COMPARISON OF SIMPLE INTERRUPTED GAMBEE AND CRUSHING TECHNIQUES FOR INTESTINAL ANASTOMOSIS IN CATTLE

A thesis submitted for the Degree of Doctor of Philosophy of the University of Nairobi

BY

STANLEY MBAKA MBIUKI, BVM(NBI), MS(C.S.U.)

Department of Clinical Studies
Faculty of Vet. Medicine
University of Nairobi
P.O. BOX 29053,
KABETE

1984

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

STANLEY MBAKA MBIUKI

This thesis has been submitted for examination with our approval as University Supervisors

PROF. G.M. MUGERA

DR. P.N. NYAGA

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

TABLE OF CONTENTS (cont'd)

LIST OF TABLES

LIST OF TABLES (cont'd)

LIST OF TABLES (cont'd)

LIST OF FIGURES

LIST OF FIGURES (cont'd)

LIST OF FIGURES (cont'd)

LIST OF FIGURES (cont'd)

ABSTRACT

End-on approximating techniques for intestinal anastomosis have been found to be more advantageous than everting and inverting techniques by different investigators. The simple interrupted, gambee and crushing anastomotic techniques when evaluated independently have usually been found to be superior to the techniques with which they were compared. Comparison of simple interrupted, gambee and crushing techniques together have not been previously reported. The purpose of this investigation is to make a comparative study of the simple interrupted, gambee and crushing approximating techniques as used for intestinal anastomosis in cattle.

Twenty four male cattle were used to perform anastomoses in the small intestine utilizing simple interrupted, gambee and crushing techniques for comparative studies. Six anastomoses were performed in each of 8 animals using any one of the techniques to make a total of 48 anastomoses per technique. Therefore one hundred fourty four anastomoses were performed using the three techniques.

Evaluation procedures were carried out at 4, 14, 28 and 56 days post-operatively to assess the efficacy of the respective techniques.

Parameters investigated were: time per anastomosis and per stitch, adhesion formation, stenosis (external and internal) and histopathologic changes. From the histologic sections, epithelialization, inflammatory reaction, muscle layer approximation, cellular intensity and cell types were evaluated. Increase in thickness of anastomotic site relative to the adjacent and normal areas were also evaluated.

Results of adhesion formation, external stenosis, epithelialization, inflammatory reaction, and muscle layer approximation were given numerical scores to make the comparison objective and easier to visualize.

The results showed that time per suture was found to be a more reliable indicator of the time taken to perform an intestinal anastomosis. The simple interrupted technique took the least mean time per suture (36.6 sec.) followed by gambee technique

(49.5 sec.) and crushing technique was last with 57.4 seconds.

There were no adhesions in 77% of gambee anastomoses, 58% of crushing anastomoses and 27% of simple interrupted anastomoses. This clearly demonstrated gambee as the technique that induces least adhesions.

Stenosis as seen externally was absent in 62% of gambee anastomoses, 53% of simple interrupted anastomoses and 40% of crushing anastomoses indicating that gambee was a better technique in this regard.

Luminal stenosis evaluated from radiographs showed a mean narrowing of 30.6% in gambee anastomoses, 36% in simple interrupted anastomoses and 38.9% in crushing anastomoses, thus implying gambee was a superior technique.

Epithelial healing was more or less complete at 4 days in simple interrupted anastomoses followed by gambee anastomoses with crushing anastomoses having poor epithelial healing. However, at 14 days, the healing in the crushing anastomoses overtook that in the other techniques. Simple inter-

rupted anastomoses were inferior in the epithelial healing to the anastomoses performed using the other two techniques at 14 days. After 14 days all the anastomoses showed a similar degree of epithelial healing. Overall, gambee anastomoses had a slightly better epithelial healing than the other two techniques.

Generally, inflammatory reaction decreased with time. There was a greater decrease in gambee anastomoses followed by crushing anastomoses and simple interrupted anastomoses showed the greatest inflammatory reaction.

Muscle layer approximation apparently did not change with time but remained as it was after anastomosis for all techniques. However, gambee anastomoses showed the best alignment followed by crushing anastomoses and simple interrupted anastomoses had least efficacy in alignment of the muscle layer.

Cellular intensity was high in all the techniques at 4 days due to the increased number of cells in the early proliferative phase and late lag phase. The higher cell

counts at 56 days were due to wound contraction in the maturation phase. Cellular intensity was lowest at 28 days. Gambee anastomoses had the least cellular intensity followed by crushing anastomoses, with simple interrupted anastomoses having the highest cellular intensity probably due to the attendant greater inflammatory reaction.

All the 7 cell types observed were present in the 3 types of anastomosis regardless of technique used. None of the techniques had any cell in great excess of what was expected at various time intervals. The cell types were therefore not important in the comparative evaluation.

The index of increase in thickness was developed for this investigation. None of the techniques had any greater index of thickness than the other. However, the coefficient of increase in thickness, demonstrated that gambee anastomoses had the least spread of increase in thickness followed by crushing anastomoses. Simple interrupted anastomoses had the most widely spread increase in thickness.

Generally, gambee technique showed superior qualities. Crushing technique was second and simple interrupted was last.

ACKNOWLEDGEMENTS

I am very grateful to my major supervisor, Prof. G.M. Mugera for his fatherly advice and guidance from the planning to the completion of the investigation.

Special thanks go to my other supervisor, Dr. P.N. Nyaga for his time, constructive scientific ideas and suggestions during the course of my investigations.

I am greatly indebted to my wife, Mwonge for her encouragement and understanding during my study.

The help of the staff of the Department of Clinical Studies and Veterinary Pathology of the Faculty of Veterinary Medicine is appreciated. Thanks are also expressed to Dr. J.C. Kiptoon for his friendly support and to Mr. James Muraguri for his tireless efforts to provide laboratory assistance.

I am grateful to Prof. L.C. Vaughan for the use of the facilities of the Royal Veterinary College, London during the preparation of the manuscript.

The help of Miss Catherine Kariuki in typing the thesis is sincerely appreciated.

This work received financial support from the National Council for Science and Technology for which I am grateful.

1 INTRODUCTION

In an attempt to find the most suitable technique for intestinal anastomosis, surgeons have investigated numerous techniques including inverting, everting, overlapping and end-on approximating techniques either in single layer or double layers (Bennett & Zydeck, 1970; McAdams et al., 1970; Schillaci et al., 1979; Ellison et al., 1982; Richardson et al., 1982). Different investigators have compared various end-on approximating techniques with inverting and everting techniques and usually found end-on approximating techniques to be more advantageous (DeHoff et al., 1973; Reinertson, 1976; Somvanshi et al., 1980; Bellenger, 1982; Stashak, 1982). Studies on simple interrupted, simple continous and crushing end-on approximating techniques have recently been reported in dogs (Ellison et al., 1982).

According to the available literature, no investigation has been reported in the past comparing simple interrupted, gambee and crushing techniques. These techniques have been found to be more desirable when compared with inverting and everting techniques by different investigators working independently (Bennett & Zydeck, 1970; DeHoff et al., 1973; Reinertson, 1976; Richardson et al., 1982).

It was therefore proposed that a comparative study of simple interrupted, gambee and crushing end-on approximating anastomotic techniques be carried out to determine the most advantageous in all aspects of the surgical intervention in the correction of intestinal problems.

The time intervals choosen for the study were 4, 14, 28 and 56 days to make sure that the three phases of healing were included in the investigation. The 4 and 14 days intervals were from the lag and proliferative phases respectively. The other two intervals were from the maturation phase becuase structural healing is slow in this phase.

Parameters to be investigated in this study were:

1) Time to perform the anastomosis and time per suture.
2) Formation of adhesions and frequency of occurence of various degrees of adhesions.
3) Stenosis as seen externally and the frequency of various degrees of stenosis.
4) Luminal stenosis using contrast radiography.
5) Degree of epithelialization
6) Degree of inflammatory reaction
7) Degree of muscle layer alignment
8) Cellular intensity and cell types in the vicinity and along the anastomotic line.
9) Increase in intestinal wall thickness.

2 LITERATURE REVIEW

2.1 ANATOMY OF THE SMALL INTESTINE

Generally, the small intestine is composed of three parts: the duodenum, jejunum and ileum. The bovine jejunum is very long and has numerous close coils arranged as a festoon around the border of the mesentery. Caudally, before it joins the ileum, the bovine jejunum is prolonged by a "U" - shaped series of loops on an extension of the mesentery (Habel, 1975). The caudal coils of the bovine jejunum are very mobile because of their long mesenteric attachment (Nickel et al., 1973) as compared with the dog's jejunum which is made up of 6-8 large loops suspended by a long mesentery (Nickel et al., 1973; Ellenport, 1975).

A typical cross-section of the mammalian small intestinal wall has the following layers from outside inwards: serosa, muscular tunic, submucosa, muscularis mucosae and mucosa. The serosa is made of loose connective tissue invested by the mesothelium of visceral peritoneum. It completely envelopes the intestine except on the mesenteric side. The

muscular tunic has an outer longitudinal and an inner circular smooth muscle layers. In the connective tissue between the two layers are numerous parasymphathetic ganglionic cells and fibres making the mesenteric plexus. The submucosa is composed of fibroblasts, collagen and elastic fibres arranged in an irregular manner in bundles. Blood vessels and lymphatics are also present in the submucosa. Submucosa is the strongest layer of the intestinal wall (Lord & Varies, 1977; Jansen et al., 1982; Stashak, 1982; Ellison et al., 1982). The ganglionic plexus of the submucosa is scattered throughout the layer. The muscularis mucosae, like the main muscle layer, is composed of an outer longitudinal layer and an inner circular layer of smooth muscle cells, but these layers are thin and incomplete except in the dog.

The mucosal layer has permanent folds of mucous membrane (plicae circularis). On the surface of the permanent folds are villi. Lining the villous surface is an epithelial layer and immediately below is the lamina propria which harbours intestinal glands (crypts of Lieberkuhn). The glands open into pits between the bases of the villi and penetrate the mucosa as far as muscularis mucosae (Bloom and Fawcett, 1970; Dellman and

Brown, 1976). The surface epithelium of the villi and the crypts of Lieberkuhn is a continous single layer of six different cell types.

Columnar cells and goblet cells line the surface of the villi while Paneth's cells, columnar secretory cells, argentaffin cells and undifferentiated cuboidal cells line the crypt's surface. The undifferentiated crypt cells usually have high mitotic activity and serve as a source of differentiated cells. Newly formed cells migrated up from the crypts and at the junction of the crypts and villi, they undergo morphologic, biochemical and functional maturation so that absorptive columnar cells, goblet cells and Paneth's cells are formed. Cells migrating upwards push older more differentiated cells towards the tip of the villi and these older cells are eventually extruded into the lumen. The mitotic activity within the crypts is constant and is not influenced by enzymatic activity or amount of ingesta. Epithelium is renewed every 2-7 days (Watson & Sodeman, 1974; Dellman & Brown, 1976; Gyton, 1976).

Lamina propria is made up of loose connective tissue containing collagen, elastic and reticular fibres. It forms the core of the

villi and surrounds the intestinal glands. In the lamina propria are blood vessels, lymph vessels, and longitudinally oriented smooth muscle cells - extending from muscularis mucosae to the tips of the villi (Dellman & Brown, 1976). Cells present in the lamina propria include: macrophages, smooth muscle cells, lymphocytes, plasma cells, eosinophils, sometimes mast cells and in the ileum Peyer's patches (Bloom and Fawcett, 1970).

Blood supply to the small intestines is by branches of the mesenteric artery which penetrate the muscular layer sending some branches to this layer before continuing to the submucosa where they form a plexus. From the plexus short arterioles supply the muscularis mucosae and mucosal gland areas. Long arterioles supply tips of the villi and form a capillary network continous with venules (arteriovenous loop). Veins from the villi and periglandular capillary bed flow back to the submucosal venous plexus. The submucosal venous plexi form veins which traverse the muscular layers parallel to the arterial supply to join larger veins (Dellman & Brown, 1976).

2.2 PHYSIOLOGY OF THE SMALL INTESTINE

The following discussion is limited to domestic mammals and man. Columnar epithelial cells of the villi are absorptive; the absorptive surface being increased by microvilli on the surface of the villi. Goblet cells are mucous secreting cells. The cuboidal undifferentiated cells of the crypts serve as a source of new cell populations. Paneth's cells are the enzyme-producing cells (Dellman & Brown, 1976). Argentaffin cells apparently produce serotonin (Hill, 1970). The cells of Brunner's glands of the duodenum secrete succus entericus (Guyton, 1976).

Intestinal movements are divisible into mixing and propulsive movements (Hill, 1970; Guyton, 1976). Mixing movements keep the intestinal contents thoroughly mixed all the time and is effected by segmentation, peristalsis and pendular (swaying) movements of the intestine. Segmentation which is local contraction of small segments of the gut, may take place in any part of the intestine and is enhanced by parasymphathetic stimuli. The ingesta, in addition to being mixed, is repeatedly exposed to the absorptive mucosa during mixing.

Peristalsis, the basic propulsive movement is an inherent property of syncytial smooth muscles. Stimulation of the gut at any point will form a contractile ring which is spread in both directions. Distension, the main stimulus of the gut, will cause a contractile ring 2-3 cm anterior to the distension and this forces contents aborally. Although the intestinal peristalsis occurs in both directions, the orad wave dies rapidly while the aborad wave travels some distance. In the dog, 6-10 waves may occur in a 15 cm long intestinal segment and each wave forces the contents a short distance (Hill, 1970). Very intense irritation of the intestinal mucosa or excessive distension can elicit a "peristaltic rush" which is a powerful peristaltic wave which forces the contents into the colon, relieving the extensive distension or expelling the irritant. This is due to enhanced electrical activity of the mesenteric plexus by a superimposed stimulus. Effectual peristalsis requires an active mesenteric plexus although the basic phenomenon does not depend on the mesenteric plexus.

Villous movements are effected by the muscularis mucosae and individual muscle fibres within the villi. Their stimulus is either local or symphathetic. The movements allow

absorption and transport of lacteal contents to lymphatics.

2.3 GENERAL CONSIDERATIONS ON INTESTINAL ANASTOMOSIS

The constant concern of the surgeon to find the most advantageous method of suturing the intestinal wall has led to the description of numerous techniques of intestinal closure including open, closed, inverting, everting, overlapping, end-on approximating, multi-layer and single layer (McAdams et al., 1970; Vaughan, 1972; Irvin et al., 1974; Necula & Mandace, 1974; Singh & Nigam, 1974; Nakanish, 1975; Wise et al., 1975; Stowater, 1978; Schillaci et al., 1979; Singh et al., 1979 a & b; Singh et al., 1981; Bellenger, 1982, Ellison et al., 1982; Stashak, 1982; Richardson et al., 1982). The principles on which techniques have been based have varied and various modifications of techniques have been undertaken (Bennett & Zydeck, 1970; DeHoff et al., 1973). There are a number of ways to anastomose the intestine effectively but there is no single technique which is 100% successful. A surgeon must alter his planned approach according to circumstances. This is in agreement with Travers (1812) who suggested that so long as secure closure and

close contact of the divided ends in their entire circumference is achieved, the particular type of suture is not important. The overall success of the intestinal anastomosis will depend upon the skill of the operating surgeon and the correct utilization of the particular technique employed (Loeb, 1969; Ott et al., 1968; DeHoff, 1975).

The technique of bowel anastomosis should be simple and safe to perform. It should anchor the two ends of the bowel together securely (Hardy, 1968). The major requirement in the technique for intestinal anastomosis is that it should render the intestinal tract ready to function in an animal that desires to eat as soon as possible following surgery reducing post-operative problems of management and nursing care. This can be accomplished by atraumatic surgery which involves gentle handling of tissues and careful attention to detail, thus reducing the chances of paralytic ileus with subsequent early return to function and improved prognosis (Cohn & Nance, 1966; DeHoff et al., 1973; DeHoff, 1975; Grier, 1975). Minimizing time is also a factor in reducing trauma. Early oral intake will promote normal intestinal motility and the animal will support its own nutritional, electrolyte and fluid

requirements rather than rely upon intravenous therapy (DeHoff, 1975; Grier, 1975). Comparison between two layer and single layer anastomotic techniques have shown that normal intestinal function, returns sooner with single layer techniques (DeHoff, 1975).

Trauma should be minimized throughout the operation. Usually the intestinal contents are milked from the area to be incised but some authors recommend no milking of the intestinal contents as it is traumatic (DeHoff et al., 1973). The mucosa naturally everts when the intestine is transected because of the smooth muscle spasm of the circular muscle fibres and the loose attachment of the mucosa (Halsted, 1887; Grier, 1975). Periodic mild digital compression with a moist sponge will reduce mucosal eversion (Grier, 1975). A taper needle with a cutting point which has less traumatic penetration of the submucosa is used. Correct suture tension is judged clinically by absence of pallor and biting into the bowel wall but allowing adjacent bowel ends to be drawn snugly together (Halsted, 1887; Hardy, 1968) except for the crushing technique (Poth & Gold, 1968). Continous swabbing of oozing material is unnecessary and traumatic.

It is desirable to use an open rather than a closed method as it permits a more careful and meticulous technique. It is more reliable and less traumatic and allows more accurate evaluation of blood supply at the point of anastomosis (Cohn & Nance, 1966; Hamilton, 1967).

2.3.1 PREVENTION OF CONTAMINATION

Risk of spillage during anastomosis can be minimized by careful draping of the intestinal segment to protect the abdominal cavity, by suction emptying of the bowel or use of non crushing clamps (Cohn & Nance, 1966). Camalt intestinal clamps have been placed 4 cm cranial and caudal to the site of anastomosis (Bennett & Zydeck, 1970) or Pearlman intestinal clamps placed 5cm from the anastomotic site (Grier, 1975). Gambee (1951); DeHoff, (1975) and Stashak, (1982) do not recommend the use of intestinal clamps as they are traumatic and can cause tissue ischaemia making it difficult to be sure that sutures are not being tied too tightly. Other authors recommend the use of warm saline soaked sponges held by an assistant 2.5cm from the cut surfaces (DeHoff et al., 1973; DeHoff, 1975). The use of occlusive ligatures tied loosely around the intestine 5cm or so from the cut surfaces has also been

suggested or used (Gambee, 1951; Gambee et al., 1956; DeHoff et al., 1971; Reinertson, 1976; Stashak, 1982). These occlusive ligatures do not compress the mesenteric vessels thus allowing slow but free bleeding which ensures viability of the anastomosis.

Regardless of technique, bacterial contamination occurs during performance of the anastomosis and escape occurs for a short time thereafter (Bromwell et al., 1967; Rusca et al., 1969; Grier, 1975; Peacok & Van Winkle, 1976). Peritoneal and omental defences play a vital role in preventing infection, suture breakdown and abscessation. There are tremendous phagocytic and walling off processes by the peritoneal surfaces (Rusca et al., 1969; Grier, 1975).

Rinsing contaminants with Ringer's Solution within a reasonable time before the intestine is returned to the abdomen decreases peritoneal reaction (DeHoff et al., 1973; DeHoff, 1975). Pre-operative antibiotics are probably indicated to reduce post-operative reaction (Canalis & Ravitch, 1968). Antibiotics following surgery reduce adhesions (Ravitch, 1969; DeHoff et al., 1971). Post-operative antibiotics have been used by others (Richardson

et al., 1982; Schillaci et al., 1979; Singh, et al., 1979 a&b; Singh et al., 1980).

Post-operative monitoring of temperature, pulse, respiration, gut motility and appetite ensures that there are no complications from unforeseen contamination. This should be carried out daily for the first two weeks (Reinertson, 1976).

2.4 END TO END SINGLE LAYER INTESTINAL ANASTOMOSIS

End to end intestinal anastomosis is the simplest to perform and most physiologically compatible to intestinal function. This avoids blind loops and side tracked bowel (Cohn & Nance, 1966; Reinertson, 1976).

Since Halstead (1887) advocated use of a single row of sutures, the single layer anastomosis has become increasingly popular. This is because of reduced tissue trauma, less compromise to the blood supply, ease of construction, rapidity and little tendency to narrowing of the lumen at the anastomosis (Gambee et al., 1956; Herrmann et al., 1964; Loeb, 1967; Hardy, 1968; Bennett & Zydeck, 1970; Nakanishi, 1975). Single layer was originally considered to enhance spreading of infection (Necula and Mandace, 1974). Today a single row of sutures is known to provide an adequate closure preventing leakage of intestinal contents because fibrin or epithelium

seals the anastomosis within four hours (Reinertson, 1976). Including submucosa in each stitch of a single row of sutures provides sufficient strength for a satisfactory anastomosis because it is the strongest layer of the intestinal wall (Halstead, 1887; Ott et al., 1968, DeHoff et al., 1973; Grier, 1975; Reinertson, 1976; Lord & Valies, 1977; Ellison et al., 1982; Stashak, 1982).

The critical point in any single layer technique is that the intestine is not "hinged" or gathered together unequally (Hardy, 1968; DeHoff, 1975). Gathering occurs when diameters of the two ends are unequal or sutures are placed closer to the last suture on one side than the other.

2.5 APPROXIMATING END TO END SUTURE TECHNIQUES

The ideal anastomosis is the one in which all layers of the tissues are approximated since this ensures anatomical alignment after healing and results in minimal scar formation (Poth & Gold, 1968, Singh & Nigam, 1972; DeHoff et al., 1973). End to end approximating techniques for intestinal anastomosis offer many advantages when compared with inverting and everting

techniques (Bellenger, 1982; Ellison et al., 1982; Stashak, 1982). The advantages include the fact that individual intestinal layers are aligned anatomically. Suture application is easy. The lumen of the bowel is not compromised. There is rapid mucosal regeneration with less collagen deposition. There is no doubt that the submucosa is engaged by the suture ensuring strong closure. Intestinal motility returns early. There is better protection against leakage and minimal adhesion formation. The main disadvantage is they take a little longer to apply.

The techniques which result in end-on approximation are: simple interrupted, crushing, where the sutures are tied tightly enough to cut through the serosa, muscular layer and mucosa to hold snugly in the submucosa; gambee placed so that the sutures pick up the mucosa and prevent its eversion and simple continous.

2.5.1. SIMPLE INTERRUPTED TECHNIQUES

This end to end approximating technique is biologically sound, simple and safe with minimal chances of obstruction (Hardy, 1968; DeHoff et al., 1973; DeHoff, 1975; Grier, 1975; Bellenger, 1982; Ellison et al., 1982;

Stashak, 1982).

The first suture is placed on the mesenteric border starting 4-5 mm from the cut edges penetrating all the layers by entering the serosal surface and exiting in the lumen of the first segment and penetrating the mucosa on the second segment and out through the serosa 4-5 mm from its cut edges. The suture is tied gently approximating the mesenteric portion of both ends making sure that the tissue is not drawn so tightly in tying as to make the tissues blanch. A four throw knot is used. A slight modification of the above (Grier, 1975) starts 3 mm from the incised edge of the mucosa and is then directed through the margins of the mucosa, angled through submucosa and muscular layer of the opposite side and is brought through the serosa 3 mm from the transected edge. Next, a similar suture is placed at the antimesenteric border dividing the circumference of the cut ends. Ends of the two stay sutures are left long to be used for traction. These stay sutures can be clamped with haemostats or hand held. Sutures are then placed evenly (3-5mm) between these stay sutures beginning with the nearest side. A conservative attempt is made to invert the

bulging mucosa as each knot is secured. When the anterior row is completed, the mesenteric and antimesenteric sutures are rotated so that the posterior side may be sutured. Only enough sutures to accomplish approximation should be placed. Checking for leakage is usually carried out and additional sutures placed if necessary.

Sutures have been placed 2-18 mm apart and it was only after 10 mm that some degree of obstruction was observed grossly but not microscopically (Hardy, 1968). The anastomotic edges may invert, evert, or approximate when brought into apposition depending on the thickness of the intestine at the anastomotic site. However, it is important that only gentle apposing force be exerted. Lembert sutures can be inserted where there is excessive eversion of the mucosa. It is worthy of mention that simple interrupted sutures can be used to perform an everting anastomosis (Ott *et al.*, 1968; Reinertson, 1976).

2.5.2 GAMBEE TECHNIQUE

Gambee suture technique for intestinal anastomosis is an interrupted inverting suture of full thickness bowel wall using a single row of sutures (Gambee *et al.*, 1956;

McAdams *et al.*, 1970; Singh *et al.*, 1980). Gambee and crushing suture techniques are modifications of simple interrupted pattern (Gambee *et al.*, 1956; Poth and Gold, 1968). The anterior row of gambee technique is similar in suture placement to a modification of the simple interrupted pattern used in the crushing technique where mucosa has a tendency to evert (Gambee *et al.*; 1956; Bennett and Zydeck, 1970). The suture placement in gambee technique is such that the mucosa is selectively picked up to prevent its eversion and Stashak (1982) feels that the gambee technique is approximating rather than inverting.

Advantages of gambee technique include:

1) precision so that it can be performed under difficult conditions like suturing within the pelvis.

2) minimal adhesions and less chances of leakage.

3) it is non-obstructive since only a narrow flange of tissue is formed.

4) it is simple and easy to construct (Gambee, 1951; Gambee *et al.*, 1956; Hamilton, 1967; McAdams *et al.*, 1970; Reinertson, 1976).

After placement of stay sutures from the mucosal side, the posterior one half of the intestinal circumference is sutured from within the lumen inversion of the edges being supplied by slight tension on the stay sutures. The anterior one half of the anastomosis is completed by simple interrupted pattern placed as follows: The needle passes from the serosa through all layers into the lumen on one side and then back through the mucosa on the same side and then crossing over to the other side. Here, suturing starts near the cut edge with the needle being introduced from the serosa to the lumen through the mucosa and about 2 mm from there, the needle is passed from the mucosa to the serosa and tied down snugly (Gambee et al., 1956). Fig. 1 shows suture placement in the posterior and anterior rows of the gambee technique. Sutures are placed 6 mm apart (Gambee, 1951). The serosal tied sutures abut mucosa to mucosa, coapt submucosa firmly and invert the serosa.

The anterior row has been described by Loeb, (1967) as being a modified Lembert. Taking small bites will result in a negligible inversion cuff. Stay sutures are removed before checking for leakage. Additional Lembert sutures may be placed where necessary to provide

Figure 1. Diagrammatic representation of technique for suture placement in gambee anastomoses.

A) The posterior row is sutured from within the lumen starting on the mucosal side to the serosal side in one end of the transected intestine and from the serosal side to the mucosal side in the other end and is tied down on the mucosal side to appose the edges.

B) The anterior row is sutured starting from the serosal side to the mucosal side. The mucosa is picked before proceeding to the other side to prevent its interposition between the muscle layer. Sutures are tied down to appose the edges.

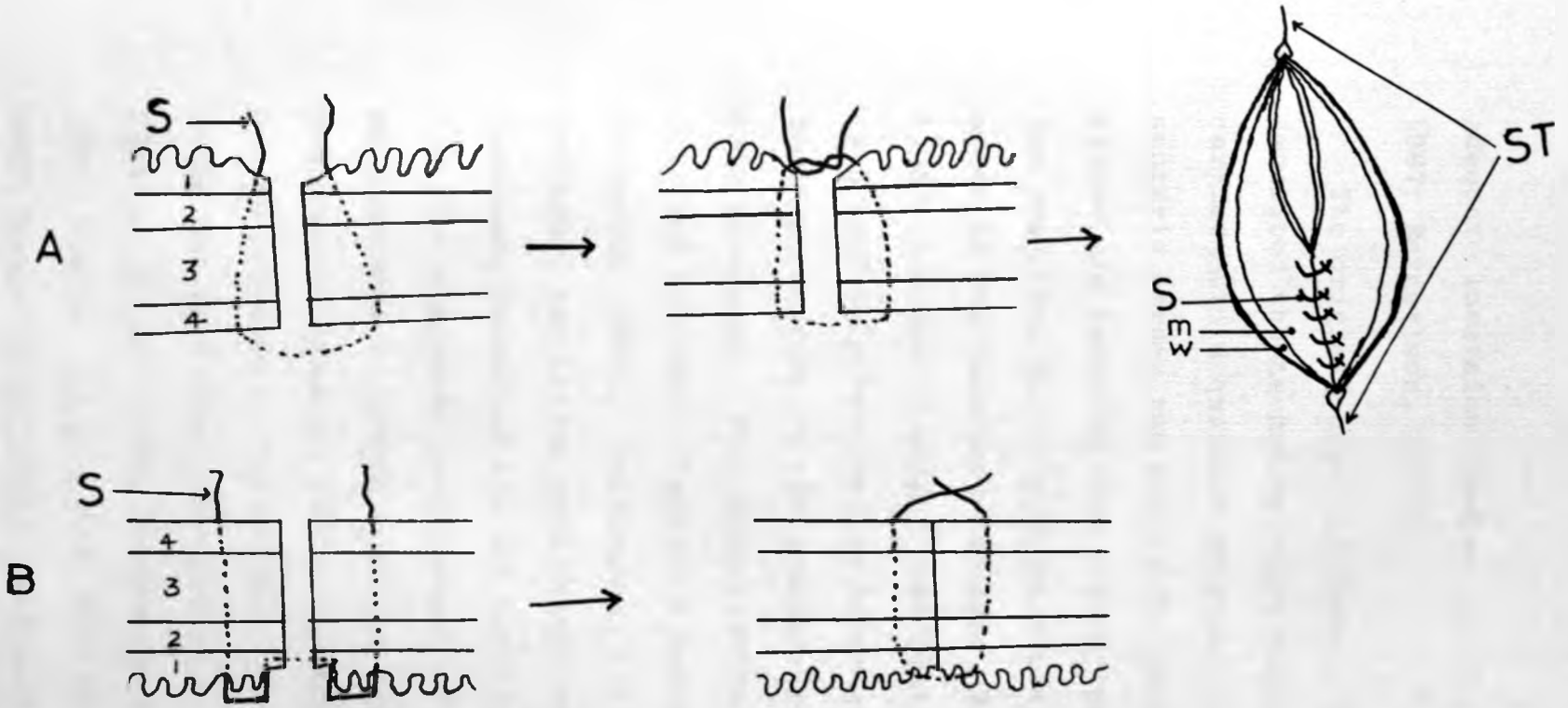


FIGURE 1.

1 - MUCOSA; 2 - SUBMUCOSA; 3 - MUSCLE LAYER; 4 - SEROSA;

S - SUTURE; ST - STAY SUTURES; M - MUCOSA; W - INTESTINAL WALL

adequate inversion (Gambee et al., 1956; Loeb, 1967; Reinertson, 1976).

The original paper of Gambee, (1951) described the technique where suturing was carried out in quarters starting from the mesenteric border and moving one quarter distance either way suturing from within the Lumen and the remaining quarters being closed as the anterior row described earlier. Hamilton, (1967) has used a slight modification where each knot is poked inwards towards the intraluminal position in the anterior row to maintain inversion. This modification has been utilized by others (Canalis & Ravitch, 1968; Nakanishi, 1975). Reinertson, (1976) has performed the entire anastomosis utilizing the technique described for the anterior row and has also suggested preplacement of the last 3 or 4 sutures to obtain accurate and consistent placement. Vaughan, (1972) has suggested the method employed by Reinertson. Gambee technique has been used experimentally by others (Getzen et al., 1966; Abramowitz & McAlister, 1969; McAdams et al., 1970; Scillaci et al., 1979; Singh et al., 1980; Somvanshi et al., 1980; Stashak, 1982).

2.5.3 CRUSHING TECHNIQUE

Crushing suture technique is an accurate end on approximating technique effected by interrupted through and through sutures incorporating all layers and appears to be well suited for use in intestinal anastomosis (Poth & Gold, 1968; Bennett & Zydeck, 1970; Meagher, 1973; Neal, 1975; Stashak, 1982). The technique is based on the fact that the submucosa is the strongest layer of the intestinal wall. According to Brunius & Zederfeldt, (1970), local circulation is influenced by technique rather than suture material. Myers (1971) and DeHoff, (1975) state that sutures should be tied not too tightly but firmly to approximate tissues. This avoids blanching of tissues and possible oedema With the crushing technique on the other hand, sutures are tied tightly so that they cut throuth all layers and gather all the collagen fibres together. This relieves pressure on microcirculation and re-establishes definite capillary blood supply to the cut edges resulting in minimum ischaemia and little postoperative oedema Microcirculation is preserved even if oedema causes tissues to swell in the area (Poth & Gold, 1968; Neal, 1975). Studies of the technique by others (Ellison et al., 1982), however, suggest

that this technique tends to lacerate and compress the microvasculature more when compared with other approximating techniques. After suture placement, the ends are approximated but there is some incidence of overlapping or eversion and mucosa may be extruded to the external surface (Mbiuki, 1977; Ellison et al., 1982). A bite of the mucosa (similar to the anterior row of gambee technique) will prevent the mucosa from bulging out. A back-and-forth pull once or twice before tying down the first throw of the knot will help the suture material to cut through the mucosa (Poth & Gold, 1968).

Crushing technique results in minimal narrowing of the lumen as compared to inverting suture techniques and is comparable to simple interrupted (DeHoff, 1975) and modified gambee techniques (Reinertson, 1976). The technique is simple to perform and results in a more comfortable patient due to less postoperative complications. However, it takes relatively more time to perform compared with single layer inverting technique (Stashak, 1982).

The cut surfaces should be properly aligned for suturing. The suture material must have adequate strength for tight tying. The sutures are placed passing through all the

layers and are tied tightly so that they cut through the mucosa on the luminal side and serosa and muscle layer on the outside. They lodge in the submucosa thus being burried in the wall. The various layers of the intestine are aligned anatomically and compression of the mucosa and submucosa prevents leakage. Sutures are placed 3-5 mm apart depending on the thickness of the wall of the viscus (the thicker the wall, the further apart the sutures can be placed). Sutures are also 3-5 mm from the cut edges. A four throw knot (double square knot) is tied. The fourth throw is for security when sutures are cut close to the knot. Suture ends when cut 2mm or more beyond the knot will become covered by the serosa.

Suture placement starts at the mesenteric border, the needle passing from the serosal surface into the lumen of one edge and from the lumen through the full thickness of the second edge. The first throw is pulled very firmly. The tissues beneath and adjacent to the suture first blanch, then turn pale as the microcirculation is compressed by pressure on soft tissues. With further tightening the suture cuts through the serosa, muscle layer and mucosa releasing the compression, so that the pale tissue becomes

pink again and the suture is completely burried into the wall of the bowel at the level of submucosa.

Occasionally, when the mucosa tends to evert, sutures are placed 2-3 mm back from the cut edges of the serosa straight through catching 4-5 mm of the mucosa due to the eversion of the mucosa. The needle is then reversed through the mucosa to complete one half of the suture placement. Suturing is continued to the other edge starting by taking a bite through the mucosa from outside into the lumen and is reversed straight through to the serosa 2-3 mm from the cut edge to complete the passage of the suture. A rounded probe or end of an haemostat is used to press the mucosa back into the lumen as the suture is pulled and tied. This modification of suture placement is similar to the anterior row of gambee technique (Gambee, et al., 1956). Additional sutures may be indicated where closure is considered to be inadequate. Bennett & Zydeck (1970) have used this modification for the entire anastomosis. Neal, (1975) has described a slightly modified technique where suturing starts 2-3 mm from the cut serosal edge through all layers emerging as close as possible to the edge of the bulging mucosa. The needle is then passed through the

opposite side in a reverse order, beginning near the cut edge of the mucosa and after emerging 2-3 mm on the serosal side from the cut edge, the knot is tied.

2.5.4 SIMPLE CONTINOUS TECHNIQUE

Simple continous technique for intestinal anastomosis has been utilized experimentally (Hardy, 1968; Ellison et al., 1982). Its advantages include: 1) Less adhesion formation. 2) Rapid healing due to proper approximation of the layers of intestinal wall and less tissue ischaemia especially in the first week. Hardy, (1968) in his technique placed sutures 2-14 mm apart. Double swaged on suture material with the initial suture being placed at the mesenteric border was used (Ellison et al., 1982). In the technique used by Ellison et al., (1982), the needle is passed through the serosa, muscularis and submucosal layers excluding the mucosa to minimize mucosal eversion. Sutures are placed 2-3 mm apart and 2-3 mm from the wound edges.

It may be worthy of mention that, although continous lock-stitch may be used to prevent a purse string effect instead of simple continous suture (Nakanishi, 1975). Some authors have

used continous lock-stitch as either everting (Singh et al., 1979 a&b; Singh et al., 1980). or as an inverting suture technique (Somvanshi et al., 1980).

2.6 HEALING OF AN INTESTINAL ANASTOMOSIS

Intestinal healing is made up of 3 overlapping phases, namely: the lag phase, the proliferative phase and the maturation phase (Herrmann et al., 1964; Necular & Mandace, 1974; DeHoff, 1975; Grier, 1975; Nakanishi, 1975; Reinertson, 1976; Stashak, 1982). This intestinal healing process is similar to that of the skin (Johnson, 1977). However, visceral wounds are metabolically more active than skin wounds (Hastings et al., 1975). For this reason, intestinal wound healing will be described together with the skin wound healing.

Immediately after injury, vasoconstriction of small blood vessels occurs in the injured area. This lasts 5-10 minutes and is followed by active vasodilation. Blood flows into the gap filling up the space and it clots providing a watertight seal of the edges of the wound within a few hours of completion of the anastomosis. Fibrin from fibrinogen is responsible for this (Cetzen et al., 1966; Peacock &

VanWinkle, 1970; Necula & Mandace, 1974; DeHoff, 1975; Grier, 1975). Neutrophil infiltration occurs within 6 hours after wounding and within 12 hours monocytes migrate into the area. Blood monocytes become macrophages after they have emigrated from the vasculature. The inflammatory exudate that forms is composed of fluid of blood origin, migrating leucytes i.e. neutrophils, monocytes, lymphocytes, plasma cells and eosinophils. Dead tissue is also a part of the inflammatory exudate. The acute inflammatory reaction reaches a peak in 24-48 hours. This inflammatory reaction is the same in all parts of the body.

The anastomosis is weakest between the 3rd and 4th day (Hamilton, 1967), due to lysis of collagen by collagenase which is present in high concentration at the anastomotic site at this time (Irvin et al., 1974; Wise et al., 1975; Scillaci et al., 1979; Singh et al., 1981; Richardson et al., 1982). The anastomotic strength at this time is provided by the suture material since the fibrin which is formed initially is lysed after 2 days (Ott et al., 1968; Stashak, 1982).

The proliferative phase is characterized by fibroplasia, re-epithelialization and

revascularization. Repair processes begin as soon as debri has been removed by the body scavenging system, and in uncomplicated simple wounds, this begins within 3-5 days of injury (Van Winkle, 1969; Peacock & Van Winkle, 1970; Heinze, 1974; Johnson, 1977). Inflammatory cells except the neutrophils are still present and may persist longer.

Fibroblasts originate from undifferentiated mesenchymal tissue in the surrounding connective tissue and initially lie on a fibrin scaffold. They start proliferating 48-72 hours after injury and peak mitosis occur in the 3rd to 5th days. Old collagen is usually destroyed in the first 3 days (Cronin et al., 1968; Peacock & Van Winkle, 1970; DeHann et al., 1974). As the fibroblasts proliferate, they manufacture and secrete glycoproteins. From the 4th day connective tissue starts being laid down. Reticulin fibres are present on the 4th day. Collagen fibres appear on the 5th day. Between 6-10 days collagen production is maximal. Collagen fibres are oriented across the incision after 6-7 days and there is fibrous union between the new and old collagen by 9-10 days. Collagen syntnesis slows down from 11-14 days. Fibroplasia may last

from 2-4 weeks (Madsen, 1953; Van Winkle, 1969; Peacock & Van Winkle, 1970; Postlethwait, 1970; Heinze, 1974; Johnson, 1977).

Revascularization occurs simultaneously with other healing processes (Sako & Wangensteen, 1951, Herrmann et al., 1964; Ott et al., 1968; Abramowitz & McAlister, 1969; Ravitch, 1969; Nakanishi, 1975; Somvanshi et al., 1980; Jansen et al., 1981; Ellison et al., 1982). There appears to be no difference between small and large intestines. Meticulous surgery enhances earlier anastomotic revascularization (Elkin, 1940; Herrmann et al., 1964; Peacock & Van Winkle, 1970; Jackson et al., 1971; DeHoff, 1975; Grier, 1975; Nakanishi, 1975).

New capillaries originate as bud-like structures on nearby vessels. These penetrate the wound and grow into loops which ramify through the wound (Herrmann et al., 1964; Necula & Mandace, 1974; Johnson, 1977; Jansen et al., 1981; Ellison et al., 1982). Some vascular supply to the anastomotic site can come from the omentum (Jansen et al., 1981). Revascularization has been shown to proceed from submucosal, muscular and serosal plexuses. Within 2-3 days it has been demonstrated that there are either lymphatic or vascular channels

across the anastomosis (Ravitch, 1969; Peacock & Van Winkle, 1970; Wise et al., 1975). Microscopic evidence of regeneration of the blood vessels around the anastomosis is present by 6-7 days and anastomotic channels are present between 7 and 9 days (Singh et al., 1980; Jansen et al., 1981; Singh et al., 1981). Functional communication of blood vessels are not present until the 14th day (Sako & Wangensteen, 1951; Nakanishi, 1975). Irregular fine vessels crossing the anastomotic site are seen in the second week. After the 3rd week, vascular patterns at the anastomotic site are more pronounced. Vascularization is complete by the 6th week when vessels are normal size and density.

Anastomotic epithelial healing seems to take longer. During days 1-7 there is progressive atrophy of the exposed mucosa. The epithelium regenerates and bridges the mucosa by 7-9 days (Sako & Wangensteen, 1951, Braucher & Kirsner, 1962; Herrmann et al., 1964; Ott et al., 1968; Necula & Mandace, 1974; Singh et al., 1979b; Jansen et al., 1981). Some studies, however, show that epithelialization may occur as early as 3 days depending on technique (Ellison et al., 1982).

The undifferentiated cells of the crypts of Lieberkuhn provide the new epithelial cover to the injured area. Regeneration of the epithelium starts as low cuboidal cells covering the defect and these eventually become columnar cells. The columnar epithelium invaginates to form new glands by 17-21 days. By 3 months, the epithelium is of normal size and appearance.

Single layer anastomoses show epithelial regeneration earlier than double layer anastomoses (Nakanishi, 1975). Everting anastomoses take one week to develop a continous mucosa and inverting anastomoses take 2 weeks (Nakanishi, 1975). End-on approximating techniques take about 3 days (Ellison et al., 1982).

Jansen et al., (1981) have observed that a definite repair of the underlying layers is necessary before restoration of the villous epithelium can take place.

There is no sharp demarcation between proliferative and maturation phases (Van Winkle, 1969). The maturation phase is characterised by a decrease in vascular and cellular elements and an increase in collagen. Those collagen fibres that are properly oriented thicken and there is a dissolution of the fibres that are improperly oriented. Maturation in the healing

intestinal wound may last up to one year (Herrmann et al., 1964; Stashak, 1982).

According to Peacock & Van Winkle (1976), chronic inflammation or a granulomatous reaction may occur especially where there is persistence of foreign material. Chronic inflammation consists mainly of macrophages, giant cells and lymphocytes. A granuloma may develop where macrophages and monocytes persist around a foreign body and fibroblasts from the local mesenchymal tissue produce collagen that becomes deposited around the foreign body.

Suture support of the anastomosis is essential before fibroplasia and thereafter sutures contribute little to the strength (Grier, 1975; Stashak, 1982). From the 4th day, granulation tissue mass occupies the anastomotic region and edema decreases. Although fusion of the muscularis propria has been noted on the 3rd day (Getzen et al.,1966), circular and longitudinal muscle layers atrophy in the anastomotic area in all techniques at 30 days (Bennett, & Zydeck, 1970). Functional healing occurs in 15 days (Necula & Mandace, 1974). At 21 days all anastomoses are secure with little individual difference (Hamilton, 1967), although DeHoff et al., (1973) have observed variation

in scar size with different techniques. Essential structural healing is complete in 6 weeks (Ravitch, 1969; Nakanishi, 1975). At 6 months there is no lumen diameter difference for different anastomotic techniques (Reinertson, 1976). Scaring may regress and the intestinal wall revert to normal preoperated structure given optimum conditions and sufficient time (Braucher & Kirsner, 1962). However, according to Herrmann et al., 1964, intestinal layers are ill-defined even at one year.

Regeneration of smooth muscle cells apparently occurs in rats and rabbits (Herrmann et al., 1964; Necular & Mandace, 1974).

2.7 METHODS OF EVALUATION OF TECHNIQUES FOR INTESTINAL ANASTOMOSIS

It is essential that all anastomoses be performed by the same surgeon for comparative study of various techniques (Abramowitz & Butcher, 1971). The small intestine is often used in anastomotic evaluation as it is more often involved in obstructive disease requiring resection and anastomosis than the large intestine (Reinertson, 1976).

Anastomotic spacing has been variable in dogs, the range has been 10-75cm (Getzen et al.,

1966; Hamilton, 1967; Bennett & Zydeck, 1970; Abramowitz & Butcher, 1971; Ellison et al., 1982) and 100 cm in the horse (Reinertson, 1976). In sheep anastomoses have been spaced 15 cm apart (Singh et al., 1979a) and 30 cm in the pig (Singh et al., 1979b).

In various comparative studies, the same technique has been performed in one animal several times. Using another animal a second technique is carried out similarly and the process is repeatedly carried out depending on the design of the experiment (Singh & Nigam, 1971; Singh & Nigam, 1974; Singh et al., 1979 a&b; Singh et al., 1980; Singh et al., 1981). Other workers have performed all the types of a anastomoses in each animal to allow intra-animal comparison (Jansen et al., 1981; Ellison et al., 1982).

Time interval for evaluation has varied from immediately after surgery to one year (Braucher & Kirsner, 1962; Herrmann et al., 1964; Getzen et al., 1966; Loeb. 1967; Canalis & Ravitch, 1968; Hardy, 1968; Ott et al., 1968; Singleton, et al., 1968; Ravitch, 1969; McAdams et al., 1970; Abramowitz & Butcher, 1971; Irvin et al., 1974; Singh & Nigam, 1974; Nakanishi, 1975; Wise et al., 1975; Reinertson, 1976;

Singh et al., 1979 a&b; Singh et al., 1980; Somvanshi et al., 1980; Jansen et al., 1981; Singh et al., 1981; Bellenger, 1982; Ellison et al., 1982; Richardson et al., 1982). From the literature cited above it appears that studies on intestinal healing should incorporate all the 3 phases of the healing process. Maturation phase which takes more time may be investigated at different stages.

Withholding feed and water before surgery is usually indicated. Cattle have been fasted for 24 - 48 hours and water has been withheld for 12-24 hours without risking serious complications (Singh & Nigam, 1972; Singh & Nigam, 1974; Mbiuki, 1977; Singh et al., 1980). Therefore it appears that the duration of withholding feed in cattle has not been standardised.

Parameters utilized in evaluation of suture techniques include: operative time; time before the animal can feed; formation of adhesions, leakage, gross intestinal obstruction or stenosis, radiographic evaluation of stenosis of intestinal lumen; and histologic studies.

2.7.1. OPERATIVE TIME

The average time required to perform each

type of anastomosis has been utilized (Hamilton, 1967; Singh *et al.*, 1979a; Singh *et al.*, 1980; Stashak, 1982). These authors generally found some difference between the average time required to perform each type of anastomosis. The time taken to place each suture of the anastomoses would have been more informative.

2.7.2 TIME BEFORE THE ANIMAL CAN FEED

Time before the animal ate voluntarily after performing the anastomosis has been evaluated in calves and dogs (Singh & Nigam, 1972; DeHoff *et al.*, 1973). This parameter does not seem to be reliable for comparative studies because the desire to feed after an operation is likely to vary with individual animals.

2.7.3 FORMATION OF ADHESIONS

Various comparative studies of anastomotic techniques have been carried out by subjective evaluation of adhesion formation (Herrmann *et al.*, 1964; Hamilton, 1967; Hardy, 1968; Ott *et al.*, 1968; Abramowitz & McAlister, 1969; Bennett & Zydeck, 1970; McAdams *et al.*, 1970; Abramowitz & Butcher, 1971; Singh & Nigam, 1972; Reinertson, 1976; Singh *et al.*,

1979a&b; Singh et al., 1980; Singh et al., 1981; Ellison et al., 1982; Richardson et al., 1982; Stashak, 1982). Adhesions can be expressed as a percentage relative to full circumference of the anastomotic site (Ellison et al., 1982; Richardson et al., 1982). Observations grossly to find the organs involved have been made (Singh & Nigam, 1972; Singh et al., 1979 a&b; Singh et al., 1980; Bellenger, 1982; Richardson et al., 1982) and then classified as either slight (mild), moderate or marked. The organs involved could have been the omentum, other loops of intestine or the mesentery. McAdams et al., 1970 have graded adhesions according to the number of quandrants involved and then averaged the quandrants for comparison. Grading of adhesions using quandrants and then allocating numerical values to the quandrants involved appears to be quite objective and comparison of these numbers is easy.

Herrmann et al., (1964) using rat colon have found adhesions to be maximal at 7 days and remained constant thereafter. Similar observations have been made in horses (Reinertson, 1976). Studies by others show that adhesions are prominent much longer and only decrease with time (Ott et al., 1968;

Singh et al., 1979a&b, Singh et al., 1980).

2.7.4 LEAKAGE

This can be determined by local abscessation, peritonitis or formation of a tract into the intestinal lumen (Herrmann et al., 1964; Hardy, 1968; Abramowitz & McAlister, 1969; Reinertson, 1976; Singh et al., 1979; Singh et al., 1980). Evaluation of leakage may not produce useful data for comparative studies when appositional techniques are utilized to perform intestinal anastomoses because these anastomoses are generally not associated with leakage.

2.7.5 GROSS INTESTINAL OBSTRUCTION OR STENOSIS

When there is obstruction, the proximal bowel loops are dilated or hypertrophic and the distal loops are collapsed or actual anastomotic narrowing is observed (Getzen et al., 1966; Hardy, 1968; Abramowitz & McAlister, 1969; McAdams et al., 1970; Reinertson, 1976). Gross stenosis is end-on approximating anastomoses is unlikely to be observed because the intestine is usually not distended with ingesta. Use of barium sulphate suspension to fill the anastomosed intestinal segment to a moderate degree may indicate presence or absence of stenosis visually.

2.7.6. STENOSIS EVALUATED BY CONTRAST RADIOGRAPHY

Countrast radiographic studies have been employed to estimate the degree of luminal stenosis using a barium sulphate suspension (Hamilton, 1967; Poth & Gold, 1968; Bennett & Zydeck, 1970; McAdams *et al.*, 1970; Reinertson, 1976; Singh *et al.*, 1979b; Singh *et al.*, 1980; Singh *et al.*, 1981; Bellenger, 1982). Another contrast medium which has been used is air (Richardson *et al.*, 1982).

Each anastomosis is isolated and ligated about 10cm on either side of the anastomosis. Barium sulphate suspension is then cautiously introduced into the lumen of the bowel taking care to create only mild distension. All anastomoses are filled to the same degree of distension. Alternatively, the entire segment containing the anastomoses or individual anastomotic segments are gravity filled with barium sulphate and the ends are tied up or clamped. The contrast medium filled anastomoses are then radiographed. The resultant radiodensity outlines clearly the lumen of the intestine at the anastomotic site.

To determine the degree of stenosis, an index is calculated using measurements of the

intestinal lumen as outlined by the radiodensity at the anastomotic site and 2 cm on either side. The formula used for calculations is:-

$$\text{Index of stenosis} = 100\left(1 - \frac{2A}{B+C}\right) = \%,$$

where A is the measurement at the anastomotic site; B&C are 2 measurements 2 cm proximal and distal to A (McAdams et al., 1970; Singh et al., 1980).

Non-radiographic evaluation of lumen narrowing has been conducted using dental modelling composition to make intraluminal casts of anastomotic segments (Getzen et al., 1968) and plaster of Paris cast (Ott et al., 1968; Singh & Nigam, 1972; Singh et al., 1979a).

2.7.7 HISTOLOGIC STUDIES

Histologic studies have been carried out after specimens are fixed in 10% buffered formalin and processed routinely before histologic sections were cut. Histologic sections used in various studies have been 5-6μ thick (Singh & Nigam, 1974; Singh et al., 1979b; Somvanshi et al., 1980; Bellenger, 1982). Serial histologic section studies of the anastomosis at the anastomotic site have been carried out (Braucher & Kirsner, 1962; Getzen et al., 1966).

Cutting specimens of varying lengths and different sites of the anastomosis is likely to reveal more information on the processes occuring during the healing of intestinal anastomosis. Several sections from each specimen may produce more complete data on each anastomosis.

Oedema can be estimated histologically by observing increases in intercellular spaces (Herrmann et al., 1964; Getzen et al., 1966; Hamilton, 1967).

Distance between various layers of the intestine wall have been measured with an ocular micrometer to determine the degree of apposition of the layers (Jansen et al., 1981).

Epithelialization, inflammatory reaction, and muscle layer alignment which influence healing if evaluated, may show the effects of different techniques on healing. This approach to comparative studies appears reasonable although it has not been previously reported.

Cellular reaction observed from histologic slides varies with time (Singh & Nigam, 1974; Singh et al., 1979a; Singh et al., 1980). Cell observed include: macrophages, lymphocytes, neutrophils, fibroblasts, and giant cells (Singh & Nigam, 1974; Richardson et al., 1982).

According to Singh et al., (1980), cellular reaction decreases with time. There is no reported method of estimating the numbers of various types of cells involved in the inflammatory reaction around the anastomotic site as an indicator of cellular reaction.

Observations of thickness of the anastomotic site have been made from histologic section (Singh & Nigam, 1972; Richardson et al., 1982). The anastomotic site is compared with a normal area of the same intestine. The increased thickness apparently decreases with time (Singh & Nigam, 1972).

Gross evaluation of thickness by palpation of anastomotic sites has been carried out (Bennett & Zydeck, 1970). The thickness was assumed to be due to fibrosis.

The above studies of the thickness of the anastomotic site relative to the normal area were subjective. The authors do not describe methods for determining the thickness. Determination of an index of increase in thickness and its use subsequently in comparative studies would make the evaluation objective. Such an index was developed in this study.

Histologic studies on intestinal anastomosis appearing in literature are generally

subjective. Conversion of abstract values in subjective evaluation to numerical values which are objective would be easier to visualize in comparative studies.

2.8 INTESTINAL ANASTOMOSIS IN CATTLE

Intestinal anastomosis is not commonly performed in the daily work of a clinical bovine veterinarian consequently, one is less adept at the actual technical procedure. Intestinal surgery requiring anastomosis is usually performed as an emergency procedure and has relatively few indications.

Indications for intestinal surgery in cattle include: 1) traumatic intestinal surgery (Frank, 1964; Kumar et al., 1982) and 2) Obstructions interfering with blood supply, especially where there is a likelihood of necrosis, as occurs with strangulating hernia, volvulus of the jejunum, intussusception, foreign body and constrictive adhesions (Payton, 1954; Zavitz & Zavitz, 1960; Frank, 1964, Berge & Westhues, 1966; Hofmeyr, 1974; Mitchel, 1975). Experimental anastomoses of the small intestine in bovine species have been performed (Singh & Nigam, 1972; Singh and Nigam, 1974; Singh et al., 1976; Mbiuki, 1977; Singh et al.,

1980; Somvanshi et al., 1980; Singh et al., 1981).

In cattle, colic occurs some hours after obstruction and signs may disappear in 9-12 hours. The course of the disease may be 8-14 days (Hofmeyr, 1974). This may imply a better prognosis in a case of delayed treatment as compared to other species.

It is therefore appropriate that whenever a bovine case requiring intestinal anastomosis arises, a veterinary surgeon is conversant with the most advantageous technique of intestinal anastomosis.

2.9 HYPOTHESES TO BE TESTED FOR COMPARISON OF SIMPLE INTERRUPTED, GAMBEE AND CRUSHING ANASTOMOTIC TECHNIQUES

It is evident from the literature review that no comparative studies on simple interrupted, gambee and crushing techniques for intestinal anastomoses together have been carried out. Since these techniques have been compared with inverting and everting techniques and found to be more desirable, it is proposed to compare the three techniques together to determine whether any of the techniques is more advantageous than the others.

The present project is therefore designed to test the hypothesis that there are no differences between simple interrupted, gambee and crushing techniques for end to end approximating intestinal anastomosis during surgery and in the post-operative healing period. In the investigation it is intended to test the sub-hypotheses:

1) That the three techniques take equal periods of time to perform.

2) that the degree of adhesion formation is the same after using the three techniques.

3) that none of the three techniques causes stenosis at the anastomotic site.

4) that the rate of epithelialization is identical.

5) that the degree of muscle layer approximation is similar.

6) that the degree of inflammatory reaction is identical.

7) that cellular intensity at the anastomotic sites is identical.

8) that the same cell types are present at the anastomotic sites.

9) that the increase in thickness of the intestinal wall around the anastomotic site does not vary with the technique.

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL ANIMALS

Twenty five young male cattle of various breeds and ages were brought from within 15 km radius from the Faculty of Veterinary Medicine, Kabete. Some bulls were intact and others were castrated. Details of the animals and the suture technique used in each animal are contained in table 1.

3.2 HOUSING AND FEEDING OF EXPERIMENTAL ANIMALS

The animals were divided into groups of three. Each group of animals was housed together in one stall and fed on a normal diet of hay, maize bran, water and mineral supplement.

3.3 PRE-OPERATIVE PREPARATION OF THE ANIMALS

Each animal was starved for 18 hours and deprived of water for the last 6 hours prior to surgery except 3 animals. One of the 3 animals was fasted for 36 hours and the other two for 24 hours.

The animals were shaved in the right paralumbar fossa. The dorsal border of the shaved area was tuber coxae along the lumbar transverse processes to thoracic vertebra 12 (T12). The caudal border

TABLE I: EXPERIMENTAL CATTLE USED IN THE EVALUATION OF END TO END APPROXIMATING TECHNIQUES OF INTESTINAL ANASTOMOSIS

NUMBER	BREED	AGE (YEARS)	WEIGHT (KG)	TECHNIQUE*
1	Friesian Cross	3½	168	SI
2	Friesian Cross	3	153	G
3**	Friesian	2½	148	C
4	Ayrshire	1½	110	C
5	Friesian Cross	1	82	SI
6	Friesian Cross	2	140	G
7	Jersey Cross	2½	110	C
8	Jersey Cross	2	125	SI
9	Ayrshire Cross	2	116	G
10	Jersey Cross	3	84	C
11	Friesian Cross	2½	154	SI
12	Friesian	2½	186	G
13	Friesian	2½	168	C
14	Ayrshire	3	152	SI
15	Jersey Cross	3	156	G
16	Ayrshire Cross	2½	84	C
17	Guernsey Cross	2½	144	SI
18	Guernsey Cross	3	124	G
19	Friesian Cross	3	134	C
20	Ayrshire	2	194	SI
21	Friesian	2	148	G
22	Friesian Cross	2	155	C
23	Friesian	2	142	SI
24	Friesian	1½	114	G
25	Hereford Cross	2	108	C

* SI = Simple interrupted;
G = Gambee; C = Crushing

** Died on the 10th post operative day due to stress.

was the tuber coxae along the cranial border of the stiffle joint ending about 5-8 cm below the level of stiffle joint. The cranial border followed the 12th rib to the point 5-8 cm below the level of the stiffle joint.

Hibiscrub^{R} (Imperial Chemical Industries Ltd) was used to scrub the shaved area 3 times and rinsed liberally with water after each scrub. Following the scrubbing, 70% ethyl alcohol was applied to the area.

The animal was tranquilized with xylazine hydrochloride (Rompun^{R} - Bayer, West Germany) at a rate of 0.1 mg/kg intramuscularly. Xylazine hydrochloride was allowed 10 minutes to take effect. The animal was cast and restrained on the operating table in left lateral recumbency. The head and limbs were tied to the table. Care was taken to avoid radial nerve paralysis by putting a cushion under the lower front limb after pulling it forwards. The upper forelimb was pulled backwards and tied. A second cushion was placed under the neck to make sure that the pharynx and larynx were at an higher level than the mouth so that saliva drained out.

Twenty millilitres of 2% lidocaine hydrochloride (Sunways - Bombay, India) was infiltrated along the line of incision in the shaved area at the level of the stiffle joint for a distance of 15 cm

70% ethyl alcohol was reapplied to the area before draping.

3.4 SURGICAL PROCEDURE

A 15 cm skin incision was made using a scalpel blade to expose the muscles which were first cut with the scalpel blade. The incision through the muscle layer was completed using a pair of mayo scissors. The peritoneum was opened with a pair of mayo scissors after a stab incision was made with a scalpel blade. The incision was 10 cm at the level of the transversalis muscle and the peritoneum.

The intestines were exteriorized and the portion of the jejunum with the longest mesentery was located. The rest of the intestines were returned into the abdominal cavity.

The majority of the ingesta was milked away from the site of incision in the jejunum. Two Doyen intestinal forceps were placed, one 4-5 cm proximal and the other the same distance distal to the site. Sterile swabs were placed under the incision site to avoid contamination of the rest of the abdominal cavity.

A number 22 scalpel blade was used to incise across the intestinal wall starting from the mesenteric side to the antimesenteric side. Wet saline

soaked swabs were used by the assistant to hold the cut ends for placement of stay sutures. Two stay sutures, one in the mesenteric border and the other in the antimesenteric border were placed using 2-0 chromic catgut with a swaged round bodied needle. The ends of the stay sutures were clamped with mosquito haemostats and held out by the assistant as sutures were placed around the circumference of the intestine.

Sutures were placed 4-5 mm from the cut edges and the same distance apart. Four throws were used for each knot to ensure uniformity and security of the knot. The suture material used throughout the project was 2-0 chromic catgut with a taper point round bodied swaged curved needle. When there was a defect in the mesentery, it was closed in a simple continous pattern using the same suture material as for the anastomosis.

Six anastomoses using the same technique were performed in each animal. The anastomoses were spaced 50 cm apart. Eight animals were used for each of the three anastomotic techniques making a toal of 48 anastomoses per technique.

The heart rate and respiratory rate were monitored every 15 minutes during the operation. The rumen was percussed at the same time to rule out bloating.

3.5 PROCEDURE FOR PERFORMING THE ANASTOMOSES USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES

Gambee technique as described by Gambee et al., (1956) was utilized (Fig. 1). The simple interrupted technique was used as described by Grier (1975) to reduce the tendency of everting of the ends (Fig. 2). The modified technique of suture placement in the crushing technique used by Bennett and Zydeck (1970) was applied (Fig. 3).

With the two cut ends of the intestine still held end to end, suturing was done between the stay sutures. After one half of the anastomosis was completed, the intestine was turned over and the process was repeated for the second half to complete the anastomosis. The anastomosis performed using gambee technique did not require turning over because the first half of the anastomosis was performed from the mucosal side. Blood clots on the mesentery were removed with swabs moistened with warm saline.

The anastomosis was returned to the abdominal cavity after measuring a distance of 50 cm from the anastomotic site. A second anastomosis was performed at this site. Four other anastomoses were performed using the 50 cm spacing to make a total of 6 anastomoses in each animal.

Figure 1. Diagrammatic representation of technique for suture placement in gambee anastomoses.

A) The posterior row is sutured from within the lumen starting on the mucosal side to the serosal side in one end of the transected intestine and from the serosal side to the mucosal side in the other end and is tied down on the mucosal side to appose the edges.

B) The anterior row is sutured starting from the serosal side to the mucosal side. The mucosa is picked before proceeding to the other side to prevent its interposition between the muscle layer. Sutures are tied down to appose the edges.

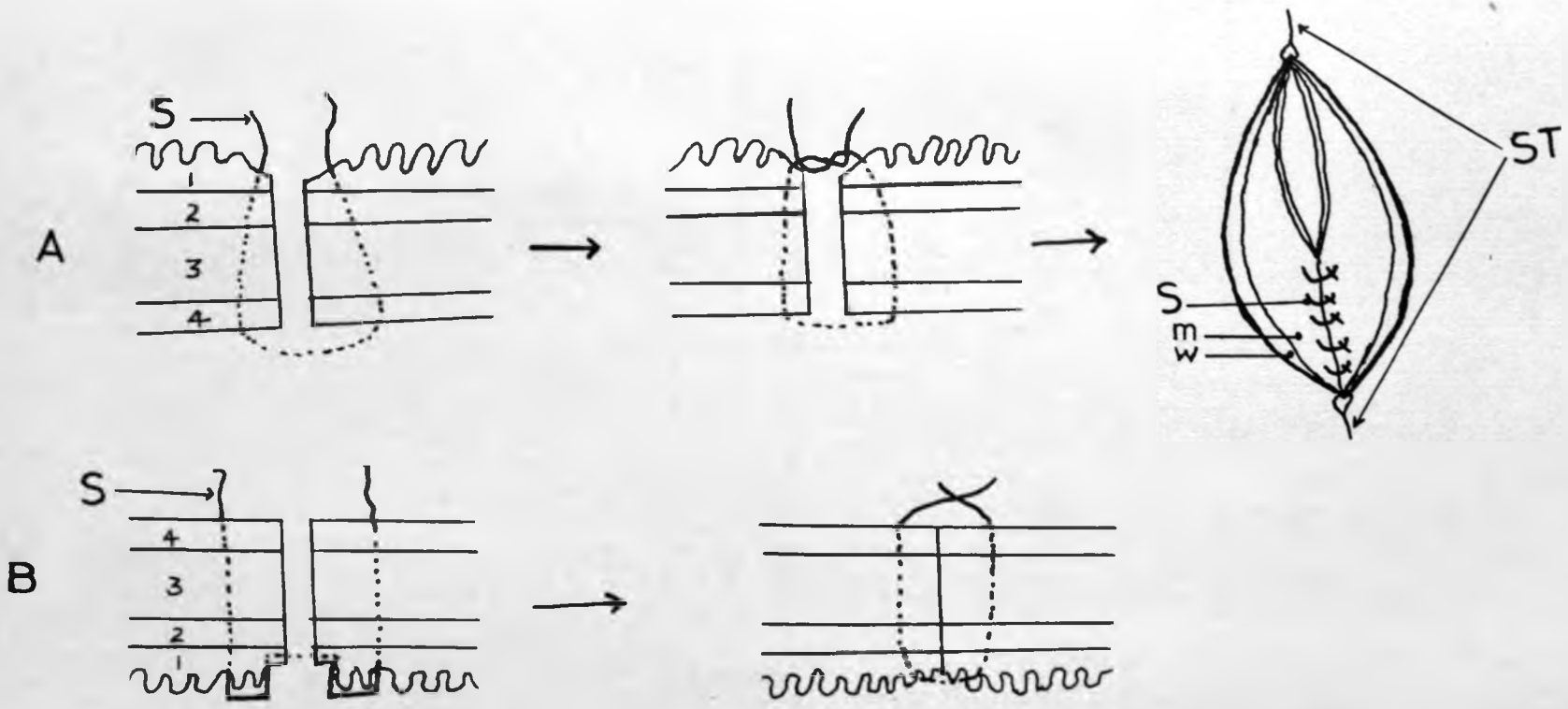


FIGURE 1.

1 - MUCOSA; 2 - SUBMUCOSA; 3 - MUSCLE LAYER; 4 - SEROSA;

S - SUTURE; ST - STAY SUTURES; M - MUCOSA; W - INTESTINAL WALL

Figure 2 - Diagrammatic representation of Technique of suture placement in simple interrupted anastomoses.

A) Suturing starts on the serosal side taking a bigger bite on the serosal side than the mucosal side to avoid interposition of mucosa between the muscle layers.

B) Completed suture placement

C) Sutures are tied down to appose the cut edges.

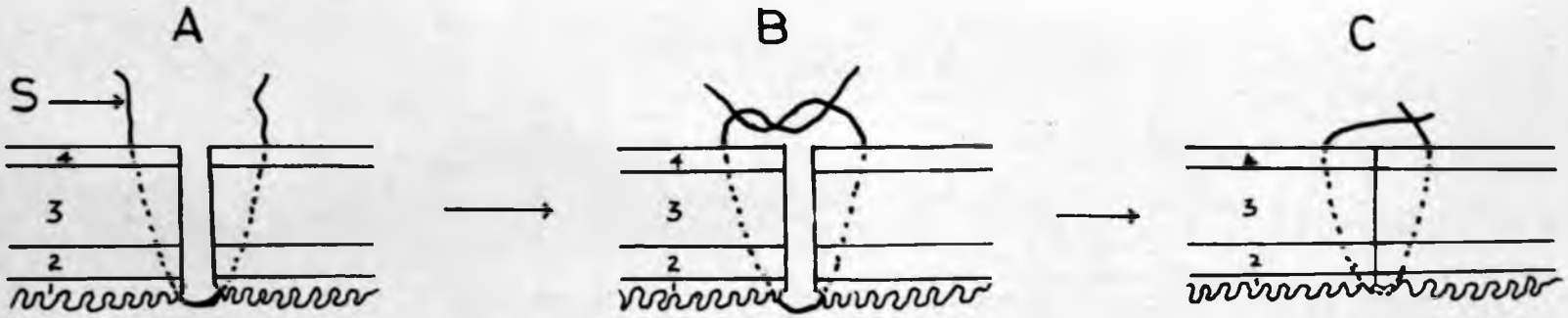


FIGURE 2:

1 -MUCOSA; 2 - SUBMUCOSA; 3 - MUSCULAR LAYER; 4 - SEROSA; S - SUTURE

Figure 3: Diagrammatic representation of technique of suture placement in crushing anastomoses.

A) Suturing starts on the serosal side through all the layers to the mucosal side. The mucosa is picked before proceeding to the other side. The suturing in the other end starts by picking the mucosa and it is continued from the mucosa to the serosa. Picking the mucosa prevents interposition of the everted mucosa between the muscle layer.

B) Completed suture ready for tying down.

C) Suture is tied down firmly to cut through the serosa, muscle layer and mucosa but not the submucosa thus leaving the suture holding only the submucosa. The suture is burried in the intestinal tissues.

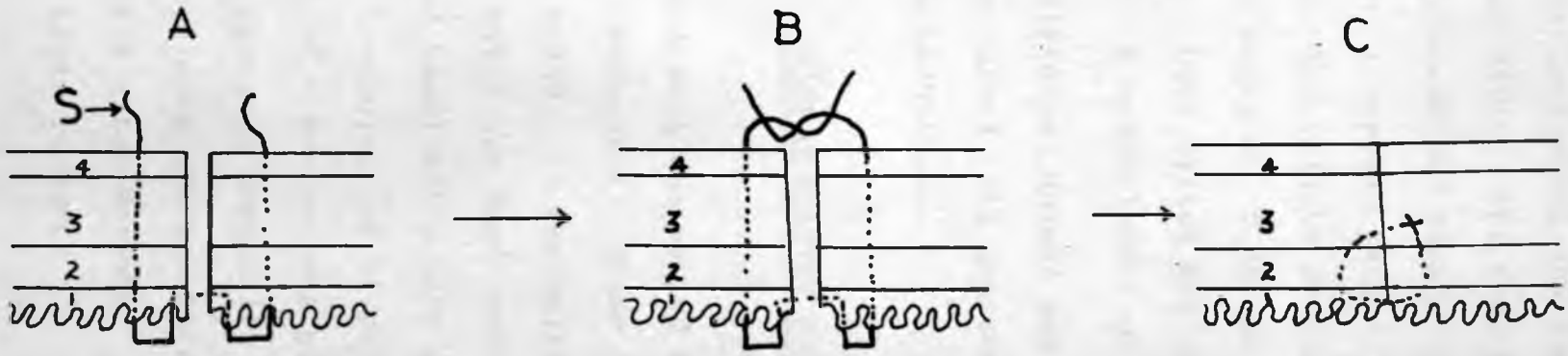


FIGURE 3.

1 - MUCOSA; 2 - SUBMUCOSA; 3 - MUSCULAR LAYER; 4 - SEROSA; S - SUTURE

After returning the last anastomosis into the abdominal cavity, the body wall was closed in layers. The peritoneum and transversalis muscle were sutured using No. 1 chromic catgut in a simple continuous pattern. The oblique abdominal muscles were sutured together using No. 2 chromic catgut in a cruciate pattern. Lock stitch was used to close the skin using No. 2 monofilament nylon.

A different animal was used for each technique. In every animal, six anastomoses were performed using the same technique.

3.6 POST-OPERATIVE CARE

The animal was untied and allowed to lie in sternal recumbency. After 10 minutes, it was assisted to stand. It was walked to a clean stall and allowed water and feed immediately. Temperature, pulse and respiration were monitored daily for 2 weeks. A combination of penicillin and streptomycin (Combiotic^{R} - Pfizer) was given at a rate of 10,000 i.u. of procaine penicillin G/kg and 0.25g dihydrostreptomycin/kg intramuscularly for 3 days starting immediately after surgery. Skin sutures were removed after 14 days.

3.7 THE SECOND OPERATION

This was carried out at 4 days, 14 days, 28 days and 56 days post operation in different animals to harvest the anastomoses performed using the three techniques for the respective time intervals.

Animals which had the second operation after 28 and 56 days were starved for 18 hours and deprived of water for 6 hours. Animals operated after 4 days were not put off feed. Feed was withdrawn for 12 hours and water was not withdrawn for animals operated after 14 days.

The surgical area used for the primary surgery, the right paralumbar fossa, was prepared as described earlier. Xylazine hydrochloride was given at the same dosage as in first operation. Twenty millilitres 2% lidocaine hydrochloride (Sunways - Bombay, India) were infiltrated about 5 cm from the last rib and parallel to it. This was slightly dorsal and cranial to the first incision.

A muscle transecting incision was made as described for the first operation. Exploration of the abdominal cavity to locate the segment of the intestine containing the anastomoses was carried out. The segment was not easy to locate by palpation and exteriorization of the jejunum was necessary to visualize the anastomoses. Care was taken to avoid disrupting any adhesions to the

adjacent intestinal loops.

The segment containing the 6 anastomoses was isolated by use of two Doyen intestinal clamps placed about 15 cm from the anastomosis on either side approaching from the antimesenteric side. The mesenteric vessels were ligated with No. 2 chromic catgut.

After completion of the ligatures, a second set of Doyen intestinal forceps were placed on the non ligated part of the intestine about 5 cm from the first set of the Doyen forceps after milking away the ingesta. The intestine was transected using a scalpel blade between the two clamps on one side starting from the antimesenteric side. The ligated blood vessels along with the mesentery was then transected making sure that the ligatures were not cut. After all the vessels were cut, the intestine was finally sectioned at the other end between the two Doyen intestinal forceps. The segment containing the anastomoses was transfered to a table ready for inspection and detailed study.

The two ends of the intestine resulting after resecting the segment with anastomoses were reanastomosed using any of the three techniques. The body wall was closed in layers as in the first operation.

Post operative care was the same as in the first operation.

3.8 PARAMETERS USED IN THE ASSESSMENT OF PERFORMANCE OF RESPECTIVE TECHNIQUES

3.8.1. TIME AND STITCHES PER ANASTOMOSIS

Timing was started using a stop-watch after the stay sutures were placed as the actual anastomosis was being performed. When the last stitch was tied down, the watch was stopped. The total time in minutes and the total number of stitches used were recorded. The process was repeated for all the anastomoses.

Average time per anastomosis and average number of stitches were calculated and recorded. Calculations were carried out of the average time to place each stitch of the 6 anastomoses performed in a sequence in each animal. This was because time taken to complete an anastomosis was not very meaningful due to variation in the number of stitches placed per anastomosis. The number of stitches varied because the intestines of different animals had different circumferences. Eight animals each with 6 anastomoses were utilized for each technique in the calculations and there were 8 of each of Nos 1-6 in the calculated averages. Calculations were based on 6 anastomoses per animal because the anastomoses were performed in a sequence of 1-6 during the same operation in each animal.

3.8.2. FORMATION OF ADHESIONS

During the second operation to harvest the segment containing the anastomoses, all the adhesions were noted as the segment containing the anastomoses was carefully isolated and exteriorized. Observations were made for sites of adhesions around the circumference of each anastomosis after the segment containing the 6 anastomoses was removed and placed on a table.

The degree of adhesion was scored as 0, 1, 2, 3 or 4 when adhesions affected 0, $\frac{1}{4}$, $\frac{2}{4}$, $\frac{3}{4}$ or $\frac{4}{4}$ of the circumference respectively. Results were recorded in a table for various time intervals studied in the 3 techniques. A total of 12 anastomoses were studied for each of the 4 time intervals. The results for the whole study were tabulated and the total scores were calculated. Total scores for each of the four time intervals and grand totals were also tabulated.

The frequency of occurence of numerical degrees of adhesion in the four time intervals were tabulated for the 3 anastomotic techniques.

3.8.3. EXTERNAL VISUAL EVALUATION OF STENOSIS

Due to the presence of variable amounts of ingesta in the segment of the intestine containing

the anastomoses, stenosis was not easy to evaluate visually. It was found necessary to fill the anastomotic segments to a moderate degree with barium sulphate suspension. The mesentery was trimmed to straighten the segment containing the 6 anastomoses after observations for adhesions were made.

All the ingesta was flushed out with cold ($4^{o}c$) physiologic saline solution. The segment was then placed in a bucket containing the cold physiologic saline. Within an hour from the time of harvesting, all the 6 anastomoses were cut to separate them. Each anastomotic segment included at least 10 cm of intestine on either side of the anastomotic site. These single anastomotic segments were stored in the cold physiologic saline solution before being filled with barium sulphate suspension. Each of the 6 anastomoses was filled with barium sulphate suspension prepared by mixing 200 g of barium sulphate in one litre of tap water. To fill a segment with barium one end was tied 2 cm from the cut edge with a string. Barium sulphate was poured into the lumen of the intestine from the open end filling it to the level of the anastomosis. The open end was then tied with a second string about 2 cm from the cut edge. The anastomotic segment was laid on a drape after filling and tying of both ends. The process was repeated for the other anastomoses.

Every anastomosis was observed for external stenosis. Stenosis was scored 0, 1, 2 or 3 depending on whether there was none, slight, moderate or marked stenosis respectively.

Total numerical scores of stenosis were calculated for each time interval and for every technique.

3.8.4 STENOSIS EVALUATED BY RADIOGRAPHY

One anastomosis which was perforated accidentally close to the anastomotic site during the trimming of the mesentery was omitted in this study.

The anastomotic segments already filled with barium sulphate suspension were divided into two groups of 3 segments for ease of taking radiographs. Three anastomotic segments were laid on a large loaded radiographic cassette (30.5 x 38.1cm) so that the outer anastomotic segments were equidistant from the middle segment. They were placed so that they faced the side of the label on the cassette and the first anastomosis was noted for identification purposes. These were exposed to x-rays from a distance of 80cm at 58kv and 8mAs (0.4sec x 20mAs). The procedure was repeated for the remaining 3 anastomoses. The films were developed, fixed and dried. They were labelled 1-6 starting with the first anastomosis. Since 2 sets of anastomoses were studied

for each time interval, the second set of radiographs were labelled 7-12.

Measurements were taken in centimetres of the radiodensity of each of the anastomotic segments. The site of anastomosis in the radiograph was usually identified as the point showing some narrowing around the centre of the radiodensity. The first measurement (A) was at the anastomotic site. Two other measurements (B&C) were taken 2 cm on either side from the anastomotic site. These measurements were used to calculate an index of stenosis using the formula

$$\left(1 - \frac{2A}{B+C}\right)100 = \% \text{ (Index.; McAdams } \underline{\text{et al.}}\text{, 1970)}$$

The calculated indices were tabulated for the different time intervals and the 3 techniques. Means for the time intervals and overall results were calculated for the 3 techniques.

3.8.5. HISTOLOGIC STUDIES

The anastomotic segments after taking x-ray pictures were emptied of barium sulphate and rinsed with cold physiologic saline solution. They were put in labelled containers with 10% buffered formalin for fixation. The segments were left in for at least 2 weeks before being trimmed for processing to make histosections. Tissue to formalin ratio was maintai-

ned 1:10 to ensure adequate fixation. The containers were stored at room temperature.

After fixing the anastomotic segments in 10% buffered formalin, they tended to shrink causing wrinkling of the whole segments. It was found necessary to open the entire anastomotic segment at the middle on the mesenteric side and fix it on a cutting board. This was to allow cutting of uniform sizes of the specimens and to facilitate indentification of various sites where specimens were to be cut. Six different specimens for histologic sectioning were cut from each anastomotic segment as shown in Fig. 4.

For each of the three anastomotic techniques, 72 specimens were cut for each time interval making a totabl of 288 specimens for the 4 time intervals. A total of 864 specimens were cut for the project. These were processed routinely and embedded in paraffin wax. Sections 5μ thick were cut and stained with haematoxylin and eosin. Five slides were prepared from each of the specimens making a total of 4,320 slides.

3.8.5.1 DEGREE OF EPITHELIAL HEALING

Each of the anastomoses was studies to determine epithelial healing using a light microscope. Observations were made to determine the extent of epi-

Figure 4: Diagrammatic representation of the sites where the six specimens for histologic studies were cut from each of the anastomotic segments.

A = Anastomotic site; 1 - 6 are the specimens.

Each of specimens 1-4 and 6 were 3-3.5cm long and 0.5cm wide. Specimen 5 was 0.5-1cm long and 0.5cm wide.

- Specimen 1 included at least one suture knot.
- Specimen 2 was similar to 1 and had not suture knot.
- Specimen 3 was 0.5 cm on one side of the anastomosis and 3 cm on the other side
- Specimen 4 was similar to 3 but from the opposite side.
- Specimen 5 was parallel to the anastomotic line and including it
- Specimen 6 was the normal specimen cut from the same side as 3 and 4 cm from the anastomotic line.

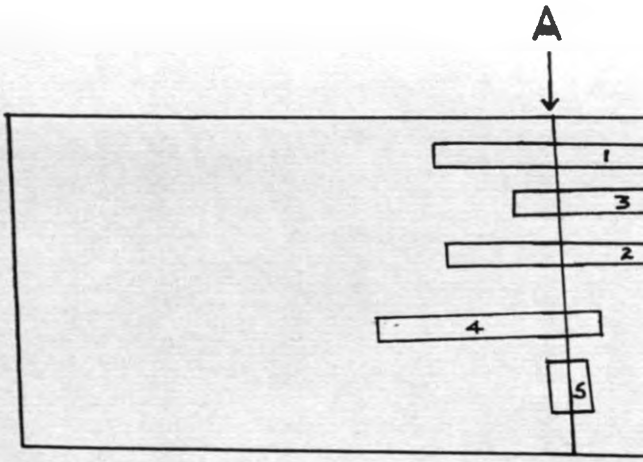
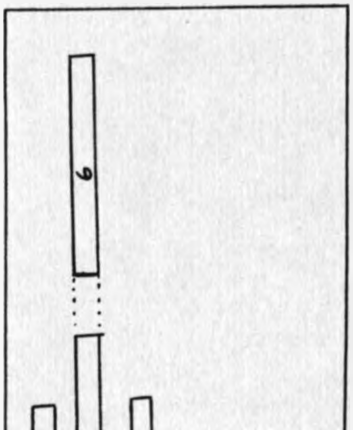


FIGURE 4.


9

thelialization using histologic sli
specimens (1) & (2). Specimen (1) h
line in the centre and included at l
knot. Specimen (2) was similar to s
except no suture knot was included.
prepared from specimens (1) & (2) we
magnifications x40, x100 and x400 in
proper appreciation of epithelial re

Healing was considered to be ei
partial or poor and given numerical
and 1 respectively. Complete healin
epithelium at the anastomotic site w
with some degree of differentiation.
recorded as being partial when there
layer of epithelial cells present acr
stomotic site. Poor epithelializatio
there were no epithelial cells but or

The inflammatory reaction was graded as 3, 2, or 1 depending on whether it was slight, moderate or marked respectively. The grading was such that a width of the inflammatory zone of up to 4 cm using magnification x40 was considered slight; up to 8cm was moderate and above 8 cm was considered marked.

The numerical grading of the inflammatory reaction was ordered in the reverse compared to the grading for epithelialization and muscle layer approximation. It was represented in this way because excessive inflammation is usually detrimental to rapid healing and as such a bigger inflammatory zone was given a low score.

3.8.5.3. INFLAMMATORY CELL INTENSITY AT THE ANASTOMOTIC SITE

This study was carried out using histologic sections from specimens (1) & (2). All the 10 slides from each anastomosis were examined at a magnification of x400. From each slide, various cell types were counted at three different levels along the anastomotic site. The three levels were: just below the muscularis mucosae on the submucosal side; at the midpoint between the muscularis mucosae and outer margin of the serosa and within the serosal area.

In order to get a suitable area for counting the cells, a paper disc with a window 0.5cm square was used. The disc which was of the same diameter as the inner circumference of the microscope's eye piece was cut from a firm sheet of white paper. A window 0.5 cm square was cut in the centre of the paper disc. The paper disc with the window cut was fitted to one of the 2 eye pieces of the microscope. Fig. 5 shows the microscopic eye piece and the paper disc used in the estimation of inflammatory cell intensity and cell types.

Cells seen through the window in the eye piece were counted. Each cell type present was counted separately and then all the cell types were added together to get one figure for each of the three levels of counting. The three counts were averaged and noted as the count for one slide. An average was calculated using the counts for all the slides from one anastomosis. This final average was recorded as the count for the anastomosis. The process was repeated for all the anastomoses.

The averages of various cell types counted were calculated for the three techniques at various time intervals.

3.8.5.4. DEGREE OF MUSCLE LAYER APPROXIMATION

Observations were carried out microscopically

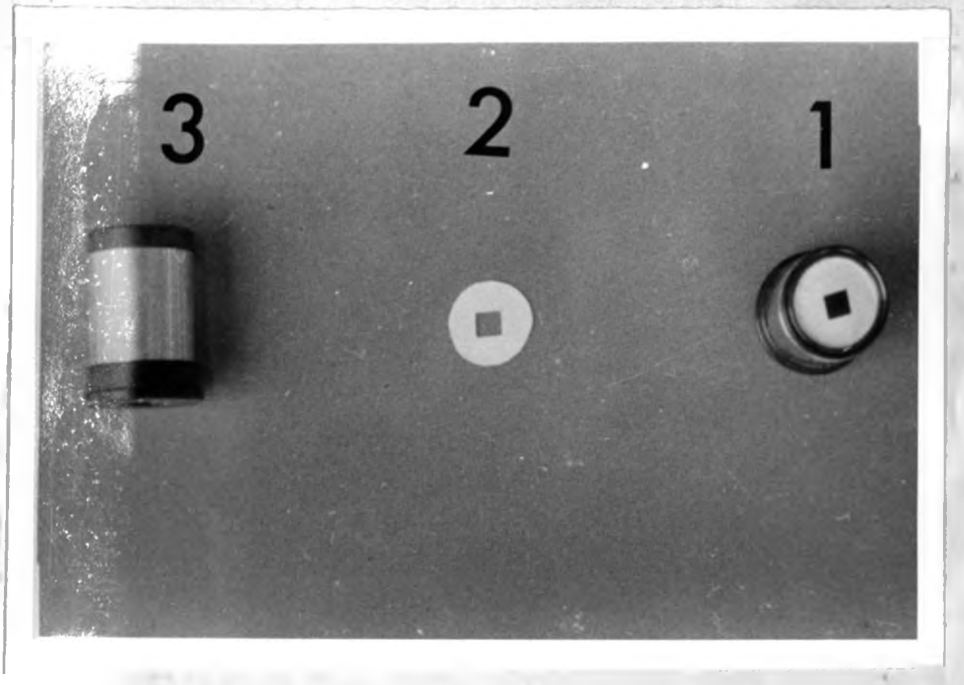


Figure 5. The microscopic eyepiece and the paper disc used in the estimation of inflammatory cell intensity and cell types. The paper disc is shown in place in the eyepiece (1) and in the centre is a similar paper disc (2). On the left side is the side view of the other eyepiece of the microscope (3). The window made in the paper disc is 5 mm square.

using histologic sections from specimens (1) and (2) at a magnification of x40. Ten slides were used per anastomosis. The two ends of the muscle layer which were cut before anastomosis were observed for proper alignment because each of the three techniques was expected to be in an end-on approximation after anastomosis.

Approximation was considered to be either good, satisfactory or poor and given numerical values of 3,2, or 1, respectively. Good approximation was when the ends were properly aligned. A satisfactory score was when the ends were slightly out of alignment. Approximation was considered poor when the two ends were either inverted or everted.

Histograms were plotted using the combined grand total results of epithelialization, inflammatory reaction and muscle layer alignment for further comparison of the techniques in regard to general healing.

3.8.5.5. INDEX OF INCREASE IN INTESTINAL WALL THICKNESS

The thickness of the intestinal wall was measured using a pair of calipers applied to each of 5 histologic sections from specimens (3), (4) and (6) per anastomosis. A strip of white paper was placed

across the anastomotic site and marked at the two points for the respective 2 edges of the thickness. The pair of calipers was then used to measure the distance between the two points. The pair of calipers and the strip of paper used in the measurements of increase in intestinal wall thickness are shown in Figure 6.

Measurements from specimens (3) and (4) were recorded as 3A and 4A respectively. Using the same histologic sections, two more measurements were made 1.5 cm from the anastomotic sites. The second set of measurements from specimen (3) histologic sections were recorded as B and those from specimen (4) as C. Measurements made at both ends of the normal histologic sections were recorded as N_1 and N_2. Figure 7 shows the various sites where measurements were made.

Two types of measurements were made at each point. One measurement included the whole thickness (WT) of the intestinal wall involving all layers, while the second measurements involved all layers except the mucosa (WM).

The following formulae were derived to calculate the indices of increase in intestinal wall thickness.

1) $\left(\frac{3A + 4A}{B + C} - 1\right)100 = \%\ (\text{Index})$

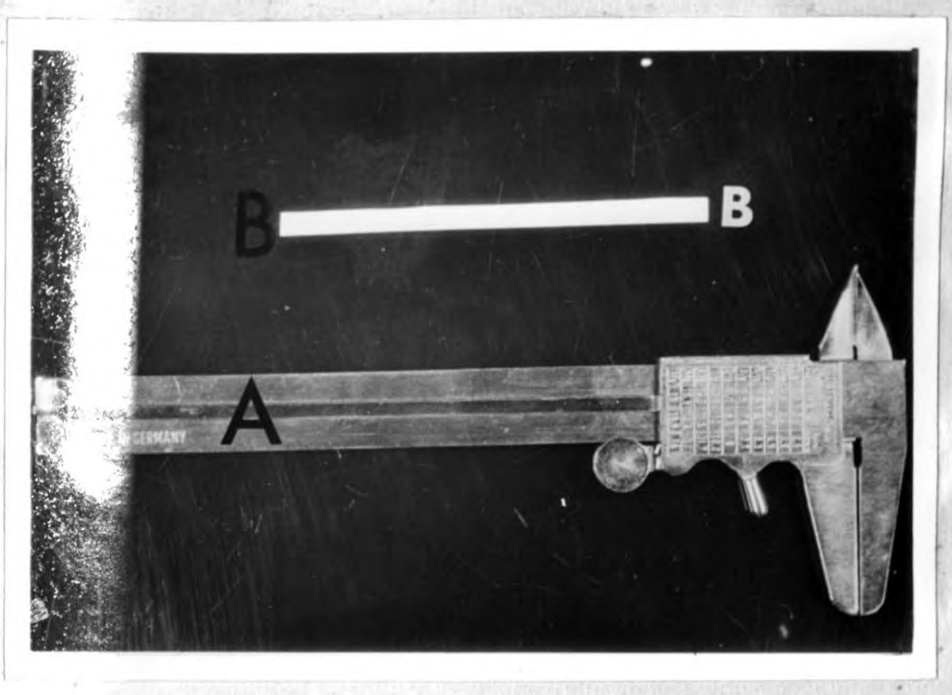


Figure 6. A pair of calipers and the strip of white paper used in the measurements of increase in intestinal wall thickness for calculation of index of increase in intestinal wall thickness

A = Calipers

B = Strip of paper 2-3 mm wide. The thickness was marked on the white paper with a pencil and the space between the two marks was measured using the calipers

Figure 7: Diagrammatic representation of sites where intestinal thickness measurements used to calculate index of increase in intestinal wall thickness were taken in specimens 3, 4 and 6. Note specimens 3 & 6 were from the same side of anastomosis.

A = Anastomotic line,

WT = Whole thickness,

WM = Thickness excluding mucosa,

M = Mucosa.

Measurements of thickness were made from histological sections at the following points:

A, B, C, N_1 & N_2

A to B & A to C = 1.5 cm

A to N1 = 4 cm; N1 to N2 = 3 cm

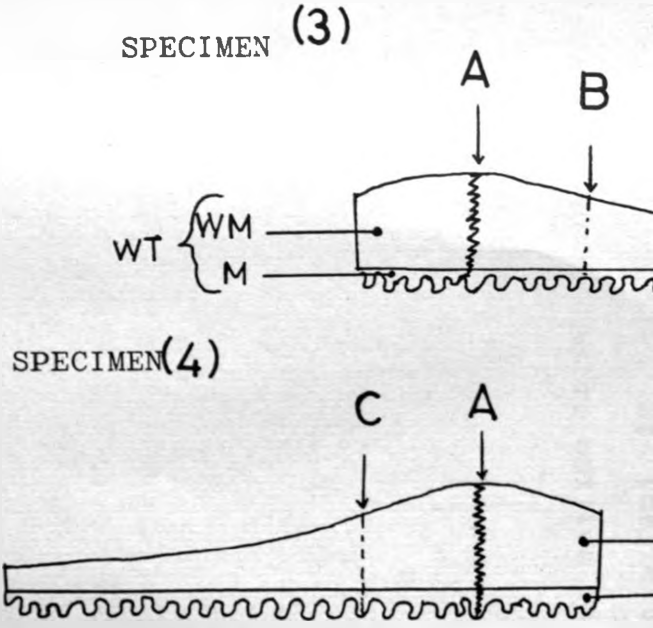
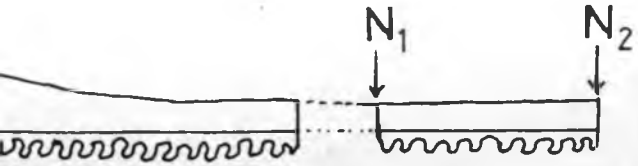


FIGURE 7

SPECIMEN (6)



WM
M
WT

2) $\left(\frac{3A + 4A}{N_1 + N_2} - 1\right)100 = \%$ (Index)

There were two indices using each formulae because there was one for the ness (WT) and one for intestinal wall e mucosa (WM). Finally the four indices from the two formulae were two for the B and C and two for normal measurements

Whole thickness measurements were most of the time at 4 days because the was either absent, one layer or too th measurable. Only a few simple interru moses were measured. All the results interval were therefore omitted in the

4 RESULTS

4.1. GENERAL OBSERVATIONS

The period of time animals were fasted influenced their behaviour post-operatively. One animal which was fasted for 36 hours was very weak. It took 24 hours to show any interest in feed and only nibbled the concentrates. It remained recumbent despite manual attempts to stand it up twice daily. When it died 10 days after the operation, the anastomoses were intact at post-mortem. Two animals fasted for 24 hours had a staggering gait immediately after the operation but fed on concentrates and hay when they were returned to their stalls. All the remaining animals which were fasted for 18 hours remained bright and strong post-operatively. They walked normally and showed interest in feed as soon as they were back in their stalls.

Bloating was not a problem at any stage during surgery.

The animals were slightly dull with reduced appetite in the first week. Generally, temperature was within normal limits (38.8 - 39.5^{o}C) at all times. Pulse and respiration remained within normal ranges.

Radial paralysis was not a major problem in these animals due to the precautions taken by tying the forelimbs such that weight was removed from the lower limb and putting a cushion under the point of the shoulder of the lower limb.

Animals passed faeces within 24 hours. The faeces were less than normal quantities. Those animals in which the second operation was performed after 4 and 14 days appeared weaker than after the first operation but they improved within a week.

During the second operation, the anastomotic sites were clearly visible at 4 and 14 days in all the anastomoses. At 28 and 56 days, the anastomotic sites were not readily seen. But in a few instances at 28 days, the simple interrupted anastomotic sites were visible because of inflammatory reaction. Fig. 8 shows a segment containing the six anastomoses exteriorised in the 2nd operation.

4.2 TIME AND STITCHES PER ANASTOMOSIS

The mean time taken to perform a simple interrupted anastomosis was 10 minutes, a gambee anastomosis took 12.5 minutes and a crushing anastomosis took 13.5 minutes.

The calculated average time per stitch for different time intervals for the three techniques are shown in table 2. Simple interrupted took the least

Figure 8. The segment containing the six anastomoses exteriorized during the second operation. The sites of anastomoses are not readily visible.

TABLE 2: AVERAGE TIME* PER STITCH (IN SECONDS) FOR EACH OF THE SIX ANASTOMOSES PERFORMED IN SEQUENCE FOR EACH OF THE ANASTOMOTIC TECHNIQUES

SEQUENCE OF ANASTOMOSES	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
1	43.9	55.2	64.5
2	39.3	51.0	58.3
3	37.8	50.7	54.5
4	36.8	48.6	57.0
5	37.9	45.6	56.8
6	33.9	47.1	54.2
Mean	36.6	49.5	57.4

*: 8 animals were used for each of simple interrupted, gambee and crushing techniques.

time all along and crushing technique took the greatest time. The gambee technique was intermediate.

4.3 FORMATION OF ADHESIONS

Adhesions were observed usually around the anastomotic site, the adjacent loop of intestine or mesentery. The adhesions did not interfere with the ligation of the mesenteric vessels before resecting the segment containing the anastomoses.

Table 3 contains the numerical scores for adhesion in respective techniques and time intervals. Generally, gambeee anastomoses had the least adhesions followed by crushing anastomoses. Most adhesions were found in the simple interrupted anastomoses. The adhesions did not seem to decrease with time except for gambee anastomoses. Simple interrupted anastomoses had the same number of adhesions at 28 and 56 days whereas adhesions increased in crushing anastomoses.

Total adhesion score showed gambee anastomoses to have the least adhesions; crushing anastomoses followed and simple interrupted anastomoses had most adhesions.

The frequency of occurence of degrees of adhesions at different time intervals are presented in table 4. Gambee anastomoses had the highest incidence of no adhesions followed by crushing anastomoses and simple

TABLE 3: TOTAL NUMERICAL SCORES* OF INTESTINAL ADHESIONS AROUND THE ANASTOMOTIC SITES AT VARIOUS TIME INTERVALS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES

TIME INTERVAL (DAYS)	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	15	4	10
14	8	10	14
28	14	2	1
56	14	1	6
Total	51	17	31

* Total scores were from scores of 12 anastomoses per time interval for each technique. Individual scores were based on the quandrants of the circumference having the adhesions where

0 = none; 1 = 1/4; 2 = 2/4; 3 = 3/4 and 4 = 4/4 respectively.

TABLE 4: FREQUENCY OF OCCURENCE OF DEGREES OF ADHESION AROUND THE ANASTOMOTIC SITES FOR SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS AFTER SURGERY

TIME INTERVAL (DAYS)	TECHNIQUE											
	SIMPLE INTERRUPTED				GAMBEE				CRUSHING			
	DEGREES* OF ADHESION				DEGREES OF ADHESION				DEGREES OF ADHESION			
	0	1	2	3	0	1	2	3	0	1	2	3
4	2	5	5	-	8	4	-	-	3	8	1	-
14	5	6	1	-	7	2	1	2	5	3	1	3
28	3	4	5	-	11	-	1	-	12	-	-	-
56	3	6	1	2	11	1	-	-	8	2	2	-
TOTAL	13	21	12	2	37	7	2	2	28	13	4	3

* DEGREES BASED ON QUANDRANTS OF CIRCUMFERENCE WITH ADHESIONS WHERE 0 = 0; 1 = 1/4; 2 = 2/4; 3 = 3/4 of the circumference. The number shown for each degree at different time intervals was the sum of the observations for that degree.

interrupted was last. None of the anastomoses had adhesions involving the entire circumference at any time.

4.4. EXTERNAL VISUAL EVALUATION OF STENOSIS

Observations of the anastomoses *in situ* did not show any indication of obstruction and in most cases, the anastomotic segments appeared like the normal portion of the intestine. Figure 9 shows the anastomotic segments after filling with barium sulphate ready for visual evaluation of stenosis.

Sums of the various scores of stenosis for the different time intervals are presented in table 5.

At 28 days, stenosis appeared to be minimum in all the anastomoses although simple interrupted anastomoses had the least stenosis followed by gambee anastomoses. Crushing anastomoses showed the greatest stenosis. The pattern was not consistent for the various time intervals. There was overall more stenosis at 56 days than at 28 days except in the gambee anastomoses. However, gambee anastomoses had the least stenosis score followed by simple interrupted and crushing anastomoses, respectively.

Frequency of occurence of degrees of stenosis at various time intervals are shown in table 6.

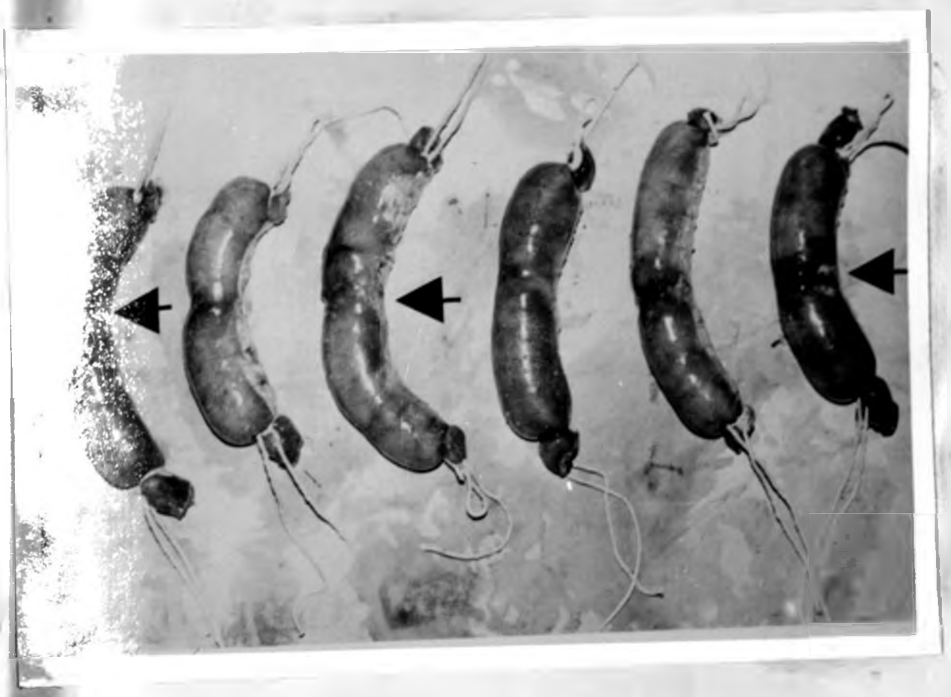


Figure 9. The six anastomotic segments after filling with barium sulphate suspension just before evaluation of external stenosis and taking of radiographs.
Arrows show the sites of anastomosis which are approximately at the middle of each segment.

TABLE 5: TOTAL NUMERICAL SCORES* OF EXTERNAL VISUAL STENOSIS OF THE INTESTINE AT THE ANASTOMOTIC SITES AT VARIOUS TIME INTERVALS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES

TIME INTERVAL (DAYS)	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	5	8	8
14	13	4	10
28	2	4	5
56	6	3	14
TOTAL	26	19	37

* Total scores shown for each time interval were from the scores of 12 anastomoses per technique. Individual Scores were:

0 = No stenosis; 1 = Slight; 2 = Moderate; 3 = Marked

TABLE 6: FREQUENCY OF OCCURENCE OF DEGREE OF EXTERNAL STENOSIS OF THE ANASTOMOTIC SITES AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	TECHNIQUE											
	SIMPLE INTERRUPTED				GAMBEE				CRUSHING			
	DEGREES* OF STENOSIS				DEGREES OF STENOSIS				DEGREES OF STENOSIS			
	0	1	2	3	0	1	2	3	0	1	2	3
4	7	5	-	-	8	6	1	-	6	4	2	1
14	1	9	2	-	5	4	-	-	4	6	2	-
28	9	2	-	-	7	4	-	-	6	5	-	-
56	8	2	2	-	9	3	-	-	3	4	5	-
TOTAL	25	18	4	-	29	17	1	-	19	19	9	-

*: - DEGREES OF STENOSIS: 0 = None; 1 = Slight; 2 = Moderate; 3 = Marked.

Note: Degrees were determined visually after anastomotic segments were filled with barium sulphate suspension. The numbers shown for each degree at different time intervals was the sum of the observations for that degree.

Gambee anastomoses showed the highest frequency of no stenosis followed by simple interrupted and crushing anastomoses were last. There was no marked stenosis in any of the anastomoses.

4.5 STENOSIS EVALUATION BY RADIOGRAPHY

The radiographs of the barium sulphate filled anastomotic segments are shown in figures 10 and 11 while the sites were measurements for calculation of index of stenosis are shown in figure 12.

The mean indices for each time interval and for all 4 time intervals are shown in table 7.

There was some degree of stenosis for all the three techniques even at 56 days. The stenosis decreased with time in gambee anastomoses. Simple interrupted anastomoses although showing less stenosis than the other techniques at 4 days, had more stenosis at 14 days than other techniques. However, the stenosis induced by simple interrupted technique decreased thereafter. Stenosis caused by crushing technique decreased initially but was constant at 28 and 56 days. The overall mean for each of the techniques indicated that gambee technique induced the least stenosis followed by simple interrupted while crushing technique induced the most stenosis.

Figure 10. The radiographs of the first 3 anastomoses per animal after filling with barium sulphate suspension. The sites of anastomosis are the constricted areas around the centres of the radiodensities.

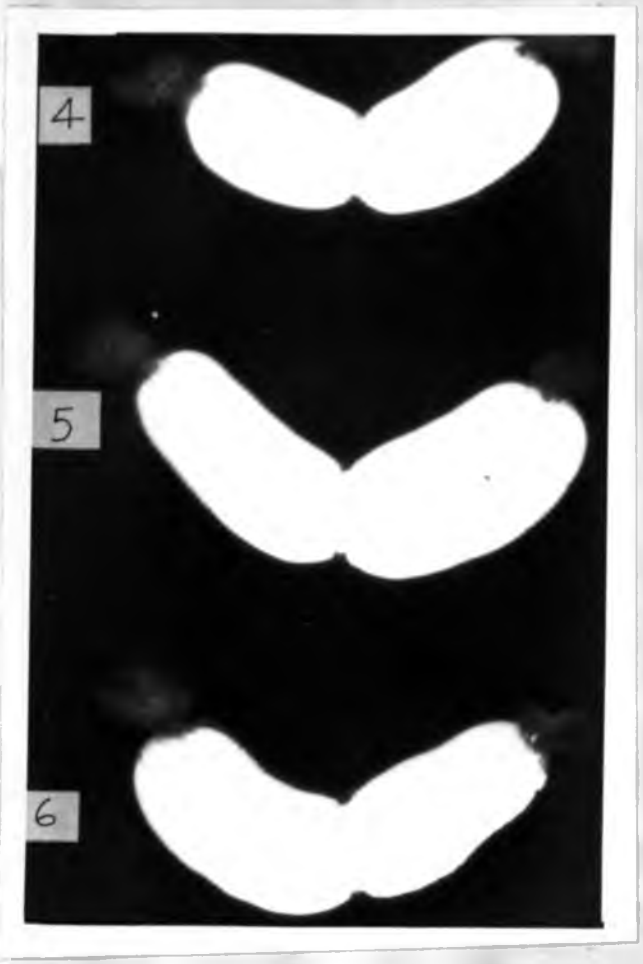


Figure 11. The radiographs of the remaining 3 of the six anastomoses from each animal after filling with barium sulphate suspension. The sites of anastomosis are the constricted areas around the centres of the radiodensities.

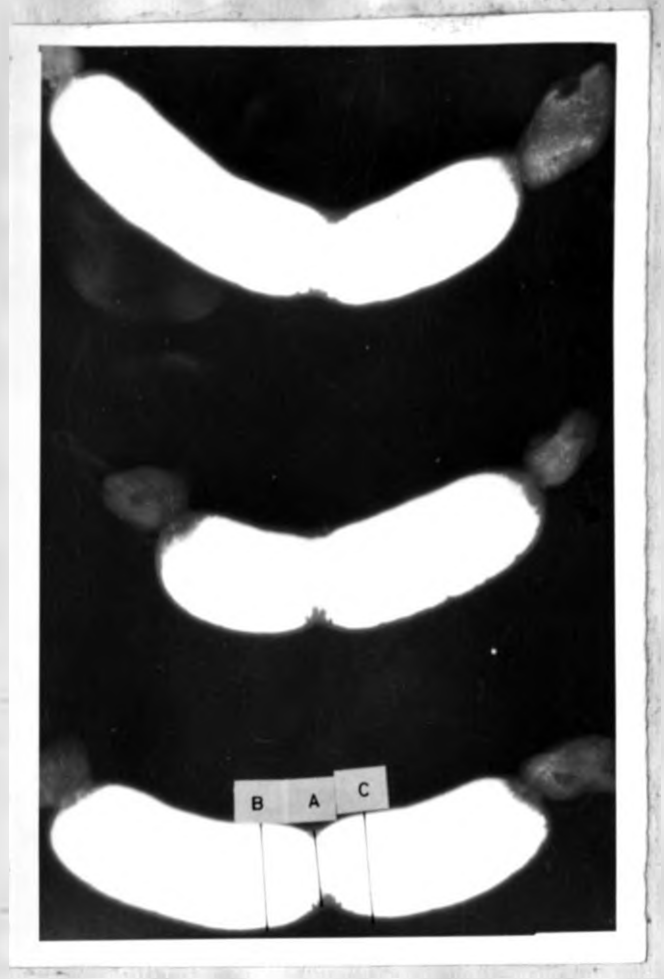


Figure 12. Sites where measurements for calculation of index of stenosis were taken on the radiographic image of intestinal lumen.

A = Anastomotic site

B&C = 2 cm from anastomosis

TABLE 7: MEAN INDICES* (PERCENTAGE) OF STENOSIS INDUCED BY SIMPLE INTERRUPTED, GAMBEE AND CRUSHING SUTURE TECHNIQUES ASSESSED BY USE OF RADIOGRAPHS AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	35.6	42.0	49.6
14	46.3	29.4	36.2
28	34.8	26.9	34.9
56	27.3	24.0	35.0
MEAN	36.0	30.6	38.9

* Calculated using the formula of McAdams et al., (1970). Each mean is from 12 indices for each time interval per technique.

4.6 DEGREE OF EPITHELIAL HEALING

The degrees of epithelial healing are shown in Figs. 13-15. Figure 13 shows complete epithelization; Fig. 14 partial epithelialization and Fig. 15 poor epithelialization.

Totals for the various time intervals and grand totals are presented in table 8. Epithelial healing was best in simple interrupted anastomoses at 4 days but thereafter, the healing in the anastomoses performed using the other two techniques overtook the simple interrupted anastomoses. Generally epithelial healing was present at 14 days and was complete at 28 days after surgery for all the anastomoses.

Overall, gambee anastomoses showed slighly better healing of the epithelium followed by crushing anastomoses. Simple interrupted anastomoses were last.

4.7 DEGREE OF INFLAMMATORY REACTION

The degree of inflammatory reaction was considered to be either slight (Fig. 16); moderate (Fig. 17) or marked (Fig. 18).

The sum of the numerical scores of inflammatory reaction at different time intervals for the three techniques are contained in table 9.

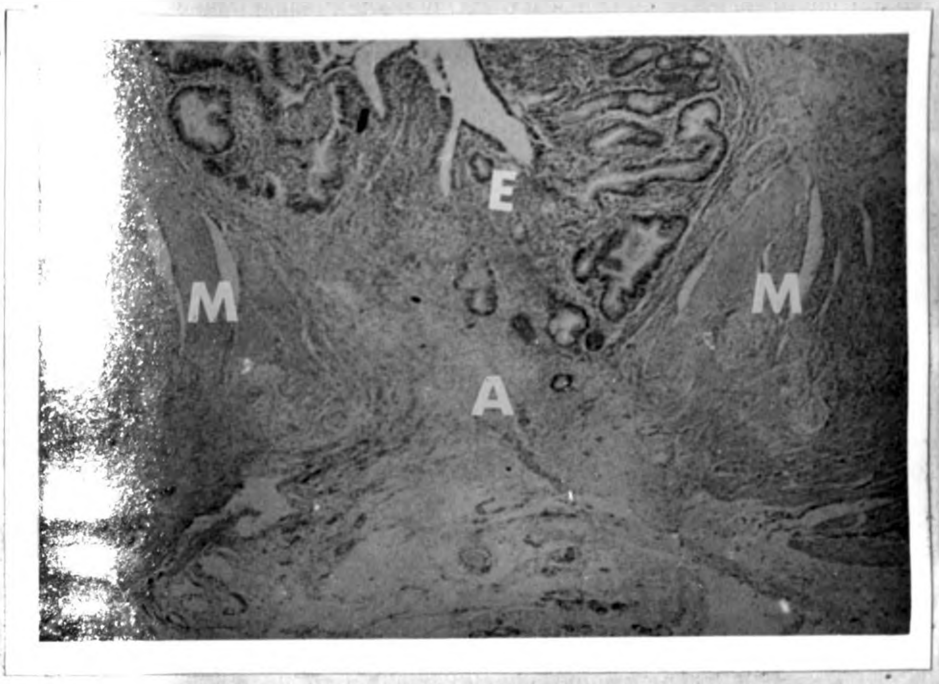


Figure 13. Photomicrograph of the bovine small intestine showing complete epithelialization after anastomosis x 50. The epithelium (E) is in many layers with some differentiation. Other layers are united by fibrous tissue (A) at the site of anastomosis. M = Muscle layer.

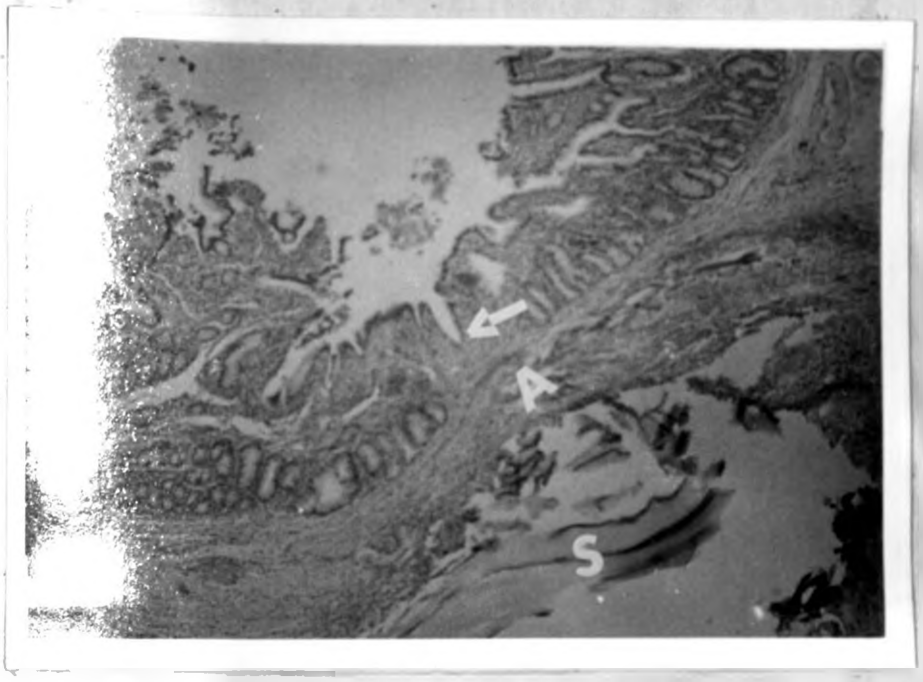


Figure 14. Photomicrograph of the bovine small intestine showing partial epithelialization after anastomosis x 50. There is only one layer of epithelial cells (Arrow). The empty space in the inflammatory zone was originally filled with suture material, part of which is present (S).

A = fibrous tissue uniting the two ends of the intestine.

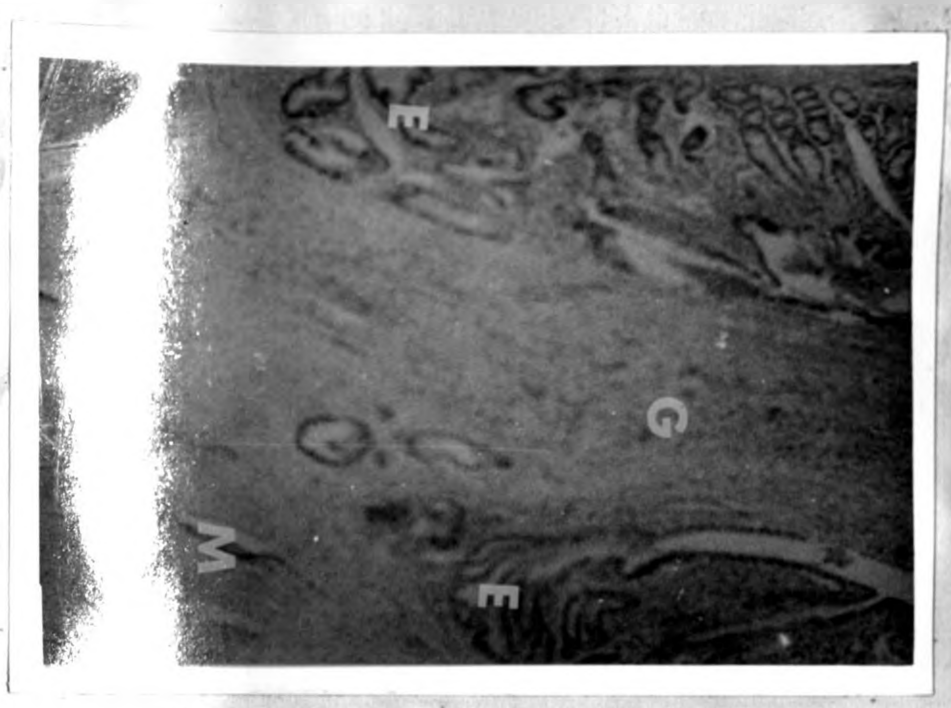


Figure 15. Photomicrograph of the bovine small intestine showing poor epithelialization after anastomosis x 50. The epithelial layer is incomplete and the space between the epithelial ends is occupied by a column of granulation tissue (G)

M = Muscle layer (one end) and

E = the ends of the epithelium

TABLE 8: TOTAL NUMERICAL SCORES* FOR HEALING OF INTESTINAL EPITHELIAL LAYER AT VARIOUS TIME INTERVALS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES

TIME INTERVAL (DAYS)	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	30	23	18
14	22	29	33
28	34	36	36
56	36	36	36
TOTAL	122	124	123

* Total scores were accumulated for 12 anastomoses in each time interval per technique. Individual scores were graded on a scale of: 3 = complete; 2 = partial; 1 = poor healing.

Figure 16. Photomicrograph of the bovine small intestine showing slight degree of inflammatory reaction after anastomosis x 50

The width of the inflammatory zone is shown by the arrows. The inflammatory zone was estimated in the area of anastomosis.

M = ends of the muscle layer

E = epithelium

Figure 17. Photomicrograph of the bovine small intestine showing moderate degree of inflammatory reaction after anastomosis x 50
The entire width of the inflammatory zone is shown only at the right lower corner (arrows)
M = one end of muscle layer
E = epithelium

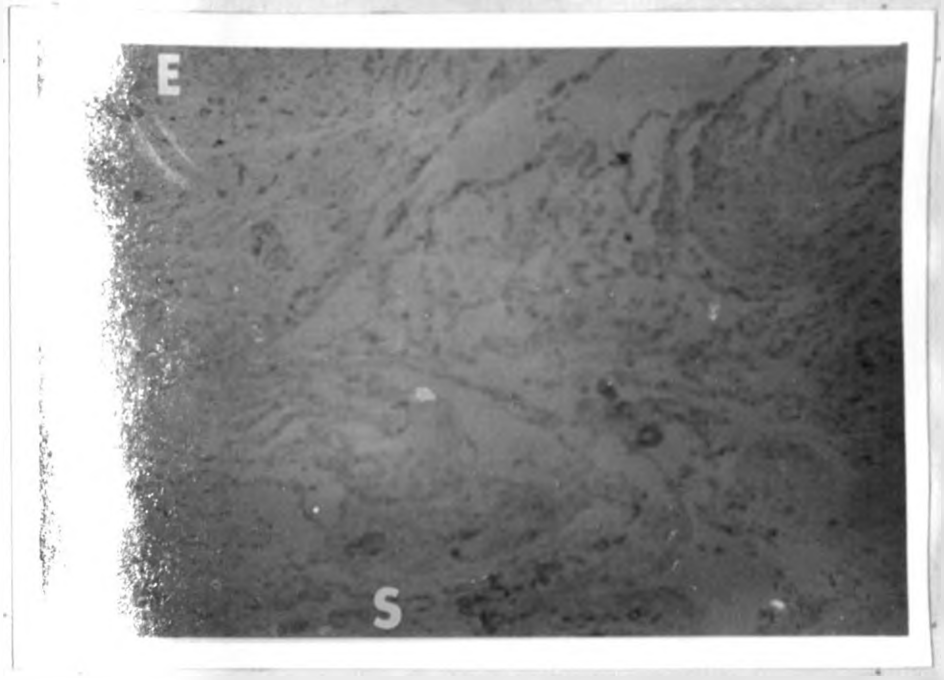


Figure 18. Photomicrograph of the bovine small intestine showing marked degree of inflammatory reaction after anastomosis x 50

The width of the inflammatory zone was much bigger than the area shown in the photograph although the picture was taken using the lowest magnification

E = epithelial side

S = serosal side

Muscle layer is not included.

TABLE 9: TOTAL NUMERICAL SCORES* FOR INFLAMMATORY REACTION AT THE ANASTOMOTIC SITES AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	18	24	29
14	17	17	13
28	19	23	21
56	22	30	27
TOTAL	76	94	90

*: Total scores were accumulated for 12 anastomoses in each time interval per technique. Scores were graded on a scale of: 3 = slight; 2 = moderate; 1 = marked inflammatory reaction.

There was a low inflammatory reaction at 4 days which increased at 14 days and decreased at 28 and 56 days. Gambee anastomoses had the least inflammatory reaction followed by crushing anastomoses. The greatest inflammatory reaction was in simple interrupted anastomoses.

4.8. INFLAMMATORY CELL INTENSITY AT THE ANASTOMOTIC SITE

Mean cells per time interval and overall means for the techniques are presented in table 10.

The cellular intensity was fairly high at 4 days and highest at 56 days. At 28 days cellular intensity was lowest except for the crushing anastomoses.

Overall means of cellular intensity showed that gambee anastomoses had the lowest cellular intensity and crushing anastomoses were second. Cellular intensity in simple interrupted anastomoses was slightly higher than that of crushing anastomoses.

The means of different cell types for the various time intervals are shown in table 11.

The main cell type was the fibroblast. The lymphocyte was the second most common cell. Other cells occuring in varying numbers were macrophages, neutrophils, eosinophils, monocytes and plasma cells.

TABLE 10: MEAN* INFLAMMATORY CELL INTENSITY ALONG THE ANASTOMOTIC SITE AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	40	36	31
14	35	32	39
28	35	22	37
56	39	45	39
MEAN	37.3	33.8	36.5

*: Based on counts from 12 anastomoses for each time interval per technique.

TABLE II: MEANS OF VARIOUS CELL TYPES ALONG THE ANASTOMOTIC SITE AT VARIOUS TIME INTERVALS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES

TIME INTERVAL (DAYS)	TECHNIQUE																				
	SIMPLE INTERRUPTED							GAMBEE							CRUSHING						
	F	Mc	L	N	E	M	P	F	Mc	L	N	E	M	P	F	Mc	L	N	E	M	P
4	26	1	9	2	1	1	-	23	2	7	2	1	1	-	19	1	9	2	1	1	-
14	26	1	4	2	1	1	1	23	1	6	1	-	-	1	26	1	7	3	1	1	-
28	27	1	4	1	-	-	1	15	1	4	-	1	-	1	26	1	6	1	-	-	1
56	33	1	3	1	-	1	-	33	2	7	1	-	1	1	30	2	6	1	-	-	-
MEAN	28	1	5	2	1	1	1	24	2	6	1	1	1	1	25	1	7	2	1	1	1

F = Fibroblast; Mc = Macrophage; L = Lymphocyte; N = Neutrophil; E = Eosinophil

M = Monocyte; P = Plasma cell

The number of fibroblasts apparently increased with time so that at 56 days, their number was highest.

Macrophages did not seem to change with time although at 56 days, gambee and crushing anastomoses had more macrophages than simple interrupted anastomoses. Generally gambee anastomoses possessed more macrophages than the other 2 types of anastomosis.

Neutrophils decreased with time except in the crushing anastomoses where at 14 days they had increased slightly suggesting probable infection.

Eosinophils were present at 4 and 14 days in simple interrupted and crushing anastomoses. These anastomoses had no eosinophils thereafter. Gambee anastomoses possessed eosinophils at 4 and 28 days.

Monocytes were observed at 4 days for all the techniques. At 14 days gambee anastomoses had no monocytes but the other anastomoses had monocytes. At 28 days no anastomosis had any monocyte. Monocytes were present in simple interrupted and gambee anastomoses at 56 days.

No plasma cells were seen at 4 days in all techniques. Simple interrupted and gambee anastomoses possessed plasma cells at 14 and 28 days but the crushing anastomoses had plasma cells only at 28 days. At 56 days only gambee anastomoses had

plasma cells.

4.9. DEGREE OF MUSCLE LAYER APPROXIMATION

End-on muscle layer approximation shown in figure 19 was considered good. Figure 20 shows satisfactory muscle layer approximation. Figures 21 and 22 show poor muscle layer approximation. There is eversion in figure 21 and inversion in figure 22.

Total numerical scores of muscle layer approximation at various time intervals are shown in table 12.

The approximation was poorest at 4 days and best at 28 days for all the techniques. Gambee anastomoses from 14 days had constantly superior degree of muscle layer approximation. Approximation for simple interrupted anastomoses fluctuated with time. Muscle layer approximation for crushing anastomoses was poorer at 56 days than at 28 days.

Generally, gambee anastomoses showed the best approximation followed by crushing anastomoses. Simple interrupted anastomoses showed less satisfactory approximation of the muscle layer.

From the histogram of combined results of epithelialization, inflammatory reaction and muscle layer approximation (Fig. 23) the gambee anastomoses had the best healing followed closely by crushing

Figure 19: Photomicrograph of the bovine small intestine showing end-on muscle layer approximation after anastomosis x 50

The two ends of the muscle layer (M) are aligned in the same plane.

A = Fibrous tissue at site of anastomosis

E = Epithelium

Figure 20. Photomicrograph of the bovine small intestine showing satisfactory muscle layer approximation after anastomosis x 50
The two ends of the muscle layer (M) are slightly off the end-on alignment

A = Fibrous tissue at the site of anastomosis

E = Epithelium

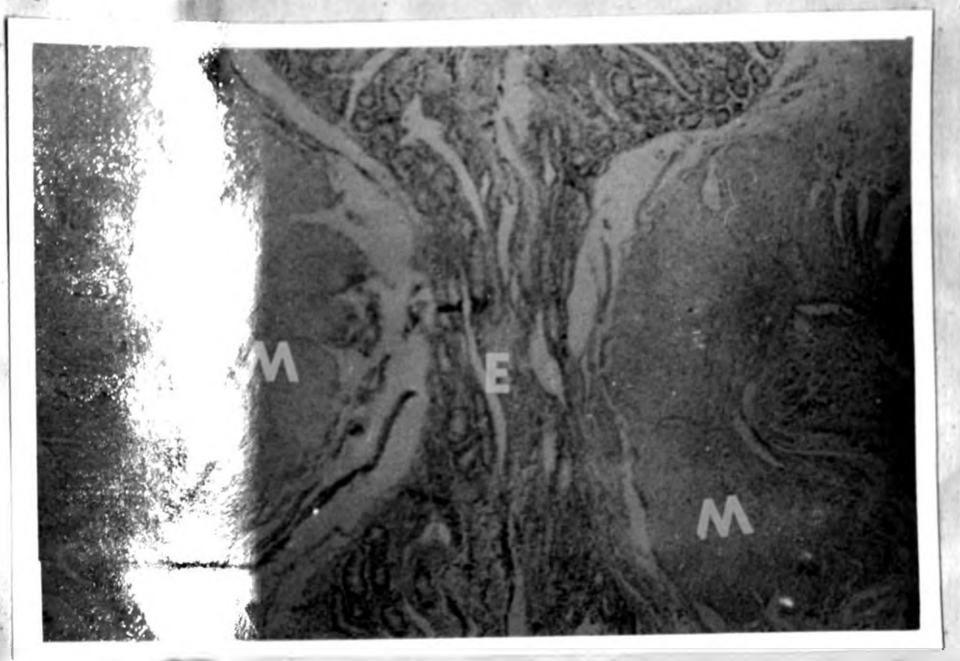


Figure 21. Photomicrograph of the bovine small intestine showing poor approximation - eversion after anastomosis x 50
The muscle layer (M) is everted and the space in between the two ends of the muscle layer is filled with the epithelium (E).
Fibrous tissue (not shown) sealed the serosal side enclosing the mucosa between the two ends of the everted muscle layer

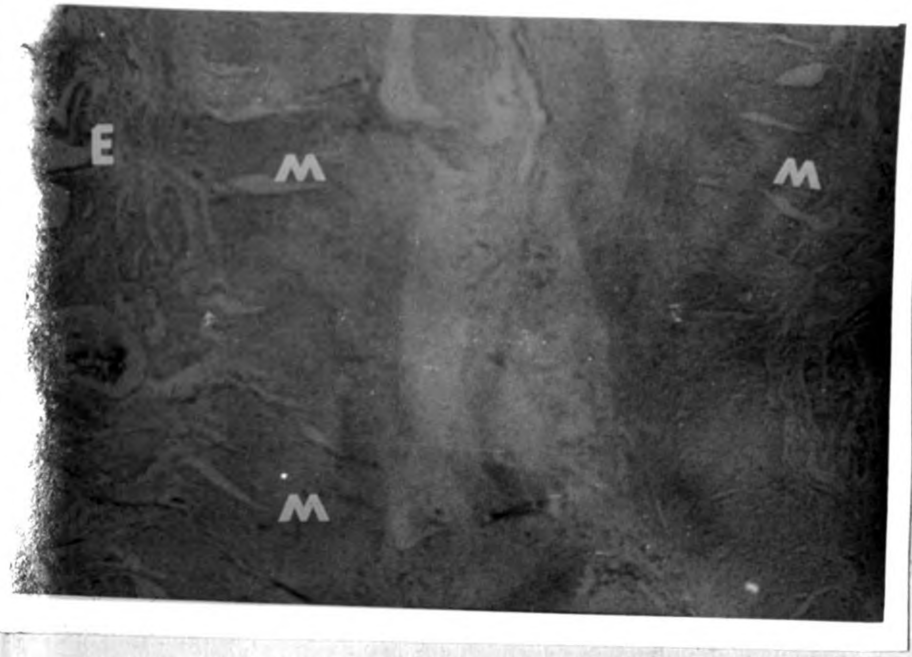
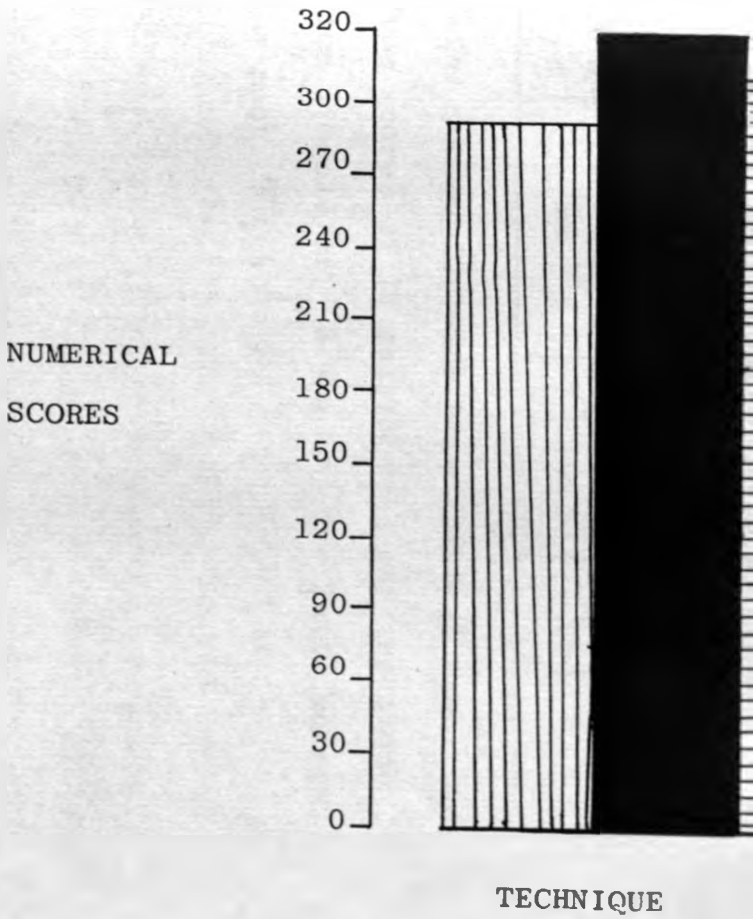


Figure 22. Photomicrograph of the bovine small intestine showing poor approximation - Inversion after anastomosis x 50
The muscle layer (M) is inverted. In between the muscle layer is fibrous tissue and inflammatory cells. The serosal side (not shown) was sealed by fibrous tissue. The epithelium (E) had bridged the two cut ends but the point of union is not shown.

Figure 23. Combined numerical scores of degrees of epithelial layer healing, inflammatory reaction and muscle layer approximation for all 4 time intervals for simple interrupted, gambee and crushing anastomotic techniques.

FIGURE 23


320
300
270
240
210
180
150
120
90
60
30
0
NUMERICAL
SCORES
TECHNIQUE

Simple interrupted

Gambee

Crushing

TABLE 12: TOTAL NUMERICAL SCORES* FOR MUSCLE APPROXIMATION DURING ANASTOMOSIS USING SIMPLE RUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VAR TIME INTERVALS

TIME INTERVAL (DAYS)	TECHNIQUE		
	SIMPLE INTERRUPTED	GAMBEE	CR
4	22	21	
14	21	27	
28	26	27	
56	25	27	
TOTAL	94	102	1

*: Total scores for each time interval

anastomoses. Simple interrupted anastomo
third.

4.10 INDEX OF INCREASE IN WHOLE INTEST THICKNESS

Table 13 contains the average indice
time intervals and their overall means.

Averages for Index I showed that alt
interrupted anastomoses had the greatest
in thickness at 14 days, it decreased dra
with time. Crushing anastomoses averages
I showed a uniform decrease in thickness
increased thickness remained generally hi
the other techniques. Gambee anastomoses
least increase in thickness initially but
in thickness was high at 28 days and decr
slightly at 56 days. The gambee anastomo
generally better than the crushing anasto
for an increase in thickness was concerne

TABLE 13: MEAN INDICES* AND COEFICIENTS** OF INCREASE IN WHOLE INTESTINAL WALL THICKNESS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	TECHNIQUE					
	SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
	INDEX I	INDEX II	INDEX I	INDEX II	INDEX I	INDEX II
4	-	-	-	-	-	-
14	61.9	69.5	24.2	33.3	56.3	78.1
28	23.1	82.8	34.8	54.0	48.0	70.1
56	4.5	14.4	31.6	27.2	33.4	27.9
MEAN	29.8	55.6	30.2	38.2	45.9	58.7
COEFICIENT	1.85		1.27		1.28	

* - Each mean index is from indices of 12 anastomoses for each time interval per technique.

** - Coeficient calculated by dividing overall mean index II by overall mean index I (I = B+C measurements used in calculation
II = Normal measurements (N_1 + N_2) used in calculation).

Overall mean index I showed that simple interrupted anastomoses had slightly less increase in thickness than gambee anastomoses. Crushing anastomoses had the greatest increase in thickness. When overall mean index II was calculated, gambee anastomoses had the least increase in thickness followed by simple interrupted anastomoses. Crushing anastomoses showed the greatest increase in thickness.

From the above presentation of the results of increase in intestinal wall thickness, there was no clear indication of the technique causing the least increase in thickness. It was decided to calculate a ratio of the indices by dividing overall mean Index II by overall mean Index I (Table 13) and the ratio was called coeficient of increase in thickness.

Using the coeficient of increase in thickness, gambee anastomoses showed the least spread out increase in thickness followed closely by crushing anastomoses. The simple interrupted anastomoses exhibited more wide spread increase in thickness.

4.11 INDEX OF INCREASE IN INTESTINAL WALL THICKNESS WITH MUCOSA EXCLUDED

The mean indices for each time interval and overall for each technique are presented in table 14.

TABLE 14: MEAN INDICES* AND COEFICIENTS** OF INCREASE IN INTESTINAL WALL THICKNESS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS WITH MUCOSA EXCLUDED IN MEASUREMENTS

TIME INTERVAL (DAYS)	TECHNIQUE					
	SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
	INDEX I	INDEX II	INDEX I	INDEX II	INDEX I	INDEX II
4	69.5	123.8	103.9	148	188.5	235.8
14	90.8	159.4	56	65.8	118.1	142.4
28	53.9	118.9	64.4	90.8	80.3	130.9
56	12.1	29.3	45.8	73.9	101.7	73.9
MEAN	56.6	107.9	67.5	94.6	83.2	145.8
COEFICIENT	1.92		1.41		1.75	

* - Each mean index is from indices of 12 anastomoses in each time interval per technique.

** - Coeficient calculated by dividing overall mean index II by overall mean index I (I = B+C measurements used in calculation II = Normal measurements used in calculation).

Averages for index I, showed crushing anastomoses had the greatest increase in thickness at all times. Simple interrupted had less increase in thickness than gambee anastomoses except at 14 days when simple interrupted anastomoses had a greater increase in thickness. Gambee anastomoses on the other hand, showed less increase in thickness than crushing anastomoses despite its failure to show a rapid decline like simple interrupted anastomoses.

The overall mean Index I (Table 14), showed that simple interrupted had the least increase in thickness followed by gambee anastomoses. Crushing anastomoses showed the greatest increase in thickness. From the overall mean index II, the positions of simple interrupted and gambee anastomoses were reversed while crushing anastomoses maintained their position.

As for the whole thickness evaluation comparison of the techniques was difficult after examining the results from the two indices. It was found necessary to calculate the coeficients of increase in thickness shown in table 14 using the overall means. The coeficients of increase in thickness showed that gambee anastomoses had the least spread out increase in thickness followed by crushing technique. Simple interrupted technique showed more wide spread increase in thickness.

4.12 SUMMARY OF THE RESULTS

Table 15 contains the summary of the various studies. For comparison of the techniques, the three techniques were ranked 1-3 and given point scores. From the scores it was evident that gambee technique scored the highest points and crushing technique was second. Simple interrupted technique scored the lowest points.

TABLE 15: SUMMARY OF THE RESULTS OF VARIOUS STUDIES FOR THE COMPARISON OF SIMPLE INTERRUPTED, GAMBEE AND CRUSHING SUTURE TECHNIQUES

STUDY	RANKING*		
	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
Time per stitch	1	2	3
Adhesions around circumference	3	1	2
Anastomotic stenosis (Visual)	2	1	3
Anastomotic stenosis (X-ray)	2	1	3
Epithelialization	3	1	2
Muscle layer approximation	3	1	2
Inflammatory Reaction	3	1	2
Increase in thickness (whole)	3	1	2
Increase in thickness (Mucosa excluded)	3	1	2
Inflammatory cell intensity	3	1	2

* Based on the numerical position after the final scores.

SCORING THE RANKINGS		Points
1 = 3 points	GAMBEE	29
2 = 2 points	CRUSHING	24
3 = 1 point	SIMPLE INTERRUPTED	14

5 DISCUSSION

The period of time animals were fasted was reduced to 18 hours after complications occured when animals were fasted for longer periods of time. However 18 hours was less than previously recommended times (Mbiuki, 1977; Walker & Vaughan, 1980). Animals used in this project might have had less feed intake because they were changed from feeding on green grass to dry grass (hay) and concentrates. Taking them off feed for long periods of time probably depleted the already reduced rumen contents and reduced their source of energy. This was apparently the cause of the weakness observed post-operatively in those animals which were fasted longer than 18 hours.

Since the animals were not to be subjected to general anaesthesia, the long period of fasting might not have been necessary because Xylazine hydrochloride which was used as a premedication does not interfere with eructation, coughing or swallowing reflexes (Hall, 1974). The 18 hours of fasting were adequate to prevent bloat.

Dullness and reduced appetite observed in the first post-operative week were expected because of the inflammation around the anastomotic sites.

The six anastomoses performed in each animal apparently did not affect adversely the gastro-intestinal physiology because the animals fed soon after the operations. This was probably due to adequate spacing (5Ocm) of the anastomoses. Feeding as soon as possible after operation has been suggested as being desirable because it promotes intestinal motility thereby reducing the chances of post-operative paralytic ileus (Bromwell et al., 1969; Defloff, 1975; Grier, 1975). It is possible that the animal which died had paralytic ileus due to failure to feed.

It might be argued that the different techniques should have been performed in every animal to eliminate individual variations. It was decided however, to perform each of the 6 anastomoses per technique in individual animals using the same technique to make sure that all the findings were associated with the particular technique. If different techniques were used in each animal, some reactions might have been difficult to associate with a particular technique. Also in practice, one would not be required to use different techniques in the same animal during one operation. Repeating the same technique in each animal was to increase the number of anastomoses studied.

AVERAGE TIME PER ANASTOMOSIS AND AVERAGE TIME PER STITCH

Average time per anastomosis had been used previously to compare anastomotic techniques (Hamilton, 1967; McAdams et al., 1970; Singh et al., 1979a; Stashak, 1982). In our studies average time per anastomosis was different between the three techniques.

Different numbers of stitches were used in performing the anastomoses because of the varying sizes of intestinal circumferences. It was better to compare time taken to place each stitch rather than time taken to perform the entire anastomosis. Time per stitch in our studies was calculated using the 6 anastomoses performed in each animal because there was in every animal anastomoses 1-6. Therefore pooling within animals for stitches gave the best indicator of time per stitch.

FORMATION OF ADHESIONS

The formation of adhesions in this study was not extensive. They were confined to the adjacent loops of intestine or mesentery. This was in agreement with observations by other workers (Trueblood et al., 1969; Singh & Nigam, 1972; Bellenger, 1982; Richardson et al., 1982). Omental

adhesions were not observed.

Grading of degrees of adhesions according to the number of quandrants involved had been reported (McAdams et al., 1970). Unlike our cases where we gave each quandrant a numerical score and added them up later, these workers averaged the quandrants for comparison.

From our studies, it appeared that the formation of adhesions was influenced by the technique used to perform the anastomoses. The degree of adhesions was probably not affected by time after the operation in the period studied and the adhesions which formed initially persisted instead of increasing or decreasing. This observation partly supported reports by Herrmann et al.,(1964) and Reinertson (1976) who reported that adhesions remain constant after 7 days. It contradicted reports by others (Ott et al.; 1968; Singh et al., 1979a&b; Singh et al., 1980) who found adhesions to decrease with time.

The frequency of occurence of various degrees of adhesions had not been previously studied. Such a study proved to be a good indicator of the suitability of a technique for performing an anastomosis.

EXTERNAL EVALUATION OF STENOSIS

Since no grossly visible obstruction was present

in situ, it was decided to fill the anastomotic segments to a moderate degree of distension with barium sulphate. This enabled an evaluation of narrowing at the anastomotic sites. This particular study had not been reported previously.

Numerical scoring of different degrees of stenosis was essential because a subjective comparison of the anastomoses using various techniques would have been difficult since large numbers of anastomoses were studied.

The technique utilized to perform the anastomoses seemed to have influenced the degree of stenosis of the anastomotic sites as seen grossly (Table 3). Stenosis decreased with time only in gambee anastomoses and not in simple interrupted and crushing anastomoses.

Frequency of occurence of various degrees of stenosis was found to be a reliable method of comparing the different techniques.

STENOSIS EVALUATED BY RADIOGRAPHY

The technique used in performing the anastomoses apparently affected the degree of intestinal luminal stenosis because the anastomoses performed utilizing the three techniques showed different levels of luminal stenosis. Generally stenosis

decreased with time in simple interrupted and gambee anastomoses but not in crushing anastomoses. This was partly in agreement with Singh et al., (1979a) who reported decrease of luminal stenosis with time.

Like earlier workers (McAdams et al., 1970, Singh et al., 1980), we found that the calculated indices of stensosis clearly demonstrated the degree of luminal stenosis.

DEGREE OF HEALING OF EPITHELIAL LAYER

The method of evaluating epithelial layer healing employed in this study was in an attempt to obtain an objective evaluation of healing to facilitate the comparative study.

Our findings were in agreement with Ellison et al.,(1982) who found that the rate of epithelial healing was dependent on technique. In this study, there was complete epithelialization at 4 days in the majority of anastomoses performed using simple interrupted technique. Epithelialization was present in gambee anastomoses while 50% of crushing anastomoses showed only granulation tissue at 4 days. Early epithelialization in this study was contrary to the findings by other workers who reported epithelialization as starting after 7 days (Herrmann

et al., 1964; Necula and Mandace, 1974; Jansen, et al., 1981). However, their findings were not directly comparable because they were comparing everting and inverting anastomoses.

Regeneration of the epithelium did not show any difference between the techniques after 14 days. However, the findings at 4 days were important due to the fact that early epithelialization prevents further contamination of the granulating wound. This would enhance laying of fibrous tissue for a rapid gain in strength of the anastomotic site (Teveney & Way, 1977).

The techniques compared in this study showed trends favouring simple interrupted anastomoses at 4 days and gambee anastomoses generally as shown in the final scores.

DEGREE OF INFLAMMATORY REACTION

The technique used to evaluate inflammatory reaction has not been previously utilized. It seemed a reliable indicator of inflammatory reaction because the width of the healing area at the point where the two ends were cut excluding the mucosa was caused by inflammatory processes. The contents of the inflammatory reaction were expected to include fibrin, necrotic tissue, oedema and granulation tissue.

As for epithelial healing, numerical scores were given to the different degrees of inflammatory reaction for ease in comparing the techniques. It was obvious from this study that inflammatory reaction decreased with time but to different degrees for the different techniques. This was in agreement with findings by Somvanshi et al., (1980).

INFLAMMATORY CELL INTENSITY AND CELL TYPES ALONG THE ANASTOMOTIC SITE

The use of the paper disc with a window in the centre was very useful in reducing the area where cells were counted. This was particularly of use in this study because the cells present in one microscopic field were usually numerous and counting them was not possible without repititions and omissions.

Studies of cellular intensity along the anastomotic line had not been carried out previously according to the available literature. It was decided to study the cellular intensity because the different techniques were expected to show varying degrees of cellular reaction.

The method used in the determining inflammatory cell intensity is applicable in studies to determine cellular intensity in other histologic

studies.

The high cell counts at 4 days with the fibroblast as the main cell was due to fibroplasia in the early proliferative phase of healing. The high lymphocyte count was probably from the blood stream in the newly formed capillaries. The cells of fibroblast origin were in large numbers at 56 days apparently because wound contraction had started and there was consolidation of the fibrous tissue. The lymphocytes which were the second most prominent cells, probably came from the lymphoid tissue of the intestinal wall.

Neutrophils were generally present in low numbers at the anastomotic site except in the crushing anastomoses at 14 days and this could have been due to localized infection.

Due to the low numbers of other cell types at the anastomotic sites, it was possible that these cells could have been the normal intestinal wall connective tissue cells (Bloom & Fawcet, 1970).

Cellular intensity did not decrease in this study as reported by Singh et al., (1980). In fact, there was a higher cellular reaction at 56 days than at other time intervals.

The presence of various cell types except the fibroblasts and lymphocytes was not consistent. It appeared unlikely that the technique utilized to

perform the anastomoses influenced the cell types present because all the cell types observed were present in all techniques.

DEGREE OF MUSCLE LAYER APPROXIMATION

In this study which was carried out for only 8 wks, muscle layer alignment was possible to determine. This was because muscle layer healing which is by fibrosis does not affect extensive areas of the muscular tissue and as such degree of muscle layer alignment can be assessed from histologic sections (Somvanshi et al., 1980; Jansen et al., 1981).

The results of degree of muscle layer alignment were suggestive of technique influencing the degree of muscle layer alignment. The approximation of the cut ends apparently remained the way it was at the time of anastomosis. It did not improve with time as demonstrated by the anastomoses performed using simple interrupted and crushing techniques.

Combination of epithelial healing, inflammatory reaction, and muscle layer approximation was logical because in the healing process, epithelialization and granulation tissue formation occur. Muscle layer alignment would affect the end-on approximation of the other intestinal wall layers. Since end-on appro-

ximation is essential for primary healing (Jansen et al., 1981; Ellison et al., 1982), inclusion of the muscle layer alignment findings in the combination allowed a broad evaluation of the healing process. The combined results showed gambee anastomoses to have better healing than the anastomoses performed using the other techniques.

INCREASE IN INTESTINAL WALL THICKNESS

A pair of calipers had been previously used to measure macroscopic thickness of the intestinal wall (Getzen et al., 1966). Use of histologic sections to estimate increase in intestinal wall thickness has been reported (Singh and Nigam, 1972; Richardson et al., 1982). The method utilized in the present study has not been reported previously.

The formulae for calculation of the index of increase in thickness were derived so that all the measurements around the anastomotic sites could be presented as percentages (indices) which was easier to compare. One index was calculated using measurements fairly close to the anastomotic site (1.5cm) and the other using measurements from a normal area, to assess whether the inflammatory reaction extended some distance from the anastomotic sites in the different techniques.

It was interesting to find that increase in thickness when whole thickness measurements were used, did not show as much difference between the techniques as when mucosa was excluded in the measurements. From this it was deduced that the mucosa somehow affected the measurements. This was probably because the degree of epithelialization differed between the techniques. Depending on the thickness of the epithelial layer, the measurements for the whole thickness were bound to be variable. Therefore it seemed like measurements of the thickness of the intestinal wall excluding the mucosa, were more informative on the increase in intestinal wall thickness.

Because the results when the indices were calculated using formulae I & II were variable. it was decided to calculate a coeficient of increase in thickness. This was done on the assumption that there was no increase in thickness in the normal specimens. It meant that the index II calculated using normal specimen measurements was high because the thickness at the anastomotic site was divided by normal thickness. The index I should also have been high if there was no increase in thickness at points B & C and vice versa. If the coeficient of increase in thickness is higher than one, then there was increase in thickness at points B & C which made the index I low and vice versa. Therefore a coeficient of increase in thickness greater than one

means wide spread increase in thickness.

It was possible using the coeficient of increase in thickness to demonstrate the difference in increase in thickness in the three techniques. In fact it was evident that gambee anastomoses had the least spread increase in thickness followed by crushing anastomoses and simple interrupted anastomoses the most wide spread increase in thickness. The wide spread increase in thickness was considered undesirable.

The increase in thickness generally decreased with time supporting findings of Singh & Nigam, (1972).

It was necessary to derive the formulae for calculating indices used in the determination of increase in thickness because the various mesurements were not very meaningful and therefore not useful in the comparative study. The calculations might seem to have included only one side of the cross section of the intestinal wall thickness and since the intestinal wall is tubular, it might be argued that both sides of the intestinal wall should have been included. This was not done on the assumption that the sections used were representative of the thickness around the circumference. It was also not necessary because our studies were comparative and indices were calculated using the same method for the three techniques.

The coeficient of increase in thickness in this study was definitely a good and reliable indicator of the spread of increase in thickness and was useful in comparing the techniques.

GENERAL DISCUSSION

Angiographic and microangiographic studies of revascularization of anastomotic sites undertaken to compare different techniques have been reported (Ravitch et al., 1967; Abramowitz et al., 1969; Wise et al., 1975; Singh et al., 1976; Ellison et al., 1982). These parameters were not used in this study but if used, they might have provided further data for comparison of the techniques. However, the parameters studied were considered adequate for the comparative studies.

Simple continous technique was the only end-on approximating technique not included in our study. Recent studies using fluorescein dye, angiographic and histologic evaluation have demonstrated the technique as being better than simple interrupted and crushing techniques in the rate of healing (Ellison et al., 1982). Since gambee technique showed the best results in this study, it might be interesting to compare simple interrupted technique with gambee technique.

Although formation of adhesions, gross stenosis as seen externally, epithelialization, inflammatory reaction and muscle layer alignment, might have been examined in previous studies, the presentation of the results was usually subjective except in one case (McAdams et al., 1970). The use of numbers rather than subjective evaluation was easier to compare. It was therefore suggested that the objective evaluation of the above parameters which was more informative than the subjective evaluation be adopted for future use.

6

CONCLUSIONS

Different investigations were carried out to compare simple interrupted, gambee and crushing anastomotic techniques. The following conclusions were made:

1) Gambee technique had superior qualities in lack of adhesion formation, absence of external stenosis, minimal intestinal lumen stenosis, epithelialization, changes in inflammatory reaction, good muscle layer approximation, least cellular intensity, and least spread increase in intestinal wall thickness. It only came second to simple interrupted technique in time taken to place a suture.

2) Crushing technique took maximum time to place a suture and caused most external and internal stenosis. However, it was second in lack of adhesions, epithelialization, inflammatory reaction, muscle layer approximation, cellular intensity and spread of increase in intestinal wall thickness.

3) Simple interrupted technique took minimum time to perform and came second in the absence of external and internal intestinal

stenosis. It was the least desirable in epithelialization, inflammatory reaction, muscle layer approximation and cellular intensity. There was the most widely spread increase in intestinal wall thickness in the simple interrupted anastomoses

4) Gambee technique was found to be the most suitable technique for end-on approximating intestinal anastomosis. The crushing technique came second and simple interrupted was the least suitable.

7 REFERENCES

ABRAMOWITZ, H.B.; BUTCHER, H.R. (1971)
Everting and Inverting Anastomoses: An Experimental Study of Comparative Safety. Am. J. Surg. 121: 52-56

ABRAMOWITZ, H.B.; McALISTER, W.H. (1969)
A Comparative Study of Small Bowel Anastomosis by Angiography and Microangiography. Surg. 66: 564-569

BELLENGER, C.R. (1982)
Comparison of Inverting and Appositional Methods for Anastomosis of the Small Intestine in Cats. Vet. Rec. 110 (2): 265-268

BENNETT, R.R.; ZYDECK, F.A. (1970)
A Comparison of Single Layer Suture Patterns for Intestinal Anastomosis. J. Am. Vet. Med. Assoc. 157 (2): 2075-2080

BERGE, E; WESTHUES, M. (1966)

Veterinary Operative Surgery. Williams & Wilkins Co. Baltimore: Pg 212

BLOOM, W.; FAWCETT, D.W. (1970)

Intestines. In "A Textbook of Histology" 9th Ed. W.B. Saunders Co. Philadelphia Pg 560-581

BRAUCHER, R.F.; KIRSNER, J.B. (1962)

Regeneration of Colon Mucosa. Gastroentol. 42: 706-717

BROMWELL, A.W.; RUTLEDGE, R.; DALTON, M.L. (1967)

Single Layer Open Gastrointestinal Anastomoses. Ann. Surg. 165: 925-930

BRUNIUS, U.; ZEDERFELDT, B. (1970)

Suture Materials in General Surgery: A Comment. Progr. Surg. 8: 38-44

BULKLEY, G.B.; WHEATON, L.G.; STRANDBERG, J.D.; SUIDEMAN, G.D. (1979)

Assessment of Intestinal Recovery from Ischaemic Injury after Segmental Arterial,

Venous and Arterio-venous Occlusion. Surg. Forum 30: 210-213

CANALIS, F.; RAVITCH, M.M. (1968)
Study of Healing of Inverting and Everting Intestinal Anastomosis. Surg. Gyn. Obstet. 126: 109-114

COHN. I.; NANCE, F.C. (1966)
Small Bowel Resection. Surg. Clin. North Am. 46: 1179-1190

CRONIN, K.; JACKSON, D.R.; DUNPHY, J.E. (1968)
Changing Bursting Strength and Collagen Content of Healing Colon. Surg. Gyn. Obstet. 126: 747-753

DEHAAN, B.B.; ELLIS, H.; WILKS, M. (1974)
The Role of Infection in Wound Healing. Surg. Gyn. Obstet. 138: 693-750

DEHOFF, W.D. (1975)
Small Intestinal Anastomosis. Vet. Clin. North Am. (Small Anim. Pract.) 5 (3); 551-556

DEHOFF, W.D.; NELSON, W.; LUMB, W.V. (1973)
Simple Interrupted Approximating Technique for Intestinal Anastomosis. J. Am. Anim. Hosp. Assoc. 9: 483-489

DELLMANN, H.D.; BROWN, E.M. (1976)
Textbook of Veterinary Histology. Lea & Febiger, Philadelphia: Pg 551-556

DEVENEY, K.E.; WAY, L.W. (1977)
Effect of Different Absorbable sutures on Healing of Gastrointestinal Anastomoses. Am. J. Surg. 133: 86-94

ELKIN, D.C. (1940)
Wound Infection: A Comparison of Silk & Catgut Sutures. Ann. Surg. 112: 280-283

ELLENPORT, C.R. (1975)
Carnivore Digestive System. In "Sisson & Grossman's: The Anatomy of Domestic Animals". 5th Ed. W.B. Saunders Co. Philadelphia: Pg 1552

ELLISON, G.W.; JOKINEN, M.P.; PARK, R.D. (1982)
End to End Approximating Intestinal Anastomosis in the Dog: A Comparative Fluorescein Dye, Angiographic and Histopathologic Evaluation. J Am. Anim. Hosp. Assoc. 18: 729-736

FRANK, E.R. (1964)
Veterinary Surgery. 7th Ed.: Pg 240-241

GAMBEE, L.P. (1951)
A Single Layer Open Intestinal Anastomosis Applicable to Small as well as Large Intestines. West. J. Surg. Obst. Gyn. 59: 1-5

GAMBEE, L.P.; GARNJOBST, W.; HARDWICK, C.E. (1956)
Ten years Experience with a Single Layer Anastomosis in Colon Surgery. Am. J. Surg. 92: 222-227

GETZEN, L.C.; ROE, R.D.; HOLLOWAY, C.K. (1966)
Comparative Study of Intestinal Anastomotic Healing in Inverted and Everted Closures. Surg. Gyn. Obstet. 123: 1219-1227

GRIER, R.L. (1975)
Techniques for Intestinal Anastomosis. In "Current Techniques in Small Animal Surgery." Edited by J.M. Bojrab. Lea & Febiger, Philadelphia: Pg 119-125

GUYTON, A.C. (1976)
Textbook of Medical Physiology. Chapter 63. 5th Ed. W.B. Saunders Co. Philadelphia: Pg 853

HABEL, R.E. (1975)
Ruminant Digestive System. In "Sisson & Grossman's: The Anatomy of Domestic Animals." 5th Ed. W.B. Saunders Co., Philadelphia: Pg 903-904

HALL, L.W. (1974)
Premedication. In "Wright's Veterinary Anaesthesia & Analgesia". 7th Ed.

Bailliere Tindall. London. Pg 161-163

HALSTEAD, W.S. (1887)
Circular Suture of the Intestine - An Experimental Study. Am. J. Med. Sci. 94: 436-460

HAMILTON, J.E. (1967)
Reappraisal of Open Intestinal Anastomosis. Ann. Surg. 165: 917-923

HARDY, K.J. (1968)
Suture Anastomosis - An Experimental Study using Limited Suturing of Small Bowel in the Dog. Arch. Surg. 97: 586-589

HASTINGS, J.C.; VAN WINKLE, W.J. (Jr): BARKER, E.; HINES, D.; NICHOLS, W. (1975)
Effects of Suture Materials on Healing Wounds of the Stomach and Colon. Surg. Gyn. Obstet. 140: 701-707

HAWLEY, P.R.; FAULK, W.P.; HUNT, T.K.
DUNPHY, J.E. (1970)
Collagenase Activity in Gastrointestinal Tract. Br. J. Surg. 57: 896-900

HEINZE, C.D. (1974)
Wound Healing and Tissue REpair. In "Textbook of Large Animal Surgery". Edited by Oehme and Prier. Williams & Wilkins Co. Baltimore: Pg 41-54

HERRMANN, J.B.; WOODWARD, S.C.; PULASKI, E.J. (1964)
Healing of Colonic Anastomoses in the Rat. Surg. Gyn.-Obstet. 119: 269-275

HILL, K.J. (1970)
In "Duke's Physiology of Domestic Animals" 8th Ed. Comstock Publishing Associates. A division of Cornell University Press. Ithaca & London: Pg 370-372

HOFMEYR, C.F. (1974)
The Digestive System. In "Textbook of Large Animal Surgery." Williams & Wilkins Co. Baltimore: Pg 423-428

IRVIN, T.T.; HUNT, T.K. (1974)
Reappraisal of the Healing Process of Anastomoses of the Colon. Surg. Gyn. Obstet. 138 : 741-746

JACKSON, D.W.; POLLOCK, A.V.; TINDAL, D.S. (1971)
The Effects of Antibiotics in Prevention of Wound Infection: A Controlled Trial. Br. J. Surg. 58: 565-566

JANSEN. A.; BECKER, A.E.; BRUMMELKAMP, W.H.; KEEMAN, J.N.; KLOPPER, P.J. (1981)
The Importance of Apposition of Submucosal Intestinal Layers for Primary Healing of Intestinal Anastomosis. Surg. Gyn. Obstet. 152: 51-58

JIBORN, H.; ABONEN, J.; ZEDERFELDT, B. (1978)
Healing of Experimental Colonic Anastomosis. The Effects of Suture Technique on Collagen Concentration in the Colonic wall. Am. J. Surg. 135: 333-340

JOHNSON, D.E. (1977)
The Process of Wound Healing. J. Am.

Anim. Hosp. Assoc. 13: 186-196

KUMAR, A.; TANWAR, R.K.; SHARMA, K.B.; SAXENA, A.K.; JOSHI, Y.; PRADHAM, P.K. (1982)
Intestinal Fistula in a Cow. Mod. Vet. Practice 63: (10): 833-834

LOEB, M.J. (1967)
Comparative Strength of Inverted, Everted, and End-on Intestinal Anastomoses. Surg. Gyn. Obstet. 125: 301-304

LORD, M.G.; VALIES, P. (1977)
A Morphologic Study of the Submucosa of the Large Intestine. Surg. Gyn. Obstet. 145: 55-60

MADSEN, E.T. (1953)
An Experimental and Clinical Evaluation of Surgical Suture Materials. Surg. Gyn. Obstet. 97: 73-80

MBIUKI, S.M. (1977)
Bovine Intestinal Anastomosis: Effects of Suture Materials on Healing. M.S. Thesis, Colorado State University,

Fort Collins Colorado, USA.

McADAMS, A.J.; MEIKLE, A.G.; TAYLOR, J.O. (1970)

One Layer or Two Layers Colonic Anastomosis? Am. J. Surg. 120: 546-550

MEAGHER, D.M. (1973)

Surgery of Small Intestines in the Horse. Arch. Am. Coll. Vet. Surg. 2: 3-9

MITCHEL, J. (1975)

Diagnosis and Correction of Some Intestinal Obstructions. Proc. 8th Annual Convention of Am. Assoc. Bovine Pract.: 53

MYERS, M.B. (1971)

Sutures and Wound Healing. Am. J. Nursing 71: 1725-1727

NAKANISHI, H. (1975)

Experimental Gut Anastomoses and Their Revascularization. Aust. Nz. J. Surg. 45:(3): 309-314

NEAL, T.M. (1975)

Crushing Technique for Closure of Incisions and Resections in Small Intestine. In "Current Techniques in Small Animal Surgery". Edited by J.B. Bojrab; Lea & Febiger, Philadelphia: Pg 125-126

NICKEL, R.; SCHUMMER, A.; SEIFERLE, E.; SACK, W.O. (1973)

The Viscera of Domestic Mammals. Verlag Paul Parcy, Berlin, New York: Pgs 127; 168

NECULA, T.; MANDACE, E. (1974)

Ultrastructural Features of the Healing area after Everting and Inverting sutures of Small Intestine in Rabbits Acta Morp. Acad. Sci. Hung. 22 (3-4): 227-239

OTT, B.S.; MOREY, D.D.; GREENAWALD, K.A. (1968)

Single Layer Everted Intestinal Anastomosis. J. Am. Vet. Med. Assoc. 153: 1742-1753

PAYTON, J. (1954)

Intestinal Resection in a Bull. J. Am. Vet. Med. Assoc. 124: 352-353

PEACOCK, E.E.; VAN WINKLE, W. (Jr). (1970)
Surgery and Biology of Wound Repair.
1st Ed. W.B. Saunders Co. Philadelphia,
London, Toronto: Pgs 34, 579

PEACOCK, E.E.; VAN WINKLE, W. (JR). (1976)
Wound Repair. 2nd Ed. W.B. Saunders Co.
Philadelphia, London, Toronto: Pgs 1-22;
40-41; 149-153; 215-221; 640-646

POTH, E.J.; GOLD, D. (1968)
Intestinal Anastomosis: A Unique
Technique. Am. J. Surg. 116: 643-647

POSTLETHWAIT, R.W. (1970)
Long Term Comparative Study of Non-
Absorbable Sutures. Ann. Surg. 171:
892-898

RAVITCH, M.M. (1969)
Some Considerations on Healing of Intes-
tinal Anastomosis. Surg. Clin. North
Am. 49: 627-635

REINERTSON, E.L. (1976)
Comparison of Three Techniques for Intestinal Anastomosis in Equidae. J. Am. Vet. Med. Assoc. 169: 208-212

RICHARDSON, D.C.; DUCKETT, K.E.; KRAHWINKEL,D.J. (Jr).; SHIPMAN, L.W. (1982)
Evaluation of End to End Crushing and Inverting Techniques. Am. J. Vet. Res. 43: 436-442

RUSCA, J.A.; BORNSIDE, G.H.; COHN, I. (1969)
Everting Versus Inverting Gastrointestinal Anastomosis: Bacterial Leakage and Anastomotic Disruption. Ann. Surg. 169: 729-735

SAKO, Y.; WANGENSTEEN, O.H. (1951)
Experimental Studies in Gastrointestinal Anastomosis. Surg. Forum 2: 117-123

SCHILLACI, A.; CAVALLARO, A.; STIPA, S. (1979)
Comparative Results of Three Different Techniques for Colonic Anastomosis in the Dog. Surg. Gyn. Obstet. 149: 238-240

SINGH, G.; MANOHAR, M.; KUMAR, R.V.; NIGAM,J.M (1976)

Continous Lock-stitch for Everted Enteroanastomosis in Bovines (Bubalus Bubalis): An Angiographic Study. J. Am. Radiol. Soc. 17 (1): 20-22

SINGH, M.M.; NIGAM, J.M. (1972)

Macroscopic Studies on the Evaluation of Different Techniques of End to End Enteroanastomosis in Buffalo calves. H.A.U. J. Res. 2: 73-79

SINGH, M.M.; NIGAM, J.M. (1974)

Evaluation of Suture Materials in Enteroanastomosis in Buffalo Calves. A Microscopic Study. H.A.U. J. Res 4: 214-218

SINGH, G.R.; SINGH, A.P.; BHARGAVA, A.K. (1981)

An Angiographic Evaluation of Double Layer Everted Intestinal Anastomoses in calves. Indian J. Anim. Sci. 51 (8): 770-774

SINGH, G.R.; SINGH, A.P.; BHARGAVA, A.K. (1981)
Studies on Intestinal Anastomosis - Comparison of Invagination and Inversion Methods of Enteroanastomosis in Pigs. Indian Vet. J. 58 (3): 227-231

SINGH, A.P.; SINGH, G.R.; BHARGAVA, A.K.; MOGHA, I.V. (1979a)
Observations on Everted Intestinal Anastomosis using Continous Lock-Stitch Sutures. Indian J. Anim. Sci. 49 (4): 286-292

SINGH, A.P.; SINGH, G.R.; BHARGAVA, A.K.; SOMVANSHI, R. (1980)
An Experimental Comparison of Single Layer Suture Patterns for Intestinal Anastomosis in Cow Calves. Indian J. Vet. Surg. 1 (2): 47-53

SINGH, G.R.; SINGH, A.P.; NIGAM, J.M. (1980)
End to End Enteroanastomosis - A Modified Invagination Technique. Indian Vet. J. 57: 1018-1019

SINGH, A.P.; SINGH, G.R.; PARIHAR, N.S.; BHARGAVA, A.K. (1979b)

Studies on Intestinal Anastomosis 2: Comparison of Single Layer Inverting and Everting Patterns for Entero-anastomosis in Pigs. Indian J. Anim. Sci. 49 (10): 812-816

SINGH, G.R.; SINGH, A.P.; SOMVANSHI, R.; BHARGAVA, A.K. (1980)

Studies on Intestinal Healing; Observations of Single and Double Layer Everting and Inverting Intestinal Anastomoses in Calves. Indian J. Anim. Sci. 50 (5): 400-406

SINGLETON, A.O.; WHITE, D.; MONTALBO, P. (1968)

A Comparative Study of Intestinal Anastomosis, Arch. Surg. 96:563-566

SOMVANSHI, R.; SINGH, A.P.; SINGH, G.R. (1980)

Histopathology of Experimental Intestinal Anastomosis using Single Layer Sutures in Calves. Indian Vet. Med. J. 4: 108-114

STASHAK, T.S. (1982)

Techniques for Enterotomy, Decompression, and Intestinal Resection/Anastomosis. Symposium on Equine Gastrointestinal Surgery. In Vet. Clin. North Am. (Large Anim. Pract.) 4 (1): 147-165

STOWATER, J.L. (1978)

Intestinal Adenocarcinoma in a Cat. Vet. Med./Small Anim. Clin. 73: 475-477

TRAVERS, B. (1812)

An Enquiry into the Process of Nature in Repairing Injuries of the Intestine. Longman. Co. London: 129

TRUMBLOOD, H.W.; NELSEN, T.S.; KOHATSU, S.; OBERHELMAN, H.A. (Jr) (1969)

Wound Healing in Colon. Comparison of Inverted and Everted Closures. Surg. 65: 919-930

VAN WINKLE, W (Jr). (1969)

The Tensile Strength of Wound and Factors that Influence It. Surg. Gyn. Obstet. 129: 819-842

VAUGHAN, J.T. (1972)

Surgical Management of Abdominal Crisis in a Horse. J. Am. Vet. Med. Assoc. 161: 1199-1211

WALKER, D.F.; VAUGHAN, J.T. (1980)

Anaesthesia. In "Bovine & Equine Urogenital Surgery". Lea & Febiger, Philadelphia: Pg 7-9

WATSON, O.W.; SODEMAN, W.A. (Jr) (1974)

The Small Intestine. In "Pathologic Physiology: Mechanisms of Disease". 5th Ed. W.B. Saunders Co. Philadelphia: Pg 735-736

WISE, L.; McALISTER, W.; STEIN, T.; SCHUCK, P. (1975)

Studies on Healing of Anastomoses of Small and Large Intestines. Surg. Gyn. Obstet. 141: 190-194

ZAVITZ, S.; ZAVITZ, V. (1960)

Rumenotomy and Intestinal Anastomosis in a Heifer. Can. Vet. J. 1: 115-118

LIST OF APPENDICES

LIST OF APPENDICES (cont'd)

LIST OF APPENDICES (cont'd)

APPENDIX 1: TIME AND STITCHES PER ANASTOMOSIS (MINUTES) FOR THE THREE ANASTOMOTIC TECHNIQUES

	TECHNIQUE					
ANA-STO-MO-SIS	SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
	TIME	STIT-CHES	TIME	STIT-CHES	TIME	STITCHES
1	24.0	17	19.82	16	23.60	16
2	15.0	16	18.88	17	18.45	18
3	15.0	16	18.97	15	17.50	16
4	13.0	16	19.73	17	20.90	16
5	13.0	16	17.92	16	16.33	14
6	12.0	17	21.32	17	16.87	13
7	9.78	14	13.13	15	18.97	15
8	10.17	14	13.30	14	12.95	14
9	9.75	14	14.12	15	12.42	14
10	9.78	14	11.10	14	13.92	14
11	11.45	14	13.25	14	16.78	14
12	8.50	14	12.75	14	14.78	14
13	14.63	15	14.92	15	15.88	12
14	12.17	14	12.83	14	16.92	13
15	7.70	14	11.17	14	13.82	12
16	10.73	15	10.67	14	12.08	12

APPENDIX I: (Continued)

ANA-STO-MO-SIS	TECHNIQUE					
	SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
	TIME	STITCHES	TIME	STITCHES	TIME	STITCHES
17	8.00	13	10.67	14	14.73	13
18	10.50	14	7.92	14	12.58	13
19	10.08	15	18.98	18	14.25	14
20	8.58	16	16.43	18	13.93	14
21	11.33	16	14.17	17	13.20	14
22	11.08	16	13.97	17	16.85	14
23	9.98	15	12.73	16	15.73	14
24	9.33	17	13.60	17	13.37	14
25	7.30	14	14.83	15	12.42	12
26	7.45	15	11.48	14	11.68	12
27	9.00	15	15.00	14	10.08	12
28	9.03	16	12.08	14	10.72	12
29	8.33	16	9.62	14	9.92	12
30	9.05	17	11.45	14	10.98	12
31	8.25	16	9.02	14	9.87	14
32	8.50	16	8.83	14	11:45	14
33	8.50	16	8.70	14	12.17	15
34	7.58	16	9.60	14	10.50	14

APPENDIX I: (Continued)

ANASTOMOSIS	TECHNIQUE					
	SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
	TIME	STITCHES	TIME	STITCHES	TIME	STITCHES
35	8.65	16	7.70	14	10.20	14
36	8.05	16	8.00	14	9.22	14
37	6.97	12	10.42	14	12.33	16
38	8.06	14	11.57	14	13.75	15
39	6.57	14	9.72	14	11.00	15
40	6.50	14	10.67	14	9.22	16
41	7.43	14	8.78	14	9.08	15
42	6.65	14	9.10	14	9.30	15
43	8.50	17	13.33	17	14.05	14
44	9.08	16	10.17	16	11.87	14
45	8.67	17	8.38	15	11.42	14
46	7.45	16	8.05	14	12.17	14
47	9.00	17	8.57	15	12.65	14
48	8.48	16	9.50	15	12.70	14
$\bar{X}$	9.97	15.18	12.50	15.03	13.45	13.45

APPENDIX II: NUMERICAL SCORES OF INTESTINAL ADHESIONS AROUND THE ANASTOMOTIC SITES AT DIFFERENT TIME INTERVAL AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNQIUES

TIME INTERVAL (DAYS)	TECHNIQUE			
	ANASTOMOSIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	1	1*	0	2
	2	2	0	1
	3	2	0	1
	4	2	0	0
	5	2	0	0
	6	0	1	1
	7	2	0	1
	8	2	1	1
	9	1	1	1
	10	1	0	0
	11	1	1	1
	12	0	0	1
14	1	0	0	0
	2	1	2	2
	3	0	1	3
	4	1	1	3
	5	0	3	3
	6	0	3	1
	7	2	0	0
	8	0	0	0
	9	1	0	0
	10	1	0	0
	11	1	0	1
	12	1	0	1

APPENDIX II: (Continued)

TIME INTERVAL (DAYS)		TECHNIQUE		
	ANASTOMOSIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	0	2	0
	2	2	0	1
	3	1	0	0
	4	1	0	0
	5	2	0	0
28	6	1	0	0
	7	1	0	0
	8	2	0	0
	9	2	0	0
	10	2	0	0
	11	1	0	0
	12	0	0	0
	1	0	0	1
	2	3	0	0
	3	1	0	0
	4	1	0	2
	5	1	0	2
56	6	1	1	1
	7	1	0	0
	8	0	0	0
	9	1	0	0
	10	0	0	0
	11	3	0	0
	12	2	0	0
TOTAL	48	51	17	30

*: Scores were based on fraction of the circumference having adhesions where 0 = 0; 1 = 1/4; 2 = 2/4; 3 = 3/4; 4 = 4/4

APPENDIX III: NUMERICAL SCORES OF DIFFERENT DEGREES OF INTESTINAL STENOSIS AT THE ANASTOMOTIC SITES AT VARIOUS TIME INTERVALS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE: SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	1	0*	1	0
	2	0	0	0
	3	0	0	2
	4	0	0	0
	5	1	0	0
	6	1	0	0
	7	0	1	1
	8	1	1	2
	9	0	2	1
	10	0	1	1
	11	1	1	0
	12	1	1	1
14	1	1	1	1
	2	1	0	2
	3	1	1	2
	4	1	0	0
	5	2	0	0
	6	1	0	1
	7	1	0	1
	8	1	0	1
	9	1	1	0
	10	0	0	0
	11	2	0	1
	12	1	1	1

APPENDIX III: (Continued)

TIME INTER-VAL (DAYS)	TECHNIQUES			
	ANA-STO-MO-SIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	0	0	1
	2	0	1	0
	3	0	0	0
	4	0	0	0
	5	0	0	1
28	6**	-	-	-
	7	0	0	1
	8	0	1	0
	9	1	0	0
	10	0	0	1
	11	0	1	1
	12	0	1	0
	1	0	0	0
	2	2	0	0
	3	2	0	1
	4	0	0	0
	5	1	0	1
56	6	0	0	1
	7	0	1	2
	8	0	1	2
	9	0	0	1
	10	0	0	2
	11	0	1	2
	12	1	0	2
TOTAL	48	26	19	37

*: Degree of stenosis: 0 = None; 1 = Slight; 2 = Moderate; 3 = Marked.

**: Ommited because simple interrupted anastomosis was perforated accidentally.

APPENDIX IV: INDEX OF STENOSIS INDUCED BY SIMPLE INTERRUPTED, GAMBEE AND CRUSHING ANASTOMOTIC TECHNIQUE ASSESSED BY USE OF RADIOGRAPHS AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE		
		SIMPLE INTEPRUPTED	GAMBEE	CRUSHING
4	1	28	52	64
	2	24	43	48
	3	37	40	64
	4	33	38	52
	5	45	27	37
	6	61	39	51
	7	23.6	49.2	44
	8	39	45.2	50
	9	35.7	43.3	46.8
	10	36.8	45.2	52.1
	11	27.7	40.7	38.0
	12	35.8	41.4	47.5
14	1	49.0	24	40
	2	39	29	42
	3	53	24	45
	4	54	12	42
	5	49	26	47
	6	49	40	35
	7	38.1	26.5	34.4
	8	53.1	27.3	27.5
	9	35.7	31.5	22.7
	10	32.5	32.4	32.3
	11	58	34.2	22.0
	12	45.2	45.4	44.0

APPENDIX IV: (Continued)

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE		
		SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	62	35	54
	2	62	34	43
	3	66	35	44
	4	52	23	46
	5	19	23	42
28	6	* -	15	55
	7	20.6	13.5	20.0
	8	27.3	22.9	20.0
	9	7.7	29.6	16.3
	10	12.5	19.4	28.6
	11	30.2	36.1	34.2
	12	6.3	35.4	16.0
	1	24	5	27
	2	37	10	27
	3	45	14	37
	4	24	11	33
	5	35	8	37
56	6	34	9	38
	7	14.9	44.7	44.6
	8	8.3	38.9	32.4
	9	22.2	30	36.4
	10	21.4	40	34.3
	11	13.8	42.5	35.3
	12	47.4	34.1	37.8
MEAN	48	36.0	30.6	38.9

*: Anastomosis perforated accidentally before filling with barium sulphate suspension for X-raying.

APPENDIX V: NUMERICAL SCORES OF DEGREES OF HEALING OF INTESTINAL EPITHELIAL LAYER AT VARIOUS TIME INTERVALS AFTER SUTURING USING SIMPLE INTERRUPTED GAMBEE AND CRUSHING TECHNIQUES

TIME INTERVAL (DAYS)	TECHNIQUE			
	ANASTOMOSIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	2*	2	1
	2	3	2	1
	3	3	2	1
	4	2	2	1
	5	2	2	2
4	6	3	2	1
	7	3	2	2
	8	3	1	1
	9	1	2	2
	10	3	2	2
	11	3	2	2
	12	2	2	2
	1	3	3	3
	2	3	3	3
	3	2	1	3
	4	1	3	3
	5	1	3	3
14	6	1	1	3
	7	3	3	3
	8	1	3	3
	9	1	3	3
	10	3	3	3
	11	3	3	3
	12**	1	-	3

APPENDIX V: (Continued)

TIME INTERVAL (DAYS)		TECHNIQUE		
	ANASTOMOSIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	3	3	3
	2	3	3	3
	3	3	3	3
	4	3	3	3
	5	1	3	3
28	6	3	3	3
	7	3	3	3
	8	3	3	3
	9	3	3	3
	10	3	3	3
	11	3	3	3
	12	3	3	3
	1	3	3	3
	2	3	3	3
	3	3	3	3
	4	3	3	3
	5	3	3	3
56	6	3	3	3
	7	3	3	3
	8	3	3	3
	9	3	3	3
	10	3	3	3
	11	3	3	3
	12	3	3	3
TOTAL	48	122	124	123

*: Scores: 3 = complete; 2 = partial; 1 = poor

**: All scores in this line excluded in the totals.

APPENDIX VI: NUMERICAL SCORES OF DEGREES OF INFLAMMATORY REACTION AT THE ANASTOMOTIC SITES AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE		
		SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	.1*	3	3
	2	1	3	3
	3	3	3	3
	4	2	2	3
	5	2.	3	3
4	6	3	3	3
	7	1	1	1
	8	1	2	2
	9	1	1	2
	10	1	1	2
	11	1	1	2
	12	1	1	3
	1	1	3	1
	2	1	2	1
	3	2	1	1
	4	1	1	2
	5	1	3	1
14	6	2	1	2
	7	2	1	2
	8	2	1	1
	9	1	1	2
	10	1	2	1
	11	3	1	2
	12**	1	-	1

APPENDIX VI: (Continued)

TIME INTERVAL (DAYS)		TECHNIQUE		
	ANASTOMOSIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	2	3	2
	2	1	3	3
	3	1	2	3
	4	3	3	3
	5	1	2	2
28	6	2	3	3
	7	2	1	1
	8	2	2	1
	9	1	1	1
	10	1	1	1
	11	2	1	1
	12	1	1	1
	1	3	3	2
	2	3	3	3
	3	2	3	3
	4	1	2	2
	5	2	2	3
56	6	1	2	3
	7	2	2	1
	8	1	3	2
	9	1	2	1
	10	2	3	3
	11	2	3	1
	12	1	3	3
TOTAL	48	76	94	90

*: Scores: 3 = slight; 2 = moderate; 1 = marked

**: All scores in this line were excluded in the totals

APPENDIX VII: NUMERICAL SCORES OF THE DEGREES OF MUSCLE LAYER APPROXIMATION DURING ANASTOMOSIS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	2*	2	1
	2	1	2	1
	3	2	3	1
	4	2	1	1
	5	1	2	1
4	6	3	3	1
	7	2	1	3
	8	2	1	1
	9	2	1	3
	10	1	1	3
	11	1	3	3
	12	2	1	3
	1	1	1	2
	2	3	3	1
	3	1	3	2
	4	2	2	2
	5	3	2	3
14	6	1	2	2
	7	2	3	3
	8	2	3	3
	9	2	2	1
	10	2	3	3
	11	2	3	3
	12**	2	-	3

APPENDIX VII: (Continued)

TIME INTERVAL (DAYS)		TECHNIQUE		
	ANASTOMOSIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	3	2	2
	2	2	3	3
	3	3	3	2
	4	1	2	1
	5	2	1	3
28	6	3	2	3
	7	3	2	3
	8	2	2	2
	9	1	2	2
	10	2	2	2
	11	1	3	2
	12	3	3	2
	1	3	3	2
	2	3	2	2
	3	2	3	3
	4	2	2	3
	5	1	3	3
56	6	2	3	3
	7	3	3	1
	8	2	2	3
	9	2	3	1
	10	2	1	2
	11	1	2	2
	12	2	2	1
TOTAL	48	94	102	100

*: Scores: 3 = good; 2 = satisfactory; 1 = poor

**: All scores in this line were excluded in the totals

APPENDIX VIII: CELLULAR INTENSITY (NUMBER OF CELLS) ALONG THE ANASTOMOTIC SITE AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS

TIME INTERVAL (DAYS)	TECHNIQUE			
	ANASTOMOSIS	SIMPLE INTERRUPTED	GAMBEE	CRUSHING
4	1	43	47	32
	2	23	50	35
	3	18	34	38
	4	35	28	19
	5	38	24	31
	6	39	28	31
	7	57	25	32
	8	40	36	32
	9	26	36	20
	10	39	28	35
	11	50	30	31
	12	43	25	28
14	1	37	52	52
	2	35	33	55
	3	41	24	44
	4	45	39	48
	5	56	36	38
	6	42	26	25
	7	20	30	30
	8	21	14	34
	9	17	30	26
	10	26	24	22
	11	29	22	25
	12	18	-	37

APPENDIX VIII: (Continued)

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE SIMPLE INTERRUPTED	GAMBEE	CRUSHING
	1	45	24	33
	2	17	33	49
	3	38	30	35
	4	23	26	26
	5	32	21	35
28	6	27	19	28
	7	51	32	22
	8	31	22	38
	9	41	17	36
	10	30	18	19
	11	23	23	30
	12	44	27	23
	1	52	72	37
	2	74	48	47
	3	53	69	47
	4	29	52	34
	5	42	45	29
56	6	51	41	71
	7	21	31	35
	8	25	43	22
	9	20	19	25
	10	27	27	35
	11	19	23	22
	12	26	28	38
MEAN		37.3	33.8	36.5

APPENDIX IX: AVERAGES OF CELL TYPES OBSERVED ALONG THE ANASTOMOTIC SITE AT DIFFERENT TIME INTERVALS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE																					
		SIMPLE INTERRUPTED							GAMBEE							CRUSHING							
		F	Mc	L	N	E	M	P	F	Mc	L	N	E	M	P	F	Mc	L	N	E	M	P
4	1	41	2	6	2	3	0	0	13	1	10	1	1	0	0	12	3	14	1	0	1	0
	2	26	1	11	1	1	0	0	24	1	10	1	1	0	0	20	1	4	4	3	0	0
	3	16	1	6	1	2	0	0	24	1	5	4	1	1		5	0	9	6	0	1	0
	4	21	0	17	1	0	0	0	17	0	12	2	1	0	0	21	1	7	6	0	0	0
	5	33	1	16	1	0	0	0	24	2	3	1	1	0	0	16	1	13	1	0	1	0
	6	26	0	3	13	0	1	0	14	1	7	3	1	0	0	17	1	10	3	1	1	0
	7	15	0	7	0	0	0	0	30	3	11	2	0	1	0	23	1	6	2	0	0	0
	8	12	1	5	1	0	0	0	39	2	7	2	0	0	0	29	0	5	2	0	0	0
	9	29	2	10	1	2	0	0	24	2	3	2	0	0	0	28	2	6	2	0	0	0
	10	29	1	6	0	0	1	0	22	1	3	2	0	0	0	14	0	5	1	0	0	0
	11	27	0	10	1	0	0	0	22	0	1	2	0	0	0	11	0	12	0	0	0	0
	12	33	1	7	2	0	0	0	19	2	5	3	0	0	0	20	1	11	0	0	0	0

F = Fibroblast; Mc = Macrophage; L = Lymphocyte; N = Neutrophil; E = Eosinophil; M = Monocyte; P = Plasma cell

APPENDIX IX: (Continued)

TIME INTERVAL (DAYS)	ANASTO-MOSIS	TECHNI											
		SIMPLE INTERRUPTED							GAMBEE				
		F	Mc	L	N	E	M	P	F	Mc	L	N	E
	1	28	0	5	0	2	0	0	24	1	8	1	0
	2	33	1	4	0	0	0	1	20	1	3	1	0
	3	36	2	3	0	1	1	0	44	2	5	1	0
	4	38	1	5	1	0	1	0	28	0	9	1	1
	5	42	0	4	11	0	0	0	28	0	8	1	0
14	6	35	0	5	2	0	0	0	22	1	4	0	0
	7	14	1	5	0	0	0	0	25	0	5	0	0
	8	16	0	3	2	0	0	1	10	1	3	0	0
	9	11	0	3	2	0	0	0	22	0	7	2	0
	10	18	1	3	5	0	0	0	19	0	5	1	0
	11	22	0	7	1	0	0	0	16	0	5	1	0
	12	13	1	1	4	0	0	0	-	-	-	-	-

Q U E

CRUSHING

M	P	F	Mc	L	N	E	M	P
0	0	33	2	16	1	0	1	0
0	1	21	3	19	12	0	0	0
0	1	39	1	4	1	0	0	0
0	0	41	1	3	1	2	0	0
0	0	35	1	2	0	0	0	0
0	0	21	0	4	1	0	0	0
0	0	22	0	8	1	0	0	0
0	0	22	1	5	5	0	0	0
0	0	20	0	4	2	0	0	0
0	0	13	0	6	3	0	0	0
0	0	17	0	5	3	0	0	0
-	-	23	1	7	6	0	0	0

TIME INTERVAL (DAYS)	ANASTO-MOSIS	SIMPLE INTERRUPTED						
		F	Mc	L	N	E	M	P
	1	14	0	2	1	0	0	0
	2	26	0	5	6	0	0	0
	3	18	0	5	1	0	0	0
	4	29	1	1	1	0	0	0
	5	23	1	3	1	0	0	0
28	6	37	0	5	3	0	0	0
	7	42	1	7	0	0	0	0
	8	28	0	2	1	0	0	0
	9	36	2	3	1	0	0	0
	10	23	0	7	0	0	0	1
	11	20	1	2	1	0	0	1
	12	39	0	5	1	0	0	0

APPENDIX IX:

TIME INTERVAL (DAYS)	ANASTO-MOSIS	SIMPLE INTERRUPTED						
		F	Mc	L	N	E	M	P
	1	48	1	3	1	0	0	0
	2	66	1	6	1	0	1	0
	3	47	1	5	0	0	1	0
	4	28	1	1	0	0	0	0
	5	39	0	1	1	0	1	1
56	6	49	1	1	1	0	0	0
	7	18	0	3	0	0	0	0
	8	20	1	5	0	0	0	0
	9	17	0	2	1	0	0	0
	10	26	1	0	0	0	0	0
	11	18	0	1	0	0	0	0
	12	23	0	3	1	0	0	0
MEAN		28	1	5	2	1	1	1

(Continued)

C H N I Q U E													
GAMBEE							CRUSHING						
F	Mc	L	N	E	M	P	F	Mc	L	N	E	M	P
5	1	16	1	0	1	0	31	3	4	0	0	0	0
36	4	7	1	0	0	0	40	2	5	0	0	0	0
61	1	7	1	0	0	0	39	1	7	0	0	0	0
42	2	8	1	0	0	0	21	1	7	0	0	0	0
37	2	6	1	0	0	0	69	1	1	1	0	0	0
27	3	9	0	0	0	0	21	0	1	12	0	0	0
21	1	9	0	0	0	0	31	0	4	0	0	0	0
32	0	10	1	0	0	0	16	0	6	0	0	0	0
14	1	4	0	0	0	0	20	1	5	1	0	0	0
25	0	2	0	0	0	0	21	0	12	0	0	0	0
18	0	4	0	0	0	0	17	0	5	1	0	0	0
27	0	2	0	0	0	0	33	0	5	0	0	0	0
24	2	6	1	1	1	1	25	1	7	2	1	1	1

APPENDIX X: INDEX OF INCREASE IN INTESTINAL WALL THICKNESS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT VARIOUS TIME INTERVALS USING WHOLE THICKNESS MEASUREMENTS

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE					
		SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I	II	I	II	I	II
	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
4	6	-	-	-	-	-	-
	7	6	83	-	-	-	-
	8	100	132	-	-	-	-
	9	-	-	-	-	-	-
	10	-3	-3	-	-	-	-
	11	-	-	-	-	-	-
	12	-	-	-	-	-	-

I: B+C Measurements used in the calculations

II: Normal measurements used in the calculations

APPENDIX X: (continued)

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE					
		SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I	II	I	II	I	II
	1	100	66	18	15	19	49
	2	83	83	-34	-5	145	207
	3	-	-	-	-	80	36
	4	-	-	38	49	49	116
	5	105	52	0	69	20	23
14	6	-	-	-	-	3	94
	7	25	29	32	13	148	71
	8	-28	-7	118	95	42	110
	9	18	54	10	26	17	40
	10	115	146	24	5	28	73
	11	5	17	30	34	31	16
	12	32	116	-	-	93	102

APPENDIX X: (Continued)

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE					
		SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I	II	I	II	I	II
	1	7	78	33	58	145	111
	2	30	127	91	166	12	51
	3	41	54	-20	-35	74	74
	4	57	57	27	121	24	112
	5	80	161	40	35	142	102
28	6	179	197	41	62	44	103
	7	18	21	37	66	10	22
	8	17	68	48	64	38	90
	9	-33	-25	27	14	49	78
	10	13	85	33	61	22	29
	11	3	28	45	38	-17	-17
	12	7	142	16	37	33	86

APPENDIX X: (Continued)

TIME INTERVAL (DAYS)	TECHNIQUE						
	ANASTOMOSIS	SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I I	II	I	II	I	II
	1	-30	-9	90	31	56	29
	2	112	71	3	36	-25	-10
	3	-6	0	23	8	-5	16
	4	20	17	22	17	29	-23
	5	20	9	63	33	113	117
56	6	-3	9	20	-9	25	38
	7	-17	5	85	138	8	2
	8	-15	-13	25	29	9	0
	9	-11	-42	57	57	91	60
	10	-7	31	21	15	49	62
	11	-29	8	-8	-17	11	9
	12	20	64	-22	-12	39	35
MEAN		30	56	30	38	46	59

APPENDIX XI: INDEX OF INCREASE IN INTESTINAL WALL THICKNESS AFTER SUTURING USING SIMPLE INTERRUPTED, GAMBEE AND CRUSHING TECHNIQUES AT DIFFERENT TIME INTERVALS WHEN MUCOSA IS EXCLUDED FROM MEASUREMENTS

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE					
		SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I	II	I	II	I	II
4	1	104	194	15	55	68	131
	2	68	176	59	115	143	240
	3	53	115	-11	41	184	74
	4	77	117	118	205	160	210
	5	74	122	16	115	347	375
	6	15	-18	61	125	143	367
	7	43	77	126	169	450	450
	8	100	236	47	75	-31	20
	9	78	116	115	79	275	275
	10	-3	25	200	253	227	324
	11	77	124	150	194	200	235
	12	74	176	250	350	95	129

I: B+C Measurements used in the calculations

II: Normal measurements used in the calculations

APPENDIX XI: CONTINUED

TIME INTERVAL (DAYS)	ANASTO-MOSIS	TECHNIQUE					
		SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I	II	I	II	I	II
	1	155	193	34	38	19	79
	2	143	386	-33	4	222	240
	3	65	211	68	79	127	42
	4	123	263	57	60	77	224
	5	205	87	8	78	28	31
14	6	96	96	51	119	-8	150
	7	31	45	70	15	195	97
	8	-34	21	149	155	118	350
	9	29	113	59	43	64	95
	10	221	307	46	23	29	113
	11	9	4	82	107	45	23
	12	46	186	-	-	210	264

APPENDIX XI: CONTINUED

TIME INTERVAL (DAYS)	ANASTOMOSIS	TECHNIQUE					
		SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I	II	I	II	I	II
	1	106	210	104	114	168	178
	2	90	171	208	221	18	59
	3	67	138	-11	-8	78	109
	4	89	96	14	120	38	165
	5	107	257	34	56	273	185
28	6	254	254	59	103	55	132
	7	18	45	64	112	45	114
	8	-4	85	84	92	86	215
	9	-44	-34	42	47	86	189
	10	13	107	70	95	57	106
	11	8	37	72	87	0	-12
	12	3	60	32	116	59	130

APPENDIX XI: (Continued)

TIME INTERVAL (DAYS)	TECHNIQUE						
	ANASTOMOSIS	SIMPLE INTERRUPTED		GAMBEE		CRUSHING	
		I	II	I	II	I	II
	1	-36	-11	29	83	41	9
	2	220	132	10	83	-4	24
	3	-20	-6	23	14	-9	18
	4	37	53	57	47	88	-21
	5	11	11	58	58	207	229
56	6	-14	36	50	24	65	133
	7	-12	30	127	293	66	60
	8	-6	0	33	41	100	47
	9	-4	-54	175	200	171	119
	10	-15	63	65	50	173	193
	11	-41	-9	-14	0	39	34
	12	25	56	-13	-7	59	42
MEAN		57	108	62	95	83	146