

Do iatrogenic serosal tears result in small bowel perforation in a rabbit model?

Dr. Ming – Chih Tsai

MB BCh, DA (SA), FCS (SA)

Student number: 00 00 123 N

Department of Surgery

University of Witwatersrand

Supervisors :

Dr. M Brand, Department of Surgery, University of Witwatersrand.

Prof GP Candy, Department of Surgery, University of Witwatersrand.

Candidate's declaration

I, Ming - Chih Tsai declare that this dissertation is my own work. It is being submitted for the degree of Master in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Dedication

This dissertation is dedicated to my family.

Abstract

Introduction

A common decision faced by surgeons during laparotomy is how to manage iatrogenic small bowel serosal tears. Surgical dogma dictates that serosal tears should be repaired; as not doing so may result in the bowel becoming ischemic and likely perforation. This would result in significant morbidity and potentially mortality and re - laparotomy with either repair of the perforation or bypass management options. However, we do not know if serosal tears result in a localised area of bowel ischaemia as no study has been performed to test this assumption.

Methods

I designed two experiments wherein I determined whether or not serosal tears result in localized bowel ischemia in a rabbit model using adult - size white rabbits. The first experiment demonstrated the intra - luminal pressure required for perforation to occur at the site of a small bowel serosal tear. In the second experiment I investigated whether delayed serosal tears occur *in-vivo*. The WITS Animal Ethics Committee approved both experiments.

The rabbit model consisted of adult - sized white rabbits were subjected to a mid – line laparotomy under ketamine - xylazine anaesthesia with the assistance of the Central Animal Service veterinary nurses and staff. Serosal tears of various lengths and circumferences were created while wearing 2.5x surgical loupes.

Rabbits were euthanized at the end of the procedure. The bowel was harvested for histology to check for viability of the mucosa.

Experiment 1. Twelve rabbits were used in this experiment. In order not to compromise the vascular supply of the bowel, the bowel was clamped approximately 3cm away on either side of the serosal tear. Two 18 gauge jelcos were inserted into the isolated bowel lumen. One jelco was used to incrementally infuse the normal saline solution. The other jelco was connected to a manometer or a pressure transducer and used to measure intraluminal pressure generated by the normal saline.

Experiment 2. A mid – line laparotomy was performed as above on another ten rabbits, of which a 4 cm x 100 % circumferential serosal tear was created on a segment of small bowel. Their abdomens were closed with PDS suture and the rabbits were observed after the operation for signs of bowel perforation and then terminated at either 72 hours or 120 hours to harvest the bowel for histological examination.

Results

The mean intraluminal pressure for bowel perforation was: 26.4 cm H₂O for sham rabbits, 23.0 cm H₂O for 1cm serosal tears in length and 23.3 cm for 4cm in length. There is no statistically difference between the mean intraluminal pressures of 1cm and 4 cm. For serosal tears involving the circumference with 1 cm in length, the intraluminal pressure of perforation was: 27.7 cm H₂O for 25 % circumference, 30.6 cm H₂O for 50 % circumference, 23.8 cm H₂O for 75 % circumference, 25.4 cm for 100 % circumference. At normal physiological intraluminal pressure of 6 – 8 cm H₂O during peristalsis, no perforation of the serosal tear present in this experiment. Findings of the second experiment also demonstrated no obvious bowel perforation at 72 hours and 120 hours after the operation. Histological examination of the small bowel serosal tear site also showed that the mucosa and the submucosal layers, at the tear sites, were intact and viable without signs of ischemia.

Conclusion

In the acute setting, small bowel serosal tears up to 4 cm in length or, up to 100% of the bowel circumference did not perforate at physiological pressures of 6 - 8 cm H₂O, which are encountered during normal peristalsis.

Furthermore, serosa of the small bowel does not appear to affect the tensile strength of the bowel wall nor does it contribute significantly to mucosal and submucosal blood supply. No small bowel perforation occurred within 72 - 120 hours after creating serosal tears.

Acknowledgements

I acknowledge Dr A. Grieve, who is currently a consultant in the Department of Paediatric Surgery at the University of Witwatersrand, contributed significantly by way of advice and technical assistance with the surgical techniques used in these experiments. I acknowledge Sr. M - A. Costello, and all the other veterinary nurses who assisted me in many aspects of the operation and care of the animals during this study.

And finally to my supervisors, Dr. M. Brand and Professor G. Candy, who helped me with concept design, statistical analysis of my data and final editing of my dissertation.

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CHAPTER ONE: INTRODUCTION

A common problem faced by surgeons during laparotomy is how to manage serosal tears of the small bowel. Our fear is that if we do not suture the serosal layer, the bowel may perforate, causing peritonitis, and require a further laparotomy for repair of the perforation. However, the practice of repairing serosal tears is not evidence based. No study has been performed to determine whether, following a serosal tear, the bowel wall is weakened; or whether the blood supply in the mucosal and submucosal layer is affected, resulting in the small bowel being more prone to perforation at physiological intraluminal bowel pressures encountered during peristalsis (1).

From anatomy textbooks we know that, within the bowel wall, the muscular and submucosal layers are strongest (2). The bowel wall receives blood, supplied in a radial manner from branches of mesenteric arteries, which penetrate the serosa and muscular layers, terminating as a submucosal plexus (2). It is hypothesized that bowel perforation may occur at the site of a serosal tear, because local ischemia weakens the area of bowel. This ischemia may be caused either by local devascularisation or by the intraluminal pressure of the bowel during peristalsis (1). There appears to be no data as to the length of serosal tear which will result in bowel perforation.

It is recommended that large serosal tears (i.e. >80% of small bowel circumference) should have a segmental bowel resection as it is assumed that the blood supply of the submucosa is inadequate and bowel perforation may occur with increased intraluminal pressure during peristalsis (3) . This assumption has not been tested in an animal or human model.

Repair of the serosal tear with sutures predisposes the patient to anastomotic leaks or delayed strictures. The risk of strictures occurring post – anastomosis is approximately 2 - 4 % (4) and the mortality associated with an anastomotic leak is 22% (5). The overall morbidity associated with a stoma, often the choice of treatment to divert bowel content away from repaired areas or exteriorize the leak once the anastomotic leak has been detected, may be as high as 41 % (6). Therefore, if we could avoid having to repair these tears, such complications may be avoided.

The aims of my research were to determine:

- the length and circumference of small bowel serosal tears which would perforate at physiological intraluminal pressures.
- whether signs of ischaemia, as assessed by histological assessment of the serosal tear, were associated perforation.
- whether such created serosal tears were associated with delayed perforation over a period of five days immediately following the tear.

CHAPTER TWO: METHODS

It would be unethical to conduct human studies to intentionally create perforations of bowel and predispose patients to anastomotic leaks, delayed strictures, or severe infection, which potentially could result in death. Therefore, it was necessary to conduct these studies in animal models. Ethics approval for the study was obtained from the Animal Ethics Committee of the University of the Witwatersrand with approval number ...(See appendix 1).

Pilot study 1. A rabbit model was tested in a pilot study to assess the extent of bowel ischemia, in relation to the size of the serosal tear 24 hours after laparotomy. A medium – sized adult rabbit weighs 3500g to 4500g, equivalent to the average weight of a new born human baby. This model was selected and used in all experiments.

General anaesthesia was induced with ketamine 100mg/kg, together with xylazine 5mg/kg administered intramuscularly by the veterinary nurses in the Central Animal Service Laboratory operating theatre. After laparotomy, the bowel was exposed and a 1cm longitudinal serosal tear was created on a segment of small bowel. The surgeon worn surgical loupes with 2.5x magnification to ensure that only the serosal layer of bowel was incised. The bowel was re - inserted and the abdomen closed. Analgesia with buprenorphine 0.05mg /kg subcutaneously, was administered 8 hourly to manage pain.

The abdomen was reopened 24 hours later and the bowel exposed and re - examined. No bowel perforation or necrosis was observed and the animal was euthanized with sodium thiopental (Euthanase) 1ml/kg intravenously at the end of the laparotomy.

Pilot study 2. This experiment was undertaken in a second rabbit, anaesthetized and prepared as described above. A serosal tear was created as described in pilot study 1. A sham animal was also used in which no serosal tears were created to assess at which pressure uninjured bowel wall perforated.

The bowel was clamped on either side of the serosal tear, approximately 3 cm away from the tear to ensure the vascular supply of the bowel was not compromised. Two 18 gauge peripheral intravenous catheter needles (Jelco), were inserted into the bowel lumen. One jelco was used to incrementally infuse normal saline solution. The second jelco was connected to a manometer and used to measure intraluminal pressure generated by the normal saline. (Figures 1 and 2) In this manner, by injecting normal saline into the isolated segment of small bowel, the intraluminal pressure created during peristalsis was simulated (4).

Once the intraluminal pressure causing bowel perforation was recorded, the length of serosal tear was increased by 1 cm. This was repeated up to a 4cm length of serosal tear. (See Figure 3). This was done to determine the minimum length of tear that perforates at normal physiological pressure.

In another segment of small bowel I created a 25% circumferential tear and measured the intraluminal pressure required to perforate the bowel. In successive tears the circumference of the tear was increased by 25% until 100% of the circumference was reached. (See Figures 4, 5, 6). This second pilot animal was euthanized, with Euthanase 1ml/kg intravenously, as for the initial pilot study.

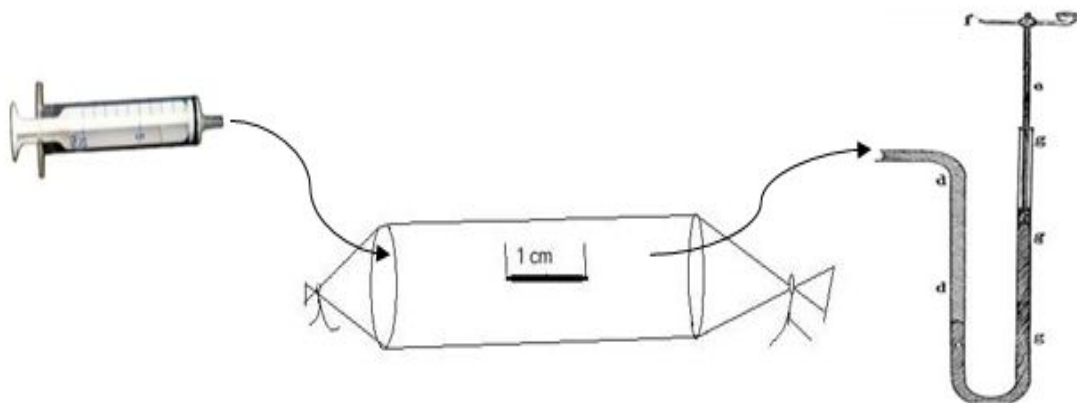


Figure 1: Experimental apparatus and set - up illustrating clamped off bowel section with a 1 cm tear of 0% circumference. The pressure was increased and pressure monitored using the water manometer shown on the right side of the Figure. See Figure 2.

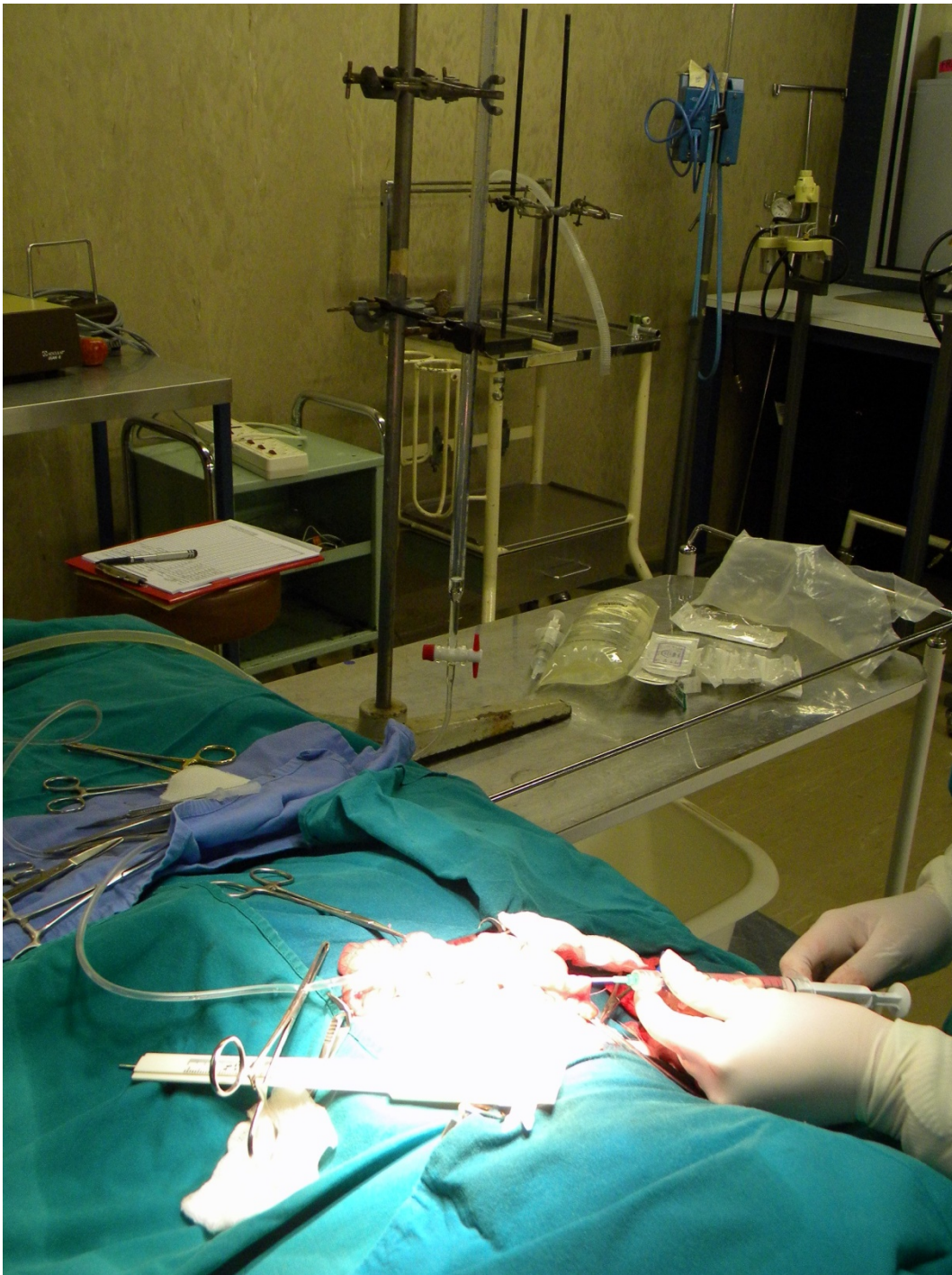


Figure 7.1 : Photograph showing a typical set - up of the experiment in the Central Animal Service laboratory, as illustrated in Figure 1.

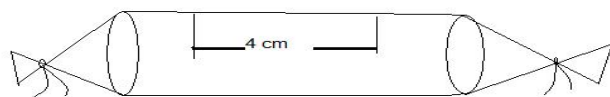
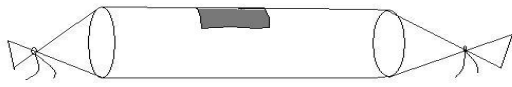


Figure 2: Section of small bowel with 4 cm serosal tear of 0% circumference.

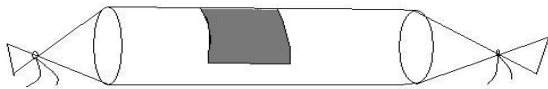
a.



b.



c.



d.



Figure 3, 4, 5, 6: Serosal tears (length and circumference (%)): a. 1cm x 25%; b. 2cm x 25%; c. 1cm x 50%; d. 1cm x 100%.



Figure 7 . 2 : Photograph showing the creation of a serosal tear created during the experiment.

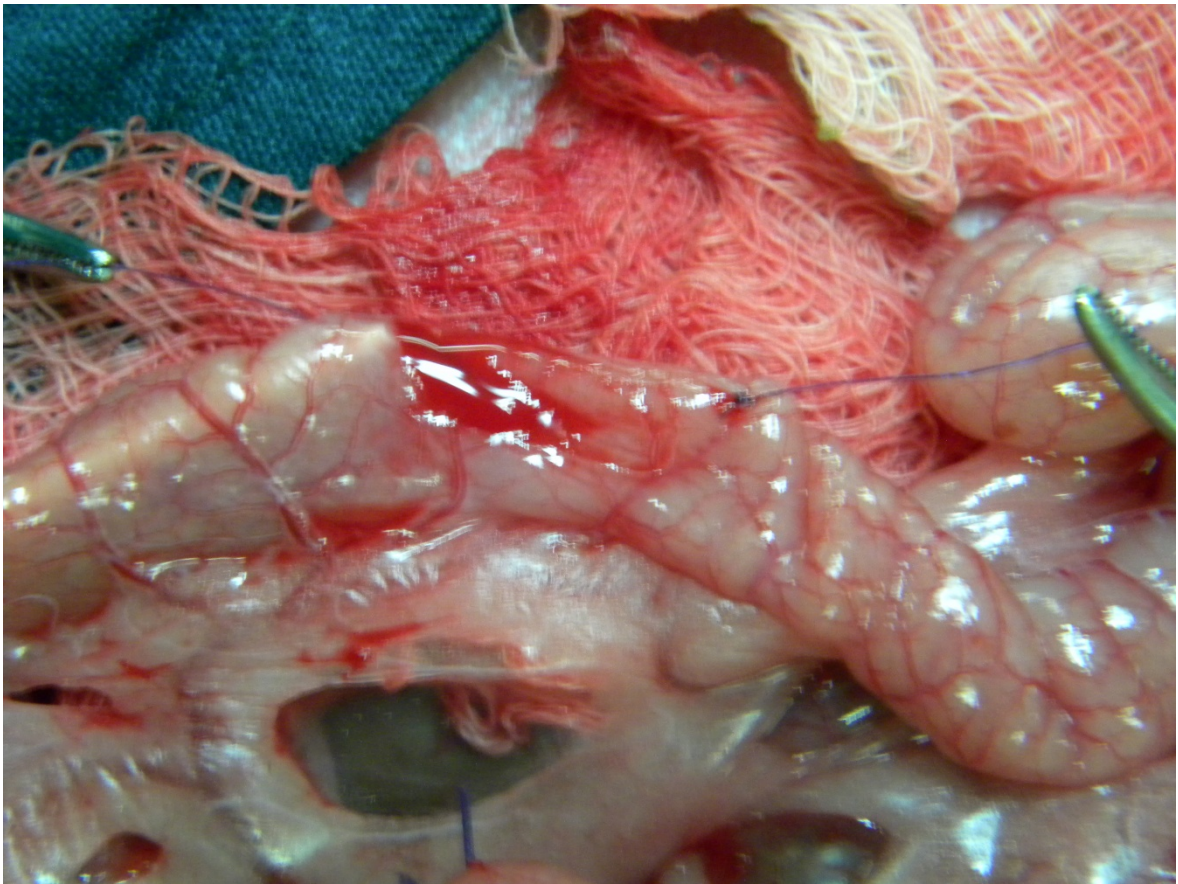


Figure 7 . 3. Photograph of a small bowel with 1cm long and 50 % circumferential serosal tears marked on either side with a stitch.

Experiment 1: Determining at which pressure serosal tears consistently perforate.

Once I determined the intraluminal pressure at which the bowel perforated, I performed the same experiment in 11 more rabbits to reach statistical significance and to confirm our pilot study findings. Normal saline was injected and the intraluminal pressure was increased incrementally. (See Figure 7).

All readings were recorded until the bowel perforated. In six of the 12 rabbits the perforation pressure was measured using both a water manometer and a pressure transducer. Their readings were the same, however the transducer was easier to use and hence was used in the remaining rabbits.

To minimise the number of animals used several isolated sections of small bowel were tested from each animal. One rabbit was used for each perforation pressure, testing different tear lengths, and the percentage circumference of tear. Various lengths of serosal tears, both circumferential and longitudinal were created in separate segments of bowel throughout the whole length of small bowel including jejunum and ileum. All tears were 1 cm long and increased from 1 cm to 4 cm long in 1 cm increments. Circumferential tears, 1cm wide, removed 0%, 25 %, 50 %, 75%, and 100% of circumference of the bowel. (Figures 3 and 4).

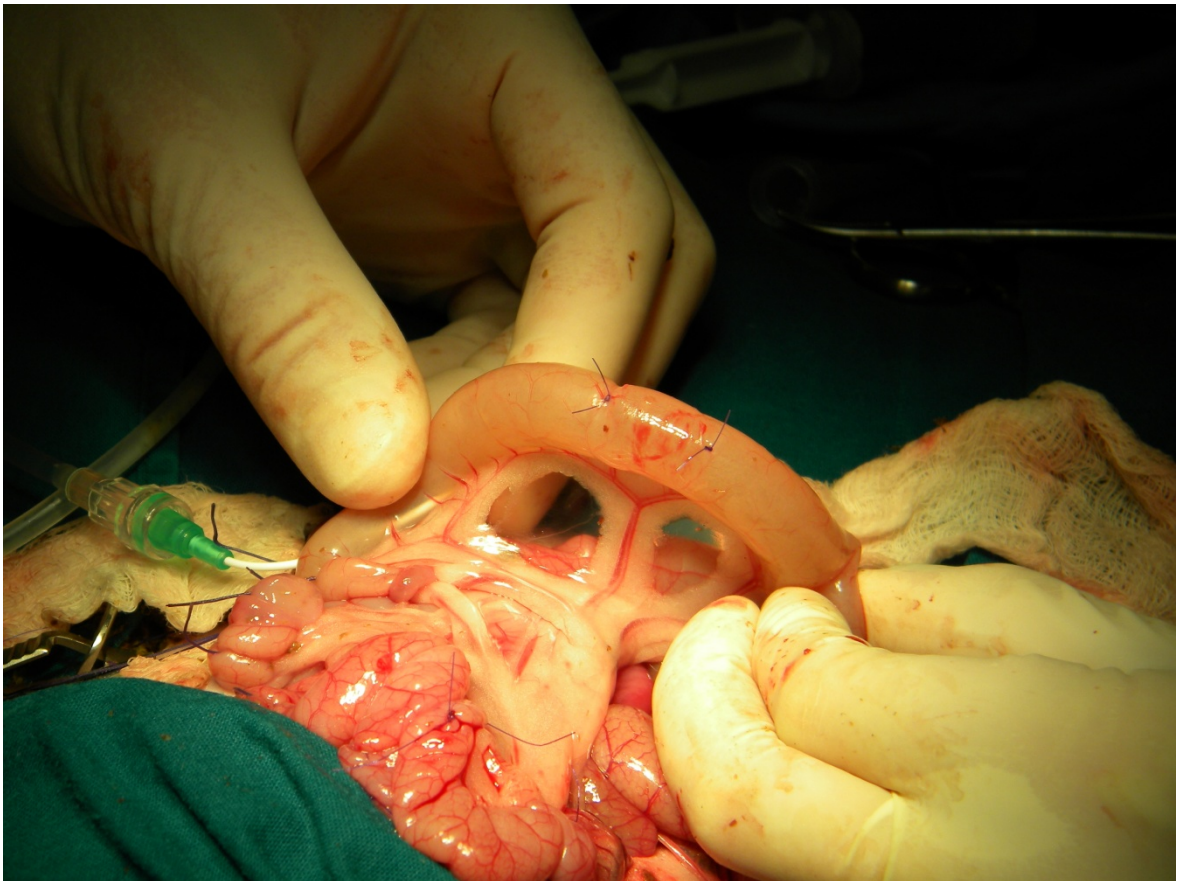


Figure 7 . 4: Photograph to illustrate the distension of the isolated segment of small bowel when injecting normal saline to determine the perforation pressure.

The rabbits were kept alive under general anaesthesia using isoflurane during the operation to ensure a continuous blood supply to the bowel and perfusion of the mucosa. At the end of the experiments the animals were euthanized with Euthanase 1ml/kg intravenously at the end of the measurements.

Specimens of the bowel were submitted to a surgical pathology laboratory to confirm that the tear only involved the serosal layer, and not the deeper layers, as well as to assess whether any signs of ischemia were present at the site of perforation.

Experiment 2: Determining whether serosal tears develop delayed bowel perforation.

The final experiment was designed to assess whether the created serosal tears resulted in the delayed perforation of small bowel, specifically at 72 and 120 hours after the laparotomy.

The largest serosal tear, 4 cm long was stripped 100% circumferentially in a single segment of bowel in 10 rabbits, as described in the previous subsections.

The rabbits were allowed to recover from general anaesthesia. They were then placed under 24 hour care by myself and the nursing staff in the Central Animal Service facility. Normal diets were given. Laparotomy pain was controlled with opioids given by the veterinary nurses. Six hourly observations were done to assess the rabbits' behaviour and to monitor the dietary intake.

Any abdominal pain that was not controlled by analgesia or signs of bowel perforation, would have lead to early termination of the animals followed by a post mortem, specifically to assess the state of the serosal tear.

Five rabbits were eventually euthanized 72 hours after their index laparotomy while the remaining five rabbits were euthanized 120 hours after the index laparotomy. All rabbits were weighed at the beginning and end of each experiment to assess the degree of weight - loss.

A repeat laparotomy was performed on the euthanized rabbits in order to assess any possibility of bowel perforation, degree of bowel obstruction, and the extent of adhesions. The small bowel segment spanning the serosal tear was submitted for histological examination to analyse the degree of ischaemia and / or healing.

Statistics

All data was collected in an excel sheet. The Student t – test was used to calculate p – values for the serosal tear length and pressure at which perforation occurs comparisons. Readings from the water manometer and the pressure transducer were correlated with one another, to allow the data to be converted from millimetre mercury to centimetre of water.

CHAPTER THREE: RESULTS

The mean intraluminal pressure for bowel perforation to occur was: 26.4 cm H₂O in the sham animal, 23.0 cm H₂O for 1 cm long serosal, 23.3 cm for 4 cm long serosal tear. For 1 cm long serosal tears involving various circumferences the intraluminal pressure of perforation is : 27.7 cm H₂O for 25 % circumference, 30.6 cm H₂O for 50 % circumference, 23.8 cm H₂O for 75 % circumference, 25.4 cm for 100 % circumference. (See Table 1).

No perforation occurred at normal physiological pressure of the small bowel. Physiological intraluminal bowel pressure is 6 to 8 cm water (1). All serosal tears perforated at supraphysiological pressures between 20 to 30 cm water. (See Table 1).

Figure 8 demonstrates the vital signs and laparotomy times for each group of animals, they were not statistically significantly different between any group.

Comparing the length of serosal tears and their perforation pressure with the student t - test demonstrated a p - value of 0.8, in other words the length or circumference of serosal tear made no significant difference to the pressure at which it perforated.

Two sets of results illustrated in the following diagrams. Raw data illustrates the actual measurements taken both by the water manometer and the pressure transducer. Both sets of results were correlated with a statistics formula and given the adjusted measurements. Subsequently, all readings have both correlated mmHg and cm H₂O values. (See Figures 9 to 12).

Size of tear	Control	1 cm length	4 cm length	25 % circumference	50 % circumference	75 % circumference	100 % circumference.
No. of tears	7	20	14	23	20	16	12
Pressure of perf.(cm H ₂ O)	26.4 (+/- 6.6)	23.0 (+/- 5.11)	23.3 (+/- 4.2)	27.7 (+/- 4.4)	30.6 (+/- 5.4)	23.8 (+/- 6.2)	25.4 (+/- 6.9)

Table 1: Table illustrating in pressure at which various size of serosal tears perforate.

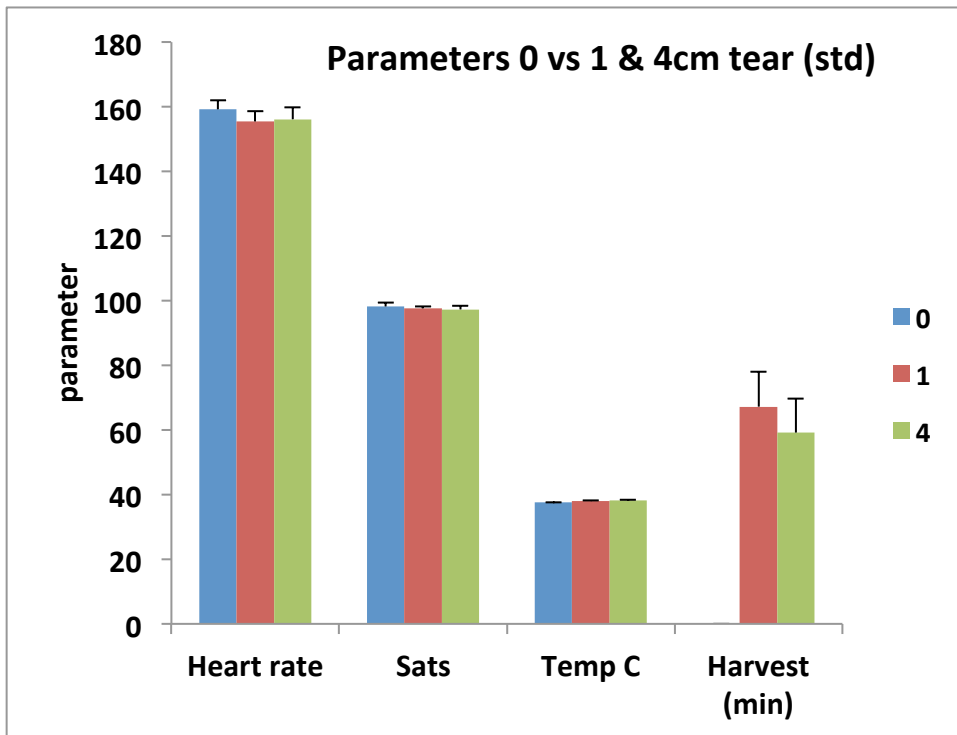


Figure 8: Graph illustrating the vital signs and the harvesting time for the animals during the experiment.

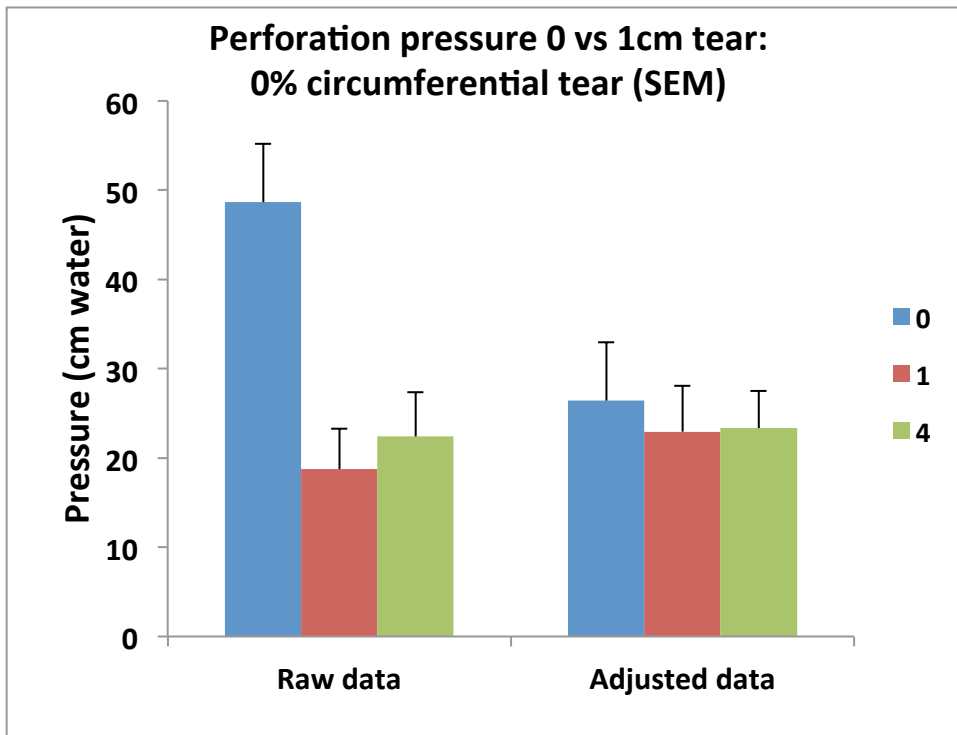


Figure 9 : Graph illustrating the pressure of perforation in the group of longitudinal serosal tears of 1 cm versus 4 cm versus control group in centimeter of water.

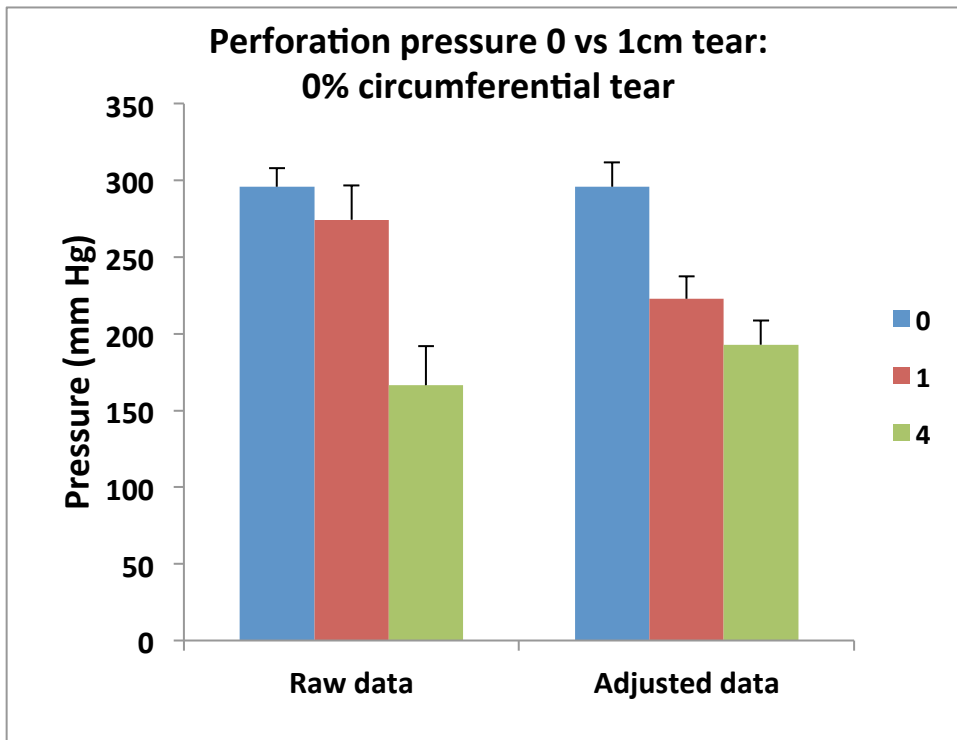


Figure 10 : Graph illustrating the pressure of perforation in the group of longitudinal serosal tears of 1 cm versus 4 cm versus control group in millimeter of mercury.

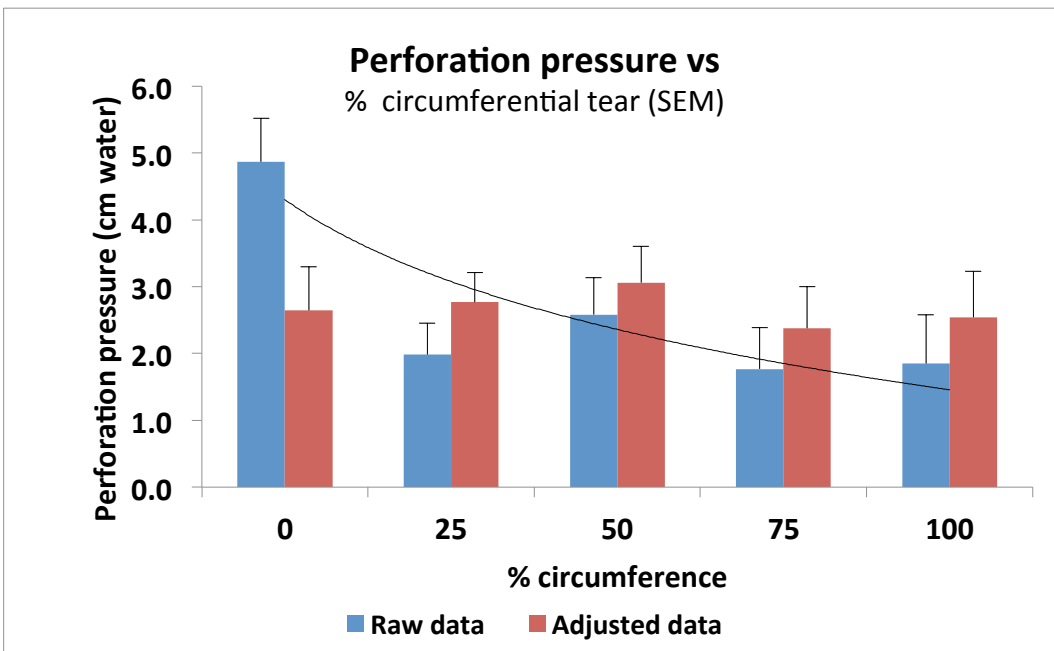


Figure 11 : Graph illustrating the pressure of perforation in the group of circumferential serosal tear of 25 % versus 50 % versus 75 % versus 100 % versus sham group in centimeter of water.

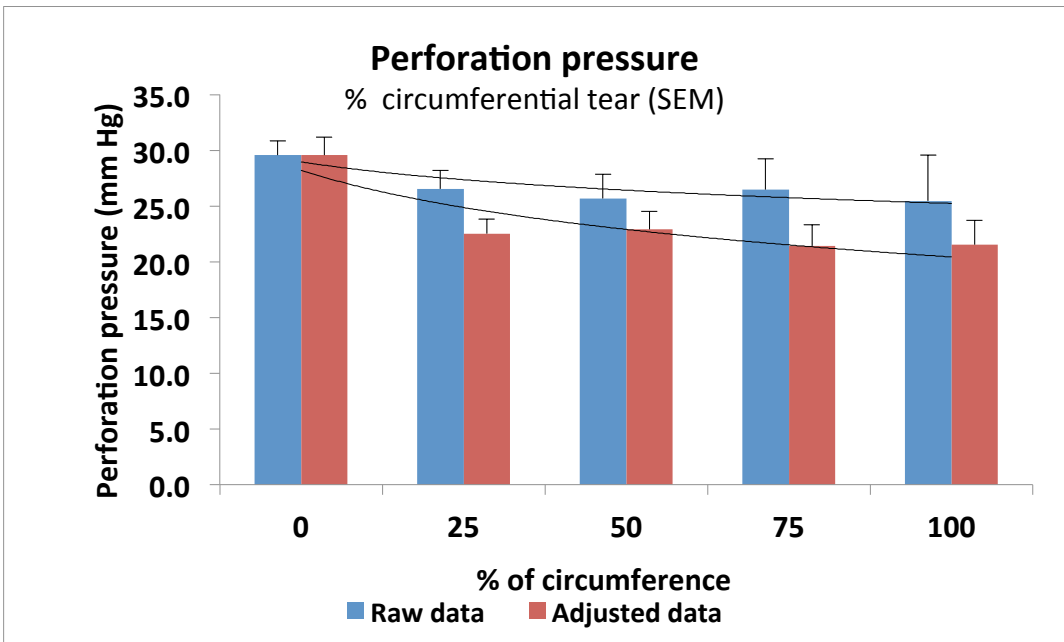


Figure 12 : Graph illustrating the pressure of perforation in the group of circumferential serosal tear of 25 % versus 50 % versus 75 % versus 100 % versus control group in millimeter of mercury.

Histologically, the control specimen demonstrated similar structure and thickness of the small bowel wall layers in rabbits compared to human small bowel. (See Figures 13 and 14). The following were noted findings:

- The serosal layer was successfully dissected off the bowel. Inflammation of the bowel wall was visualized as mild infiltration of inflammatory cells in the mucosa and submucosa area.
- No obvious signs of ischemia were reported by the pathologists.
- During repeat operation in 72 and 120 hours, no signs of small bowel perforated noted. The degree of adhesion was similar to the control group, and no obvious dilated loops of bowel showing any possibilities of bowel obstruction.

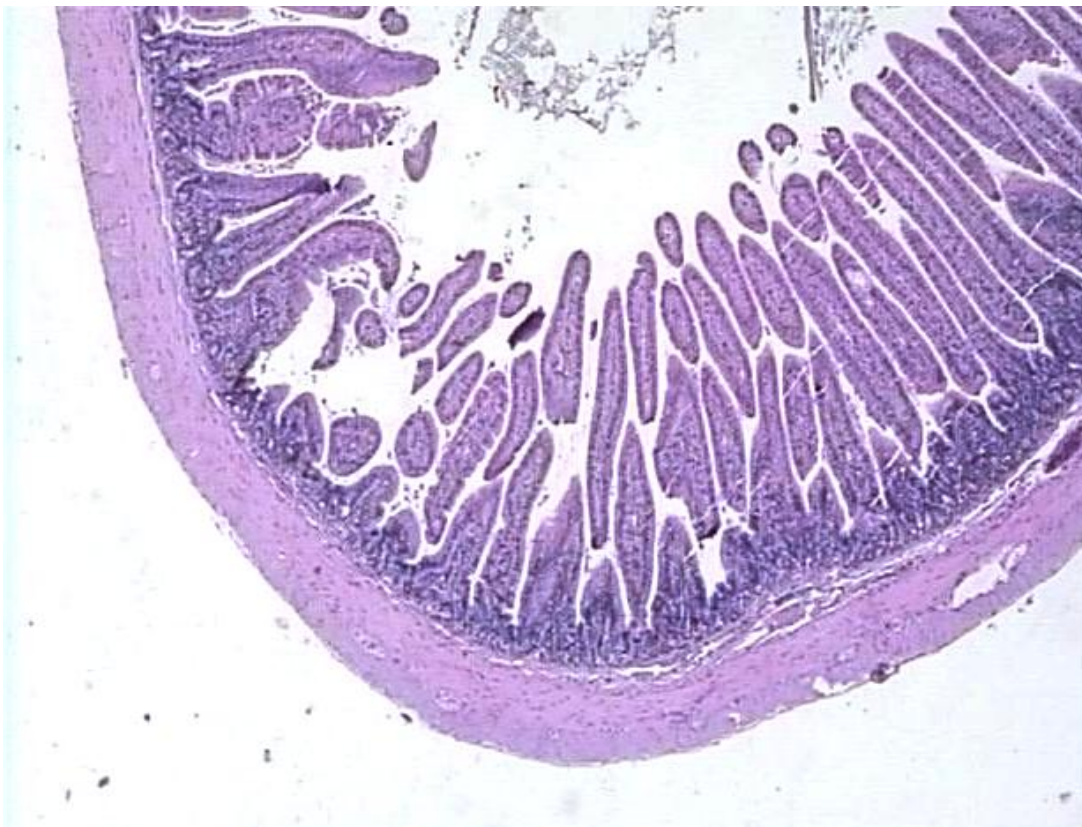


Figure 13: Histology illustrating the cross – section of the rabbit small bowel in the sham group.

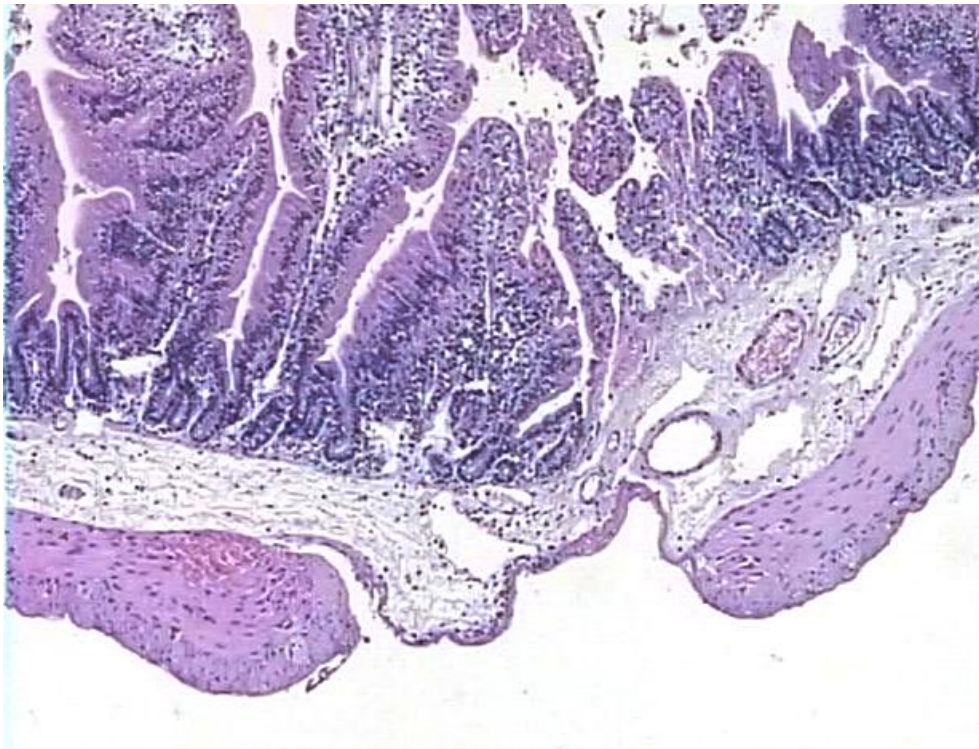


Figure 14: Histology of the rabbit small bowel illustrating the presence of serosal tears created during the experiment.

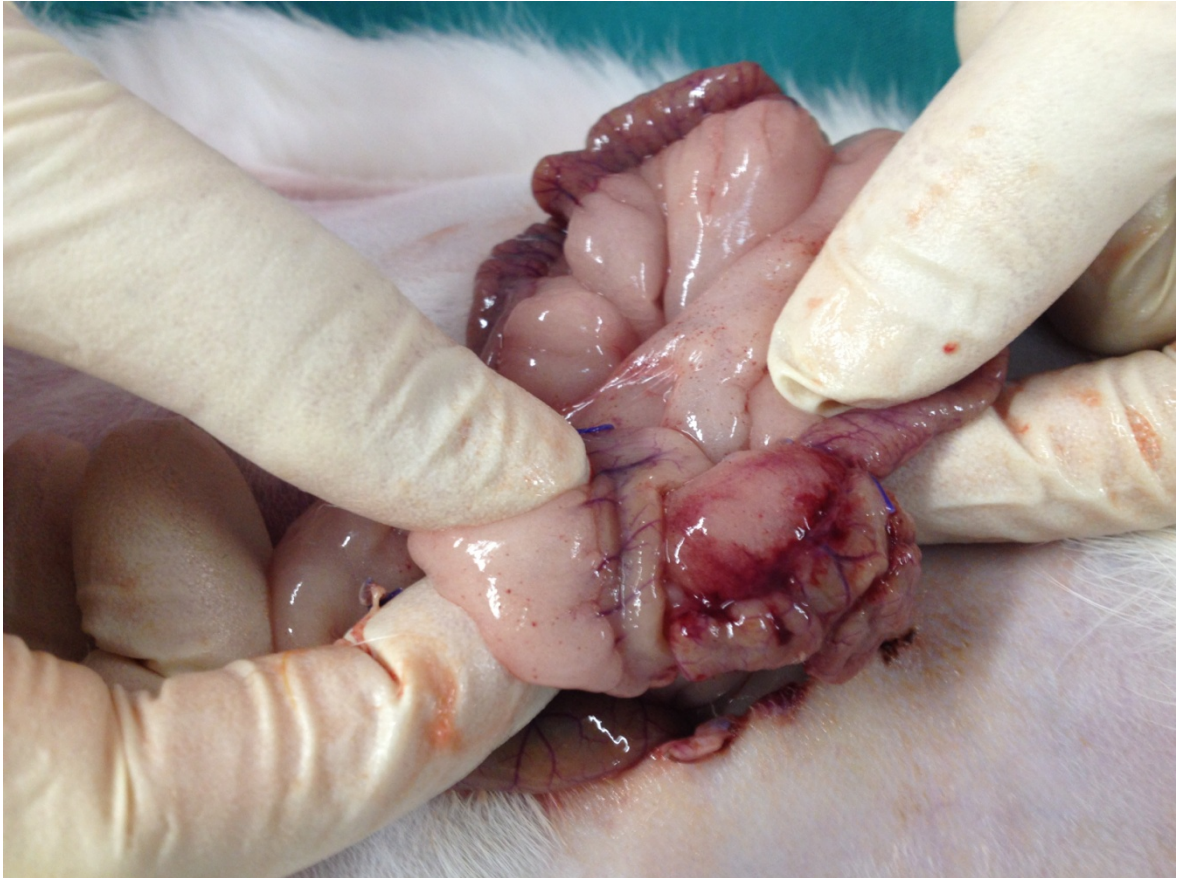


Figure 15: Illustration of the repeat laparotomy without signs of bowel perforation or obstruction.

Chapter 4: Discussion

The rationale of using sentient animals is that the small bowel of all mammals consists of 5 layers (Figure 16):

1. Mucosa - as the lining of the bowel lumen.
2. Submucosa - containing capillaries responsible for the blood supply of the bowel.
3. Muscularis propria interna – inner circular muscular layer which functions as the pump to push food forward during peristalsis.
4. Muscularis propria externa – outer longitudinal muscular layer also responsible for peristalsis.
5. Serosa - condensed connective tissue that provides the semi – rigid covering of small bowel.

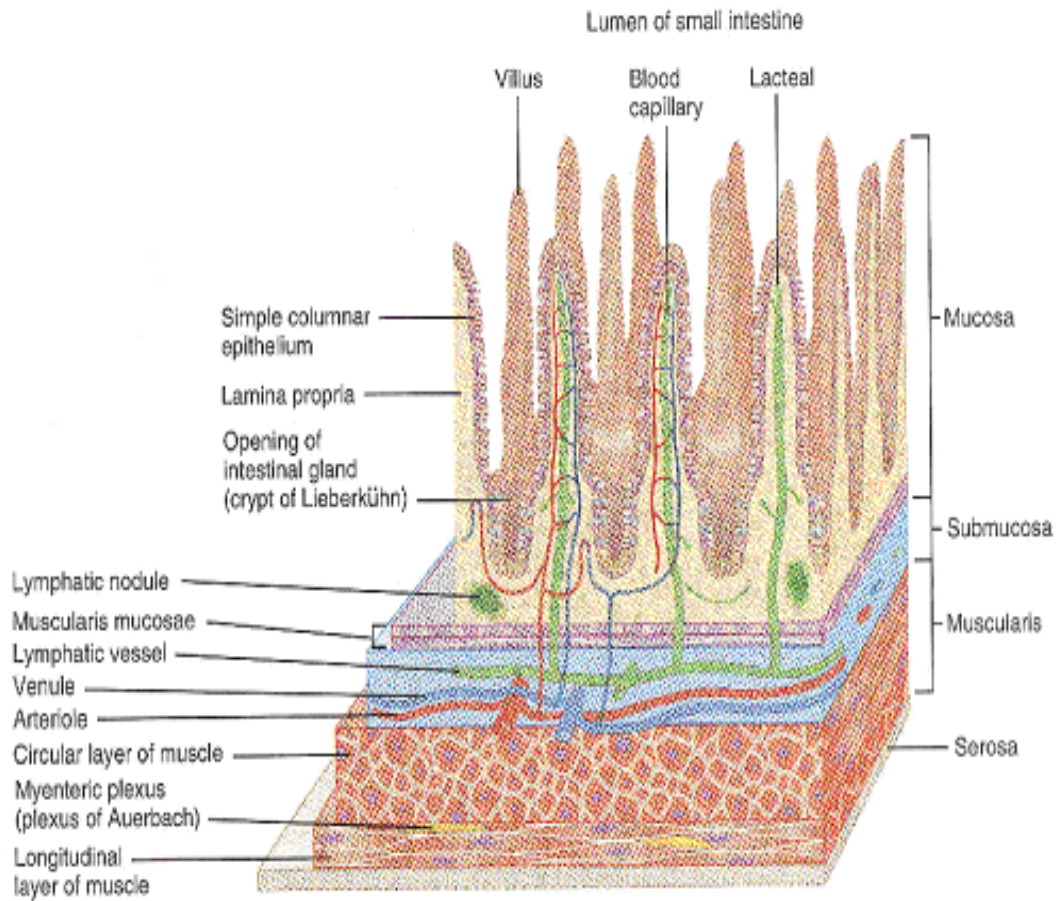


Figure 16. Histology of the small intestine a three – dimensional view of layers of the small intestine showing villi (7).

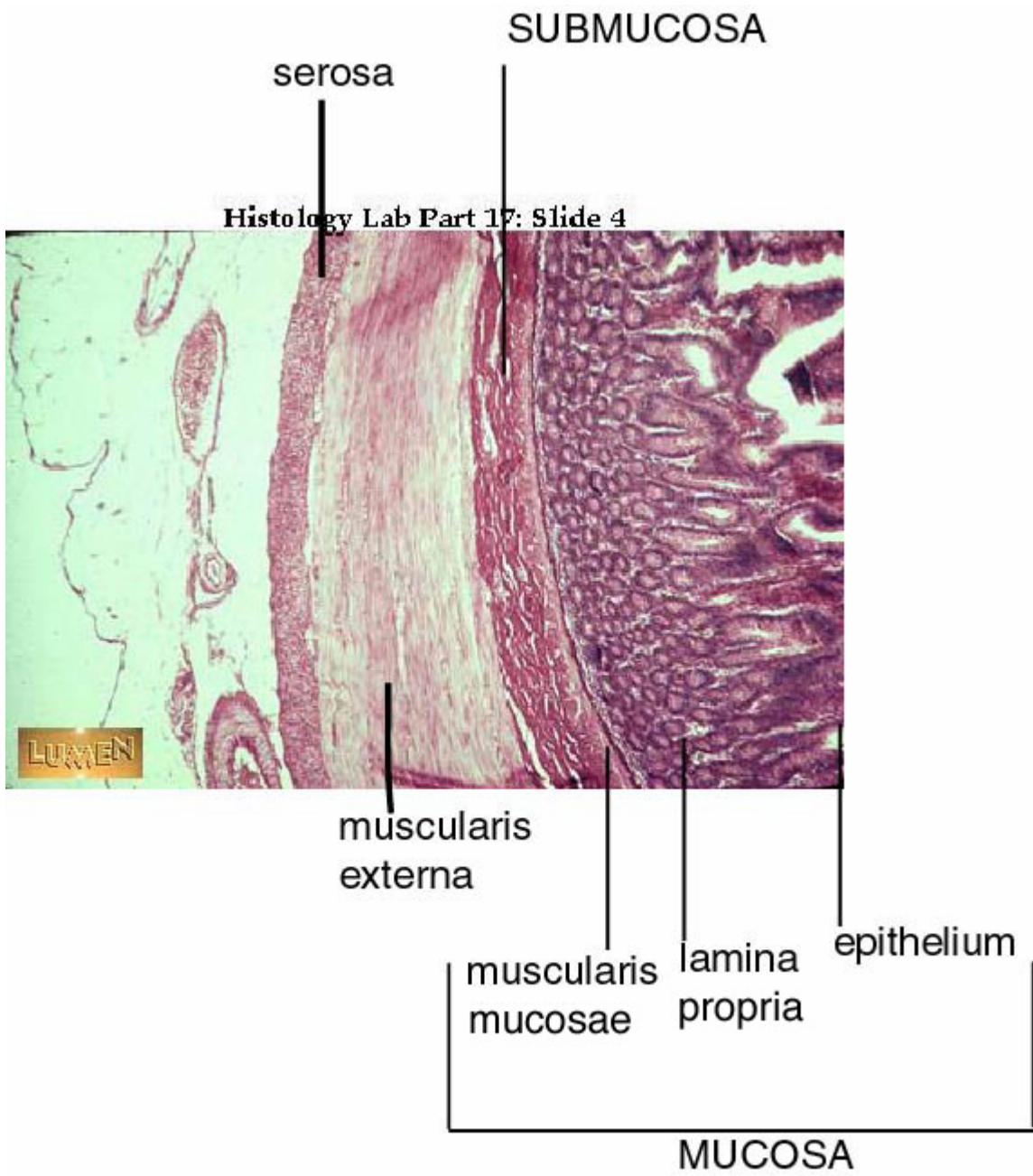


Figure 17: Histology of small bowel under low – field power microscopy (8).

Hence an animal model is an accurate reflection of human anatomy and function.

The data from the first experiment have shown that serosal tears perforate at intraluminal pressures beyond the physiological level. The histology of the small bowel demonstrated that the mucosal and submucosal layers were not ischaemic at the sites of perforation, in other words the tensile strength of the bowel was overcome to result in perforation rather than ischaemic changes resulting in areas of weakness.

The second in - vivo experiment demonstrated no adverse outcome with presence of large serosal tears on day three to day five post - operation. In fact the small bowel started to show clinical presence of fibrosis and adhesion representing a process of healing.

The limitation of this experiment was that the rabbits used in this animal model were all healthy animals without any risk factors for poor wound healing, such as sepsis, malnutrition, immune suppression etc.. This is a very specific situation that may not be representative of humans that have to undergo laparotomies.

Chapter 5: Conclusions

I have designed a rabbit model to assess whether or not serosal tears result in perforation at physiological intraluminal pressures and once perforation does occur, at which length and at which intra - luminal pressures it occurs. Furthermore, in a clinical setting where a serosal tear is likely to perforate at day 3 to day 5 after initial laparotomy, a second rabbit model was created in order to assess the small bowel at 72 hours and 120 hours after operation. This experiment demonstrated that delayed perforation does not occur.

It has been assumed that the longer the small bowel serosal tear the more likely the bowel is to perforate, and at lower intraluminal pressures, but this was not demonstrated in these animal studies.

My conclusions from this study are:

1. The intraluminal pressure at which serosal tears perforate is far beyond the physiological pressure of 6 - 8 cm H₂O.
2. The histology reports have shown that the submucosal and mucosal layers at the site of perforation showed no signs of ischemia.

Thus, I have demonstrated that serosal tears up to 4 cm in length or 100 % circumference do not perforate at a physiological intraluminal bowel pressures in an acute setting as well as after day 3 to day 5 post - op.

The serosal layer of the small bowel does not seem to contribute any tensile strength to the small bowel, nor do tears affect blood supply of the bowel in the mucosal and

submucosal layers.

In a clinical setting, there may be no indication to repair any size of serosal tears in a healthy individual. However this must be confirmed in human studies.

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8) UNIVERITY OF COLORADO BOULDER .Updated 11 / 09 / 2006 Digestive histology :
general characteristics, esophagus, stomach, small bowel.

Appendix – letter of approval from animal ethics committee.



STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2013/38/2B

APPLICANT: Dr M-C Tsai

SCHOOL: Clinical Medicine

DEPARTMENT: Surgery

LOCATION: Medical School

PROJECT TITLE: How long must a serosal tear be in a small bowel for it to perforate in a medium-sized adult rabbit model?


Number and Species

6 adult rabbits


Approval was given for the use of animals for the project described above at an AESC meeting held on 20110726. This approval remains valid until 20130725.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

1. The applicant must discuss the appropriate anaesthetic with the CAS Director

Signed:  Date: 01/08/2011
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed:  Date: 03/08/2011
(Registered Veterinarian)

cc: Supervisor: Dr M Brand
Director: CAS

Works 2000/1ain0015/AESCcert.wps



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ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2012/04/05

APPLICANT: Ms M C Tsai

DEPARTMENT: Department of General Surgery

PROJECT TITLE: Effect of the serosal tears on ischemia and necrosis of small bowel in rabbits after 72 hours

Number and Species

10 Rabbits

Approval was given for to the use of animals for the project described above at an AESC meeting held on **28 February 2012**. This approval remains valid until **28 February 2014**.

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

Conditions:

- Rabbits must be habituated to the experimental equipment to the satisfaction of the CAS Director before experimentation is started.
- Daily health evaluations of rabbits should be conducted using a 'Pain Assessment Score Sheet', rather than by means of measuring blood pressure.
- Blood pressure and other metrics should be taken only when rabbits show signs of sickness.
- The procedure for taking blood pressure should be reported to the Screening Committee before experimentation starts.

Signed: _____

(Chairperson, AESC)

Date: _____

13/3/2012.

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____

(Registered Veterinarian)

Date: _____

13/03/2012

cc: Supervisor:
Director: CAS