balance if nutritional support is to be provided via the intraperitoneal route. Little attention has been given to the complexity of fluid balance in experimental animals receiving intraperitoneal nutrition. In addition to the influence of normal physiological processes and the impact of a disease condition, the capacity of the peritoneal cavity to absorb fluid as part of a nutrient solution is unclear. The intraperitoneal route has been used clinically by veterinary practitioners for administering crystalloid fluids to a number of species. Osmotic forces exerted across the peritoneum by hypertonic nutrient solutions are, however, likely to influence intraperitoneal volume and free water absorption.¹¹ Given sufficient dwell time in the peritoneal cavity, osmolar equilibrium will be reached and fluid absorption/resorption should start to occur.¹¹ Another possible influence on patient fluid balance is diuresis, which may occur secondary to absorption of nutrient solutions. Rapid absorption of glucose and amino acids can lead to hyperglycaemia, glucosuria and an osmotic diuresis.² The extent to which this occurs, and its regulation by endogenous or exogenous insulin, is not well evaluated.

Intraperitoneal nutrition has been provided by either continuous rate infusions or by bolus delivery. Continuous rate infusion requires placement and maintenance of indwelling peritoneal catheters. Specialised infusion equipment is required, financial outlay is increased and the potential for mechanical problems is greater.^{6,7} Additionally, the fluid lines and attachments restrict patient movement and accessibility. Should the animal disrupt or dislodge the infusion line or catheter the potential exists for free access to be created from the peritoneal cavity to the exterior.

A number of investigators have delivered intraperitoneal nutrient solutions in a bolus fashion, either twice or three times daily.^{4,19,23,25,27} The ability to provide parenteral nutrition as intermittent boluses and avoid the need for expensive infusion equipment are advantages for intraperitoneal nutrition over intravenous nutrition in veterinary medicine.

Conventional parenteral nutrient solutions are hypertonic with respect to plasma. One impetus for the use of continuous rate infusions is the avoidance of delivering large volumes of hypertonic fluid into the peritoneal cavity over a short period of time. Such an event can result in major fluid shifts from the interstitial and intravascular into the peritoneal cavity. Hypovolaemic shock has been reported secondary to intraperitoneal infusion of large volumes of hyperosmolar nutrient solutions.^{6,7} The risk to patient safety as a result of such fluid shifts has been recognised in intraperitoneal nutrition research but safety guidelines have not been established. This should be an important safety consideration when attempting to provide nutritional support via this route.

Indwelling peritoneal catheters have been placed by some groups of investigators to allow both intermittent and constant delivery of intraperitoneal nutrient solutions.^{7,23,27} Indwelling catheters provide ongoing and easily available peritoneal access but can be problematic and have provided some frustration to veterinary investigators in the past.²⁸ Most intraperitoneal catheters are a straight or curved fenestrated tube designed to float free in the ventral abdomen of humans.²⁹ Placed in veterinary patients, these catheters are prone to occlusion with omentum, intestine or fibrin plugs.^{28,30} The normal posture of dogs and cats may aggravate outflow obstructions with this style of peritoneal catheter.²⁹ Column-disk catheters have been designed for peritoneal dialysis of veterinary patients and may be the most suitable for intraperitoneal nutrition purposes.^{28,29,30} Another disadvantage of indwelling catheters is that general anaesthesia and minor surgery are required for placement and removal.^{28,29,30}

Fenestrated tube indwelling peritoneal catheters have been used to supply intraperitoneal nutrition in dogs and rabbits. In one report, several dogs required re-operation for replacement of their intraperitoneal catheters because they had become dislodged or blocked.²⁷ Some authors recommend omentectomy prior to, or at the time of, catheter placement to avoid omental wrapping of indwelling peritoneal catheters.²⁹ This has been performed by some intraperitoneal nutrition investigators, whilst others have placed catheters inside mesh pouches to obviate the problem.^{24,31} Another reported catheter-related problem involved one incident of nutrient solution leaking around the catheter.⁶ This occurred in one of twelve rabbits fed for 21 days. One group of investigators described 30 days of intraperitoneal nutrition in 24 dogs and made no mention of catheter-related problems.²³

3.6 MEASURABLE EFFECTS OF INTRAPERITONEAL NUTRITION ON PATIENTS

A few abnormalities in blood cell parameters have been reported in animals involved in long term intraperitoneal nutrition studies. Transient leukocytosis was noted in two dogs in one study associated with infectious peritonitis.²³ A trend towards an increase in packed cell volume, thought to reflect mild volume depletion, was noted in 10 rats given seven days of intraperitoneal nutrition.¹⁹ No other haematological parameters were measured in that study to give support to the volume depletion hypothesis. A 5% decrease in packed cell volume in both intraperitoneal nutrition and intraperitoneal saline control groups was noted in a 22-day study in dogs.²⁵ The authors attributed this alteration to the effects, over time, of repeated venepuncture. The decreased packed cell volume was accompanied by a decrease in serum albumin concentration and may have been, in part, the result of haemodilution following absorption of the intraperitoneal fluid.

Decreases in serum albumin or total serum protein concentration have been reported commonly in long-term intraperitoneal nutrition studies. Reductions of 20% to 30% are reported in studies involving rabbits and dogs.^{4,23,25,7} Proposed mechanisms are discussed infrequently. Suggestions have been made that excessively rapid absorption of amino acids from the peritoneal cavity may prevent their anabolic utilisation by the animal.²³ In addition, investigators have found supplying sufficient protein and calories through the intraperitoneal route a difficult task.^{23,25} Some investigators have drained residual intraperitoneal fluid prior to delivering further doses of intraperitoneal nutrition. This would also contribute to hypoproteinaemia as albumin diffuses across the peritoneal membrane and would be lost in the fluid effusate. This problem is seen in patients undergoing chronic peritoneal dialysis.^{2,9} Reductions in blood urea levels reported in three canine intraperitoneal nutrition studies, have also been attributed to the supply of insufficient quantities of amino acids.^{7,23,27} However, haemodilution, high fluid delivery and polyuria may have contributed to reduced serum urea concentrations.

Hyperphosphataemia was observed in four of seven dogs in one 30-day intraperitoneal nutrition experiment.⁷ The hyperphosphataemia developed during the first week of intraperitoneal nutrition and persisted at mildly elevated levels for the remaining three weeks. No convincing explanation was offered by the authors. Blood urea nitrogen and creatinine levels were considered normal to low and urine output normal, thus making renal failure an unlikely cause. A mild reduction in glomerular filtration rate with haemodilution obscuring the azotaemia could not be ruled out. Phospholipids were present in the nutrient solution but the concentration delivered considered too low to have resulted in hyperphosphataemia. The acid-base status of the dogs was not evaluated.

Hyperglycaemia can occur secondarily to rapid absorption of large amounts of glucose or amino acids contained in intraperitoneal nutrient solutions.^{8,23} A variety of ways to avoid hyperglycaemia have been reported. Some studies gradually increase the concentration of nutrient solutions and rely on increased endogenous insulin secretion to maintain normoglycaemia.⁷ Others investigators have supplied exogenous insulin either by injection or intraperitoneally with the nutrient solution.^{6,23,25} Normoglycaemia was more easily maintained in one long-term canine intraperitoneal nutrition study when exogenous insulin was included in the nutrient solution, rather than administered as intermittent injections.²³

The effect of long-term intraperitoneal nutrition on the gross appearance of the peritoneum and internal organs has been assessed at necropsy or laparotomy in most studies investigating the effects of long-term intraperitoneal nutrition.^{6,7,23,25} Findings vary from no detectable lesions to obliteration of the peritoneal cavity by dense adhesions. Most commonly, erythema and thickening of the peritoneum is reported.^{6,7,23} Investigators propose these changes are due to the irritant nature of hypertonic solutions and a compensatory hypertrophy of the peritoneum in response to increased absorptive demands.

Dense intra-abdominal adhesions were noted at post mortem examination of eleven dogs which had undergone four weeks of intraperitoneal nutrition.²⁷ The dogs apparently showed no signs of ill health and there was no change in the absorption of nutrient solution from the peritoneal cavity.²⁷ This observation is supported by human medical experience where people with dense intra-abdominal adhesions are still able to be effectively managed with peritoneal dialysis.

No gross abnormalities of other internal organs have been documented. Samples of omentum, liver, diaphragm, small intestine, kidney and occasionally spleen and lung have been collected at necropsy or laparotomy after completion of the intraperitoneal nutrition. Reported histological changes vary. Hypertrophy and hyperplasia of the peritoneal mesothelial cell layer is a common finding and has been attributed to the increased absorptive demand placed on the peritoneum.^{6,7,23,25} Other investigators report conclusions such as "mild to moderate inflammation" of serosa and peritoneum.^{24,27}

Hepatocellular microvacuolation and fatty infiltration were noted following intraperitoneal nutritional support in rabbits and dogs.^{6,27} These changes are consistent with those seen in patients that have received intravenous nutritional support. In one study direct comparisons were made between rabbits on intraperitoneal nutrition and another group of rabbits who received the same nutrient solution intravenously.⁶ Blinded histological examination of hepatic biopsies was conducted and microscopic changes were graded. Both fatty infiltration and microvacuolation were significantly greater in the rabbits who received intravenous nutrition compared to the intraperitoneal nutrition group. Liver biopsy specimens obtained three weeks after concluding parenteral nutritional support were normal. In another study, no pathological changes were reportedly found in peritoneal, liver, spleen, kidney or intestinal biopsy specimens after 28 days of intraperitoneal nutrition in rabbits.⁴ It is unclear in this report whether no changes at all were observed or if changes were seen but deemed insignificant and therefore not mentioned.

Both gross and microscopic peritoneal changes seen after three to four weeks of intraperitoneal nutrition in rabbits and dogs have resolved after 30 days of enteral nutrition.^{6,7} This indicates that the changes which occur are reversible in nature.

3.7 SAFETY AND POSSIBLE COMPLICATIONS OF INTRAPERITONEAL NUTRITION

Administration of hypertonic solutions into the peritoneal cavity results in a shift of fluid from the interstitial and intravascular space into the cavity. If the fluid shifts are of sufficient magnitude, the potential exists for circulatory shock to occur. This is a reported complication in some intraperitoneal nutrition studies. Three of ten dogs in one study died from circulatory collapse in the first week of the trial.⁷ In this study, it was planned that 96ml/kg/day of a nutrient solution with an osmolality of 1290mOsmol/L would be delivered intraperitoneally by constant rate infusion. Mechanical problems with the infusion systems resulted in 12 to 23 hours of missed nutrient delivery which investigators attempted to "catch-up" with bolus administration. Dogs in this study had been maintained nil per os and could potentially have been dehydrated prior to receiving a bolus of hypertonic nutrient solution intraperitoneally. In another study, 20 rabbits were to be fed intraperitoneally for a period of 21 days, but five rabbits died in the first week presumably from hypovolaemic shock.⁶ The nutrient solution had an osmolality of 965mOsmol/L and was infused at a rate of 150ml/kg/day. No mention was made of altering infusion rates although the authors do mention experiencing mechanical difficulties.

In the aforementioned studies, it would appear that an unfortunate combination of circumstances led to the overly rapid administration of a large volume of high osmolality solution into the peritoneal cavity. Solutions with osmolalities similar to those quoted above have been used without reported incident in other studies. Nutrient solutions with osmolalities of 1100 and 1250 mOsmol/L delivered at lower rates, 33 and 37 ml/kg/day respectively, have been administered intraperitoneally to dogs.²³ Investigators in one rat study made a stepwise increase from 20ml/kg/day of 850mOsmol/L to 30ml/kg/day of

1200mOsmol/L solution without incident.¹⁹ Other studies have delivered nutrients at infusion volumes higher than those mentioned above but the solutions were approximately iso-osmolar with blood.²⁷ The exact effect of solution volume or osmolality on the magnitude of fluid shifts into the peritoneal cavity is not known. Variations in peritoneal permeability may play a role in susceptibility of individual animals or species to peritoneal fluid shifts.

Most studies of long-term intraperitoneal nutrition have uncovered some degree of peritoneal hypertrophy and hyperplasia.^{7,23,25} This change is assumed to be in response to the increased absorptive demands being placed on the peritoneum by intraperitoneal nutrient administration. Sterile inflammation could be a consequence of placing irritant hypertonic solutions into the peritoneal cavity. Inflammation may lead to hypertrophy or hyperplasia and it would seem likely that these processes could be present concurrently in the peritoneum of animals receiving intraperitoneal nutritional support.

The delivery of nutrient solutions into the peritoneal cavity carries with it the risk of introducing microorganisms with subsequent development of septic peritonitis. This is potentially a life-threatening complication. Fortunately, septic peritonitis appears to be an uncommon sequelae to intraperitoneal nutrition and reported cases have been easily managed.^{23,25} Microbial contamination of the peritoneal cavity could occur in a number of ways including: contamination of the nutrient solution prior to, or at the time of, administration; entry through an indwelling catheter; contamination during peritoneal puncture or catheter placement; haematogenous spread; or translocation across the gastrointestinal mucosa in a debilitated patient. Parenteral nutrition solutions are excellent growth media for micro-organisms. Strict sterility is recommended when compounding, handling and storing solutions. Asepsis should also be observed when handling and placing catheters and attaching infusion systems. Other precautions used by some

intraperitoneal nutrition investigators have included placement of in-line bacterial filters in the infusion systems and inclusion of antibiotics in the nutrient mixture.^{6,7,23}

Clinical episodes of peritonitis have been documented in two of eight long-term intraperitoneal nutrition studies.^{23,26} Two dogs receiving 30 days of intraperitoneal nutrition exhibited clinical episodes of depression and displayed transient leukocytosis.²³ *Klebsiella*-like organisms were detected in the peritoneal fluid drained from both dogs. During these episodes the dogs were temporarily removed from the study whilst being treated with parenteral antibiotics and peritoneal lavage. They successfully re-entered the study two days later. These episodes of peritonitis represent four days out of a total of 360 during which intraperitoneal nutritional support was provided to dogs in this study. In another study, clinical deterioration and an elevated rectal temperature was experienced by one dog out of 13 maintained on intraperitoneal nutrition for 22 days.²⁶ This dog recovered with administration of systemic antibiotics and cessation of intraperitoneal nutrient infusion for one day. These are the only reports of septic peritonitis occurring in intraperitoneal nutrition studies. A total of five days of peritonitis were detected in approximately 1,300 days of nutritional support in four reports of long-term intraperitoneal nutrition in dogs.^{7,23,25,27}

Vomiting and increased thirst were observed subsequent to intraperitoneal nutrient infusion in one study in dogs.²³ Thirst was attributed to fluid shifts into the abdomen after infusing large volumes of hyperosmolar solutions into the peritoneal cavity. The vomiting may have been due to fluid distension of the peritoneal cavity or peritoneal irritation.

After maintaining nine rabbits on intraperitoneal nutrition for 22 days, one study reports two rabbits dying as a result of failure to resume oral food intake.⁶ It is unclear if this was a direct result of receiving intraperitoneal nutrition. Other studies have either not mentioned experiencing problems or not returned animals to oral food intake. Some

investigators, who returned a proportion of their experimental subjects to oral food intake in order to assess the reversibility of peritoneal changes induced by intraperitoneal nutrition, make no mention of difficulty in getting those dogs to eat.⁷ Voluntary food intake has been shown to be reduced in rabbits whilst receiving intraperitoneal infusions of large volumes of hypertonic solutions.³¹ Whether this effect persists beyond administration of infusions is not known. Rabbits may be more difficult to return to a normal eating pattern than dogs.

4.8 CLINICAL USE OF INTRAPERITONEAL NUTRITION

In the 1960s, intraperitoneal lipids were administered to human patients for nutritional purposes.^{14,32} One report described the provision, on average, of 320 kilocalories per day to 34 critically ill patients who were unable to receive nutrition by other means.¹⁴ Caloric supplementation in some patients described in this report exceeded 800 kilocalories per day.¹⁴

The next study, published in 1980, described the intraperitoneal administration of all elemental nutrients used in intravenous nutrition to three patients.¹ These patients were receiving peritoneal dialysis and were provided with peritoneal nutrition during episodes of catabolic stress associated with uraemia.

Partial peritoneal nutrition has also been provided to an infant intolerant of enteral feedings and with no venous access.²² This infant received peritoneal nutrition for four weeks, substantially increasing his total energy intake. His body weight, serum albumin concentration and overall clinical condition improved such that he was eventually able to tolerate enteral feedings.

These limited clinical reports, and the published success of experimental intraperitoneal nutrition, suggest this method of nutrient delivery may be a simple and effective route to

provide nutritional support. Much remains to be evaluated with regard to suitability of specific nutrients, tolerable volumes and osmolalities and effects upon the patient. In addition, practicalities of the technique to suit various species and hospital situations need to be examined.

REFERENCES

1. Giordano C, Capodicasa G, De Santo NG. Artificial gut for total parenteral nutrition through the peritoneal cavity. *International Journal of Artificial Organs*. 1980;**3**:326-330
2. Gilsdorf RB, Selby R and Tillach P. Systemic appearance of nutrients placed into the peritoneal cavity. *Journal of Parenteral and Enteral Nutrition*. 1985;**9**:148-152
3. Albert A, Takamatsu H, Fonkalsrud EW. Absorption of glucose solutions from the peritoneal cavity in rabbits. *Archives of Surgery*. 1984; **119**:1247-1251
4. Kalfarentzos F, Spiliotis J, Christopolous D *et al*. Total parenteral nutrition by intraperitoneal feeding in rabbits. *European Journal of Surgical Research*. 1988;**20**:352-357
5. Stabile BE, Borzatta M. Transperitoneal absorption of glucose and amino acids for nutritional support. *Archives of Surgery*. 1987;**122**:344-348
6. Stone MM, Mulvihill SJ, Lewin KJ *et al*. Long-term total intraperitoneal nutrition in a rabbit model. *Journal of Pediatric Surgery*. 1986;**21**:267-270
7. Gilsdorf RB, Selby RR, Schon DA *et al*. Total nutritional support through the peritoneal cavity. *Journal of the American College of Nutrition*. 1985;**4**:461-469
8. Moran JM, Mahedero G, Salas J *et al*. A pharmacokinetic study of peritoneal absorption of glucose and alanine in rats: Nutritional implications. *Journal of Parenteral and Enteral Nutrition*. 1998;**22**:72-76
9. Berlyne GW, Jones JH, Hewitt V *et al*. Protein loss in peritoneal dialysis. *Lancet*. 1964;**1**:738-741
10. Oren A, Wren G, Anderson GH *et al*. Effective use of amino acid dialysate over four weeks in CAPD patients. *Peritoneal Dialysis Bulletin*. 1983;**3**:66-73
11. Flessner MF. Peritoneal transport physiology: Insights from basic research. *Journal of the American Society of Nephrology*. 1991;**16**:122-135
12. Wretlind A. Modern principles of the use of fat emulsion in parenteral nutrition. In: *Parenteral Nutrition*. Wilkinson AW, Ed.. Churchill Livingstone, Edinburgh. 1972, pp 160-171

13. Paulsrud JR, Pensler L, Whitten CF *et al.* Essential fatty acid deficiency in infants induced by fat-free intravenous feeding. *American Journal of Clinical Nutrition.* 1972;**25**:897-904
14. Lindqvist B. Nutrition by intraperitoneal fat infusion. *Acta Medica Scandinavia.* 1969;**185**:323-325
15. Adkins ES, Salman FT, Fonkalsrud EW. Triglyceride absorption in transperitoneal alimentation. *American Journal of Surgery.* 1990;**159**:237-240
16. Klein MD, Coran AG, Drongowski RA *et al.* The quantitative transperitoneal absorption of a fat emulsion: Implications for intraperitoneal nutrition. *Journal of Pediatric Surgery.* 1983;**18**:724-731
17. Mahedero G, Moran JM, Salas J *et al.* Absorption of Intralipid and interferences from nutrients infused into the peritoneal cavity of the rat. *American Journal of Surgery.* 1992;**164**:45-50
18. Moran J, Cruz G, Nogue-Rales F *et al.* Transperitoneal absorption of Intralipid in rats: Total serum fatty acids and triglyceride after absorption. *Journal of Parenteral and Enteral Nutrition.* 1986;**10**:604-608
19. Rubin J, Jones Q, Planch A *et al.* Intraperitoneal feeding. *Transactions of the American Society of Artificial Internal Organs.* 1988;**34**:125-130
20. Fischer GW, Hunter KW, Wislon SR *et al.* Diminished bacterial defences with Intralipid. *Lancet.* 1980;**2**:819-820
21. Wan JM, Tea TC, Babayan VK *et al.* Invited comments: Lipids and the development of immunodysfunction and infection. *Journal of Parenteral and Enteral Nutrition.* 1988;**12**:43S-52S
22. Merrit RJ, Atkinson JB, Whalen TV *et al.* Partial peritoneal alimentation in an infant. *Journal of Parenteral and Enteral Nutrition.* 1988;**12**:621-625
23. Garcia-Gamito FJ, Moran JM, Mahedero G *et al.* Long-term peritoneal nutrition in dogs, both normal and after intestinal resection. *International Surgery.* 1991;**76**:235-240
24. Pessa ME, Sitren HS, Copeland EM *et al.* Nutritional support by intraperitoneal dialysis in the rat: Maintenance of body weight with normal liver and plasma chemistries. *Journal of Parenteral and Enteral Nutrition.* 1988;**12**:63-67

25. Moran JM, Limon M, Mahedero G *et al.* Long-term peritoneal nutrition in dogs: metabolic and histopathologic results. *Nutrition*. 1989;**5**:89-93
26. Moran JM, Garcia-Gamito FJ, Mahedero G *et al.* Peritoneal nutrition in dogs after intestinal resection: Comparative study. *Nutrition* 1989;**5**:315-320
27. Klein MD, Coran AG, Drongowski RA *et al.* Long-term survival of dogs maintained solely on intraperitoneal nutrition. *Journal of Pediatric Surgery* 1985;**20**:765-771
28. Thornhill JA, Hartman J, Boon GD *et al.* Support of an anephric dog for 54 days with ambulatory peritoneal dialysis and a newly designed peritoneal catheter. *American Journal of Veterinary Research*. 1984;**45**:1156-1161
29. Carter LJ, Wingfield WE, Allen TA. Clinical experience with peritoneal dialysis in small animals. *Compendium on Continuing Education for the Practicing Veterinarian*. 1989;**11**:1335-1343
30. Thornhill JA. Peritoneal dialysis in the dog and cat: An update. *Compendium on Continuing Education for the Practicing Veterinarian*. 1981;**3**:20-34
31. Balaskas EV, Rodela H, Oreopoulos DG. Effects of intraperitoneal infusion of dextrose and amino acids on the appetite of rabbits. *Peritoneal Dialysis International*. 1993;**13**:S490-S498
32. Gjessing J. Absorption of amino acids and fat from the peritoneum. *Opuscula Medica*. 1968;**13**:251-252

CHAPTER 4

DIAGNOSTIC PERITONEAL LAVAGE : A REVIEW

4.1 INTRODUCTION

Abdominocentesis and peritoneal lavage have been utilised extensively in small animal medicine as diagnostic procedures for investigating cases of abdominal disease and trauma. A variety of methods for performing the procedure are described. Technique has changed as more experience is gained in both human and veterinary medicine. Diagnostic peritoneal lavage (DPL), once used solely to diagnose intra-abdominal haemorrhage, is now used to aid the diagnosis of a variety of medical and surgical conditions of the abdomen. A number of potential complications of DPL are reported; however, the majority are of minor consequence. The technique remains a relatively safe procedure with a high level of accuracy in appropriately selected patients.

4.2 HISTORY OF DEVELOPMENT AND TECHNIQUES

Needle aspiration of the peritoneal cavity (abdominocentesis) has been used in the diagnosis of intra-abdominal pathology since early this century and is considered the precursor to diagnostic peritoneal lavage. Attempts to improve the sensitivity of the procedure, resulted in recommendations to aspirate four quadrants of the abdomen but still false negative results were common.¹ It was then demonstrated that after instilling five millilitres of blood per kilogram body weight into the peritoneal cavity of a dog, abdominocentesis resulted in a positive return of blood in only 15% of attempts.² On opening the abdominal cavity, direct aspiration of blood remained difficult as omentum and bowel loops occluded the needle aperture.² Larger amounts of blood were necessary to increase the chance of obtaining a positive return.

The technique of DPL was developed with the aim of improving the diagnostic value of abdominocentesis.³ Reports of the use of this procedure as a diagnostic technique in veterinary medicine soon followed.⁴ The value of this procedure in the assessment of abdominal injuries and pathology in companion animals is now well established.^{4,5,6}

The technique, as first described in humans, involved insertion of a dialysis catheter into the peritoneal cavity under direct visualisation. Using local anaesthesia, a small skin incision was made below the umbilicus to the peritoneum. Sharp entry into the peritoneal cavity was then achieved using a trochar. If aspiration didn't reveal free fluid, one litre of sterile saline was infused under gravity, allowed to dwell, then withdrawn.³

Variations of this technique have been reported in both human and veterinary literature and have been classified as open, semi-open or closed.⁷ The open technique involves incision through the abdominal wall and peritoneum followed by insertion of the catheter, under direct visualisation, into the peritoneal cavity.^{8,9} The semi-open technique differs only in that the peritoneum is punctured by the catheter and stylet.³ The closed technique involves blunt puncture of the skin, subcutaneous tissue, muscle and peritoneum with the needle, trochar, or catheter and stylet.¹⁰

Positioning the patient to undergo DPL and selection of a site for catheter placement demands consideration of the anatomy of the abdominal cavity. The urinary bladder should be emptied to reduce the risk of iatrogenic injury occurring when the needle or catheter enters the abdomen. For the same reasons, gastric decompression has sometimes been recommended in people prior to undergoing DPL.^{8,11,12} In companion animals, the most common recommendations are for patients to be placed in dorsal recumbency and the abdomen entered on the ventral midline slightly caudal to the umbilicus.^{4,5} Entry into the abdomen at this site has a number of advantages. Major abdominal organs are not close to the abdominal wall in this area and the peritoneum is

closely adherent to fascia. In addition, the risk of trauma to abdominal musculature is lower compared to paramedian catheter insertion.⁶

The suspicion of intra-abdominal disease may influence the technique chosen and site of catheter insertion into the abdomen. Distended, enlarged or abnormal intra-abdominal organs may require the clinician to choose a different site of entry into the abdominal cavity. Risk of iatrogenic bowel perforation is reported to be higher in people with intra-abdominal adhesions from previous abdominal surgery.^{13,14} Use of an open technique in these circumstances may reduce the risk of iatrogenic injury. Some authors recommend routine use of an open technique to reduce the likelihood of iatrogenic intra-abdominal injury.^{14,15}

Once the needle or catheter has entered the abdomen, general recommendations are that it then be directed caudally. Experimentally, fluid introduced into the peritoneal cavity of a dog positioned in dorsal recumbency preferentially migrates into the pelvic cavity and gutters of the caudodorsal abdomen.²

Performing DPL using a multi-fenestrated catheter gives greater accuracy and enables recovery of a larger volume of lavage fluid when compared with the use of a needle or single holed catheter. Support for this is obtained from both clinical experience and experimental studies.^{2,5,16} The single aperture of a needle or catheter is quickly occluded by bowel loops and omentum and requires a greater volume of free fluid to be present before a diagnostic sample can be obtained.^{2,5}

4.3 INDICATIONS AND LIMITATIONS OF DIAGNOSTIC PERITONEAL LAVAGE

Abdominocentesis and peritoneal lavage are indicated in cases where intra-abdominal injury or disease is suspected on the basis of history, physical examination findings,

laboratory or radiographic data but in which the diagnosis is equivocal.¹⁷ Clinical indications for performing DPL in veterinary medicine include: blunt abdominal trauma; penetrating abdominal wounds; trauma patients in which response to abdominal palpation is difficult to assess due to the patient's demeanor or mental status; patients with signs of hypovolaemic shock not responding to medical therapy; assessment of acute abdominal pain; suspected peritonitis or peritoneal fluid accumulation; and undiagnosed abdominal disease.^{13,16}

Diagnostic peritoneal lavage has been used in people as an aid to the diagnosis of: conditions resulting from blunt trauma to the abdomen (such organ damage and vessel rupture), peritonitis, acute pancreatitis, viscus perforation, strangulated bowel, ascities of undetermined origin, some neoplastic conditions and ectopic pregnancies.¹⁸ In the past, if clear-cut indications for exploratory surgery were lacking, patients underwent serial monitoring until their condition either improved or worsened.¹⁹ Diagnostic peritoneal lavage has improved diagnostic accuracy, shortened the time to definitive treatment, and reduced the number of patients who have to undergo unnecessary exploratory surgery or diagnostic procedures.^{11,19,20} The use of peritoneal lavage has been cited to have reduced the number of patients undergoing unnecessary laparotomies by up to 50%.²¹

Claims have been made that diagnostic peritoneal lavage is limited in its ability to detect isolated intra-abdominal pathology or pathology limited to the retroperitoneal area. Indeed, this inadequacy was noted in a veterinary case series where DPL failed to reveal any abnormalities in two animals with isolated retroperitoneal injuries.¹⁶ Similar occurrences have been reported elsewhere by the same author and others.^{4,14} Others however, report detection of injuries to retroperitoneal structures by the return of haemorrhagic lavage fluid.⁸ These authors suggest the trauma required to produce retroperitoneal injury frequently results in multiple abdominal injuries which aid their

detection by DPL. Reports in human literature of localised injuries or pathology not detected by peritoneal lavage include appendiceal rupture and local abscessation, and splenic injury isolated from the general abdominal cavity by adhesions from previous abdominal surgery.^{14,22}

Diagnostic peritoneal lavage may fail to reveal abnormalities in patients with diaphragmatic tears or hernias if there are minimal concurrent injuries.¹⁴ In one case series, however, two dogs with diaphragmatic tears, unassociated with herniation, were diagnosed by aspiration of peritoneal lavage fluid from the thorax.¹⁶ The diagnosis of generalised bacterial peritonitis by DPL based upon the presence of toxic, degenerative neutrophils and intracellular bacteria has been reported to be 100% accurate.¹⁶

4.4 INTERPRETATION OF RESULTS

In an early report describing the use of DPL in people, lavage fluid which had more than "a faint salmon pink tinge" was taken to indicate the need for exploratory laparotomy.³ Assessment of the colour of DPL fluid, however, is largely subjective. In addition, small volumes of blood are capable of imparting a colour change that could be deemed clinically significant. A quarter of a millilitre of blood per litre of saline imparts a faint pink hue and two millilitres of blood per litre of saline results in a grossly bloody sample.²³ Such volumes of blood could easily result from the procedure itself or following minor intra-abdominal injury. The diagnostic dilemma was how to avoid patients with trivial injuries undergoing exploratory laparotomy.^{20,24} Attempts to address this problem led to the introduction of qualitative and quantitative analysis of peritoneal lavage fluid. Colour charts, verbal colour descriptions and total red cell counts have all been used to assess the blood content of returned lavage fluid.^{8,9,23,25}

Olsen and colleagues described analysis of DPL fluid from 232 human patients who had suffered blunt abdominal trauma.²⁰ A lavage return positive for blood was obtained in 80 of the first 100 patients and categorised from trace to ++++ on a visual basis. One third of patients with a bloodied lavage fluid underwent exploratory laparotomy unnecessarily. Consequently, the next 132 patients were arbitrarily divided into two groups: those with a large volume of blood in the lavage fluid (+++ or greater) and those with smaller volumes (trace to ++). Newsprint could not be read through plastic tubing containing DPL fluid with +++ or greater blood contamination. Of those patients considered to have +++ or greater intra-abdominal haemorrhage, 98% had significant intra-abdominal injuries detected on exploratory laparotomy. In the second group, 30% had significant intra-abdominal injury and monitoring or repeat lavage were used to identify the need for surgery in these patients. A score of +++ was attributed to intra-abdominal haemorrhage of greater than 25 millilitres.

A packed cell volume of 1% is equivalent to 100,000 cells per millilitre. In the dog, one millilitre of blood contains approximately 5,000 to 7,000 red blood cells. Diagnostic peritoneal lavage fluid with a PCV of 1% from a dog may therefore contain as little as 15 to 20 mls of blood per litre.²⁴ That level of red cells in returned lavage fluid considered indicative of serious intra-abdominal haemorrhage varies between reports. The incidence of iatrogenic injury and the volume of lavage fluid infused will influence the red cell count. Authors in the human medical literature report the finding of greater than 100,000 red cells per litre as an indicator of intra-abdominal injury requiring exploratory surgery.^{15,20} In the veterinary literature, similar red cell counts have been quoted, however, others suggest higher red cell numbers.^{6,16} In the case of patients with equivocal red blood cell counts in the DPL effusate the peritoneal lavage catheter can be left in place and repeat lavages performed. A consistent or increasing number of red cells on repeat lavage would support ongoing intra-abdominal haemorrhage and the need for exploratory laparotomy.¹⁷

Diagnostic peritoneal lavage fluid free from blood has been obtained from patients with intra-abdominal injuries requiring surgery.²⁶ Additional evaluation of DPL fluid is necessary to detect injuries in some patients. Such evaluations include: assessment of colour and turbidity, total nucleated cell counts, various enzyme activities and cytological evaluation.²⁷ Peritoneal lavage performed on healthy animals returns fluid that is clear to straw coloured with little or no turbidity.²⁷ Quantitative assessment of total nucleated cell counts in returned peritoneal lavage fluid has been used to indicate the presence of inflammation or infection within the peritoneal cavity. Total nucleated cell counts in returned lavage fluid from healthy dogs and cats are less than 500 cells per microlitre with a predominance of mononuclear cells and segmented neutrophils (Allan FJ 1999 *pers comm*).²⁷ In people, clinical application of 'normal' reference ranges for DPL lavage fluid has been questioned.^{24,28} Both false negative and false positive results have been reported based on the total nucleated cell count of returned DPL fluid.^{24,28}

The length of time between injury and diagnostic peritoneal lavage has been shown to influence peritoneal lavage total nucleated cell counts.²⁸ Diagnostic peritoneal lavage performed immediately after injury may return fluid with negligible white cell counts.²⁹ The appearance of leukocytes in DPL fluid begins to increase two hours after injury in dogs and people.²⁹ Experimentally, the extent and speed of this elevation varies with the cause of the injury.²⁹ Abdominal contamination with gastric juice, pancreatic juice and bile resulted in rapid and marked elevations in peritoneal leukocyte counts, where as urine contamination of the abdominal cavity resulted in much slower elevations.²⁹ Blunt abdominal trauma, unassociated with visceral damage, did not result in elevations in total nucleated cell counts over 500 cells per microlitre.²⁹

In an early clinical evaluation of the use of DPL in people having suffered blunt abdominal trauma, eight patients with blood in the peritoneal cavity, in the absence of gastrointestinal injury, had DPL fluid white cell counts from 20 to 3160 cells/mm³.²⁹ Investigators concluded that the presence of blood in the peritoneal cavity induced peritoneal leukocytosis.²⁹ Concerns regarding false positive results continued to be raised. As the time since abdominal injury increases, there is an increasing difference between the DPL fluid nucleated cell counts of patients with serious intra-abdominal injuries (such as intestinal perforations) and those without.²⁸ Timing of DPL post-injury should be factored into the interpretation of DPL nucleated cell count.

Diagnostic peritoneal lavage performed after abdominal surgery, uncomplicated by abdominal contamination or wound dehiscence, has been associated with white cell counts above 500 cells/ μ L.²⁷ This has been attributed to migration of leukocytes into inflamed and healing tissues.²⁶ Diagnostic peritoneal lavage performed in 38 dogs 48-hours prior to abdominal surgery returned fluid with average total nucleated cell counts of 213/ μ L with the predominant cells types being segmented neutrophils and macrophages.²⁷ Two days post-surgically there was a significant increase in total nucleated cell counts to an average of 7049 cells/ μ L. Neutrophils predominated in the post-operative DPL fluid, displaying degenerative and toxic changes.²⁷ Similar findings have been reported elsewhere.³⁰ Two of the 38 dogs in the previously mentioned study required re-operation, one for leakage of an intestinal anastomosis and another for dehiscence of the abdominal wall wound. A third dog was noted at the time of surgery to have a pre-existing traumatic diaphragmatic hernia. Remaining dogs were followed for 14 days post surgery prior to euthanasia and necropsy. These findings would indicate that a moderate leukocytosis in peritoneal lavage fluid, consisting predominantly of neutrophils which display mild toxic changes, should be considered a normal finding following abdominal surgery.

Unless spectacular, an elevated white cell count in returned DPL fluid interpreted in isolation may falsely indicate the need for surgical exploration of the abdomen. Cytological evaluation of lavage fluid can be helpful in assessing the importance of equivocal nucleated cell counts. The presence of particulate matter or bacteria on cytology is considered abnormal.⁶ Bacteria have not been noted in lavage fluid from healthy patients, even post-surgically.^{27,30} Recent experimental findings have, however, raised the possibility of obtaining a positive bacterial culture from peritoneal lavage fluid in apparently healthy cats and rats (Allan FJ *pers comm*, Chapter 6). Cytological changes, such as the finding of a predominance of neutrophils, degenerative or toxic cellular changes, as well as the presence of bacteria (particularly intracellular bacteria) is suggestive of intra-abdominal sepsis.²⁷ Leukocyte morphology and presence or absence of bacteria may be more important than total leukocyte counts in diagnosing peritonitis by DPL.²⁷

Bile contamination of lavage effluent can easily be detected by chemical tests. Any level of bile is considered abnormal and may represent rupture of the biliary tree or upper intestinal leakage into the peritoneal cavity.^{2,17} Bile content of lavage fluid is unreliable if the patient is jaundiced. Urea, creatinine and potassium concentrations in lavage fluid have been used to provide evidence of urinary tract disruption. Urea may be unreliable as it is freely diffusible and peritoneal fluid urea concentration equilibrates rapidly with serum urea concentration. Evaluation of a variety of enzymes has also been recommended to aid in the detection of intra-abdominal pathology. The type of enzymes, suggested abnormal activities and their significance can be found in Table 4.1.

Transudation of amylase-containing fluid into the peritoneal cavity occurs early during acute pancreatitis and persists longer than elevations in serum amylase activity.³¹ The presence of amylase activity in peritoneal fluid has also been demonstrated to occur

following experimentally induced duodenal perforations in the dog.²⁷ In people with acute pancreatitis, peritoneal amylase and lipase activities provide good indications of the severity of disease and have been used to establish a prognosis.^{32,33} Measuring the concentration of trypsinogen activation peptides in peritoneal fluid or DPL fluid has been utilised in the diagnosis of pancreatic necrosis in people.³⁴ Measurement of DPL trypsinogen activation peptide concentrations may prove a useful diagnostic tool in cats with suspected pancreatitis.

The determination of peritoneal lactic acid concentrations may be helpful in the evaluation of patients with acute abdominal pain as an aid in the discrimination between those patients which require surgery and those which do not. In a series of human cases, lactic acid concentrations were significantly higher in the peritoneal fluid than serum in patients with viscus perforation, gangrenous intestine, peritonitis or intra-abdominal abscessation.³⁵ There are no reports of the clinical use of this measurement in veterinary medicine.

Table 4.1: Assessment of fluid obtained from diagnostic peritoneal lavage in the dog: A summary of the literature

Lavage Fluid Finding	Interpretation
Gross appearance	
Clear fluid	Normal finding
Opaque blood tinged fluid	Intra-abdominal haemorrhage ^{6,20}
Darker on repeat examination	Continuing intra-abdominal haemorrhage
Turbid/ cloudy fluid	Inflammatory disease, evaluate cytologically
Packed cell volume	
<5%	Mild-moderate intra-abdominal haemorrhage ⁶
>10%	Significant intra-abdominal haemorrhage
Nucleated cell count	
> 500 / μ L	Considered abnormal by many ⁶
> 1000 / μ L	Mild to moderate peritoneal inflammation ¹⁶
Amylase activity	
> 200 caraway units	Pancreatic trauma/ injury ^{6,16,29}
Bile	
any presence	Biliary tree or proximal small intestinal leakage (not reliable in icteric animals)
Creatinine	
> serum creatinine levels	Urine leakage, uroabdomen
Alkaline phosphatase	
> serum alkaline phosphatase	Intestinal trauma/ ischaemia
Cytology	
Presence of bacteria	Bacterial peritonitis, particularly if intracellular or accompanied by neutrophilia
Neutrophilia, neutrophils with toxic changes	Suggestive of peritoneal inflammation
Vegetable fibres	Gastrointestinal leakage
Neoplastic cells	Intra-abdominal neoplasia

Note: Reference values based on the volume of lavage fluid infused being 20 ml/kg

4.5 COMPLICATIONS OF DIAGNOSTIC PERITONEAL LAVAGE

Potential complications arising from abdominocentesis and peritoneal lavage include haemorrhage, iatrogenic abdominal organ damage or viscus perforation, and herniation or fluid leakage from around the catheter site. In a case series describing the use of DPL in 129 dogs and cats, a complication rate of 4.6% was reported.¹⁶ Of the six animals that suffered complications, three cats had subcutaneous haematoma formation with fluid leakage at the site of catheter placement, which spontaneously resolved. Another had a small hole in the mesocolon, noticed incidentally at laparotomy, which was presumed to have occurred during DPL catheter placement. In the other two animals, one had a ruptured bladder, presumably because it had not been emptied prior to the procedure, and the other iatrogenic splenic rupture due to incorrect technique. Bjorling *et al*²⁷ found a slightly higher complication rate of 13% from 76 DPLs performed in 38 dogs. Seven of the ten complications seen in this study were iatrogenic haemorrhage. The haemorrhage was thought to have resulted from trauma to abdominal musculature involved with paramedian catheter insertion and may have been avoidable had midline catheter placement been used. One animal each suffered from: subcutaneous swelling due to leakage of lavage fluid; herniation of omentum through an abdominal incision for catheter placement; and splenic laceration when a left paramedian approach was inadvertently used. Haemorrhage is usually self-limiting and of minor consequence to the patient. More serious complications tend to have occurred in patients where recommended protocols were not followed.^{16,27}

The use of a closed technique is potentially contraindicated in patients with obvious organomegaly, distended intestinal loops or viscera, and any obvious indication for surgery. Controversy exists over performance of the procedure during gestation. Adherence to strict protocol when performing the technique will reduce the chances of

iatrogenic abdominal injury.^{16,27} The incidence of iatrogenically-induced haemorrhage is reduced when catheters are inserted on the ventral midline rather than a paramedian location.²⁷ Performing DPL using an open technique is recommended when there is an increased risk of organ or viscus damage.^{9,14,19}

REFERENCES

1. Wright LT, Prigot A, Hill LM. Traumatic rupture of the liver without penetrating wounds. *Archives of Surgery*. 1947;**54**:613-632
2. Giacobine JW, Siler VE. Evaluation of diagnostic abdominal paracentesis with experimental and clinical studies. *Surgery Gynecology and Obstetrics*. 1960;**110**:676-685
3. Root HD, Hauser CW, McKinley CR *et al*. Diagnostic peritoneal lavage. *Surgery*. 1965;**57**:633-637
4. Crowe DT, Crane SW. Diagnostic abdominal paracentesis and lavage in the evaluation of abdominal injuries in dogs and cats: Clinical and experimental investigations. *Journal of the American Veterinary Medical Association*. 1976;**168**:700-705
5. Kolata RJ. Diagnostic abdominal paracentesis and lavage: Experimental and clinical evaluations in the dog. *Journal of the American Veterinary Medical Association*. 1976;**168**:697-699
6. Bjorling DE, Crowe DT, Kolata RJ *et al*. Penetrating abdominal wounds in dogs and cats. *Journal of the American Animal Hospital Association*. 1982;**18**:742-748
7. Manganaro AJ, Patcher HL, Spencer FC. Experience with routine open abdominal paracentesis. *Surgery, Gynecology and Obstetrics*. 1978;**146**:795-796
8. Gill W, Champion HR, Long WB *et al*. Abdominal lavage in blunt trauma. *British Journal of Surgery*. 1975;**62**:121-124
9. DuPriest RW, Rodriguez A, Khaneja SC *et al*. Open diagnostic peritoneal lavage in blunt trauma victims. *Surgery, Gynecology and Obstetrics*. 1979;**148**:890-894
10. Sachatello CR, Bivins BA. Technique for peritoneal dialysis and diagnostic peritoneal lavage. *American Journal of Surgery*. 1976;**131**:637-640
11. Engrav LH, Benjamin CI, Strate RG *et al*. Diagnostic peritoneal lavage in blunt abdominal trauma. *Journal of Trauma*. 1975;**15**:854-859
12. Powell DC, Bell RM. Diagnostic peritoneal lavage. *Surgery, Gynecology and Obstetrics*. 1982;**155**:257-264
13. Hunt CA. Diagnostic peritoneal paracentesis and lavage. *Compendium on Continuing Education for the Practicing Veterinarian*. 1980;**11**:449-453

14. Soderstrom CA, DuPriest RW, Cowley RA. Pitfalls of peritoneal lavage in blunt abdominal trauma. *Surgery, Gynecology and Obstetrics*. 1980;**151**:513-518
15. Fischer RP, Beverlin BC, Engrav LH *et al*. Diagnostic peritoneal lavage. Fourteen years and 2,586 patients later. *American Journal of Surgery*. 1978;**136**:701-704
16. Crowe DT. Diagnostic abdominal paracentesis techniques: Clinical evaluation in 129 dogs and cats. *Journal of the American Animal Hospital Association*. 1984;**20**:223-230
17. Crowe DT. Abdominocentesis and diagnostic peritoneal lavage in small animals. *Modern Veterinary Practice*. 1984;**65**:877-882
18. Hickman TC. Abdominal paracentesis. *Surgery Clinics of North America*. 1969;**49**:1409-1412
19. Shaw JH. Diagnostic peritoneal lavage: Technical aspects, and its place in blunt abdominal trauma. *Australian and New Zealand Journal of Surgery*. 1980;**50**:503-505
20. Olsen WR, Redman HC, Hildreth DH. Quantitative peritoneal lavage in blunt abdominal trauma. *Archives of Surgery*. 1972;**104**:536-543
21. Ahmad W, Polk HC. Blunt abdominal trauma. *Archives of Surgery*. 1976;**111**:489-492
22. Barbee CL, Gilsdorf RB. Diagnostic peritoneal lavage in evaluating acute abdominal pain. *Annals of Surgery*. 1975;**181**:853-856
23. Caffee HH, Benfield JR. Is peritoneal lavage for the diagnosis of haemoperitoneum safe? *Archives of Surgery*. 1971;**103**:4-7
24. Thal ER, Shires GT. Peritoneal lavage in blunt abdominal trauma. *American Journal of Surgery*. 1973;**125**:64-69
25. Jacob ET, Cantor E. Discriminate diagnostic peritoneal lavage in blunt abdominal injuries: Accuracy and hazards. *American Surgeon*. 1979;**45**:11-14
26. Root HD, Keizer PJ, Perry JF. The clinical and experimental aspects of peritoneal response to injury. *Archives of Surgery*. 1967;**95**:531-537
27. Bjorling DE, Latimer KS, Rawlings CA *et al*. Diagnostic peritoneal lavage before and after abdominal surgery in dogs. *American Journal of Veterinary Research*. 1983;**44**:816-820
28. Feliciano DV, Bitondo-Dyer CG. Vagaries of the lavage white blood cell count in evaluating abdominal stab wounds. *American Journal of Surgery*. 1994;**168**:680-684

29. Root HD, Keizer PJ, Perry JF. Peritoneal trauma, experimental and clinical studies. *Surgery*. 1967;**62**:679-685
30. Botte RJ, Rosin E. Cytology of peritoneal effusion following intestinal anastomosis and experimental peritonitis. *Veterinary Surgery*. 1983;**12**:20-23
31. Keith LM, Zollinger RM, McCleery RS. Peritoneal fluid amylase determinations as an aid in diagnosis of acute pancreatitis. *Archives of Surgery*. 1950;**61**:930-936
32. Robbert JH, Meyer P, Rohner A. Can serum and peritoneal amylase and lipase determinations help in the early prognosis of acute pancreatitis? *Annals of Surgery*. 1986;**203**:163-168
33. Pickford IR, Blackett RL, McMahon MJ. Early assessment of severity of acute pancreatitis using peritoneal lavage. *British Medical Journal*. 1977;**2**:1377-1379
34. Heath DI, Wislon C, Gudgeon AM. Trypsinogen activation peptide (TAP) concentrations in the peritoneal fluid of patients with acute pancreatitis and their relation to the presence of histologically confirmed pancreatic necrosis. *Gut*. 1994;**35**:1311-1315
35. DeLaurier GA, Ivey RK, Johnson RH. Peritoneal fluid lactic acid and diagnostic dilemmas in acute abdominal disease. *American Journal of Surgery*. 1994;**167**:302-305

CHAPTER 5:**PILOT STUDY: SUITABILITY OF INTRAPERITONEAL
NUTRIENT DELIVERY IN THE CAT****5.1 INTRODUCTION**

Crystalloid fluids and blood products have been successfully delivered via the intraperitoneal route for many years. In more recent times, the peritoneal cavity has also been utilised to dialyse veterinary patients in renal failure.^{1,2} Peritoneal dialysis has been used to maintain people with chronic renal failure for over sixty years. Osmotic agents in common use in dialysis solutions include glucose and amino acids. High levels of these substances are detected in the systemic circulation of dialysis patients.^{3,4} The intraperitoneal route may therefore serve as a means to provide supplemental nutrition to dialysis, and perhaps other patients.^{5,6}

Nutrient absorption across the peritoneum has been investigated in a number of animal species. Systemic absorption of lipid, amino acid and glucose solutions after intraperitoneal administration has been demonstrated to occur in dogs, rats and rabbits.^{7,8,9} It has been suggested that nutritional support via this route is safe and efficacious.^{10,11,12}

Total parenteral nutrition (TPN) solutions are usually composed of amino acids, dextrose and lipids.¹³ Amino acids are a source of protein and provide some calories. Dextrose is cheap, easily available and commonly used as a calorie source in TPN solutions. Use of glucose as the sole energy source can result in greatly elevated blood glucose levels, osmotic diuresis and dehydration. Such glucose intolerance is a frequent metabolic complication of TPN.¹⁴ When glucose and amino acids are compounded to provide sufficient calories, the resultant solution is hyperosmolar.¹⁵ Lipids are a concentrated source of calories and exert little osmotic pressure.¹⁶ They have been used to lower the incidence of glucose intolerance and to reduce the osmolality of parenteral nutrition

solutions. If metabolic energy requirements are to be met using this form of nutrition high osmolality solutions are required necessitating placement and maintenance of central venous catheters. Solutions are delivered at a constant rate requiring mechanical infusion devices, adding expense and introducing the potential for equipment failure or malfunction. Catheter-related problems are numerous and include difficulties in placement, sepsis, air embolism, patient interference, accidental removal and haemorrhage.¹⁷ Other problems encountered with intravenous TPN include metabolic derangements and sepsis.¹⁷ Intravenous TPN is rarely undertaken by veterinarians outside referral institutions. Intraperitoneal nutrition may be a simple, more accessible and less problematic method to provide parenteral nutrition.

This pilot study was designed to whether healthy adult cats would tolerate this technique of nutrient delivery, and the intraperitoneal provision of dextrose, amino acid and lipid solutions.

5.2 MATERIALS AND METHODS

Animals

Four, two and a half year old female neutered cats supplied by the Animal Health Services Centre of Massey University were used in this project. Cats were free from clinical signs of disease and no abnormalities had been detected on physical examination prior to commencement of the experiment. Massey University Animal Ethics Committee approval had been granted prior to commencement of the experiment.

Feeding and Housing

Cats were housed individually in cages measuring 0.6 by 0.65 by 0.65 metres and allowed free exercise within a closed room twice daily. Water was provided ad libitum and intake was measured daily. Cats were fed Hills Science Diet[®] Feline Maintenance (Hills Pet Nutrition Pty Ltd) to meet maintenance energy requirements of $100 \times \text{bodyweight (kg)}^{0.75}$ kcal/day.¹⁸ This was divided and fed twice daily at four and ten hours after intraperitoneal

nutrient delivery. Food intake was recorded and residual food removed twelve hours prior to the following day's sedation and nutrient infusion.

Planned Experimental protocol

Cats were to be sedated at the same time every day for six days. An intraperitoneal catheter was to be placed on each of the 6 days. On Days 1 through to 5, intraperitoneal infusion of nutrient solutions would take place, and on Days 2 through to 6, diagnostic peritoneal lavage performed. Blood and urine sampling was planned for Days 1, 3 and 5. A schematic representation of the planned experimental protocol is shown in Figure 5.1.

Figure 5.1 : Schematic representation of planned experimental protocol

Procedure	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
IPN	x	x	x	x	x	
DPL		x	x	x	x	x
Haematology	x		x		x	
Urinalysis	x		x		x	

Legend:

- IPN = intraperitoneal nutrient delivery
- DPL = diagnostic peritoneal lavage

Nutrient solutions

Three nutrient solutions were used in this study: 20% glucose, 10% amino acids and 20% lipid solution. Product specifications for each nutrient solution are shown in Table 5.1. It was planned that each cat would receive 20ml/kg of a single nutrient solution every day for five days. Cats would then have a four week rest period before similarly receiving one of the other nutrient solutions. The rest period would then be repeated and cats receive the final of the three nutrient solutions. Of the six possible permutations for order of deliver of three different nutrient solutions, four combinations were randomly selected and allocated to each of the four cats. All three solutions were commercially pre-formulated and provided in individually sealed sterile containers or syringes ready for administration (McGaw Biomed Ltd, Auckland, New Zealand).

Table 5.1 : Nutrient solution specifications

Nutrient solution	Concentration (gm/L)	Osmolality (mOsmol/l)	Caloric density (kcal/L)	pH (range)
Dextrose ^a	200	1010	680	4.6 (3.5 - 6.5)
Amino acids ^b	100	950	400	6.5 (6.0-7.0)
Lipid ^c	200	380	1908	6.5 - 8.5

Chemical restraint

Heavy sedation was provided by premedication with a combination of acetylpromazine^d 0.04mg/kg, buprenorphine^e 0.01mg/kg plus atropine^f 0.04mg/kg administered subcutaneously and followed forty minutes later by ketamine^g (5mg/kg) and diazepam^h (0.5mg/kg) given intravenously. Thermal support during this period was provided by circulating warm water blankets, heat lamps and ensuring nutrient solutions were warmed prior to intraperitoneal infusion. Additional thermal support was provided from Day 2 by placing cats in passive insulation suits.¹⁹

Technique for aseptic delivery of nutrients into the peritoneal cavity

Once sedated, the urinary bladder was expressed and cats were placed in dorsal recumbency. Hair was clipped from the ventral abdominal midline and skin aseptically prepared in a standard technique using aqueous chlorhexidine followed by chlorhexidine tincture. An 18 gauge, 32mm, over-the-needle catheter (Optiva™ intravenous catheter, Critikon, Italy) was inserted along the ventral midline, approximately 2cm caudal to the umbilicus. Using sterile technique, an intravenous fluid extension set was attached to the catheter and nutrient solutions were infused over a 15-minute period. Once completed,

^a 20% Dextrose Injection USP, McGaw Inc, Irvine CA USA

^b 10% FreAmine® III, McGaw Inc, Irvine CA USA

^c 20% Lipofundin® MCT/LCT, B. Braun, Melsungen, Germany

^d ACP injection, Novartis New Zealand Ltd, N.Z.

^e Temgesic® injection, Reckitt & Colman Pty Ltd, U.K.

^f Phoenix Atropine Injection. Phoenix Pharmaceutical Distributors, Auckland, N.Z.

^g Ketamine Injection, Pamell Laboratories, N.Z.

^h Pamlin Injection, Pamell Laboratories, N.Z.

the catheter was withdrawn from the peritoneal cavity and pressure applied to the puncture site for two minutes.

Technique for performing diagnostic peritoneal lavage

It was planned that DPL would be performed immediately prior to nutrient infusion on Days 2 to 5. Both procedures would therefore be performed under the same anaesthetic and utilise the same intraperitoneal catheter. For DPLs performed on Day 6 chemical restraint and intraperitoneal catheter placement as described above were to be used. Using sterile technique a 3-way stop-cock was attached to the catheter. Sterile isotonic saline (22 ml/kg) was infused into the peritoneal cavity via an intravenous fluid administration set with in-line buretteⁱ, connected to the stop-cock and catheter. After infusion of the required volume, the 3-way stop-cock was closed to prevent back leakage of fluid. Cats were gently rolled from side to side for two minutes to facilitate widespread contact of the fluid throughout the peritoneal cavity. After a 10-minute dwell time, peritoneal fluid was removed through the catheter by gentle aspiration using a 50ml syringe.

Modified diagnostic peritoneal lavage technique

Due to difficulties with recovering sufficient volumes of lavage fluid with the above technique, modifications were made for the DPL's performed on cats 3 and 4 on Day 4 of the study. After sedation, cats were positioned in dorsal recumbency, the urinary bladder expressed and ventral abdomen aseptically prepared. A small stab incision was made in the ventral midline, 2cm caudal to the umbilicus. An 8.3 French, 35cm long, sterile polyurethane catheter with multiple side holes was introduced into the abdomen guided by a central stylet (Cook Pneumothorax Set, product number V-TPT-815U). The procedure was continued as described above. After sampling, the catheter was withdrawn from the abdominal cavity and pressure applied to the puncture site for two minutes.

Assessment of diagnostic peritoneal lavage fluid

A five-ml fluid sample was collected into a sterile container with no additives and assessed grossly for colour, turbidity and presence of particulate matter. Total red cell and nucleated cell counts were performed manually using a haemocytometer^j. A direct smear and 100µl cytopsin preparation of the fluid was examined by a veterinary clinical pathologist for a differential nucleated cell count and assessment of red blood cell and nucleated cell morphology, as well as for the presence of any particulate matter or bacteria. A five-ml sample of the diagnostic peritoneal lavage fluid was submitted within ten minutes of collection in a capped syringe for aerobic and anaerobic culture. Plates were read daily for five days and a negative report given if no growth had occurred after 14 days.

Blood sampling and analysis

Blood samples were taken from the jugular vein immediately prior to nutrient infusion. A two-ml sample was collected into EDTA (final concentration EDTA 1.5mg/ml) and a direct smear was made immediately from fresh blood. Total red and white blood cell counts were assessed using an automated Cobas Vet Haematology Analyser^k. The blood smear was stained by Wright's method and examined for a differential white cell count and red and white blood cell morphology. Serum biochemical and electrolyte analysis^l was planned to be run on Days 1, 3 and 5 on each cat.

ⁱ Buretrol® Solution Set, Baxter Healthcare Corporation, Deerfield, IL, U.S.A.

^j Kova®Glasstic® Slide 10 with grids, Hycor Biomedical Inc., CA, U.S.A.

^k Minos Vet, ABX, France

^l Urea, creatinine, alanine aminotransferase (ALT), alkaline phosphatase (AP), glucose, total protein, sodium, potassium, chloride

Urine collection and analysis

Voided urine samples were to be collected from each cat on Days 1, 3 and 5. These were to be subjected to dipstick analysis for pH, protein, bilirubin, blood, glucose and ketones and measurement of specific gravity.

baseline value of 38.6°C to 36.6°C measured after nutrient infusion. Two hours after completion of nutrient infusion the cat was recovering well and active. The cat was uncooperative for further temperature measurements so none were taken. Food, provided four and ten hours post infusion, was consumed willingly. This cat was given free access to water four hours after infusion.

Sedation was repeated on the second day of the study and additional thermal support was provided in the form of passive insulation suits.¹⁹ Rectal temperature was recorded pre-infusion and hourly post-infusion. A nadir of 37.6°C was reached at the time of first temperature recording. As planned, prior to nutrient infusion, sterile saline was instilled into the peritoneal cavity for diagnostic lavage at the rate of 20ml/kg. No lavage fluid was able to be collected and DPL was aborted pending re-evaluation of the technique. Lipid solution was infused as planned. Two hours post-infusion, subcutaneous leakage of the lipid solution was noted. Moderate swelling of subcutaneous tissues extending a radius of approximately three centimeters from the abdominal puncture site accompanied the leakage. Aspiration of this area was attempted and no fluid returned. The cat resented abdominal palpation at this time. An abdominal compression bandage was placed in an attempt to reduce subcutaneous leakage of fluid and the cat given ketoprofenⁿ at 2.0mg/kg subcutaneously. Buprenorphine^o was administered at 0.01mg/kg subcutaneously an hour later for additional analgesia. Four hours after the infusion this cat was extremely agitated, manifested by vocalising and rolling around its cage. The abdominal wrap was removed and the cat sedated with 0.02mg/kg acetylpromazine administered intramuscularly. The cat was closely monitored for the next three hours.

ⁿ Ketofen[®] 1%, Rhône Mérieux, Animal Health Division Rhône-Poulenc, N.Z.

^o Temgesic[®] injection, Reckitt & Colman Pty Ltd, U.K.

An elevated rectal temperature was noted on a number of occasions over the following two days, reaching a maximum of 40.2°C. Rectal temperature consistently dropped to between 39.0°C and 39.5°C following the administration of ketoprofen and buprenorphine. No further lipid infusions were given. Abdominal discomfort was managed symptomatically with continued analgesia. The crusted areas of skin around the abdominal puncture site were cleaned twice daily with aqueous chlorhexidine (0.5%).

On Day 4 the cat was sedated and diagnostic peritoneal lavage performed to evaluate persistent abdominal discomfort and pyrexia. Results are summarised in Table 5.2. Neutrophilic inflammation was seen on cytology but no infectious organisms identified. Total nucleated cell count was 5.1×10^9 cells/L with 90% mildly to moderately degenerate neutrophils, 2% lymphocytes, 8% mononuclear cells and occasional mast cells. Mononuclear cells frequently contained vacuolated cytoplasm consistent with intracellular lipid. Aerobic and anaerobic bacterial culture yielded no growth. There was no evidence of a systemic white cell response. A complete blood count performed concurrently with DPL returned values all within reference range for our laboratory.

This cat remained under observation for a further three days prior to discharge. During this time receiving analgesia as above and oral food and water ad libitum.

Cat 4

This cat received 20% lipid solution. Hypothermia occurred after sedation, with rectal temperature reaching a nadir of 34°C two hours post-sedation. Nutrient infusion was without incident and the cat was recovering well four hours later. The cat was given food and water at this time and fed again six hours later. All food offered was consumed.

Sedation and intraperitoneal catheter placement was repeated on Day 2. Due to difficulties experienced with the DPL procedure in cat 3, saline infusion was not

performed on this cat pending re-evaluation of the technique. Lipid solution was administered as planned. A passive insulation suit was used to provide additional thermal support and rectal temperature reached a nadir of 37.0°C two hours post infusion. The cat appeared to be recovering well at this time. Four hours after the infusion the cat subjectively appeared quieter and demonstrated tenderness upon abdominal palpation. Analgesia was provided in the form of Ketoprofen, 2.0mg/kg administered subcutaneously. Buprenorphine, which had been given as part of premedication regimen, was repeated that evening (0.01mg/kg subcutaneously).

On Day 3 the cat had a heart rate of 240 bpm, respiratory rate of 40 bpm and rectal temperature of 39.2°C. The cat was becoming increasingly sensitive to abdominal palpation, walked slowly when out of its cage and kept its body close to the ground. When sitting the cat held its hind quarters elevated. This cat continued to receive buprenorphine and ketoprofen for analgesia. Food and water were provided ad libitum. Later that day the cat stopped eating and vomited a number of times overnight.

Sedation and DPL were undertaken on Day 4 in an attempt to gain a better understanding of the cat's response to intraperitoneal lipid infusion. Results are summarised in Table 5.2. Placement of the peritoneal lavage catheter in this cat resulted in a moderate degree of iatrogenic haemorrhage. Cytology revealed large numbers of erythrocytes ($256.4 \times 10^9/L$) but no erythrophagocytosis, consistent with recent haemorrhage. Total white cell count was elevated at 3.2×10^9 cells/L (Table 5.2). Neutrophils were the predominant cell, comprising 95% of the total nucleated cell population and exhibiting mild to moderate degenerative changes. The majority of mononuclear cells had vacuolated cytoplasm. No evidence of potential aetiological agents was identified. Aerobic and anaerobic bacterial culture revealed no growth. Complete blood cell count showed no abnormalities.

Intravenous fluids were started on Day 4, administering an isotonic crystalloid solution (Hartmann's solution) at 25ml/hr for 6 hours then 12ml/hr for another 24 hours. Analgesia was provided as above for a further 48 hours and the cat discharged 6 days after beginning the study.

Table 5.2 : Diagnostic peritoneal lavage results from Cats 3 and 4, Day 4

Parameter	Cat 3	Cat 4	Normal Range ‡
Colour	Milky white	Pink	Clear to straw
Turbidity	Opaque	Opaque	Clear
TNCC (10^9 cells/L)	5.10	3.20	.04 – 0.39
RBCC (10^9 cells/L)	1.41	256.40	0.11 – 327.60
Neutrophils (10^9 cells/L)	4.59	3.04	0.00 – 0.11
Lge Mononuclear cells (10^9 cells /L)	0.41	0.13	0.02 – 0.22
Lymphocytes (10^9 cells/L)	0.10	0.03	0.00 – 0.03
Bacterial culture	Negative	Negative	

TNCC = total nucleated cell count

RBCC = red blood cell count

‡ (Allan FJ 1999 *pers comm*) Based upon data collected from 13 healthy cats using the same DPL protocol and Veterinary Pathology Laboratory

5.4 DISCUSSION

Two cats that received intraperitoneal amino acid or glucose infusion exhibited clinical signs consistent with hypovolaemic shock approximately five to six hours after nutrient infusion. This was characterised by elevated heart rates, weak peripheral pulses, and dry, tacky mucous membranes. These solutions had a high osmolality and were likely to have exerted a direct osmotic effect, drawing water through the permeable peritoneal membrane into the peritoneal space resulting in hypovolaemia.

Anaesthesia and hypothermia are both known to depress baroreceptor response to a changing intravascular volume.²⁰ The sedation and resultant hypothermia experienced by these cats may have contributed to the hypovolaemia by obtunding normal, physiological, compensatory responses to the fluid shifts. If this was indeed the case, early responses to a decreasing intravascular volume such as elevation in heart rate and peripheral vasoconstriction may not have occurred. What was initially attributed to a prolonged anaesthetic recovery may have been early, undetected, hypovolaemic shock.

Although healthy, cats may have been better able to compensate for such fluid shifts had they not been heavily sedated. Patients requiring intraperitoneal nutrition are likely to be systemically ill. Creating fluid shifts in these patients is likely to be poorly tolerated. Consequently, no further attempts at infusing these solutions intraperitoneally were made.

Nutrient solutions of similar osmolality to the amino acid and dextrose infusions used in this study have been delivered into the peritoneal cavity of rats and dogs without apparent incident.^{11,12,21} However, one study in dogs and another in rabbits reported life-threatening fluid shifts after intraperitoneal nutrient instillation.^{10,22} In both studies, nutrient solutions were being delivered as constant rate infusions through indwelling intraperitoneal catheters. Mechanical problems resulted in 12 to 23 hours of missed

nutrient infusion in the canine study which investigators then attempted to 'catch-up' with bolus infusions.²² Three fatalities resulted.

Species or individual variation in peritoneal membrane permeability may exist, predisposing one species to greater, or more rapid, fluid shifts into the peritoneal space. The hypertonicity of the solution may have resulted in tissue dehydration and an increase in effective pore size within the peritoneal membrane. The solutions may have been irritant to the peritoneum. An inflammatory response by the peritoneum may have increased the area of blood vessel contact with peritoneum, thereby facilitating fluid shifts.

Clinically healthy cats used in this study did not tolerate infusion of the hypertonic amino acid or glucose solutions. Although cats in this study were sedated, similar fluid shifts are likely to occur in unsedated cats. A number of changes could be proposed to minimise the risk of such fluid shifts occurring in future investigations. Use of solutions with lower osmolalities may reduce fluid shifts but larger volumes would be required to meet daily protein and caloric requirements. A larger overall volume would require more frequent administration, or potentially, delivery via constant rate infusion. Caution would need to be exercised to avoid volume overload. The use of smaller volumes of the high osmolality solutions used in this study may reduce the magnitude of the fluid shifts to a clinically tolerable level but again would necessitate frequent delivery or constant rate infusion.

For practical reasons it was decided not to utilise continuous infusions in this study nor place indwelling catheters, which avoided problems associated with catheter maintenance. Intraperitoneal catheters can become occluded rapidly by omentum and this has proven problematic in veterinary peritoneal dialysis.^{23,24} Continuous infusions require mechanical infusion pumps and nutrient delivery can be interrupted if pump failure occurs. Mechanical problems with catheters, infusion lines and pumps are

frequently reported in intraperitoneal nutrition studies and reports of intravenous total parenteral nutrition.^{10,22}

Both cats which received intraperitoneal lipid developed clinical and cytological findings consistent with sterile peritonitis. This was an unexpected finding as such reactions had not previously been documented in animals after intraperitoneal lipid infusion.^{25,26} Intraperitoneal lipid infusions have however, been reported to cause pain on administration to critically ill human patients. In one report of 34 patients who received intraperitoneal lipid infusions, three experienced what investigators termed "great discomfort" on infusion of the lipid.²⁷ All three received their infusions directly after a peritoneal dialysis cycle and their discomfort was, in part, attributed to this. A further eight patients experienced slight but transient abdominal pain for which no explanations were offered. Pain in response to intraperitoneal lipid infusion has not been reported in previous studies in rats, rabbits and dogs. Either it did not occur or animals were not being observed closely. A lack of familiarity with animal examination and behaviour by investigators may have overlooked transient discomfort. Alternatively, there may be a species difference in response to intraperitoneal lipid infusion.

The peritonitis in these cats was unlikely to have been infectious in origin. Diagnostic peritoneal lavage is thought to have a high sensitivity for detection of septic peritonitis based upon the presence of bacteria in association with high nucleated cell counts. Up to 100% sensitivity is reported.²⁸ In both cats, neutrophils, often showing degenerate changes, predominated on cytological evaluation of DPL fluid. This could be indicative of sepsis, however, no infectious organisms were visualised cytologically and bacterial culture of DPL fluid yielded no growth. It has been suggested that neutrophils displaying degenerative or toxic changes may be a normal cytological finding in fluid obtained from DPL performed on an animal after abdominal surgery not associated with sepsis.³⁴ In the

human medical literature there are reports of patients with isolated areas of septic peritoneal inflammation remaining undetected following DPL.²⁹ Focal areas of infection could not be definitively ruled out in these cats but seem unlikely. The generalised abdominal discomfort exhibited by both cats would be more consistent with diffuse peritoneal disease.

Increased thickness and mild hypertrophy of the peritoneal mesothelium is often reported in intraperitoneal nutrition studies.^{10,11,12,30} These changes are presumed to occur in response to the increased absorptive demand. Mild to moderate serosal and peritoneal inflammation have been documented by other investigators.^{31,32} Diagnostic peritoneal lavage has not previously been utilised to assess the peritoneal response to nutrient instillation, however, should a hypertrophied mesothelium contribute to an elevated cell count in returned peritoneal lavage fluid, it would be expected that mononuclear cells would predominate.³³ Given the predominance of neutrophils in the DPL fluid from the cats in the present study, it seems unlikely that mesothelial hypertrophy would have contributed greatly to the elevated nucleated cell counts detected in the lavage fluid from these cats.

Iatrogenic haemorrhage complicated the diagnostic peritoneal lavage procedure in Cat 4 on Day 4. Haemorrhage was observed to occur during catheter placement and cytological appearance of the returned lavage fluid was consistent with recent haemorrhage. Haemorrhage, usually minor and self-resolving, is a reported complication of diagnostic peritoneal lavage.^{28,34} Haemorrhage will interfere with assessment of nucleated cell numbers in the returned lavage fluid as the blood contamination will have contributed white cells in addition to erythrocytes. There are approximately 500 red blood cells to one white blood cell in peripheral blood.³⁵ These will be mostly neutrophils as they are the predominant circulating white cell in the cat. Correcting DPL results for the degree of

haemorrhage seen in the cats from the present study, gives a white cell count of approximately 2.7×10^9 cells/L. The magnitude of this elevation and predominance of degenerate neutrophils remains supportive of neutrophilic peritoneal inflammation.

The lipid solution used in this study, Lipofundin[®] MCT/LCT is a commercially available mixture of equal proportions of medium-chain triglycerides (MCT) and long-chain triglycerides (LCT). The Lipofundin[®] MCT/LCT differs from those lipid solutions utilised in previous intraperitoneal nutrition studies which have been composed primarily of LCTs. The immediate availability of LCTs for oxidation has been questioned. Medium-chain triglycerides (MCT) are cleared from circulation more rapidly, oxidise more completely and are less likely to be incorporated into tissue lipids than LCTs.³⁶ For those reasons it was decided to use this lipid formulation in the current study. This lipid solution, or a component of it, may be directly irritant to the peritoneum. Alternatively, the ability of this lipid solution to incite significant peritoneal inflammation may be related to the species under investigation. Intraperitoneal nutrition has not previously been investigated in cats. They may be less tolerant of intraperitoneal nutrition than other species.

Subcutaneous leakage of intraperitoneally delivered fluids in Cat 3 may have contributed to its apparent abdominal discomfort. This cat appeared to be more uncomfortable than Cat 4. Subcutaneous leakage of fluid is a reported complication of diagnostic peritoneal lavage.²⁸ This cat received 20ml/kg of saline intraperitoneally as part of a diagnostic peritoneal lavage procedure immediately prior to lipid infusion. None of this fluid was successfully collected. The increased intra-peritoneal fluid volume may have predisposed this cat to fluid leakage. Cat 4 did not undergo DPL at this time due to the unsuccessful attempts at recovering fluid from Cat 3.

Diagnostic peritoneal lavage technique required modification during the study due to an inability to retrieve adequate volume of infusate. This problem has previously been

reported with single-holed needles or catheters.^{28,37} The single entry point to the catheter may have become obstructed by omentum or the lavage fluid may have collected in pockets within the abdomen and was not in contact with the end of the catheter. Modification of the technique to use a larger bore, multi-holed catheter successfully rectified the problem. This modified technique is analogous with the technique used by Allan *et al* (*pers com*) to establish reference ranges for DPL fluid analysis in healthy adult cats.

Not unexpectedly, hypothermia was a consistent complication of heavy sedation in this study. Hypothermia is a well recognised complication of sedation and general anaesthesia in companion animals.³⁸ Moderate hypothermia may have contributed to the prolonged recoveries experienced by all cats on Day 1 of the study. Initial methods implemented to minimise hypothermia, such as circulating warm water blankets, heat lamps and warm lavage or nutrient solutions, were supplemented by the use of passive insulation suits on the second and subsequent days.¹⁹ This appeared to successfully reduce the degree of hypothermia.

In summary, in the present study, two cats which received either 10% amino acid or 20% dextrose intra^{per}itoneally developed clinical signs consistent with hypovolaemic shock and another two cats developed clinical evidence of peritonitis after receiving two intra^{per}itoneal infusions of a 20%, 50:50 medium-chain and long-chain triglyceride solution. Clearly, intra^{per}itoneal delivery of solutions with high osmolality, at the volumes used, are not tolerated by cats. Further research in this area will need to evaluate solutions with lower osmolalities, or smaller volumes of high osmolality solutions. Less osmotically active glucose polymers, if tolerated and absorbed after intra^{per}itoneal administration to cats, may provide the key. Cats may be particularly sensitive to intra^{per}itoneal lipid infusion. Alternatively, this type of lipid formulation may

not be suitable for intraperitoneal administration in any species. Further investigation is needed to assess the response of cats to infusion of other lipid formulations or the response of a different species to intraperitoneal administration of the current lipid formulation. Chapter 6 presents DPL and post-mortem findings after three days of intraperitoneal injection of Lipofundin® MCT/LCT in rats.

REFERENCES

1. Thornhill JA, Hartman J, Boon GD et al. Support of an anephric dog for 54 days with ambulatory peritoneal dialysis and a newly designed peritoneal dialysis catheter. *American Journal of Veterinary Research*. 1984;**45**:1156-1161
2. Carter LJ, Wingfield WE, Allen TA. Clinical experience with peritoneal dialysis in small animals. *Compendium on Continuing Education for the Practicing Veterinarian*. 1989;**11**:1335-1343
3. Salusky IB, Fine RN, Nelson P et al. Nutritional status of children undergoing continuous ambulatory peritoneal dialysis. *American Journal of Clinical Nutrition*. 1983;**38**:599-611
4. Hanning RM, Balfe JW, Zlotkin SH. The effect of amino acid containing dialysis solutions on plasma amino acid profiles in children with chronic renal failure. *Journal of Pediatric Gastroenterology and Nutrition*. 1987;**6**:942-947
5. Giordano C, Capodicasa G, De Santo NG. Artificial gut for total parenteral nutrition through the peritoneal cavity. *International Journal of Artificial Organs*. 1980;**3**:326-330
6. Kopple JD, Bernard D, Messana J et al. Treatment of malnourished CAPD patients with an amino acid based dialysate. *Kidney International*. 1995;**47**:1148-1157
7. Gilsdorf RB, Selby R, Tillach P. Systemic appearance of nutrients placed into the peritoneal cavity. *Journal of Parenteral and Enteral Nutrition*. 1985;**9**:148-152
8. Bryant MS, Sinclair KGA, Copeland EM et al. Absorption of a parenteral diet from the peritoneal cavity. *Current Surgery*. 1987;**44**:382-384
9. Kalfarentzos F, Spiliotis J, Christopoulos D et al. Total parenteral nutrition by intraperitoneal feeding in rabbits. *European Surgical Research*. 1988;**20**:352-357
10. Stone MM, Mulvihill SJ, Lewin KJ et al. Long-term total intraperitoneal nutrition in a rabbit model. *Journal of Pediatric Surgery*. 1986;**21**:267-270
11. Moran JM, Limon M, Mahedero G et al. Long-term peritoneal nutrition in dogs: Metabolic and histopathologic results. *Nutrition*. 1989;**5**:89-93
12. Garcia-Gamito FJ, Moran JM, Mahedero G et al. Long-term peritoneal nutrition in dogs, both normal and after intestinal resection. *International Surgery*. 1991;**76**:235-240

13. Lippert AC, Faulkner JE, Evans AT *et al.* Total parenteral nutrition in clinically normal cats. *Journal of the American Veterinary Medical Association.* 1989;**194**:669-676
14. Buffington CAT. Parenteral Nutrition. Proceedings: International Veterinary Emergency and Critical Care Society. 1994 pp 339-341
15. Raffe MR. Total Parenteral Nutrition. In: *Textbook of Small Animal Surgery.* First Edition. Slatter D Ed. WB Saunders & Co. 1985 Chapter 17, pp 225-241
16. Wretling A. Complete intravenous nutrition. Theoretical and experimental background. *Nutrition and Metabolism.* 1972;**14**Sup: 1-57
17. Lippert AC, Fulton RB, Parr AM. A retrospective study of the use of total parenteral nutrition in dogs and cats. *Journal of Veterinary Internal Medicine.* 1993;**7**:52-64
18. Lewis LD, Morris ML, Hand MS. Small Animal Clinical Nutrition. Mark Morris associates, Kansas, US. 1987, pp 1-6
19. Walsh VP. Unpublished data. Massey University 1999
20. Muir WW, Mason D. Chap 5 Cardiovascular system physiology. In: *Lumb & Jones Veterinary Anaesthesia 3rd edition.* Thurmon JC, Tranquilli WJ, Benson GJ Editors. Williams and Wilkins, Baltimore, USA. 1996, pp 62-114
21. Rubin J, Jones Q, Planch A *et al.* Intraperitoneal feeding. *Transactions of the American Society of Artificial Organs.* 1988;**34**:125-130
22. Gilsdorf RB, Selby RR, Schon DA *et al.* Total nutritional support through the peritoneal cavity. *Journal of the American College of Nutrition.* 1985;**4**:461-469
23. Carter W, Wingfield WE, Allen TA. Clinical experience with peritoneal dialysis in small animals. *Compendium on Continuing Education for the Practicing Veterinarian.* 1989;**11**:1335-1343
24. Thornhill JA. Peritoneal dialysis in the dog and cat: An update. *Compendium on Continuing Education for the Practicing Veterinarian.* 1981;**3**:20-34
25. Klein MD, Coran AG, Drongowski RA *et al.* The quantitative transperitoneal absorption of a fat emulsion: Implications for intraperitoneal nutrition. *Journal of Pediatric Surgery.* 1983;**18**:724-731

26. Moran JM, Limon M, Mahedero G *et al.* Biochemical and histopathological findings after a long-term intraperitoneal fat infusion. An experimental study in rats. *Journal of Parenteral and Enteral Nutrition.* 1986;**10**:609-613
27. Lindqvist B. Nutrition by intraperitoneal fat infusion. *Acta Medica Scandinavia.* 1969;**185**:323-325
28. Crowe DT. Diagnostic abdominal paracentesis techniques: Clinical evaluation in 129 dogs and cats. *Journal of the American Animal Hospital Association.* 1984;**20**:223-230
29. Soderstrom CA, DuPriest RW, Cowley RA. Pitfalls of peritoneal lavage in blunt abdominal trauma. *Surgery, Gynecology and Obstetrics.* 1980;**151**:513-518
30. Gilsdorf RB, Selby RR, Schon DA *et al.* Total nutritional support through the peritoneal cavity. *Journal of the American College of Nutrition.* 1985;**4**:461-469
31. Klein MD, Coran AG, Drongowski RA *et al.* Long-term survival of dogs maintained solely on intraperitoneal nutrition. *Journal of Pediatric Surgery.* 1985;**20**:765-771
32. Pessa ME, Sitren HS, Copeland EM *et al.* Nutritional support by intraperitoneal dialysis in the rat: Maintenance of body weight with normal liver and plasma chemistries. *Journal of Parenteral and Enteral Nutrition.* 1988;**12**:63-67
33. Barter LS. Cytological and histological evaluation of the rat peritoneal cavity after three days of intraperitoneal lipid infusions. Chapter 6, MVSc thesis. Massey University 2000
34. Bjorling DE, Latimer KS, Rawlings CA *et al.* Diagnostic peritoneal lavage before and after abdominal surgery in dogs. *American Journal of Veterinary Research.* 1983;**44**:816-820
35. Feldman BF, Thomason KJ. Useful indexes, formulas, and ratios in veterinary laboratory diagnostics. *Compendium on the Continuing Education for the Practicing Veterinarian.* 1989;**11**:169-180
36. Dennison AR, Ball M, Hands LJ *et al.* Total parenteral nutrition using conventional and medium chain triglycerides: Effect on liver function tests, complement and nitrogen balance. *Journal of Parenteral and Enteral Nutrition.* 1988;**12**:15-19
37. Hunt CA. Diagnostic peritoneal paracentesis and lavage. *Compendium on Continuing Education for the Practicing Veterinarian.* 1980;**11**:449-453

38. Evans AT. Anaesthetic emergencies and accidents. In: *Lumb & Jones Veterinary Anaesthesia 3rd edition*. Thurmon JC, Tranquilli WJ, Benson GJ Editors. Williams and Wilkins, 1996, pp 849-860

CHAPTER 6

**CYTOLOGICAL AND HISTOLOGICAL EVALUATION OF
THE RAT PERITONEAL CAVITY AFTER THREE DAYS OF
INTRAPERITONEAL LIPID INFUSIONS**

6.1 INTRODUCTION

The peritoneal cavity has been used in veterinary medicine as a site for administration of crystalloid fluids and blood products and for dialysis of renal failure patients.¹ In human medicine, peritoneal dialysis has been used for over sixty years in the management of patients with chronic renal failure. Glucose and amino acids are the osmotic agents commonly utilised in dialysis fluids. Large amounts of these substances are absorbed and detected in the blood after intraperitoneal delivery.² This observation led to the theory that the intraperitoneal instillation of nutrient solutions may provide an alternative route for the provision of nutritional support.

Animal models have been used to investigate nutrient absorption after intraperitoneal administration and the feasibility of providing total nutritional support via this route. Lipid, amino acid and glucose solutions are absorbed after intraperitoneal delivery to dogs, rabbits and rats.^{3,4,5} Dogs and rabbits have been maintained for periods of up to thirty days on intraperitoneal nutrition.^{3,5,6}

In the pilot study described in Chapter 5, two infusions, 24 hours apart, of 20% lipid emulsion^a at a dose of 20 ml/kg bodyweight into the peritoneal cavity of two cats resulted in clinical and laboratory evidence of sterile peritonitis. Diagnostic peritoneal lavage performed two days after the second intraperitoneal lipid infusion in these cats revealed

^a Lipofundin® MCT/LCT, B.Braun Melsungen, Germany

markedly elevated total nucleated cell counts. Values were 5.1 and 3.2×10^9 cells/ μL , with the upper normal in our laboratory being 0.8×10^9 cells/ μL (Allan FJ *pers comm*). In addition to the elevation in overall cell count there was an increase in the proportion of neutrophils present. No evidence of an infectious origin was detected cytologically and bacterial culture, performed aerobically and anaerobically, yielded no growth.

These findings were unexpected. Intraperitoneal nutrition has not previously been investigated in the cat, nor has this specific lipid emulsion been used in intraperitoneal nutrition studies. Reaction of the feline peritoneum to infusion of this lipid emulsion may be species-specific, idiosyncratic or product related. Due to the discrepancy between our experiences and the current literature, this study was designed to investigate the response of a previously studied species, the rat, to infusion of the same lipid emulsion used in the feline pilot study at an equivalent volume per kilogram bodyweight.

6.2 MATERIALS AND METHOD

Animals

Five eight-week-old male Sprague Dawley rats from two related litters were obtained from a known source^b. Average weight at commencement of the study was 233gms (range = 207 to 262gms). All rats were bred and raised on the premises and had no history of illness. Prior to commencement of the study, no abnormalities were detected on routine physical examination of any rat.

Housing

Rats were housed individually in wire bottomed cages. Food^c and water were provided *ad libitum* and intake recorded daily. Daily food intake was calculated by weighing food in and out and subtracting spillage. Spillage was estimated by daily collection of under cage

^b Crop and Food, AgResearch, Fitzherbert Science Centre, Palmerston North, New Zealand

debris and sifting to remove hair and faeces. A four-day acclimatisation period allowed rats to become accustomed to housing, diet and handling prior to commencement of the study.

Experimental protocol

Rats were examined and weighed at the beginning of each day throughout the study. Commencing on Day 2, and continuing daily for the entire study period, food intake for the preceding 24 hours was calculated. Five rats were randomly allocated to either the control or treatment group.

On Day 5, rats underwent anaesthesia for diagnostic peritoneal lavage (DPL). On Days 6, 7 and 8, rats were anaesthetised for intraperitoneal infusions and on Day 9 they were anaesthetised for repeat DPL and euthanasia.

The five rats in the control group received intraperitoneal 0.9% sodium chloride at 20ml/kg and the remaining five rats received 20% Lipid solution^d at 20ml/kg. Each rat received the same infusion daily for three consecutive days.

After performing DPL on Day 9, rats were euthanased by anaesthetic overdose and a post mortem examination carried out. The peritoneal cavity and its contents were visually inspected and samples of diaphragm, body wall, liver, spleen, kidney and small intestine taken for histological evaluation. A schematic representation of the overall experimental protocol is shown in Figure 6.1.

^c Rat Chow, Diet 86, Sharpes Grain & Seed Ltd, Lower Hutt, New Zealand

^d Lipofundin[®] MCT/LCT, B.Braun Melsungen, Germany

Figure 6.1: Planned experimental protocol

Procedure	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Acclimatisation	✓	✓	✓	✓					
Pain assessment	✓	✓	✓	✓	✓	✓	✓	✓	✓
Anaesthesia					✓	✓	✓	✓	✓
DPL					✓				✓
IP infusion						✓	✓	✓	
Euthanasia									✓

Legend: DPL = diagnostic peritoneal lavage
 IP = intraperitoneal
 ✓ = procedure performed

Anaesthetic protocol

Anaesthesia was induced with halothane delivered into an induction chamber and maintained using a tight fitting facemask attached to a Bain, non-rebreathing anaesthetic circuit with fresh gas flow rates in excess of two L/min. Halothane was delivered via a calibrated vaporiser. Rats were maintained at a plane of anaesthesia such that a withdrawal response to deep toe pinch was present but subdued. This depth was sufficient for intraperitoneal puncture without movement. Thermal support was provided by insulating rats from the metal table, use of a heat lamp during the anaesthetic and recovery period and warming of fluids to be given intraperitoneally. After anaesthesia, rats were placed in individual housing boxes and observed until able to walk normally at which time they were returned to their cages and given food and water.

Technique for performing diagnostic peritoneal lavage

Diagnostic peritoneal lavage was performed using a modification of a previously described protocol.⁷ Anaesthetised rats were positioned in dorsal recumbency, hair from the ventral

abdomen shaved and skin aseptically prepared using aqueous chlorhexidine^e followed by chlorhexidine tincture^f. A 2.5 inch 18 gauge over-the-needle-catheter^g with added side holes was introduced into the lower left quadrant of the abdomen as per recommendations for intraperitoneal injection in this species.⁸ Sterile isotonic saline, warmed to 37°C, was then infused into the abdomen by syringe at 20ml/kg. The syringe was left in place to maintain sterility and prevent back-leakage of fluid. The rat was gently rolled from side to side to distribute fluid throughout the abdomen. After a dwell time of ten minutes, as much fluid as possible was gently aspirated from the peritoneal cavity and the catheter withdrawn. Due to difficulties with recovery of sufficient volume of lavage fluid, the saline volume infused was increased to 40ml/kg for DPL performed on Day 9 of the study.

Assessment of diagnostic peritoneal lavage fluid

A one-ml sample of the DPL fluid was collected into a sterile vacutainer^{®h} (with no additives) for assessment of colour, turbidity and presence of particulate matter. Total nucleated cell counts were performed manually using a haemocytometerⁱ. A direct smear and a 100µl cytospin preparation of the DPL fluid was examined by the author to assess cellular morphology and to perform a differential count of 200 nucleated cells. A separate one ml aliquot of the diagnostic peritoneal lavage samples from Day 9 was sent, within 2 hours, for aerobic and anaerobic bacterial culture.

^e 0.1% solution prepared from Hibiclens Skin Cleanser, ICI Pharmaceuticals, ICI Australia Operations Pty. Ltd., Australia

^f 0.5% solution in alcohol prepared from Hibitane concentrate, Zeneca Pharmaceuticals Pty. Ltd., Australia

^g Optiva™ intravenous catheter, Critikon, Italy

^h Becton Dickson and Co. New Jersey, USA

ⁱ Kova®Glasstic® Slide 10 with grids, Hycor Biomedical Inc., California, USA

Technique for intraperitoneal infusion

Rats were anaesthetised, positioned and aseptically prepared as described above. The solutions (Lipofundin or saline) were warmed to 37°C and injected over a five minute period into the lower left quadrant of the abdomen using a syringe and 22 gauge needle.

Abdominal pain assessment

Due to the concern that this lipid solution may incite some degree of inflammation when delivered intraperitoneally, a pain assessment scoring system was used.⁹ Five parameters were assessed: change in body weight, appearance, clinical signs, unprovoked behaviour and response to abdominal palpation. Scores from 0 to 3 were given for each variable. A score of 0 corresponded to no obvious deviation from normal, score 1 possibly abnormal, score 2 a definite change, and a score 3 a marked change from normal. If a score 3 was given in the same assessment of an animal more than once, all scores of 3 for that assessment were given an extra mark. Total scores were added and interpreted according to Figure 6.2. A copy of the pain assessment score sheet used in this study can be found in Appendix I. If required, analgesia was to be provided in the form of subcutaneous buprenorphine. If any score of 15 or greater was given it was planned for that rat to proceed immediately to anaesthesia and DPL followed by euthanasia and post mortem.

Figure 6.2: Interpretation of pain assessment scores

Score	Assessment
0 to 4	Normal
5 to 9	Monitor carefully Consider the use of analgesics
10 to 14	Evidence of suffering, pain relief should be provided Animals should be regularly checked Consider termination of experiment
15 to 20	Evidence of severe pain Experiment of questionable value due to physiological abnormalities Terminate experiment if conditions likely to persist

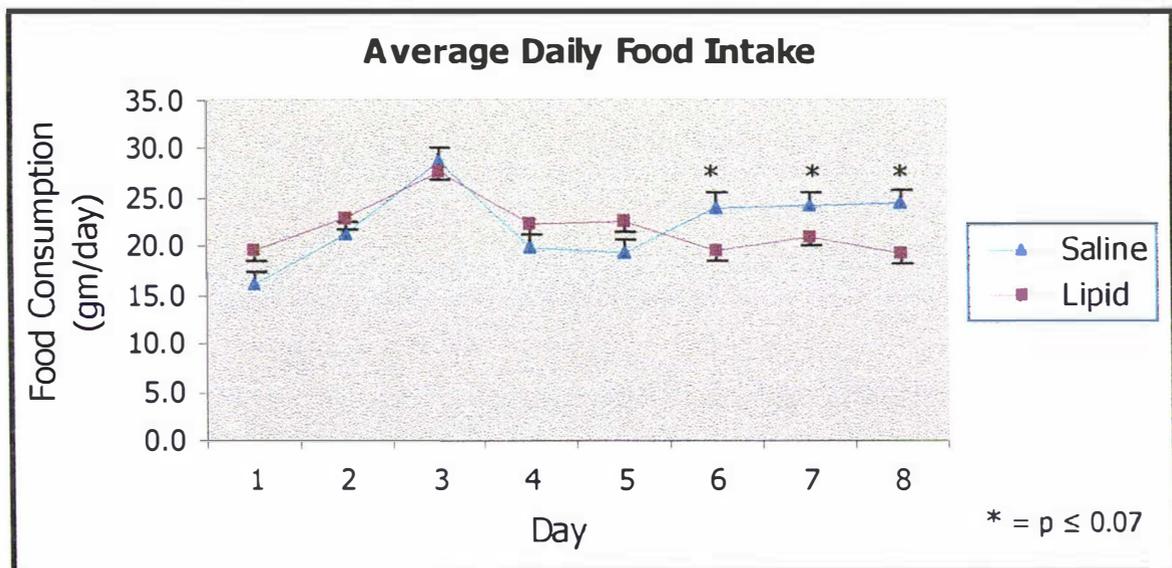
Statistical analysis

Data was log transformed due to high variance which occurred as the cell counts increased. Log transformed total nucleated cell counts, large mononuclear cell and neutrophil counts from Day 9 DPL fluid were compared between treatment groups using single factor ANOVA. Within group comparisons of the Day 5 DPL fluid cell counts and comparisons of Day 5 and Day 9 DPL fluid analysis were not made due to the inconsistent yields obtained from Day 5 DPL and consequent necessity to increase the volume of DPL infusate from 20 to 40ml/kg for Day 9 DPL. Daily food intake on Days 6, 7 and 8 were compared between treatment groups using repeated measures ANOVA. For all statistical evaluations, $p \leq 0.05$ was considered significant.

6.3 RESULTS

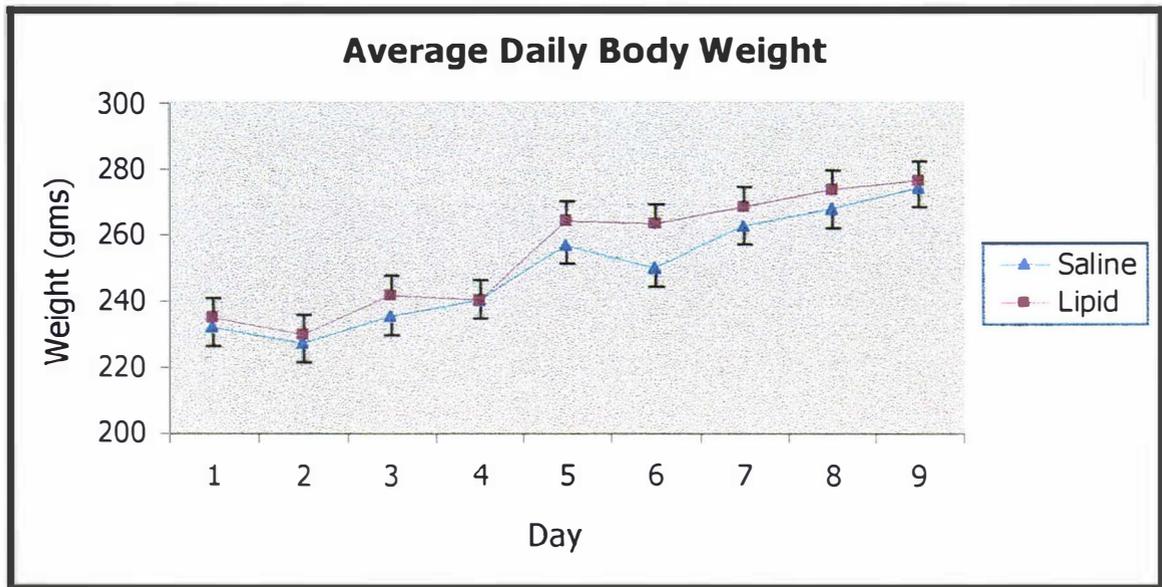
Daily food intake fluctuated in all rats throughout the experimental period. No rat ceased eating at any stage of the experiment. The lowest average daily food intake for all rats occurred in the first 24-hour period (Figure 6.3). Food intake declined throughout the treatment period in the lipid treatment group, averaging 5.3gm less during the final 24-hour period than the saline treatment group. The difference in food intake between the two groups on Days 6, 7 and 8 approached statistical significance ($p \leq 0.07$).

Figure 6.3: Daily food intake, mean and standard error, for the five rats in each treatment group



Average bodyweight of the ten rats on entry into the study was 233.4gms (range = 207.3 to 261.6). All rats gained weight over the experimental period (Figure 6.4). This weight gain averaged 42.3gms in the saline group and 41.7gms in the lipid group. Average bodyweight of both groups decreased twice during the study, on Days 2 and 6, corresponding to the day after arrival and the day after commencing experimental manipulations.

Figure 6.4: Daily body weight, mean and standard error, for the five rats in each treatment group.



Total nucleated and differential cell counts of the fluid obtained from DPL performed on Day 5 of the study are shown in Table 6.1. Samples were recovered from all rats but volumes were small (in some cases less than 0.1ml). Gross evaluation of the DPL samples revealed clear to straw coloured fluid free from particulate matter in six of the ten rats. Slightly cloudy, pink fluid was noted in two, and grossly haemorrhagic fluid from another. One sample contained aggregated red cells, which precluded any assessment of nucleated cells. Total nucleated cell counts were performed on nine samples. These samples averaged 0.897×10^9 nucleated cells/L (range = 0.002 to 3.899×10^9 cells/L). Three of the nine samples contained too few cells to perform differential counts. Of the six samples in which differential cell counts were performed, large mononuclear cells and lymphocytes predominated. Neutrophils, eosinophils and mast cells were also noted.

Table 6.1: Diagnostic peritoneal lavage results Day 5

Cell Type	Units	Saline			Lipid		
		n.	Mean	S.D.	n.	Mean	S.D.
Total nucleated	10 ⁹ cells/L	4	0.975	1.633	5	0.835	1.714
Large mononuclear	10 ⁹ cells/L	3	0.839	0.168	3	1.009	1.726
Lymphocytes	10 ⁹ cells/L	3	0.239	0.048	3	0.122	0.110
Neutrophils	10 ⁹ cells/L	3	0.009	0.017	3	0.014	0.023
Eosinophils	10 ⁹ cells/L	3	0.155	0.112	3	0.183	0.314
Mast cells	10 ⁹ cells/L	3	0.058	0.004	3	0.039	0.068

n = number of rats from which this data was collected

S.D. = standard deviation

Diagnostic peritoneal lavage performed on Day 9 returned fluid volumes of greater than three ml from each rat. In the saline treatment group, gross evaluation of the returned fluid revealed clear to straw coloured fluid with no obvious particulate matter. Two samples were slightly cloudy. In the lipid treatment group, lavage returned a white opaque fluid in four rats and a cloudy straw-coloured fluid from another. Clear gelatinous clots were noted in three samples whilst white particulate material was detected in another.

Quantitative assessment of Day 9 lavage fluid revealed an average total nucleated cell count of 3.328×10^9 cells per litre in the saline treatment group and 10.933×10^9 cells per litre in the lipid treatment group. This trend for the lipid treatment group to have higher total nucleated cell counts was not statistically significant ($p=0.066$). Associated with this elevation in nucleated cell count in the lipid treatment group was an increased proportion of neutrophils. Statistically significant differences existed between saline and lipid treatment groups in the absolute neutrophil and large mononuclear cell counts. Neutrophil count was higher ($p=0.012$) and large mononuclear cell count lower ($p=0.035$) in the lipid treated group. Absolute total and differential nucleated cell counts for both treatments groups are shown in Table 6.2.

Table 6.2: Diagnostic peritoneal lavage results Day 9

Cell Type	Units	Saline		Lipid		p value
		Mean	S.D.	Mean	S.D.	
Total nucleated	10 ⁹ cells/L	3.328	1.075	10.933	9.097	0.066
Large mononuclear	10 ⁹ cells/L	1.830	0.615	5.868	4.825	0.035
Lymphocytes	10 ⁹ cells/L	0.441	0.257	0.462	0.365	
Neutrophils	10 ⁹ cells/L	0.325	0.640	3.714	4.187	0.012
Eosinophils	10 ⁹ cells/L	0.635	0.300	0.796	0.803	
Mast cells	10 ⁹ cells/L	0.098	0.073	0.094	0.120	

S.D. = standard deviation

Cytologically, DPL fluid from rats in the saline treatment group consisted mainly of large mononuclear cells (Table 6.2). Eosinophils were the next most prominent cell type, with smaller numbers of lymphocytes and neutrophils and occasional mast cells (Figures 6.5 and 6.6). Cytology performed on DPL fluid from lipid treated rats revealed greater numbers of neutrophils, some of which displayed degenerative changes (Figure 6.7). Neutrophils, large mononuclear cells and lymphocytes contained vacuolated cytoplasm (Figures 6.7 and 6.8). Sudan III staining demonstrated these vacuoles to contain lipid (Figure 6.9). No evidence of infectious organisms was seen on cytological evaluation of any DPL sample.

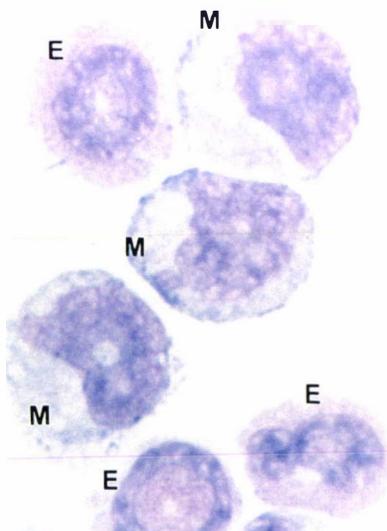


Figure 6.5: Photomicrograph (original magnification x 500) of a cytospin preparation, stained with Diff-Quik®, of diagnostic peritoneal lavage fluid from a rat following three days of intraperitoneal saline infusions. Typical rat eosinophils (E) are demonstrated with orange-red staining rod-like cytoplasmic granules and ring shaped nucleus. In addition, a number of larger mononuclear cells (M) can be seen.

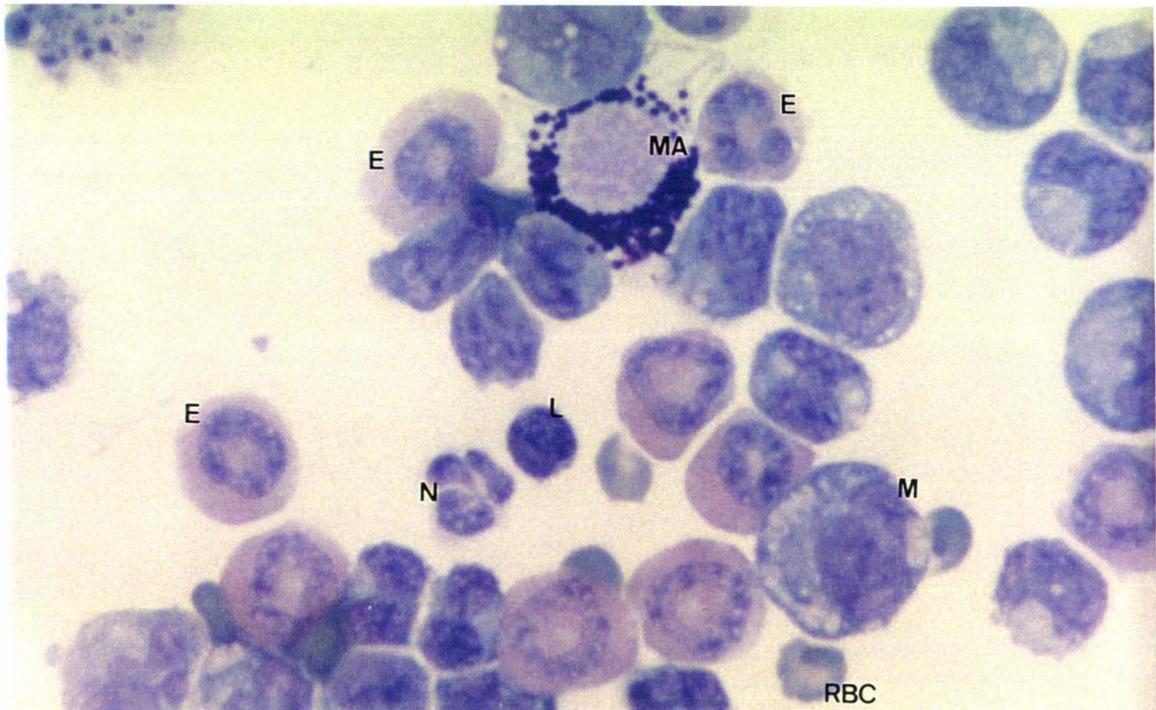


Figure 6.6: Photomicrograph of a Diff-Quik[®] stained cytospin preparation (original magnification x 250) from diagnostic peritoneal lavage performed on a rat following three days of intraperitoneal saline infusion. Typical murine eosinophils (E), with their ring shaped nucleus and orange-red staining rod-like cytoplasmic granules, are demonstrated. A mast cell (MA) can be seen along with large mononuclear cells (M), lymphocytes (L), occasional neutrophils (N) and red blood cells (RBC).

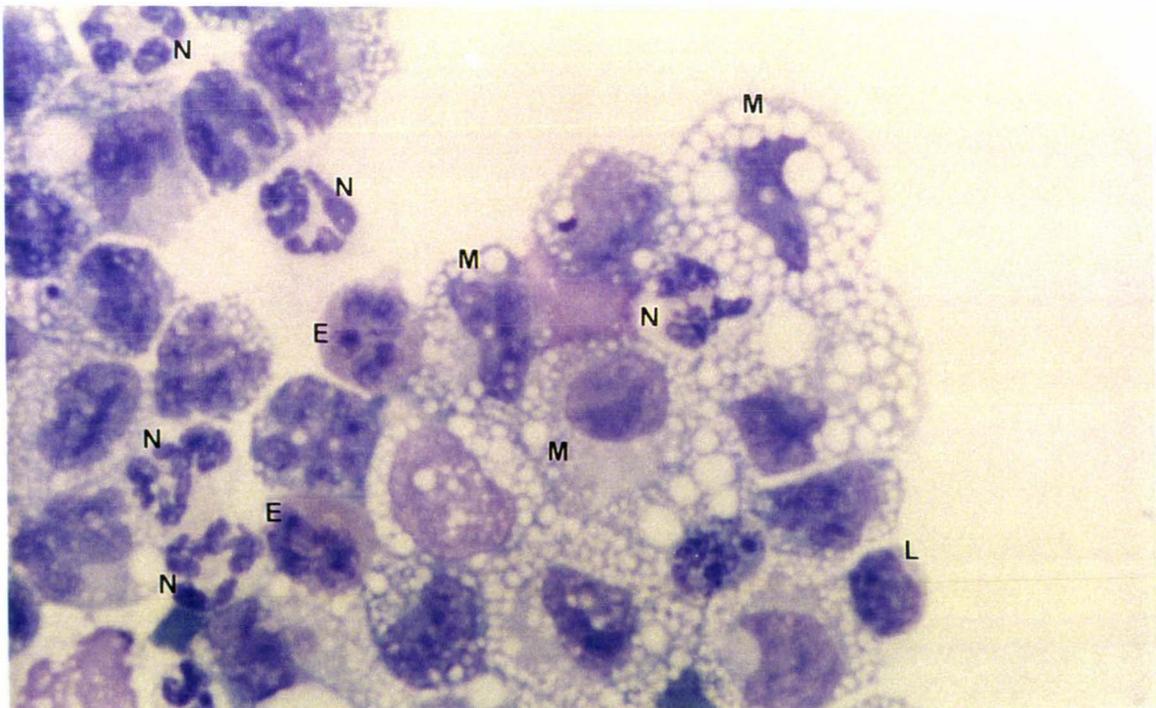


Figure 6.7: Photomicrograph of a Diff-Quik[®] stained cytospin preparation (original magnification x 250) of fluid obtained from diagnostic peritoneal lavage performed on a rat after three days of intraperitoneal lipid infusions. Neutrophils (N) and particularly the mononuclear cells (M) contain vacuolated cytoplasm as a result of lipid phagocytosis. Occasional lymphocytes (L) and eosinophils can be seen (E).

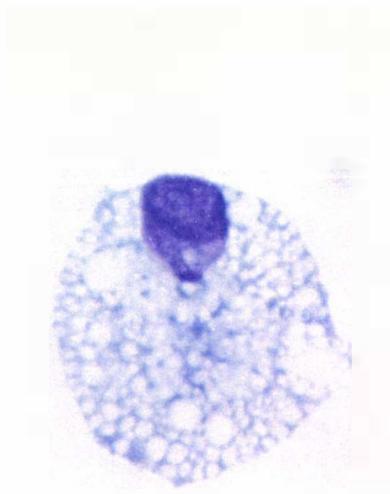


Figure 6.8: Photomicrograph (original magnification $\times 500$) of a large mononuclear cell from a Diff-Quik[®] stained cytospin preparation of fluid obtained from diagnostic peritoneal lavage in a rat after three days of intraperitoneal lipid infusion. Cytoplasm is vacuolated and the eccentric nucleus displays a prominent nucleolus. Sudan III staining demonstrated many of these cytoplasmic vacuoles contained lipid.

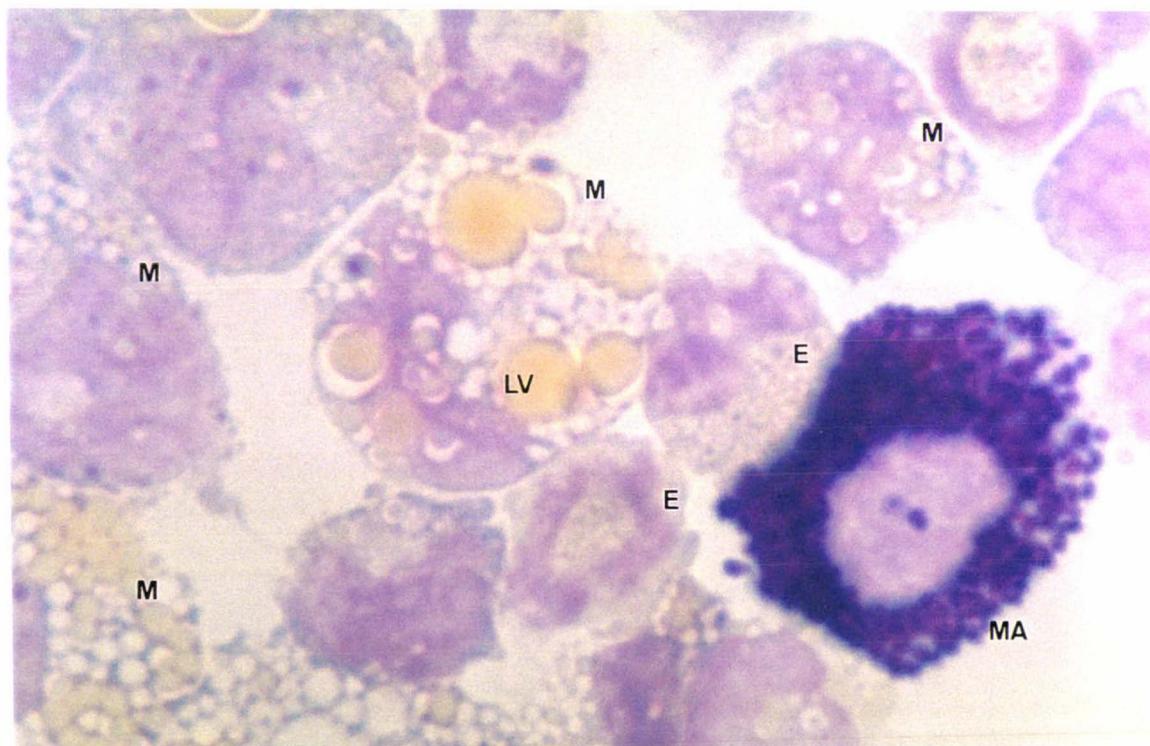


Figure 6.9: Photomicrograph of a cytospin preparation (original magnification $\times 500$) of fluid obtained from diagnostic peritoneal lavage in a rat after three days of intraperitoneal lipid infusion. The wet mounted slide was stained using Sudan III followed by New Methylene blue. Numerous mononuclear cells (M) with vacuolated cytoplasm are present. These vacuoles stained with Sudan III (LV), demonstrating the presence of lipid. Eosinophils (E) and a mast cell (MA) are also present.

One Day 9 DPL fluid sample from a saline treated rat was direct culture positive, producing a scanty growth of *Escherischia coli*. A variety of organisms were grown on enrichment broth from another six samples. Bacterial culture results from each rat are given in Table 6.3.

Table 6.3: Bacterial culture results from diagnostic peritoneal lavage performed after three intraperitoneal infusions of either lipid or saline.

Rat	Treatment Group	Direct culture	Enrichment broth
1	Saline	No growth	No growth
2	Lipid	No growth	Streptococcus sp
3	Saline	No growth	Coryneform organism
4	Saline	No growth	No growth
5	Lipid	No growth	Streptococcus sp and coliform
6	Lipid	No growth	Streptococcus sp
7	Lipid	No growth	Unidentified gram positive bacillus & coagulase negative Staphylococcus
8	Saline	Scanty growth of <i>Escherischia Coli</i>	Streptococcus sp
9	Saline	No growth	No growth
10	Lipid	No growth	Coryneform organism

Two rats suffered minor, self-resolving haemorrhage as a result of DPL catheter placement. Subcutaneous leakage of fluid from the site of abdominal puncture occurred in one rat after its second lipid infusion, which resolved after brief application of digital pressure to the site. Abdominal distension was noted in two rats from the lipid treatment group from Day 7 until the end of the study. The degree of distension did not appear to cause discomfort or impair respiration. Abdominal muscle fasciculation occurred in two rats during their initial intraperitoneal lipid infusions. Both rats appeared adequately anaesthetised and made no gross movements. In one of the two rats, a 10-20% increase in both heart and respiratory rate was noticed to occur during lipid infusion.

No obvious signs of distress or discomfort were detected in any rat during this study. Pain assessment scores, separated by treatment group, can be found in Table 6.4. No total pain score greater than a 3 was recorded on any day.

Table 6.4: Pain assessment scores for each treatment group throughout the experimental period

Score	Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9	
	Saline	Lipid														
0			2	3	2	1	10	10	2				2	3	4	4
1	2	1	3	2	2	4			6	10	9	9	4	4	1	
2	1	2			1				2		1	1	3	1		1
3	2	2											1	2		

Each box represents the number of animals in that treatment group which received the specific score on that day. Pain assessment scores were generated for each rat, once daily during the acclimatisation period (Days 2 to 4) and the final day (Day 9), and twice daily on the remaining days. Scores were based upon deviation from normal behaviour, physical examination, changes in food and water intake and bodyweight.

At post mortem, one rat from the saline treatment group (Rat 1) had a 1mm diameter adhesion noted on the ventral surface of the caecum. This may have been due to damage caused by intraperitoneal injection. No other abnormalities were detected on gross post mortem examination in the saline treated rats. Of the five rats which received intraperitoneal lipid, three were noted to have milky fluid within the peritoneal cavity with white fatty deposits on the serosal surfaces of intraperitoneal structures. One of these rats had generalised inspissated lipid throughout the peritoneal cavity. All of the rats in the lipid treatment group displayed generalised erythema of the peritoneal surfaces.

Histological examination of liver, gut, body wall and diaphragm taken from saline treated rats displayed no abnormalities in four of the five rats. One rat displayed mild hyperplasia and inflammation of the peritoneum. Figure 6.10 is a typical photomicrograph of a section through the diaphragm of a saline treatment rat stained with hematoxylin and eosin.

Histological examination of those samples from the lipid treatment group displayed hyperplasia and hypertrophy of the mesothelial layer. A mixed inflammatory infiltrate in the mesothelial and submesothelial layers was present (see Figure 6.11). Vacuolation was noted in the cytoplasm of neutrophils and monuclear cells and there was increased vascularity within the peritoneal sub-mesothelial layer. Early peritoneal fibroplasia was also present in two of the five rats. Changes were most marked in the diaphragm and perirenal tissues but degree of change varied between rats. Figure 6.11 is a section of the diaphragm from a lipid treated rat stained with hematoxylin and eosin.

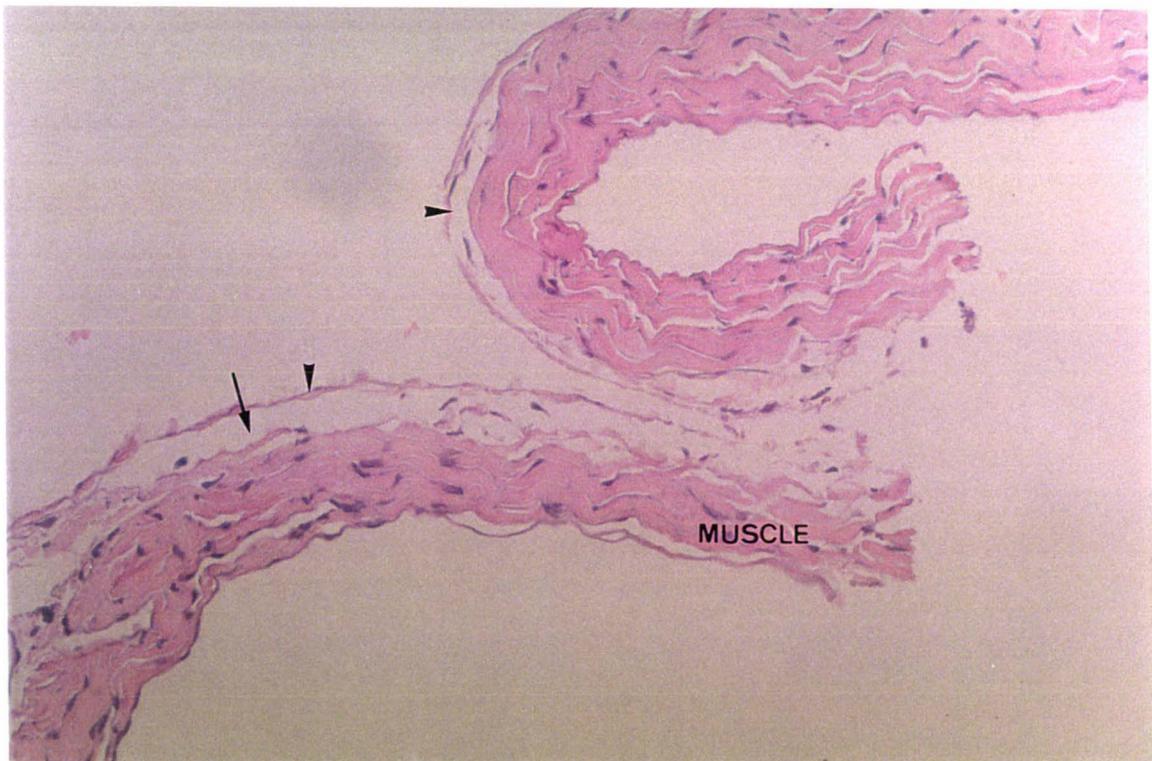


Figure 6.10: Photomicrograph (original magnification x 40) of rat diaphragm stained with hematoxylin and eosin. This rat had received three intraperitoneal saline injections and two diagnostic peritoneal lavages. Histological appearance is normal, a single mesothelial cell layer can be visualised (arrowhead). Separation of the peritoneum from the underlying muscular tissue (arrow) is artifactual.

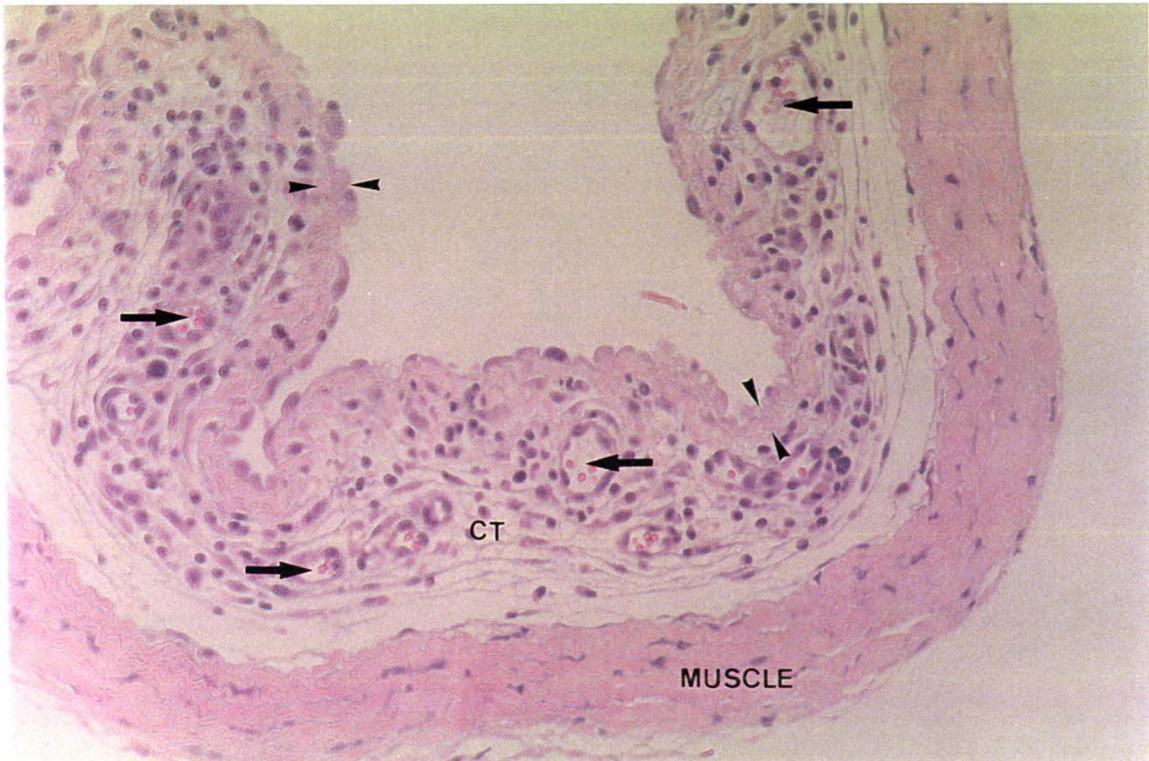


Figure 6.11: Photomicrograph (original magnification x 40) of rat diaphragm stained with hematoxylin and eosin. This rat had received three intraperitoneal lipid injections and two diagnostic peritoneal lavages. The peritoneal mesothelial layer (between the arrowheads) displays hypertrophy and hyperplasia. Submesothelial connective tissue (CT) layer is increased in thickness and has a marked inflammatory cell infiltrate. Early fibroplastic changes are present as are an increased number of blood vessels or lymphatic channels (arrows).

6.4 DISCUSSION

Although not statistically significant, there was a trend for rats in the lipid treatment group to eat less. This observation has been reported previously in rats receiving intraperitoneal lipid infusions.¹⁰ Rats in the current study were still growing and it was expected that food intake would increase over the study period. Multiple factors may have played a role in the decline in appetite of the lipid treated rats. Rats are reported to eat sufficient calories to meet energy requirements.¹¹ The calories supplied by the intraperitoneal lipid may have reduced voluntary food intake, however, the reduction in caloric intake seen was slightly greater than the number of calories supplied by the intraperitoneal lipid. Alternatively, the abdominal distention resulting from intraperitoneal infusion may have reduced appetite. A fluid volume of 30 ml/kg into the peritoneal cavity of rabbits has been shown to reduce voluntary food intake.¹² Although both treatment groups in the present study received the same volume of fluid intraperitoneally, saline is absorbed more rapidly than lipid. It is, therefore, likely that intraperitoneal fluid volumes persisted longer in the lipid treated rats. Lastly, the peritoneal inflammation seen in the lipid treated rats may have inhibited appetite. The exact reasons for peritonitis inhibiting appetite remain unclear but may include abdominal discomfort or release of inflammatory mediators.

Diagnostic peritoneal lavage performed after the intraperitoneal lipid infusions revealed a trend towards an elevation in total nucleated cell numbers in the returned lavage fluid compared to saline treated rats. Baseline peritoneal lavage data was considered unreliable due to very poor fluid returns. In addition, increase in the volume of fluid infused for DPL on Day 9 makes comparison difficult. Therefore, in this study, a comparison between DPL results from healthy rats and rats after peritoneal infusions, is lacking. Repeated percutaneous abdominal punctures may themselves have resulted in some peritoneal inflammation secondary to introduction of small numbers of bacteria, trauma to body wall

or internal structures, and healing processes. An elevation in nucleated cell numbers in diagnostic peritoneal lavage fluid could accompany such processes. Given the lack of reliable baseline DPL data, it is difficult to rule this out as a possible contributing factor to the total nucleated cell counts in Day 9 DPL fluid from both saline and lipid treated groups.

Importantly, a significant shift in the DPL fluid cell population from mononuclear cells to neutrophils was seen in lipid treated rats. This suggests that the lipid emulsion infused intraperitoneally in these rats incited an acute inflammatory response. Such an inflammatory reaction would also explain the trend towards higher total nucleated cell counts in DPL fluid from lipid treated rats versus saline treated rats. The lipid solution used in this study differs in its formulation from those used in previous intraperitoneal nutrition studies. Lipofundin® MCT/LCT is composed of a 50:50 mixture of medium and long chain triglycerides, whereas previously utilised solutions have been based primarily on long chain triglycerides (LCT).^{5,13,14} It is possible that MCTs incite an inflammatory reaction but any component of the solution, including emulsifiers, preservatives or excipients may have contributed. Lipid solutions, composed primarily of long-chain triglycerides, have been administered intraperitoneally to a variety of animal species without reported incident.^{5,13,14} In one study, however, rats died suddenly following intraperitoneal administration of 200ml/kg bolus of lipid solution.¹⁵ Death of rats in this study was likely the result of the large volume of fluid placed into their peritoneal cavities and subsequent effects on cardiac and respiratory function, rather than a specific effect of the lipid solution.

Low numbers of *Escherichia coli* were grown from direct bacterial culture of DPL fluid collected on Day 9 from a saline treated rat (Rat 8). Diagnostic peritoneal lavage fluid obtained from this rat on Day 9 had the highest total nucleated cell count of the control group as well as the highest percentage of neutrophils. Histological examination of this

rat's peritoneum revealed mild mesothelial hypertrophy accompanied by inflammatory cell infiltration. No evidence of infectious organisms was detected on DPL cytology and this rat had no clinical signs of peritonitis. The possibility remains, however, that this rat may have had low-grade, sub-clinical septic peritonitis. A cause of septic peritonitis was not definitively identified but may have resulted from perforation of the gastrointestinal tract by needle or catheter, or contamination of the abdomen with organisms from the skin at the time of abdominal punctures.

Six of the remaining nine diagnostic peritoneal lavage samples grew one or more bacterial species on enrichment broth. No infectious organisms were detected cytologically or histologically to support infectious peritonitis. The significance of organisms grown on enrichment broth is uncertain. Bacterial numbers would have been small and it is possible that they could have been contaminants. Contamination of the DPL sample may have occurred at any stage of the procedure despite precautions with skin preparation, technique, sample handling and shipment. Bacterial contamination of the peritoneal cavity is a risk of percutaneous abdominal puncture and infusion of nutrient solution, although the risk of septic peritonitis occurring during IPN appears small if precautions are taken to maintain sterility.^{3,6,16}

Bacteria normally resident in the intestinal tract have been demonstrated to traverse the gastrointestinal mucosal barrier and may explain the presence of low numbers of bacteria in the peritoneal cavity.¹⁷ Numerous factors have been reported to facilitate bacterial translocation including: abnormal bacterial proliferation, host stress or immune dysfunction, physical insults to mucosal integrity and altered nutrition status.^{17,18} A number of these factors would have been present in the rats in this study. Bacteria have been cultured from mesenteric lymph nodes, presumably translocated from the gut, in healthy rats and dogs.^{19,20} One previous study has examined bacterial culture results from DPL performed in healthy animals and reports positive bacterial cultures in a number of

these cats (Allan FJ 1999 *pers comm*). The placement of a solution known to support bacterial growth, such as a lipid emulsion, may also have improved bacterial survival, thus facilitating their detection. Alternatively, lipids have been thought to impair immune system function which may have facilitated bacterial survival in the peritoneal cavity.²¹

Iatrogenic haemorrhage was the most common complication of abdominal punctures performed in this study. Haemorrhage is a potential complication of DPL with an increased incidence reported in association with paramedian catheter insertion in dogs and cats.^{22,23} Despite this, a paramedian approach was used in the present study according to recommendations for intraperitoneal injection in this species in order to avoid damaging intra-abdominal structures.⁸ Technique for performing DPL in rats is not reported in the literature. Other complications encountered, also previously reported, were subcutaneous fluid leakage and iatrogenic bowel injury.²² The small adhesion noted on the surface of the caecum in one rat may have occurred secondary to serosal damage or penetration of the bowel lumen, but did not appear to result in any clinical or laboratory abnormalities.

Despite finding cytological and histological evidence of peritoneal inflammation in lipid treated rats the pain scores indicated that any discomfort experienced by the rats was minor. Although pain score assessment is subjective, it was considered our best clinical means of assessing pain or distress. Transient fasciculation of abdominal musculature, noted to occur in two rats during their initial intraperitoneal lipid infusions, were also noted in a previous pilot study involving infusion of the same lipid solution to two cats (Chapter 5). The fasciculations may have been a result of peritoneal irritation by the lipid solution. Pain on intraperitoneal lipid infusion has been reported in a study involving a group of human patients.²⁴ It has not, however, been a feature noted in previous animal studies.^{5,13,14,25}

The inflammation, hyperaemia and thickening of the peritoneum noted at post-mortem examination of the lipid treated rats has been previously documented to occur post infusion of a variety of nutrient solutions into the peritoneal cavity.^{3,6,26} As has also been previously reported,¹⁵ a moderate amount of inspissated lipid was present throughout the peritoneal cavity of one rat occasional lipid granulomas noted in others. Presumably, inspissation occurred because the solution in which the lipid was emulsified was absorbed but the lipid was not. What caused failure of lipid absorption was not determined. A difference may exist in an individual's ability to absorb fatty acids from the peritoneal cavity. The granulomatous lesions could have resulted from inflammation secondary to localised areas of inspissated lipid.

Increased thickness of the mesothelial cell layer, neutrophilic infiltration, neovascularisation and fibrosis support the presence of an inflammatory peritoneal reaction in lipid treated rats. Mesothelial hypertrophy and hyperplasia have previously been documented to occur as a result of intraperitoneal nutrient infusion.^{6,10} It has been suggested these changes may occur secondary to the increased absorptive demands placed on the peritoneum. The marked difference between the two treatment groups, however, would lend support to the hypothesis that this peritoneal inflammatory reaction was a result of the lipid infusion.

In summary, this study in rats confirms our previous observations in cats: 20 ml/kg of 20% Lipofundin® MCT/LCT delivered intraperitoneally results in peritoneal inflammation. In contrast to the cats in the pilot study, rats did not display signs of pain or systemic illness. A species variation may, therefore, exist in the response to peritoneal irritation or inflammation. At this time it would seem unwise to recommend this lipid solution for intraperitoneal administration. Further studies are necessary to determine which component(s) of this lipid solution are responsible for inciting the inflammation, and whether other lipid solutions cause a similar response.

REFERENCES

1. Carter LJ, Sinclair KGA, Copeland EM *et al.* Clinical experience with peritoneal dialysis in small animals. *Compendium on Continuing Education for the Practicing Veterinarian*. 1989;**11**:1335-1343
2. Hanning RM, Balfe JW, Zlotkin SH. The effect of amino acid containing dialysis solutions on plasma amino acid profiles in children with chronic renal failure. *Journal of Pediatric Gastroenterology and Nutrition*. 1987;**6**:942-947
3. Gilsdorf RB, Selby RR, Schon DA *et al.* Total nutritional support through the peritoneal cavity. *Journal of the American College of Nutrition*. 1985;**4**:461-469
4. Bryant MS, Sinclair KGA, Copeland EM *et al.* Absorption of a parenteral diet from the peritoneal cavity. *Current Surgery*. 1987;**44**:382-384
5. Kalfarentzos F, Spiliotis J, Christopoulos D *et al.* Total parenteral nutrition by intraperitoneal feeding in rabbits. *European Surgical Research*. 1988;**20**:352-357
6. Garcia-Gamito FJ, Moran JM, Mahedero G *et al.* Long-term peritoneal nutrition in dogs, both normal and after intestinal resection. *International Surgery*. 1991;**76**:235-240
7. Crowe DT. Abdominocentesis and diagnostic peritoneal lavage in small animals. *Modern Veterinary Practice*. 1984;**65**:877-882
8. Waynforth HB, Flechnell PA. Administration of substances. In: *Experimental and surgical technique in the rat*. Harcourt Brace. 1992; pp21-22
9. Morton DB, Griffiths PHM. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record*. 1985;**116**:431-436
10. Moran JM, Limon M, Mahedero G *et al.* Biochemical and histological findings after a long-term intraperitoneal fat infusion. An experimental study in rats. *Journal of Parenteral and Enteral Nutrition*. 1986;**10**:609-613
11. Anon. Nutrient requirements of the laboratory rat. In: *Nutrient requirements of laboratory animals*. Third edition. National Research Council. National Academy of Sciences, Washington, United States. 1978; pp 7-37

12. Balaskas EV, Rodela H, Oreopoulos DG. Effects of intraperitoneal infusions of dextrose and amino acids on the appetite of rabbits. *Peritoneal Dialysis International*. 1993;**13**
S2:S490-S498
13. Klein MD, Coran AG, Drongowski RA *et al*. The quantitative transperitoneal absorption of a fat emulsion: Implications for intraperitoneal nutrition. *Journal of Pediatric Surgery*. 1983;**18**:724-731
14. Mahedero G, Moran JM, Salas J *et al*. Absorption of intralipid and interferences from nutrients infused into the peritoneal cavity of the rat. *American Journal of Surgery*. 1992;**164**:45-50
15. Rubin J, Jones Q, Planch A *et al*. Intraperitoneal feeding. *Transactions of the American Society of Artificial Internal Organs*. 1988;**34**:125-130
16. Moran JM, Limon M, Mahedero G *et al*. Long-term peritoneal nutrition in dogs: Metabolic and histopathologic results. *Nutrition*. 1989;**5**:89-93
17. Barber AE, Jones WG, Minei JP *et al*. Bacterial overgrowth and intestinal atrophy in the etiology of gut barrier failure in the rat. *American Journal of Surgery*. 1991;**161**:300-304
18. Nakasaki H, Mitomi T, Tajima T *et al*. Gut bacterial translocation during total parenteral nutrition in experimental rats and its countermeasure. *American Journal of Surgery*. 1998;**175**:38-43
19. Spaeth G, Gottwald T, Hirner A. Fibre is an essential ingredient of enteral diets to limit bacterial translocation in rats. *European Journal of Surgery*. 1995;**161**:513-518
20. Dahlinger J, Marks SL, Hirsh DW. Prevalence and identity of translocating bacteria in healthy dogs. *Journal of Veterinary Internal Medicine*. 1997;**11**:319-322
21. Wan JM, Tea TC, Babayan VK *et al*. Invited comments: Lipids and the development of immunodysfunction and infection. *Journal of Parenteral and Enteral Nutrition*. 1988;**12**:43S-52S
22. Crowe DT. Diagnostic abdominal paracentesis techniques: Clinical evaluation in 129 dogs and cats. *Journal of the American Animal Hospital Association*. 1984;**20**:223-230
23. Bjorling DE, Latimer KS, Rawlings CA *et al*. Diagnostic peritoneal lavage before and after abdominal surgery in dogs. *American Journal of Veterinary Research*. 1983;**44**:816-820

24. Lindqvist B. Nutrition by intraperitoneal fat infusion. *Acta Medica Scandinavia*. 1969;**185**:323-325
25. Moran J, Cruz G, Nogue-Rales F *et al*. Transperitoneal absorption of Intralipid in rats: Total serum fatty acids and triglyceride after absorption. *Journal of Parenteral and Enteral Nutrition*. 1986;**10**:604-608
26. Stone MM, Mulvihill SJ, Lewin KJ *et al*. Long-term total intraperitoneal nutrition in a rabbit model. *Journal of Pediatric Surgery*. 1986;**21**:267-270

CHAPTER 7

GENERAL CONCLUSIONS

A review of the published literature suggests intraperitoneal nutrition is an inexpensive and practical alternative to intravenous parenteral nutrition. It has been convincingly demonstrated that glucose and amino acid solutions are absorbed after intraperitoneal administration.^{1,2,3,4} Many studies have shown lipid solutions are also absorbed from the peritoneal cavity, albeit at slower rates.^{5,6,7,8} Few side effects have been encountered in previously published intraperitoneal nutrition studies, however, infection and hypovolaemic shock have been reported. Septic peritonitis has been reported infrequently and where it has occurred, has been easily treated.^{9,10} Hypovolaemic shock has occurred secondarily to the intraperitoneal instillation of large volumes of hypertonic solution.^{11,12} This body of work has uncovered a number of problems with both the technique of intraperitoneal nutrition and the nutrient solutions used, particularly in relation to feline patients.

Due to their inherent disposition, most cats are unlikely to tolerate the restraint and positioning necessary to perform intraperitoneal puncture in a safe and sterile fashion without heavy sedation or anaesthesia. Current recommendation for intraperitoneal catheter placement requires dogs and cats to be positioned in dorsal recumbency to minimise the risk of iatrogenic injury. Potentially, cats would require sedation for every intraperitoneal nutrient delivery, which could occur multiple times each day. Repeated use of chemical restraint in debilitated patients would need careful consideration. The added cost and deleterious effects of sedation, such as hypothermia and prolonged recoveries, detract from the practicality of intraperitoneal nutrition.

An indwelling intraperitoneal catheter would obviate the need for repeated sedation. These catheters have been used successfully in previous intraperitoneal nutrition experiments; however, a number of problems have been reported.^{9,12,13} Intraperitoneal catheters can rapidly obstruct with omentum.¹⁴ Other problems reported, include fluid leakage from around the catheter entry site and catheter dislodgement.^{11,13} In addition, the placement and removal of indwelling peritoneal catheters requires general anaesthesia. These considerations detract from the practical advantages of intraperitoneal nutrition.

In the pilot study reported in Chapter 5, intraperitoneal infusion of hypertonic glucose and amino acid solutions results in clinical signs of hypovolaemia. Movement of fluid from the vascular compartment into the abdomen can be readily explained by the tonicity of the fluids infused intraperitoneally. Such events were, however, unexpected as administration of solutions with similar osmolalities has been previously reported in other species without mention of hypovolaemia.^{9,15} In the clinical setting, cats which would receive intraperitoneal nutrition are likely to be debilitated and fluid shifts in these patients would be of particular concern. Further research is required to determine what osmolality of solution is safely tolerated in the peritoneal cavity. This may vary with nutrient solution, disease states, as well as within and between species.

Nutrient solutions of high osmolality are often utilised in attempts to provide sufficient protein and calories. Currently available proprietary intravenous solutions of sugars and amino acids are hyperosmolar, resulting in total parenteral nutrition solutions that are markedly hypertonic. Diluting such solutions reduces osmolality but raises the problem of having to administer large volumes of fluid to meet nutrient requirements. Use of polymers, peptides and polypeptides may provide a solution to this problem as solutions have lower osmolalities and lower volumes could be delivered. Their use has already been

investigated in some intraperitoneal nutrition studies and they appear to be the future substrates of choice for this form of nutrition.^{1,16}

The use of lipids in total parenteral nutrition solutions is desirable because they are a concentrated form of calories, exert little osmotic force and provide a source of essential fatty acids.¹⁷ Compared to long-chain triglycerides, medium chain triglycerides (MCT) are cleared more rapidly from systemic circulation and are oxidised more completely.¹⁸ The use of MCT-based lipid solutions for intravenous nutrition is becoming more popular.¹⁸ In this study, a proprietary mixture of medium and long chain triglycerides (Lipofundin[®] MCT/LCT) was selected for investigation. To our knowledge, the lipid solution investigated in this study has never before been used in intraperitoneal nutrition research.

Lipofundin[®] MCT/ LCT infused intraperitoneally resulted in peritoneal inflammation in both rats and cats. In two cats, clinical signs of abdominal pain, pyrexia, anorexia and vomiting were accompanied by laboratory findings of sterile peritonitis. The same solution produced subclinical peritoneal inflammation in rats. Despite careful attempts to do so, the author may have not detected mild clinical signs of pain or discomfort resulting from the peritonitis in these rats. Alternatively, there may be a species difference in the reaction of the peritoneum to intraperitoneal lipid infusion or in the animal's response to peritoneal inflammation. Lipofundin[®] MCT/LCT does not appear to be a suitable lipid source for intraperitoneal nutrition. Further research is required to determine what constituent/s of this lipid emulsion are responsible for inciting peritoneal inflammation.

Many questions regarding intraperitoneal nutrition remain unanswered. What are the exact mechanisms and rates of nutrient absorption? What nutrient solutions are suitable for intraperitoneal administration? What tonicity and volume of solution can safely be administered intraperitoneally? What changes are induced in the peritoneal cavity by intraperitoneal nutrition and what impact do they have on the subject?

Despite the literature published in the 1980s suggesting great promise for the technique, little has been published since. In a recent paper from a leading group of intraperitoneal nutrition investigator the suggestion was made that "preparations designed for intravenous nutrition may not be suitable for intraperitoneal nutrition".¹⁶ Although not specifically mentioned, it appears that this group may have experienced similar problems or concerns with intraperitoneal nutrient instillation as those experienced in the current research and have returned to answer more fundamental questions regarding this form of nutritional support. Until the many unanswered questions are addressed, the technique of intraperitoneal nutrition does not appear to provide any easy solutions for the provision of parenteral nutritional support, particularly for feline patients.

REFERENCES

1. Gilsdorf RB, Selby R, Tillach P. Systemic appearance of nutrients placed into the peritoneal cavity. *Journal of Parenteral and Enteral Nutrition*. 1985;**9**:148-152
2. Albert A, Takamatsu H, Fonkalsrud EW. Absorption of glucose solutions from the peritoneal cavity in rabbits. *Archives of Surgery*. 1984; **119**:1247-1251
3. Kalfarentzos F, Spiliotis J, Christopolous D *et al*. Total parenteral nutrition by intraperitoneal feeding in rabbits. *European Journal of Surgical Research*. 1988;**20**:352-357
4. Stabile BE, Borzatta M. Transperitoneal absorption of glucose and amino acids for nutritional support. *Archives of Surgery*. 1987;**122**:344-348
5. Adkins ES, Salman FT, Fonkalsrud EW. Triglyceride absorption in transperitoneal alimentation. *American Journal of Surgery*. 1990;**159**:237-240
6. Klein MD, Coran AG, Drongowski RA *et al*. The quantitative transperitoneal absorption of a fat emulsion: Implications for intraperitoneal nutrition. *Journal of Pediatric Surgery*. 1983;**18**:724-731
7. Mahedero G, Moran JM, Salas J *et al*. Absorption of Intralipid and interferences from nutrients infused into the peritoneal cavity of the rat. *American Journal of Surgery*. 1992;**164**:45-50
8. Moran J, Cruz G, Nogue-Rales F *et al*. Transperitoneal absorption of Intralipid in rats: Total serum fatty acids and triglyceride after absorption. *Journal of Parenteral and Enteral Nutrition*. 1986;**10**:604-608
9. Garcia-Gamito FJ, Moran JM, Mahedero G *et al*. Long-term peritoneal nutrition in dogs, both normal and after intestinal resection. *International Surgery*. 1991;**76**:235-240
10. Moran JM, Garcia-Gamito FJ, Mahedero G *et al*. Peritoneal nutrition in dogs after intestinal resection: Comparative study. *Nutrition* 1989;**5**:315-320
11. Stone MM, Mulvihill SJ, Lewin KJ *et al*. Long-term total intraperitoneal nutrition in a rabbit model. *Journal of Pediatric Surgery*. 1986;**21**:267-270
12. Gilsdorf RB, Selby RR, Schon DA *et al*. Total nutritional support through the peritoneal cavity. *Journal of the American College of Nutrition*. 1985;**4**:461-469

13. Klein MD, Coran AG, Drongowski RA *et al.* Long-term survival of dogs maintained solely on intraperitoneal nutrition. *Journal of Pediatric Surgery*. 1985;**20**:765-771
14. Thornhill JA. Peritoneal dialysis in the dog and cat: An update. *Compendium on Continuing Education for the Practicing Veterinarian*. 1981;**3**:20-34
15. Rubin J, Jones Q, Planch A *et al.* Intraperitoneal feeding. *Transactions of the American Society of Artificial Internal Organs*. 1988;**34**:125-130
16. Moran JM, Mahedero G, Salas J *et al.* A pharmacokinetic study of peritoneal absorption of glucose and alanine in rats: Nutritional implications. *Journal of Parenteral and Enteral Nutrition*. 1998;**22**:72-76
17. Wretling A. Modern principles of the use of fat emulsion in parenteral nutrition. In: *Parenteral Nutrition*. Wilkinson AW, Ed.. Churchill Livingstone, Edinburgh. 1972, pp 160-171
18. Bach AC, Frey A, Lutz O. Clinical and experimental effects of medium-chain-triglyceride-based fat emulsions – A review. *Clinical Nutrition*. 1989;**8**:223-235

APPENDIX I - Pain Assessment Score Sheet

Modification of previously published assessment scheme. (Morton DB, Griffiths PHM. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record*. 1985;116:431-436)

Variable Assessed	Score 0 Responses	Score 1 Responses	Score 2 Responses	Score 3 Responses
Body Weight	<ul style="list-style-type: none"> ● Maintained or increased ● Normal food & water consumption 	<ul style="list-style-type: none"> ● < 5% decrease in body weight ● Normal food & water consumption 	<ul style="list-style-type: none"> ● 10-15% decrease in body weight ● Reduced food and water consumption 	<ul style="list-style-type: none"> ● >15% decrease in body weight ● Not eating or drinking
Appearance	<ul style="list-style-type: none"> ● Smooth, flat shiny coat ● Clear bright eyes 	<ul style="list-style-type: none"> ● Lack of grooming 	<ul style="list-style-type: none"> ● Rough coat ● Ocular or nasal discharge 	<ul style="list-style-type: none"> ● Ungroomed coat, external orifices ● Abnormal posture ● Eyes pale, mydriasis
Clinical Signs	<ul style="list-style-type: none"> ● Temp., HR, RR within normal limits 	<ul style="list-style-type: none"> ● Small changes in Temp., HR, RR 	<ul style="list-style-type: none"> ● Change in temp. +/- 1-2°C ● HR & RR elevated >30% 	<ul style="list-style-type: none"> ● Change in temp. +/- 2°C ● HR & RR elevated >50% or shallow RR ● Auscultatable abnormalities
Unprovoked Behaviour (ie. dull, hiding, immobile, pica, self injury etc.)	<ul style="list-style-type: none"> ● Normal behaviour 	<ul style="list-style-type: none"> ● Minor changes in behaviour 	<ul style="list-style-type: none"> ● Abnormal behaviour ie. less mobile, less alert or inactive 	<ul style="list-style-type: none"> ● Markedly abnormal ie. self mutilation, vocalisation, restlessness, immobility
Response to Stimuli (abdominal palpation)	<ul style="list-style-type: none"> ● Expected behavioural responses 	<ul style="list-style-type: none"> ● Minor exaggeration in response 	<ul style="list-style-type: none"> ● Moderate increased response or change in behaviour 	<ul style="list-style-type: none"> ● Violent reaction (unless very weak)

Note: If more than one score 3 is given to an animal then each score 3 is given an extra mark.

Interpretation of assessed scores

0 to 4	Normal
5 to 9	Monitor carefully
10 to 14	Consider use of analgesics Evidence of suffering, pain relief should be provided Animals should be under regular supervision Consider termination of experiment
15 to 20	Evidence of severe pain, relief must be provided Value of experiment questionable due to likely physiological abnormalities Experiment should be terminated if conditions likely to endure
