



**TUMOUR METASTASIS AND
DISSEMINATION DURING LAPAROSCOPIC
SURGERY**

Thesis submitted for the degree of Doctor of Philosophy

in the University of Adelaide

by

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This work is dedicated to my husband Peter to whom
I am forever indebted for his ongoing patience,
considerable sacrifices, and unending faith
which has enabled this work to be completed and to my friends and
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ABSTRACT

Recent applications of laparoscopy to the resection of abdominal and thoracic malignancy have been followed by a burgeoning literature which describes cases of metastatic involvement of laparoscopic port sites, not only in patients with advanced tumours but in patients with early stage carcinoma, and even in patients following laparoscopic procedures during which tumours were not disturbed. The development of a port site metastasis in a patient following laparoscopic tumour resection with curative intent or the 'upstaging' of tumour stage, constitutes a failure of treatment.

Experimental studies incorporating bench top and large animal models have confirmed that tumour cells are redistributed to port sites during laparoscopic surgery directly from contaminated instruments, or indirectly in the insufflation gas. Of particular concern, a large number of experimental studies have demonstrated an increase in tumour implantation and metastasis to wounds following laparoscopic as compared to laparotomy techniques. Previous work by the Royal Adelaide Centre for Endoscopic Surgery suggests that the addition of a pneumoperitoneum may increase the rate of tumour implantation five-fold. Of pivotal importance is the question of what contribution the laparoscopic environment plays in the process of tumour dissemination and whether these effects can be modulated.

This thesis utilised an established small animal model to investigate the aetiology of port site metastases and the efficacy of preventive strategies in reducing tumour implantation following laparoscopy.

The role of the insufflation gas was investigated using both benchtop and small animal models. Exclusion of carbon dioxide from the laparoscopic environment by replacement with the inert gas helium, was associated with a reduction in port site metastases and tumour implantation. Further work investigated the effect of carbon dioxide on the peritoneal immune environment and suggests that port site metastases can be influenced by alterations in the immune environment, particularly at the local level of the peritoneal membrane. Carbon dioxide induced depression of macrophage activity, possibly mediated by a decrease in intraperitoneal pH, is a contributing factor to the development of port site metastases.

Further studies investigating the role of insufflation pressure also support the concept that development of port site metastases is not solely due to mechanical factors. In particular, the development of port site metastases following laparoscopic surgery was not determined by the pressure of the insufflation gas.

The use of preventive strategies such as intraperitoneal cytotoxics at the time of surgery has been proposed by some authors and was investigated in this thesis. The findings support the use of appropriate intraperitoneal cytotoxic agents to reduce viable tumour cells and therefore tumour implantation in wounds. In particular the efficacy of intraperitoneal povidone iodine has been demonstrated. The role of tumour specific intraperitoneal chemotherapy has potential but requires further investigation.

The use of intraperitoneal heparin was demonstrated to reduce port site metastases whilst the presence of intraperitoneal blood promoted tumour implantation and growth. These findings suggest a possible role for the use of intraperitoneal heparin and reinforce the importance of meticulous surgical haemostasis.

Excision of port site wounds following laparoscopy resulted in an increase in port site tumours and is not an effective strategy, causing a potentially adverse outcome. The use of carbon dioxide laser or localised irrigation with povidone iodine were also not effective strategies to reduce tumour implantation in wounds following laparoscopy.

The work presented in this thesis suggests that the aetiology of this problem is likely to be multifactorial and that the development of port site metastases not only depends on physical redistribution of tumour cells, but also on the specific insufflation gas used, possibly due to influences on local metabolic or immune factors acting within the peritoneum. Effective strategies to reduce tumour implantation have been demonstrated, in particular the use helium insufflation, and intraperitoneal povidone iodine require further clinical evaluation.

Further research to clarify this problem is urgent. Until the issue is understood better, only patients entered into clinical trials should undergo laparoscopic surgery for malignancy.

DECLARATION

I declare that this thesis contains no material which has been accepted
for the award of any other degree or diploma in any University
and that to the best of my knowledge and belief,
the thesis contains no material previously published or written by another person,
except where due reference is made in the text of the thesis.
I further consent to this copy of my thesis, when deposited in the University
Library, being made available for loan and photocopying.

Susan J. Neuhaus

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The large number of rats that were subjects for these investigations into the mechanism of tumour metastasis following laparoscopy

‘Primum non nocere’

First do no harm

PREFACE

Part of the work described in this thesis has been published or accepted for publication. These publications are listed below in the order in which they were submitted. Copies of the front pages of these papers are included in the appendix.

Neuhaus SJ, Watson DI, Ellis T, Rowland R, Rofe AM, Mathew G, Jamieson GG. Wound metastasis after laparoscopy with different insufflation gases. *Surgery* May 1998 ; 579-583

Neuhaus SJ, Watson DI, Ellis T, Dodd T, Rofe AM, Jamieson GG. Efficacy of cytotoxic agents for the prevention of port site metastases. *Archives of Surgery* 1998, **133**;762-766

SJ Neuhaus, M Texler, PJ Hewett , DI Watson. Port site metastases following laparoscopic surgery - A Review *The British Journal of Surgery* 1998, **85**,735-741

Neuhaus SJ, Ellis T, Pike GK, Rofe AM, Jamieson GG, Watson DI. Tumour implantation following laparoscopy using different insufflation gases. *Surgical Endoscopy* 1998;**12**:1300-1302

Neuhaus SJ, Ellis T, Jamieson GG, Watson DI. An experimental study of intraperitoneal heparin on tumour implantation following laparoscopy. *The British Journal of Surgery* 1999, **86**, 400-404

SJ Neuhaus, DI Watson, T Ellis, AM Rofe, GG Jamieson. Influence of cytotoxic agents on intraperitoneal tumour implantation following laparoscopic surgery. *Diseases of the Colon and Rectum* 1999; **42**:10-15

Neuhaus SJ, Ellis T, Barrett MW, Rofe AM, Jamieson GG, Watson DI. In vitro inhibition of tumour growth in a helium rich environment - Implications for laparoscopic surgery. *Australian and New Zealand Journal of Surgery* 1999 ; **69**: 52-55

Neuhaus SJ, Watson DI, Ellis T, Dodd T, Jamieson GG. Port site metastases are not increased by high pressure insufflation *Minimally Invasive Technology and Allied Therapy* 1999, **8** (2): 117-121

Neuhaus SJ, Watson DI, Ellis T, Rofe AM, Mathew G, Jamieson GG. The influence of different gases on intraperitoneal immunity in tumour bearing rats. *World Journal of Surgery* 2000, **24**(10): 1227-1231

Neuhaus SJ, Watson DI, Ellis T, Rofe AM, Jamieson GG. The effect of immune enhancement and suppression on the development of laparoscopic port site metastases. *Surgical Endoscopy* 2000, **14** (5): 439-443

Neuhaus SJ, Hewett PJ, Disney . An unusual case of port site seeding. *Surgical Endoscopy* - (in press)

Neuhaus SJ, Watson DI. 'Strategies for prevention of port site metastases: laparoscopy without carbon dioxide' in "Port site recurrences in cancer surgery", Reymond MA, Bonjer HJ, Kockerling F (eds.), Springer Verlag, Hiedelberg/Berlin/New York 1999

SECTION I

INTRODUCTION



1.1 HISTORICAL OVERVIEW

Laparoscopy, the examination of the peritoneal cavity by means of endoscopes, is a recent addition to the surgical armamentarium but one which has revolutionised the art of general surgery.

Although attempts to visualise internal organs date back to the 9th Century ⁸⁴, it was the development of the cystoscope in the 19th Century that provided the precursor for modern laparoscopy. Early cystoscopes utilised crude lighting methods initially using candle light and mirrors (Bozzini) ¹⁶² and then later kerosene lanterns (Desormais) ¹⁵⁹. It was not until after Thomas Eddison developed the incandescent bulb in 1880 that a more contemporary light source was combined with an optical viewing instrument by Newman in 1883 ¹⁶².

The earliest attempt at human laparoscopy was performed by Jacobaeus, a Swedish physician. This was a purely diagnostic procedure with no pneumoperitoneum. The following year, in 1902 Kelling combined the use of a cystoscope and the insufflation of air via a syringe into the peritoneal cavity of a dog, to perform what he described as 'coelioscopy' ¹⁴⁹.

It was Zollkoffer, in 1926 that first proposed the use of carbon dioxide as an insufflating agent ¹⁶² after concerns about the potential for explosion with the combined use of electrocautery and oxygen. The efficacy and safety of carbon dioxide was established by Fevers in 1933 ⁶⁵. It was however, the development of the automated peritoneal insufflator by Kurt Semm in the 1960's that established the use of carbon dioxide as the gas of choice for peritoneal insufflation. The creation of a pneumoperitoneum with regulated, automated gas flow provided a viable working space ²³⁹, which, combined with the development of fibreoptics, made it possible to expand diagnostic uses of laparoscopy to therapeutic interventions within the

peritoneal cavity. Kurt Semm was also responsible for the development of many instrumentation advances in use during laparoscopy today.

Over the subsequent 20 years, laparoscopy was to remain largely in the domain of gynaecologist with little or no enthusiasm for its application to general surgery. During this time however, Kurt Semm demonstrated the feasibility of laparoscopic appendicectomy, ²⁴⁰ whilst advocates such as George Berci were utilising laparoscopy for diagnosis in abdominal trauma ¹⁶². This period also marked a time during which developing computer technology and advances in the computer-chip camera and in video imaging significantly improved vision within the abdominal cavity.

It was not however, until 1987 that a new dimension in laparoscopic surgery began when the French surgeon, Phillipe Mouret in Lyon removed a gallbladder at laparoscopy. Rapidly others also performed laparoscopic cholecystectomy. Credit for the first operation remains contested although two years earlier Muhe in Germany removed a gallbladder using a modified optical rectoscope and carbon dioxide insufflation ^{173,195}. Nonetheless, it was Mouret's procedure that demonstrated laparoscopy is a valuable tool for the general surgeon and within a few years of 1987 its use was firmly established into routine surgical practice ¹.

Now laparoscopic techniques have been used in an ever increasing list of procedures and there are few intraabdominal operations which have been free from attempts at achieving them laparoscopically. Laparoscopy is now the most frequently performed gynaecological procedure in the United States ¹¹⁸ and laparoscopic cholecystectomy one of the most commonly performed general surgical operations. It has been predicted that 80% of abdominal operations will be soon performed through the laparoscope ^{93,207}.

TABLE 1.1**MILESTONES IN LAPAROSCOPY**

936-1013	Arabian physician Abulkasim first uses reflected light to examine an internal organ; the cervix
19thC	Cystoscopy developed (also early urology and ENT examinations)
1901	Jacobaeus first to perform human 'ventroscopy'
1902	Kelling performs diagnostic 'coelioscopy' with air pneumoperitoneum
1926	Zollikoffer proposed the use of carbon dioxide as an insufflating agent
1952	Hopkins, a British physicist, developed the rod lens
1960's	The development of peritoneal insufflation by Semm
1982	Semm describes laparoscopic appendicectomy
1985	Muhe performs 'laparoscopic' cholecystectomy using a modified rectoscope
1987	Mouret performs laparoscopic cholecystectomy
1987 - present	"The laparoscopic revolution"

1.1.1 **Recent developments - consequences of a paradigm shift**

Since 1987 there has been an exponential growth in the number of procedures now being performed laparoscopically. Some of these procedures, although enthusiastically embraced initially have not proven cost effective or beneficial.

Clinical laparoscopy is a recent phenomenon, having been accepted only in the last decade in general surgery and yet in this time it has completely changed the practice of abdominal surgery. Developments in laparoscopy have been driven largely by parallel interests. First was the overwhelming public enthusiasm for surgical techniques that are associated with improved cosmesis, less post operative pain and the economic advantage of an early return to work. Industry also rapidly associated itself with laparoscopy with the development of new instruments, improved video-cameras and insufflators all of which facilitated the expansion of these techniques. Health authorities too embraced the emergent laparoscopic technology seeing increased cost benefit and reduced length of hospital stay.

With any new surgical technique critical and scientific appraisal of the advantages and disadvantages must be undertaken before their acceptance into routine practice. One of the basic tenets of medicine is "*primum non nocere*" ("first, do no harm"). There is now debate about the relative merit of laparoscopic techniques in some areas of surgery, particularly the application of laparoscopy in patients with intra-abdominal malignancies.

It is important to remember that laparoscopic operations must maintain the same standards as the equivalent operation performed via laparotomy. For benign disease key parameters include postoperative pain, length of hospital stay, morbidity and intra-operative and post-operative complications. When applied to malignant disease this includes not just the adequacy of anastomoses and adequacy of tumour resection

but overall survival and recurrence rates. For this reason when treating malignancy, the short term benefits of laparoscopy are overshadowed by the desire to cure the disease with the least possible chance of recurrence and the greatest possible chance of long term survival 286. Laparoscopic resection techniques differ subtly from open techniques, not only due to the addition of a pneumoperitoneum, but also as a result of the use of different instruments, an alteration in tactile and visual information for the surgeon and the need to extract specimens through small abdominal wounds 31.

Concern about the possible facilitation of tumour growth by laparoscopic techniques resulted in a moratorium by some surgeons on the use of laparoscopic resection for cancer outside of randomised clinical trials 25,31,287.

1.1.2 **Laparoscopy and cancer surgery**

Laparoscopic surgery has moved beyond the treatment of benign disease and is increasingly used for the diagnosis, staging and resection of intraabdominal malignancy 46,253,280.

1.1.2.1 ***Diagnostic laparoscopy and staging***

The role of laparoscopic staging is currently considered complimentary to other minimally invasive imaging modalities such as arteriography and non-invasive modalities such as computerised tomography, magnetic resonance imaging and ultrasound 37. Diagnostic laparoscopy may provide more accurate information that could allow the patient to avoid an unnecessary laparotomy and provide tissue samples for histology with relatively little morbidity 46. In the management of pancreatic cancer, diagnostic laparoscopy has provided a means of accurately staging those patients in whom laparotomy and resection would not be of benefit 83,280. In particular, the use of laparoscopy for staging of oesophagogastric carcinoma 192,227 and lymphomas 37,178 is likely to become a more widespread practice. As new

technologies become available it is also likely that interest in the use of laparoscopic techniques will extend into new areas of cancer management . These areas include the use of combination diagnostic localisation and therapy procedures such as laparoscopic ultrasound localisation of liver tumours and the delivery of laparoscopic guided cryotherapy or photodynamic therapy to the common bile duct, pancreatic duct or liver 103.

1.1.2.2 *Laparoscopic cancer resections*

The earliest use of laparoscopy in cancer surgery has been for ovarian tumours 157. Laparoscopic gastric and oesophageal resections, whilst technically feasible are not widely practised. Laparoscopic hemicolectomy was first reported in 1990 25 and is now routinely performed operation in some centres. Its use for colon carcinoma however, has been clouded in controversy 25,31,33,290.

Whilst early debate centred around technical issues such as the adequacy of distal resection margins and extent of lymphadenectomy, these issues have largely been resolved 286. New questions arose about the adequacy of laparoscopic techniques to cancer surgery following early reports of tumour metastases to port sites. Initially it was thought that this represented a technical problem due to direct contamination from viable tumour cells. However, as reports occurred of port site tumours in patients with otherwise curable disease occurred, it became necessary reassess the impact of laparoscopic techniques on tumour cell growth, spread and implantation 33.

If laparoscopic colectomy is to replace open colectomy for cancer, it must be demonstrated that it is equivalent to open colectomy in terms of out disease-free and overall survival. The choice between the two procedures will then be guided by an assessment of endpoints such as health care costs and quality of life 209. These questions can only be answered by the results from randomised controlled trials.

Trials are currently underway in several centres 21,92,151,160 and although early results from retrospective and non-randomised series suggest an advantage to laparoscopic colectomy, medium and long-term survival and recurrence rates are not yet available 151,154,160.

1.1.2.3 *Evidence based medicine*

Early enthusiasm for laparoscopic techniques has been tempered by the appearance in the literature of laparoscopic-specific complications 267. It must be recognised that the use of new techniques *per se* may critically alter not only operative complications but also the natural history of the disease process itself 289. New techniques in surgery should not be introduced merely because of technical feasibility but need to be assessed against objective measures. It is only by addressing these concerns that the use of laparoscopic techniques can be optimised to provide a means by which the advantages of minimal access techniques can be combined with the highest standards of available cancer treatment.

‘when operating for cure....we cannot condone compromise’

O’Rourke

1.2 LITERATURE REVIEW

The following section is a review of the current literature relating to studies in this dissertation. A modification of this review has been published as: SJ Neuhaus, M Texler, PJ Hewett and DI Watson, *Port site metastases following laparoscopic surgery: A review*, Br J Surg 1998; **85**,735-741

Since the first report of metastasis to a laparoscopic port site by Dobronte in 1978⁷⁵, it has become clear that the laparoscopic manipulation of both abdominal and thoracic malignancies can be followed by metastasis to surgical access wounds. Whether this is more likely following laparoscopic than conventional open surgery is controversial^{201,243,294}. However, individual case reports, as well as the results of recent experimental studies^{57,61,128,131,184} suggest that this problem is significant.

A critical review of this issue is warranted, as laparoscopic techniques, whilst attractive in terms of cosmesis, shorter hospital stay, and reduced postoperative pain,¹ cannot be justified if they result in an incidence of recurrent malignant disease in surgical access wounds which compromises long term outcomes. Wound seeding of potentially curable malignancies is a disaster which may change a potentially curable cancer into an incurable lesion. Recurrence after palliative laparoscopic resections may also compromise quality of life. Consequently, many surgeons have expressed concern about the appropriateness of the application of laparoscopic techniques to the surgical treatment of malignancy^{163,184,201,294}.

1.2.1 Evidence from clinical cases

Since 1978, in excess of 150 reports of metastasis to laparoscopy wounds have appeared in the surgical and gynaecological literature^{181,236} (see appendix I). These include instances of metastasis following diagnostic laparoscopy or

laparoscopic resection of carcinoma of the gallbladder 58,214,277,279,294 and from ovary 2,75,250, pancreas 243,281, colon 5,57,208,217,279, bladder 15,251, prostate 22, stomach 52, cervix 213 and liver 232 (see appendix I). Of concern are reported instances of metastasis following laparoscopy when it has been documented that the primary tumour was not manipulated at laparoscopy (Table 1.2.1.1) as well as metastasis following laparoscopic colectomy for Duke's A colon lesions 55,85,163,217, mucosal gall bladder cancer 128 and carcinoma-in-situ 294. It is also important to realise that this problem is not exclusive to laparoscopy, with an incidence seen following thoracoscopic cancer resection as well 48,68,73,94.

Following the early case reports of port site metastasis, *Nduka et al* 201 published the first review of this problem in 1994, describing a collected series of 20 cases, all adenocarcinomas. *Wibbenmeyer et al* 294 in 1995 reviewed a population undergoing laparoscopic cholecystectomy, and reported a 1% incidence of gallbladder cancer in the patient group studied. Laparoscopic cholecystectomy was associated with an increased risk of dissemination and diffuse peritoneal seeding of tumour, compared to historical experience with open cholecystectomy. A retrospective analysis of 419 patients with ovarian cancer was reported by *Kruitwagen et al* 157 in 1996. Forty three of these patients developed tumours secondaries at trocar sites following diagnostic laparoscopy, giving an overall incidence of port site metastasis of 16%. *Martinez et al* also reviewed the clinical literature in 1995 181. This series comprises 104 cases (diagnostic laparoscopy- 5 cases; laparoscopic cholecystectomy - 42; gynaecologic surgery-13; colorectal surgery- 34; thoracoscopic-7; and urologic-4) 181.

TABLE 1.2.1.1**CASE REPORTS OF PORT SITE METASTASES WITH NO MANIPULATION OF TUMOUR**

	procedure	tumour type
<i>Siriwardena et al</i> 243	laparoscopic cholecystectomy	pancreatic adenocarcinoma
<i>Watson et al</i> 281	laparoscopic gastroenterostomy	pancreatic adenocarcinoma
<i>Nieveen et al</i> 205	diagnostic laparoscopy	pancreatic adenocarcinoma
	diagnostic laparoscopy	pancreatic adenocarcinoma
	diagnostic laparoscopy	gastric adenocarcinoma
	diagnostic laparoscopy	liver
<i>Aractingi et al</i> 17	diagnostic laparoscopy	Burkitt's lymphoma
<i>Ugarte et al</i> 271	laparoscopic cholecystectomy	colonic adenocarcinoma
<i>Neuhaus et al</i> (unpub)	laparoscopic cholestectomy	colonic adenocarcinoma

TABLE 1.2.1.2**CASE REPORTS OF PORT SITE METASTASES FOLLOWING POTENTIALLY CURABLE RESECTIONS**

<i>Prasad et al</i> 217	colon adenocarcinoma	Dukes A
<i>Lauroy et al</i> 163	colon adenocarcinoma	Dukes A (polypectomy)
<i>Fingerhut et al</i> 85	colon adenocarcinoma	Dukes A
<i>Champault et al</i> 55	colon adenocarcinoma	Dukes A
<i>Shepherd et al</i> 241	ovarian	IA -borderline
<i>Fry et al</i> 94	adenocarcinoma lung	T1N0M0
<i>Gleeson et al</i> 100	ovarian	low malignant pot
<i>Patsner and Damien</i> 213	cervical	IB

Implantation of tumour following conventional thoracic tumour resection is rarely reported ^{73,190}, and usually involves surgery for mesothelioma. Wound metastases following oesophagectomy are rare ²²¹. Tumour implantation following mediastinoscopic tumour extraction which is a minimal access technique, similar to thoracoscopy is similarly rare ⁷³ although a high proportion of these patients go on to postoperative radiotherapy which, combined with their limited life expectancy, may limit the number of tumour implants which become clinically apparent. Following the advent of thoracoscopic lung resection for malignancy, however, an increasing number of implantation metastases have been reported ^{48,73,94,139,300}. In 1996 *Downey et al* described 21 cases of wound dissemination of malignant tumours following video-assisted thoracic surgery ⁷³. These occurred as a result of surgery for both primary and secondary lung tumours. The majority of the port site metastases were adenocarcinomas, although squamous cell carcinoma, small cell carcinoma, medullary carcinoma of the thyroid and melanoma were also reported. Port site metastasis has also been reported following oesophagectomy ^{68,73}. This provides evidence that the presence of a pneumoperitoneum is not necessary for the occurrence of port site metastases ²²⁵.

Tumour implantation into sites of percutaneous biopsy and drainage have been reported ^{102,137,189}. However, *de novo* cutaneous or subcutaneous metastases from carcinomas of internal organs are relatively rare ²⁴⁹ and most commonly involve the abdominal wall. The reported incidence of tumour involvement of the laparotomy wound in patients undergoing conventional open colectomy for carcinoma was reported to be less than 1% by both *Hewett et al* and *Hughes et al* ^{113,121} and 1% for potentially curative resection of rectal carcinoma ¹⁸⁶. *Fortner et al* report an incidence of 1.5% of wound recurrence following potentially curable resection for gastric cancer ⁸⁹. By contrast, both *Wexner et al* ²⁸⁹ and *Prasad et al* ²¹⁷ estimate that the incidence of wound metastasis following laparoscopic colectomy is

approximately 4%. In a prospective study of 1711 patients undergoing potentially curative colon cancer resection by *Reilly et al* the incidence of this complication was shown to be up to approximately 3.5%, depending on the method of diagnosis but only 3 of the 1,711 patients were found (0.2%) to have an isolated scar recurrence without other metastatic foci ²²⁴.

In contrast *Anderson et al* ¹⁶ have in an multicentre analysis of 1,333 diagnostic and therapeutic laparoscopies, demonstrated a 6.7% rate of port site metastases after gallbladder and bile duct surgery and a 1.8% rate after colonic surgery. A retrospective review conducted by *Yamaguchi et al* ²⁹⁹ of 2616 laparoscopic cholecystectomies found that, of the 24 patients with gallbladder cancer there was a 19% rate of recurrence at trocar sites in the absence of distant metastases.

Differences in tumour biology and aggressiveness may explain the different incidence of port site tumours seen in these studies. Overall it would appear that port site tumours are more commonly associated with tumours such as ovarian and gallbladder cancer that are known to have a poor outcome than with colorectal cancer (Table 1.2.1.3). *Fleshman et al* ⁸⁸ reviewed 372 patients following laparoscopic resection of adenocarcinoma of the colon or rectum. There were 4 cases of port site metastases (1.3%) and all four occurred in patients who were potentially curable patients. This incidence of wound implantation is not significantly different incidence than from some series of open surgery ⁸⁸. It is possible therefore that laparoscopic surgery has merely altered the presentation rather than the pattern of the disease recurrence ²²⁴.

However, these estimates are crude, and in the absence of better data which defines both the actual number of port site recurrences in the community (numerator) and the number of laparoscopic resections (denominator), the true incidence remains unknown. Due to limitations of data collection methodology in these studies, it is

also possible that historical estimates of the incidence of wound metastasis following open surgery could have been underestimated.

TABLE 1.2.1.3

INCIDENCE OF WOUND INVOLVEMENT FOLLOWING RESECTION OF MALIGNANCY FROM PUBLISHED SERIES

	<i>laparotomy wound</i>	<i>port site wound</i>	<i>site of primary tumour</i>
<i>van Dijkum 274</i>		1.6%	diagnostic laparoscopy group
<i>Kriutwagen et al 157</i>		16%	ovarian
<i>Fortner et al 89</i>	1.5%		stomach
<i>Yamaguchi et al 299</i>		19%	gallbladder
<i>Anderson et al 16</i>		6.7%	gallbladder
		1.8%	colon
<i>Reilly et al 224</i>	0.6%		colo-rectal
<i>Fleshman et al 88</i>		1.3%	colon
<i>Wexner et al 289</i>			colon
<i>Hughes et al 113</i>	<1%		colon
	1%		rectum
<i>Fingerhut 85</i>		3.2%	colon
<i>Cass et al 51</i>	9%		colon
<i>Prasad et al 217</i>		4%	colon

1.2.2 **Clinical presentation of port site metastases and their prognostic significance**

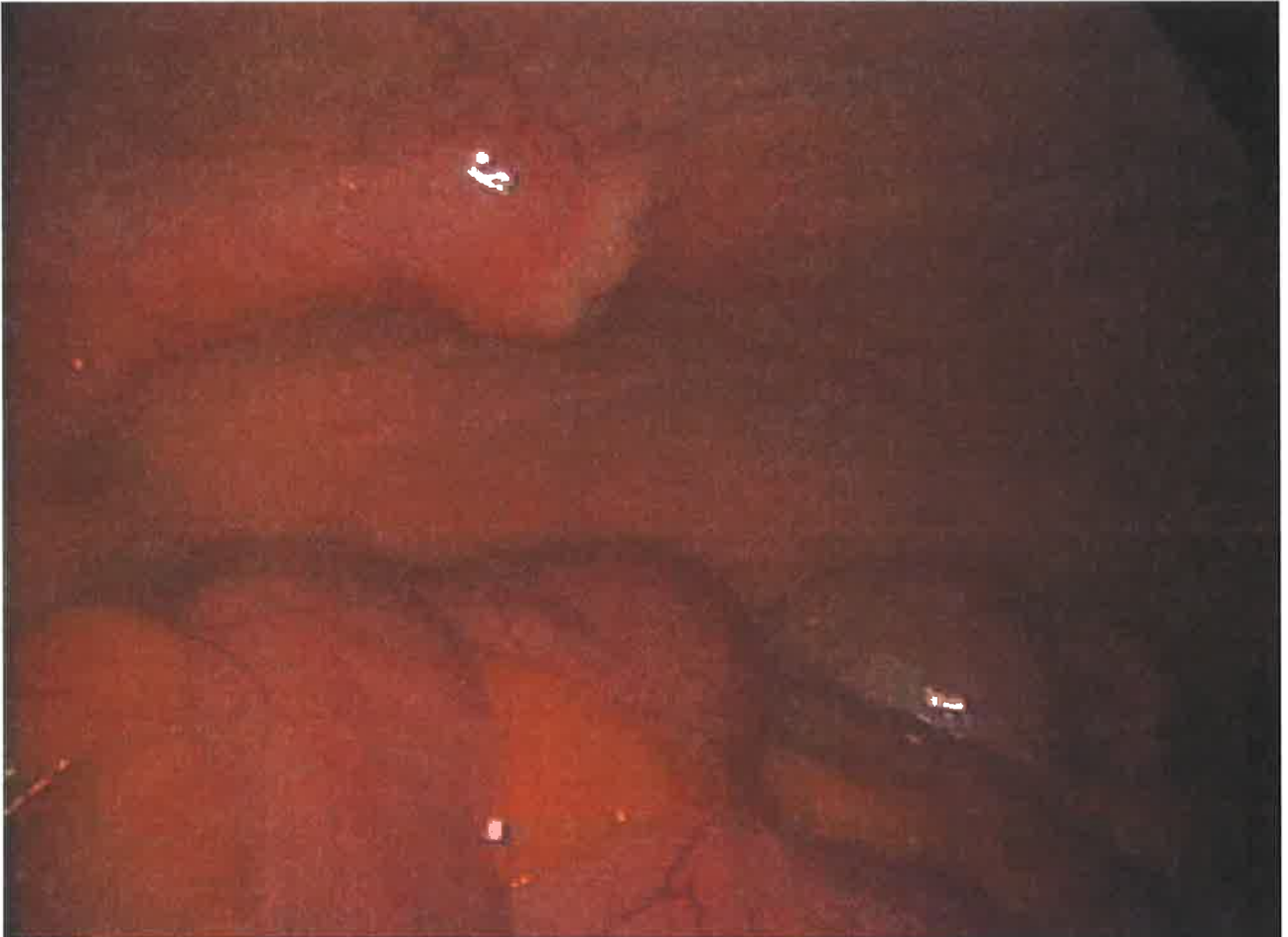
The period elapsing between laparoscopic surgery and the clinical finding of a wound metastasis is variable. It has occurred as early as 7 days following surgery 52,152, although most lesions are reported to arise between 3 and 9 months following initial surgery 157,181,201,288. Metastases typically present as hard, painful nodules at the previous placement site of one or more of the laparoscopic cannulae 181,201.

Histologically the incisional recurrence centres around the incisional scar and involves the dermis and subcutaneous fat but not the muscle 225.

Wound recurrence of cancer after conventional curative resection via laparotomy or thoracotomy is a devastating complication often requiring extensive abdominal wall or thoracic wall resection and/or composite repair and often postoperative radio- or chemotherapy 139,165. Experience with abdominal wall recurrences after curative resection following laparotomy suggests that the outcome for patients with recurrence in incisional scar tissue is poor. More than 50 % of patients in *Hughes et al* 121 series of patients with recurrent colon tumour died of disseminated tumour within 6 months and all patients were dead from their disease within four years.

Kruitwagen et al 157 assessed the effect of ovarian port-site metastasis on patient survival, and found that the presence of a metastasis at the site of a trocar or paracentesis access wound adversely correlated (although not statistically significantly) with survival. This analysis was performed following adjustment of data for factors such as age, tumour stage and pre-existing illness and tumour histology.

FIGURE 1.2.1
LAPAROSCOPIC APPEARANCE OF PORT SITE TUMOUR METASTASIS



It has been proposed that the development of port site metastases correlates with the aggressiveness of the primary tumour and/or its stage ^{157,281} that is to say it is a prognostic indicator which is independent of the surgical approach. Certainly the majority of cases reported have involved carcinomas with a known predisposition to unfavourable outcome such as gallbladder adenocarcinoma ^{228,294}, ovarian tumours ^{157,191}, Dukes C colon adenocarcinoma ^{85,88,90,217,220} and transitional cell carcinoma of the bladder ¹⁵. However, laparoscopic surgery for tumours with low metastatic potential, such as a borderline ovarian tumour ²⁴¹, as well as Dukes A colon carcinoma ^{85,217} has resulted in this phenomenon, suggesting that outcome may be adversely affected. The development of port site tumours in patients with diffuse carcinomatosis or positive peritoneal washings, as reported by *Miralles et al* ¹⁹¹ is probably a reflection of the disease process *per se* rather than the laparoscopic environment ²²⁰. Most reported cases of clinical port site tumours have not been associated with disseminated peritoneal disease ²³⁶. Currently, however, there is inadequate clinical follow-up to accurately assess the overall survival and morbidity impact of developing a port site tumours following laparoscopic resection with curative intent.

1.2.3 Experimental models

A number of experimental studies which seek to improve our understanding of port site metastasis have been reported recently (Tables 1.2.3.1 and 1.2.3.2). The models described are usually one of three types. 'Bench top' in vitro studies have been used to evaluate cell movement during laparoscopy ^{72,265}. These models are somewhat artificial, and it may be difficult to extrapolate results obtained in a rigid plastic pseudo-abdomen to clinical settings. Animal models are more analogous to clinical situations. Large animals (usually pig) have been used to test for acute cell movement during laparoscopy, by using radiolabelling techniques, or micropore filters

combined with cytological examination 6,116,265. While the internal volume of the pig abdomen closely resembles the human situation, the lack of native tumour lines precludes testing for the endpoint of tumour growth. Nevertheless, these models may be useful for studies which map the intra-operative movement of tumour cells during laparoscopy.

Small animals (mice, rats, hamsters) have also been used 6,44,120,143. The use of inbred, immune competent strains with native tumour lines 120,184,185, or immune compromised strains with human cancer cells 6,131 allow the endpoint of tumour growth to be tested. Whilst the peritoneal cavity is much smaller than in humans, these models can be used to test variations in the laparoscopic environment apart from just the simple mechanical manipulations tested in larger animals.

Evidence from bench top and large animal models confirms that tumour cells are delivered to port sites by manipulation within the laparoscopic environment 72,116,185,265. There is little doubt that direct spread by contaminated instruments may account for this 98,116,235,265. The role of aerosolisation of tumour cells and indirect transfer to port sites is more controversial. Only one research group 155 has demonstrated aerosolisation of tumour cell in an *in vitro* model. In a pig model aerosolisation of tumour cells has been demonstrated to occur infrequently 262 but aerosolisation per se may not be responsible for the development of tumour metastasis 293. *Whelan et al* 293 were unable to reproduce aerosolisation of viable tumour cells when either solid tumours or liquid suspensions were subjected to high pressure carbon dioxide environments either *in vitro* or *in vivo*. However, under certain circumstances, desufflation may transport cells in a liquid suspension and this may be a mechanism by which tumour cells are transported to port sites 182,293.

Experimental studies have demonstrated that port site metastases occur following laparoscopy and that the incidence of tumour implantation in wounds is higher than following laparotomy (Table 1.2.3.1). Studies in small animal models have demonstrated a 3 to 5 fold increase in the incidence of wound metastasis following laparoscopy with carbon dioxide compared to laparotomy ¹⁸⁴, and adverse patterns of tumour growth following carbon dioxide pneumoperitoneum ¹⁸⁵. *Jones et al* ¹⁴³ reported an increase in implantation free colon cancer cells using a rat model in which tumour cells were injected in to the caecal mesentery to simulate invasion of tumour through the serosa as may occur with Dukes B carcinoma. In this study the addition of a pneumoperitoneum with carbon dioxide more than doubled the incidence of tumour at 6 weeks at both port sites and peritoneal surfaces compared with the non-pneumoperitoneum group.

Le Moine et al ¹⁶⁴ have developed a model of laparoscopic caecal resection in a rat utilising a colon cancer line (DHK/K12) that simulates a solid colon adenocarcinoma, by implanting disc of tumour and extracellular matrix into the caecal serosa (orthotopic neoplastic inoculation). The rate of diffuse carcinomatosis at 8 weeks was 70% for the laparoscopic group compared to 23% in the open laparotomy group. Of particular note when considering only those tumours which had not involved serosa (i.e. not associated with deposits on the surface of the caecum), the frequency of carcinomatosis was 0% (of 6) in the open groups compared with 75% (6 of 8) in the laparoscopic group. In regard to wound metastases no difference was observed between groups in the incidence of tumour implantation, however it should be noted that all port sites were treated with povidone-iodine at the time of surgery. This study is important because it shows that a laparoscopic approach to a potentially curable cancer can have a deleterious effect. Other studies demonstrating an increase in the incidence of tumour implantation following laparoscopy compared with laparotomy are tabulated in Table 1.2.3.1.

TABLE 1.2.3.1

**EXPERIMENTAL MODELS DEMONSTRATING INCREASED TUMOUR
IMPLANTATION/ WOUND INVOLVEMENT AFTER CO₂
PNEUMOPERITONEUM vs LAPAROTOMY**

<i>Author</i>	<i>laparotomy</i>	<i>CO₂ pneumoperitoneum</i>	<i>species</i>	<i>tumour cell line</i>
<i>Mathew et al</i> 184	2/12 rats	10/12 rats	rat	DAMA
<i>Mathew et al</i> 185	33/72 sectors	64/72 sectors	rat	DAMA
<i>Jones et al</i> 143	23/41 (56%) wounds	49/50 (98%) wounds	hamster	CW-39 colon
<i>LeMoine et al</i> 164	23% † 0% *	70% † 75%*		DHK/K12 colon
<i>Lee</i> 167	10%	13% 41% if tumour crushed	mice	C-26 colon
<i>Bouvey et al</i> 44	6/8 rats	8/8 rats	rat	CC-531 colon
<i>Jacobi et al</i> 131	7/25	20/25	rat	DHD/K12/TRb colon

* when tumour did not involve serosa

† carcinomatosis

In contradistinction to the above findings *Hubens et al* 120, also using a rat model with the introduction into the peritoneum of free cancer cells has reported that although the presence of a port may predispose to port site metastases, the presence of a pneumoperitoneum did not enhance the implantation of free cancer cells *per se*. It only led to the localisation of tumour at the port sites. In this study a short

insufflation time (15 minutes) was used, which limits the time that pneumoperitoneum related effects may be seen. *Paik et al* 211 utilising a DHD/K12 rat colon carcinoma cell line also found that a laparoscopic procedure with pneumoperitoneum was less likely than laparotomy to result in wound implantation. In this study 26/50 (50%) of rats undergoing laparotomy developed tumour implantation in their midline wound compared with only 25% of rats (14/57) who developed at least one trocar site wound implantation after laparoscopy. No tumour growth was noted on the peritoneal surfaces in either group. This study also used only a short time of laparotomy and insufflation (10 minutes). *Canis et al* 49 utilised a rat ovarian tumour model of laparoscopy and reported that the incidence of wound metastases was 96% in the laparotomy groups and 55% in the laparoscopy groups. In this model, although tumour growth was greatest after laparotomy, peritoneal tumour dissemination was more severe after carbon dioxide pneumoperitoneum 49.

TABLE 1.2.3.2

**EXPERIMENTAL MODELS DEMONSTRATING DECREASED TUMOUR
IMPLANTATION/ WOUND INVOLVEMENT AFTER CO₂
PNEUMOPERITONEUM vs LAPAROTOMY**

<i>Author</i>	<i>laparotomy</i>	<i>CO₂ pneumoperitoneum</i>	<i>species</i>	<i>tumour cell line</i>
<i>Canis et al</i> 49	96% (implantation)	55%	rat	ovarian
<i>Paik et al</i> 211	26/50 (50%)	14/57 (25%)	rat	DHD/K12 colon
<i>Hubens et al</i> 120	60%	50%	rat	CC-531 colon

1.2.4 **Effect of laparoscopy on tumour growth**

It is believed that primary tumour growth rates are influenced by the degree of operative trauma^{141,196}, and the depressant effect that this may have on systemic immunity^{11,59,171,172,268,276}. Consequently, it has been proposed that laparoscopy, because it creates less access related trauma, results in less suppression of immune function, and therefore laparoscopy is less likely to facilitate tumour growth^{43,59}. *Allendorf et al's* work^{11,12,14}, which utilised a murine model, supports this concept. They demonstrated that tumour cells inoculated into the dorsal skin grew more easily and aggressively after laparotomy than after laparoscopy with carbon dioxide insufflation. This implies a benefit for laparoscopy in treatment of malignant conditions. However, it is important to note that this study looked at extra peritoneal tumour growth. *Voltz et al*²⁷⁶ has demonstrated that the induction of a carbon dioxide pneumoperitoneum resulted in an increased seeding rate of intraperitoneal tumour cells and also increased both the number and size of intra-abdominal metastases. This suggests that laparoscopy could result in a worsening of prognosis compared to laparotomy.

*Mathew et al*¹⁸⁴ also demonstrated increased growth of an implanted flank tumour in rats following laparotomy compared to laparoscopy, but despite this there were significantly more wound metastases in the laparoscopic group, negating the benefit of decreased 'primary' tumour growth. Thus, despite an overall immune benefit, laparoscopy may still result in seeding of intraabdominal tumour cells, worsening the overall outcome.

It remains to be proven that systemic immunological benefits for laparoscopic approaches are of benefit as it is possible that adverse local factors lead to early tumour recurrence in wounds.

1.2.5 Mechanisms of metastasis

A range of possible explanations for the occurrence of port site metastases have been proposed, and it is likely that the aetiology is multifactorial, with a range of possible contributing factors leading to the development of this problem. These possible mechanisms are summarised in Table 1.2.5.

TABLE 1.2.5

POSSIBLE MECHANISMS WHICH LEAD TO PORT SITE METASTASIS

Mechanical

Direct contamination

- a) tumour seeded during extraction of tumour through a small wound.
- b) tumour seeded by contact with instruments contaminated with tumour cells .

Indirect contamination

- c) tumour cells are seeded into the wound during episodes of desufflation of the pneumoperitoneum .
- d) tumour cells are aerosolised by the pneumoperitoneum and transferred to wounds and trocars without direct contamination ("chimney effect").

Metabolic/Immunological

- e) tumour cells are seeded in both open and laparoscopic wounds, but are more likely to form metastases after laparoscopy due to locally acting immunological and/or metabolic factors.

Haematogenous

- f) tumour is seeded by haematogenous spread during surgery.

Most contributing factors are specific to the laparoscopic environment. Evidence supporting our current understanding of this problem has been derived exclusively from research applied in animal models.

1.2.5.1 *Contamination*

Cells may implant in wounds due to direct contact with contaminated laparoscopic instruments 91,116,201. Frequent changes of instruments may predispose to this 98,116, and extraction of the tumour through a port wound may lead to coating of the port site with viable tumour cells. 90,98,116,201 This may be particularly relevant when attempting to extract a relatively large tumour through a small access wound, where traumatic manipulation of the tumour may predispose to a greater release of viable tumour cells at that site. Studies by *Allardyce et al* 243 demonstrate that, although carbon dioxide may enhance tumour implantation, the major variable influencing tumour cell deposition is whether or not the port is used by the surgeon. It has also been proposed that direct contact between the abdominal wall and tumour may occur during periods of temporary loss of pneumoperitoneum 6,116.

The indirect spread of viable tumour cells to surgical access wounds has also been proposed as an explanation for this phenomenon. Studies have established that viable tumour cells are liberated into the peritoneal cavity during the resection of malignancy by conventional techniques 64,91,123,272, and many researchers believe that, in the presence of a pneumoperitoneum, these viable exfoliated cells can be aerosolised, resulting in implantation in the laparoscopy access wounds. Implantation of exfoliated cells has been demonstrated to occur during open surgery 91,121. Laparoscopic techniques are inherently less dextrous than open surgery, and this may predispose to an increased burden of exfoliated tumour cells.

However, careful study of evidence from clinical case reports suggests that it is unlikely that instrument contamination or the direct implantation of exfoliated tumour cells is the sole mechanism for this phenomenon. Port-site tumours have been reported at sites where direct contamination is unlikely⁹⁶, and in cases in which the primary tumour was not manipulated^{243,281}. *Watson*²⁸¹ reports a case involving the development of multiple abdominal wall metastases from a pancreatic carcinoma following a laparoscopic gastroenterostomy. In this instance the theory of direct implantation is not feasible as the lesser sac was not opened, and the tumour was not manipulated. Similar cases following laparoscopic cholecystectomy have been reported by *Jorgensen et al*¹⁴⁴ and following diagnostic laparoscopy by *Sirirwardena et al and Samarji*²⁴³ (Table 1.2.1.1).

1.2.5.2 *Haematogenous spread*

Haematogenous spread of tumour from the primary lesion to the port site has also been proposed. However, this is an unlikely mechanism. A study by *Murthy et al* demonstrated that when tumour cells are injected intravenously, wound metastases are unlikely to occur, although metastases did arise at sites of intraabdominal surgical trauma¹⁹⁶. In this study tumour implants occurred in skin in 15% of cases and peritoneum in 38.5%. Most gastrointestinal tumours preferentially spread to the resection site/tumour bed following resection with peritoneal surfaces as the second most common site of involvement²⁵⁵. It is possible that the positive pressure environment in the peritoneal cavity during pneumoperitoneum, along with the spillage of cancer cells into the peritoneal cavity and associated raw surfaces may lead to viable tumour emboli entering the circulation. However, if this were the case, one would expect to see an increase in the rate of tumour metastasis to the usual sites of secondary involvement as well as to the previously rarely involved access wounds.

1.2.5.3 *Local factors*

Local factors at the sites of trocar placement may contribute to the localisation of tumour metastases to these wounds. It is known that tumour preferentially spreads to recently traumatised tissues and that malignant cells grow more easily in areas of high cellular proliferation¹⁹⁶. Whilst the intact peritoneum is resistant to tumour cell implantation^{143,184}, the port site provides a localised peritoneal breach and an area of high cellular proliferation associated with the healing wound. The associated local release of inflammatory regulators^{141,172,275} may generate an environment which facilitates the subsequent development of tumour metastasis. This mechanism alone, however, does not provide a complete explanation for an increased incidence of port site metastases associated with laparoscopic surgery. It is, however, possible that small wounds, such as those created by laparoscopic trocars, create a different environment for healing than large laparotomy wounds. If the immunologic or inflammatory response in these two different classes of wounds were different, then it may contribute to the problem.

1.2.6 **Pneumoperitoneum specific factors**

The fundamental difference between an open laparotomy and a resection performed laparoscopically, other than the size of the wounds, is the need to provide access space for both vision and operative manipulation. This is achieved in all cases (except when performed with a gasless technique) by the creation of a pneumoperitoneum, involving the introduction of gas under pressure into the peritoneal cavity. Factors specific to the pneumoperitoneum which may play a role in the development of port site metastases include; mechanical effects of gas under pressure, potential metabolic and immunological effects due to the insufflating gas, alterations in peritoneal humidity, stretching of the abdominal wall and peritoneal lining, possible electrostatic interactions with trocars and pressure/flow effects related to the use of insufflating gas.

1.2.6.1 *Aerosolisation*

Nduka et al 201 have suggested that the pneumoperitoneum acts as a closed system through which air-borne particulate matter must circulate whereas during open surgery this particulate matter, tends to be drawn away by the theatre ventilation system. This may result in 'trapping of viable tumour cells by moist peritoneal surfaces' 201. A so called "chimney phenomenon" has also been proposed by *Kazemier et al* 147. This is associated with the development of a Venturi phenomenon, whereby the insufflation of carbon dioxide causes turbulence which displaces tumour cells. At the port sites, these cells are concentrated as a result of the leakage of carbon dioxide alongside trocars leading to high local gas flows at the trocar sites. This gas may contain an aerosol of viable tumour cells which are 'delivered to the port sites, resulting in tumour implantation 44,45,143,185.

Aerosolised tumour cells may adhere to trocars via attractive forces (electrostatic or molecular) or deposit on the trocar sleeves from instruments. When a trocar is removed, these tumour cells may then be wiped off the trocar and deposited within the port site wound. It is also possible that carbon dioxide gas under pressure dissects between tissue layers at the port site, thereby causing additional trauma and allowing tumour cells to lodge between muscle, fascial and peritoneal surfaces.

The presence of a pneumoperitoneum also results in stretching of the abdominal wall. This may augment the release of inflammatory mediators, such as transforming growth factor alpha, which is involved in the healing of wounds, and may also promote tumour proliferation 134,143,275,283. Smoke produced by diathermy is toxic and dangerous and, in a closed space such as the peritoneal cavity, absorption of toxic chemicals may occur. This gas has been demonstrated to contain potential mutagens and carcinogens 210. Changes in the local environment as a result of desiccation and hypothermia may adversely alter the peritoneal milieu 32.

Similarly, it is also possible that alterations to pressure and flow rates during laparoscopy could affect a series of complex local and systemic metabolic and immune responses which may in turn facilitate tumour survival and implantation.

1.2.7 **Influence of specific insufflation gases**

Experimental studies using small animal models have demonstrated that the incidence of wound metastasis is reduced by eliminating carbon dioxide insufflation by using gasless laparoscopy 42,44,182,282. Studies by *Watson et al* 282 suggest that the rate following gasless laparoscopy is similar to the incidence of wound metastasis following laparotomy. These experiments suggest that an effect specific to laparoscopic insufflation accounts for the increased incidence of wound metastases proposed from clinical reports.

Most studies have utilised carbon dioxide as the insufflation gas, and have assumed that the physical effects of carbon dioxide pneumoperitoneum account for wound metastasis. However, two recently reported experimental studies have investigated the effect of different insufflation gases on tumour behaviour during laparoscopy. These studies report somewhat conflicting data. *Jacobi et al* 131 report a series of *in vitro*, *in vivo* and *ex vivo* experiments using a rat model and colonic tumour line. Whilst the insufflation of carbon dioxide resulted in greater tumour growth than a control group, helium had no tumour enhancing effect. *Dorrance et al* 70, however, using a rat intraperitoneal tumour cell line, report that the promotion of tumour growth by laparoscopy is independent of the insufflating gas. It remains possible therefore that the development of port site metastases may be influenced by metabolic determinants as well as the physical redistribution of tumour cells by the laparoscopic environment. Table 1.2.7 summarises the evidence from experimental models suggesting possible mechanisms for port site metastases.

TABLE 1.2.7**INFORMATION OBTAINED FROM EXPERIMENTAL MODELS****Bench top studies**

- a) Trocars and instruments are easily contaminated with tumour cells during laparoscopic surgery by direct contact 265
- b) Aerosolisation of tumour cells has not been demonstrated 293

Large animal studies

- c) Trocars and instruments are easily contaminated with tumour cells by direct contact 6,8,98,116,265
- d) Aerosolisation of tumour cells has been demonstrated in some studies 262

Small animal studies

- e) Laparoscopy is associated with less growth of 'primary' tumours, and less overall immune suppression 11,44
- f) carbon dioxide pneumoperitoneum results in adverse patterns of intraperitoneal tumour growth 44,120,143,185
- g) The likelihood of wound metastasis is increased 3 to 5 times by carbon dioxide pneumoperitoneum 184
- h) Wound metastasis is less likely following gasless laparoscopy 44,282
- i) Laparoscopy using insufflation with helium may reduce the risk of wound metastasis 131

1.2.8 **Prevention of cutaneous metastases**

Although the cause of port site metastases is far from clear, and is almost certainly multifactorial, many investigators have suggested preventive strategies to avoid this problem. Peritoneal tumour dissemination requires a number of 'metastatic steps' to occur; the detachment of tumour cells from the serosal surface of the primary tumour, movement of cells through the abdominal cavity, attachment to the port sites and invasion and subsequent proliferation. Each of these steps offer a potential site for an interventional strategy to prevent port site tumours. These include; port site protection ^{60,234} the use of prophylactic tumoricidal agents, ^{69,135,136,181,201} wound 'sterilisation' ¹⁶¹ and gasless laparoscopy ²⁸².

1.2.8.1 ***Wound protection***

Port site protection involves the use of plastic bags or wound protectors to prevent direct contact between a resected specimen and the port site ^{60,201,234,277}. It is also sensible to avoid traumatic manipulation of tumours which may shed tumour cells into the peritoneal cavity, and to ensure that the incision through which the specimen is retrieved is wide enough to avoid further inadvertent shedding of tumour cells ^{36,181}. As clinical cases have been reported where tumour recurrence has occurred at port sites other than those used to deliver the tumour, this strategy alone is unlikely to prevent all port-site tumours ^{57,193}.

1.2.8.2 ***Intraperitoneal cytotoxic agents***

The use of tumoricidal agents to destroy viable intraperitoneal cells, and therefore prevent their implantation, was originally proposed for open surgery more than 30 years ago ¹⁹⁴. It has more recently been proposed as a strategy to prevent port-site metastases ^{136,181,201}. Tumoricidal agents can be used either via parenteral or intraperitoneal routes ^{91,194,255,256}. The efficacy of intraperitoneal chemotherapy, at the time of surgery, for gastric cancer and ovarian carcinoma have been

demonstrated for conventional surgery 255-257. Lavage of the peritoneal cavity with large volumes of 0.9% saline has also been proposed 272 as a preventive strategy. At present is no supporting experimental or clinical evidence has been published which supports the routine use of tumoricidal agents during laparoscopic surgery.

1.2.8.3 *Treatment of the port site wound*

Excision of laparoscopic port-sites following procedures which involve malignancy has been proposed 61,277,294. However, the disadvantage of this is that it may negate the principal benefit of minimal access surgery (i.e. small wounds), and it is not certain that the resultant wounds are any less likely to become involved with metastatic tumour. The use of low-energy carbon dioxide laser light 161 has been described for the 'sterilisation' of mastectomy fields to prevent wound recurrence. This has been proposed for use in laparoscopic surgery also, although supporting data is yet to be published 201.

1.2.8.4 *Exclusion of carbon dioxide - gasless laparoscopy*

Gasless laparoscopy, which involves the provision of laparoscopic access without the use of positive pressure pneumoperitoneum is feasible 176,282, although existing devices used to maintain a working space, may provide poorer access in the abdominal flanks where good access may be needed for colonic mobilisation during laparoscopic colectomy. Because experimental evidence suggests that the exclusion of a pneumoperitoneum may significantly reduce the risk of port site tumours 44,282 this has been suggested as a strategy in the resection of malignant disease. However, it has been demonstrated in a porcine model by *Allardyce* 6 that gasless laparoscopy does not prevent port site contamination by tumour cells so this strategy may not be effective in isolation. Alternatively, exclusion of carbon dioxide from the pneumoperitoneum can be achieved by the use of inert insufflating agents such as helium.

1.2.9 Conclusions

Recent evidence from clinical cases and experimental studies confirms that the incidence of wound metastasis following cancer surgery is increased by laparoscopic approaches, and its occurrence following early stage cancers suggests that prognosis may be adversely influenced. This is despite the apparently beneficial reduction in systemic immune suppression and reduced primary tumour growth following laparoscopy for cancer in experimental models. It is likely that the mechanism which accounts for this problem is multifactorial. Laparoscopy can result in the mechanical redistribution of tumour cells by direct spread from contaminated instruments and indirectly due to the mechanical effect of the insufflation gas. Metabolic and immunological factors specific to the choice of carbon dioxide insufflation, acting locally at the port site may also be important. Until the issue of laparoscopy associated wound metastasis is fully understood, laparoscopic cancer surgery should only take place within the context of clinical trials.

1.3 **SUMMARY OF PREVIOUS WORK USING THE DA RAT MODEL OF LAPAROSCOPY SURGERY**

The following section is a summary of work performed by Dr George Mathew in the University of Adelaide Department of Surgery. This work utilised the DA rat laparoscopic surgery model that was developed by Dr Mathew. Details of the model are at sections 2.1 to 2.4. This work forms the foundation on which the current dissertation is based, although not all of his studies were complete at the time of commencing the current work. Dr Mathew's published articles are referred to as appropriate in the text.

1.3.1 **Laparoscopy vs open surgery in a solid tumour model** 184

In this study, using the DA rat tumour model, an implanted tumour was lacerated at laparoscopy or laparotomy to investigate whether application of laparoscopic techniques for malignant abdominal disease led to an increased risk of tumour dissemination and implantation within the peritoneal cavity, and abdominal wall wounds.

Growth of the primary tumour was greatest in rats that had undergone surgery compared with controls (no surgery), and was greatest after laparotomy. However, wound metastases were five times more likely after laparoscopic tumour laceration than after the same procedure through an open incision (10/12 rats versus 2/12 rats).

This high rate of wound metastases in rats undergoing laparoscopic tumour laceration compared with rats having an identical procedure performed via a laparotomy suggests that the presence of a carbon dioxide pneumoperitoneum in some way facilitates the development of port site tumours. Tumour was present in wounds that were used for the passage of the laparoscope as well as those used for tumour

laceration, suggesting that direct implantation of tumour from a tumour-laden instrument or during specimen extraction does not account for all wound metastases.

The differential growth of the primary tumour in this study supports the observation that laparoscopy may have beneficial systemic immunological effects compared with laparotomy. However, this may be of no practical benefit when a breach of the tumour capsule has occurred or when there are free intraperitoneal tumour cells.

1.3.2 **Laparoscopy with carbon dioxide vs without carbon dioxide in a solid tumour model** 282

The aim of this study was to compare the incidence of port site metastases in an experimental tumour model following tumour manipulation of an implanted tumour during laparoscopy aided by conventional insufflation, with laparoscopy using a gasless technique.

All implanted primary tumours enlarged in the week following surgery, with tumour growth rates comparable following laparoscopy with and without gas. This suggested that growth of the primary tumour may be related to wound size alone, and not some other factor inherent in the laparoscopic environment.

Tumour metastases were found significantly more often in the wounds of rats undergoing laparoscopic tumour laceration during carbon dioxide insufflation, than following comparable gasless surgery (3/12 vs 10/12 rats). The distribution of tumour metastases found in the port wounds demonstrated no predilection for a particular trocar site in either group. No tumour dissemination or peritoneal invasion was evident beyond the site of primary tumour laceration and the abdominal wounds in any of the 24 experimental animals.

Of major importance is the fact that the incidence of wound metastasis was significantly reduced by eliminating the use of carbon dioxide insufflation under pressure. This reduction was comparable to the rate seen following the equivalent open surgical procedure reported in the earlier study (i.e. 3/12 vs 2/12), suggesting that the use of carbon dioxide insufflation gas is associated with the movement of cells to laparoscopic trocar wounds followed by implantation and growth.

1.3.3 **The effect of laparoscopy on the movement of radiolabelled tumour cells** 182

This study used two experimental models to determine whether carbon dioxide insufflation during laparoscopic surgery influences the movement of tumour cells, and whether it results in tumour implantation and growth in surgical wounds.

In one study 'the abdominal cavity of recipient ' rats were connected via a length of plastic tubing to rats undergoing laparoscopy with carbon dioxide insufflation or gasless laparoscopy. A tumour cell suspension was introduced into the abdominal cavity of the 'donor' rat only. Seven days later nodular tumour metastases were found in 5 out of 6 of the recipient rats in the group which underwent carbon dioxide insufflation. None of the recipient rats in the gasless laparoscopy group developed metastatic tumour. This provides additional evidence that insufflation is essential for the promotion of wound metastases.

A similar 'donor -recipient' model was also used to assess the movement of radio-labelled tumour cells during laparoscopy with carbon dioxide insufflation and gasless laparoscopy. In this study vented gas was delivered into a solution of phosphate buffered saline which was then assessed for radio-activity counts. A significant transfer of tumour cells to the vented gas occurred in rats undergoing laparoscopy with insufflation but not when using gasless laparoscopy. Although this model does

not entirely mimic clinical laparoscopy, it does suggest that inadvertent liberation of large numbers of tumour cells into the peritoneal cavity can occur. Whilst it is possible that careful laparoscopic technique may avoid the liberation of tumour cells, this cannot be guaranteed pre-operatively, and tumour cell spillage remains a real possibility. If this is combined with carbon dioxide insufflation and a significant gas leak, then the necessary environment for clinically important port site metastases may be created.

Both models therefore support the concept that carbon dioxide insufflation results in tumour dissemination during laparoscopy, leading to port site metastasis and that gasless laparoscopy may prevent this problem.

1.3.4 **Adverse Impact of pneumoperitoneum on intraperitoneal implantation and growth of tumour cell suspension in an experimental model** 185

In this study a tumour cell suspension was introduced into the peritoneal cavity of rats at laparotomy, laparoscopy with carbon dioxide insufflation, and gasless laparoscopy to investigate the effects on peritoneal tumour implantation.

The tumour implantation pattern of tumour in the peritoneal cavity of rats undergoing laparoscopy with carbon dioxide insufflation was significantly different from that observed following laparotomy or gasless laparoscopy. Insufflation resulted in tumour redistribution and spread to all sectors and port sites, suggesting that the insufflation of carbon dioxide gas during laparoscopy leads to more widespread tumour deposition and growth, compared to gasless laparoscopy and laparotomy. In these groups tumour implantation was predominantly on the left side of the abdominal cavity, near the site of introduction of the tumour cell suspension. Port site tumours

were more likely in rats undergoing laparoscopy with carbon dioxide insufflation compared to rats in the gasless laparoscopy group.

It is not clear from this study whether this phenomenon is due solely to the physical effects of the insufflation gas or whether carbon dioxide induced metabolic effects have a role.

1.3.5 **The role of peritoneal immunity and the tumour-bearing state on the development of wound and peritoneal metastases after laparoscopy** 183

This study investigated the effect of the tumour-bearing state and alteration in peritoneal immune function on the incidence of port site and peritoneal metastases after laparoscopy with and without a carbon dioxide pneumoperitoneum.

Laparoscopy with carbon dioxide resulted in a significant increase in port-site metastases and peritoneal spread compared to laparotomy and gasless laparoscopy groups. The presence of a pre-existing tumour was associated with increased tumour spread in all treatment groups and at most sites. Injection of endotoxin also resulted in increased tumour spread. This indicates that the tumour bearing state with established effects on the immune and metabolic status of the host, can influence the pattern of spread of tumour in the peritoneal cavity. The increased spread of tumour within the peritoneal cavity without any surgical intervention indicates that changes in peritoneal environment alone can facilitate implantation of tumour.

Peritoneal macrophages harvested from rats who underwent either laparotomy or gasless laparoscopy showed increased activation while those from the carbon dioxide laparoscopy group, whether tumour bearing or non-tumour bearing, had markedly reduced ability to produce TNF- α after LPS stimulation *in vitro*. The markedly

decreased response in the carbon dioxide laparoscopy group implicates carbon dioxide as the primary modulating influence.

The results of this study suggest that the underlying immune or metabolic status of the host, as influenced by the tumour-bearing state or modification of the peritoneal environment, has a marked independent effect on tumour spread and implantation and that carbon dioxide can modulate this effect.

1.3.6 **Questions raised by this work - that will be addressed in this dissertation**

1. Does the use of different insufflation gases affect the incidence of port site metastases and tumour implantation.?
2. What are the metabolic and immune effects of different insufflation gases on peritoneal immunity?
3. What are the effects of immune suppression and enhancement on tumour implantation and port site tumour formation?.
4. Does the use of different insufflation pressures affect the incidence or distribution of port site metastases?
5. What is the effect of removing the primary tumour on the development of port site tumours?

6. Can preventive strategies, such as the use of intraperitoneal cytotoxics prevent port site metastases ?

7. Can treating port site wounds e.g. by excision or local irrigation with cytotoxics alter the rates of port site tumour development?

SECTION II

METHODOLOGY

2.1 THE DA RAT MODEL

The Dark Agouti (DA) rat model for the investigation of laparoscopic port site metastases was developed by Dr George Mathew in the University of Adelaide Department of Surgery, Royal Adelaide Hospital. This tumour model was chosen for the work described in this thesis for the following reasons:

1. The DA rat laparoscopic tumour model has consistently produced a high incidence of metastases to wounds following laparoscopy with carbon dioxide compared to laparotomy and gasless laparoscopy 184,185,282.
2. The DA rat is an immune-competent species 54. This provides a model which can be better extrapolated to a clinical setting, as immune suppression is not needed to achieve tumour growth.
3. The DA mammary adenocarcinoma (DAMA) is native to the strain of rat 54 and the tumour line was readily available within the Royal Adelaide Hospital campus.
4. Previous work in the Royal Adelaide Hospital / Institute of Medical and Veterinary Science campus has defined the baseline metabolic and physiological responses of the DA rat to the presence of DAMA 63,229-231.
5. The tumour has been characterised as a 'non- metastasising' tumour, in that it does not metastasise to liver, lungs or brain until late in the its growth and death of the rat is usually secondary to advanced local disease 54.

2.1.1. **Animal details**

Dark Agouti (DA) rats are an inbred immunocompetent species of rat. Inbred male and female rats were obtained from the Institute of Medical and Veterinary Science (IMVS) animal breeding facility at Gillies Plains, South Australia. The genetic profile of the inbred strain is checked yearly by the supplier. All studies were performed using male DA rats. Female rats were used as carriers. The rats weighed 200-250g.

2.1.2. **Animal maintenance**

The rats were housed on sawdust in perspex cages in an animal house maintained at 22±1 degrees Celsius with a 14hr light /10hr dark cycle. The rats were caged in groups of two, and food and water were available *ad libitum* throughout the study period (M&V mouse cubes, Milling Industries Adelaide). The animals were studied in the fed state.

2.2. **TUMOUR CELL DETAILS**

2.2.1. **Tumour model**

The tumour, a DA rat mammary adenocarcinoma (DAMA or MRMC2) arose spontaneously in female DA rats in 1972 and had been maintained by transplanting tissue from animal to animal and by storage in liquid nitrogen⁶³. The tumour was a gift from the Department of Pathology and Immunology, Monash Medical School, Alfred Hospital, Melbourne and has been maintained in Adelaide and used in the IMVS Department of Clinical Biochemistry. Hereafter the tumour is referred to as DAMA.

2.2.2. **Morphology**

The DAMA tumour contains nests of large, polymorphic cells surrounded by vascular stroma⁶³. Neoplastic cells are surrounded by abundant eosinophilic cytoplasm and exhibit a lack of glandular differentiation. The nuclei are large, vesicular, and contain prominent nucleoli. The mitotic index is 3.95 ± 1.35 cell per high power field (x100). The ultrastructure of DAMA is consistent with that of an adenocarcinoma arising from a mammary gland.

Cytogenic studies performed on cultured DAMA cells show that the cell line is characterised by near tetraploidy (83-85 chromosomes) with trisomy of pair 2 chromosomes and a metacentric marker formed by centric fusion of two pairs of 3 chromosomes. The findings are constant features of the karyotype. The DNA concentration of DAMA cells is 22.9 ± 0.01 ng/cell⁶³.

The tumour associated vasculature consists of irregular blood vessels, forming extensive networks, distributed irregularly through the tumour mass. The typical histological appearance of the DAMA tumour is illustrated in Fig 2.2.2.

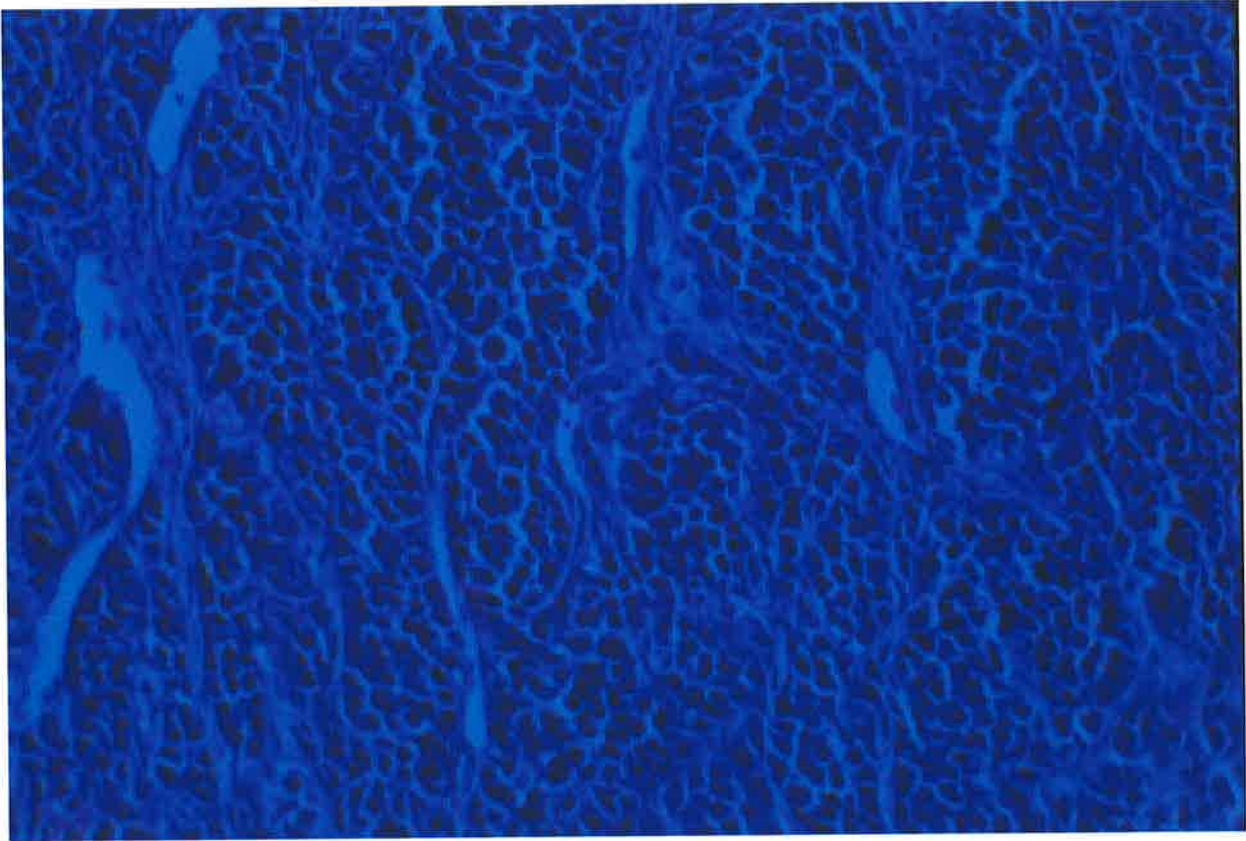
2.2.3. **Immune composition of DAMA**

Immunohistochemical characterisation of immune cell infiltrates of the DAMA showed that macrophages (ca 140) and CD4 (ca 130) positive cells were the most prominent infiltrates. B cell staining was absent in the primary tumours.

2.2.4. **Maintenance of tumour**

Viable DAMA cells were prepared using a standardised technique by homogenising fresh tumours propagated in, and resected from, female carrier rats. Subcutaneous tumours of approximately 5g were excised from the carriers and dissected from any surrounding necrotic or connective tissue. The tumour was

FIGURE 2.2.2
HISTOLOGICAL APPEARANCE OF DAMA TUMOUR



then diced and washed in phosphate buffered saline (PBS, sterile 10 mmol/l sodium phosphate buffer, pH 7.0, containing 0.15 mmol/l sodium chloride). The tissue was homogenised in a motor driven Potter Elvehjen homogeniser (Wheaton, Millville, NJ, USA; radial clearance 0.5 mm) and the crude debris removed by filtration through sterile gauze. The cell suspension was next centrifuged four times in 10 volumes of PBS at 400g, each for 1 minute. The viability of the suspension was assessed by trypan blue (Hopkins & Williams, Essex, UK) exclusion (trypan blue in 0.5% saline), and the cell number determined using a Neubauer counting chamber (Improved Neubauer, Weber, UK, depth 0.1mm, 1/400m²). The final concentration was adjusted to give 2×10^7 viable cells in 200 μ l of sterile PBS. The DAMA was passaged in syngeneic rats by injecting a sterile suspension of tumour cells as described above. The tumour can also be transplanted directly by transplantation of small pieces of tumour tissue. However this was not used in these studies due to an increased risk of infection ⁶³.

DAMA cells can be propagated by tissue culture and when cultured cells were injected into the flank of a DA rat they grew with identical growth characteristics as the original transplanted tumour. Moreover, tumours propagated by passaging the cells from rat to rat and those derived from cultured DAMA cells have been shown to have similar histological appearances ⁶³.

2.2.5. **Preparation of tumour cell suspension for cell culture studies**

Tumour cell suspension was prepared as above. The cells were then washed in phosphate buffered saline (PBS), centrifuged and added to a culture media containing 10% v/v foetal calf serum (RPMI-1640 with Herpes buffer (20 mmol/l), benzylpenicillin (60 micrograms per ml), gentamycin (40 micrograms per ml) and foetal calf serum (10%v/v)). The cells were then incubated overnight in 50 ml flasks at 37 degrees Celsius. The next day the cells were washed in PBS and

exposed to trypsin solution (0.01% w/v) and 0.002% (w/v) disodium EDTA for 10 minutes. Media was then added and the resulting suspension centrifuged at 250g for 10 min . The cells were then re-suspended in the media. Cell numbers were determined using a Coulter counter (Improved Neubauer, Weber, England) (trypan blue exclusion).

2.2.6. **Solid flank tumour**

Solid flank tumours were implanted by injecting the tumour cell suspension subcutaneously into the left flank abdominal wall of Dark Agouti (DA) rats . Previous work using this model has confirmed that the tumour grows reliably to form a mass approximately 20 to 25 mm diameter after 7 days , and that this extends into the abdominal cavity, whilst still covered by a thin layer of peritoneum. The tumour is readily visible at laparoscopy 184,282.

2.2.7 **Natural history of tumour growth**

The injection of the tumour cell suspension into the abdominal wall resulted in a solid flank tumour, which became palpable 7 to 8 days after implantation. Growth was exponential and by 12 days post tumour implantation the tumour represented 5-10% of body weight. A tumour cell suspension of 2×10^7 tumour cells as described above consistently produced a tumour of 20-25 mm. This tumour was originally described as a 'non-metastasising' tumour. In previous studies and in this work there was no evidence of metastatic spread to liver or lungs at autopsy of any animal. However, involvement of draining lymph glands was noted during this study under some circumstances (see sections 3.4 and 4.1).

2.2.8. **Appropriateness of the DAMA model**

The DAMA cell line demonstrates histological characteristics similar to an adenocarcinoma. These include a lack of glandular differentiation, high mitotic

index, tetraploidy and a high rate of glycolysis. The absence of distant metastases in the host ensures that in single lesion studies, findings are not compounded by the effect of secondary tumour deposits.

The findings using this cell line may not be applicable to all tumour cell lines, but it provides a useful and reliable model to investigate the effects of the laparoscopic environment on tumour dissemination and implantation.

In addition, the previously reported outcomes using this model (see section 1.3) are consistent with outcomes from the majority of studies reported by other groups 44,120,131,143,143,164 and observations from clinical practice 181,201,236, confirming that this model can provide valuable insights into the aetiology and prevention of laparoscopy associated tumour metastasis.

2.3. OPERATIVE TECHNIQUES

2.3.1. Anaesthesia

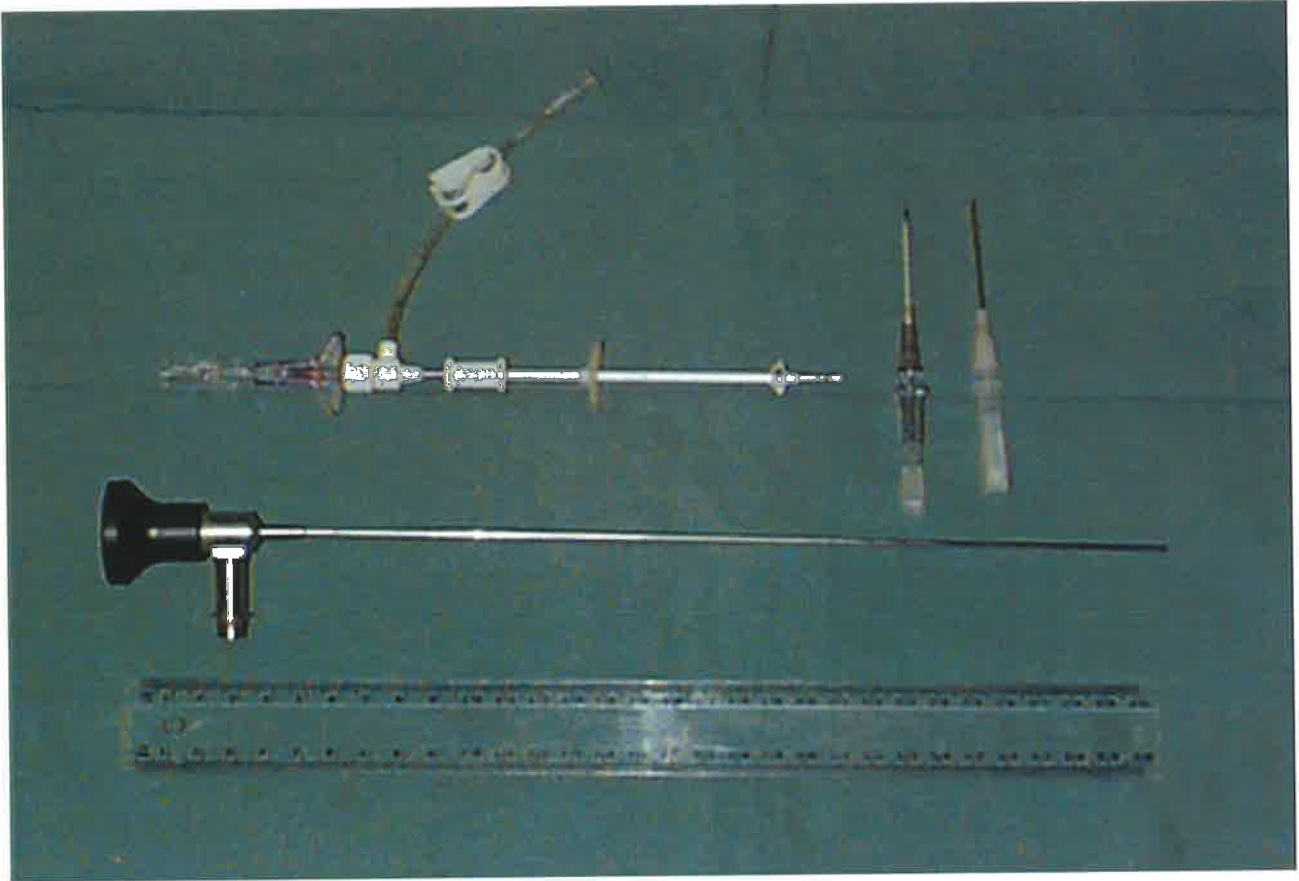
Rats were anaesthetised during all procedures with a combination of halothane (Fluothane, Zeneca Ltd, UK) and nitrous oxide supplemented by oxygen via a close fitting mask (0.3L/min oxygen, 7 L/min nitrous oxide and 2L/min halothane). Gas delivery was titrated and controlled by using a direct circuit to a CIG Midget-III anaesthetic machine, incorporating a Cryopane Fluothane Vapouriser. The respiratory status of the animals was monitored throughout anaesthesia, and all surgical procedures were performed under sterile operating conditions.

2.3.2. Laparoscopy

Pneumoperitoneum was achieved using a conventional Veress needle (Surgineedle 18G, 150mm long, Autosuture, Australia) placed through a right hypochondrial stab wound. A disposable mini-laparoscopy cannula (Microlap Introducer, Model LT 1500-MF-Imagyn Medical, Laguna Niguel, California, USA) was used to provide access for a 2 mm mini-laparoscope (focal length 1cm, length 275mm, Imagyn Medical) with attached conventional laparoscopy camera (Panasonic WV=KS152, Japan). Light was provided by a cold light fountain (450V, 250Watt metal halide illumination - Karl Storz, Germany) and the system connected to a conventional video monitor (Panasonic, Japan). The Storz insufflator was chosen for these studies as it reliably insufflates gas at the low pressure and low flow rates required in rat studies.

The mini- laparoscopy cannula is fitted with a side port that was connected to 5mm diameter tubing to an insufflator (Karl Storz model 26012 B, Germany) to provide a pneumoperitoneum (Figure 2.3.2). .

FIGURE 2.3.2
MINI LAPAROSCOPY CANNULA AND 'PORTS'



Two additional "ports" were inserted; an 18 gauge cannula in the left hypochondrium which was left open throughout the procedure to vent the insufflation gas, and a 16 gauge cannula in the left lower quadrant which was used to provide access for a needle used for tumour laceration or for the introduction of tumour cell suspension (Figure 2.3.3). All procedures were performed by the same investigator (S. J. Neuhaus).

2.3.3. **Tumour models**

Two established experimental tumour models of laparoscopic surgery were used for these studies 184,185.

2.3.3.1. *Solid tumour model*

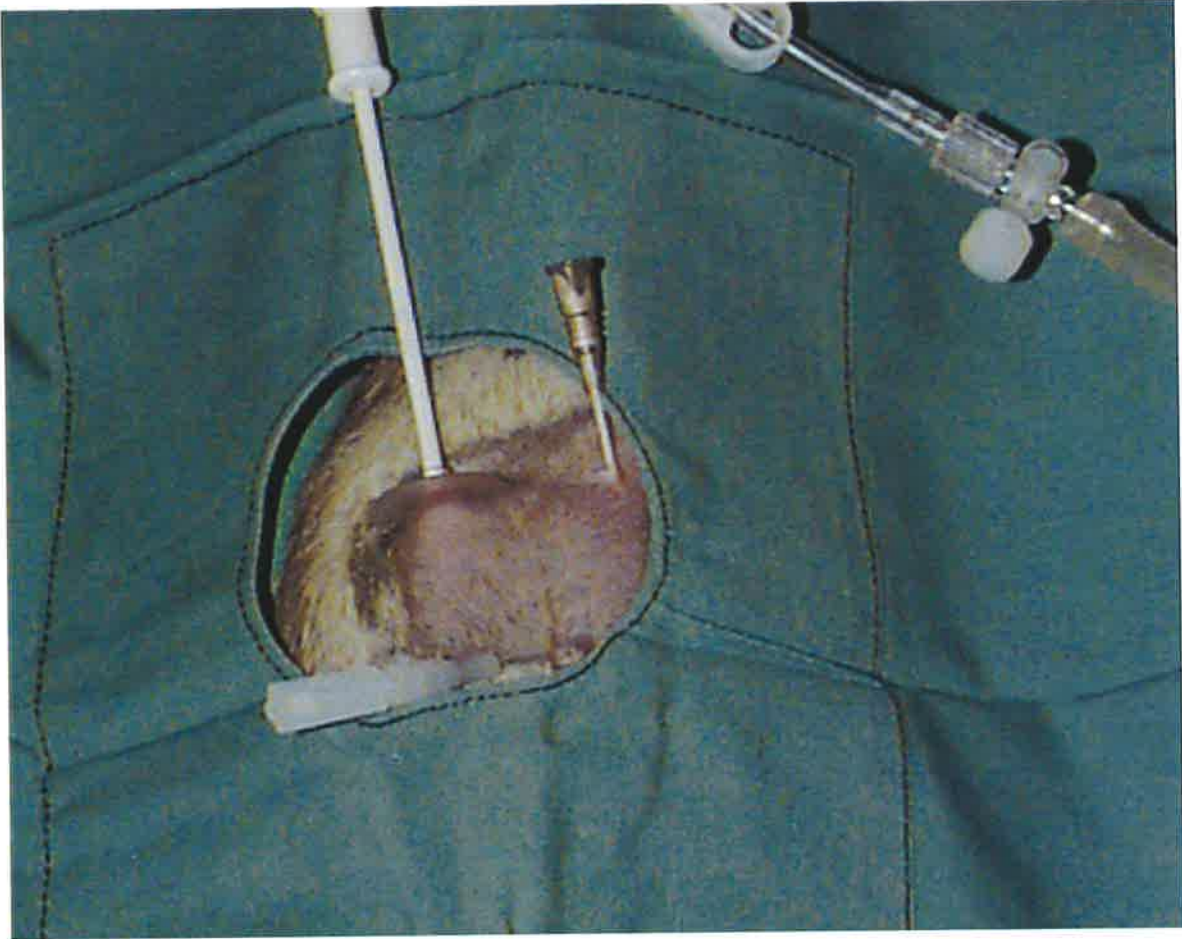
After commencing gas insufflation and placing all "ports", the tumour capsule and overlying peritoneum was lacerated in a standardised fashion under laparoscopic vision using an 18 gauge needle inserted through the 16 gauge cannula. This created a direct communication between the tumour and the peritoneal cavity, simulating the surgical manipulation of an intraperitoneal tumour.

The 16G cannula was then sealed to prevent gas leakage. Gas continued to be insufflated at a rate of 0.4 litres/min and a pressure of 2 mmHg for 40 minutes after tumour laceration, with a constant gas flow maintained through the venting cannula. After 40 minutes the "ports" were removed, and the puncture sites were closed with sutures.

2.3.3.1. *Free cell suspension model*

Tumour cell suspension was prepared as described above. After commencing gas insufflation and placing all "ports" , a 200µl volume of tumour cell suspension was introduced under laparoscopic vision through the 16G cannula. The tumour cell

FIGURE 2.3.3
RAT UNDERGOING LAPAROSCOPIC INSUFFLATION



dose was determined by pilot studies performed by *Mathew et al* in the University of Adelaide Department of Surgery at the Royal Adelaide Hospital. Tumour cell doses are specified for each study.

The 16G cannula was then sealed to prevent gas leakage. Gas continued to be insufflated at a rate of 0.4 litres/min and a pressure of 2 mmHg for 40 minutes after introduction of the tumour cell suspension. After 40 minutes the "ports" were removed, and the puncture sites were closed with sutures.

2.3.4. Gasless laparoscopy

In rats undergoing gasless laparoscopy, no gas insufflation was used. A Veress needle was inserted into the peritoneal cavity using the technique described above. Intraperitoneal position was determined by visualising the cavity with the laparoscope. The anterior abdominal wall of the rat was then suspended to a frame to provide adequate exposure. This was achieved by passing multiple sutures through the overlying skin and anchoring them to the frame. Ports were placed as described above. At the end of the procedure, these sutures were removed. In some later studies the anterior abdominal wall was elevated using a lifting device. This device comprised a curved stainless steel blade, similar to a Nathanson liver retractor, which was inserted into the peritoneal cavity and anchored to a retort stand. Where this device has been used is specified in the relevant studies.

2.3.5. Perioperative monitoring

Previous experience with the DA rat tumour model had demonstrated that laparoscopic surgery and tumour induction is well tolerated and results in no discernible problems providing the primary tumour does not grow too large
63,184,229.

Both open abdominal surgery and laparoscopy are well tolerated by the DA rat and are believed to result in mild pain only. All animals were monitored daily for signs of discomfort or distress. All animals were weighed preoperatively and at the time of autopsy. The amount of feed allowed to the animals in the intervening seven days was monitored and their food weighed. Any animal unwell or distressed was killed.

2.4. AUTOPSY PROCEDURES

2.4.1. Killing of animals

All animals were killed by cervical dislocation under general anaesthesia.

2.4.2. Tumour size (solid tumour model)

Tumour size was assessed by measurement of the maximum and minimum dimensions of the primary tumour. In some studies the flank tumour was also dissected free and weighed.

2.4.3. Histopathological examination of port sites

The abdominal cavity and the surgical access wounds were examined for the presence of macroscopic tumour metastasis, before excising the port site wounds for histopathological examination. Excised specimens were fixed and stored in buffered formalin. The fur was removed from all specimens and they were cut into 5x5mm pieces and processed in tissue cassettes. After embedding in paraffin the specimens were cut with a microtome to a thickness of 5 μ m and placed on glass slides. The slides were dried overnight at 37 degrees Celsius and then dewaxed.

Haematoxylin and eosin staining was performed by placing the slides into two washes of Histoclear or xylene for 2 minutes each. Slides were then re-hydrated by dipping in three successive baths of alcohol. They were then rinsed in distilled water and PBS before being placed in haematoxylin for three minutes. Slides were rinsed in water and then acetyl alcohol to remove excess stain before fixing in lithium carbonate for one minute. The counter staining with eosin was performed for one minute. Excess eosin was removed by rinsing in water. The slides were then soaked in PBS for 30 seconds.

Following staining, the slides were placed in successively three alcohol and three HistoClear baths for a minute each. A coverslide was then placed on the stained tissue section using Dipex mounting medium and the slides allowed to dry overnight.

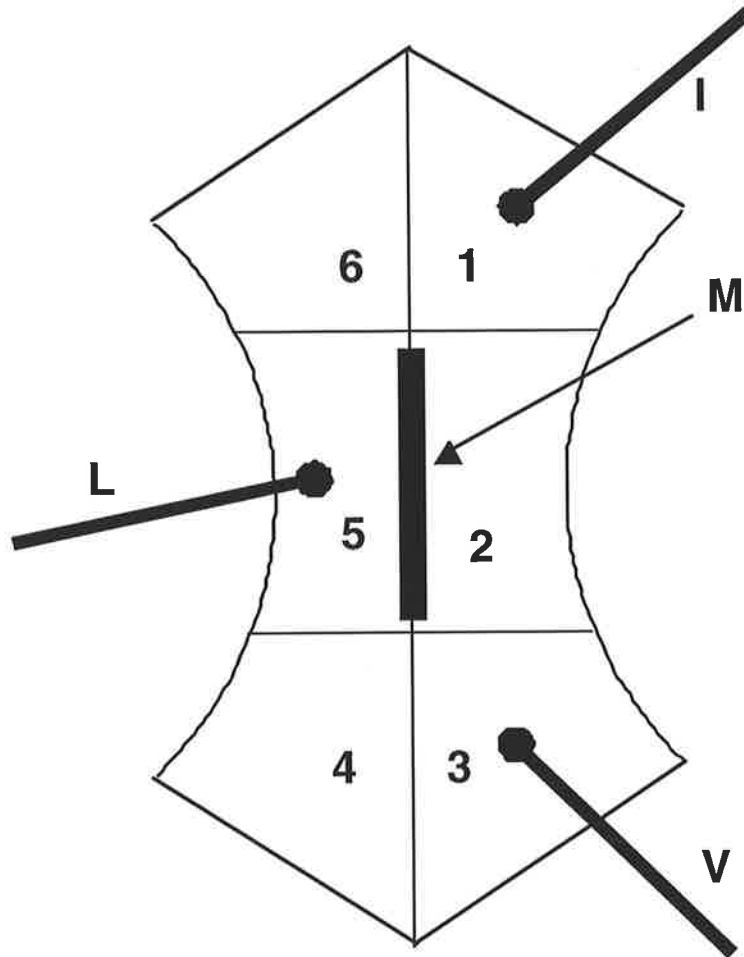
All specimens were examined in a blinded fashion by a histopathologist who was unaware of the study group or the anatomical site of origin of the specimens .

2.4.4. **Peritoneal cancer index (free cell suspension model)**

Peritoneal tumour deposits in each of 6 abdominal sectors (Fig 2.4.4) were assessed using the peritoneal cancer index proposed by *Eggermont et al* 76 .

- 0 - no intraperitoneal tumour;
- 1 - less than three minute tumour foci;
- 2 - moderate tumour;
- 3 - abundant or confluent tumour.

Each sector was scored for the presence or absence of tumour deposits, and for tumour density. Representative samples were examined histologically to confirm the macroscopic assessment. All port-sites were also specifically examined for macroscopic evidence of tumour implantation.

FIGURE 2.4.4.**ABDOMINAL CAVITY DIVIDED INTO SIX SECTORS****FIGURE LEGEND**

- I - Injection cannula
- V - Venting cannula
- L - Laparoscopic cannula
- M - Midline incision

2.4.5. **Lymph node dissection/extended autopsy**

At autopsy lymph node dissection was performed in study 3.4. Bilateral axillary and groin node groups were harvested from rats. Lymph nodes were then examined histologically with haemotoxylin and eosin staining as described above to determine the presence or absence of tumour and the presence or absence of reactive change. Serial 2.0mm sections of liver and lung, were examined for the presence of visible or palpable macroscopic tumour metastases.

2.4.6. **Peritoneal macrophage harvest and function assessment**

Peritoneal macrophage harvest and function assessment was used in study 3.2.3. Peritoneal macrophages were collected and their function was assessed using a Tumour Necrosis Factor alpha (TNF- α) assay. This was performed using the following technique:

2.4.6.1. ***Macrophage harvest and culture***

A small area of fur was dissected free from the underlying muscle, and the exposed muscle was swabbed with 70% alcohol solution. Peritoneal lavage was then performed by introducing 10 mls of sterile phosphate buffered saline (PBS) through a 21 gauge needle placed into the peritoneal cavity through the exposed muscle. The abdomen of the rat was gently agitated to mix the fluid with the contents of the peritoneal cavity. Two minutes later the abdominal cavity was opened using a small incision and the lavage fluid was aspirated using a sterile 14 gauge intravenous cannula and a syringe.

The lavage fluid was centrifuged for 7 minutes at 500 G to form a pellet of cells. The supernatant was removed. The cell pellet was then resuspended in 1 ml of RPMI medium (Trace Bioscience Pty Ltd) containing antibiotics and 10% foetal calf serum. A cell count was performed using trypan blue exclusion, and the cells

were plated in a 6 well plate tray, with 4 wells used per rat. The cell suspension was diluted to make up a final concentration of 1×10^6 cells in each well. All the groups had an initial macrophage viability of greater than 95%, as determined by trypan blue exclusion. Macrophages were incubated for 3 hours in 5% carbon dioxide at 37° degrees Celsius to promote adhesion to the wells. The wells were then washed twice with PBS to remove any non-adherent cells, leaving a purified population of macrophages attached to the wells.

2.4.6.2. *TNF- α measurement*

Activation of the macrophages was estimated by determining TNF- α production. Three mls of RPMI media was added to the washed macrophages and they were incubated overnight. Each rat had 4 wells, of which two wells contained no lipopolysaccharide (LPS; E Coli:B4, Sigma) and two contained LPS (final concentration 2 μ g/ml). The trays were incubated overnight in an incubator at 37 degrees Celsius, and the supernatants were collected from each well, spun to remove cell debris and then stored at -70 degrees Celsius until assayed. The degree of activation of the macrophages was determined by estimating the levels of TNF- α production by LPS stimulated macrophages in culture. The TNF- α in the supernatants was measured using a L929 bioassay.

2.4.6.3. *L929 bioassay*

TNF sensitive L929 cells seeded in 96 well plates (8×10^4 cells in each well) were incubated overnight. 50 μ l of 8 x 1:10 serial dilution's of a known concentration of mouse TNF- α was added as a positive control and some wells contained only RPMI media as a standard as negative controls. 50 μ l of 6 x 1:4 dilutions of macrophage solutions were added to the wells. Trays were incubated overnight, media removed and 0.9% NaCl added. Saline was replaced with 50 μ l of crystal violet stain in each well. Trays were left for 10 minutes to allow absorption of the

stain into live cells. The excess stain was rinsed away and the plates were left to dry. 33% acetic acid was used to dissolve the cells and release the stain. Trays were then placed in a microplate reader (Biorad 3550-UV, Japan) to obtain the optical density values of each well.

2.5. **STATISTICS AND ETHICS**

InStat Biostatistics software programme (Graph-Pad Software Inc., San Diego, CA, USA) was used to calculate statistics. Individual statistical methods are referred to in the text.

Approval for animal experimentation was given by the Institute of Medical and Veterinary Science Animal Ethics Committee (Application Numbers 33/97, 55/97, 19/98, 31/97 and 25/97) and The University of Adelaide Animal Ethics Committee (Application Nos. M/095/95, M/017/96, M/064/97).

SECTION III

**INFLUENCES ON
TUMOUR IMPLANTATION AND METASTASIS
FOLLOWING LAPAROSCOPY**

3.1. THE EFFECT OF DIFFERENT INSUFFLATION GASES ON TUMOUR METASTASIS FOLLOWING LAPAROSCOPY

3.1.1. OVERVIEW

In 1926 Zollkoffer first proposed the use of carbon dioxide as an insufflation agent⁹⁵. Now, carbon dioxide is almost universally used as the gas to create a laparoscopic pneumoperitoneum, with its role firmly established in the 1960's when Kurt Semm developed the peritoneal insufflator¹⁶². Carbon dioxide has properties which make it a very suitable insufflation agent. It is cheap, non combustible and colourless. Furthermore, it is excreted by the lungs during normal respiration, and it is highly soluble in water which reduces the risk of gas embolism impairing cardiac function. More recently, however, as the physiological effects of laparoscopy have become better understood, debate has arisen about the metabolic and oncologic consequences of insufflating the peritoneal cavity with carbon dioxide. This has resulted in some surgeons re-evaluating the effects of a carbon dioxide pneumoperitoneum, or perhaps more accurately; capnoperitoneum¹⁸⁰. Alternatives to carbon dioxide insufflation are gasless laparoscopy exposure techniques, as well as the use of other insufflation gases such as helium or argon.

Previous work from the University of Adelaide Department of Surgery has demonstrated that the incidence of port site metastases could be reduced by the exclusion of carbon dioxide from the laparoscopic environment^{184,282}. This suggests that some insufflation gases, carbon dioxide in particular, may exert an adverse metabolic effect on tumour cells thereby facilitating tumour implantation and growth, and that laparoscopy utilising alternate insufflation gases may be beneficial.

To investigate this possibility further, the following studies were performed:

1. Investigation of the in vitro growth of the DAMA tumour cells in a helium or carbon dioxide rich environment.
2. Investigation of the use of different insufflation gases on port site metastases using the solid tumour laparoscopic model.
3. Investigation of the use of different insufflation gases on tumour implantation using the tumour cell suspension model.

3.1.2 EFFECTS OF A HELIUM OR CARBON DIOXIDE RICH ENVIRONMENT ON IN VITRO TUMOUR GROWTH

This experiment has been published as: Neuhaus SJ, Ellis TS, Barrett MW, Rofe AM, Jamieson GG and Watson DI. *In vitro inhibition of tumour growth in a helium rich environment - implications for laparoscopic surgery.* Aust. N.Z.J. Surg. (1999) **69**,52-55

3.1.2.1. AIM

This study was performed to compare the growth of cultured DAMA tumour cells following exposure to simulated laparoscopic environments, rich in helium, carbon dioxide or air.

3.1.2.2. METHODS

For this study the DAMA cell suspension (prepared as per section 2.2.5) was divided equally between 3 sterile containers (5 ml per container) and allocated to one of 3 study groups:

- 1. control (atmospheric air)**
- 2. CO₂ rich environment**
- 3. helium rich environment**

The suspension was exposed to the gas environment for 40 minutes. This was achieved by insufflating gas into a chamber at low pressure (5 mmHg) and flow (5l/min) using a laparoscopic insufflator (Storz Electronic Laparoflator 26012, West Germany). The insufflation gas was filtered through sterile filters to prevent any microbial contamination of the culture media. The culture chamber, a 70 ml sterile

plastic container, provided a sealed and sterile environment. Two 19 gauge cannulae were inserted through the seal (Nesco film, Bando Chemical Ltd, Japan) at the top of the chamber, to provide access for the gas insufflation and gas venting. In the control group, insufflation was not performed, with the cannulae left open to allow room air to passively enter the chamber (Fig 3.1.2.2).

FIGURE 3.1.2.2.

CLOSED CHAMBER FOR EXPOSURE OF ALL CULTURES TO VARIOUS GASES

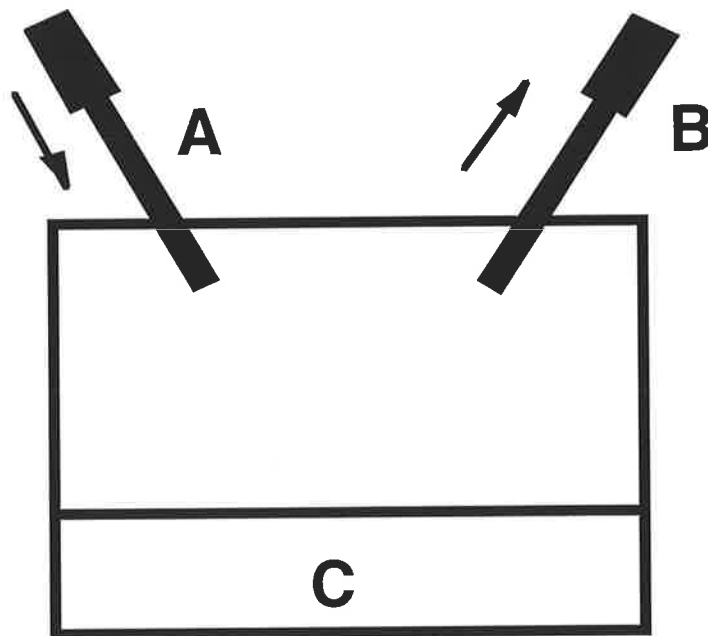


FIGURE LEGEND

A = "insufflation" cannula

B = "venting" cannula

C = cell culture

3.1.2.2.1 Cell culture studies

Following the 40 minute exposure period, the cells in each culture flask were reassessed for cell viability using trypan blue exclusion and recounted using the Coulter counter. Cells were then resuspended in the growth media and the concentrations were adjusted to 3×10^5 cells per ml, and then plated into a 96 well plate. A single channel pipette was used to place 100 μ l of cells in each of the inner wells. The outer wells were filled with PBS to prevent any edge effects which might occur due to dehydration or evaporation.

Cells were then cultured for 18 hours. Optical density readings were used to assess the number of viable tumour cells at the end of this time period. This technique is similar to that described by *Löwik et al* 175. The cells were incorporated with neutral red (3-amino-7-dimethylamino-2-methyl-phenazine hydrochloride) for the last 1 1/2 hours of culture by adding 50 μ l of neutral red solution to each well. Following incubation the cells were centrifuged at 100 rpm for 10 minutes. The dye was extracted from the cells by the addition of 100 μ l of 0.05M Na₂PO₄ in 50% ethanol. The plates were inverted and blotted dry with filter paper. Optical density was read, using a microplate reader (Biorad 3550-UV, Japan), with 540 nm used as the absorbance wavelength, and 645 nm as the reference wavelength. The spectrophotometer was blanked on the first column of control wells which contained PBS alone. This study was repeated to ensure consistency of the results.

3.1.2.2.2. pH studies

A further study was performed using 15 mls of culture media, prepared using the technique described above. Again this was divided equally between 3 sterile containers (5 ml per container), and exposed to control, carbon dioxide and helium environments. During the 40 minute exposure period and for 10 minutes following insufflation, pH was continuously measured using an antimony probe and Digitrapp

Mark II pH recorder (Synectics, Sweden). This equipment is normally used for assessment of 24hr pH in patients being investigated for gastro-oesophageal reflux. The pH probe was calibrated before all studies using commercially prepared buffer solutions (Synectics, Sweden) of pH 7 and pH 1 respectively. The study period was divided into 2 minute time periods, and the median pH for each period was determined using a computerised analysis programme (*EsopHogram* version 5.107c, Synectics Medical, Sweden). Recording of pH was not performed during the specific cell culture experiments in order to minimise any risk of contamination of the sterile environment.

3.1.2.3 RESULTS

The results of the cell culture experiments are summarised in Table 3.1.2.3.1 and Figures 3.1.2.3.1 and 3.1.2.3.2. Cell growth was demonstrated in all culture wells, with growth greatest in the control group in each of the three experiments. Cell growth was significantly less following incubation in the helium rich environment compared to both the carbon dioxide and control groups (Table 3.1.2.3.1; $p < 0.001$, Kruskal-Wallis test).

Continuous pH recording of the culture media during insufflation demonstrated a significant decrease in pH in the carbon dioxide group which was not observed during exposure to either air or helium (Figure 3.1.2.3.3; $p < 0.001$: Kruskal-Wallis test). The pH fell to 5.9 following insufflation of carbon dioxide, whilst the minimum pH value in the control and helium groups was 7.6 and 7.7 respectively.

TABLE 3.1.2.3.1**OPTICAL DENSITY READINGS : MEASUREMENT OF CELL GROWTH**

(all measurements are median and range)

	experiment 1	experiment 2
control	0.183 (0.096-0.213)	0.324 (0.242-0.387)
carbon dioxide	0.140 (0.101-0.177)	0.268 (0.236-0.348)
helium	0.077 (0.053-0.127)	0.174 (0.102 - 0.250)

Kruskal-Wallis Test $p < 0.001$

Dunn's Multiple comparisons: control vs carbon dioxide; $p < 0.01$
control vs helium; $p < 0.001$
carbon dioxide vs helium; $p < 0.001$

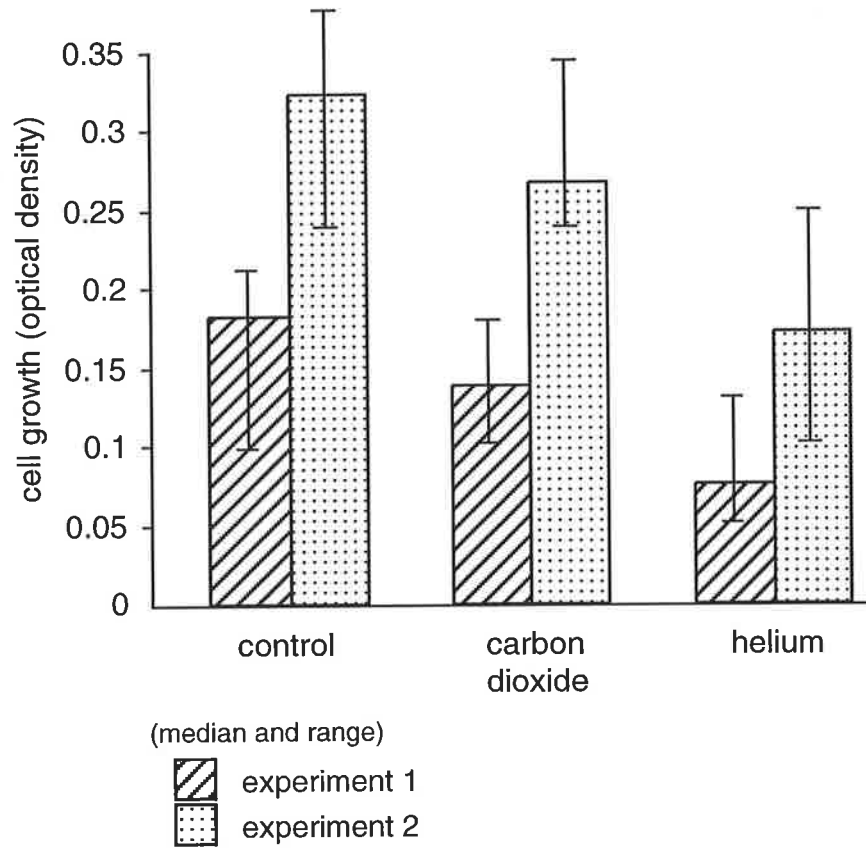
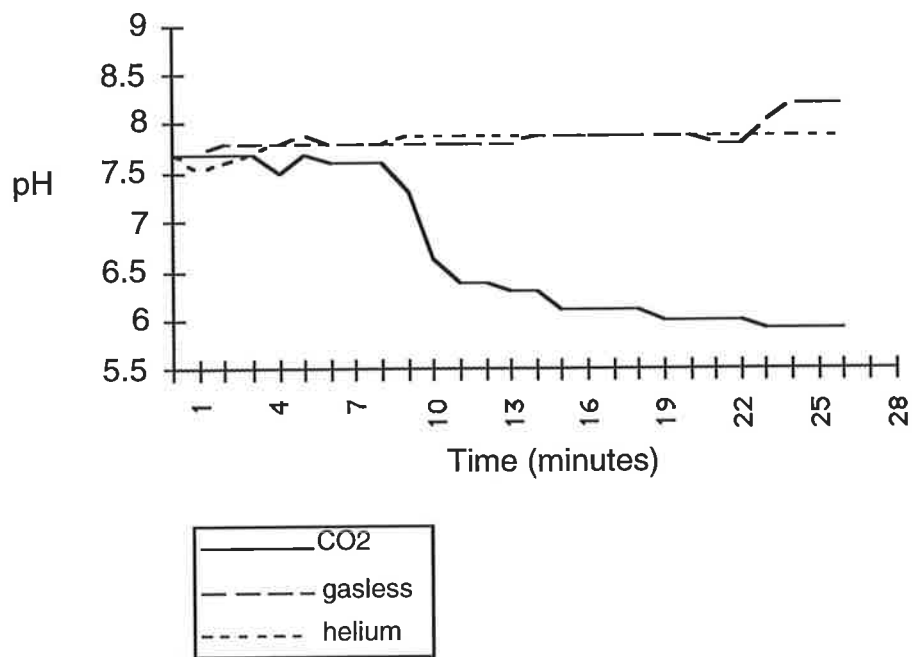
FIGURE 3.1.2.3.1***IN VITRO* CELL EXPERIMENTS 1 AND 2: CELL GROWTH AS DETERMINED BY OPTICAL DENSITY**

FIGURE 3.1.2.3.2**PH OF CULTURE SOLUTIONS**

(all points are median values pH data from consecutive 2 minute time intervals)



control vs carbon dioxide; $p < 0.001$

control vs helium $p > 0.05$

3.1.3 THE EFFECT OF DIFFERENT INSUFFLATION GASES USING THE SOLID TUMOUR MODEL

This experiment has been published as: Neuhaus SJ, Watson DI, Ellis T, Rowland R, Rofe AM, Mathew G and Jamieson GG. *Wound metastasis after laparoscopy with different insufflation gases.* Surgery 1998;123 (5):579-583

3.1.3.1 AIM

The aim of this study was to determine the effect of different insufflation gases on the incidence of port site tumours and on the growth of the primary flank tumour.

3.1.3.2 METHODS

Forty eight male Dark Agouti (DA) rats were injected in the left flank abdominal musculature with tumour cell suspension as described at section 2.2.5.

Seven days after tumour implantation the rats underwent laparoscopy with tumour laceration, using insufflation with one of the following gases (12 rats in each group):

- 1. Carbon dioxide (CO₂)**
- 2. Nitrous oxide (N₂O)**
- 3. Helium**
- 4. Air**

The choice of insufflation gas was allocated randomly.

After commencing gas insufflation and placing all "ports", the tumour capsule was lacerated in the standardised fashion (as described at section 2.3.3.1). Gas continued to be insufflated at a rate of 0.4 litres/min and a pressure of 2 mmHg for 40 minutes

after tumour laceration, with a constant gas flow maintained through the venting cannula. The same insufflator (Karl Storz model 26012 B, Germany) was used for all procedures, with its coupling connections modified to allow insufflation with nitrous oxide, helium and air. After 40 minutes the "ports" were removed, and the puncture sites were closed with sutures.

Seven days after the operative procedure all rats were killed and underwent autopsy as described at section 2.4.

3.1.3.3. RESULTS

The measured size of the primary tumour at the time of surgery was similar for all study groups ($P=0.12$; Kruskal-Wallis test), and the growth of the implanted tumour following surgery was independent of the insufflation gas used ($P=0.50$; Table 3.1.3.3.1: Kruskal-Wallis test).

TABLE 3.1.3.3.1

TUMOUR GROWTH FOLLOWING LAPAROSCOPY

(maximum dimension measured in mm - all figures are median and range)

	Air	CO2	Helium	N2O
Size at surgery	29 (20-41)	28 (15-38)	32 (15-36)	25 (16-32)
Size at day 7	49 (30-70)	49(40-60)	56 (35-96)	47 (3-75)
*%increase in median size	62 (42-131)	72 (52-200)	88 (45-166)	88 (45-200)

* $p=0.50$

At autopsy examination, with the exception of the surgical wounds, the tumour was not disseminated beyond the site where the tumour was lacerated in any animal. At the site of intraperitoneal tumour laceration, however, tumour was seen growing as a nodule at the site of the previous peritoneal breach.

The development of macroscopic and microscopic metastases at the laparoscopy trocar wounds is summarised in Tables 3.1.3.3.2 and 3.1.3.3.3 below.

TABLE 3.1.3.3.2

NUMBER OF RATS WITH METASTASES IN TROCAR WOUNDS

(12 rats per group)

	Air	CO2	Helium	N2O
Macroscopic	5	5	1	7
Microscopic*	8	7	1	10

*p=0.0017

TABLE 3.1.3.3.3

NUMBER OF WOUNDS WITH METASTATIC TUMOUR PRESENT

(36 wounds per group)

	Air	CO2	Helium	N2O
Macroscopic	5	8	1	7
Microscopic*	12	15	1	20

*p<0.0001

Tumour involvement of the surgical wounds was less likely in the helium insufflation group. Only 1 rat (8%) in the helium insufflation group developed histologically proven wound metastases compared to eight (66%), seven (58%) and ten (83%) in the air, carbon dioxide and N₂O groups respectively (Table 3.1.3.3.2). Chi-squared analysis of the likelihood of metastasis per wound confirmed a significantly lower incidence following helium insufflation (Table 3.1.3.3.3; $p < 0.001$; Chi squared test). Tumour metastasis occurred significantly less often in the laceration port compared to the laparoscope port (Table 3.1.3.3.4, $p = 0.0280$, Fishers' exact test).

TABLE 3.1.3.3.4

LOCATION OF METASTASES (microscopic)

(45 wounds per group)

	Air	CO₂	Helium	N₂O	Total
Laparoscope port	7	6	1	8	22*
Venting port	3	5	0	7	15
Lacerating port	2	4	0	5	11*

$p = 0.0495$

*laceration port vs laparoscope port $p = 0.0280$ (Fishers' exact test)

3.1.4. THE EFFECT OF DIFFERENT INSUFFLATION GASES ON TUMOUR IMPLANTATION

This study has been published as: SJ Neuhaus, T Ellis, AM Rofe, GK Pike, GG Jamieson and DI Watson. *Tumour implantation following laparoscopy using different insufflation gases.* Surg Endosc (1998) **12**:1300-1302

3.1.4.1. AIM

The aim of this study was to assess the influence of different insufflation gases on the extent of tumour cell implantation in the abdominal cavity.

3.1.4.2. METHODS

This study was performed using the introduction of DAMA suspension into the peritoneal cavity of Dark Agouti (DA) rats.

Forty DA rats were randomised to undergo laparoscopic insufflation with one of the following insufflation gases (10 rats in each group):

1. *Carbon dioxide (CO₂)*
2. *Nitrous oxide (N₂O)*
3. *Helium*
4. *Medical Air*

After commencing gas insufflation and placing all "ports", a 200 µl volume of DAMA cell suspension (containing 2×10^7 tumour cells) was introduced under laparoscopic vision through the left iliac fossa port as per section 2.3.3.1. Gas continued to be insufflated at a rate of 0.4 litres/min and a pressure of 2 mmHg for a further 40

minutes. A constant gas flow was maintained through the venting cannula. The "ports" were then removed, and the puncture sites closed with sutures.

Seven days later all rats were killed and autopsied as described at section 2.4

3.1.4.3. RESULTS

Preoperatively the rats in each insufflation group were of similar weight. All rats gained a similar percentage of weight postoperatively (Table 3.1.4.3.1, $p=0.49$; Kruskal-Wallis test).

TABLE 3.1.4.3.1

RAT WEIGHT FOLLOWING LAPAROSCOPY

(measured in grams - all figures are median and range)

	CO2	Helium	N2O	Air
Weight at surgery	281 (182-335)	289 (159-330)	248 (160-299)	241 (228-364)
Weight at day 7	298 (258-383)	302 (253-351)	268 (248-318)	253 (239-329)
%increase in weight (median*)	6.2	3.3	7.6	7.9

* $p= 0.49$

All rats developed tumour. Five of the 10 rats (50%) in the helium group had at least one peritoneal sector free from tumour growth, compared with 1, 2 and 3 rats in the carbon dioxide, nitrous and air groups respectively. In the helium group 18 sectors (30%) had no visible tumour growth. This was highly significant ($p < 0.0001$; Chi squared test) when compared to 1 (1.6%), 2 (3%) and 3 (5%) in the carbon dioxide, nitrous and air groups respectively. Tumour growth occurred in most sectors in the carbon dioxide, nitrous and air insufflation groups. Rats were less likely to develop tumour following helium insufflation, and tumour was seen evenly distributed to all sectors of the peritoneal cavity. There was no predilection for tumour growth at the site of introduction of the tumour cell suspension (Table 3.1.4.3.3, $p = 1.00$; Chi squared test).

TABLE 3.1.4.3.2

PERITONEAL TUMOUR INDEX

(number of sectors involved with each tumour grade)

Grade	CO2	helium	N20	air
0	1	18	2	3
I	15	14	13	12
II	20	10	18	15
III	24	18	27	30

$p = < 0.0001$

TABLE 3.1.4.3.3.**SECTOR ANALYSIS OF TUMOUR INVOLVEMENT**

(number of sectors containing macroscopic tumour, regardless of grade)

Sector	CO2	helium	N2O	air
1	10	6	9	8
2	10	9	10	9
3	10	7	10	10
4	10	5	10	10
5	9	8	10	10
6	10	7	9	10

p=1.00

Tumour involvement of the port sites occurred less frequently in the helium group (Table 3.1.4.3.4, p=0.02; Chi squared test). Thirteen port sites in the helium group were involved with tumour, either as plaques or discrete nodules, compared with 20, 22 and 24 in the carbon dioxide, nitrous and air groups respectively. No plaques were seen at port sites in the carbon dioxide group. Instead tumour involvement at these sites always occurred as solid nodules.

TABLE 3.1.4.3.4.**NUMBER OF WOUNDS WITH METASTATIC TUMOUR PRESENT**

(30 wounds per group)

	CO2	helium	nitrous	air
discrete nodules	20	12	17	19
plaques	0	1	5	5
TOTAL	20	13	22	24

p = 0.02 (Tumour vs no tumour)

3.1.5. DISCUSSION

Most studies assessing the phenomenon of port-site metastases have utilised carbon dioxide as the insufflation gas and have assumed that the physical effects of carbon dioxide pneumoperitoneum is the causative factor. Preliminary results from other groups have described conflicting data about the influence of the insufflation gas on the development of port-site metastases 120. *Jacobi et al* 131 report a series of *in vitro*, *in vivo* and *ex vivo* experiments using a rat model and colon adenocarcinoma. The insufflation of carbon dioxide was seen to promote tumour growth compared to the control group whereas helium had no tumour enhancing effect. *Dorrance et al* 70 however report that, using a rat intraperitoneal tumour cell line, the promotion of tumour growth by laparoscopy is independent of the insufflating gas. Differences between helium effects and carbon dioxide effects these models could be related to the use of cell lines with different degrees of carbon dioxide dependence or possibly an inhibitory effect of carbon dioxide at high concentrations 225.

Although it is likely that the aetiology of this phenomenon is multifactorial, the carbon dioxide pneumoperitoneum appears to play an important role 59,131,282.

3.1.5.1. Gasless laparoscopy

The removal of the insufflation gas may eliminate one of the essential environmental components necessary for laparoscopic wound metastasis. Previous experimental studies suggest that gasless laparoscopy confers an advantage over carbon dioxide pneumoperitoneum by reducing the incidence of abdominal wall metastasis 44,129,282, providing strong evidence that either the mechanical effect generated by the use of gas under pressure or some factor inherent in carbon dioxide gas *per se* plays a role in the development of metastases in the laparoscopy wounds 6,131,134.

Laparoscopy with insufflation gas may transport tumour cells to laparoscopic access wounds and result in the growth of metastases. *Mathew et al* reported, using an intraperitoneal suspension of tumour cells in a rat model of laparoscopy, that in gasless laparoscopy tumour deposits grew largely around the site where tumour was introduced into the peritoneal cavity, rather than evenly throughout the peritoneal cavity as seen following conventional laparoscopic insufflation¹⁸². A similar study which involved the laparoscopic laceration of the capsule of an implanted flank tumour, demonstrated a much higher incidence of port site metastases following carbon dioxide laparoscopy compared to gasless laparoscopy²⁸². In this study wound metastases were noted in only 3 of 12 rats in the gasless group compared to 10 of 12 rats in the carbon dioxide group. Gasless laparoscopy was achieved in both studies by suspending the abdominal wall using subcutaneously placed sutures, and no lifting device was inserted into the peritoneal cavity.

In a further study *Mathew et al* introduced viable tumour cells into the upper abdomen of rats undergoing either gasless or conventional laparoscopy¹⁸², following the introduction of a length of plastic tubing through the anterolateral aspect of the rat's left lower abdominal wall. This tubing was used to vent the insufflation gas through the abdomen of a further 'recipient' rat for 30 minutes. After 21 days the peritoneal cavity and surgical wound of the recipient rat were examined, and nodular tumour metastases were found around the site of both the venting port and the inflow tubing site in five out of six of the 'recipient' rats in the group that underwent carbon dioxide insufflation. However, no tumour was found in any 'recipient' rat in the gasless group. These studies suggest that insufflation (of carbon dioxide) is important for the promotion of wound metastases.

Other studies utilising real time imaging of radiolabelled cells instilled into the peritoneal cavity of pigs undergoing laparoscopy demonstrate widespread cell movement throughout the abdominal cavity during carbon dioxide pneumoperitoneum²⁶¹, supporting the postulate that carbon dioxide pneumoperitoneum facilitates tumour cell dispersal throughout the abdominal cavity, thereby bringing cells into contact with potential implantation sites.

*Bouvy et al*⁴⁴ have also reported that gasless laparoscopy is associated with decreased tumour implantation and decreased growth of a solid tumour (CC-531 colon adenocarcinoma). In a subsequent study they confirmed this result by demonstrating decreased tumour growth of an implanted renal subcapsular tumour in rats undergoing laparoscopy using a gasless technique compared to air or carbon dioxide pneumoperitoneum (ROS-1 osteosarcoma)⁴². As operative trauma and techniques were similar in both the gasless and carbon dioxide groups in each of these studies, differences in tumour implantation and metastasis may be due to either the mechanical effects of insufflation, a metabolic effect specific to carbon dioxide gas, or a combination of both. However, tumour growth in their second study was similar in rats undergoing insufflation with air compared to carbon dioxide, suggesting that increased intra-abdominal pressure may be a key factor. Mechanical stretching of the peritoneum secondary to high pressure pneumoperitoneum is associated with an increase in free radical production, and the production of inflammatory mediators in response to local ischaemia⁸⁰. Table 3.1.5.1 summarises the experimental studies which have compared carbon dioxide insufflation with gasless laparoscopy.

TABLE 3.1.5.1.

EXPERIMENTAL MODELS CO₂ vs GASLESS LAPAROSCOPY

<i>Author</i>	<i>model</i>		<i>tumours in CO₂</i>	<i>tumours in gasless</i>
<i>Watson et al 282</i>	mammary adenocarcinoma	solid flank tumour	10/12 wounds	3/12 wounds
<i>Mathew et al 185</i>	mammary adenocarcinoma	tumour cell suspension	64/72 sectors	37/72 sectors
<i>Mathew et al 182</i>	mammary adenocarcinoma	tumour cell suspension	5/6 recipient rats	nil/6 recipient rats
<i>Bouvy et al 44</i>	CC-531 colon adenocarcinoma	solid tumour	increased tumour growth in CO ₂ group; p<0.01	
<i>Bouvy et al 44</i>	CC-531 colon adenocarcinoma	cell seeding model	increased tumour seeding in CO ₂ group; p<0.01	
<i>Bouvy et al 42</i>	ROS-1 osteosarcoma	solid bolus	increased subrenal tumour growth in CO ₂ group; p=0.04	

3.1.5.2. **Physiological effects carbon dioxide**3.1.5.2.1. *metabolic*

There is now evidence from clinical and experimental studies that carbon dioxide pneumoperitoneum is associated with adverse physiological effects ^{275,276}, which result in alterations to ventilatory, cellular, hormonal and immunologic parameters. The metabolic changes associated with a carbon dioxide pneumoperitoneum include the development of systemic acidosis and hypercapnea secondary to absorption of carbon dioxide across the peritoneal surface. In addition, animal models using carbon dioxide pneumoperitoneum have demonstrated a decrease in PO₂ and increases in

heart rate and cardiac output which are not observed with gasless laparoscopy ¹⁸⁷ or the use of helium ^{87,242} *Neuberger et al* ²⁰³ have reported that in 20 patients undergoing laparoscopic cholecystectomy, the use of carbon dioxide insufflation was associated with significant hypercapnea and pH changes which were reversed when the carbon dioxide was discontinued and the pneumoperitoneum was re-established with helium. Experimental models also confirm these findings. *Mc Dermott et al* ¹⁸⁷ and *Fitzgerald et al* ⁸⁷ both report hypercapnea and acidosis following laparoscopy with carbon dioxide, which does not occur following either gasless laparoscopy or laparoscopy with helium insufflation .

Carbon dioxide, in addition to being insufflated is also constantly being produced by the normal metabolic processes in the body, and during laparoscopy substantial carbon dioxide load is presented to the body. Carbon dioxide can be stored in bone skeletal muscle and other tissues ¹⁹⁹. There is a direct correlation between the amount of carbon dioxide used in the procedure and the increase in the arterial PCO₂ ³⁹. The time taken to eliminate the excess carbon dioxide is directly proportional to the time taken for the procedure and carbon dioxide may be present in large quantities at the conclusion of the procedure. This residual carbon dioxide load can be significantly altered by the anaesthetist during the procedure by modulating the ventilation rates but patients may be left with a significant load that must be released post ventilation. This can be a problem especially in the elderly or those with a low cardiorespiratory reserve. In addition it has been suggested that the use of carbon dioxide as an insufflating agent promotes the development of postoperative pain ¹³⁸, and that this may occur due to the build-up of carbonic acid at the peritoneal membrane surface.

3.1.5.2.2. *cardiorespiratory*

Most pathophysiologic alterations occurring during laparoscopy are secondary to the insufflation of gas under pressure. Significant alterations in central venous pressure, cardiac output, cardiac rhythm and splanchnic and mesenteric blood flow have all been identified 180. Direct pressure from the use of insufflated gas also results in decreased flow in the inferior vena cava and decreased venous return from the lower limbs and pelvis 180. Studies which have compared the physiological effects of a pneumoperitoneum using carbon dioxide with helium and argon suggest that whilst hypercapnea is a carbon dioxide specific effect, the metabolic acidosis observed during pneumoperitoneum is not dependent on the type of gas used, but it is in fact a function of impaired tissue perfusion due to pressure in the peritoneal cavity, which results in an accumulation of the end products of hypoxic metabolism 242. Carbon dioxide has a vasodilatory effect on the blood vessels and a depressant effect on the heart, causing bradycardia and decreased contractility. Sympathetic stimulation due to elevated PCO₂ caused increased levels of vasoactive compounds such as adrenaline, nor adrenaline and angiotensin, resulting in tachycardia, increased cardiac output increased diastolic and systolic blood pressure and vasoconstriction of vascular beds. Sustained high levels of PCO₂ result in arrhythmias, depressed cardiac output and death 199.

3.1.5.2.3. *local effects*

It is known that the presence of a carbon dioxide pneumoperitoneum also alters the peritoneal milieu and results in localised metabolic changes 275. These changes include severe acidosis, not only of the peritoneal surface, but also in the underlying connective tissue, disturbance in the electrical surface charge and the release of various mediators such as endotoxin. *Voltz et al* 275 propose that 'severe acidosis in connection with an elevated intraabdominal pressure may alter or impair macrophage function'. Carbon dioxide is also irritant to the peritoneum, and this, via the release

of inflammatory mediators such as transforming growth factor alpha, may favour tumour implantation at raw peritoneal surfaces such as those created by the access ports 275.

Nduka et al 200 investigated the effect of incubating colonic cancer cells in carbon dioxide on cellular adhesiveness and report that carbon dioxide may directly influence the metastatic cascade by reducing cell to cell adhesion of tumour cells and promoting cell detachment and impairment of cell binding to immune effector cells.

The creation of a capnoperitoneum has also been shown to be associated with structural changes in the peritoneal mesothelial surface layer which are visible with electron microscopy 79. This may reflect changes secondary to carbon dioxide *per se* or it might be due to desiccation of the peritoneal surface by cold insufflation gas (independent of the gas type). Carbon dioxide is irritant to the peritoneum, and the subsequent release of inflammatory mediators such as transforming growth factor alpha, may favour tumour implantation at raw peritoneal surfaces such as those created by laparoscopy access ports 275. This effect is eliminated when a gasless exposure technique is used 44,185,282.

3.1.5.2. **Alternative insufflation gases**

Whilst carbon dioxide is the most frequently used gas to create a pneumoperitoneum, helium, argon, nitrous oxide and air have all been used clinically and experimentally 38,78,203,216,219. Noble gases like helium and argon have been suggested previously as alternative insufflation gases due to their inert nature, non flammability, and low cost 38,78. Argon gas is readily available in operating theatres and is inexpensive. Studies by *Eisnehauer et al* 78 demonstrate that it may not be entirely inert as its use as an insufflation gas in pigs was associated with cardiac depression. Helium is an inert gas and has no pharmacological or metabolic effects. In addition it

is not associated with systemic acid-base changes seen as a result of carbon dioxide retention 203,219. Because of possible adverse effects associated with the use of carbon dioxide insufflation 97,187,199,275, helium has recently been suggested as an alternative insufflation gas for laparoscopic surgery 145,203,219. Physiological studies in both animals and humans have demonstrated that the helium insufflation does not cause systemic or peritoneal acid-base changes which are associated with the use of carbon dioxide 203,242. PCO₂ and pH are not altered during insufflation with nitrous oxide suggesting that the hypercarbia is due to transperitoneal absorption of carbon dioxide rather than a decrease in tidal volume and increased dead space due to the presence of a pneumoperitoneum.

It is reasonable, therefore, to hypothesise that a helium pneumoperitoneum may also not cause depression of immune responses and the release of inflammatory mediators which occurs with carbon dioxide insufflation 283. If the release of inflammatory mediators or disturbance to the acid-base balance (either local or systemic) is responsible for the phenomenon of port-site metastases, then it would be expected that there would be a reduction in their incidence when using helium pneumoperitoneum in clinical and experimental studies. On the other hand, if the occurrence of port site tumours is not affected by the choice of insufflating gas, then it must be assumed that the phenomenon is related to a physical rather than chemical property of the gas.

It is also possible that carbon dioxide insufflation exerts an adverse metabolic effect on cells which facilitate tumour implantation and metastasis and that this effect may not be seen with helium 131. A similar phenomenon may occur with nitrous oxide and air insufflation, as these gases are also metabolically active 3.

3.1.5.4. Discussion of results

The results of **Study 3.1.2** suggest that helium does have a direct cytotoxic effect on cultured tumour cells. Tumour cell growth was least in the helium-rich environment and greatest when exposed to carbon dioxide. *Jacobi et al* ¹²⁹ have demonstrated a similar tumour growth enhancement effect of carbon dioxide *in vitro*. In this experiment the number of viable tumour cells was greatest after incubation with air rather than with carbon dioxide. It has been suggested that differences in tumour growth may be caused by gas acting on the tumour cells themselves by changing the extra- and intracellular hydrogen ion concentration, switching aerobic into anaerobic cellular metabolism ¹³².

The *in vitro* pH changes observed in this study are consistent with physiological data available from human and animal studies, in which the insufflation of helium or the use of gasless laparoscopy, unlike carbon dioxide, has not been associated with the development of intraperitoneal acidosis ^{38,169,170,187,270}. It is possible that the acidosis observed with the insufflation of carbon dioxide may stimulate tumour growth. However, this effect is unlikely to be the only factor determining tumour growth, as growth was greatest in the control group, rather than in the carbon dioxide group.

The pH changes observed in this study were lower than observed using the same tumour line *in vivo* (**Study 3.2.3**). This may reflect altered tumour cell metabolism in the peritoneal environment. Alternatively, tumour cells may be more severely affected by low pH conditions *in vivo*.

Tumour cell growth in **Study 3.1.2** was greatest following incubation in air. This is consistent with the findings of *Jacobi et al* ¹²⁹ and suggests that the reduction in tumour cell implantation observed in experimental models of gasless laparoscopy

reported previously ²⁸² is due to differences in cell distribution, rather than local acid-base or immunological changes.

Whilst the mechanism for the reduction in cell growth observed in this study is not yet understood, the study's results are supported by those *Jacobi et al* ¹³¹ who also developed a similar conclusion. They report, using an adenocarcinoma tumour cells line, an increase in the frequency of tumour growth at abdominal incision sites in carbon dioxide groups as compared to helium and control groups. In addition tumour of subcutaneous tumours and intraperitoneal tumour mass was also increased by exposure to carbon dioxide. Their *in vitro* studies demonstrated an increase in *in vitro* tumour cell growth following insufflation with carbon dioxide and an acidosis of the medium which was not observed in the helium or control group ¹³¹.

The results of **Study 3.3.3** demonstrate a significant reduction in the incidence of wound metastases following helium insufflation. This suggests that the chemical properties of some insufflation gases, via an alteration in the peritoneal milieu, facilitate the development of port-metastasis, and that this is reduced in the presence of a chemically non-reactive insufflation gas. The fact that metastases did occur in the helium group suggest that the gas alone is not the sole causative agent, but that an interaction between the chemical properties of the gas and the physical presence of the pneumoperitoneum is responsible. Nevertheless, the incidence of metastases following helium insufflation was similar to that reported following laparotomy and gasless laparoscopy in previous studies from the Royal Adelaide Hospital ^{184,282}. The results of **Study 3.1.3** also demonstrates a reduction in incidence of wound metastases at the site of the lacerating port compared to the laparoscope port. This finding suggests that contamination alone is not responsible for the phenomenon of wound metastases.

One possible problem with this study is that, because of the relative density of helium, the flow of gas through the insufflator is greater than that indicated by the insufflators' gas flow meter. Therefore, the decreased incidence of port site tumours could be due to increased flow of insufflation gas. However, one would logically anticipate that increased flow should increase the rate of metastasis if this was a purely mechanical phenomenon, leading therefore to an increased incidence of metastases in the helium insufflation group rather than the decrease seen in this study. The influence of flow and pressure on the development of port-site tumours is investigated at section 3.3.

The results of **Study 3.1.4** demonstrate a significant reduction in peritoneal tumour implantation and growth following helium insufflation. The fact that implantation occurred evenly in all sectors is also significant. Tumour cells are likely to be evenly distributed throughout the peritoneal cavity during laparoscopy, due to the mechanical effect of insufflation. The reduced rate of tumour growth in all sectors also supports the hypothesis of a metabolic or cytotoxic role for helium insufflation. In contrast, gasless laparoscopy reduces the likelihood of port site metastases and tumour implantation in experimental studies by greatly reducing the likelihood of tumour spread to areas in the peritoneal cavity distant to the operative field 282.

The mechanism for these findings is not yet understood, but may be related in part to specific effects of carbon dioxide on the peritoneal milieu discussed above, such as impairment of macrophage function and alterations in intraperitoneal pH 275.

Helium, on the other hand, because of its inert nature may prevent the "permissive" effect that carbon dioxide has on tumour implantation in experimental studies.

Alternatively, it is possible that the reduction in tumour metastases found in these studies, may be due to helium exerting a specific cytotoxic effect on tumour cells, as observed in **Study 3.1.2**.

3.1.5.5. Conclusions

The inhibition of tumour growth in a helium rich environment demonstrated in **Study 3.1.2** as well as the reduced incidence of port site metastases and tumour implantation observed in **Studies 3.1.3.** and **3.1.4,** have demonstrated that the development of port site metastases is related, at least in part to the chemical composition of the insufflation gas used to create a pneumoperitoneum. In particular, the choice of helium rather than carbon dioxide insufflation appears to be advantageous. This suggests that the clinical use of helium as an insufflation gas may have important advantages over carbon dioxide.

It is certainly interesting that the incidence of metastases following helium insufflation is similar to that following laparotomy and gasless laparoscopy using the similar small animal models 184,268, and these findings warrant close evaluation in the clinical setting, as substituting carbon dioxide with helium insufflation is a potentially a very simple strategy for the prevention of port site metastases. Clinical trials investigating the role of helium laparoscopy are now underway in several centres.

3.2. THE INFLUENCE OF IMMUNE FUNCTION ON TUMOUR GROWTH FOLLOWING LAPAROSCOPY

3.2.1. OVERVIEW

Evidence suggesting that the incidence of metastasis to surgical wounds following cancer resection is increased by laparoscopic approaches 181,184,236,289, conflicts with that from other experimental studies, which suggest a beneficial effect due to less systemic immune suppression and reduced primary tumour growth following laparoscopy for cancer 11,14,43,59,106.

Insufflation of carbon dioxide has been shown *in vitro* to depress peritoneal macrophage function 283 and to cause systemic immune disturbances 132. It is possible that either immune or metabolic disturbances, due to the use of a pneumoperitoneum, could contribute to the development of port site metastases and that this is mediated at the local peritoneal environment and not systemically.

To investigate this further, the following studies were performed:

1. Investigation of the effect of immune enhancement and suppression on tumour implantation.
2. Investigation of the influence of different insufflation gases on intraperitoneal immunity (*in vivo* peritoneal macrophage function and intraperitoneal pH) during laparoscopy in tumour bearing rats.

3.2.2. THE EFFECT OF IMMUNE ENHANCEMENT AND SUPPRESSION ON THE DEVELOPMENT OF PORT SITE METASTASES

This experiment has been published as: Neuhaus SJ, Watson DI, Rofe AM, Jamieson GG. *The effect of immune enhancement and suppression on the development of port site metastases* Surg Endosc 2000, **14** (5): 439-443

3.2.3.1. AIM

The aim of this study was to investigate the effect of systemic immune enhancement and suppression on tumour implantation using the free cell suspension model.

3.2.2.2. METHODS

Eighteen DA rats underwent modulation of their immune system, followed 18 hours later by laparoscopy with the introduction of a tumour cell suspension (as per section 2.3.3.1) into the peritoneal cavity. Endotoxin, which increases production of Tumour necrosis factor alpha (TNF- α) by peritoneal macrophages was used to simulate immune enhancement ²²⁹, and Cyclosporin A which produces a decrease in TNF- α production was used to suppress immunity ²³¹.

Rats were randomly allocated to one of the following groups:

- 1. control**

In this group, no immune enhancement or suppression was used.

- 2. cyclosporin**

Rats in this group were injected subcutaneously with 50 mg/kg of cyclosporin (a suppresser of the immune system) 18 hours before surgery.

3. *endotoxin*

Rats in this group were injected intraperitoneally with endotoxin (*E. Coli* 0111 β - a nonspecific stimulator of immune system) 18 hours before surgery.

Feed was withheld from all rats from the time of injection until surgery. Food was also withheld from rats in the control group for a similar time period.

Once all ports were placed, a carbon dioxide pneumoperitoneum established (as per section 2.3.2), and a 200 μ l volume of a mammary adenocarcinoma cell suspension (containing 2.5×10^5 tumour cells) was introduced under laparoscopic vision through the 16 gauge cannula which was then sealed. Seven days later all rats were killed, and underwent autopsy as per section 2.4.

3.2.2.3. RESULTS

The mean weight of the rats in each group were similar preoperatively, and all rats gained similar amounts of weight during the postoperative period ($p=0.83$, Kruskal-Wallis test; Table 3.2.2.3.1).

Tables 3.2.2.3.2 and 3.2.2.3.3 summarise the pattern of tumour implantation found in each study group. One rat in the group receiving intraperitoneal endotoxin did not develop any intra-abdominal tumour, whereas all rats in the other study groups developed tumour somewhere in their peritoneal cavity.

TABLE 3.2.2.3.1**RAT WEIGHT BEFORE AND AFTER SURGERY**

(all figures are median and range with weight measured in gms)

	Control	Cyclosporin	Endotoxin
Weight at surgery	224 (203-301)	210 (200-255)	205 (162-235)
Weight at autopsy	250 (222-310)	227 (218-271)	218 (176-256)
% increase in weight	9.5(2.6-13.1)	7.2 (3.6-10.9)	8.7 (-8.9-11.9)

(*median)

p=0.83

Tumour growth occurred in an equal number of sectors in the control and cyclosporin groups, but was less common following endotoxin administration (P= 0.008; Chi-squared test; Table 3.2.2.3.2). The density of tumour growth was also less following endotoxin (Table 3.2.2.3.2).

TABLE 3.2.2.3.2**NUMBER OF SECTORS INVOLVED WITH EACH PERITONEAL TUMOUR INDEX GRADE**

(36 sectors per study group)

Grade	control	cyclosporin	endotoxin
0	4	4	23
I	6	7	7
II	12	10	3
III	14	15	3

Total number of sectors with tumour **32 (89%)** **32 (89%)** **13 (36%)**

TABLE 3.2.2.3.3**NUMBER OF SECTORS WITH MACROSCOPIC TUMOUR GROWTH OF ANY GRADE**

Sector	control	cyclosporin	endotoxin	total
1	5	5	1	11
2	6	6	1	13
3	6	6	2	14
4	5	5	3	13
5	5	5	3	13
6	5	5	3	13

Total number of **32 (89%)** **32 (89%)** **13 (36%)**

sectors with tumour

Port-site metastases were significantly less common following the administration of intraperitoneal endotoxin (Table 3.2.2.3.4, $P < 0.0001$; Chi-squared test). There was no overall predilection for tumour growth at the site where the tumour cell suspension had been introduced (sector 3), and tumour was evenly distributed to all sectors (Table 3.2.2.3.3).

TABLE 3.2.2.3.4**NUMBER OF PORT SITES DEVELOPING TUMOUR METASTASES**

(18 port sites per study group)

Control	Cyclosporin	Endotoxin
16 (89%)	15 (83%)	2 (11%)

3.2.3. THE INFLUENCE OF DIFFERENT GASES ON INTRAPERITONEAL IMMUNITY DURING LAPAROSCOPY IN TUMOUR BEARING RATS

This experiment has been published as: Neuhaus SJ, Watson DI, Ellis T, Rofe AM, Mathew G and Jamieson GG. *The influence of different gases on intraperitoneal immunity during laparoscopy in tumour bearing rats.* World J. Surg 2000, **24** (10): 1227-1231

3.2.3.1. AIM

The aim of this study was to investigate the effect of different insufflation gases and gasless laparoscopy on *in vivo* peritoneal macrophage function and intraperitoneal pH.

3.2.3.2. METHODS

A solid flank carcinoma was implanted into the flank of 32 experimental rats (as per section 2.2.6) which underwent laparoscopic surgery within one of four treatment groups:

- 1. control (anaesthesia alone),**
- 2. gasless laparoscopy,**
- 3. helium insufflation, and**
- 4. carbon dioxide insufflation.**

Intraperitoneal pH was monitored during surgery, and peritoneal macrophage function was determined 3 days following surgery by harvesting peritoneal macrophages and then examining their ability to produce TNF- α . The study end points were intraperitoneal pH during laparoscopic surgery, and peritoneal macrophage function.

Pneumoperitoneum was established as per section 2.3.2. In the carbon dioxide and helium laparoscopy groups gas was insufflated at a rate of 0.4 litres/min and a pressure of 2 mmHg for 40 minutes with a constant gas flow maintained through the venting cannula. In the gasless laparoscopy group, pneumoperitoneum was achieved by suspending the abdominal wall from a metal frame, as described at section 2.3.4. Ports were placed in an identical manner and exposure was maintained for 40 minutes. The control group was anaesthetised for 40 minutes, but underwent no surgical intervention during this time period. Tumours in all groups were not manipulated during surgery.

3.2.3.2.1. pH studies

Throughout the 40 minute laparoscopic exposure period, and for a further 10 minutes after this, intraperitoneal pH was recorded continuously using equipment normally used for the assessment of gastro-oesophageal reflux disease. This comprised an antimony pH electrode (Synectics, Sweden) connected to a Digitrappor Mark II pH recorder (Synectics, Sweden). The system was calibrated before each study by immersing the tip of the pH electrode and the reference electrode in commercially prepared buffer solutions (Synectics) of pH 7 and pH 1. The tip of the electrode was positioned so that it was in contact with the peritoneal surface of the small bowel or its mesentery at all times. This was ensured in all studies as failure to maintain direct tissue contact in earlier studies was shown to result in erroneous measurements. From the continuous pH measurement, the median pH for consecutive two minute measurement periods was calculated using a computer programme (EsopHogram

version 5.107c, Synectics Medical, Sweden). This facilitated data analysis, and minimised artefactual errors. After 50 minutes all ports were removed, and the surgical wounds were closed with sutures. Because laparoscopy was not performed in the control group, a continuous recording of pH was not obtained in this group.

3.2.3.2.2. **Peritoneal macrophage harvest and function assessment**

Three days after surgery the animals were killed and the dimensions of the primary tumour were measured as per section 2.4. Peritoneal macrophages were collected and their function was assessed using a TNF- α assay as described at section 2.4.6.

3.2.3.2. **RESULTS**

The preoperative weights for the rats in each study group were similar, and weight gain following surgery was similar for all groups (Table 3.2.3.3.1; $p=0.15$, Kruskal-Wallis test). The overall growth of the implanted tumour was also similar for all groups during the 3 days following surgery (Table 3.2.3.3.2, $p=0.77$, Kruskal-Wallis test).

Continuous pH recording in the abdominal cavity revealed a consistent fall in intraperitoneal pH below pH 6 during carbon dioxide insufflation (Figure 3.2.3.3.1). No significant pH fall was observed during either gasless or helium laparoscopy. Mean pH declined to 5.4 forty minutes following the commencement of carbon dioxide insufflation. The minimum pH values during gasless and helium laparoscopy were 6.7 and 6.6 respectively.

TABLE 3.2.3.3.1.**RAT WEIGHT FOLLOWING LAPAROSCOPY**

(all measurements are median and range in grams)

	control	gasless	helium	CO2
wt at surgery	277.5 (251-318.5)	305 (222-322)	277.25 (246.5-287)	273.75 (229.5-313.5)
wt at day 3	274 (192-307)	308.5 (222-340)	277.5 (250-290)	266 (233.5-308)
% weight change (*median)	2 (-9-31)	-2 (-12-6.9)	0.9 (-3-13)	-1 (-7-18)

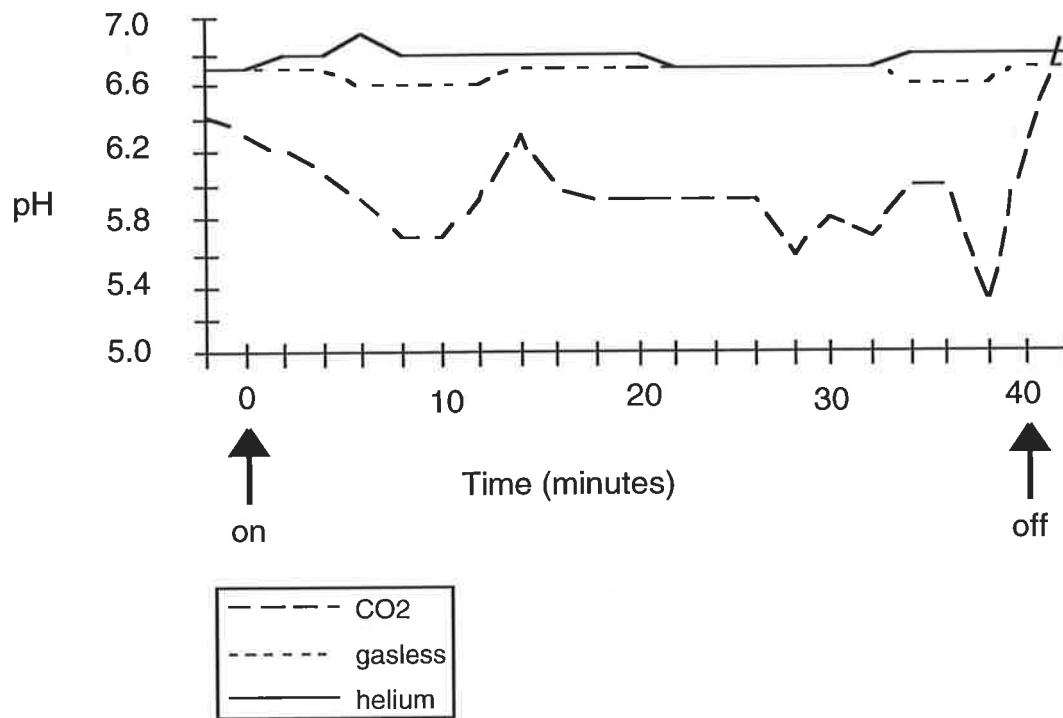
*p=0.15

TABLE 3.2.3.3.2.**TUMOUR GROWTH FOLLOWING LAPAROSCOPY**

(maximum dimension measured in mm - all figures are median and range)

	control	gasless	helium	CO2
Size at surgery	22(11-30)	24.5(11-20)	14(9-30)	20(11-24)
Size at day 3	30(19-41)	23(20-27)	24(19-38)	26.5(20-35)
%increase in size (*median)	36 (14-164)	58 (25-91)	48.5 (-9-131)	43 (5-127)

*p=0.77

FIGURE 3.2.3.3.1**pH CHANGES DURING LAPAROSCOPY.**

Prior exposure to carbon dioxide insufflation in vivo was associated with a marked decrease (>90%) in the ability of peritoneal macrophages to produce TNF- α . This effect was not seen with helium or gasless laparoscopy techniques (Table 3.2.3.3.3; $p=0.001$, Kruskal-Wallis test).

**TABLE 3.2.3.3.3**

**TUMOUR NECROSIS FACTOR ALPHA PRODUCTION FOLLOWING
LAPAROSCOPY**

(TNF production measured in "units of biological activity", all figures are median and range)

	Control	Gasless	Helium	CO2
TNF-alpha production	55	144	128	3
	(30-500)	(9-1100)	(9-1050)	(1-9)

p= 0.001, Kruskal-Wallis test; Post-testing with Dunn's multiple comparisons; control vs CO2, P<0.05; helium vs CO2, P<0.05; gasless laparoscopy vs CO2, P<0.01; all other comparisons P> 0.05)

3.2.4. DISCUSSION

3.2.4.1. Systemic immune modulation

Surgical intervention is known to impair systemic immune function, with the degree of effect proportional to the extent of operative trauma 41,171,172,196. This generalised state of immunosuppression following surgery has been implicated in the development of tumour metastases 177 and it has been suggested to be related to a defect in natural and lymphokine activated killer cell function in the postoperative period 66. Because of this, it has been claimed that laparoscopic techniques, because they result in less wound related trauma and cause less disturbance of systemic immune function, might be advantageous for patients undergoing cancer surgery 43,59.

3.2.4.1.1. *Delayed type hypersensitivity responses*

This proposal is supported by clinical and experimental studies which have demonstrated that postoperative immune function is better preserved following laparoscopy than laparotomy 11,12,14,41,43,47,59,104,268. *Trokel et al* reported that rats undergoing laparotomy had significant impairment of delayed type hypersensitivity (DTH) responses when challenged with keyhole limpet haemocyanin antigen or phytohaemmagglutinin (PTH) post operatively following laparotomy but that DTH was preserved after laparoscopy with carbon dioxide insufflation 268. Rats undergoing insufflation and laparoscopy had unchanged DTH responses from preoperative responses. *Bessler et al* 35 also examined the response of pigs to sow Bac-e antigen after either laparoscopic or open colectomy and found that pigs undergoing laparoscopic resection had a 20% increase in their post operative responses in comparison with pigs in the laparotomy group .

Berguer et al have also demonstrated better preservation of cell mediated immunity in the early postoperative period in rats undergoing laparoscopic compared with open laparoscopic fundoplication 29.

Iwanaka et al 125 report that laparotomy was associated with a reduction in peripheral macrophage numbers and viability in mice undergoing laparotomy compared with minimally invasive and control groups. In addition, serum macrophage production of tumour necrosis factor alpha and nitric oxide, two markers of macrophage stress, were significantly increased in the laparotomy group 24 hours post surgery compared with control groups. Gasless suspension and pneumoperitoneum did not lead to an increase in TNF or nitric oxide production by peritoneal macrophages. This study endorses the possible physiologic benefit of minimally invasive surgical techniques and preservation of immune mediated defences.

3.2.4.1.2. ***Growth of primary tumours***

Studies comparing the growth of tumour following laparotomy compared pneumoperitoneum demonstrate that laparotomy is associated with an enhancement of growth of the primary tumour. The Columbia group using MMC MC2 tumour line demonstrating increased tumour growth in rats undergoing laparotomy compared with pneumoperitoneum groups 11. Tumour growth was least in controls. This finding was consistent when repeated with a colon-26 adenocarcinoma and B-16 melanoma cell lines 247.

Allendorf et al also observed that the differences in tumour growth between the open and pneumoperitoneum groups found in the above study was lost in athymic mice 9. Tumours in both surgical groups were similar and larger than in anaesthesia control

groups, suggesting that post operative immune function plays a role in limiting the rate of tumour growth in immunocompetent mice.

Allendorf et al 11,12,14 have also demonstrated in a murine model that tumour cells inoculated into the dorsal skin of laboratory mice grow more easily and aggressively following laparotomy than laparoscopy with carbon dioxide insufflation, and that post operative immune function is better preserved after laparoscopic-assisted bowel resection than after open resection suggesting a systemic benefit for laparoscopic treatment of malignancy, presumably due to less systemic immune suppression.

Bouvey et al 41 demonstrated using a rat model and an CC-531 colonic cancer line and both subcapsular renal implantation and intraperitoneal tumour cell suspension that laparoscopy and small bowel resection was associated with less tumour growth compared with the same procedure performed via laparotomy. This study is important because unlike *Allendorf et al*'s it demonstrated a decrease in intraperitoneal tumour load in the laparoscopic groups.

In other words, laparotomy appears to have a significantly greater effect than laparoscopy in encouraging tumour growth. Whether this is due to increased tumour cell proliferation or decreased cell death is unclear. It has been suggested that tumour cell proliferation rates may be higher following laparotomy compared with laparoscopy 10. *Allendorf et al* assessed tumour cell proliferation rates by determining the level of proliferating cell nuclear antigen (PCNA) via immunohistochemical staining of tumour sections. The PCNA is an intranuclear protein essential for DNA synthesis that is maximally expressed in the S-phase of the cell cycle. The proliferation index of the open groups was significantly higher than the other two groups (carbon dioxide pneumoperitoneum and anaesthesia alone).

Interestingly in the carbon dioxide pneumoperitoneum group the PCNA index was significantly higher than the control group both at 6 and 12 days postoperatively.

Given that systemic immune suppression or anergy is associated with an increased rate of tumour recurrence following resection⁷⁷ it would be expected that laparoscopy would provide an advantage to patients with cancer. However, because these studies investigated the growth of an implanted tumour located outside the abdominal cavity or abdominal wall during laparoscopic and open surgical exposure, and the issue of peritoneal (local) immunity and port site metastases was not addressed, the results may not be directly applicable to the clinical situation. Their findings of reduced primary tumour growth have also been consistently replicated in studies from our department using the same model as that used in the current study¹⁸⁴. However, despite the reduced growth of the primary tumour, the risk of tumour metastasis to port sites and implantation elsewhere in the peritoneal cavity was adversely affected by laparoscopic insufflation with carbon dioxide in our studies^{131,184,185}, suggesting that the local intraperitoneal environment is likely to be of more importance for the development of port site metastases than general systemic immune status.

3.2.4.1.3. *Systemic versus local effect?*

However, these potential benefits for laparoscopy conflict with the risk of port site metastases. One possible explanation for this apparent discrepancy is that the development of port site metastases might at least in part be influenced by locally acting factors which influence immune function at the level of the peritoneal membrane. This is supported by work reported by *Volz et al*²⁷⁵ who have demonstrated that carbon dioxide pneumoperitoneum results in significant changes to mechanical, ventilatory, cellular, hormonal and immunologic parameters including acidosis involving the peritoneal surface and the underlying connective tissue,

disturbances in electrical surface charges, and the release of various mediators such as endotoxin.

Collet et al ⁵⁹ investigated the effect of laparoscopy and laparotomy on peritoneal host defences in swine and report that peritoneal and systemic monocyte class II antigen expression and serum TNF- α activity was greater following laparotomy but that peritoneal bacterial clearance was more efficient in the laparoscopic groups. This suggests that there may be a potential immune benefit of laparoscopic surgery, at least in terms of the ability to clear bacterial contamination. It is not clear however, if this proposed immune benefit also extends to clearing malignant cells from the peritoneal cavity.

One must therefore consider when attempting to understand the phenomenon of port site metastases, that whilst it is possible that at least some systemic immune defence mechanisms may be better preserved following laparoscopy compared with laparotomy ^{59,268}, this may not reflect the local response to tumour cells at the level of the peritoneal membrane.

3.2.4.2. Discussion of results

Study 3.2.2 sought to further investigate the possible influence of immune function on the development of port site metastases. Endotoxin (lipopolysaccharide *E. Coli* 0111 β) was used to stimulate peritoneal immune function. It is derived from the cell wall of Gram negative bacteria and has been previously shown to be a potent stimulator of peritoneal macrophage function, with its intraperitoneal administration resulting in stimulation of mononuclear phagocytes and increased release of a number of inflammatory mediators including TNF- α , and IL-1, IL-6, IL-8, the reactive oxygen and nitrogen intermediaries superoxide, hydrogen peroxide and nitric oxide and lipid derivatives, thromboxane A₂ and platelet activating factor ^{229,283}.

Endotoxin administration resulted in a significant reduction in the incidence of tumour implantation and port site metastases. The most likely explanation for this finding is that the reduction in tumour implantation was mediated by intraperitoneal immune function enhancement, stimulated by endotoxin administration. A direct toxic effect of endotoxin on the tumour cells is unlikely to account for the findings in this study, as the endotoxin was administered into the peritoneal cavity 18 hours before the tumour cells were introduced at laparoscopy.

Cyclosporin, has been shown to produce immune suppression in previous studies using the DA rat 229,231. However, it failed to significantly increase tumour implantation or port site metastases compared to rats in the control group. Whilst this lack of difference could be explained by proposing that immune suppression does not influence the risk of tumour dissemination, the similar pattern of metastases observed in the cyclosporin and control groups may also be related to the high rate of port site metastases found in the control group. To demonstrate a significant increase in the rate of metastases, a much larger number of rats would have been needed for this experiment.

Nevertheless, the results of **Study 3.2.2** do support the hypothesis that the incidence of port site metastases can be influenced by alterations in the immune environment, particularly at the local level of the peritoneal membrane.

3.2.4.3. **Peritoneal immune environment**

The normal peritoneal cavity contains less than 100 ml of serous fluid, which is essentially an ultrafiltrate of plasma with a protein concentration lower than 3g/dl 112. The peritoneum usually contains fewer than 300 cells per mm³, mostly macrophages plus some desquamated mesothelial cells and lymphocytes but the ability of the peritoneal cavity to generate polymorphonuclear leucocytes (PMNs) and macrophages

is prodigious¹¹². Abdominal surgery, even in the absence of gross inflammation elicits a rapid and transient influx of polymorphs which are soon followed by macrophages. Degranulation of peritoneal mast cells releases vasoactive substances (increasing vascular permeability), complement (components of which are chemotactic for macrophages) and opsins. In addition cytokines secreted by polymorphs up regulate macrophage phagocytic functions¹¹².

Peritoneal macrophages are integral to the primary inflammatory response generated in the abdominal cavity in response to infection and cancer²²². Pathogens and foreign cells are phagocytosed in opsonised and non-opsonised forms through Fc, C3b and mannose fucose receptor binding sites and subsequently killed by mechanisms that include reactive oxygen intermediates. Their scavenging action is mediated in part by the production of inflammatory cytokines such as TNF- α , which may be important for the effective killing of tumour cells^{142,222,229}. Macrophages are also implicated in regulation of the acute phase response and the release of the monokines IL-1, IL-6, TNF- α and arachadonic acid metabolites. In keeping with the immunosuppressive effects of surgery, *Redmond et al* have reported a temporary but significant impairment in both local and systemic macrophage function following laparotomy²²². Depression of peritoneal macrophage function may therefore prevent effective “scavenging” of viable tumour cells liberated during laparoscopic cancer surgery.

3.2.4.3.1. *Effect of carbon dioxide on peritoneal macrophage function*

The results of our current study have demonstrated a significant *in vivo* reduction in TNF- α production from intraperitoneal macrophages harvested three days after insufflation with carbon dioxide. This effect was not seen following gasless or helium laparoscopy. TNF- α was used in this study as it is a “marker” for cytokines produced by macrophages. TNF- α is cytostatic to neoplastic cells *in vitro* and *in*

vivo 50 and acts as an activating agent for macrophages by increasing tumour cell lysis and increased production of other biologically active cytokines. In addition TNF is a cytoattractant for the recruitment of activated macrophages 198,252. Tumour bearing rats were selected for this study as it has previously been demonstrated that macrophage reactivity (measured by macrophage ability to produce TNF- α varies in the tumour and non-tumour bearing state 252. The tumour bearing state more accurately reflects the clinical situation.

West et al 285 reported a similar reduction in TNF- α and IL-1 production in murine peritoneal macrophages incubated *in vitro* with carbon dioxide, but not when incubated in a helium rich environment 284,285. In West's study, the depression of macrophage cytokine production by exposure to carbon dioxide was reversible following incubation for 24 hours in a control atmosphere. Of interest in this study marked inhibition of IL-1 was seen after as little as 15 minutes of exposure to carbon dioxide whereas a minimum of 30 minutes was required to inhibit TNF. Maximal TNF inhibition was not observed until after 4 hours of test gas incubation 284. *West et al* propose that the mechanism of IL-1 inhibition induced by carbon dioxide is via a transcriptional control mechanisms whilst intracellular acidosis induced by the carbon dioxide rich environment may contribute to the inhibition of TNF- α . *West et al* also suggest that because of this macrophages incubated with carbon dioxide may be unable to generate a normal oxidative burst response. A similar toxic effect of carbon dioxide on *in vitro* lymphocyte function has also been reported 81. In addition, *Puttick et al* 218 have reported that exposure of peritoneal macrophages to carbon dioxide pneumoperitoneum diminishes anti-tumour cell cytotoxicity.

Recent studies by *Jacobi et al* 132 also suggest that the use of a carbon dioxide pneumoperitoneum is associated with adverse systemic alterations to the experimental immune environment. They demonstrated a decrease in plasma TNF- α levels and an

increase in plasma IL-10 in rats undergoing laparoscopy with carbon dioxide insufflation compared to control rats, and rats undergoing helium laparoscopy. *Redmond et al* 223 reported a reduced release of TNF by inflammatory cells from patients after laparoscopic cholecystectomy compared to those following open surgery. *West et al* hypothesise that this carbon dioxide mediated inhibition of inflammatory cells function may, in part, explain why laparoscopic surgery is so well tolerated 285. Our findings complement these studies as they have demonstrated that similar *in vivo* depression of macrophage function occurs within the peritoneal cavity itself. In contradistinction to this, *Evrard et al* 81 demonstrated no impairment of peritoneal lymphocyte function in patients undergoing pneumoperitoneum. This study however, did not assess the effect of the pneumoperitoneum on peritoneal macrophages, and it may be that it is macrophages which provide the important initial response to tumour cells.

Previous work from our department has demonstrated a reduction in TNF- α one day after tumour bearing DA rats underwent carbon dioxide laparoscopy compared to both gasless laparoscopy and to anaesthesia alone 183. Helium insufflation was not investigated in this study. When these results are examined alongside the results of the current study, it appears likely that 40 minutes of carbon dioxide insufflation results in adverse disturbances to the local intraperitoneal immune environment, and that these disturbances can persist for at least 3 days in our experimental model.

3.2.4.3.2. *pH changes*

The pH changes observed in the current study are consistent with other reports 275,276 and with the results of the *in vitro* gas studies in section 3.1.2. *In vitro* studies by *West et al* 285 also demonstrated that the use of carbon dioxide, but not helium or air, is associated with marked “cytosolic acidification of macrophages” and lowered tissue pH. It is possible that these two findings are linked; i.e. the observed

impairment in peritoneal macrophage function could be due to the reduction in intraperitoneal pH during laparoscopy with carbon dioxide. *West et al* 285 reported that a similar reduction in peritoneal macrophage production of TNF- α can be induced by pharmacologically induced acidification.

3.2.4.4. **Conclusions**

The discrepancy between the overall systemic benefit conferred by laparoscopy compared to laparotomy contrasts with the increased incidence of port site tumours and intraperitoneal tumour seeding observed in experimental models following laparoscopy. This suggests that the local intraperitoneal environment is likely to be of more importance for the development of port site metastases than general systemic immune status.

Alterations to local peritoneal immune function induced by carbon dioxide may contribute to the development of port-site metastases. The hypothesis generated by the results of the above studies is that carbon dioxide induced depression of macrophage activity, possibly mediated by pH changes, is a contributing factor to the development of port site metastases. Certainly the lack of adverse finding in rats undergoing helium and gasless laparoscopy is consistent with previous studies which have demonstrated reduced rates of port site metastases in animals undergoing laparoscopy using these exposure techniques, compared to laparoscopy with carbon dioxide 131,282.

3.3. THE EFFECT OF INSUFFLATION PRESSURE ON TUMOUR METASTASIS FOLLOWING LAPAROSCOPY

3.2.1. OVERVIEW

Previous experimental studies using the DA rat laparoscopy model have demonstrated that laparoscopy with carbon dioxide insufflation is associated with wider dissemination and implantation of intra-abdominal tumour compared to both laparotomy and gasless laparoscopy. This suggests that the adverse effects of pneumoperitoneum may be influenced by physical properties of the insufflation gas and its use in a positive pressure environment. To investigate this further, the likelihood of wound metastasis following laparoscopic surgery in an experimental model utilising different insufflation pressures for the carbon dioxide pneumoperitoneum was assessed.

To investigate this possibility further, the following study was performed:

Investigation of the role of insufflation pressure on the incidence of port site tumours using the solid tumour model and a carbon dioxide pneumoperitoneum .

3.3.2. INVESTIGATION OF THE EFFECTS OF INSUFFLATION PRESSURE ON THE DEVELOPMENT OF PORT SITE METASTASES SUMMARY

This study has been published as: SJ Neuhaus, DI Watson, T Ellis, T Dodd and GG Jamieson. *Port site metastases are not increased by high pressure insufflation.* MITAT 1999 8 : 2 ; 117-121

3.3.2.1. AIM

The aim of this study was to investigate the effect of different insufflation pressures on the incidence of port site metastases using the solid tumour model.

3.3.2.2. METHODS

24 DA rats with implanted flank tumours underwent laparoscopy with intraperitoneal tumour laceration (as described at section 2.3.3.1). All rats underwent laparoscopy with a carbon dioxide pneumoperitoneum using a Storz insufflator . To investigate the influence of insufflation pressure the rats were randomly allocated to one of the following groups (12 rats in each study group):

- 1. *low insufflation pressure (2 mmHg)***
- 2. *high insufflation pressure (6 mmHg)***

For both groups the flow rate which was regulated by the insufflator was set to a maximum of 0.4 l/min.

All rats were killed 7 days after the procedure and the wounds were examined for the presence of tumour metastasis as described at section 2.4.

3.3.2.3. RESULTS

The size of the primary tumour was similar in the two groups preoperatively ($p=0.82$, Table 3.3.2.3.1, Mann-Whitney test). There was no significant difference in the percentage growth of the implanted tumour following surgery between the two groups ($p=0.90$, Mann-Whitney test) and in addition the rats gained a similar amount of weight during the postoperative period ($p=0.42$; Mann-Whitney test).

TABLE 3.3.2.3.1.

TUMOUR GROWTH FOLLOWING LAPAROSCOPY

(maximum dimension measured in mm - all figures are median and range)

	low pressure	high pressure
	2mm Hg	6mmHg
Size at surgery	34(15-47)	33(20-48)
Size at day 7	55(45-74)	52(44-75)
*% increase median in size	63(29-200)	67(24-157)

* $p= 0.90$

TABLE 3.3.2.3.2

ANIMAL WEIGHT CHANGE FOLLOWING LAPAROSCOPY

(in grams - all figures are median and range)

	low pressure	high pressure
	2mmHg	6mmHg
Weight at surgery	225(196-254)	241(206-294)
Weight at day 7	264(226-280)	262(215-325)
*% increase in median weight	13(-2-23)	9.5(-22-33)

$p=0.42$

The median volume of gas used per rat was significantly higher in the high pressure group; 15 l (range 5.1-26l) compared to 11.7 l (range 0.7-17l ; p=0.01; Fisher's exact test) in the low pressure group. The median flow rate (calculated by dividing insufflation volume by the duration of each experiment) was also greater following high pressure insufflation (0.38 l/min) than following low pressure insufflation (0.29 l/min)

Three rats developed histologically confirmed port site metastases in the low pressure group compared to five rats in the high pressure group (p=0.67;Fisher's exact test). The number of wounds in which tumour was found was identical for the two study groups. Each study group had 36 wounds. Eight port sites developed tumour in each group (p=1.0;Fisher's exact test). Metastases were more common at the port site which contained the cannula used to vent the insufflation gas (Table 3.3.2.3.3). Tumour was found at the port sites only, with no of metastases occurring to any other abdominal sites.

TABLE 3.3.2.3.3

LOCATION OF METASTASES (microscopic)

(36 wounds per group)

	low pressure 2mm Hg	high pressure 6mmHg	total
Laparoscope port	3	2	5
Venting port	4	4	8
Lacerating port	1	2	3
TOTAL	8	8	16

3.3.3. DISCUSSION

Mechanical factors specific to the pneumoperitoneum may play a role in the development of port site metastases. These include the mechanical effects of gas under pressure such as stretching of the abdominal wall and peritoneal lining, and pressure/flow effects related to the use of insufflation to create an operating space.

Mechanical factors which may play a role include the so called 'chimney phenomenon' or Venturi effect proposed by *Kazemier* 6,44, whereby the insufflation of gas causes turbulence which displaces tumour cells. At the port sites, these cells are concentrated as a result of the leakage of gas alongside trocars resulting in high local gas flows at the trocar sites. This gas may contain an aerosol of viable tumour cells which are 'delivered to the port sites, resulting in tumour implantation 44,201. Viable tumour cells have also been demonstrated on filters attached to port sites during laparoscopy which suggests that tumour cells can be aerosolised or at least circulated to port sites 116,265. If this 'chimney phenomenon' was responsible for localising tumour at the trocar sites, it would be expected that alterations to the pressure of the insufflating gas might alter the number of viable tumour cells being deposited at these sites and therefore the incidence of tumour metastasis. It is also possible that carbon dioxide gas under pressure dissects between tissue layers at the port site, thereby causing additional trauma and allowing tumour cells to lodge between muscle, fascial and peritoneal surfaces.

The presence of a pneumoperitoneum results in stretching of the abdominal wall. This may augment the release of inflammatory mediators, such as transforming growth factor alpha, which is involved in the healing of wounds, and may also promote tumour proliferation 143,275. Studies in patients suggest that increased intraabdominal insufflation pressures are associated with a number of adverse physiological changes 24,260. Using an intraabdominal pressure of 15 mm Hg

pushes the diaphragm upwards leading to reduction in pulmonary function ²⁴ and increased postoperative pain compared to low pressure insufflation ²⁷⁸. Elevated intraabdominal pressure also results in splanchnic vasoconstriction and reduction in caval, renal, hepatic and portal vein blood flow ^{24,146}. This situation is aggravated by the commonly used reverse-Trendelenburg position ²⁴. *Taura et al* ²⁶⁰ demonstrated in patients undergoing laparoscopic sigmoidectomy that elevations in intraabdominal insufflation pressure with carbon dioxide were associated with an decrease in arterial pH and accumulation of lactic acid. Arterial lactate levels result from the degradation of adenine nucleotides and provide a marker of critical regional hypoperfusion. Whilst this may not be problematic with short laparoscopic procedures, with more difficult procedures such as colon resections, a prolonged pneumoperitoneum and high intraabdominal pressure may predispose the patient to increased metabolic acidosis ²⁶⁰. *Schilling et al* ²³⁷ have also demonstrated that increasing insufflation pressure was associated with pressure dependent reduction in mesenteric arterial blood flow and decreased gastric perfusion as measured by intraoperative laser Doppler probes in patients undergoing laparoscopy. They recommend that laparoscopic procedures should be performed at pressures of 10mm Hg or lower to prevent these microcirculatory changes. Further the authors hypothesise that the significant reduction in abdominal wall perfusion during pneumoperitoneum may be one reason for the development of implantation metastases as a result of localised ischaemia ²³⁷.

It is therefore possible that alterations to pressure and flow rates during laparoscopy could affect a series of complex local and systemic metabolic and immune responses which may in turn facilitate tumour survival and implantation.

3.3.3.1 Discussion of results

The aim of **Study 3.3.2** was to investigate the effect of increased pressure of the insufflation gas on the development of port site metastases. Tumour development was similar in both groups. This suggests that the mechanism of development of port site metastases is not solely related to mechanical effects of gas under pressure and that other factors are probably more important. This supports the finding in section 3.1 suggesting that tumour growth and implantation in the laparoscopic environment is influenced by the metabolic and chemical effects of carbon dioxide on the peritoneal milieu 131,275.

A possible limitation of this study is the 4 mm Hg difference in the intra-abdominal pressures generated between the two groups. Ideally it would have been desirable to test a larger difference such as 2 vs 12 mmHg. However, the difference tested was restricted by the physiological tolerance of the rat to increased intraabdominal pressure 28. Intraabdominal pressures in excess of 6 mmHg prevented adequate respiration by the rats during laparoscopy in preliminary studies in our laboratory, and if used might have resulted in unwanted mortality. This limitation is recognised by other authors utilising small animal models of laparoscopy in that at lower insufflation pressures it is difficult to establish a satisfactory pneumoperitoneum and higher pressures result in respiratory difficulties 49,124.

The issue of increased flow rates was not specifically addressed in **Study 3.3.2**. This is of potential interest and could be examined in future studies. Nevertheless, the higher volume of gas used in the high pressure group, presumably due to increased gas leakage, suggests that the flow rate in this group was also greater. As both groups developed the same number of port site metastases, high flow may not be an important issue.

The results of **Study 3.3.2** suggest that the development of port site metastases following laparoscopic surgery is not determined by the pressure of the insufflation gas. This finding adds weight to the argument that mechanical factors related to the use of positive pressure insufflation are not the sole cause of port site metastases. It is likely that other factors, such as the effects of carbon dioxide insufflation altering the metabolic and immunologic environment, are equally, or more important ¹³¹.

The work of *Jacobi et al* ¹³³ supports the argument that intraabdominal pressure alone is not a mediator of tumour implantation and demonstrated that *in vivo* intraperitoneal tumour growth is stimulated by carbon dioxide independently of the intraperitoneal pressure. In addition *Jacobi et al* ¹³³ report that mean subcutaneous tumour growth was promoted after laparoscopy with carbon dioxide and was independent of the pressure used.

In contrast however, raised intraperitoneal pressure led to suppression of *in vitro* tumour growth. In this study ¹³³ tumour growth of the DHD/K12/TRb adenocarcinoma cells *in vitro* was significantly decreased after incubation with carbon dioxide at 10 and 15mm Hg compared with a pressure of 0 or 5 mmHg. This effect may be due to a direct pressure-damage related effect on the tumour cells. In contrast, *in vivo* intraperitoneal tumour weight was significantly increased after both laparoscopy at 5mm Hg and 10 mmHg compared to a pressure of 0mmHg . Interestingly, the intraperitoneal tumour weight decreased again after laparoscopy with an intraperitoneal pressure of 15 mmHg compared with control groups. The authors propose that this may possibly be due to alterations in immunosuppression or due to a direct tumour cell damaging effect as observed in the *in vitro* experiments.

Contrary to the findings of *Jacobi et al* 133 and in **Study 3.3.2** above, *Canis et al* 49 reported that increased insufflation pressure may be a possible cause of peritoneal tumour cell dissemination. In their study utilising an ovarian tumour cell line and a rat model of laparoscopy, the peritoneal tumour implantation score was significantly higher in rats undergoing laparoscopy utilising carbon dioxide with a pressure of 10 mm Hg than in a group where an insufflation pressure of 4 mm Hg was used.

The discrepancies between these experimental findings may be a reflection of the different insufflation pressures used. Equally, given the finding of *Jacobi et al* 133 there may be different susceptibilities of tumour cell lines to the effects of pressure.

3.3.3.2. **Conclusions**

These findings support the concept that mechanical factors are not the sole contributors to the development of port site metastases. Further studies need to be done to assess the role of increased insufflation pressure on intraperitoneal immunity. Due the limitation in the pressure differences able to be generated in the DA rat model, an alternate model would be required. It would appear however, that at pressures lower than 10mmHg, adverse effects on both physiology and tumour implantation are limited and that other mechanisms are more important in the generation of port site metastases.

3.4. **EXTENDED SURVIVAL STUDIES**

3.4.1. **OVERVIEW**

It has been argued that port site metastases may reflect tumour stage. In this case it would be expected that extended survival of rats with implanted DAMA tumours would be associated with an increase in the incidence of port site tumours. However, clinical case reports indicate that port site tumours can also occur with early, potentially curable disease 55,85,163,217.

To investigate this further the following study was performed:

Investigation of tumour resection and extended survival on the development of port site tumours.

3.4.2. INVESTIGATION OF TUMOUR RESECTION AND EXTENDED SURVIVAL ON THE DEVELOPMENT OF PORT SITE TUMOURS.

3.4.2.1. AIM

The aim of this study was to assess the influence of tumour resection and extended survival on the incidence of port site tumours.

3.4.2.2. METHODS

Flank tumours were implanted into 24 male DA rats with tumour cell suspension as per section 2.2.6 .

Rats were randomly allocated to one of the following groups (6 rats per group):

1. control (sham port sites)

In this group rats underwent anaesthesia for 40min and sham port sites without laceration of the primary tumour.

2. laparotomy

In this group a 3cm midline laparotomy incision was made. The tumour was lacerated in a standardised manner, using an 18G needle. The abdomen remained open for 40 minutes and was then closed in two layers with sutures.

3. laparoscopy with carbon dioxide

In this group pneumoperitoneum was established using the technique described at section 2.3.2. The tumour capsule and overlying peritoneum was lacerated in the standardised fashion (section 2.3.3.1) under laparoscopic vision. Gas continued to be insufflated for 40

minutes after tumour laceration, with a constant gas flow maintained through the venting cannula.

4. *gasless laparoscopy*

In the gasless group, pneumoperitoneum was established by suspension of the rats' anterior abdominal wall from a metal frame with sutures as described at section 2.3.4. No gas was insufflated in this group.

3.4.2.2.1 *Tumour resection*

In all groups the primary tumour was resected 30 minutes after laceration of the tumour. This was performed via a conventional elliptical 2cm long incision over the tumour in the left flank. Tumour was dissected free from the underlying muscle and a 2mm gross clearance in the subcutaneous layers was obtained. The wound was then closed with a single layer of interrupted sutures. The resected tumour specimen was weighed.

Fourteen days after the operative procedure the animals were killed and underwent autopsy as per section 2.4. Bilateral axillary and groin node groups were harvested from rats, examined histologically to determine the presence / absence of tumour and the presence / absence of reactive change. Serial sections of liver and lung (2.0mm slices) were examined for the presence of macroscopic or palpable tumour metastases. Animals were weighed preoperatively and at the time of autopsy.

3.4.2.3. RESULTS

Animals were of similar weight preoperatively and at 14 days ($p=0.13$, Kruskal-Wallis test; Table 3.4.2.3.2). The weight of resected tumour in each group was comparable ($p=0.11$, Kruskal-Wallis test; Table 3.4.2.3.1)

TABLE 3.4.2.3.1.**RESECTED TUMOUR WEIGHTS**

(all figures are median and range - in grams)

	control (sham)	laparotomy	CO2	gasless
weight	10.5(3-24)	4(2-7.5)	5(1.5-11.5)	3(1-5)

p=0.22

TABLE 3.4.2.3.2**RAT WEIGHT FOLLOWING LAPAROSCOPY**

(in grams- all figures are median and range)

	control (sham)	laparotomy	CO2	gasless
Weight at surgery	294 (253-338)	300.5 (267-320)	316.5 (263-353)	289 (261.5-338)
Weight at day 14	302.5 (242-353.5)	315.5 (281-331)	325 (290.5-365.5)	311 (287.5-362)
%increase in weight (*median)	7.7 (-7.6-12.6)	4.5 (0.63-8.2)	12.1 (-14.9-22.7)	0.5 (-4.9-4.5)

p= 0.13

Tables 3.4.2.3.3 and 3.4.2.3.4 summarise the development tumours at wound sites.

Tumour implantation into wounds occurred in all groups. One rat in the control group developed tumour at the sham 'venting' port. Five of six rats in the laparotomy group developed tumour in the midline wound. Port site tumours developed in three of six rats in both laparoscopy groups.

TABLE 3.4.2.3.3**NUMBER OF RATS WITH METASTASES IN TROCAR WOUNDS**

(6 rats per group)

	control (sham)	laparotomy	CO2	gasless
Microscopic				
tumour	1	5	3	3

TABLE 3.4.2.3.4**NUMBER OF WOUNDS WITH METASTATIC TUMOUR PRESENT**

(18 wounds per group- excluding laparotomy group)

	control (sham)	laparotomy	CO2	gasless
Laparoscope port	-	N/A	2	2
Venting port	1	N/A	1	1
Lacerating port	-	N/A	3	2
TOTAL	1	5/6	6	5

No spread to liver or lung was evident in any group. Multiple peritoneal tumour nodules were observed in rats in all treatment groups but not in controls. Tumour seeding was most dramatic in the laparotomy group. Three rats in this group developed malignant ascites and tumour nodules were also observed on omentum, bowel and renal surfaces in this group.

FIGURE 3.4.2.3.1
LYMPH NODE GROUPS

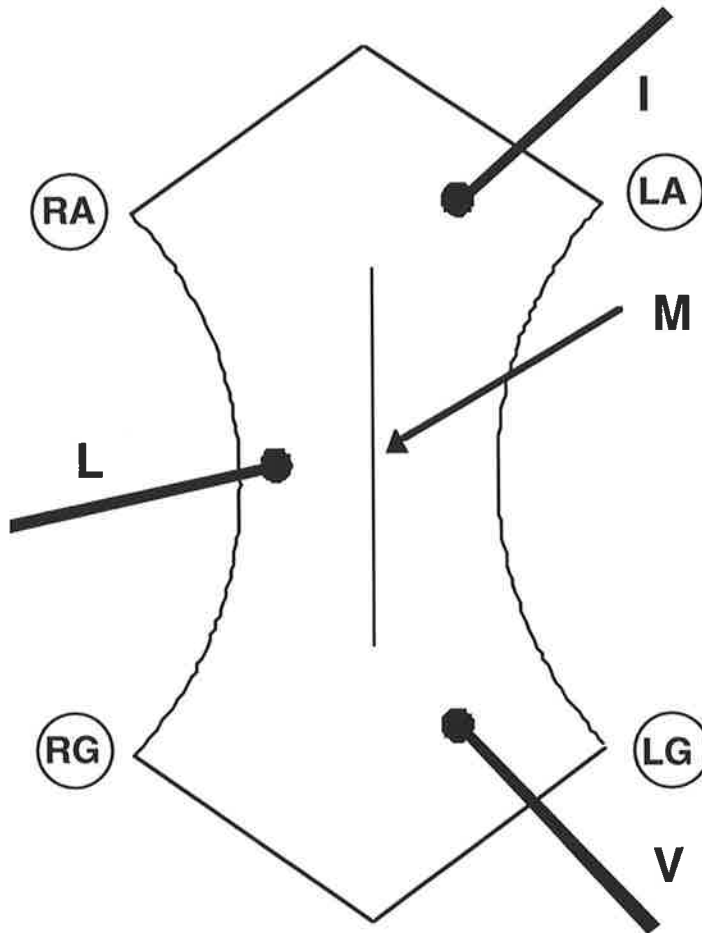


FIGURE LEGEND

I - Injection cannula	RA - Right axillary lymph node group
V - Venting cannula	LA - Left axillary lymph node group
L - Laparoscopic cannula	RG - Right groin lymph node group
M - Midline incision	LG - Left groin lymph node group

Tumour involvement of draining lymph node groups is summarised in table 3.4.2.3.5. Reactive changes were observed in all lymph node groups. There was no evidence of tumour involvement in any nodes of the right side in any of the study groups.

TABLE 3.4.2.3.5

NUMBER OF LYMPH NODE GROUPS WITH METASTATIC TUMOUR PRESENT

	control	laparotomy	CO2	gasless
LA	3	2	4	4
LG	2	1	3	2
RA	-	-	-	-
RG	-	-	-	-

3.4.3. DISCUSSION

Although the development of tumour implantation in wounds following abdominal resection of cancer is considered to be a grave prognostic indicator^{121,157,281}, the effect of port site metastases on survival is not known. Clinical port site tumours have occurred more commonly in tumours considered to be biologically 'aggressive' such as gallbladder carcinoma, and may therefore represent an altered presentation of advanced disease, rather than affect the survival outcome²⁸⁸. It is possible that rather than adversely affecting patient outcome *per se*, port site tumours act merely as a prognostic marker of the biological aggressiveness of the tumour¹⁵⁷. This is also consistent with experimental study reported by *Lee et al*¹⁶⁷ who demonstrated in a rat splenic tumour model that the incidence of liver metastases was the same in both laparoscopic and open groups and also in rats with and without port site metastases and that there was no survival difference or difference in tumour stage between these groups.

The aim **Study 3.4.2** was to investigate the effect of extended survival on tumour metastasis and involvement of regional and distant lymph nodes. Flank tumours were resected in all rats at initial surgery. This simulated the removal of the primary tumour at laparoscopic resection. Survival of animals with the tumour still present to 14 days would have been ethically precluded due to the relative tumour size and tumour burden on the animal. Excision of the primary tumour at the time of surgery was not intended to be a curative resection as this would have required major surgery and flap rotation that was not viable in this rat model. Initially it was proposed to have 12 rats in each study group. However, numbers were restricted to 6 in each group for ethical reasons as rats developed substantial tumour recurrence at their excision site. In addition, rats in the laparotomy group, whilst still gaining weight, appeared unwell due to their burden of disseminated disease.

In this study the development of port site tumours in the carbon dioxide and gasless laparoscopy groups was similar. This is not consistent with previous work which suggests a beneficial effect of gasless laparoscopy ^{42,44,182,185,282}. However the finding in this study may be a consequence of the small numbers in each group. Alternatively it may reflect late disease. Each of the rats in the gasless group that developed port site tumours also had diffuse peritoneal tumour seeding.

The rate of growth of tumour metastases is dependent on the tumour cell line and degree of differentiation. In many clinical cases the interval between laparoscopy and tumour recurrence has been sufficiently short to suggest that either a large tumour inoculum, consistent with disruption of the tumour whilst extracting it, has been deposited at the port site ⁷³ or that the tumour cell line has an inherently high metastatic potential. Similarly it has been demonstrated that the rate of growth of metastases varies depending on the site of implantation and that some cancers may progress at a faster rate in the peritoneal cavity than elsewhere ¹¹¹.

O'Rourke et al ²⁰⁸ cite two cases of aggressive port site recurrences occurring soon after laparoscopy. In one patient a gallbladder recurrence developed at a port site two weeks post laparoscopic cholecystectomy, two recurrences at three weeks and a third port site tumour by 3 months. A second patient developed two port site tumours at 10 weeks, suggesting that port site tumours are rapid and aggressive. This is supported by *Wade et al* ²⁷⁷ who reported a case of port site tumour developing after laparoscopic cholecystectomy for gallbladder at 21 days. The authors postulated that the umbilical recurrence was a single cell implantation at the time of laparoscopy. It is unclear whether this high doubling time represents that of the primary tumour or if the metastases are somehow 'enhanced' by an effect of laparoscopy. In many animal models, primary tumour removal produces increased proliferation of cells in metastatic foci ^{67,150}. This acceleration of tumour cell kinetics is attributed to an

increase in the number of non-cycling cells becoming proliferative during the first 3 to 4 days following removal of the primary tumour ⁸⁶.

In **Study 3.4.2** one port site tumour developed in a sham port in the control (anaesthesia alone) group. This site was closest to the tumour. The most likely explanation is direct spread of tumour although haematogenous spread cannot be excluded. Direct introduction of tumour cells intravenously has been experimentally associated with tumour localisation at the site of wounds ^{196,245}.

Spread to regional draining lymph nodes was comparable in all groups. Tumour to the lymph nodes was most likely via contiguous spread or seeded haematogeneously at the time surgery. The absence of spread to lymph nodes on the right side or to liver or lung in any of the rats, including those with disseminated peritoneal disease, supports previous observations that the DAMA cell line is tumour line that spreads to distant organs late in its growth and that rats implanted with DAMA tumours die preferentially from aggressive local disease.

3.4.3.1 **Conclusions**

The implications of port site tumours on survival is unknown. Many of the case reports have involved gall bladder adenocarcinoma which carries a poor prognosis from diagnosis. It would appear from some reports that complete excision of the port site tumour may not adversely affect outcome, provided there is no other evidence of disseminated disease ²³⁶. This requires further investigation. The results of the current study, utilising the DAMA tumour line, suggest that rats would have died as a result of aggressive local disease rather than widespread tumour metastases.

SECTION IV

**STRATEGIES TO REDUCE TUMOUR
IMPLANTATION AND PORT SITE TUMOURS
FOLLOWING LAPAROSCOPY**

4.1. **THE EFFECT OF CYTOTOXIC AGENTS ON TUMOUR IMPLANTATION AND METASTASES FOLLOWING LAPAROSCOPY**

4.1.1. **OVERVIEW**

Irrespective of whether wound contamination with tumour cells occurs due to direct contamination from laparoscopic instruments which have been in contact with malignant tissue, or due to indirect contamination from tumour cells aerosolised in the insufflation gas 6,184,184,265,282,293, it is possible that the application of topical intraperitoneal cytotoxic agents, or the use of parenteral agents as adjuvant therapy, may prevent metastasis by killing tumour cells which have been liberated by laparoscopic manipulation 136,181,201,255. This would then overcome one of the principal impediments to laparoscopic cancer surgery 55,181,184,201,290,294. For this reason some authors have suggested that the use of intraperitoneal cytotoxic agents, or parenteral agents as adjuvant therapy, may prevent tumour implantation by killing tumour cells which have been liberated by laparoscopic manipulation 136,181,201,255.

To investigate this possibility further, the following studies were performed:

1. Investigation of the *in vitro* growth of the DAMA tumour cells in a culture with cytotoxic agents.
2. Investigation of the use of intraperitoneal cytotoxics on port site metastases using the solid tumour laparoscopic model.
3. Investigation of the use of intraperitoneal cytotoxics on tumour implantation using the tumour cell suspension model.

4.1.2. EFFECTS OF CYTOTOXIC AGENTS ON IN VITRO TUMOUR GROWTH

4.1.2.1. AIM

This study was performed to compare the growth of cultured DAMA tumour cells following exposure to culture in media to which cytotoxics were added.

4.1.2.2. METHODS

For this study the DAMA cell suspension (prepared as per section 2.2.5). The suspension was exposed to a simulated laparoscopic environment as described at section 3.1.2. Carbon dioxide was used as the insufflation gas and this environment was maintained for 40 minutes.

4.1.2.2.1. Cell culture studies

Following the 40 minute exposure period, the cells were reassessed for cell viability, resuspended in the growth media, the concentrations were adjusted to 3×10^5 cells per ml, and then plated into a 96 well plate as described at 3.1.2.2.1. Plates were previously prepared with the addition of 50µl of cytotoxic agent to each of the inner wells. The outer wells were filled with PBS to prevent any edge effects which might occur due to dehydration or evaporation. The following agents were used (12 wells in each group).

- 1. control**
- 2. 0.9% 'normal' saline**
- 3. povidone-iodine 1 in 10 dilution with 0.9% Saline**
- 4. povidone-iodine 1 in 50 dilution with 0.9% Saline**
- 5. methotrexate 0.25 mg**
- 6. methotrexate 0.125 mg**
- 7. aqueous chlorhexidine**

Cells were then cultured for 18 hours. Optical density readings were used to assess the number of viable tumour cells at the end of this time period as per 3.1.2.2.1. This study was repeated to ensure consistency of the results.

4.1.2.3. **RESULTS**

The results of the cell culture experiments are summarised in Table 4.1.2.3.1 and Figures 4.1.2.3.1 and 4.1.2.3.2. Cell growth was demonstrated in all culture wells, with growth greatest in the control group in each of the three experiments. Cell growth was significantly less following incubation with 1/10 strength povidone iodine (Table 4.1.2.3.1; $p < 0.01$, Kruskal-Wallis test, Dunn's post test comparisons between pairs). This effect was not observed when povidone iodine was used in greater dilution. Chlorhexidine was also cytotoxic *in vitro*. Methotrexate had no effect on tumour cell growth following incubation with carbon dioxide.

TABLE 4.1.2.3.1**OPTICAL DENSITY READINGS (MEASUREMENT OF CELL GROWTH)**

(all measurements are median and range)

	<i>Experiment 1</i>	<i>Experiment 2</i>
control	1.852 (1.7-2.19)	1.283 (1.117-1.423)
betadine 1/10	0.654 (0.525-0.697)	0.451 (0.436-0.489)
betadine 1/50	0.994 (0.818-1.06)	0.821 (0.737-0.925)
MTX 0.25mg	1.923 (1.87-2.16)	0.792 (0.678-0.930)
MTX 0.125 mg	1.969 (1.81-2.02)	0.675 (0.643-0.831)
saline	2.016 (1.68-2.18)	
chlorhexidine	0.556 (0.448-0.658)	

EXPERIMENT 1

control vs betadine 1/10 *p<0.01

control vs betadine 1/50 p>0.05

control vs MTX 0.25 mg p>0.05

control vs MTX 0.125 mg p>0.05

control vs saline p>0.05

control vs chlorhexidine *p<0.001

EXPERIMENT 2

p<0.01

p>0.05

p>0.05

p>0.05

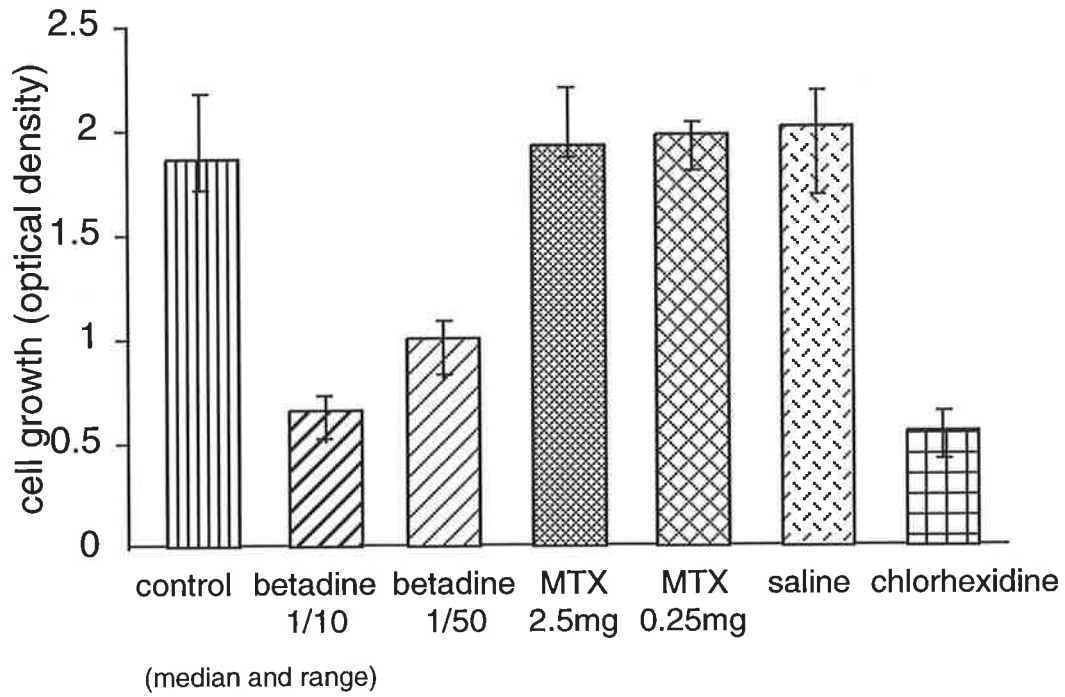
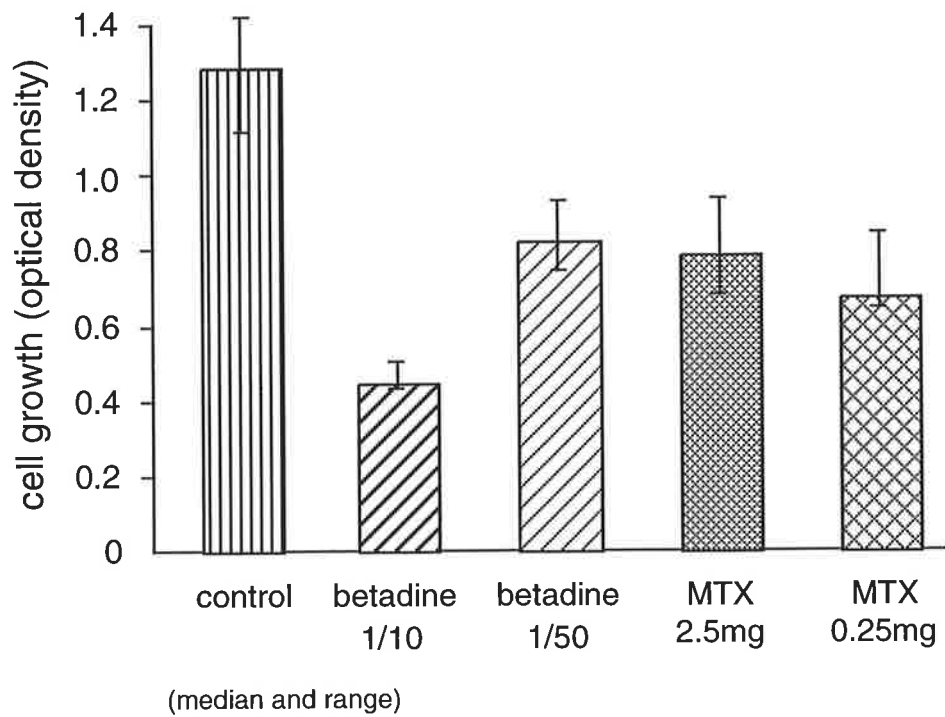
FIGURE 4.1.2.3.1**CELL GROWTH IN VARIOUS CYTOTOXIC MEDIA - EXPERIMENT I**

FIGURE 4.1.2.3.2

CELL GROWTH IN VARIOUS CYTOTOXIC MEDIA - EXPERIMENT II



4.1.3. EFFICACY OF CYTOTOXIC AGENTS FOR THE PREVENTION OF LAPAROSCOPIC PORT SITE METASTASES USING THE SOLID TUMOUR MODEL

This experiment has been published as: Neuhaus SJ, Watson DI, Ellis TS, Dodd T, Rofe AM and Jamieson GG. *Efficacy of cytotoxic agents for the prevention of laparoscopic port site metastases using the solid tumour model.* Arch Surg 1998;**133**:762-766

4.1.3.1. AIM

This study was performed to investigate the effect of intraperitoneal or parenteral cytotoxic agents on the incidence of port site metastases following laparoscopic surgery.

4.1.3.2. METHODS

Seventy two male DA rats were implanted with left flank tumours as described at section 2.2.6. Seven days after tumour implantation the rats underwent laparoscopic laceration of an implanted tumour with carbon dioxide insufflation (as per section 2.3.2) and instillation of an intraperitoneal agent.

Rats were randomised to one of the following study groups (12 rats per group):

1. Controls (non intraperitoneal instillation)

Control rats underwent laparoscopy and laceration of the tumour without instillation of an intraperitoneal agent.

2. Intraperitoneal saline (laparoscopy control group)

Three mls of 0.9% saline was introduced into the peritoneal cavity .

3. Intraperitoneal Betadine

Three mls of povidone iodine solution (Betadine), diluted 1:10 with normal saline, was introduced into the peritoneal cavity.

4. Intraperitoneal Methotrexate

Methotrexate was introduced into the peritoneal cavity at a dosage of 0.5 mg/kg body weight in a solution of 4 mls of 0.15M NaCl.

5. Intraperitoneal Chlorhexidine

Three mls of aqueous Chlorhexidine undiluted was introduced into the peritoneal cavity.

6. Intramuscular Methotrexate

Methotrexate (0.5 mg/kg body weight) was administered by intramuscular injection 30 minutes before conventional carbon dioxide insufflation commenced. Intraperitoneal agents were not used in this group.

Earlier work using the tumour cell line had confirmed the *in vivo* sensitivity of the tumour cells to methotrexate with complete inhibition of *in vivo* tumour growth following a single injection of methotrexate (1.25mg/kg body weight) 230.

Various solutions were introduced into the peritoneal cavity through the 16 gauge cannula immediately after laceration of the tumour. These solutions remained within the peritoneal cavity during the entire period of laparoscopic exposure and following surgery. No attempt was made to aspirate them from the peritoneal cavity.

Seven days after the operative procedure the animals were killed, and rats underwent autopsy as per section 2.4. The presence or absence of intraperitoneal adhesions was also noted, and the rats were examined for tumour involvement of the axillary and femoral lymph node groups.

4.1.3.3. RESULTS

The measured size of the primary tumour at the time of surgery was similar in all study groups (Table 4.1.3.3.1, $p=0.63$ Kruskal-Wallis test), and the growth of the implanted tumour following surgery was similar in all study groups ($P=0.42$; Table 4.1.3.3.1;Kruskal-Wallis test).

TABLE 4.1.3.3.1.

TUMOUR GROWTH FOLLOWING LAPAROSCOPY

(maximum dimension in mm - all figures are median and range)

	control	saline	betadine	chlorhex*	IP MTX†	imMTX#
size at	34.5	30	37	42	30	30
surgery	(15-47)	(15-41)	(24-48)	(30-57)	(21-40)	(20-46)
size at	55	48	58	59	51	50
day 7	(45-74)	(12-69)	(52-72)	(48-81)	(41-62)	(40-60)
%increase	63	76	58	52	81	65
in size*	(30-200)	(-20-87.5)	(43-125)	(10-127)	(27.5-108)	(30-100)

* $p=0.42$

LEGEND

chlorhex* = aqueous chlorhexidine acetate

IP MTX†= intraperitoneal methotrexate

imMTX# = intramuscular methotrexate

In addition, the body weight of the rats was similar in all groups preoperatively. Rats in all groups gained a similar percentage weight when compared to controls (Table 4.1.3.3.2, $p=0.05$ Fisher's exact test). A statistical difference was observed between weight gain in the saline and betadine groups (Dunn's multiple comparisons- % change in weight control vs other groups $p >0.05$; saline vs betadine $p <0.05$).

TABLE 4.1.3.3.2.

RAT WEIGHT POSTOPERATIVELY

(median and range in grams)

	control	saline	betadine	chlorhex*	IP MTX†	imMTX#
wt at	225	244	220	225	253	239
surgery	(196-254)	(222-287)	(203-255)	(178-290)	(225-283)	(215-276)
weight at	264	244	267	257	273	265
day 7	(226-280)	(166-330)	(225-288)	(181-321)	(245-328)	(233-305)
%change	13(-2-23)	7(-26-15)	16(4-31)	16(-7-25)	9(2-16)	9(3-15)

in wt

$p=0.05$

LEGEND

chlorhex* = aqueous chlorhexidine acetate

IP MTX† = intraperitoneal methotrexate

imMTX# = intramuscular methotrexate

At autopsy tumour was visible as a nodule at the site of intraperitoneal tumour laceration. There was however, no evidence of tumour dissemination to any site other than the surgical wounds. The development of macroscopic and microscopic metastases at the laparoscopy trocar wounds is summarised in Tables 4.1.3.3.3 and 4.1.3.3.4. Tumour involvement of the surgical wounds did not occur following the intraperitoneal administration of betadine (Table 4.1.3.3.2; Fisher's exact test; $p=0.037$). In contrast 41.7%, 33.3%, 33.3%, 16.7% and 41.7% of the rats in the control, saline, chlorhexidine, intraperitoneal methotrexate and parenteral methotrexate groups respectively developed port site metastases (Table 4.1.3.3.2). The results of an analysis of the occurrence of metastases in individual wounds mirrored the findings of a significantly lower risk of wound metastasis following the instillation of intraperitoneal betadine (Table 4.1.3.3.4; $p=0.011$, Fisher's exact test). One rat in the saline group developed bacterial peritonitis.

TABLE 4.1.3.3.3.

NUMBER OF RATS WITH METASTASES IN TROCAR WOUNDS

(12 rats per group)

	control	saline	betadine	chlorhex*	IP MTX	imMTX
Macroscopic	1	1	0	2	2	3
Microscopic*	5	4	0	4	2	5
p value		1.0	0.04	1.0	0.37	1.0

(Fisher's exact test; no. of microscopic metastases compared to control)

LEGEND

chlorhex* = aqueous chlorhexidine acetate

IP MTX† = intraperitoneal methotrexate

imMTX# = intramuscular methotrexate

TABLE 4.1.3.3.4**NUMBER OF WOUNDS WITH METASTATIC TUMOUR PRESENT**

(36 wounds per group)

	control	saline	betadine	chlorhex*	IP MTX†	imMTX#
Macroscopic	1	1	0	3	3	3
Microscopic*	7	4	0	5	3	5
p value		0.51	0.011	0.75	0.31	0.75

(Fisher's exact test, no. of microscopic metastases compared to control)

LEGEND

chlorhex* = aqueous chlorhexidine acetate

IP MTX† = intraperitoneal methotrexate

imMTX# = intramuscular methotrexate

The development of a metastasis to the left axillary lymph nodes occurred in 5 rats from the intraperitoneal methotrexate group and 6 from the parenteral methotrexate group (Table 4.1.3.3.5). No other palpable lymph nodes were noted. This did not occur in either the control group or the betadine group.

TABLE 4.1.3.3.5**PRESENCE OF LYMPHADENOPATHY**

(Left Axillary Palpable Lymphadenopathy)

	control	saline	betadine	chlorhex*	IP MTX†	imMTX#
palpable axillary	0	2	0	3	5	6
lymph nodes						
p value	-	0.48	1.0	0.22	0.037	0.014

(Fisher's exact test; compared to controls)

LEGEND

chlorhex* = aqueous chlorhexidine acetate

IP MTX† = intraperitoneal methotrexate

imMTX# = intramuscular methotrexate

4.1.4. **INFLUENCE OF CYTOTOXIC AGENTS ON INTRAPERITONEAL TUMOUR IMPLANTATION**

This experiment has been published as: Neuhaus SJ, Watson DI, Ellis TS, Rofe AM and Jamieson GG. *Influence of cytotoxic agents on intraperitoneal tumour implantation following laparoscopy.* Dis Colon & Rectum 1999 **42**: 10-15

4.1.4.1. **AIM**

To investigate the cytotoxic agents on tumour dissemination and implantation following laparoscopy. This study utilised the tumour cell suspension model.

4.1.4.2. **METHODS**

Thirty three DA rats underwent laparoscopic insufflation and instillation of a suspension of 2.5×10^5 tumour cells. Rats were randomised to one of the following groups:

1. *Controls (n=9)*

These rats underwent laparoscopy and injection of a tumour cell suspension only, without the instillation of any agent.

2. *Intraperitoneal saline (n=6)*

Three mls of 0.9% saline was introduced into the peritoneal cavity.

3. *Intraperitoneal betadine (n=6)*

Three mls of povidone iodine solution (betadine), diluted 1:10 with normal saline, was introduced into the peritoneal cavity.

4. *Intraperitoneal methotrexate (n=6)*

Methotrexate was introduced into the peritoneal cavity (dosage 0.125 mg/kg body weight in a solution of 3 mls of 0.15M NaCl). These rats received a further dose (0.125 mg/kg body weight) of methotrexate administered by direct intraperitoneal injection 24 hours after laparoscopy.

5. *Intramuscular methotrexate (n=6)*

Methotrexate (0.125 mg/kg body weight) was administered by intramuscular injection 30 minutes before conventional carbon dioxide insufflation commenced. Intraperitoneal agents were not used in this group. Rats received a further dose (0.125 mg/kg body weight) of methotrexate administered intramuscularly 24 hours after laparoscopy.

Pneumoperitoneum was established with carbon dioxide in all rats and maintained for 40 minutes as per section 2.3.2. Various solutions were introduced into the peritoneal cavity through the 16 gauge cannula 30 minutes after instillation of the tumour suspension. These solutions remained within the peritoneal cavity during the remaining 10 minutes of laparoscopic exposure and following surgery. No attempt was made to aspirate them from the peritoneal cavity. The "ports" were then removed, and the puncture sites were closed with sutures.

Seven days later the rats were killed and underwent autopsy as per section 2.4.

4.1.4.3. RESULTS

The preoperative weight of the rats in each group were similar ($p=0.42$; Kruskal-Wallis test). Rats in all "treatment" groups gained less percentage weight compared the control group ($p=0.03$; Table 4.1.4.3.1, Kruskal-Wallis test).

TABLE 4.1.4.3.1**RAT WEIGHT FOLLOWING LAPAROSCOPY**

(measured in grams - all figures are median and range)

	control	saline	pov-iod*	IP MTX †	IM MTX#
Weight at surgery	305 (192-342)	291.5 (200.5-356)	264 (221.5-327)	236 (223-253.5)	234 (203-268)
Weight at day 7	312 (199.5-343)	264 (202-394)	259.5 (227-324)	230 (221-250.5)	237 (204-267.5)
% increase in size	2.3	0.7	-0.9	-1.5	0.2

(median*)

p=0.03

LEGEND

pov-iod* = povidone iodine

IP MTX † = intraperitoneal methotrexate

IM MTX# = intramuscular methotrexate

Tumour implantation is summarised in Tables 4.1.4.3.2 and 4.1.4.3.3. Three rats in the povidone-iodine group and one rat in the intraperitoneal methotrexate group were totally free of tumour. All rats in the other groups developed tumour somewhere within their peritoneal cavity.

A reduction in tumour growth was observed in all treatment groups; povidone-iodine, intraperitoneal and intramuscular methotrexate (Table 4.1.4.3.2; Chi squared, $P < 0.0001$). In the povidone-iodine group 32/36 (89%) of sectors had no visible tumour growth compared to 10/54 (19%) in the control group ($p < 0.0001$; Fisher's

exact test). Both the intraperitoneal (83%, $P < 0.0001$; Fisher's exact test), and intramuscular (61%, $P < 0.0001$; Fisher's exact test) methotrexate groups also had significantly more sectors free of visible tumour than controls. Tumour growth was not significantly reduced in the saline group (27% of sectors tumour free, $p = 0.44$; Fisher's exact test). There was no predilection for tumour growth to occur around the site of introduction of the tumour cell suspension, and tumour distribution was similar in all sectors (Table 4.1.4.3.3).

TABLE 4.1.4.3.2.

PERITONEAL TUMOUR INDEX

(number of sectors involved with each tumour grade)

Grade	control n=54	saline	povidone iodine	IP MTX†	IM MTX#
0 (no tumour)	10	10	32	30	22
I	14	12	3	6	9
II	17	11	1	0	5
III	13	3	0	0	0
total No of sectors with tumour (all grades)	44/54 (81%)	26/36 (72%)	4/36 (11%)	6/36 (17%)	14/36 (39%)

LEGEND

IP MTX † = intraperitoneal methotrexate

IM MTX# = intramuscular methotrexate

TABLE 4.1.4.3.3**SECTOR ANALYSIS OF TUMOUR INVOLVEMENT**

(number of sectors containing macroscopic tumour, regardless of grade)

Sector	control (n=9)	saline (n=6)	povidone iodine (n=6)	IP MTX† high dose (n=6)	IM MTX# high dose (n=6)	Total (n=33)
1	6	6	0	1	1	14
2	8	4	1	1	3	17
3	9	5	1	1	4	20
4	6	3	1	0	1	11
5	8	4	1	3	4	20
6	7	4	0	0	1	14
Total	44/54	26/36	4/36	6/36	14/36	96/198

LEGEND

IP MTX † = intraperitoneal methotrexate

IM MTX# = intramuscular methotrexate

Tumour metastasis to the port sites occurred less frequently in the povidone-iodine, intraperitoneal and intramuscular methotrexate groups (Table 4.1.4.3.4; Chi squared, $P < 0.0001$). Tumour was found in only one port site in the povidone-iodine group (6%: $P < 0.0001$, Fisher's exact test; treatment group vs control). In the intraperitoneal and intramuscular methotrexate groups 17% ($P = 0.0002$) and 39% ($P = 0.002$) of port sites respectively were involved with tumour, compared with 72% ($P = 1.0$) in the saline and 74% in the control groups.

TABLE 4.1.4.3.4**NUMBER OF PORT SITES WITH TUMOUR PRESENT**

control	saline	pov-iod*	IP MTX†	IM MTX#
20/27	13/24	1/24	3/24	7/24
(74%)	(72%)	(6%)	(17%)	(39%)

LEGEND

pov-iod* = povidone iodine

IP MTX † = intraperitoneal methotrexate

IM MTX# = intramuscular methotrexate

4.1.5. DISCUSSION

Previous studies have established that viable cells are liberated into the peritoneal cavity during the resection of malignant tumours ^{123,272} and many authors believe that, in the presence of a pneumoperitoneum, these viable cells can be aerosolised, resulting in implantation in the laparoscopy access wounds ⁴⁴. Alternatively cells may implant in wounds due to direct contamination from laparoscopic instruments ^{91,116,264}.

Tumour cells may be shed as a result of surgical trauma, resulting in the spillage of cells into the operative field, or they could leak indirectly from raw vascular and lymphatic channels. These viable cells may then implant preferentially in raw peritoneal surfaces and in sites of peritoneal abrasion, in particular the surgical access wounds ^{91,135,277}. Three factors are probably related to the presence of free cancer cells in the peritoneal cavity: the degree of cancer infiltration through the muscle wall of the affected organ (stomach, colon etc), the histological type of cancer and the involvement of the serosa ¹²³.

Wade et al ²⁷⁷ have demonstrated that as little as one single viable tumour cell implanting at the port site may result in a metastasis. Studies by *Hansen et al* ¹⁰⁷ and *Tanida et al* ²⁵⁹ have demonstrated that tumour cells harvested from peritoneal fluid can retain the ability to replicate in culture and induce tumours in experimental animals. Exfoliated colon cancer cells obtained from the bowel lumen by colonic lavage at surgery have also been demonstrated to retain their capacity to replicate following implantation into immune deprived mice ²⁵⁸. Recurrence in the port sites is therefore likely to reflect not only the number of free cancer cells but also their viability ¹²³.

Some authors have suggested that as laparoscopic surgery is associated with a reduction in manual dexterity, laparoscopy may result in a greater load of tumour cells shed into the peritoneal cavity at the time of surgery 167,263,291. Studies by *Kim et al* suggest that this is not the case and that the use of laparoscopic techniques *per se* is not associated with an increase in the number of tumour cells in peritoneal washings 153. However historical studies on tumour cell numbers obtained from washings may significantly underestimate the extent of tumour cell shedding 244. New genetic markers and other techniques to detect tumour cells in washes, combined with the unreliability of previous detection methods such as cytology 153, suggest that the incidence of tumour cells in peritoneal fluid is significantly higher than has previously been reported and that

tumour cells may be present in up to 30% of pre resection washings. Following a curative laparoscopic resection, it is anticipated that only a small number of cells remain in the peritoneal cavity and that these tumour cells would therefore be vulnerable to intraperitoneal agents.

Many surgeons routinely use peritoneal lavage at the end of surgical procedures involving resection of malignancy 69,257,273. Cytotoxic agents such as povidone iodine and aqueous chlorhexidine have been commonly used as lavage agents during open surgery for cancer 69. Intraoperative irrigation of 0.9% saline may remove some spilt tumour cells. However, it is not possible to adequately irrigate all peritoneal surfaces 257, resulting in viable tumour cells potentially remaining within the peritoneal cavity. *Allardyce et al* demonstrated using a porcine laparoscopic colectomy model that, whilst preoperative lavage reduced the presence of tumour cells (HeLa cells) at ports by 61 %, contamination of the port sites was not eliminated 8. For this reason, some authors have proposed the use of intraperitoneal chemotherapy as an adjuvant strategy to decrease the incidence of port site metastases following laparoscopic surgery 135,136.

Intraperitoneal chemotherapy has been used clinically for the treatment of gastrointestinal cancers 194,254-256. It has also been demonstrated that there may be a pharmacological advantage to the administration of cytotoxic agents intraperitoneally at the time of surgery 255, and that this may result in a reduction in the risk of peritoneal tumour spread. This advantage is due to direct contact between tumour cells and the tumoricidal agent 136, and due to beneficial changes in cell kinetics 86. The removal of the primary tumour is thought to stimulate the proliferation of non-cycling cells, thereby rendering them more vulnerable to the effects of cytotoxic agents. Also, peritoneal clearance of a drug is slower than plasma clearance, prolonging exposure time. Other advantages of intraperitoneal chemotherapy administered at the time of surgery include an even distribution of the agent throughout the peritoneal cavity before the development of any adhesions, concurrent recovery from both the operation and chemotherapy 179,238 and the possibility of decreased systemic side effects.

Experimental studies also suggest that for maximum efficacy, chemotherapy is most successful in controlling micro-metastases at the time of, or before primary tumour removal 86. Skipper *et al* have demonstrate that the timing of tumour cells being delivered to sites of potential implantation may be important 245. They demonstrated in an *in vivo* model of colonic anastomoses in rats that growth of tumour at the anastomosis or in the wound was maximally enhanced if tumour cells were introduced into the circulation when healing of these wounds was in progress (i.e. between 2 and 5 days). In addition there was a minor peak of enhancement if tumour cells arrived at the colonic anastomosis within 2 hours of its formation. This suggests that there may be a critical 'window' during which cytotoxics would be most beneficial.

Another cytotoxic approach utilised in colorectal surgery is to introduce a cytotoxic agent such as dilute formaldehyde *per rectum* before the resection commences. This strategy destroys potentially viable tumour cells in the bowel lumen and avoids the use of peritoneal cytotoxics ¹⁷⁴. This strategy may be appropriate for early stage/ mucosal disease but is unlikely to deal with serosal disease.

4.1.5.1. *In vitro* studies

The results of **Study 4.1.2** suggest that povidone iodine has a direct cytotoxic effect on cultured DAMA cells. The study also demonstrates that this effect is dose dependent with varying concentrations of povidone-iodine.

Povidone iodine (polyvinylpyrrolidone-iodine) is a combination of molecular iodine and polyvinylpyrrolidone. A 10% solution is the most commonly used and has 1% available iodine. The cytotoxic effect of povidone iodine is related to delivery of free iodine directly to the cell surface and its killing action takes place in a matter of seconds. Povidone-iodine has been demonstrated to be significantly more cytotoxic to breast cancer cells *in vitro* ²¹² when compared to bleomycin, hydrogen peroxide and water. The efficacy of povidone-iodine in killing colon cancer cells in a rat model has been demonstrated by *Docherty et al* ⁶⁹. In this study the use of intraluminal povidone-iodine significantly reduced the incidence of tumour recurrence whilst chlorhexidine-cetrimide had no effect.

Of particular interest methotrexate did not result in decreased tumour growth in this study. This was not an expected finding as previous work with this tumour line *in vivo* has demonstrated the efficacy of methotrexate in producing tumour regression ²³⁰. This finding may represent the variability between *in vitro* and *in vivo* effects. Alternatively it may be related to the reduced effect of antifolate cytotoxics when administered as a single dose. Similarly, chlorhexidine produced *in vitro* reduction

in tumour cell growth which was not reproduced in the *in vivo* studies . This is interesting because *Docherty et al* ⁶⁹ found both povidone iodine and chlorhexidine-cetrimide worked *in vitro* on a breast cancer cell line but only povidone-iodine worked *in vivo*. However, regardless of this it is the *in vivo* rather than the *in vitro* results which are most clinically relevant.

4.1.5.2. *In vivo* studies - solid tumour model

The results of **Study 4.1.3** demonstrated a significant reduction in the incidence of laparoscopy associated wound metastases following the use of intraperitoneal povidone-iodine. This suggests that the tumoricidal effects of povidone-iodine, may reduce the number of viable tumour cells in the peritoneal cavity, and prevent the implantation of viable cells into port site wounds. This effect was not seen with any of the other agents tested in this study. There was no reduction in the incidence of port site metastases following the administration of either intraperitoneal or parenteral methotrexate. This was also unexpected as methotrexate was chosen for our study because previous work has confirmed that this tumour line is sensitive to methotrexate, and that tumour progression can be halted with a dose of 1mg/kg ²³⁰. It is possible that the dose used was not adequate to produce effective cell killing or that it was ineffective as a single dose. Antimetabolite substances, such as methotrexate may require administration of more than one dose to affect cells not in the active cell cycle. Alternatively, the dose given suppressed the rats' systemic immunity system, thus negating any beneficial effects. The later explanation seems likely as spread of tumour to lymph nodes was more common following methotrexate administration. Another possible factor is that folate/ leucovorin rescue was not used in this study. The addition of this may have improved both the efficacy of the methotrexate and the animals nutritional/ immune state ²⁶⁶.

In this study the tumour size was unaffected by the agent used. This suggests that there has been no, or minimal, systemic effect of the agent used. The percentage gain in rat weight was also unaffected by the agent used. This also suggests that there was no significant systemic toxicity effect of the agents on the rat. Left axillary lymph nodes were noted to be palpable in significantly more rats in the methotrexate groups, suggesting that there may be a suppression of the rats' systemic immunity with this agent. The left axillary lymph node is the most proximal to the primary tumour site and is probably involved as the initial draining lymph group.

It is likely that the correct dose of an intraperitoneal agent is critical to the success or failure of this modality of treatment. During initial pilot studies, inadvertent administration of a higher dose of intraperitoneal methotrexate resulted in one rat developing increased tumour growth and spread, including malignant ascites. Similarly, variation in the concentration of povidone-iodine administered was required in pilot studies to establish a concentration which was not toxic to the rats. Maintaining the correct dose of a cytotoxic agent within the 'therapeutic window' is also essential to prevent immune depression which may not only enhance tumour implantation but also increase infection and post operative sepsis.

4.1.5.3. *In vivo* studies - tumour implantation model

Whilst in the solid tumour model (**Study 4.1.3**), intraperitoneal or intramuscular methotrexate, administered as a single dose was not associated with a reduction in port site metastases, the results of **Study 4.1.4** demonstrate a significant reduction in both peritoneal tumour implantation and port site metastases following the use of an appropriate dose of intraperitoneal or systemic methotrexate repeated at 24 hours postoperatively. In addition, the study also demonstrates a reduction in peritoneal tumour implantation and the incidence of port site metastases following administration of intraperitoneal povidone iodine.

Importantly, this was achieved even though the agents used were administered 30 minutes after introducing tumour cells into the peritoneal cavity. This suggests that the tumoricidal effects of these agents in appropriate dose, may reduce the number of viable tumour cells in the peritoneal cavity, and prevent the implantation of viable cells, and that this strategy may be applicable even when unexpected malignancy is encountered at laparoscopic surgery.

Interestingly the use of Intramuscular methotrexate was associated with a significant reduction in tumour implantation and port site tumours compared to control groups, the incidence of tumour implantation and port site tumours in this group was double that observed in the intraperitoneal methotrexate group, suggesting an advantage to the intraperitoneal approach. This contrasts with recent work published by *Iwakana et al* 126. Their study using murine model of laparoscopic tumour biopsy of a chemotherapy sensitive neuroblastoma demonstrated a reduction in port site tumours following administration of intravenous or intraperitoneal cyclophosphamide. In this study there was no difference in the incidence of tumour implantation with either route of administration. This may be a reflection of a different tumour line or of a difference in the numbers of tumour cells liberated into the peritoneal cavity at the time of experimentation.

The apparent discrepancy in the effect of methotrexate in the **Study 4.1.3 and 4.1.4** is most likely due to the administration of a second dose of agent was administered at 24 hours in **Study 4.1.4**. Alternatively, it is possible that a previously implanted and well established solid tumour might influence tumour implantation and growth in abdominal wall wounds, thereby modulating the response of methotrexate seen in the different models. Nevertheless, the clinical implications of these findings are that if a prophylactic chemotherapy regime is to be used for the prevention of port site

metastases, then it may be necessary for both an appropriate operative, as well as additional postoperative, dose of the chemotherapeutic agent to be given. This potentially complicates its clinical application.

4.1.5.4. **Conclusions**

These studies suggest that the incidence of port site metastases and tumour implantation following laparoscopic surgery can be reduced by the intraperitoneal administration of dilute povidone iodine or the use of a suitable intraperitoneal or systemic cytotoxic agent. The correct dose of the intraperitoneal chemotherapeutic is likely to be particularly important. Further clinical investigation of the efficacy of intraperitoneal povidone iodine and intraperitoneal cytotoxics following laparoscopy for malignancy is indicated.

4.2. THE EFFECT OF INTRAPERITONEAL BLOOD AND ANTICOAGULANTS ON TUMOUR IMPLANTATION FOLLOWING LAPAROSCOPY

4.2.1. OVERVIEW

The use of intraperitoneal heparin to reduce tumour cell adhesion and consequent implantation has been proposed as a preventive strategy for port site metastases 101,130. *Goldstein et al* 101 has proposed that prophylactic irrigation of the peritoneal cavity with substances that decrease cell adherence might also prevent tumour implantation.

To further evaluate the preventive possibilities of heparin, the following studies were performed:

1. Investigation of the growth of DAMA cells in a culture with heparin
2. Investigation of the effect of intraperitoneal heparin on tumour implantation using the tumour cell suspension model.

4.2.2. IN VITRO EFFECTS OF HEPARIN ON DAMA CELLS

4.2.2.1. AIM

In vitro cell culture studies were performed to determine whether heparin had any direct cytotoxic effect on the tumour cell line used so that any differences in the *in vivo* studies would be better understood.

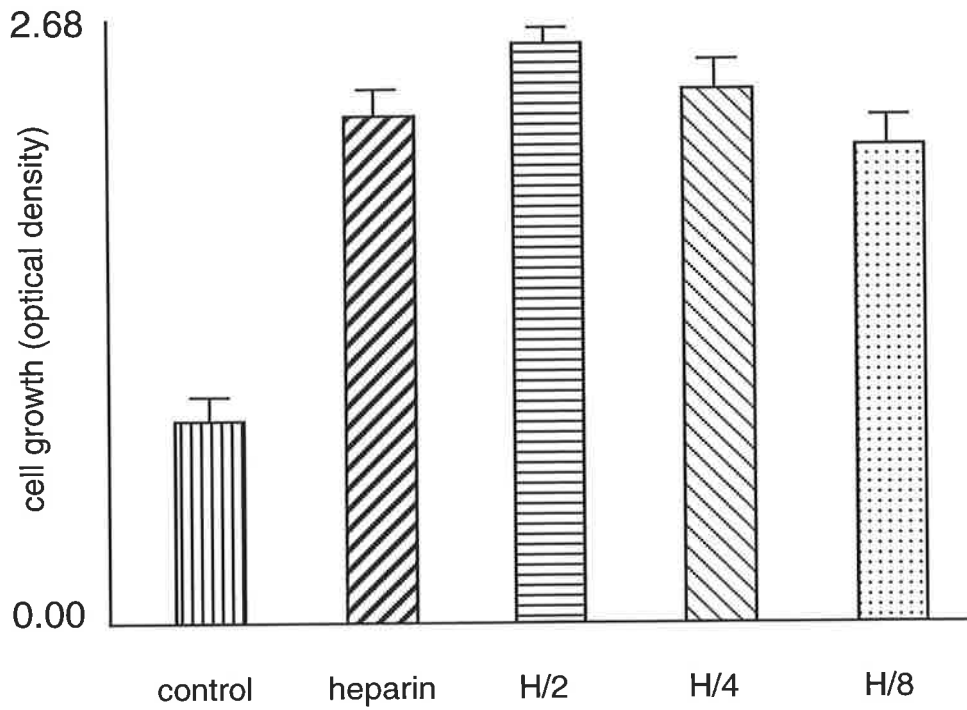
4.2.2.3. METHODS

The DAMA cell suspension was prepared as per section 2.2.5. The inner 6x6 wells of a 96 well culture plate were prepared with 100 µl of serially diluted heparin (heparin sodium, porcine mucous; David Bull laboratories) at strengths of 1000U/ml, 500U/ml, 250U/ml and 125U/ml. There were twelve control wells on each plate to which heparin was not added. The tumour cell suspension was then resuspended in the growth media and the concentration adjusted to 3×10^5 cells per ml. A single channel pipette was used to place 100 µl of cells (i.e. 3×10^4 cells) in each of the inner wells. The outer row of wells contained PBS to prevent any edge effects which might occur due to dehydration or evaporation.

Cells were then cultured for 18 hours as per section 3.1.2.2.1. This study was performed twice to ensure consistency of the results.

4.2.2.3. RESULTS

Figures 4.2.2.3.1 and 4.2.2.3.2 summarise the results of the two cell culture studies. Cell growth occurred in all culture wells. However, growth was significantly lower in the control well (no heparin) compared to the heparin culture wells of all dilutions ($p < 0.001$: Figs 4.2.2.3.1 and 4.2.2.3.2, Kruskal-Wallis test). Within the heparin group cell growth was not influenced by varying the dilutions.

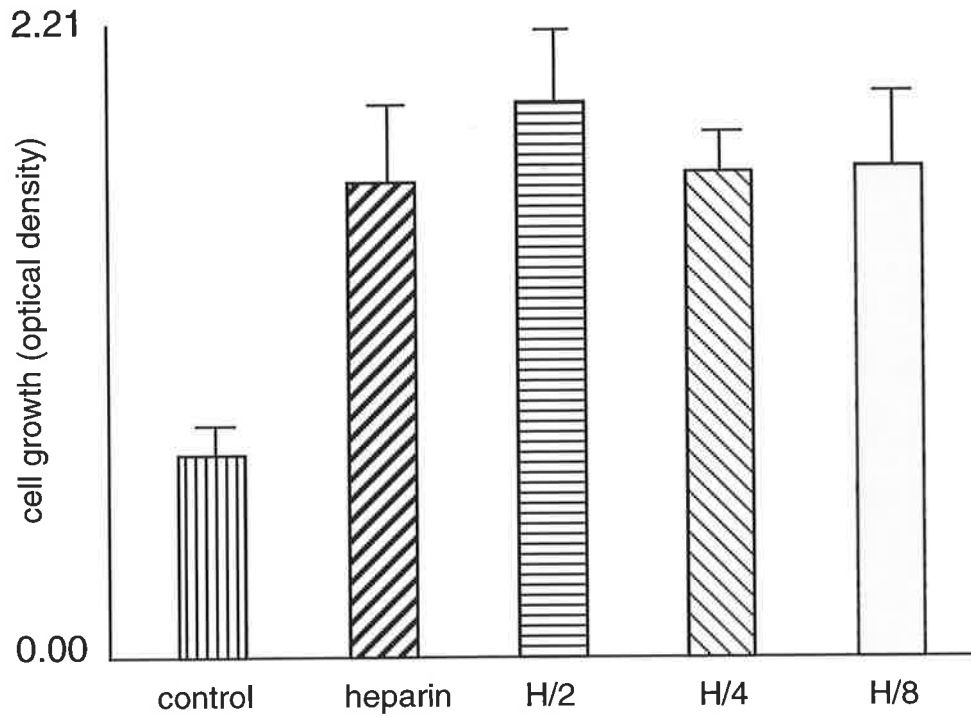
FIGURE 4.2.2.3.1***IN VITRO* CELL STUDY 1: CELL GROWTH AS DETERMINED BY OPTICAL DENSITY**

heparin = heparin sodium 1000 units/ml

H/2 = heparin sodium 500 units/ml

H/4 = heparin sodium 250 units/ml

H/8 = heparin sodium 125 units/ml)

FIGURE 4.2.2.3.2.***IN VITRO* CELL STUDY 1: CELL GROWTH AS DETERMINED BY OPTICAL DENSITY**

heparin = heparin sodium 1000 units/ml

H/2 = heparin sodium 500 units/ml

H/4 = heparin sodium 250 units/ml

H/8 = heparin sodium 125 units/ml)

4.2.3. **THE EFFECT OF INTRAPERITONEAL HEPARIN ON TUMOUR IMPLANTATION FOLLOWING LAPAROSCOPY**

This experiment has been published as: Neuhaus SJ, Ellis TS, Jamieson GG and Watson DI. *An experimental study of intraperitoneal heparin on tumour implantation following laparoscopy* Br J. Surg 1999, **86**, 400-404

4.2.3.1. **AIM**

The aim of this study was to investigate the effect of intraperitoneal blood and heparin on the incidence of tumour cell implantation and port site metastases.

4.2.3.2. **METHODS**

Twenty four Dark Agouti rats underwent laparoscopy with carbon dioxide insufflation and the instillation of intraperitoneal instillation of a suspension of 2×10^7 tumour cells (as per section 2.3.3.1). They were then randomised to one of the following study groups (6 rats per group):

1. Control

Rats in this group had no further injection.

2. Blood

Rats in this group in addition were injected intraperitoneally with 2 ml of fresh blood (obtained from a donor DA rat)

3. Heparin

Rats in this group in addition were injected intraperitoneally with 200 units of heparin

4. Blood + Heparin

Rats in this group in addition were injected intraperitoneally with 2 ml of fresh blood (obtained from a donor DA rat), and 200 units of heparin

Donor blood for rats in groups 2 and 4 was obtained from a donor DA rat by cardiac puncture under general anaesthesia. In groups 2 and 4 intraperitoneal blood was administered 2 minutes after the tumour cell suspension (Fig 4.2.3.2). Heparin (heparin sodium, porcine mucous; David Bull laboratories) was administered intraperitoneally to rats in groups 3 and 4 four minutes after the tumour cell suspension (Fig 4.2.3.2). Gas was insufflated at a rate of 0.4 litres/min and a pressure of 2 mmHg for a further 40 minutes, with a constant gas flow maintained through the venting cannula. The "ports" were then removed, and the puncture sites closed with sutures.

Rats were killed 7 days after the procedure, and the peritoneal cavity and port sites were examined for the presence of tumour (as per section 2.4).

FIGURE 4.2.3.2

FLOW CHART ILLUSTRATING TIMING OF ADMINISTRATION OF TUMOUR CELL SUSPENSION, INTRAPERITONEAL BLOOD AND HEPARIN.

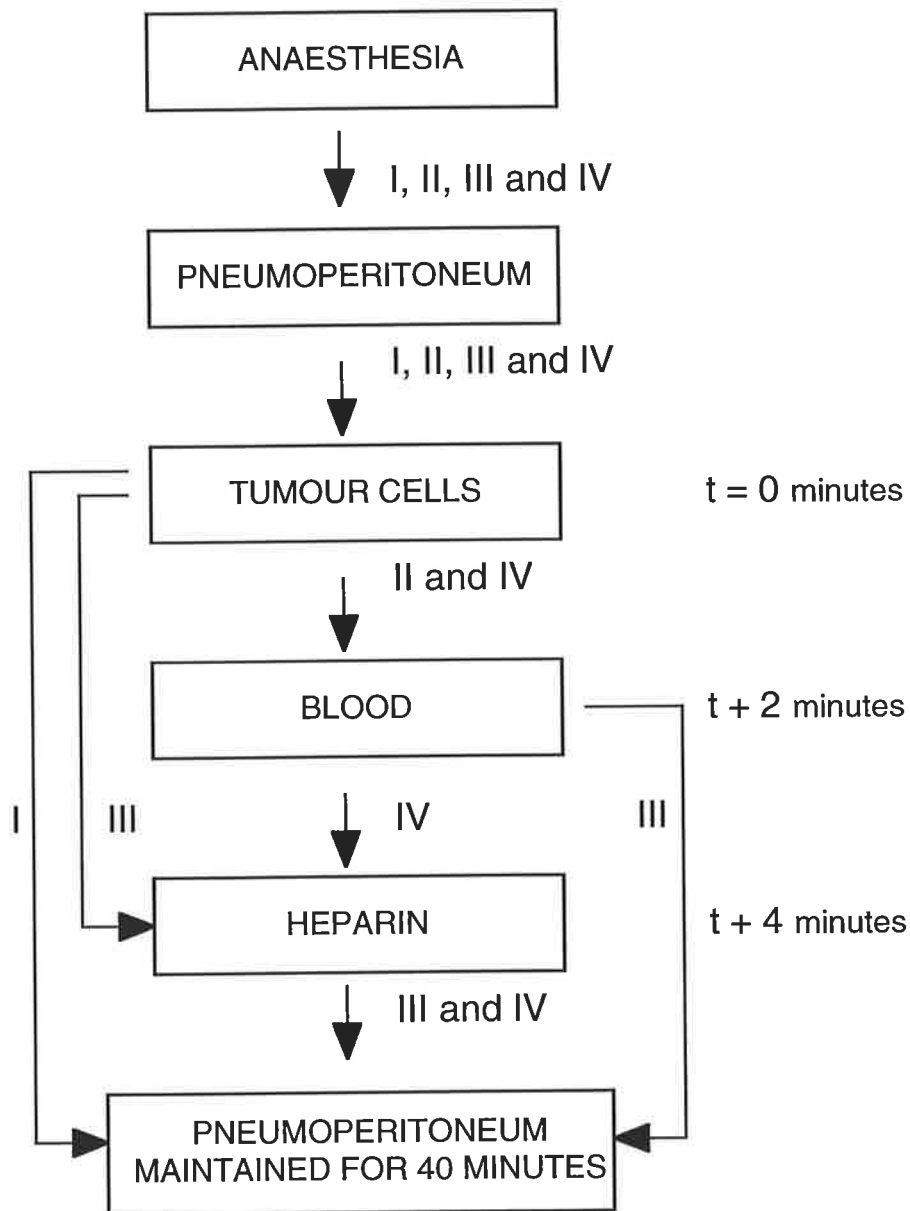


FIGURE LEGEND

Group I Control

Group II - Blood

Group III - Heparin

Group IV - Blood + Heparin

4.2.3.3. RESULTS

Preoperatively the rats in each insufflation group were of a similar weight and all rats gained weight postoperatively (Table 4.2.3.3.; $p=0.32$, Kruskal-Wallis test).

TABLE 4.2.3.3.1

RAT WEIGHTS FOLLOWING LAPAROSCOPY

(grams - all figures are median and range)

	control	blood	heparin	blood +heparin
Weight at surgery	252 (221-283)	255 (219-302.5)	251 (240-261)	248 (220-260)
Weight at day 7	288.5 (247-315)	267.5 (237-330)	258 (247-279)	280 (261-346)
%increase in weight (*median)	6.0 (0-11.7)	2.5 (-1.7-13.6)	1.5 (-1.9-5.7)	5.6 (0-18.6)

 $p=0.32$

Tables 4.2.3.3.2 and 4.2.3.3.3 summarise tumour implantation. One rat in each of the heparin and blood + heparin instilled groups was totally free of tumour. All other rats had some tumour implantation in the peritoneal cavity. All rats in the blood instilled

group had tumour in all sectors (100%) compared with 3 rats (50%) in both the control and heparin + blood instilled groups. In the heparin instilled group no rats (0%) had tumour in all sectors.

TABLE 4.2.3.3.2**PERITONEAL TUMOUR INDEX**

(number of sectors involved with each tumour grade)

Grade	control	blood	heparin	blood+ heparin
0	4	0	27	10
I	5	8	6	8
II	15	16	3	8
III	12	12	0	10

TABLE 4.2.3.3.3**SECTOR ANALYSIS OF TUMOUR INVOLVEMENT**

(number of sectors containing macroscopic tumour, regardless of grade)

Sector	control	blood	heparin	blood + heparin	Total
1	4	6	0	4	14
2	6	6	3	5	20
3	6	6	1	5	18
4	4	6	0	4	14
5	6	6	3	4	19
6	6	6	2	4	18

In the blood only instillation group tumour implantation was enhanced compared to controls (Table 4.2.3.3.2). A decrease in tumour growth was observed in the heparin and blood + heparin groups compared to controls. In the heparin group 27 sectors (75%) had no visible tumour growth. This was significant ($p < 0.0001$; Fisher's exact test) when compared to 10 (27%), 4 (11%) and 0 (0%) in the blood + heparin instilled, control and blood alone instilled groups respectively. The reduction in tumour implantation in the blood + heparin group compared to the blood alone instilled group was statistically significant ($p = 0.001$; Fisher's exact test), but not when compared to controls ($p = 0.13$; Fisher's exact test).

All port sites (100%) in the blood alone instilled group were involved with tumour (Table 4.2.3.3.4). Tumour involvement of the port sites occurred significantly less frequently following heparin administration ($p < 0.001$; Fisher's exact test). Five port sites (33%) in the heparin group were involved with tumour, compared with 10 (56%) and 17 (94%) in the blood + heparin instilled and control groups respectively. Port site metastases were significantly reduced in the blood + heparin group compared to the blood group ($p = 0.003$; Fisher's exact test). There was no predilection for tumour growth at the site of introduction of the tumour cell suspension and tumour distribution was similar in all sectors (Table 4.2.3.3.3).

TABLE 4.2.3.3.4

NUMBER OF PORT SITES WITH TUMOUR PRESENT

(18 wounds per group)

control	blood	heparin	blood + heparin
17	18	5	10
(94%)	(100%)	(28%)	(55%)

4.2.4. DISCUSSION

Tumour cells may be aerosolised during the period of pneumoperitoneum and then adhere to laparoscopic ports or they may be deposited on the port sleeves by instruments directly contaminated with tumour cells. When a port is removed, these tumour cells may deposit within a wound and tumour development can subsequently occur.

Golstein et al ¹⁰¹ reported an increase in tumour burden in mice when the peritoneum was disrupted before introducing tumour cells into the abdomen. This effect was reduced in mice receiving prophylactic intraperitoneal irrigation with heparin. They reported a reduction in tumour implantation when heparin was administered intraperitoneally along with a murine bladder tumour cell suspension in a sham laparoscopy model. They also demonstrated that this effect was not due to direct toxicity of heparin on the tumour cells *in vivo*. *Jacobi et al* also reported a beneficial effect on intraperitoneal tumour cell growth following heparin administration in a rat laparoscopy model which used a colon adenocarcinoma cell suspension ¹³⁰. In this study intraperitoneal tumour weight was lower in rats receiving heparin or taurolidine administered intraperitoneally but even lower when both substances were combined.

Heparin is a naturally occurring anticoagulant derived from bovine or porcine mucosa. Heparin binds to fibronectin, decreasing tumour cell adherence by competitive inhibition ¹⁰¹ and may therefore inhibit tumour growth by blocking tumour cell attachment to areas of peritoneal injury ¹³⁰. As well as decreasing tumour cell adherence, heparin may act by inhibition of the formation of thrombin. Thrombin in turn may play a role in the activation of progelatinase A to MMP-2 which is thought to promote tumour angiogenesis and tumour invasion ^{114,303}. Thrombin also has direct angiogenic effects, by inducing differentiation of endothelial cells into capillary

structures 108. Alternatively, heparin may inhibit tumour cell attachment by restoring the normally negative surface charge of peritoneum which is altered by injury 18.

4.2.2.1 Discussion of results

The results of **Study 4.2.2** suggest that the mechanism of reduction of tumour implantation is not by a direct cytotoxic effect of heparin on the tumour cell line used in these studies. This is consistent with the findings of *Goldstein et al* 101. In fact in **Study 4.2.2** *in vitro* heparin had the opposite effect by promoting tumour cell growth.

In **Study 4.2.3**, the use of intraperitoneal heparin was associated with a significant reduction in both tumour implantation and port site metastases. The presence of intraperitoneal blood, on the other hand, resulted in a significant increase in tumour implantation. Despite the *in vivo* results from **Study 4.2.2.**, the effect of heparin was advantageous *in vivo*. One possible explanation for this is that tumour cell adherence and implantation is facilitated by components of blood, possibly the fibrin matrix, and that this can be reversed in part by the use of heparin. Supporting this hypothesis is the adverse impact of blood in the peritoneal cavity, which promoted tumour implantation and growth. It is important to realise that even though the blood used in this study was from another rat, the DA strain is inbred, with all the DA rats effectively identical genetically to each other. This enabled the influence of a controlled amount of blood on tumour growth to be determined. Blood collection for the experimental rat rather than a donor rat may have added further physiological stress to the animal studied, which may have added a potential confounding variable to the study design.

As all surgeons know, bleeding occurs during all surgery. This bleeding is predominantly venous blood from the tumour specimen which may therefore contain a high number of tumour emboli increasing the risk of tumour seeding 107,255.

Hansen et al 107 have demonstrated the presence of tumour cells in blood shed during surgery in 93% of patients undergoing cancer surgery. In addition some of these tumour cells demonstrated proliferative capability by forming colonies in cell cultures or by inducing tumour growth after implantation into experimental animals. Bleeding can be prevented in part, but never completely, by meticulous surgical technique. Hence the adverse effect of blood demonstrated in this study is unlikely to be reliably eliminated by even the most meticulous laparoscopic technique. It is possible also that tumour cells trapped in blood clot might be afforded protection from scavenger mechanisms. This might then be in part reversed or prevented by heparin. Alternatively the reduction in port site metastases could be due to a heparin-induced decrease in tumour cell adherence to the laparoscopic cannulas.

These findings are supported by work of *Jacobi et al* 130 in which a decrease in intraperitoneal tumour weight was observed in rats receiving intraperitoneal heparin. This effect was enhanced with the addition of intraperitoneal taurolidine, an amino acid derivative which has significant anti-adherence activities on pathogenic organisms.

4.2.1.2. **Conclusions**

This study is potentially important in the context of laparoscopic cancer surgery. The risk of intraoperative bleeding can never be completely eliminated from any surgical endeavour, and if it occurs during laparoscopic surgery it may be of greater importance due to its potential to promoted tumour metastasis. Under these circumstances it is possible that intraperitoneal irrigation with heparin might reverse

or prevent these effects. Further investigation of the clinical efficacy of intraperitoneal heparin is indicated, as it has potential for reducing the risks of tumour spread in patients undergoing laparoscopic cancer surgery.

4.3. WOUND TREATMENT STRATEGIES

4.3.1. OVERVIEW

Following initial case reports of port site metastases, several authors proposed prophylactic wound treatment strategies. These include excision of the port site wound 61,294, the use of low energy carbon dioxide laser to 'sterilise' the wounds 201 or postoperative wound irradiation 20. The use of cytotoxic agents used both locally and intraperitoneally has also been advocated 136,181,201.

To investigate the efficacy of these strategies further, the following study was performed:

Investigation of the effect of various treatments of the port site wounds on the incidence of port site tumours.

4.3.2. INVESTIGATION OF THE EFFECT OF WOUND TREATMENT ON THE DEVELOPMENT OF PORT SITE METASTASES

4.3.2.1. AIM

The aim of this study was to investigate the effect of treating the port site wounds with different modalities to reduce the incidence of tumour formation.

4.3.2.2. METHODS

Twenty four rats were implanted with a solid flank tumour as per section 2.2.6 and underwent laparoscopy with laceration of primary tumour. Rats were randomised to treatment in the following groups:

1. *controls*

In this group no port sites were treated with the strategies tested in groups 2, 3 and 4.

2. *excision*

In this group the procedure the 'venting' port (LUQ) only was excised and the 1cm wound closed with sutures. Other wounds were not excised

3. *laser sterilisation*

In this group the venting port (LUQ) only was treated with carbon dioxide laser (Sharplan 743, Laser Industries Ltd, Israel) sterilisation to a 'burn' depth of 0.5mm following which the wound was closed with sutures.

4. *wound irrigation with betadine -*

In this group the 'venting' port only (LUQ) was treated by irrigation with 3ml of povidone iodine diluted 1:1 with normal saline. This was achieved by the external application of povidone iodine to the wound which was then closed

with sutures . Povidone iodine was not administered intraperitoneally or to the other wound sites.

In all rats laparoscopic tumour laceration was performed as per section 2.3.3.1. Pneumoperitoneum was maintained for with carbon dioxide as described at 2.3.2.1. At the completion of the 40 minute pneumoperitoneum, the venting (LUQ) port was treated as described above. Following surgery rats were allowed to recover. After a further seven days all rats were killed and port sites excised for histological examination (as per section 2.4).

4.3.2.3. RESULTS

Preoperatively the rats in each group were of similar weight. All rats gained a similar percentage weight postoperatively (Table 4.3.2.3.1, $p= 0.69$; Kruskal-Wallis test)

TABLE 4.3.2.3.1

RAT WEIGHT FOLLOWING LAPAROSCOPY

(measured in grams - all figures are median and range)

	control	betadine	excision	laser
Weight at surgery	285 (231-334)	284.5 (245-245)	278 (247-331)	283 (250-321)
Weight at day 7	311.5 (251-347.5)	306 (270-333)	288 (259-359)	305.5 (259.5 333.5)
%increase in weight (median*)	6.5 (3.6-11.6)	7.7 (-2.4-17)	4.7 (-4.4-13)	5.2 (3.8-12.3)

* $p=0.69$

Growth of the primary tumour was similar in all groups (Table 4.3.2.3.2, $p=0.17$; Kruskal-Wallis test). At autopsy examination the tumour was not disseminated beyond the site where the tumour was lacerated in any animal. At the site of laceration however, tumour was seen growing as a nodule at the site of the previous peritoneal breach.

TABLE 4.3.2.3.2

TUMOUR GROWTH FOLLOWING LAPAROSCOPY

(maximum dimension measured in mm - all figures are median and range)

	control	betadine	excision	laser
Size at surgery	24 (18-28)	24 (15-28)	25.5 (18-33)	25 (17-35)
Size at day 7	43 (32-50)	41 (29-60)	42 (30-50)	46 (32-60)
%increase in size	78.6	88.9	66.7	78.9
(*median)	(33.3-111)	(46.4-140)	(9.1-121)	(44-150)

$p=0.17$

The development of microscopic and macroscopic metastases at the laparoscopy trocar wounds is summarised in Table 4.3.2.3.3. Port site metastases occurred more frequently at the excision site compared to controls, however this difference was not statistically significant ($p=0.14$, Fisher's exact test). No significant differences were observed between the other treatment groups.

TABLE 4.3.2.3.3**NUMBER OF TREATED WOUNDS (LUQ WOUNDS) WITH METASTATIC
TUMOUR PRESENT**

(6 wounds per group)

	control	excision	laser	betadine
Macroscopic	1	5	0	0
Microscopic*	1	5	1	3

*p= 0.12

4.3.3. DISCUSSION

Contamination of the port sites with viable tumour cells is a *sine qua non* for the development of a port site tumour. As discussed earlier, tumour cells can be brought to the port site by either direct contact with tumour contaminated instruments, peritoneal fluid containing tumour cells, contamination of peritoneal surfaces on desufflation, or possibly indirectly by aerosolisation of tumour cells in the insufflation gas. Once at the port site, and given suitable conditions, the tumour cells can implant and grow. Fresh wound are ideal sites of tumour implantation due to the localised abundance of platelets, red blood cells, fibrin and tissue substrates 74,264.

Direct contamination is the most likely means of delivery of cancer cells to the port sites. Despite the most meticulous surgical technique it is likely that some tumour cell spillage and contamination will occur. It is well established that malignant cells collect on laparoscopic instruments and frequent passages of these instruments through trocars results in contamination of the port site wound 6,98. Bouvy *et al* 44 demonstrated in a rat model using a solid CC-531 colon adenocarcinoma which was inserted and subsequently extracted through a designated excision port that this site was at increased risk of tumour implantation, with this site having a greater tumour burden than other port wounds. This suggests that direct implantation of tumour cells was the dominant cause of port site tumours at the excision site, transferred either by the grasping instruments or by direct contact with the tumour specimen.

Removal of specimens through inadequate sized openings may increase the probability of tumour cells being implanted in the wound. The exteriorisation and resection performed in the Paul-Mikulicz extracorporeal technique of colectomy was popularised at the turn of the century because of a reduction in operative mortality but became outmoded for malignant disease because of the high incidence of local recurrence with wound recurrence being particularly common. This has been

attributed to the effect of dragging a cancerous colon through a small incision 5,57. This mechanism has also been suggested as the most likely explanation of thoracoscopic recurrences 73,94 in which insufflation gas is not used and therefore cannot be implicated. Port site protection by the use of impermeable bags does not guarantee against the development of port site metastases 288 as cases have been reported where a bag has been used to retrieve the specimen and where the port site involved was not the retrieval site 193.

Other strategies that have been proposed include closing the port site wounds to minimise the peritoneal defect 128 and irradiation of the port sites postoperatively 20, although *Baker et al* reported that irradiation delivered to the wound sites immediately after surgical excision of a KHT tumour in mice failed to result in a significant reduction in the incidence of local recurrences, suggesting that this strategy may not be effective.

4.3.3.1. Carbon dioxide laser treatment

Lanzafame et al 161 described the use of low energy carbon dioxide laser wound sterilisation as an effective experimental treatment following excision of rodent mammary carcinoma. The carbon dioxide laser permits surgical excision with the simultaneous sealing of small blood vessels and lymphatics and destruction of cells, nuclei and bacteria in the path of the beam which occurs by the flash boiling and vaporisation of cellular water 161. Surgical procedures with the carbon dioxide laser do not involve direct tissue contact, thereby preventing the inoculation of clean areas by surgical instruments. It also has the potential benefit of decreasing wound infection rates by destroying bacteria in the path of the beam and, as opposed to electrocautery, produces no thermal necrosis.

The KTP 532/YAG laser laparoscope (Laserscope) has already been used in clinical practice for cholecystectomy ²⁴⁸. It is quite possible then that laser technology can be linked with laparoscopic instrumentation resulting in the development of the laser-laparoscope which can be used both for primary laparoscopic resection and for 'sterilising' the port sites at the conclusion of the procedure.

In **Study 4.3.2** above one port site tumour occurred in one of six wounds treated with the carbon dioxide laser. Given the low incidence of tumour involvement in the control group this strategy did not demonstrate any advantage over the control (no treatment) group.

4.3.3.2. **Port site excision**

Excision of port sites following laparoscopic resections has also been suggested ²⁷⁷. The rationale for port site excision is to reduce the number of viable tumour cells at the port site and thereby decrease the likelihood of tumour development. *Wu et al* ²⁹⁶ using a GW-39 human colon cancer suspension in a hamster model demonstrated a significant reduction in the incidence of palpable tumours following excision of port wounds compared with simple wound closure (44 vs 61 percent). They concluded that wound excision following laparoscopy can significantly reduce, but not eliminate, tumour implantation rates.

Excision of port site wounds however, negates one of the principal advantages of laparoscopic surgery i.e. the small size of the access wounds. As laparoscopy disseminates tumour cells throughout the abdominal cavity, increasing the size of the wound may also increase the potential site for which tumour cells can implant following contact of the abdominal surface with peritoneal fluid on desufflation of the abdomen ¹⁸².

Whilst excision of all trocar sites at a 'second look' operation as suggested by *Pezet et al* 214 and *Sailer et al* 233 makes good sense, whether this is applicable to laparoscopy at the time is unclear. **Study 4.3.2** demonstrated a significant increase in the incidence of tumour involvement of the excised wound (5 of 6) in comparison with the control group (1 of 6). This suggests that this strategy would not be successful in clinical practice and may adversely affect the patients outcome even further.

4.3.3.3. **Local treatment with cytocidals**

Topical chemotherapy of wounds in patients undergoing resection for cancer was suggested in 1961 by *Thomas et al* 264 and wound irrigation with povidone iodine was suggested by *Akle et al* 4 as a possible strategy for the prevention of port site metastases.

Povidone iodine was chosen for **Study 4.3.2** as this substance is cytotoxic to DAMA tumour cell line *in vitro* and *in vivo* (sections 4.1.2, 4.1.3 and 4.1.4). Povidone iodine irrigation of wounds is also used in clinical practice and is one of the techniques being utilised in the current clinical trials of laparoscopic vs open colectomy for colon cancer.

Wu et al successfully decreased the incidence of port site metastases in an hamster model of laparoscopy using a GW-39 human colon cancer cell line, by the treatment of laparoscopic trocars 298. The rate of tumour cell implantation at trocar sites, and the presence of palpable port site metastases was significantly decreased by the use of cannulas dipped in 10 percent povidone- iodine or silver sulfadiazine compared with controls (untreated wounds and trocars).

In **Study 4.3.2** the use of local irrigation with povidone iodine failed to decrease the incidence of tumour implantation at this site compared with the control group. This may be because of the low incidence of tumour implantation in the control group or it may represent a failure of local treatment. The latter is more likely as this strategy is directed only at the port wound and not at any remaining viable tumour cells within the peritoneal cavity which can be contaminated the wound following desufflation or after wound treatment.

4.3.3.4. **Conclusions**

The results of this study suggest that local treatment of the port site wounds by carbon dioxide laser may not be effective in reducing the incidence of port site tumours. The use of local irrigation of port sites with povidone iodine also failed to decrease the incidence of tumour implantation. Excision of port sites is associated with not only an increase in wound length but confers a disadvantage in terms of an increased incidence of tumour implantation at this site and should therefore not be recommended as a preventive strategy.

SECTION V

CONCLUSIONS

TABLE 5.1**SUMMARY OF FINDINGS FROM STUDIES PRESENTED IN THIS WORK****The effect of different insufflation gases (section 3.1)**

1. Helium insufflation has a direct cytotoxic effect on cultured DAMA cells *in vitro*.
2. Incubation of DAMA cells in a carbon dioxide rich *in vitro* environment was associated with a significant decrease in pH. This effect was not observed with either air or helium.
3. The use of helium insufflation, compared to carbon dioxide, was associated with a significant reduction in the incidence of wound metastases and peritoneal tumour implantation following laparoscopy.

- Implications :**
1. The development of port site metastases is influenced by the nature of the insufflating gas.
 2. The development of port site metastases is not solely due to mechanical factors.
 3. Carbon dioxide, possibly through metabolic perturbations in the peritoneal environment facilitates tumour implantation and growth following laparoscopy.

The influence of immune function on tumour growth (section 3.2)

1. Port site metastases were significantly less common following laparoscopy in rats treated with endotoxin (systemic immune enhancement).
2. Peritoneal tumour implantation was significantly less likely following laparoscopy in rats treated with endotoxin.

3. The use of carbon dioxide as an insufflating agent during laparoscopy was associated with a significant reduction in intraperitoneal pH. This effect was not observed with either the use of helium insufflation or during gasless laparoscopy.
4. The use of carbon dioxide as an insufflating agent during laparoscopy was associated with a marked reduction in peritoneal macrophage production of TNF- α . This effect was not observed with helium or gasless laparoscopy techniques.

- Implications:**
1. Port site metastases can be influenced by alterations in the immune environment, particularly at the local level of the peritoneal membrane.
 2. Carbon dioxide induced depression of macrophage activity, possibly mediated by pH changes, is a contributing factor to the development of port site metastases.

The effect of insufflation pressure (section3.3)

1. The development of port site metastases was similar in rats undergoing laparoscopy with low pressure or high pressure insufflation.

- Implications:**
1. The development of port site metastases following laparoscopic surgery is not determined by the pressure of the insufflation gas.

Extended survival studies (section 3.4)

1. Survival of rats implanted with a DAMA tumour, resected at the time of surgery was associated with aggressive local disease recurrence, but not spread to distant sites such as liver or lung.

The effects of intraperitoneal cytotoxics (section 4.1)

1. Povidone-iodine is cytotoxic to DAMA cells *in vitro* in dilution of 1:10 (povidone iodine/0.9% saline). This effect was not observed when povidone iodine was used in greater dilution.
2. Chlorhexidine is cytotoxic to DAMA cells *in vitro*.
3. The administration of intraperitoneal povidone-iodine resulted in a significant reduction in the incidence of port site tumours and peritoneal tumour implantation.
4. The administration of intraperitoneal methotrexate resulted in a significant reduction in the incidence of port site tumours and peritoneal tumour implantation following laparoscopy when administered as an initial and subsequent dose. This effect was not observed following administration of a single dose.

- Implications:**
1. The use of intraperitoneal povidone-iodine or an appropriate chemotherapeutic agent can reduce the incidence of port site metastases.

The effect of intraperitoneal blood and anticoagulant (section 4.2)

1. Heparin is not cytotoxic to DAMA cells *in vitro*.
2. The administration of intraperitoneal heparin was associated with a significant reduction in peritoneal tumour implantation and port site metastases.

3. The administration of intraperitoneal blood was associated with a significant increase in tumour cell implantation and port site metastases, which was only partially reduced with concomitant administration of heparin.

- Implications:**
1. The use of intraperitoneal heparin may reduce port site metastases.
 2. Intraperitoneal blood promotes tumour implantation and growth.

The effect of wound treatment strategies (section 4.3)

1. Treatment of port sites with low energy carbon dioxide laser did not reduce the incidence of port site tumours.
2. Treatment of port sites by localised (as opposed to intraperitoneal) irrigation with povidone iodine did not reduce the incidence of port site tumours.
3. Excision of the port sites following laparoscopic tumour manipulation was associated with an increased rate of tumour implantation.

- Implications:**
1. The use of carbon dioxide laser or localised irrigation with povidone iodine is not an effective strategy to reduce tumour implantation in wounds following laparoscopy.
 2. Excision of port site wounds following laparoscopy increases the incidence of tumour involvement and is not an effective strategy for the reduction of tumour implantation in wounds. Excision of port sites may adversely affect patient outcome.

5.1. TUMOUR METASTASIS AND DISSEMINATION DURING LAPAROSCOPIC SURGERY - A THEORY AND PREVENTIVE STRATEGIES

The studies described in this thesis have investigated the mechanisms of port site metastasis following laparoscopic surgery and the efficacy of preventive strategies. The results of this work support a multifactorial aetiology of port site tumours and suggest that some preventive strategies which have proved effective in an experimental model warrant clinical investigation.

5.1.1. IS IT A REAL PROBLEM?

Since the commencement of this thesis, several large multicentre trials of laparoscopic versus open colon resection for cancer have commenced^{21,92}. Whilst it will be necessary to await the outcome of these trials to determine the true incidence of port site tumours following laparoscopic colectomy for cancer, early results from groups such as *Lacy et al*¹⁶⁰ in which no port site metastases were reported following 31 laparoscopic resections (91 segmental colectomies randomised to open or laparoscopic assisted) must be interpreted with caution as the total patient number is low and follow-up is not yet long enough to determine differences in overall survival. In this series recurrence rates are similar for the two groups (16.1% laparoscopic; 15% open). Median follow up is 21 months¹⁶² but the numbers are small and it is too early to draw any definitive conclusions from their results. These early clinical results have led some authors to infer that port site and open wounds are at equal risk of tumour implantation⁷. However, the long term results of these trials are not yet available and the weight of experimental evidence continues to support the increased risk of tumour implantation following laparoscopy.

Franklin et al report the results of a 5 year non-randomised trial of open vs laparoscopic colon resection for carcinoma of 415 patients in which there were no port site metastases in the laparoscopic group (191 patients) and survival recurrence and death rates were essentially the same between the two groups⁹². In this study however it should be noted that the protocol for the laparoscopic group included the following:

1. all trocars were sutured in place to prevent dislodgement and contaminated instruments contacting the port site wound
2. all cancer specimens were retrieved in non-porous bags
3. trocars were washed with 5% povidone iodine or distilled water prior to removal
4. an attempt was made to remove all abdominal fluid prior to desufflation and trocar removal to prevent potentially tumour laden fluid from contacting the wounds
5. all trocar sites were closed in layers
6. all insufflated gas was removed prior to removal of the trocars.

Because of the modifications used in the laparoscopy group it is not possible to assess the 'native' rate of port site tumour development. Equally, it is not possible to determine which of the methods used may have decreased the incidence of tumour implantation which might otherwise have occurred. Although the numbers in the laparoscopic group are small, the results of this study do suggest that the incidence of port site metastases may be less than first proposed and that this phenomenon may be prevented by utilising some of the strategies outlined in this thesis.

5.2. CAUSES OF PORT SITE TUMOURS

5.2.1. Contamination

There is little doubt that port site metastases can result from direct transfer and subsequent implantation of viable tumour cells borne on contaminated instruments or during extraction of specimens through the abdominal wall. Transient periods of desufflation may bring the anterior abdominal wall into contact with tumour cells during resection ¹¹⁶. However, as noted previously, this mechanism does not explain all cases of port site tumours as there have been documented clinical cases where port site tumours have occurred following laparoscopy in which the primary tumour was neither visualised or manipulated ^{17,144,205,243,271,281}.

Contamination may also occur during periods of retrograde gas flow. *Couper et al* ⁶² demonstrated that *Staphylococci*, blood particles and debris could be detected in the gas tubing and internal insufflator tubing following laparoscopy. This suggests that, although differential pressures favour the flow of gas from the insufflator to the patient, there are periods when retrograde flow of gas may occur. Similarly *Oshidi et al* ¹¹⁷ demonstrated positive microbiological cultures derived from pneumoperitoneum gas evacuated from patients during laparoscopic cholecystectomy which suggests that the evacuated peritoneal air is a potential source of contamination and raises the possibility that it may also contain tumour cells. The routine use of a filter between the insufflator and patient should prevent this.

5.2.1.1. *Bad surgery*

Some authors have suggested that port site tumours are a phenomenon related to the learning curve of laparoscopic surgical technique and therefore their incidence will decrease with time ⁴⁰. This is supported by the recent revision in the believed incidence of port site tumours. The pessimistic early suggestions of an incidence as

high as 21%²⁸⁸ have recently been revised by recent studies of larger populations to approximately 2%^{21,160}. This suggests that the early high rates were due to poor surgical technique during this early experience⁴⁰. Certainly laparoscopy is associated with some reduction in manual dexterity, which is highly dependent on the surgeons' experience. The loss in tactile sensation may be offset however by improved visualisation of the operative field afforded by magnified images and the ability to perform precise dissection.

The occurrence of port site metastases may be a surgeon related variable, related to experience and expertise. It has been demonstrated in series of open colorectal surgery and pancreatic surgery that patient outcome is dependent on surgical prowess, with recurrence rates related to the seniority of the surgeon and the number of procedures done in the institution^{139,215}.

Traumatic manipulation of tumours during surgery or during specimen extraction may increase exfoliation of malignant cells into the peritoneal cavity¹⁵³. *Krähenbühl et al* reported that in a model of laparoscopic biopsy of an implanted liver tumour, tumour growth was greatest at the site used to remove the specimen or perform the biopsy¹⁵⁶. Similarly, a study by *Bouvy et al* recorded increased tumour growth at the port used to extract a lump of CC531 colon cancer from the peritoneal cavity. However, metastatic growth at sites other than extraction sites in both clinical case reports and experimental models suggest that factors other than direct contact with the specimen are also responsible²⁶⁹. *Lee et al* report that deliberate crushing of an implanted splenic tumour before resection resulted in significantly more port wound and incisional tumours. In contrast, in this study the addition of a 20min carbon dioxide pneumoperitoneum after splenectomy did not increase the incidence of port site tumours, suggesting that the role of surgical technique is more significant than that of the pneumoperitoneum¹⁶⁷.

Other studies by *Lee et al* ¹⁶⁸ also suggest that surgical technique is a factor in the development of port site tumours. They performed two trials of laparoscopic and open mobilisation of splenic tumours followed by extracorporeal resection. Overall the incidence of port site tumours and incisional tumour recurrence was higher in the laparoscopic assisted group. However, when the two trials were compared, there was a significant decrease in the number of port site tumours, but not incisional tumour recurrence, in the second trial. This suggests that with increased experience, less manipulation of the spleen was required, resulting in a decrease in the liberation of viable tumour cells into the peritoneum.

Similarly *Mutter et al* ¹⁹⁷ demonstrated in an experimental model of pancreatic tumour that manipulation of the tumour significantly increased tumour growth and spread in the laparoscopic group compared to laparotomy, although when manipulation of the tumour was not performed tumour growth and metastatic spread was comparable. *Allardyce et al* ⁸ using a porcine laparoscopic colectomy model and HeLa cells demonstrated that the greatest contamination of ports as measured by Gamma cell counting occurred in ports used by the operating surgeon, suggesting that technical aspects of surgery may also influence the distribution of tumour cells ⁸.

Kim et al ¹⁵³ report a study comparing the number of free peritoneal cancer cells obtained from peritoneal lavage fluid in patients undergoing open and laparoscopic colon resections for cancer and conclude that when performed to strict criteria and surgical principles, laparoscopic surgery was not associated with an increased risk of tumour cell spillage compared with conventional techniques.

5.2.1.2. *Bad instruments*

It has been suggested that tumour cells may adhere more easily to laparoscopic than conventional instruments or that tumour cells may migrate up contaminated ports via capillary action ³⁰. As ports are withdrawn at the end of a procedure, tumour cells may be seeded from the instrument sleeve into the wound. In addition laparoscopic instruments such as graspers may result in more tissue trauma than conventional instruments increasing the number of exfoliated tumour cells ^{26,263}. Laparoscopic grasping instruments may produce gross or microscopic perforations or and seromuscular visceral tears, exposing the underlying mucosa to the peritoneal cavity ³³. *Texler et al* demonstrated the liberation of mucosal gallbladder cells by the use of grasping endoscopic instruments ²⁶³ and propose a mechanism for the liberation of malignant cells during laparoscopic procedures for cancer.

The studies in section 4.2 which demonstrate an advantage by using intraperitoneal solution containing heparin may also be related to decreasing cell adhesion to trocars. This is supported by the findings reported by *Jacobi et al* ¹³⁰ demonstrating a beneficial effect of taurolidine in an experimental model. Clinical trials involving heparin and taurolidine are awaited with interest.

Trocar sites themselves may also provide sites ideal for tumour cell implantation ^{26,269}. *Tseng et al* report a study in which tumour implantation was greater at deliberately traumatised trocar sites than at non/ traumatised sites. These wounds are localised areas of high cellular proliferation and may provide optimal conditions for tumour cell growth.

5.2.1.3. *Haematogenous or from the peritoneum*

Most authors believe that port site metastases occur as a result of direct seeding of exfoliated tumour cells from within the peritoneal cavity and that haematogenous spread is an unlikely mechanism. This is supported by a study by *Iwanaka et al* 127 who implanted mice with subcutaneous neuroblastomas. Mice were then randomised to receive a subsequent tumour dose, either intravenously or intraperitoneally before undergoing laparoscopy. The incidence of port site metastases was 0% in the intravenously injected mice versus 63% in mice injected intraperitoneally. This supports the theory that it is direct seeding, rather than haematogenous spread that results in the development of port site tumours.

5.2.2. **Role of laparoscopic insufflation**

Early experimental studies into the phenomena of port site metastases confirmed the pivotal role of the pneumoperitoneum 44,143,184. Studies that demonstrate no apparent increase in the rate of tumour implantation when gasless techniques are used 42,44,182,185,282 also support the concept that port site metastases are related to something inherent in the laparoscopic as opposed to the open surgical environment. Of particular interest, *Wu et al* 297 recently reported a study in which the effect of the pneumoperitoneum related significantly to the size of the tumour inoculum. These findings suggested a synergistic effect between tumour cell contamination and the pneumoperitoneum. At a dose of 3.2×10^5 tumour cells the addition of a pneumoperitoneum resulted in a significant increase in tumour implantation and tumour mass. When the tumour cell dose was decreased by half, the addition of a pneumoperitoneum had no significantly adverse effect. This suggests that both contamination and the pneumoperitoneum play a role in the development of port site metastases.

5.2.2.1. *Aerosolisation?*

The role of possible aerosolisation of tumour cells remains controversial. As originally described by *Kazemier et al*, gas insufflation during laparoscopy leaking around and through ports (the “chimney effect”) may result in an accumulation of tumour cells at the trocar sites ¹⁴⁷. *Tseng et al* observed increased tumour growth of CC531 tumour cells which had been injected intraperitoneally at a ‘leaking’ port compared to control non-leaking trocar sites ²⁶⁹. *Hewett et al* have confirmed that tumour cells can be retrieved from washings of laparoscopic instruments and trocar wounds that have not been in direct contact with tumour ¹¹⁶. *Hubens et al* and *Whelan et al* ^{119,293} however have been unable to demonstrate that tumour cells aerosolisation occurs and therefore its role remains unclear.

5.2.2.2. *Gasless laparoscopy and port site metastases*

The removal of the insufflation gas appears to eliminate one of the essential environmental components necessary for laparoscopic wound metastasis. In contradistinction to the theory of aerosolisation, laparoscopy with insufflation gas may transport tumour cells to laparoscopic access wounds and result in the growth of metastases. The study by *Mathew et al* in which 'recipient' rats developed port site tumour in the carbon dioxide group but not in the gasless group also supports the theory that carbon dioxide insufflation allows cells to be transported to and may then exert a permissive effect on tumour growth ¹⁸².

Other studies utilising real time imaging of radiolabelled cells instilled into the peritoneal cavity of pigs undergoing laparoscopy demonstrate widespread cell movement throughout the abdominal cavity during carbon dioxide pneumoperitoneum ²⁶¹, supporting the postulate that carbon dioxide pneumoperitoneum facilitates tumour cell dispersal throughout the abdominal cavity, thereby bringing cells into contact with potential implantation sites. This phenomenon is not observed when

gasless laparoscopy is used 182,185. *Cavina et al* performed a similar study in which labelled red blood cells were injected into the gallbladder bed of patients undergoing laparoscopic cholecystectomy using both gasless and carbon dioxide pneumoperitoneum techniques 53. They demonstrated increased tracer uptake in the region of the umbilical port in patients undergoing laparoscopy with a carbon dioxide pneumoperitoneum compared to patients in the gasless group. Of particular note, the use of a protective specimen retrieval bag did not significantly modify the results. It was also noted that wide intraperitoneal diffusion of tracer was observed in the pneumoperitoneum, but not in the gasless group. This suggests that the positive intraabdominal pressure induced by insufflation distributes cells to the port sites where, given an appropriate tumour load and conditions, a port site tumour can develop.

These studies in pigs and humans are particularly important as one of the major criticisms of work done in small animal models relates to the relatively large dose of tumour cells in comparison to a small peritoneal volume 292. In small animal models therefore it is possible that high rates of tumour metastases may be the consequence of increased contact between the peritoneal fluid containing viable tumour cells and the undersurface of the port wound where these cells can implant. However these studies confirm the role of the pneumoperitoneum in disseminating cells throughout the peritoneal cavity.

5.2.3. **Mechanical vs metabolic**

The studies in section 3.1 in which the replacement of carbon dioxide with the inert gas helium was associated with a reduction in the incidence of port site metastases and tumour implantation, provides evidence that it is the presence of carbon dioxide rather than the use of a pneumoperitoneum *per se* which facilitates this phenomenon.

Previous work by *Mathew et al* and *Texler et al* has demonstrated the effect of mechanical distribution of tumour cells following laparoscopic insufflation in experimental models 182,185,261, but it is unlikely that this is the sole determinant of the development of port site tumours. The findings in section 3.3.2 demonstrate that this phenomenon is independent of the insufflation pressure used and that the nature of the insufflating gas is a more important factor.

Whilst carbon dioxide has properties which make it a very suitable insufflation agent, the metabolic and oncologic consequences of insufflating the peritoneal cavity with carbon dioxide may not be advantageous.

5.2.3.1. *Metabolic*

The alterations to ventilatory, cellular, hormonal and immunologic parameters 275,276 that occur with the insufflation of carbon dioxide may facilitate tumour implantation and growth. Carbon dioxide induced acidosis resulted in increased tumour growth of the DAMA cell line *in vitro* and increased tumour implantation at port sites and peritoneally in our rat model (section 3.1). In addition carbon dioxide induced depression of macrophage activity, possibly mediated by the observed pH changes, would appear to be a contributing factor to the development of port site metastases (section 3.2.3).

The use of a prolonged carbon dioxide pneumoperitoneum also results in a lactic acidosis and increased markers of non-oxidative metabolism 260. Due to the brevity of many laparoscopic procedures, such metabolic derangements may not be clinically relevant. However, in extended procedures such as colonic resection for malignancy, changes in tissue metabolism may be potentially important in the presence of a significant tumour load.

Work in this thesis reports a reduction in the incidence of port site metastases when helium is used as the insufflation agent rather than carbon dioxide using both a solid tumour and tumour cell suspension model (sections 3.1.3 and 3.1.4). This effect was not observed when either medical air or nitrous oxide was used as the insufflation agent.

Because helium is a metabolically inert gas ^{203,219}, these studies suggest that the development of port site metastases may be dependent at least in part on metabolic determinants, and that the physical redistribution of tumour cells alone is insufficient to account for the issue of port site metastases. Possible explanations for the reduced rate of tumour metastasis following helium insufflation include this beneficial effect due to the lack of adverse metabolic activity during helium insufflation.

Alternatively, but perhaps less likely, is the possibility that this effect is due to helium gas exerting a cytotoxic effect on cancer cells.

5.2.3.2. *Immunological*

Although recent studies have suggested that minimal access surgery is associated with less overall suppression of the systemic immune system compared to conventional open surgery ^{11,14,43,59}, this systemic benefit may not necessarily be acting at the level of peritoneal membrane interface, as the insufflation of carbon dioxide under pressure has been shown to adversely modulate the local immune environment ^{275,283}. In our studies immune enhancement with the administration of endotoxin reduced the incidence of port site tumours. This is presumably related to overall enhancement of the rats' immune status. However, the use of carbon dioxide as the insufflating agent was associated with impaired ability of peritoneal macrophages to produce TNF- α and this effect persisted for three days post surgery. This effect was not observed in rats undergoing laparoscopy using helium or a gasless technique and this suggests that it is not solely due to the effects of elevated intra-abdominal

pressure causing intraperitoneal acidosis but is related to a metabolic effect of carbon dioxide *per se*. Peritoneal macrophages play an integral role in the primary inflammatory response to infection and cancer within the abdominal cavity. The scavenging action of these macrophages is mediated in part by the production of inflammatory cytokines such as TNF- α , which may have a role in the effective killing of tumour cells 142,229. We hypothesise that carbon dioxide induced depression of macrophage activity, possibly mediated by pH changes, is a contributing factor to the development of port site metastases. The depression of peritoneal macrophage function observed with carbon dioxide insufflation may inhibit effective "scavenging" of viable tumour cells liberated during laparoscopic cancer surgery.

5.3. **STRATEGIES FOR THE PREVENTION TUMOUR DISSEMINATION AND IMPLANTATION FOLLOWING LAPAROSCOPY**

5.3.1. **Better surgical technique**

Meticulous laparoscopic techniques with minimisation of handling of the primary tumour, and the avoidance of the use of crushing instruments⁴ may reduce the incidence of tumour implantation at port sites. Technological advances such as the use of ultrasonically activate scalpels and ultrasonic dissection (such as with the Harmonic Scalpel) may reduce trauma, cause less cellular damage²⁰² and therefore reduce the number of viable tumour cells liberated into the peritoneal cavity.

Developments in the simulation technology and robotics are likely to influence the laparoscopic environment and may lead to overcoming reductions in tactile input and streamline dissection. Similarly advances in 3-dimensional vision may improve depth of view. Radiological improvements offer the potential to enable dissection planes to be guided more accurately, with the detection of minuscule deposits of disease¹⁴⁰. Each of these advances may refine laparoscopic resections but technical feasibility alone does not justify the use of a new technology and each of these introductions will require critical evaluation¹⁵⁴.

However, despite advances in technology the role of tumour cell contamination in the development of port site metastases underlines the requirement for meticulous surgical technique. In particular, emphasis on improved tissue handling, dissection planes and the maintenance of haemostasis will continue to be factors of paramount importance in determining post operative tumour recurrence rates.

The use of protective non porous bags to retrieve specimens may also reduce the risk of contamination of the abdominal cavity. However, reports of wound involvement in cases where these techniques have been used 26,193,288 and the study by *Cavina et al* in which the use of a non porous bag did not prevent contamination of the port sites 53 emphasise that such measures will not compensate for surgical technique.

5.3.2. DEALING WITH 'SPILT CELLS'

5.3.2.1. Cytotoxics

Strategies such as the use of intraperitoneal cytotoxics may be clinically applicable to deal with any viable tumour cells remaining in the peritoneal cavity following a laparoscopic procedure. The routine use of intraperitoneal cytotoxics has been advocated for all laparoscopic colorectal resections 136,255. However, the use of these agents is not without morbidity and it may therefore be more appropriate to restrict their use to patients with positive peritoneal lavage cytology. A study by *Hase et al* 109 examining pre- and post-procedure cytology in patients with colorectal cancer demonstrated that positive lavage was associated with a worse outcome. In particular all patients with cancer cells in the peritoneal cavity at the end of surgery subsequently developed recurrence. In these patients, regardless of tumour stage or curative intent, the use of perioperative intraperitoneal cytotoxics may offer an advantage.

A reduction in the incidence of port site metastases was observed in section 4.1 following an appropriate dose of intraperitoneal methotrexate. The implications however, of using antimetabolite agents such as methotrexate, are that the dose and frequency of administration may be critical. Intraperitoneal chemotherapy is usually given in large volume to ensure widespread dissemination and continued over several days to ensure that all cycling cells are exposed to the agent 255. For maximum

effectiveness, intraperitoneal chemotherapy needs to be combined with complete cytoreduction and the tumour cells need to be bathed in the cytotoxic agent ²⁵⁷. If repeated doses of such an agent are required a postoperative intraabdominal drain would be required following the procedure. The correct chemotherapeutic agent, dose and dose interval is likely to be critical to the success of intraperitoneal chemotherapy and it is perhaps best reserved for patients who have aggressive cancers found unexpectedly at the time of laparoscopy.

Whilst this work has demonstrated advantages for chemotherapy, the consistent results achieved by a single dose of dilute povidone-iodine administered intraperitoneally suggest that the latter strategy is likely to be more suitable for routine clinical use.

Studies at section 4.1 also demonstrated the efficacy of povidone-iodine administered intraperitoneally to prevent port site tumour using both the solid and free cell tumour models. This work is supported by the recent findings of *Lee et al* ¹⁶⁶ utilising a murine splenectomy model. The dilution used in these studies (1:10 povidone iodine:0.9% saline) would appear safe in clinical use. Some concerns however have been raised regarding the possibility of intraperitoneal adhesion formation and sclerosis following the use of povidone iodine and this needs to be clinically addressed.

Povidone iodine is readily available, cheap and in current clinical use. Clinical concern exists however, about the use of povidone iodine intraperitoneally. Two cases of sclerosing encapsulating peritonitis have been described following the intraperitoneal use of undiluted povidone-iodine ¹⁴⁸. In addition there is a potential problem with iodine administration in large doses in patients with abnormal renal function ³⁰². Experimental studies suggest that intraperitoneal povidone iodine may

have increased levels of toxicity in patients with concurrent inflammation. *Gilmore et al* ⁹⁹ report that the lethal dose of intraperitoneal povidone iodine in dogs was 8ml/kg administered intraperitoneally, however, in the presence of peritonitis 2mg/kg over 24hrs was associated with a 100% mortality. Another study have demonstrated that intraperitoneal irrigation of povidone iodine in rats is associated with increased protein levels in peritoneal washings due to serious peritoneal cell damage and a chemical peritonitis ¹⁵⁸. For this reason the intraperitoneal use of solutions containing more than 1% povidone iodine (i.e. 1:10 dilution of commercially available Betadine) should be avoided ¹⁴⁸. Toxicity following a single intraperitoneal lavage with povidone iodine is however rare ¹⁶⁶ and studies in section 4.1.2 and by *Lee et al* ¹⁶⁶ suggest that the efficacy of povidone iodine persists to a dilution of 1:50.

The use of such agents, whilst a viable strategy is not addressing the underlying cause of the problem. Just as the cause of port site tumours is likely to be multifactorial, it would be appropriate to apply a multidirectional prevention strategy that includes the minimisation of the number of viable tumour cells remaining in the abdominal cavity at the end of the procedure. None the less, adjuvant intraperitoneal cytotoxic agents may eliminate spilled tumour cells that occur following surgery, despite the most meticulous techniques ¹⁹.

5.3.2.2. **Heparin**

The use of intraperitoneal heparin, as demonstrated in section 4.2.3 following laparoscopic cancer surgery is also a potentially viable strategy to reduce tumour implantation. The safety of intraperitoneal heparin has been established in patients undergoing peritoneal dialysis where it is utilised to prevent adhesion from limiting the peritoneal space.

It is not clear whether the beneficial effect of heparin demonstrated in study 4.2.3 is related to its anticoagulant or an antiadhesive properties. The results of clinical trials underway by *Jacobi et al* are expected to help clarify this issue.

The implications of the findings in study 4.2.3 also relate to the significant increase in the incidence of port site metastases in the experimental group that received intraperitoneal blood. There is no substitute for meticulous surgery, but even the best surgery will result in some intraperitoneal blood loss. This blood may provide a medium that facilitates tumour survival and therefore careful haemostasis and lavage of blood and fluids in the operative field is to be recommended.

5.3.2.3. **Treatment of trocars**

Experimental models confirm that tumour cells adhere to laparoscopic instruments 116,265. This may occur as a result of electrostatic interaction between the trocar and the tumour cells and may vary in degree depending on the type of trocar used (metal or plastic). Adherent tumour cells can then be deposited at the trocar sites on extraction of the trocar sleeve. *Wu et al* successfully decreased the incidence of port site metastases in an hamster model of laparoscopy using a GW-39 human colon cancer cell line, by the treatment of laparoscopic trocars. The rate of tumour cell implantation at trocar sites, and the presence of palpable port site metastases was significantly decreased by the use of cannulas dipped in povidone iodine or silver sulfadiazine 298.

Trocars that minimise gas leakage around and through the port site and cause minimal trauma may offer an advantage. Possibly trocars with balloon fixation on both external and peritoneal surfaces will offer an atraumatic, non leaking fixation 40,269.

5.3.2.4. **Treatment of wounds**

The use of the carbon dioxide laser in study 4.3.2 was shown not to be an effective strategy for treating the port wounds in the DA rat model. Lasers are increasing in use in surgery and do offer the advantage of not requiring contact with the port wound, potentially minimising the risk of contamination. Further studies with other frequency lasers is indicated. This is a relatively simple strategy but would require the development of a suitable 'laserscope' for clinical application in laparoscopy.

Excision of the port site wounds at the conclusion of a laparoscopic procedure for malignancy has been advocated by some authors^{61,294}. The results of study 4.3.2 demonstrate that in the DA model this was an ineffective strategy that resulting in an increase, rather than a decrease, in the incidence of tumour implantation in the wounds. This strategy also results in a larger incision thereby negating one of the principal benefits of minimal access surgery and is not recommended.

5.3.2.5. **Immune modulation**

The studies at section 3.2 demonstrate a decreased incidence of tumour implantation following immune enhancement. Whilst immune modulating/ immunoprophylactic substances such as taurolidine which has bactericidal antiendotoxic effects has been demonstrated to enhance survival by preventing cellular immune depression following surgery in experimental model¹⁷² the role for such agents including interferon and the interleukins¹⁷¹ in clinical practice remains speculative.

5.3.3. **ALTERNATIVES TO CARBON DIOXIDE**

5.3.3.1. **Gasless laparoscopy**

Gasless laparoscopy, which involves the creation of a laparoscopic working space without using positive pressure pneumoperitoneum, is readily achieved and various abdominal wall lifting devices are commercially available 176,180,206,301. Gasless laparoscopy may offer some advantages over the use of carbon dioxide insufflation, including the avoidance of various pneumoperitoneum specific problems such as; cardiopulmonary compromise, hypothermia due to cold insufflation gas, peripheral venous stasis and gas embolism. In addition the ability to use continuous suction and conventional surgical instruments (there is no requirement to maintain airtight ports) may also be useful. There are two different methods for achieving abdominal wall elevation. This can be achieved by traction of the skin and subcutaneous tissues, e.g. by inserting subcutaneous wires 110, or by inserting devices into the peritoneal cavity which are attached to external retraction frames 56,206. In addition hybrid systems are available which utilise a combination of elevation and low pressure (6 to 8 mmHg) gas insufflation 23,180.

However, some problems have yet to be overcome with gasless laparoscopy. Principal amongst these is that conventional lifting devices, such as "Laparolift", which involves the use of a fan shaped retractor which is inserted intraperitoneally and an external mechanical arm, do not uniformly elevate the abdominal wall in the same fashion as a pneumoperitoneum. Therefore exposure to some areas of the abdomen, particularly the flanks, can be reduced. This may be particularly relevant in laparoscopic colectomy where reduced access to the abdominal flanks may impede colonic mobilisation and compromise resection margins by making surgery more difficult. In a randomised trial of gasless vs laparoscopy with carbon dioxide in gynaecological surgery, the disadvantages of gasless laparoscopy in terms of impaired

exposure, the requirement to convert to conventional pneumoperitoneum and technical difficulty outweighed the potential advantages¹³⁸. In contrast to this, *Smith et al*²⁴⁶ report a series of 58 patients in whom gasless techniques were used, without significant technical difficulties. It should be noted however that a large percentage of the procedures performed were diagnostic laparoscopies, in which exposure and access may not be as problematic as in colonic mobilisation.

Many of the current gasless laparoscopy devices also require complex assembly and require additional incisions, which may increase the likelihood of postoperative pain and also offer another potential site for tumour implantation. In theory compression of the abdominal wall, which can occur when using intraperitoneally sited lifting devices, may also cause local ischaemia and/or trauma which may result in the release of inflammatory mediators. It is possible, but not proven, that a local environment conducive to tumour implantation may even be created¹⁴¹.

5.3.3.2. **Helium pneumoperitoneum**

Exclusion of carbon dioxide can be achieved by the use of an inert gas such as helium or argon. Helium and argon offer the advantages of a pneumoperitoneum, i.e. exposure and technical access, without the inherent metabolic disadvantages of carbon dioxide. Helium has been shown in laboratory studies not to be associated with many of the adverse physiological effects of carbon dioxide^{199,203}. Helium has been used clinically for laparoscopic surgery without adverse effects^{38,39,82,188,203}, and it is relatively inexpensive (approximately A\$90 per cylinder). However, the use of helium as an insufflation agent requires modifications to the insufflator, as flow regulators in automatic insufflators are gas specific. Nevertheless, initial clinical trials suggest that helium may offer some advantages over carbon dioxide as an insufflation agent²⁰³. In particular, patients with borderline cardiorespiratory function, and those undergoing prolonged procedures may benefit from the reduced

physiological disturbances associated with helium insufflation 71,145,170. It has also been proposed that postoperative shoulder tip pain following laparoscopy may be related to irritation of the peritoneal surface by carbonic acid, which forms as a result of the dissociation of carbon dioxide in water. Theoretically, the use of an inert gas such as helium could achieve a reduction in postoperative pain. This hypothesis is yet to be formally tested in the clinical situation.

A theoretical concern about the use of helium as an insufflation agent is that it might increase in the risk an adverse outcome following venous gas embolism 295. This is because helium is less soluble than carbon dioxide, and therefore a given volume of gas entering the venous system is likely to be eliminated more slowly if it is helium, compared to carbon dioxide, with possible more serious consequences if a sufficient volume becomes trapped in the right ventricle of the heart, thereby impairing cardiac output. Gas embolism is one of the most feared complications of laparoscopy and carries a mortality rate of 50% 24. However, gas embolism during laparoscopy is a rare event with an incidence of 0.01% 215 and there are no reports of helium gas embolism following laparoscopy. Furthermore, it is likely that several hundred mls of gas must be introduced into a major vein for cardiac arrest to result. This is only likely to occur if a Veress needle punctures an iliac vein or a vein of similar size, and insufflation occurs directly into the venous lumen, rather than the peritoneal cavity. If this occurs, the choice of insufflation gas is unlikely to make a critical difference to the likely disastrous outcome. Prevention of this scenario is achieved by using an open technique for insertion of the first laparoscopic trocar. Whilst clinical use has been limited, several trials of helium laparoscopy have recently been reported in the international literature 39,188. No complications specific to the use of helium insufflation have been reported although *Neuberger et al* have described a 15% incidence of protracted subcutaneous emphysema in patients undergoing laparoscopy with helium which required several weeks to resolve 203.

5.3.3.3. **Insufflation using other gases**

The use of other inert gases may offer the similar benefits, and such gases warrant investigation. Nitrogen and argon have both been proposed ¹²⁰. Argon is the least expensive but may not be entirely physiologically inert and in patients with cardiovascular disease its effects may be clinically important ⁷⁸. It is also relatively insoluble in blood and therefore carries a theoretical potential for gas embolism ^{78,295}.

Nitrous oxide was the gas preferred for pneumoperitoneum in the 1970s and 1980s ³ although concerns about the fear of combustion in the case of colon perforation and cases of explosion during female sterilisation procedures led to a decline in its use as an insufflation agent in surgical laparoscopy ¹²². Nitrous oxide is therefore unsuitable for use in therapeutic laparoscopy where the use of diathermy is necessary to complete resections ²⁰⁴. Nitrous oxide is still routinely used by gynaecologists for diagnostic laparoscopy ³ and has some advantages in terms of improved patients tolerance ^{3,216}. However, nitrous oxide can also be taken up into the bloodstream and may reach levels that can prolong anaesthesia or result in hypoxia. The use of nitrous oxide in diagnostic laparoscopy for intraabdominal malignancy must also be cautioned against, as experimental studies demonstrate no advantage over carbon dioxide in reducing tumour implantation rates. More recently nitrogen has been proposed, but there is currently little evidence to support its use. Further, the effects, if any, of N₂O or nitrogen on intraperitoneal immunity are not known.

5.4. CONCLUSIONS

The work presented in this thesis suggests that the aetiology of this problem is likely to be multifactorial. The development of port site metastases is dependent on the presence of viable tumour cells at the port site following laparoscopy for malignant disease. This is affected by the cell line, stage and biological aggressiveness of the tumour. Surgeon factors such as experience and degree of tumour manipulation also appear to play a role but mechanical effects such as insufflation pressure are unlikely to be the sole determinants of increased tumour implantation.

The weight of experimental evidence suggests that the use of carbon dioxide as an insufflation agent is associated with facilitation of tumour growth and implantation and that exclusion of carbon dioxide from the pneumoperitoneum is advantageous. The effect of carbon dioxide appears to be at the local level of the peritoneal membrane resulting in alterations in the immune environment. Carbon dioxide induced depression of macrophage activity, possibly mediated by a decrease in intraperitoneal pH, may be a contributing factor to the development of port site metastases.

Exclusion of carbon dioxide can be achieved either by the use of gasless exposure techniques or by using an inert gas for insufflation. Which of these options proves to be best will depend on the outcome of further clinical studies. Nevertheless gasless laparoscopy is limited by the fact that poor lateral exposure is achieved, whereas helium insufflation may be a better strategy for further clinical investigation.

Surgical technique is an important factor determining the likelihood of tumour implantation. Attention to surgical principles of experienced surgeons, minimal trauma, and meticulous haemostasis are as important in laparoscopic as in open

surgery. Such principles are likely to be more important than treatment of the wounds post procedure. In particular excision of port site wounds is unlikely to be an effective strategy, and may result in a potentially adverse outcome.

Strategies outlined in this thesis such as the use of intraperitoneal cytotoxic agents and intraperitoneal heparin may be useful clinical methods to decrease the number of viable tumour cells and therefore reduce the risk of tumour cell implantation. The role of tumour specific intraperitoneal chemotherapy has potential but requires further investigation. There is also a possible role for the use of intraperitoneal heparin

These strategies, in particular the use helium insufflation, and intraperitoneal povidone iodine, require clinical evaluation but may offer a practical method by which the incidence of port site metastasis and tumour dissemination following laparoscopy can be minimised. This will enable patients with malignant disease to benefit from the systemic immune enhancement and reduction in post operative morbidity that accompanies the use of laparoscopic techniques, without compromising their prospect of 'surgical cure'.

*“The cleaner and gentler the act of operation, the less the patient suffers,
the smoother and quicker his convalescence
(and) the more exquisite his healed wound”*

Lord Moynihan

TABLE 5.4.1.**SUMMARY OF MECHANISMS OF PORT SITE TUMOURS****patient factors**

immune competence

tumour factors

tumour cell biology

aggressiveness

stage

adhesiveness

pneumoperitoneum factors

mechanical effects

modifications in splanchnic venous flow

reduction in hepatic arterial flow

gas turbulence

gas specific effects

modification of tumour cell biology by carbon dioxide

alterations to peritoneal macrophage function

peritoneal pH changes

operative/surgeon factors

surgeon experience

degree of tumour manipulation and exfoliation of tumour cells

haematogenous dissemination of tumour emboli from cut surfaces of

veins/lymphatics

wound factors

direct cell implantation in trocar wound

non protected and forced extraction of tissue through wound

instrument contamination with viable tumour cells

5.5. FUTURE DIRECTIONS

It is important to confirm the oncological outcomes of laparoscopic tumour surgery using further small animal models, ideally incorporating a tumour cell line derived from an intraabdominal tumour such as a colon adenocarcinoma. The possibility of haematogenous spread to port sites also requires further investigation.

5.5.1. CURRENT MODELS

Current experimental models have helped to elucidate many of the mechanisms that may contribute to the development of port site metastases. Each of these models however has significant limitations, and the applicability of the findings to a clinical setting remains uncertain.

5.5.1.1. Limitations of small animal models

Small animal models (rat, hamster, mice) of laparoscopy are limited predominantly by size. This prevents the generation of significant differences in intraabdominal pressures and prohibits all but the most simple of laparoscopic procedures (or mobilisations with extracorporeal procedures). In addition there is relatively little real distance between the sites at which trocars are placed. This implies that direct contamination by instruments, or direct tumour spread may result in an artificially high incidence of port site tumours.

Despite these problems, in murine models of syngeneic species members are genetically equivalent to other members of the same species and therefore suitable model for investigation of the role of immune modulation in tumour studies ¹³.

The interpretation of experimental studies is difficult because of the different numbers of tumour cells used and the different tumour cell lines. Tumour cell lines used in most studies are also not directly applicable to humans. Most authors have used a

cell suspension model however, intraperitoneal injection of cells results in spread out irregular growth, making accurate assessment of peritoneal tumour implantation difficult 40. One other limitation of the cell suspension model is that it assumes that a high number of intraperitoneal tumour cells is present in all cases. The large inoculum often results in a very high incidence of abdominal wound tumours and metastases at other intraperitoneal sites. This is emphasised by the findings of *Wu et al* who demonstrated that there is a relationship between the size of the tumour inoculum and the effects of the pneumoperitoneum in determining tumour cell growth rates 115. Furthermore the cell suspension model does not allow for assessment of how tumour cells become liberated from the primary tumour 168.

New models such as that described by *Allendorf et al* 13 and *Berger et al* 27 in which a laparoscopic assisted caecectomy is performed in rats may provide a more realistic, and yet cost effective, model in which to test these strategies. If a suitable colon cancer line can be developed this would make the ideal small animal model, although attempts to induce such a tumour have been unsuccessful to date. This is because although colonic cancers chemo-induced by subcutaneous chemical carcinogens come close to the natural evolution of human colonic cancer 164 there are significant (>6 month) delays in tumour development and tumour growth is not guaranteed in 100% of animals or necessarily consistent. Similarly, *Gutt et al* have described a number of laparoscopic procedures in rats which could make valuable models of laparoscopic tumour surgery if a suitable native tumour cell line was identified 105.

Studies from this thesis which require further investigation in a small animal model include the effects of tumour cell adhesion. This would include the effect of different ports (plastic or metal) on tumour cell adhesion *per se* and the *in vivo* effects of manipulating tumour cell adhesiveness. Collaboration with the Queensland Institute

of Medical Research is currently being undertaken to enable the original DA rat tumour line to undergo modification so that it no longer expresses tumour cell adhesion molecule (CD44). This will allow further studies to investigate the role of tumour cell adhesion in the development of port site tumours. Small animal models are also appropriate for the investigation of haematogenous spread to port sites.

5.5.1.2. **Large animal models**

Large animal models such as the porcine models of laparoscopic assisted colon resection described by *Bessler et al* ³⁴ and *Reymond et al* ²²⁶ offer more realistic surgical procedures for research than small animal models. They are also however, limited by the current lack of a native porcine tumour which would be necessary to investigate the effects of the pneumoperitoneum on tumour biology. For this reason such models are best suited for the investigation of physiological effects of the pneumoperitoneum and are ideal for studies investigating the dispersion of cells during laparoscopy. Large animal studies are needed to further investigate the immunological and physiological consequences of helium vs carbon dioxide insufflation and to confirm that outcomes from small animal studies can be extrapolated to a more human-like species. Further, large animal studies should be performed to confirm the safety of helium insufflation and in particular to investigate the possibility of gas embolism. Specifically the effect and incidence of venous gas embolism during laparoscopy needs to be determined and the effect of developing a postoperative pneumothorax/ subcutaneous emphysema post laparoscopy. Clinical studies are required to confirm where possible that the outcomes of various experimental studies can be applied to clinical practice. Finally it should be remembered that helium is only one inert gas and other gases such as argon and nitrogen should also be investigated as they may offer cost or other advantages hitherto unknown over helium for laparoscopic insufflation.

5.5.2 CLINICAL STUDIES

Ultimately verification of these findings can only be made by clinical studies. There is sufficient evidence to progress to investigation of the influence of different insufflation gases on physiological processes during laparoscopic surgery and potential advantages of inert gas (helium) insufflation. In particular parameters such as post operative pain, clinical recovery and peritoneal immunity require investigation. Because of the low incidence of port site tumours randomised controlled multicentre trials would be required to assess the incidence of tumour implantation post laparoscopy with inert gas.

Clinical trials are also necessary to establish the efficacy of other preventive strategies outlined in this thesis. This includes the efficacy and safety of the use of intraperitoneal agents such as povidone iodine and the efficacy of port site wound treatments.

SECTION V

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