THESIS

BOVINE INTESTINAL ANASTOMOSIS: EFFECTS OF SUTURE MATERIALS ON HEALING

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY STANLEY M. MBIUKI ENTITLED BOVINE INTESTINAL ANASTOMOSIS: EFFECTS OF SUTURE MATERIALS ON HEALING BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

BOVINE INTESTINAL ANASTOMOSIS: EFFECTS OF SUTURE MATERIALS ON HEALING

Two commonly used suture materials (chromic catgut and monofilament nylon) and one relatively new synthetic suture material - polyglactin 910 (vicryl) were used to investigate the effects of suture materials on healing of small intestinal anastomoses in cattle.

A total of 120 anastomoses were performed in 20 cows (6 anastomoses per cow). Each of the 3 suture materials (chromic catgut, polyglactin 910, and monofilament nylon) was used to perform 40 anastomoses utilizing the crushing technic. The anastomoses were spaced 30 cm apart. Random distribution of the suture materials was exercised for all the 20 cows.

Biophysical (bursting pressure and breaking strength) and morphologic (histopathology) parameters were evaluated at 7 days post-operatively. Twenty one days post-operatively only breaking strength and histopathology were evaluated. For the evaluation, the entire intestinal segment containing the 6 anastomoses plus about 30 cm of normal gut on either side of the anastomoses was harvested surgically at the end of the healing period.

There was marked variation within the results of both the bursting pressure and breaking strength. Breaking strength results

were variable in the same animal and even the same anastomosis. Not every anastomosis disrupted at the anastomotic site. In fact, there was considerable variation of the point of disruption.

Accurate alignment of all tissue layers of the cut ends was present in 32.4% of the 78 histologic sections studied. The other anastomoses had one or more layers everting. Frequently there was one or more mucosal islands in the collagenous tissue on the serosal side. Chromic catgut had the highest percentage of the anastomoses with the expected apposition of the intestinal ends; nylon was the second and vicryl had the lowest.

Polyglactin 910 (vicryl) was no better than chromic catgut regarding intensity of cellular reaction at 7 and 21 days. There was no statistically significant difference in the results of bursting pressure and breaking strength but generally there were trends favoring one suture material or the other.

Anastomoses with chromic catgut were the best overall in healing properties. Nylon followed and vicryl was the last.

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INTRODUCTION

An ever-widening array of absorbable and non-absorbable suture materials is available to the surgeon today but no suture material developed to date meets all the established criteria of an ideal suture material.

Choosing suture material is best done on the basis of mechanical properties of the suture and the biologic interaction between the suture and the tissue since these factors can alter the mechanical properties of the suture material and the physical properties of the wound. In most instances choice of suture material is made on considerations other than biologic interaction of suture materials and tissue partly because there is relatively little published data about biological reactions of the suture materials in various tissues. Choice should also be made for a particular clinical situation, because tissue pathology such as inflammation, infection, necrosis or metabolic disorders, will influence the healing of a wound.

In the literature it has been pointed out that it is more informative to evaluate the morphological and biophysical changes in a healing wound. These parameters are useful in determining the influence of suture materials on healing and are applicable to the gastrointestinal tract.

Various studies have been done on healing of intestinal anastomoses in different species. Literature available to us, however, had no reference to any study on suture materials and healing of intestinal anastomoses in cattle. Extrapolation of information from one species to the other is not desirable because species variation is expected.

Although intestinal anastomosis in cattle is rarely done, it is important that whenever a situation arises, the veterinary surgeon has reliable information on which to base his choice of suture materials.

This investigation was conducted to determine the effects of two commonly used suture materials (chromic catgut and monofilament nylon) and one relatively new synthetic absorbable suture material - Polyglactin 910 (vicryl) on healing of bovine jejunal anastomosis. Morphological and biphysical parameters were evaluated at 7 and 21 days postoperatively.

LITERATURE REVIEW

Anatomy of the Small Intestine

Generally the small intestine is made 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 more mobile because of their longer mesenteric attachment (Nickel et al., 1973) as compared to 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 inward: serosa, muscular tunic, submucosa, muscularis mucosae, and mucosa. The serosa is made of loose connective tissue invested by the mesothelium of visceral peritoneum. The muscular tunic has an outer longitudinal and an inner circular smooth muscle layers. In the connective tissue between the two muscle layers are numerous parasympathetic ganglionic cells and fibers making the myenteric plexus. The submucosa is composed of fibroblasts, blood vessels, lymphatics and collagen and elastic fiber

bundles of connective tissue. Submucosal ganglionic plexus is scattered throughout the submucosa. The muscularis mucosae, like the main muscle layer, is made up of an outer longitudinal layer and an inner 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 Lieberkühn). The glands open into pits between the bases of the villi and penetrate the mucosa as far as the muscularis mucosae (Dellman & Brown, 1976). The surface epithelium of the villi and the crypts of Lieberkuhn is a continuous 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 surface. The undifferentiated crypt cells usually have high mitotic activity and serve as a source of differentiated cells. Newly formed cells migrate up 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; Guyton, 1976).

Lamina propria is made up of loose connective tissue containing collagen, elastic and reticular fibers. It forms the core of the villi and surrounds intestinal glands. In the lamina propria are blood vessels, lymph vessels and logitudinally oriented smooth muscle cells extending from muscularis muscosae to the tip of the villi (Dellman & Brown, 1976).

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 continuous with venules (arteriovenous loop). Veins from the villi and periglandular capillary bed flow back to submucosal venous plexus. The submucosal venous plexi form veins that traverse the muscular layers parallel to the arterial supply to join larger veins (Dellman & Brown, 1976).

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 enzyme-producing cells (Dellman & Brown, 1976). Argentaffin cells apparently produce serotonin (Hill, 1970). The cells of Brünner'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 parasympathetic 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 muscle. 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 analward. Although the peristalsis occurs in both directions, the orad wave dies rapidly while the analward wave travels some distance. In the dog, six to ten 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 excessive distension or expelling the irritant. This is due to enhanced electrical activity of myenteric plexus by a superimposed stimulus. Effectual peristalsis requires an active myenteric plexus although the basic phenomenon does not depend on myenteric plexus.

Villous movements are effected by the muscularis mucosae and individual muscle fibers within the villi. Their stimulus is either local or sympathetic. These movements allow absorption and transport of lacteal contents to lymphatics.

Intestinal Anastomosis

Intestinal anastomosis is not commonly performed in the daily work of a clinical bovine veterinarian and as such, 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 injury (Frank, 1964); and 2) obstructions interfering with blood supply, especially where there is a likelihood of necrosis, as occurs with strangulating hernias, volvulus of the jejunum, intussusception, foreign body, and constrictive adhesions (Frank, 1964; Berge & Westhues, 1966; Hofmeyr, 1974; Mitchell, 1975).

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.

All anastomotic techniques are surgically successful from a mechanical standpoint but the ideal anastomosis is one where all layers are perfectly aligned as seen histologically. This is because it will allow perfect healing with minimal scar (DeHoff et al., 1973). Meticulous surgical technique is essential for rapid healing of surgical wounds including intestinal anastomotic wounds (Elkin, 1940; Herman et al., 1964; Peacock & VanWinkle, 1970; Jackson et al., 1971; DeHoff, 1975; Grier, 1975; Nakanishi, 1975). According to Bromwell et al. (1967), Peacock and VanWinkle (1976) and Grier (1975), all anastomoses apparently leak to some extent and successful healing depends on natural resistance provided by the peritoneum and the omental action against bacterial infections.

Crushing Suture Technique

"Crushing" suture technic appears to be well-suited for use in intestinal anastomosis (Poth & Gold, 1968; Bennet & Zadeck, 1970; Neal, 1975). The technic is based on the fact that the submucosa is the strongest layer of the intestinal wall (Halstead, 1887; Bennet & Zadeck, 1970; Grier, 1975). According to Brunius and Zederfeldt (1970), local circulation is more influenced by technic than suture. Myers (1971) and DeHoff (1975) state that sutures should be tied out not too tightly but firmly to approximate tissues. This avoids blanching of tissues and possible edema. With the "crushing" technic, on the other hand, sutures are tied tightly so that they cut through all layers and gather all the collagen fibers together. This relieves pressure on the microcirculation and re-establishes definite capillary blood supply resulting in little post-operative edema (Poth & Gold, 1968; Neal, 1975). After suture placement the ends are approximated but some mucosa may extrude to the external surface. A bite of the mucosa (similar to Gambee technic) will prevent the mucosa from bulging out. A back-and-forth pull one or two times before making the first throw of the knot will help in cutting through the mucosa by the suture material (Poth & Gold, 1968).

Crushing suture technic results in minimal narrowing of the lumen as compared to the inverting suture technic and is comparable

to simple interrupted (DeHoff, 1975) and modified Gambee (Reinertson, 1976) technics.

Clinical Evaluation of Intestinal Anastomosis

Relatively little is written about clinical evaluation of intestinal anastomosis. All anastomotic technics function and that clinical variation is not easy to determine (Bennet & Zadeck, 1970). It appears that eating as soon as possible post-operatively will promote intestinal movements and this will reduce chances of post-operative paralytic ileus (Bromwell et al., 1967; DeHoff, 1975; Grier, 1975). Gentle handling of the intestines will also reduce chances of ileus and enhances rapid return to normal function (DeHoff et al., 1973). Desire to eat is apparently a useful observation, clinically, for evaluating intestinal anastomosis.

Healing Processes

Intestinal healing is similar to that of the skin (DeHoff, 1975) but visceral wounds are metabolically more active than skin wounds (Hastings et al., 1975). For this reason, intestinal wound healing will be described along with 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 flow into the gap will fill up

the space and clot, sealing the edges of the wound. Fibrin from fibrinogen is responsible for this. 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 leukocytes and dead tissue. This inflammatory reaction is the same in all sites of the body. Repair processes begin as soon as debris has been removed by the body scavenging system and in uncomplicated simple wounds this begins within 3-5 days of injury (VanWinkle, 1969; Peacock & VanWinkle, 1970; Heinze, 1974; Johnson, 1977).

The healing process is divisible into three parts: fibroblastic proliferation, capillary infiltration, and epithelial proliferation. Fibrin strands are present in the first 3-4 days.

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 mitoses occur in the third to fifth days. Old collagen is usually destroyed in the first 3 days (Cronin et al., 1968; DeHaan et al., 1974; Peacock & VanWinkle, 1970). As the fibroblasts proliferate, they manufacture and secrete protein polysaccharides and glycoproteins.

From the fourth day connective tissue starts being laid down. Reticulum fibers are present on the 4th day. Collagen fibers appear

on the fifth day. Between 6-10 days collagen production is maximum. Collagen fibers are oriented across the incision after 6-7 days and there is fibrous union between the new and older collagen by 9-10 days. Collagen synthesis slows down from 11-14 days. Fibroplasia may last from 2-4 weeks (Madsen, 1953; VanWinkle, 1969; Peacock & VanWinkle, 1970; Postlethwait, 1970; Heinze, 1974; Johnson, 1977).

New capillaries originate as bud-like structures on nearby vessels. These penetrate the wound and grow into loops which ramify through the wound (Johnson, 1977).

Intestinal wounds have a similar reaction (Herrmann et al., 1964; Necular & Mandace, 1974). According to Herrmann et al. (1964) the lag phase lasts 0-4 days and the proliferative phase lasts 3-14 days just like the skin. Vascular regeneration occurs within 6-9 days and is functional by 14th day (Sako & Wangensteen, 1951; Nakanishi, 1975).

Epithelialization of the skin wound occurs by epithelial migration and proliferation. Peri-incisional epithelium on either side react in hours of injury by thickening and subsequently there is mobilization of basal cells from their dermal attachment and these cells migrate as a sheet to the place of cell deficit. In an incised sutured wound it takes 24-48 hours to bridge the defect. Migration of cells is assisted by the increasing cell proliferation. After the epithelial sheet has covered the defect, there is differentiation to restore

function in the new cells (Peacock & VanWinkle, 1970; Heinze, 1974; Johnson, 1977).

In the healing intestinal wound, the undifferentiated cells of the crypts of Lieberkuhn provide the new epithelial cover to the injured area. The undifferentiated cells undergo mitosis at a constant rate all the time and this provides continuous re-epithelialization of the intestinal villi. The intestinal epithelium is renewed every 2 1/4-2 3/4 days. A defect in the intestinal mucosa is covered by low cuboidal cells and around the 4th day these start differentiating for various functions. Intestinal mitotic activity is maximum at all times and does not increase around the site of injury like the skin (Peacock & VanWinkle, 1976). Regeneration of the epithelium to bridge the mucosa occurred by 7-9 days in intestinal anastomosis (Sako & Wangensteen, 1951). According to Herrmann et al. (1964), differentiated mucosa is present by 17-21 days.

There is no sharp demarcation between proliferative and maturation phases (VanWinkle, 1969). The maturation phase is characterized by a decrease in vascular and cellular elements and an increase in collagen. Those collagen fibers that are properly oriented thicken and there is a dissolution of the fibers that are improperly oriented. This may last up to two years (Heinze, 1974; Johnson, 1977). Intestinal wounds have a similar maturation process starting from the third week and may last up to one year (Herrmann et al., 1964).

According to Peacock and 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 or monocytes persist around a foreign body. Fibroblasts from the local mesenchymal tissue may produce collagen that becomes deposited around the foreign body.

Bursting Pressure

Bursting pressure is defined as pressure required to break a viscus (VanWinkle & Hastings, 1972). It has been used by various investigators to evaluate anastomotic competence in the gastrointestinal tract (Fellows et al., 1951; Sako & Wangensteen, 1951; Nelsen & Anders, 1966; Herrmann et al., 1964; Nelson & Dudley, 1967; DeHaan et al., 1974; Cronin et al., 1968; Hastings et al., 1975). The apparatus used varied from the simple set-up used by Nelson and Dudley (1967) and Cronin et al. (1968) to a more sophisticated system used by Nelsen and Anders (1966). The test segment was inflated at a constant rate of 5 mm Hg/Sec (Fellows et al., 1951), 10 mm Hg/Sec (Herrmann et al., 1964), 10 mm Hg/15 Sec (Cronin et al., 1968).

Fellows et al. (1951) found that within 15-30 minutes jejunal anastomoses were air and water tight due to their being sealed by exudates. At 24 hours there was 50 mm Hg due to a fibrin seal, yet

there was no additional increase in strength up to 72 hours. After this, there was a rapid increase in strength up to the 7th or 8th day. Strength increased slowly after this to the 14th day. After the 14th day there was a slow increase in strength up to that of normal, unwounded tissue. Nelsen and Anders (1966) found that by day 7 the anastomosis had 50-60% of the eventual maximum strength and that there was a rapid increase in strength up to 10 days when it was the same as that of normal ileum. Herrmann et al. (1964) found that rat colon, 3 hours after anastomosis, acquired some strength which remained constant for up to days 4-5 after which there was a rapid increase up to day 7 when it was the same strength as unoperated intestine. Sako and Wangensteen (1951), using dog's gastrointestinal tract, showed a similar trend, but normal strength was not achieved until days 23-25. They also noticed that leakage occurred at exit or entrance of sutures. Cronin et al. (1968) observed that for rat colonic anastomoses there was a rapid increase in strength and after day 10 rupture occurred at sites other than at the anastomoses. Nelsen and Anders (1966) also observed that the ruputures occurred in areas other than the anastomosis at post-operative day 7 although, the strength was 50-60% of eventual maximum strength.

Hastings et al. (1975) using dog's stomach, found that normal strength was attained by day 8. DeHaan et al. (1974) observed that bursting pressure of stomach of rat reached a maximum value in the

first week after repair (3-5 days). Localio et al. (1943) using the stomach of a rat, and without removing sutures, found that wounds sutured with catgut suture were significantly weaker than wounds sutured with other suture materials in the first 7 days but thereafter there was no difference.

Normal bursting pressure values were variable. Dogs' jejunum had an average of 430 mm Hg (Fellows et al., 1951). Sako and Wangensteen (1951) stated that the normal values for dogs was 450-500 mm Hg. Cronin et al. (1968) had 150 mm Hg as normal rupturing pressure for rat's colon.

Bursting pressure is not the best criterion for testing strength of a wound and is, in fact, not an accurate measurement of wound strength (Peters, 1972; Hastings et al., 1975). Considering Laplace Law concerning force producing a rupture of a cylinder -- $\frac{\text{Force} = \text{Pressure} \times \text{radius}}{2}$ -- the degree of distension has a direct influence on pressure applied to the wall of the intestine. The anastomosis is more rigid compared to the rest of the gut wall and will tend to stretch less than adjacent tissues in response to circular stretch. When pressure is applied, rupture occurs in adjacent areas of the bowel due to high intraluminal pressure due to their distensibility increasing the radius of the bowel (Nelsen & Anders, 1966; Cronin et al., 1968; Peacock & VanWinkle, 1976; VanWinkle & Hastings, 1972; Hastings et al., 1975).

According to VanWinkle and Hastings (1972) the measurement of bursting pressure is not precise because even a pinhole leak will cause a decrease in applied intraluminal pressure and this will produce a different result than when a major disruption occurs. Pressure attained also depends on circumference of the intestine and its distensibility in relation to the rate at which air is infused into the system.

The rate of distension and shape the viscus have to be controlled. Slow distension is better tolerated at higher pressures (Peacock & VanWinkle, 1970). Presence or absence of adhesions influence the bursting pressure.

Breaking Strength

Breaking strength is the force required to break a wound or tissue regardless of its cross-section area. Tensile strength is load per unit cross-section area.

Tensile strength measurement is not easy due to technical problems in measuring cross-section area. It can be measured for homogenous materials but it is not easy to determine true tensile strength of composite non-homogenous material. Breaking strength would be of value where thickness of the material being used cannot be measured accurately. It appears that breaking strength is of more clinical significance, while tensile strength is important from a mechanical standpoint (VanWinkle, 1969; VanWinkle & Hastings, 1972).

Development of strength of a healing wound has been evaluated by measuring breaking strength by many investigators. Adler et al. (1967) worked with colon of the dog. Herrmann et al. (1964) tested the colon of a rat. Nelson and Dudley (1967) used small intestines of the dog. Adamsons and Kahan (1970) used skin, muscle and stomach of rabbits. Hastings et al. (1975) tested dog's stomach and colon. VanWinkle et al. (1975) tested dog's skin.

The size of the strips used varied. Adamsons and Enquist (1963) used 1 cm wide strips (including two stitches) from guinea pig abdominal wall. Herrmann et al. (1964) used 3 mm wide strips and stretched them at 5 mm/Sec. Adler et al. (1967) tested 2 cm wide strips and these were clamped 2 cm from the anastomosis. One centimetre wide strips were used by Nelson and Dudley (1967). Adamsons and Kahan (1970) tested 1 cm wide by 4 cm long strips. Hastings et al. (1975) and VanWinkle et al. (1975) cut strips 0.5 cm wide.

According to Everett (1970), the effect of the inflammatory reaction to different suture materials on tissue strength can be assessed experimentally by wound tensile strength with stitches in situ. Non-absorbable sutures have stronger wounds the first 5-10 days after absorption catgut sutured wounds are stronger.

Hastings et al. (1975) observed that the dog's stomach had an increase in strength up to 21 days. The colon had a rapid gain

between 5 and 10 days after which this strength was gained slowly up to 14 days. The colon had attained 70% of normal colon strength at 120 days. They found that wounds closed with absorbable suture materials were weaker than those with non-absorbable sutures between 5th and 28th days. Absorbable suture material closed wounds were also associated with a decrease in strength in the gut wall up to 6 cm from the wound. For skin wounds, VanWinkle et al. (1975) found no statistically significant difference between suture materials but, at 70 days, wounds closed with absorbable sutures were stronger. Using the colon of rats Herrmann et al. (1964) showed that normal strength was approximated by 17 days and after 3 months rupture of the gut occurred in areas other than the anastomosis.

There is a lag phase in the healing wound lasting between 4 and 6 days. After this there is a rapid increase in strength up to the 14th or 16th day due to rapid fibroplasia and production of collagen (Van Winkle, 1969; Adamsons & Kahan, 1970; Hastings & VanWinkle, 1972; Johnson, 1977). Most rapid gain in strength with or without sutures is between 3 and 15 days (Adamsons & Kahan, 1970). According to Getzen et al. (1966) breaking strength is more influenced by edema than fibroplasia.

Gain in strength after collagen content is stabilized is due to the cross-linkage and re-orientation of the already formed fibers. Ultimate strength is usually due to interaction of collagen and components of ground substance (VanWinkle, 1969; Johnson, 1977).

Herrmann et al. (1964) used a statham load cell and a Sanborn recording system. Nelson (1967) described a tensiometer for measuring wound strength where distraction was produced by a uniform motor drive. A pen recorder was used. Simultaneously line elongation of the tissue was measured by a potentiometer recording on a parallel trace. A constant rate motor driven tensiometer was used by Adamsons and Enquist (1963). Lichtensteen et al. (1970) used an electronic strain gauge tensiometer.

Adler et al. (1967) used rigid plastic templates 2 cm wide with fine needles projecting at its edges so designed to ensure uniformity in size and number of sutures in each strip subjected to tensile strength testing. They also used specifically designed clamps with serrated jaws to eliminate slippage and tearing of the intestine to hold the test pieces of colon.

Breaking strength results are more meaningful if expressed as a percentage of normal control from the same individual due to wide variations in the readings. Absolute results are less useful (Lichtensteen et al., 1970; Hastings et al., 1975; VanWinkle et al., 1975).

According to Peters (1972) and Lichtensteen et al. (1970) "yield point" is a more valuable determinant of wound integrity compared to rupture point. This is more accurate and reproducible end point.

Yield point is characterized by an abrupt fall in the curve. Any pull beyond this will result in decreased resistance.

Lichtensteen et al. (1970) stated that clamped tissue was weakest at the points where it was clamped. They indicated that sutured wounds yielded only when the sutures broke or tore through tissues. At 2 months wounds with sutures were 70% normal and those without sutures were 41% of normal. Adamsons and Enquist (1963) found that at 15 days wounds with sutures were 85% normal strength and without sutures were 76% of normal. This implied that sutures contribute to the would strength. Nagy and Zingg (1971) indicated that tensile strength depends not only on the load but also on the rate at which it is applied. Stress should be the same at each point of the wound during testing.

Living tissue and dead tissue are biologically and physically different and that in vivo measurement would be more meaningful (VanWinkle, 1969).

Adler et al. (1967), using the colon of a dog, found large animal to animal variation at any stage. There were also species variations (Hastings et al., 1975).

Suture Material and Tissue Reaction

An ideal all-purpose sture is one which will meet the following criteria: 1) should permit use in any tissue, the only limiting factor

being suture size as determined by tissue, 2) excellent handling characteristics, 3) good knot security, 4) minimal tissue reaction, 5) resistant to infection, 6) non-capillary, 7) non-electrolytic, 8) non-allergic, 9) easily sterilized, 10) predictably absorbable without inflammation, 11) easily manufactured at low cost, 12) easy to thread, and 13) should not shrink in tissues (Grier, 1971; Varma, 1973; Herrmann et al., 1970; Postlethwait et al., 1959). To date no suture material meets all the above criteria and probably there will not be such a suture in the foreseeable future.

With a few notable exceptions the surgeon's choice of suture materials has been based on considerations other than biologic interaction of suture and tissue. This has not been due to the ignorance of the surgeon but due to the dearth of knowledge of sound biological data (VanWinkle & Hastings, 1972).

While the choice of suture material is far less important than surgical technique, the actual suture material used may contribute to complications such as "cutting out," infection, and chronic sinus formation. There are no rigid rules that can be established as to which type of suture material should be used for each and every situation but knowledge of biological reactions of various suture materials in tissue will usually indicate that one material is superior to the others (Grier, 1972).

Initial tissue reaction is a reflection of the injury inflicted by the passage of needle and suture material, assuming the same technique, tissue, surgeon and absence of infection. This is the same for all sutures in the first 5-7 days but chronic reactions vary (Madsen, 1953; Postlethwait et al., 1975). All sutures have a peak reaction in 2-7 days and thereafter the reaction to non-absorbable sutures subsides to minimal but for absorbable sutures the reaction is greater until absorption is complete (VanWinkle & Hastings, 1972).

In the first week, the reaction on the 2nd day is mainly made up of red blood cells, fibrin strands, and white blood cells. By the 4th day, there are lymphocytes and polymorphonuclear cells. Fibroblasts, fibrous tissue and mononuclear cells are present on the 7th day. After the 7th day, non-absorbable sutures are encased in a fibrous capsule with an inner younger fibrous tissue. The absorbable sutures show a similar reaction until absorption begins and then the inflammation reaction intensifies (Postlethwait et al., 1959; Madsen, 1953).

Generally speaking, all suture materials induce varying degrees of inflammatory reaction related to trauma of insertion and their physicochemical properties (Kovacs & Somagyvari, 1969; Brunius & Zederfeldt, 1970; Everett, 1970). Non-absorbable monofilament sutures stimulate little if any reaction. Absorbable sutures are more reactive. Natural non-absorbable sutures are more reactive than synthetic non-absorbables (Myers, 1971).

Excessive inflammatory reaction has disadvantages. "Cutting out" is influenced by increased inflammatory reaction since there is increased collagen breakdown and softening of tissues. Therefore, the extent of inflammatory reaction is the decisive factor of holding of sutured tissue. This is particularly so because it occurs during the lag phase when suture support is essential (Everett, 1970; Adamsons & Enquist, 1963; Grier, 1975).

Edema delays healing and sutures that induce exudation cause a delay in healing. The delay in healing appears to be due to a delay in fibroplasia (Everett, 1970; Madsen, 1953) but the delayed fibroplasia may be purely localized around the suture.

Sutures causing more exudative reaction and tissue necrosis leading to local tissue autolysis provide a protein rich medium for bacterial growth. Physical structure of the suture may protect bacteria providing an environment for bacterial proliferation. For these reasons, sutures influence infection (Haxton, 1965; Alexander et al., 1967; Brunius and Zederfeldt, 1970; Everett, 1970; Edlich et al., 1973; Varma, 1973). Physical configuration of the suture is relatively unimportant in early infection (Edlich et al., 1973).

Cochrane (1969) stated that suture intolerance or sensitivity may result in adverse reactions leading to wound breakdown.

Reactions to suture materials varies with species and there is variation within the same species. Rats have an intense neutrophilic

and lymphocytic reaction initially which progresses to mononuclear phagocytes and lasts until suture absorption is complete. Rabbits on the other hand have an initial lymphocytic reaction that progresses to phagocytic reaction (VanWinkle & Hastings, 1972). According to Postlethwait et al.(1975), tissue reaction is more severe in rats than in dogs and rabbits. According to Ulin (1971), suture materials display different reactions in different tissues. Although clinical significance of tissue reaction to suture material is doubtful, the reaction will influence rate and characteristics of repair (VanWinkle & Hastings, 1972).

Hastings et al. (1975) found that histological reaction between various suture materials and tissue was essentially the same. Madsen (1953) indicated that most non-absorbable sutures had minor variations histologically.

Various suture materials have been used for intestinal anastomoses. Grier (1972) stated that absorbable sutures should be used for gastrointestinal surgery since the gastrointestinal sterilization is never complete. This was mainly because absorbable sutures harbour bacteria for a relatively short time before they are absorbed. In 1975, Grier also suggested that catgut should be the suture of choice for intestinal closure and non-absorbable monofilaments in cases with severe abdominal sepsis. VanWinkle and Hastings (1972) recommended chromic catgut which is eventually absorbed and monofilament

suture, like steel, nylon and prolene for gastrointestinal surgery since there is a potential for contamination. Kratzer and Onsanit (1974) used stainless steel and did not penetrate into the lumen. Staples were used by Ravitch (1975). Singleton et al. (1968) used silk sutures. Other sutures indicated for intestinal surgery include: polyglycolic acid and polyglactin 910 synthetic absorbable sutures and non-absorbable monofilament dacron.

It is apparent that although considerable work has been done with various suture materials in intestinal anastomosis, little attention has been paid to comparative studies on suture materials. Yet suture material along with ischaemia, and infection are the main causes of anastomotic failure. Suture material will influence the outcome of an infection because infection occurs around sutures and not the cut surfaces. "Cutting out" which is associated with suture materials is more important for the intestinal anastomosis than with other tissues because there is relatively little collagen and small bites are usually made during intestinal anastomosis.

Sutures

Catgut

This is a material made from treated collagen from sheep or beef intestines. It is made of several plies which are twisted, machine ground and polished to give a relatively smooth surface and uniform diameter. It is a monofilament-like suture and does not

harbour bacteria. The processing results in weak spots due to tearing of fibrils.

Chromic acid tanning promotes cross linkage of triple helix structures of collagen. This makes the suture less reactive and more resistant to enzymatic degradation. Smaller size suture materials are better chromicized and are as such less reactive than larger sized sutures (Edlich et al., 1973; Frank, 1964; Grier, 1971).

Catgut sutures have secure knots only when dry and when wet the knots tend to unravel and as such at least 3 throws should be made (Everett, 1970).

Absorption is by enzymatic degradation and phagocytosis (Horton et al., 1974). The rate of absorption is variable. It is more rapid in more vascular tissues, septic areas, areas of high inflammatory reaction and areas with high enzymatic activity (Grier, 1972). Sutures implanted in the stomach disappear in 3 days (Postlethwait & Smith, 1975). Absorption of sutures in the intestinal tract is influenced by enzymatic activity in the ileum and by increased vascularity and high micro-organism population in the colon (Everett, 1970).

Literature on the loss of strength of chromic catgut suture is confusing. One author states that loss of strength takes 2-3 weeks (Myers, 1971). According to Postlethwait (1959) and VanWinkle and Hastings (1972), 2-0 cutgut suture in the abdominal wall muscle of rabbits retains its strength up to 28 days and thereafter there is a

rapid loss in strength. Catgut suture loses 1/2 of its strength in the first 2 days of implantation and retains 1/3 of its strength to 60 days (Wandall et al., 1972).

Everett (1970) using baboons observed that 2-0 catgut suture when implanted in the rectus sheath retained all its strength for 10 days while for the same suture and time interval in the ileum, there was total loss of strength. In the colon 15-20% strength of the suture was left at 10 days.

Catgut suture material is associated with intense inflammatory reaction, especially plain catgut. This is accompanied by tissue necrosis around the suture resulting in a sterile abscess around the suture. The reaction is relatively intense until absorption is complete (Haxton, 1965; Everett, 1970). According to Postlethwait et al. (1975), chronic reaction to chromic catgut sture is composed of an initial thin connective tissue capsule with few histocytes, fibroblasts, lymphocytes and foreign body giant cells. This reaction is consistent until absorption begins and then the cellular reaction is composed of monocytes, a few lymphocytes and few or no neutrophils and eosinophils. After absorption only monocytes with characteristically brown foamy cytoplasm remain.

Vetafil

Vetafil suture material is a multifilament nylon suture surrounded by a smooth proteinacious sheath. It is used for skin closure but if it is autoclaved, it can be buried (Grier, 1972).

Nylon

This is a polyamide polymer monofilament suture made from condensing 1,6,Hexanediamine and adipic acid to form polyhexamethylene adipamide (Edlich et al., 1973). It has a poor knot security and at least 4 throws are necessary for adequate setting of the knot (Everett, 1970; Ferguson, 1971; Postlethwait et al., 1959). Although nylon is non-absorbable, it tends to lose strength with time when it is implanted in tissues (Postlethwait, 1970). Two years after implantation nylon loses 25% of its strength (VanWinkle & Hastings, 1972). Nylon undergoes some chemical degradation after 6 months to degradation products which are antibacterial (Edlich et al., 1973).

Nylon suture causes little to minimal reaction compared to other suture materials (Postlethwait et al., 1959; Postlethwait, 1970; Postlethwait et al., 1975). According to Postlethwait (1970), in the first week of implantation, the reaction is made of histocytes, fibroblasts, and a few lymphocytes. In the 2nd week, there is a clear zone around the suture material with unattached histiocytes. By the fourth week, the reaction is similar to that of the 2nd week but there is a fibrous capsule. The reaction around nylon suture is a narrow

compact fibrous tissue capsule with histiocytes around the suture. It is only rarely that some giant cells are observed (Postlethwait et al., 1975).

Vicryl (Polyglactin 910)

Vicryl is a co-polymer of glycolide and lactide in the ratio 90:10. This is made into fibers that are braided to form the suture material. The suture material is ethylene oxide sterilized and vacuum-packed (Horton et al., 1974; Craig et al., 1975; Postlethwait & Smith, 1975). It has good handling quality with secure knots (Horton et al., 1974). Vicryl loses strength at a rate parallel to that of catgut but retains strength longer than catgut (Horton et al., 1974). According to figures given by Craig et al. (1975), the strength of vicryl was 1/3 of normal 21 days after implantation.

Vicryl absorption is by hydrolysis of ester bonds and is not size dependent. pH and enzymatic activity do not influence absorption. The hydrolysed products are natural body metabolites (Peacock & VanWinkle, 1970). The suture is absorbed by 60 days in rabbits and 90 days in rats.

Reaction to vicryl is described as minimum to slight. It is less than that of catgut. Five days after implantation, macrophages are predominant. There are few neutrophils, fibroblasts, and multi-nucleated giant cells and minimal cellular infiltration between interstices of the suture. By day 14 the cellular reaction completely

infiltrates the interstices between the fibers. All the cells seen at day 5 are present except the neutrophils. The same cells persists until absorption is complete. After absorption the site is identified by presence of a few enlarged fibroblasts or macrophages or both. Frequently clusters of fat cells fill the area (Craig et al., 1975).

According to VanWinkle et al. (1975), vicryl is more reactive than chromic gut. This conflicts with the findings of Craig et al. (1975) cited in the above paragraph.

MATERIALS AND METHODS

Experimental Animals

Twenty female cattle of mixed breeds weighing between 500 and 1000 lbs (227.3 kg to 454.5 kg) (see Table 1) were purchased from a local auction and transported to Colorado State University Foothills Campus. They were divided into two groups: 1) with horns and 2) without horns, and put in separate corrals. A thorough physical examination was performed on each to insure that they were healthy three weeks before the first surgery. Each cow was ear tagged for identification. They were fed good quality hay consisting of 3/4 alfalfa hay and 1/4 local grass hay.

Pre-operative Preparation

Each cow was starved for 48 hours and deprived of water for 24 hours before surgery. The animal's weight was taken during the starvation period. An antibiotic[1/] (5000 units penicillin and 5 mg - streptomycin per lb of body weight) was administered intramuscularly 24 hours before surgery and the dose repeated just before pre-anaesthetic agents were given.

[1/] Distrycillin® - Procaine penicillin G in Dihydrostreptomycin sulphate solution. E. R. Squibb and Sons, Inc., Princeton, N.J. 08540.

Table 1. Cows used in the study of effects of sutures on healing of small intestinal anastomosis.

Cow No.	Body Wt (kg)	Breed
2	307	Holstein
3452	345.4	Holstein
3400	254.5	Holstein Cross
3285	281.8	Holstein Cross
3431	213.6	Angus Cross
3106	272.7	Angus Cross
1	281.2	Holstein
16	277.3	Chalolais Cross
3436	286.4	Angus Cross
3115	334.1	Angus Cross
19	309.1	Holstein
20	413.6	Holstein
7	379.6	Holstein
3293	402.3	Angus Cross
3442	363.6	Angus Cross
3121	375	Angus Cross
2N	386.4	Holstein
3	336.4	Hereford
17	257.7	Hereford
3321	293.2	Angus Cross

Anaesthesia

Atropine Sulphate[2] (0.044 mg/kg, I/m) was given 10-20 minutes before induction of anesthesia. Thiamylal Sodium[3] (0.01 g/kg, I/v) was given to induce anaesthesia.

Three liters of Ringer's Solution to which 50 milliequivalents of Sodium Bicarbonate had been added per liter was infused intravenously during the surgical procedure.

The cow was put on a left lateral recumbency and intubated as soon as possible after induction with Surital®. A mouth gag[4] was placed between the molar teeth of the lower and upper jaws of the lower left side. The right hand was introduced into the oral cavity up to the pharynx and the epiglottis was reflected forwards with fingers. The endotracheal tube[5] which had already been lubricated was introduced into the mouth and was guided with the right hand into the larynx. Contact was made on all sides of the laryngeal wall by pushing the tube as far as it could go and tight fit was ensured by the shoulder of the endotracheal tube. Outflow of expired air through the

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[2] Atropine injectible. LA. Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501.

[3] Surital®, Parke, Davis; Parke-Davis & Co., Detroit, MI 48232.

[4] Drinkwater Mouth Gag. Arnold-Nasco, Guelph, Ontario, Canada.

[5] Cole Endotracheal Tubes, P.O. Box 1124, Fort Collins, Colo. 80521.

tube verified the position of the tube. The animal was then connected to the anaesthetic machine.

Anaesthesia was maintained with halothane[6/] and oxygen in a semi-closed circle system. A Fraser Sweatman or Drager anaesthetic machine was used. Initially the oxygen was set at 4-5 liters per minute and halothane at maximum (about 5%). The re-breathing bag was emptied a few times to ensure denitrogenation of the animal. When the animal was deeply anaesthetized and stable, the anaesthesia was maintained at 2-3% halothane and oxygen at 2.5 liters per minute.

Vital signs were monitored every 15 minutes. Heart rate was maintained between 60-100 per minute. Any rise above 100/minute indicated that the anaesthesia was getting too deep. Respiratory rate was maintained around 20/minute. If the respiratory rate fell below 12/minute and was shallow, the anesthesia was too deep.

A cushion was placed under the neck to assure that the pharynx and larynx were higher than the mouth to prevent pharyngeal pooling of saliva. The lower (left) front leg which was pulled forward had a cushion placed under it to help avoid radial nerve paralysis.

Surgical Procedure

The right flank was prepared for aseptic surgery, the dorsal border of which extended from the tuber coxae along the transverse

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[6/] Fluothane® - Ayerst Laboratories, Inc., New York, N.Y.

processes cranially to T10. The caudal border was from the tuber coxae along the cranial border of the stifle joint ending at the ventral midline while the cranial border followed the 10th rib to the ventral midline. The surgical area was clipped and alternately scrubbed with surgical soap[7] and with 70% ethyl alcohol. Three soap and 3 alcohol scrubs were done, followed by air drying and the surgical area was sprayed with an antiseptic[8] and again allowed to dry.

The surgical area was draped first with an 18" x 36" sterile adhesive plastic drape,[9] and then with towels around the incision site. Before putting on a 15" fenestrated drape, orthosheets were used to cover the hind and fore parts of the cow except for the head.

A 15-20 cm vertical incision was made in the lower part of the right flank, one-half the distance between the costochondral junction and the stifle joint. An electroscalpel was used to cut through the skin and the cutaneous trunci muscle. A pair of Mayo scissors was used to incise the tunica flava and the oblique abdominal muscles. The transverse abdominal muscle was split along its fibers and Mayo scissors were used to open the peritoneum.

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[7] Iodine surgical detergent. Huntington Laboratories, Inc., Huntington, Indiana.

[8] Betadine Solution. The Purdue Frederick Co., Yonkers, N.Y.

[9] Steri-drape® - Medical products division; Minnesota Mining and Manufacturing Co., 3M Center, St. Paul, MN 55101.

Once the laparotomy incision was made, a hand was introduced into the abdomen and caudal to the omentum. Exploration of the abdomen was done to identify the jejunum. Loops of jejunum were exteriorised to expose the area with the longest mesentery allowing for minimal tension on the exposed portion. The rest of the small intestines were replaced into the abdominal cavity. An effort was made to exteriorize only the amount of intestines necessary for making the anastomosis which, along with use of saline moistened towels, helped avoid drying of the intestine.

Test sutures were inserted longitudinally in the intestinal wall for a distance of 7-8 cm using a 10 cm long 19 gauge needle. Each test suture was fixed to the intestinal wall by taking a bite before making a knot on both ends. The 3 test sutures were placed about 1 cm apart and parallel to each other. The first suture was on the antimesenteric border and the other two were placed one on either side. These test sutures were placed in the jejunum between the ileum and the first anastomosis. The site for the first anastomosis was 15-20 cm from the test sutures. The majority of the ingesta was milked out of the site of anastomosis and a bull dog intestinal clamp[10/] was placed 4-5 cm proximal and another distal to the site. A #10 or #20 Bard Parker blade was used to incise across the intestinal wall,

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[10/] Bull dog clamp. Lawton, Germany.

starting from the mesenteric side. The assistant then held the cut ends of the intestine ready for suture placement. A caliper[11] set of 6 mm was used to ensure that suture placement was evenly spaced. Measurement was made by the assistant while the surgeon did the suturing.

Suturing

The "crushing" suture was used in a simple interrupted pattern and each suture was placed on 6 mm intervals and about 5 mm from the cut edges. Swedged-on 2-0 suture materials were used for each anastomosis and a double square knot was used to secure each suture placed. A taper point needle was used with medium chromic catgut and polyglactin 910[12]. A cutting edge needle was used with the monofilament nylon suture material. All the suture materials were made by Ethicon Inc.

An initial stay suture was placed in the mesenteric border with the intestinal ends held end to end. A second stay suture was placed in the antimesenteric side. These two stay stitches were clamped with haemostatic forceps to stabilize the intestine during the anastomosis. With the cut ends of the intestine still held end to end by the

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[11] Caliper. Storz, Germany.

[12] Vicryl® - Polyglactin 910 Suture. Ethicon, Inc. Somerville, N.J. 08876.

stay sutures, simple interrupted stitches were placed making sure that the spacing was uniform by using the caliper. After one side was completed, the intestine was turned over and the process was repeated on the other side.

It is important to note that the first throw of each crushing stitch had to be as tight as possible without cutting through the gathered collagen fibers. The other 3 throws were mainly to insure security of the knots and uniformity. If there was any defect in the mesentery, it was closed using the same suture material used in the anastomosis.

Blood clots on the mesentery and the small intestines were cleaned with warm saline moistened sponges. Any mucosa protruding to the serosal surface was removed using a sponge. Full strength betadine solution was used to clean the intestine around the anastomosis.

A 30 cm long 1/4" umbilical tape (sterile) was cut and was used to measure the 30 cm spacing between the anastomoses. The suturing technique and 30 cm spacing was repeated for all the 6 anastomoses. The suture materials for the first 3 anastomoses were determined randomly, while for the next 3 anastomoses, the order of the suture materials was reversed. The result was 2 anastomoses with each suture material and 6 anastomoses per animal. Table 2 shows suture placement for all the 20 cows.

Table 2. Suture placement.

Cow Number	1	2	3	4	5	6
2 *	Vicryl	nylon	gut	nylon	gut	Vicryl
3452	Vicryl	nylon	gut	gut	nylon	Vicryl
3400	nylon	gut	Vicryl	Vicryl	gut	nylon
3285	gut	nylon	Vicryl	Vicryl	nylon	gut
3431	gut	nylon	Vicryl	Vicryl	nylon	gut
3106	nylon	gut	Vicryl	Vicryl	gut	nylon
1 *	nylon	Vicryl	gut	nylon	gut	Vicryl
16	gut	Vicryl	nylon	nylon	Vicryl	gut
3436	nylon	gut	Vicryl	Vicryl	gut	nylon
3115	Vicryl	gut	nylon	nylon	gut	Vicryl
19	gut	Vicryl	nylon	nylon	Vicryl	gut
20	nylon	gut	Vicryl	Vicryl	gut	nylon
7	gut	Vicryl	nylon	nylon	Vicryl	gut
3293	nylon	gut	Vicryl	Vicryl	gut	nylon
3442	Vicryl	nylon	gut	gut	nylon	Vicryl
3121	nylon	Vicryl	gut	gut	Vicryl	nylon
2 (NC)	gut	nylon	Vicryl	Vicryl	nylon	gut
3	nylon	Vicryl	gut	gut	Vicryl	nylon
17	Vicryl	nylon	gut	gut	nylon	Vicryl
3321	gut	Vicryl	nylon	nylon	Vicryl	gut
	V5	N7	G7	N7	G8	V6
	N8	G6	V8	G5	N6	N7
	G7	V7	N5	V8	V6	G7

*Suture materials not placed in the order described.

Closure of the Abdominal Wall

The abdominal wall was closed in 4 layers. These included: 1) the peritoneum and transverse abdominal muscle; 2) oblique abdominal muscles and tunica flava; 3) cutaneous trunci muscle; and 4) skin.

All layers except the skin were closed using #2 chromic catgut with a cruciate pattern. The skin was closed with medium size vetafil (autoclaved) using a cruciate pattern.

Recovery from Anaesthesia

Anaesthesia was lightened gradually towards the end of abdominal wall closure and the animal was allowed to breathe pure oxygen for a few minutes. Disconnection of the anaesthetic machine was done just before the animal was moved to the recovery stall. The endotracheal tube and mouth gag were removed as soon as the animal was able to elicit a swallowing reflex and this was checked by moving the endotracheal tube several cm to stimulate the swallowing reflex. The animal was observed until it could maintain a sternal recumbency to avoid possible regurgitation and aspiration while in lateral recumbency. Recovery took 20-60 minutes.

Post-operative Care

Water was given after the animal was standing for about one hour. Good quality hay (3/4 alfalfa and 1/4 local grass) was given to the animal 3-4 hours after recovery from the anaesthesia. Antibiotic

treatment was continued for 2 days post operatively. Temperature and vital signs were checked every day for the first 3 days. Skin sutures were removed during the second operation for the 21-day animals.

Second Operation

Anaesthesia

A right paravertebral block was done, and to accomplish this the method described by Cakala (1961) was used. The thirteenth thoracic nerve and first and second lumbar nerves were blocked close to the end of the first, second and fourth transverse processes by infiltrating 20 ml of 2% lidocaine[13/] per site using a 6.35 cm 16 G or 18 G hypodermic needle. This was done observing aseptic technique. Xylazine (Rompun®)[14/] was electively used as a sedative during the paravertebral block and as necessary in repeated small doses during the surgery. This was done for cow numbers 2, 3452, 3400, 3285, 3431, 3106, 1, 3436 and 3321, due to their relative intractability.

General anaesthesia using atropine as a pre-anaesthetic, Surital for induction and halthane for maintenance was used for numbers 3115, 20, 3293, 3121, 3, and 17. Chloral hydrate (7%) to effect was used for cow numbers 7 and 2N.

[13/] Xylocaine®. 2% Lidocaine hydrochloride. Astra; Worcester, Mass. 01606.

[14/] Xylazine (Rompun®). Chemagro Corp., Kansas City, MO 64120.

T61 euthanasia solution[15/] was used for cow #16 and cow #19. Cow #3442 was electrocuted. The segment with the anastomoses was harvested within 10 minutes of euthanasia for the 3 cows which were euthanatized.

Surgical Procedure

A right laparotomy incision was made after proper aseptic preparation. A twenty-five centimeter muscle transecting incision was made dorsal to the original incision in the paralumbar fossa. The layers cut through included: skin, oblique abdominal muscles, transverse abdominal muscle and the peritoneum. The transverse abdominal muscle was the only layer which was split along it fibers.

An exploration of the abdomen was done to locate the piece of intestine with the anastomoses. The segment was exteriorised after it was located.

The mesenteric blood supply to the segment containing the anastomoses was doubly ligated with 2-0 silk suture material and the mesentery was cut between the two ligatures. After the necessary ligations were made, the entire segment containing the 6 anastomoses plus 30 cm of normal gut on either side was resected.

The ends of the intestine were re-anastomosed using 2-0 polyglactin 910 (Vicryl) suture material and a "crushing" pattern. A

[15/] T61. Euthanasia Solution. National Lab. Corp., Subsidiary of Am. Hoechst Corp. Somerville, N.J. 08876.

betadine abdominal infusion was done after cleaning the intestine and the mesentery around the anastomosis with warm saline soaked sponges.

Closure of the abdominal wall was done layer by layer using the same suture materials and suture patterns as for the first operation.

Aftercare

No antibiotics were used unless it was felt that they were absolutely indicated. The animal was twice daily observed until complete recovery. Skin sutures were removed after 14 days.

Handling of the Segment with the Anastomoses

The ingesta was milked out of the whole segment and the segment was put into an insulated 1-gallon jug containing 2 liters of iced lactated Ringer's Solution ($4^{o}C$). The time at which the segment was stored in the iced lactated Ringer's solution testing was recorded.

The stored segment was handled one anastomosis at a time. Time from resection until the measurement of the bursting pressure or breaking strength was recorded. Timing began immediately after harvesting, when the intestinal segment was stored in cold lactated Ringer's Solution.

Bursting Pressure

A 20 cm long segment (10 cm on each of the anastomosis) with about 2.5 cm mesenteric attachment left intact was used in each test.

The mesentery was left intact because it was observed that bursting occurred in the area where the mesentery had been dissected out. Figures 1 and 2 show the system used for measuring bursting pressure. The test segment was attached to the bursting pressure system (Fig. 3). This was secured in place using plastic tie bands[16] which were tightened using an extracorporeal gun[17] (Fig. 4). The part of the bursting pressure system holding the anastomotic segment was then immersed in an aquarium-like tank made of glass and of about 40 liters capacity filled with about 38 liters of physiologic saline solution and maintained at 38°C (Fig. 5). Weights were used to keep the frame holding the anastomotic segment on the bottom of the tank. The segment was allowed 5 minutes to warm to 38°C.

Oxygen passing through a line pressure transducer was turned on at a valve and the oxygen passed slowly through a hole bored through the cylindrical plexiglass to which the intestinal segment was attached (part of the bursting pressure system) to the anastomotic segment lumen. Pressure was measured within the lumen of the segment of gut through a hole in the other side of the system which was connected to a Statham pressure transducer calibrated to 300 mm Hg using a

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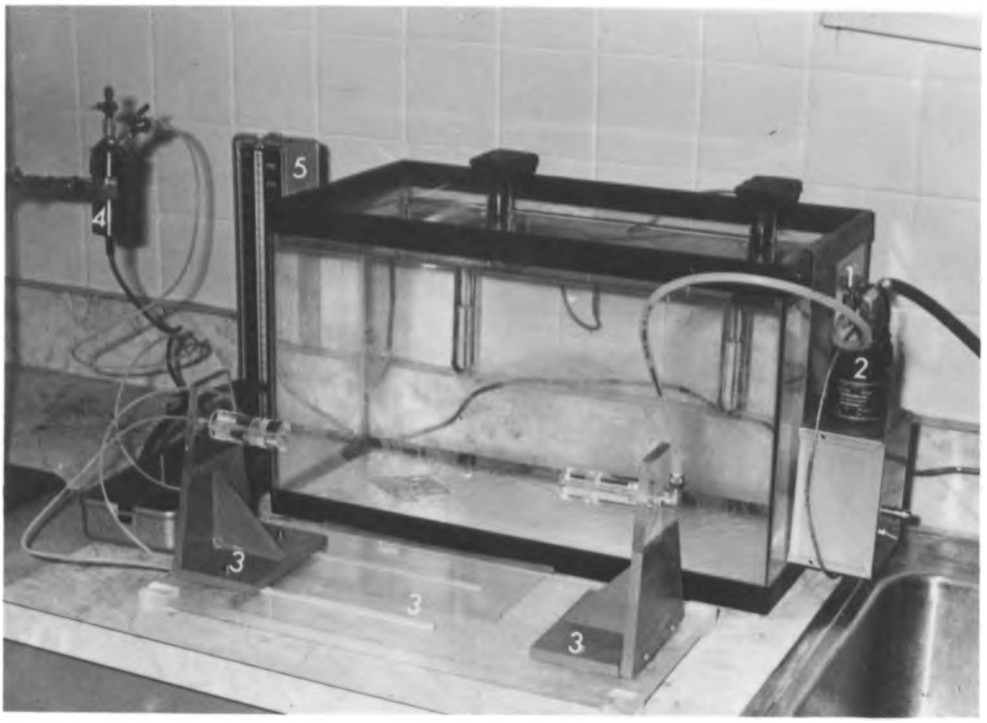
[16] Tie bands: Cole Laboratories, Inc., Lakewood, Colo. 80215.

[17] Extracorporeal gun. Extra corporeal and Medical Specialties Co., Inc., Mt. Laurel, TWP., N.J.

Figure 1. System for measuring bursting pressure (oxygen tank not shown).
(1) Pressure control valve.
(2) Line pressure transducer.
(3) Part of the system to which the test segment is attached.
(4) Statham pressure transducer.
(5) Mercury manometer.

Figure 2. Close up photograph of that of the bursting pressure system to which the segment of gut is attached.
(1) Fixed part.
(2) Mobile part.
(3) Cylindrical plexiglass grooved (arrow) about 1 cm from the free edges. Free edges are rounded.
(4) Grooves (arrows) in the base of the flatform made so that the mobile part of the part to which the test segment is attached can be moved back and forth for each attachment.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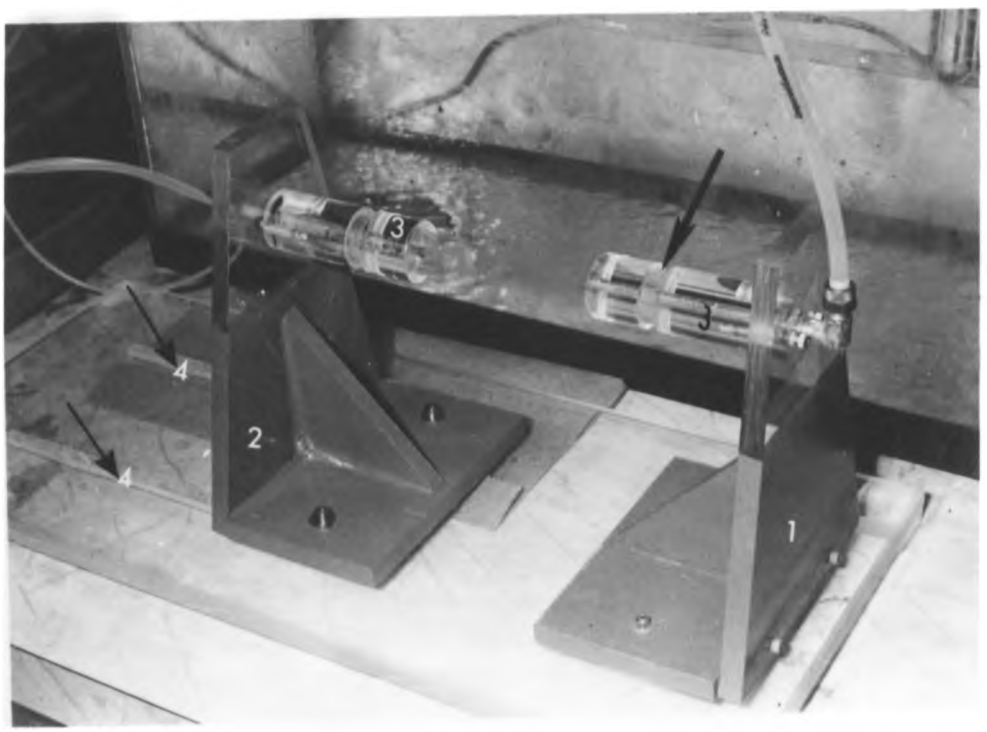
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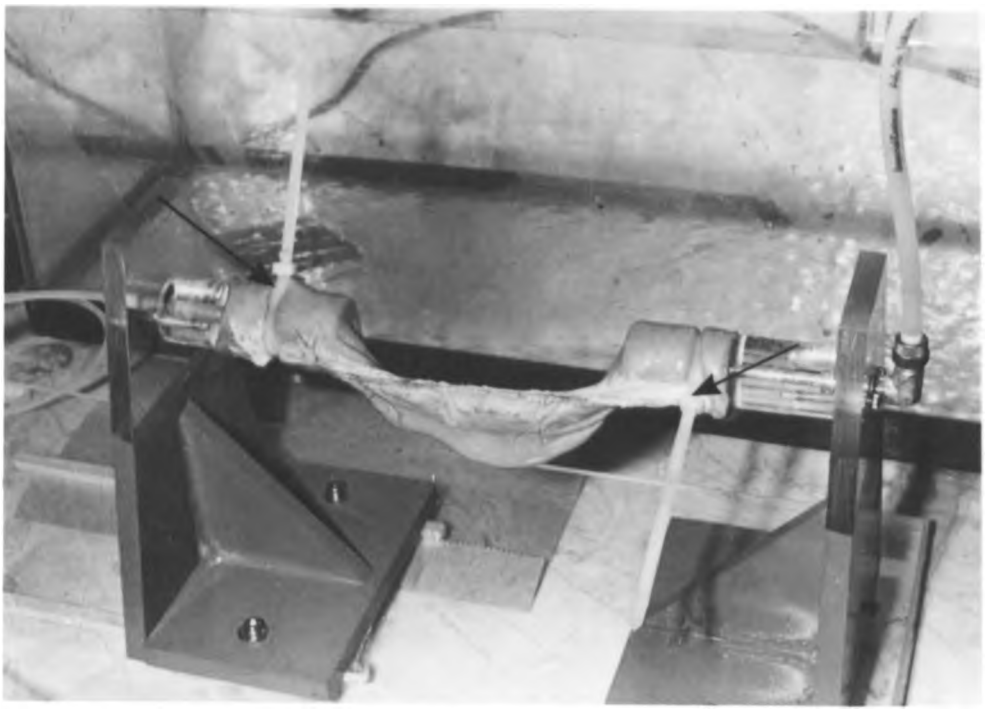
Figure 3. Part of the system shown in Figure 2 with the gut section attached. Arrows show the plastic tie bands in place. Notice the slight sagging of the test segment due to the presence of mesenteric attachment.

Figure 4. Equipment used during bursting pressure measurement to attach section of gut to pressurizing system.
(1) Extracorporeal gun.
(2) Tie bands.



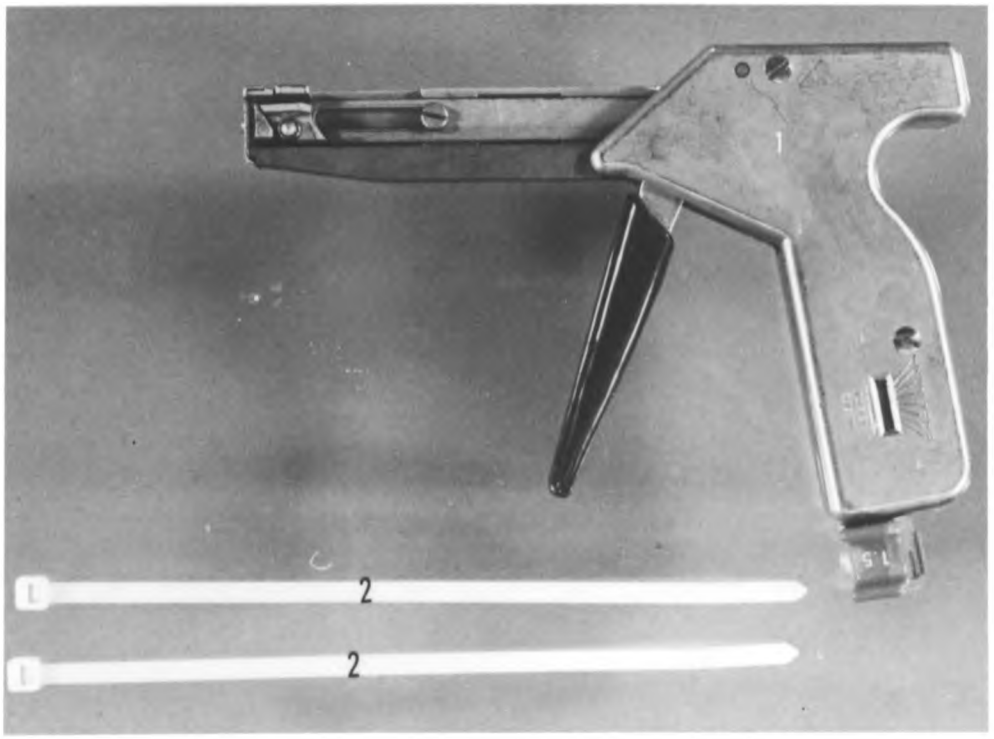

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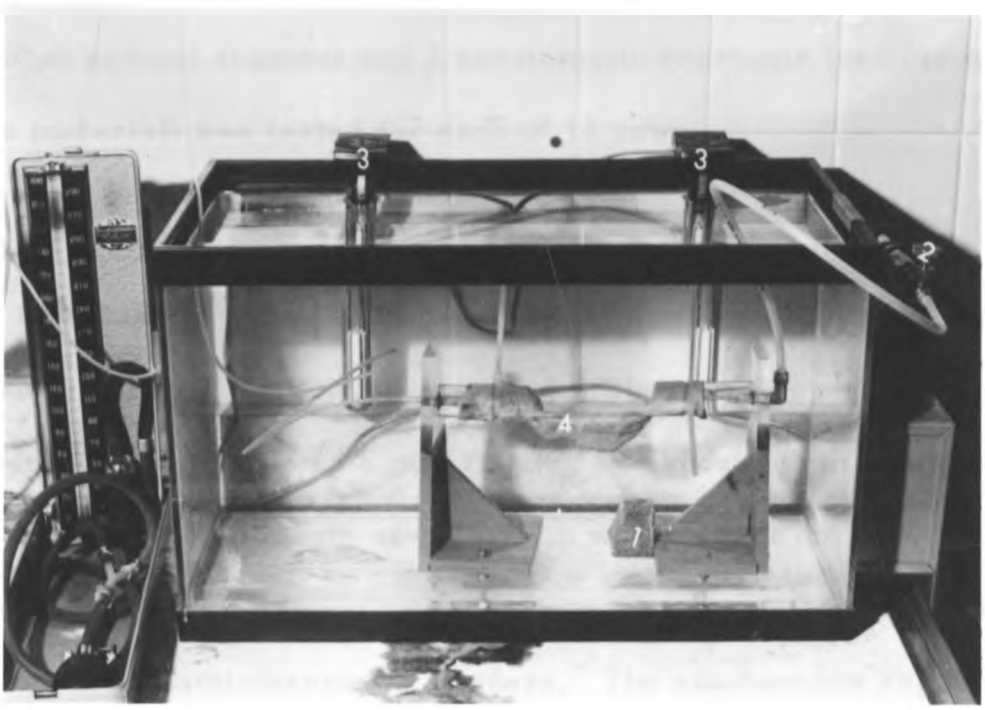
Figure 5. Bursting pressure system ready to test gut section.

(1) Weights to keep the part holding the test segment at the bottom of the tank.
(2) Pressure control valve.
(3) Thermisters.
(4) Test segment just before inflating with oxygen.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mercury manometer. The output electric signal from the Statham pressure transducer was transmitted to a bridge amplifier and recorded on a strip chart in a Sanborn 7700 unit.

As the anastomotic segment slowly inflated, it was observed to note the point of first leakage of oxygen (bubbles). This point was recorded and the strip chart was marked at the moment the bubbles were observed.

One normal segment and 3 anastomotic segments (one for each suture material) was tested for each of 12 cows.

Breaking Strength

Longitudinal strips of intestine at the anastomotic site, 1 cm wide and including 2 stitches were used. These strips were such that between the 2 stitches was 6 mm spacing and about 2 mm on either side of the stitches. The length of each strip was 6 cm (3 cm on either side of the anastomosis). Strips were cut from the gut wall between the mesenteric and antimesenteric borders. The anastomotic segment was opened on the mesenteric side and spread out on a cutting board, using pins to hold it in place. Excess mesenteric tissues were trimmed off.

A plexiglass template (10 cm x 1.0 cm x 0.6 cm) with perpendicular edges was used to aid in cutting the required sections. The template had holes drilled at the corners so that pins were passed through these holes to secure the template over the area where the

intended sections had to be made. The template was ground to about 3 mm thickness in the middle to accommodate any tissue thickening at the site of the anastomosis. Cuts were made along the long side of the template (10 cm side) using a steel blade[18] 11.75 cm (4 5/8") long (Fig. 6). Using a centimeter calibrated plastic ruler, 6 cm were measured (3 cm on either side of the anastomosis) and the strip was cut out. Measurement of the strip was made before cutting it out to avoid any variations in length due to contraction. This strip was kept moist with cold lactated Ringer's Solution before it was used.

A pair of Brown Adson's forceps was used to hold the test strip. One end of the strip was placed on the roughened surface of one side of the clamp and was fixed in place by tightening the clamp using a small wrench. The other side of the test strip was fixed to the opposite side of the clamp. About 1 cm of the test strip was clamped on either side. For all strips, the mucosal surface was facing up.

The clamp attached to the Statham load cell was fixed and the other clamp could move horizontally on a flatform (Fig. 7). The flatform was moved by a driving screw with 40 threads per inch. A motor, driven by a Sorenson D.C. power supply, was set to run at a slow constant speed (1.5 mm/sec) moving the mobile clamp backwards as the flatform moved on the driving screw. This distracted

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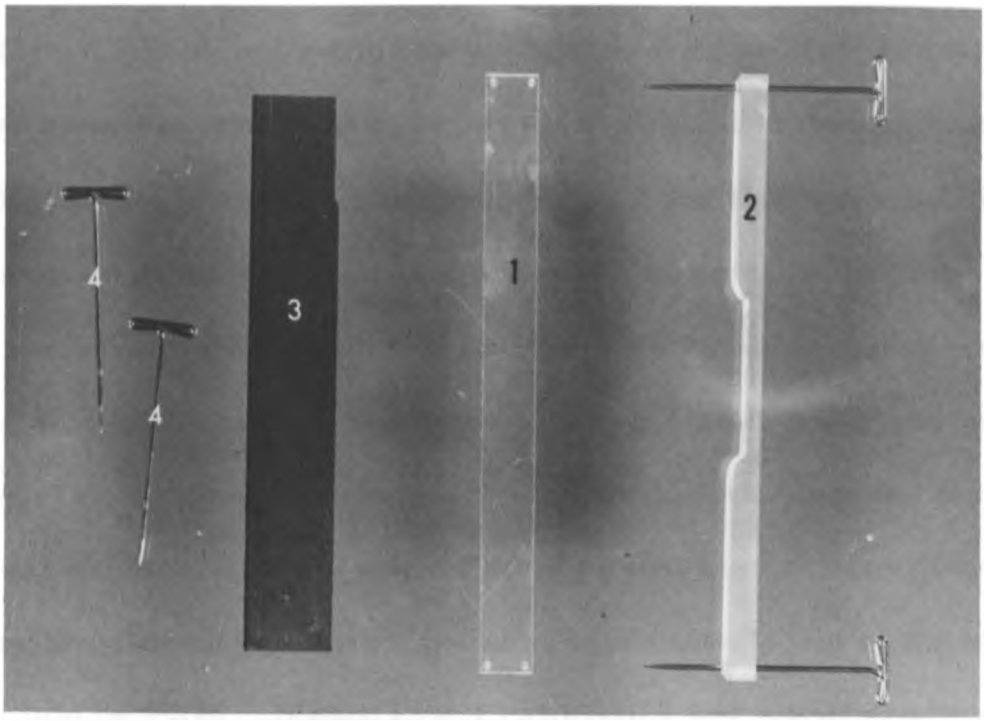
[18] Steel blades. Arthur H. Thomas Company. Philadelphia, PA.

Figure 6. Equipment used to cut uniform strips of gut wall.
(1) Plexiglass glass template (Notice the holes drilled at the corners).
(2) Plexiglass template with pins in place. Middle part of the template was ground to 0.3 cm thickness.
(3) Steel blade used for making longitudinal cuts.
(4) Pins.

Figure 7. Major parts of the breaking strength apparatus.
(1) Statham load cell.
(2) Fixed clamp.
(3) Mobile clamp.
(4) Displacement transducer (Arrows show points of attachment).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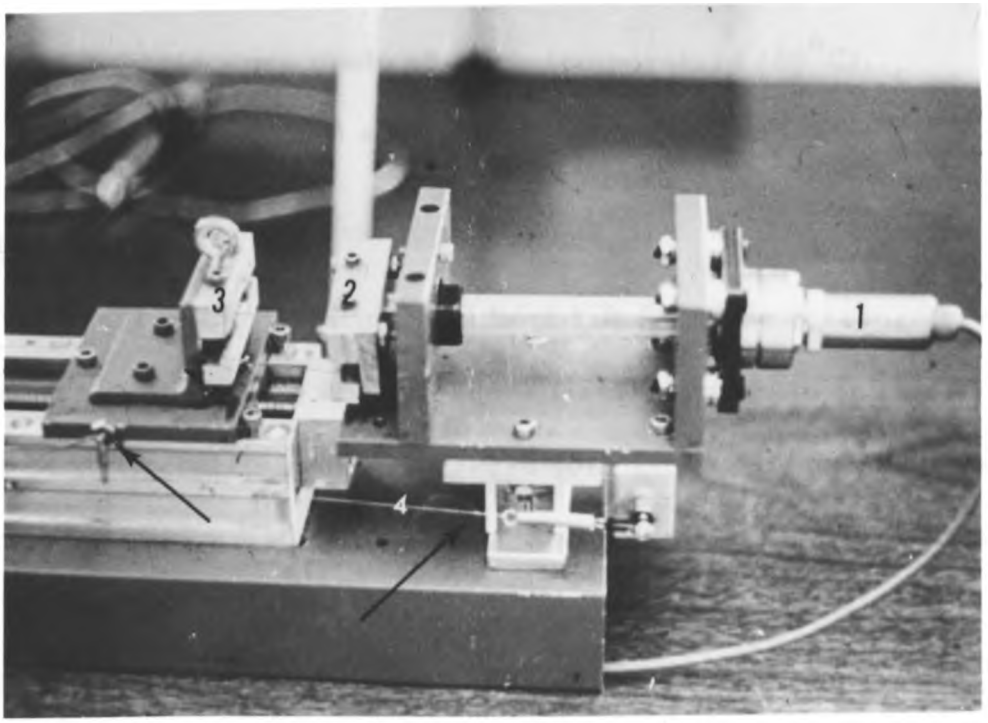
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the attached test strip longitudinally and the force required to tear the test strip was sensed by a Statham load cell calibrated in grams (0-1000g). An electrical signal was produced and this was amplified and recorded on a strip chart in a Sanborn 7700 unit. If the test strip broke away from the anastomosis and there was a big enough piece left for re-attachment, the motor was reversed so that the jaws of the clamps came close enough for re-attachment. Each time an attachment was made, the motor was switched so that the flatform reversed to bring the clamping jaws to a position where attachment of the test strip could be made. Displacement calibration and signal conditioning were done.

Two normal samples and 2 samples from each of the 6 anastomoses were tested for the 21-day animals. Three anastomoses; one for each suture material were tested for the 7-day animals. All the measurements were made at room temperature (20^{o}C).

Six cows were tested at 21 days and 12 cows at 7 days.

Histopathology

The rest of the intestinal segment left from the measurement of breaking strength had all the excess tissues trimmed and was stored in a jar containing 10% buffered formalin. Formalin tissue ratio was maintained 10:1 for proper tissue fixation. Each jar was labelled with animal number, suture material and duration of the anastomosis (7 or 21 days). All the jars were stored at 5^{o}C.

From the fixed specimens, tissue blocks about 4 cm long (2 cm on either side of the anastomosis) and about 0.6 cm wide were cut and put in tissue cassettes. These were embedded in paraffin and 10 μ sections were cut.

Two stained slides were prepared from each of the 78 tissue blocks from the 20 cows. Thirty-six tissue blocks were from 21-day animals and 42 tissue blocks from 7-day animals. One slide was stained with Hematoxylin and Eosin stain for routine histopathologic study, and the other with Masson's stain to demonstrate collagen production. These slides were studied for inflammatory response and healing.

RESULTS

General Observations

Three cows (#s 3452, 20 and 17) developed transient radial paralysis of the left foreleg post-operatively despite the precautions but in all cases this was resolved within 3 days.

Animals were able to drink water within 2 hours of recovery from anaesthesia and ate hay when it was provided.

Most cows developed diarrhea on the 3rd post-operative day but by the 5th day feces started firming.

Two cows (#s 3293 and 3442) had, in addition to diarrhea, a mucoid nasal discharge and an increased respiratory rate but the temperature was normal (38.8^{o}C). For these animals, penicillin-streptomycin was continued twice a day up to the 5th post-operative day, when the nasal discharge subsided and respiratory rate was normal.

At both the 7 day and 21 day retrieval surgeries, the cows had many adhesions. The anastomotic sites were adhered to the adjacent intestinal surfaces and some adhesions involved the omentum. Adhesions were carefully broken with fingers, avoiding breaking the anastomoses. One cow had a localized abscess of about 3 cm diameter around an anastomosis which had a small leakage between 2 stitches.

Bursting Pressure

Most of the segments had an obvious drop in pressure at disruption and maximum pressure was determined from the pressure tracing. A few segments had small bubbles leaking and for these the pressure at which the small bubbles were observed was noted on the tracing. Figure 8 shows some of the typical tracings. It was observed that bursting occurred either at the anastomosis, between anastomosis and the clamp or less than 1 cm from the clamp. Table 3 contains the bursting pressure results.

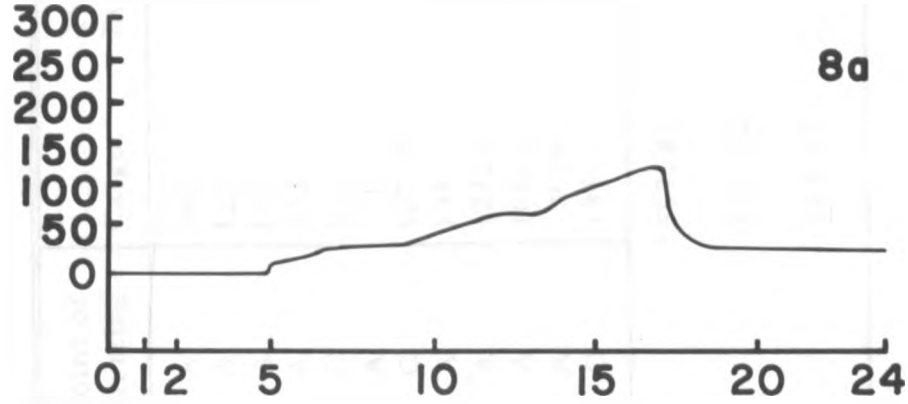
Breaking Strength

It was noted that some strips broke about 2 cm from the anastomosis. These strips were long enough for re-attachment to the clamp without getting too close to the anastomosis. For these a re-run of the breaking strength was done. Occasionally a second re-run was indicated. For each test strip, the highest reading of the breaking strength was recorded. The point of breaking where the highest reading occurred was noted. This was either at the anastomosis, next to the anastomosis (less than 1 cm from the anastomosis); between the anastomosis and the clamp (more than 1 cm from the anastomosis and more than 1 cm from the clamp) or at the clamp (less than 1 cm from the clamp). Those strips which broke through the anastomotic site tore through leaving the knot intact for all the 3 suture materials.

Figure 8. Typical bursting pressure tracings. The three tracings represent three different test segments.
Vertical axis = Tension (Bursting pressure = mm Hg)
Horizontal axis = Distension

Note: (1) A similar pattern of increase in tension characterized by a gradual rise in pressure (mm Hg) and an abrupt drop at disruption.
(2) Distension to the point of disruption was variable.
(3) Peak tension (just before disruption) was recorded.

BURSTING PRESSURE TRACINGS
(IN mmHg)



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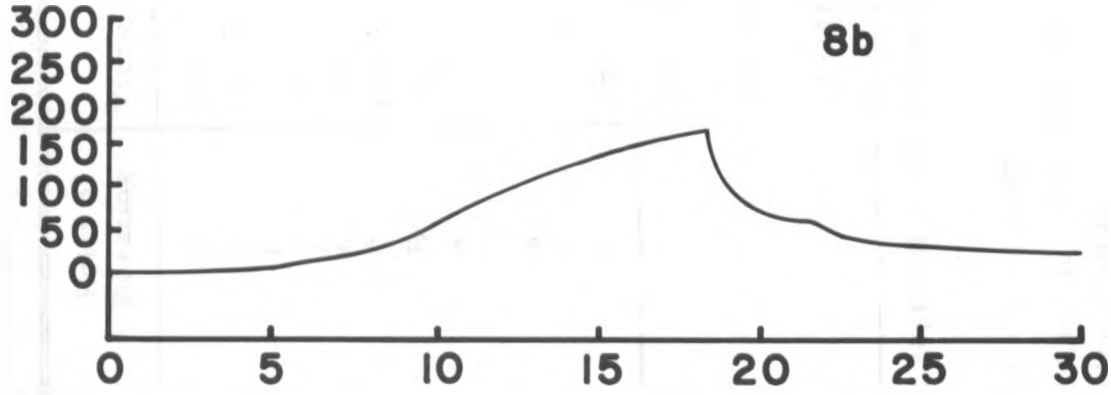

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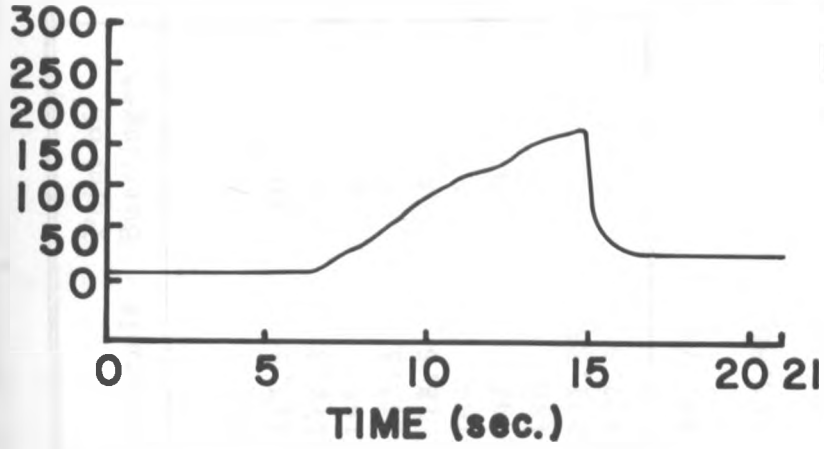

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Table 3. Bursting pressure: 7 days - measurements in mm Hg.

Cow #	Normal*	Point of Bursting**	Nylon	Point of Bursting	Vicryl	Point of Bursting	Catgut	Point of Bursting
3431			165	A	102.5	A	142.5	A
1	210	C	165	C	112.5	A	135	B
3436	247.5	B	322.5	B	232	A	153	A
7	172.5	C	127.5	C	52.5	A	153	A
19	165	C	157.5	A	180	C	120	A
3293	142.5	C	127.5	C	127.5	A	90	A
3442	78.5	A	142.5	B	135	C	127.5	C
3121	165	B	135	A	92.5	A	135	A
2N	92	B	127.5	A	75	A	127.5	A
3	180	C	60	A	112.5	A	162.5	C
17	221.25	C	177.5	C	172.5	A	86.4	A
3321	127.5	C	139	C	150	C	135	C
Total	1801.75		1846.50		1544.50		1567.40	
Mean	163.80		153.84		128.71		130.62	
SD	±55.34		±60.95		±49.48		±23.27	

* Median value for normal intestinal test segment = 128.5 mm Hg
SD = standard deviation

** Sections from normal gut (no anastomosis in section)

Point of Bursting: A - Anastomosis (middle); B - Between Anastomosis (middle) and clamp;
C - Within 1 cm from the clamp

Typical tracings are shown in Figure 9 and Tables 4 and 5 have the results.

From the absolute data of bursting pressure and breaking strength it was noted that, even at one standard deviation (SD), there was a substantial amount of variation in the results. Completely Randomised Design (CRD) analysis of variance was performed (Table 6). There was no statistically significant difference at 0.05 alpha level for both bursting pressure and breaking strength.

Percentage of Normal Mean Results for the Three Sutures

Results are shown in Tables 7, 8 and 9.

The mean values of bursting pressure or breaking strength for each suture material was expressed as a percentage of the normal mean. These percentage values were based on the absolute results and as such did not essentially tell anything more than the absolute results.

Point of disruption is shown expressed as a percentage of total observations. The point of disruption is, however, worth noting because it was anticipated that disruption would invariably occur at the anastomosis.

Figure 9. Typical breaking strength tracings. a, b, c are different test strips.
Vertical axis = Tension (g)
Horizontal axis = Stretch

Note: (1) Degree of stretch varied from section to section and the yield point was not dependent on degree of stretch.
(2) General tendency to increase in breaking strength to "yield point" although the pattern varied depending on the way the section tore. The tracings do not represent any specific point of breaking.
(3) Peak force (g) was recorded since it was the yield point.

BREAKING STRENGTH TRACINGS

(In grams)

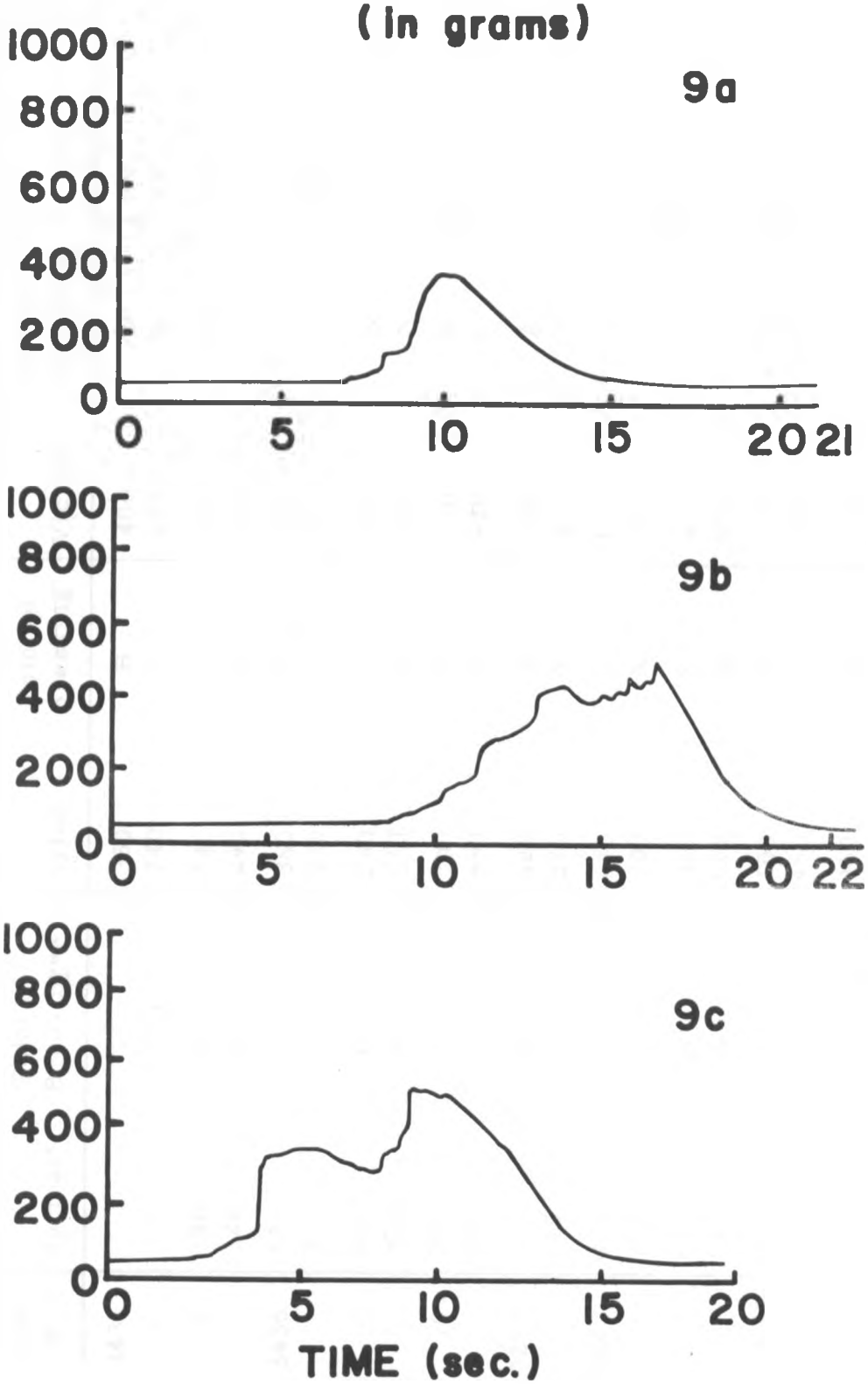


Table 4. Breaking strength: 7 days - measurements in grams.

Cow #	Normal*	Point of Breaking**	Nylon	Point of Breaking	Vicryl	Point of Breaking	Catgut	Point of Breaking
3431			350	B	400	B	700	B
			740	A	470	B	520	B
1	180	A	540	B	540	B	340	C
	320	B	240	B	280	A	340	B
3436	520	A	380	A	380	N	470	N
	400	A	430	N	300	N	640	A
7	480	C	280	C	580	N	210	N
	370	A	540	N	380	N	340	N
19	580	A	700	N	520	N	610	N
	600	A	550	A	520	C	480	N
3293	570	A	420	A	540	A	420	N
	610	A	520	N	450	N	700	N
3442	490	A	640	C	610	N	460	N
	360	A	600	A	680	B	720	N
3121	630	A	360	N	405	N	605	A
	625	A	615	N	410	A	240	N
2N	620	A	500	N	460	N	305	B
	640	C	460	N	400	N	430	N
3	615	A	460	N	730	N	730	A
	670	A	500	N	360	A	500	N

Table 4 (Continued).

Cow #	Normal*	Point of Breaking**	Nylon	Point of Breaking	Vicryl	Point of Breaking	Catgut	Point of Breaking
17	600	A	360	N	340	N	405	C
	425	A	480	N	450	B	420	N
3321	360	A	450	N	345	C	540	N
	790	A	520	N	748	N	500	B
Total	11,465		11,635		11,298		11,625	
Mean	521.14		484.79		470.75		484.38	
SD	±142.051		±123.51		±128.51		±149.83	

* Median value for normal intestinal test strip = 580 g
SD = standard deviation

** Normal sections (no anastomosis)

Point of Breaking: A - Anastomosis (middle); N - Next to the Anastomosis (< 1 cm from Anastomosis); B - Between Anastomosis and Clamp (> 1 cm from Anastomosis) or more to one side; C - At the clamp (< 1 cm from the clamp)

Table 5. Breaking strength: 21 days - measurements in grams.

Cow #	Normal*	Point of Breaking**	Nylon	Point of Breaking	Vicryl	Point of Breaking	Catgut	Point of Breaking
3452a			410	A	520	B	450	C
	625	A	420	B	480	N	840	C
3452b	430	A	480	C	380	A	420	B
			420	B	480	A	460	N
3285a			320	B	400	N	380	N
	460	A	520	B	340	C	420	B
3285b	600	A	510	B	640	A	420	B
			400	B	300	A	360	C
3106a			380	N	740	N	560	N
	500	A	590	N	540	A	600	N
3106b	460	A	540	N	430	N	860	N
			620	N	680	N	660	N
16a			680	N	580	N	460	N
	950	A	290	N	540	N	940	B
16b	380	A	330	A	420	B	420	N
			360	B	600	N	910	B
3115a			560	B	420	A	540	N
	620	A	450	N	400	N	510	B
3115b	510	A	520	N	250	A	520	N
			260	N	600	B	360	N

Table 5 (Continued).

Cow #	Normal*	Point of Breaking**	Nylon	Point of Breaking	Vicryl	Point of Breaking	Catgut	Point of Breaking
20a			420	N	370	A	580	N
	490	A	370	B	240	N	430	A
20b	520	A	475	N	340	B	280	N
			380	N	390	N	370	N
Total	6545		10,725		11,080		12,700	
Mean	545.42		446.88		461.667		529.167	
SD	±147.98		±106.24		±133.12		±194.17	

* Median value for normal intestinal test strip = 505 g
SD = standard deviation

** Normal sections (no anastomosis)

Point of Breaking: A - Anastomosis (middle); N - Next to Anastomosis (< 1 cm from Anastomosis); B - Between Anastomosis and clamp (> 1 cm from Anastomosis); C - At the clamp (< 1 cm from the clamp)

Table 6. Completely randomized design analysis of variance.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Bursting Pressure - 7 Days				
Treatments	3	10434.72	3478.24	1.48**
Error	43	100726.07	2342.47	
Total	46	111160.79		
Breaking Strength - 7 Days				
Treatments	3	30990.644	10330.215	0.5735**
Error	90	1621174.855	18013.054	
Total	93	1652165.499		
Breaking Strength - 21 Days				
Treatments	3	137425.75	45808.58	2.15**
Error	80	1704780.2	21309.75	
Total	83	1842205.95		

**Not significant at $\alpha = 0.05$ significance level.

Table 7. Bursting pressure (% of normal mean value) 7 days.

Suture	Total Observations	% Bursting Pressure	Point of Bursting (% Total Observations) A	B	C
Nylon	12	93.9	8.3	50.0	41.7
Vicryl	12	78.6	66.7	16.66	16.66
Catgut	12	79.7	66.7	8.3	25.0
Normal	11	100.0		36.4	63.6

Table 8. Breaking strength (% of normal mean value) 7 days.

Suture	Total Observations	% Breaking Strength	Point of Breaking (% Total Observations) A	N	B	C
Nylon	24	93.0	20.8	58.3	12.5	8.3
Vicryl	24	90.0	16.7	54.2	20.8	8.3
Catgut	24	92.8	12.5	58.3	20.8	8.3
Normal	22	100.0		80.9		9.1

Table 9. Breaking strength (% of normal mean value) 21 days.

Suture	Total Observations	% Breaking Strength	Point of Breaking (% Total Observations) A	N	B	C
Nylon	24	81.2	4.2	54.2	37.5	4.2
Vicryl	24	84.3	33.3	45.8	16.7	4.2
Catgut	24	96.3	8.3	54.2	25.0	12.5
Normal	12	100.0		100.0		0

Sample Median: A Measure of Sample Average

Median value was chosen because it was a more meaningful indicator of sample average since there were many variables. Variation in bursting pressure measurement was in point of disruption, manner in which the leakage of oxygen occurred, and rate of inflation with oxygen not being precisely controlled. There was variation in point of disruption and number of runs for each test strip for breaking strength measurement.

Results were expressed as percentages for those readings above and below normal median value because actual readings were difficult to interpret.

Since it was expected that both bursting and breaking of normal intestine would occur in the middle of the test segment, all readings where bursting or breaking was next to the clamp were omitted in determination of normal median value. This was because the clamp may have had some influence.

All values for each suture material above the median value irrespective of where bursting or breaking occurred were included on the assumption that in these instances the anastomosis was at least stronger than normal intestinal wall.

Bursting pressure values below the median value included were only those where bursting occurred at the anastomosis due to the possibility of suture material effect on healing. Breaking strength

Table 10. Bursting pressure - 7 days. Results expressed as a % of the median for normal gut (128.5 mm Hg).

	Nylon	Vicryl	Catgut
Above Normal Median	80	41.7	63.6
Below Normal Median	20	58.3	36.4
Total Number of Observations	10	12	11

Table 11. Breaking strength - 7 days. Results expressed as a % of the median for the normal gut (580 g).

	Nylon	Vicryl	Catgut
Above Normal Median	20	27.8	38.9
Below Normal Median	80	72.2	61.1
Total Number of Observations	20	18	18

Table 12. Breaking strength - 21 days. Results expressed as a % of the median for normal gut (505 g).

	Nylon	Vicryl	Catgut
Above Normal Median	47.1	42.9	57.9
Below Normal Median	52.9	57.1	42.1
Total Number of Observations	17	21	18

values below the median included were those where breaking occurred at the anastomosis. Those breaking next to the anastomosis were also included on the assumption that suture material somehow affected the strength of the intestinal wall next to the anastomosis.

Tables 10, 11 and 12 have the results.

Histologic Observations

Anastomosis

The ideal "crushing" sture technique would have all the intestinal layers accurately appositioned but in our anastomoses there were variations.

The anastomoses were divisible into 3 main groups based on the manner in which the layers were appositioned (Figures 10, 11, 12, 13, 14, 15 and 16).

These groups were:

1) All layers appositioning.

1a) All layers appositioning but with one or more epithelial islands on the serosal side.

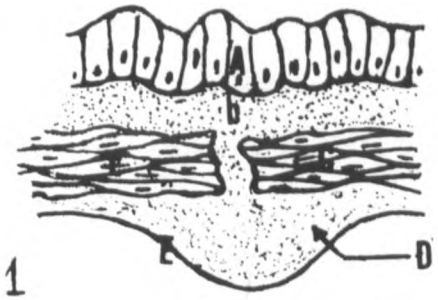
2) Epithelium and submucosa layers continuous but the muscular layer somewhat everting.

2a) Like (2) but with mucosal islands on the serosal side.

3) All layers everting with continuity of the mucosa. Fibrous tissue formed a bridge on the outside.

3a) Like (3) with mucosal islands on the outside.

Figure 10. Quality of anastomosis.
A = mucosa; B = submucosa; C = muscle layer; D = subserosal scar; E = serosa; F = epithelial island.
1 = expected apposition
2 = muscle layer everting
3 = all layers everting



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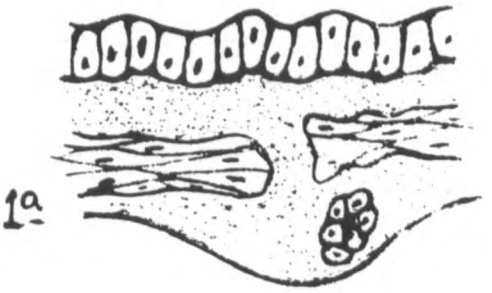

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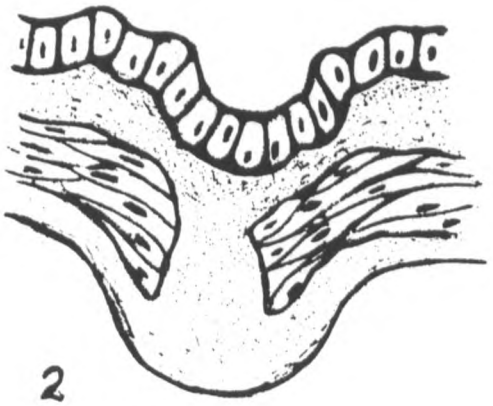

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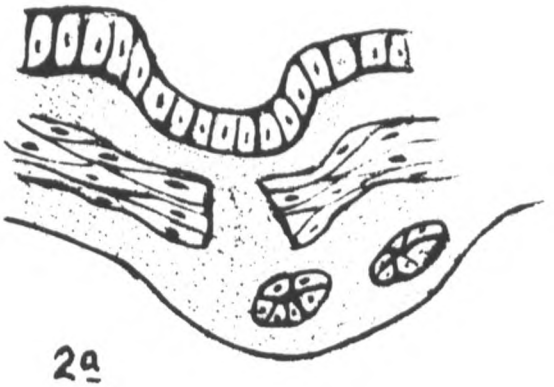

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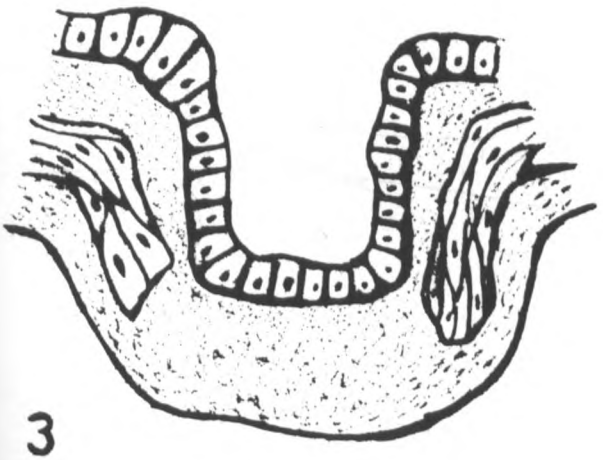

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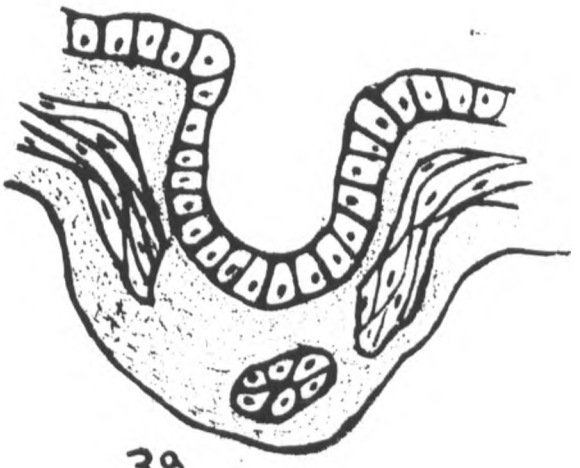

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Figure 11. Type 1 approximation of the cut ends. Suture material is seen at the site of anastomosis (arrow). H/E stain x 5.

Figure 12. Type 1a approximation. Arrow shows epithelial island. H/E stain x 5.

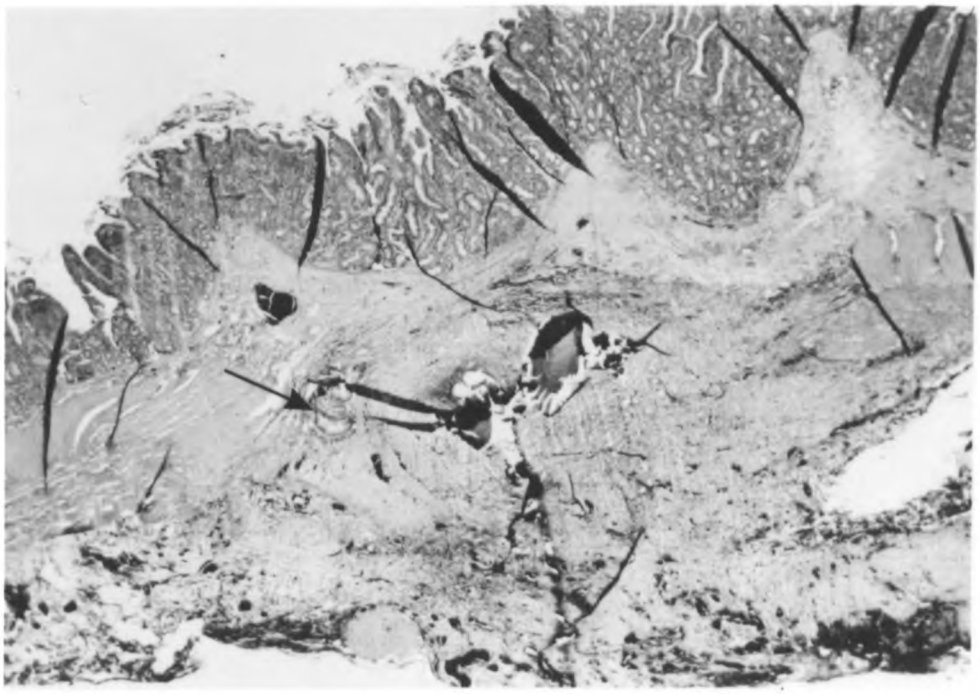


Figure 13. Type 2 approximation. The apparent tract (arrow) is an artifact. H/E stain x 5.

Figure 14. Type 2a approximation. Arrows show epithelial islands. H/E stain x 5.

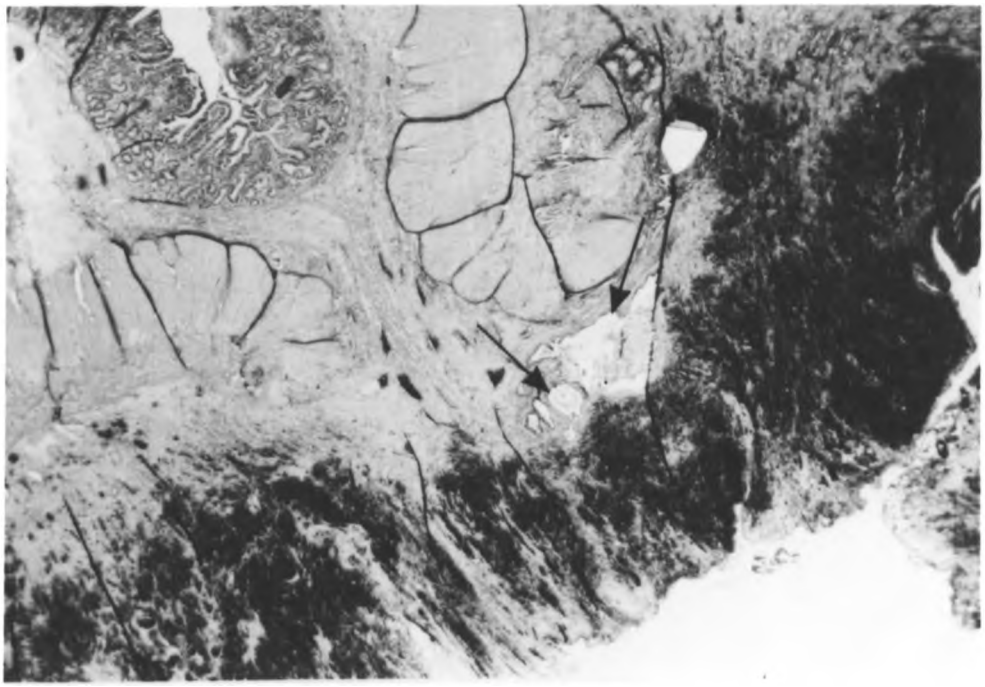
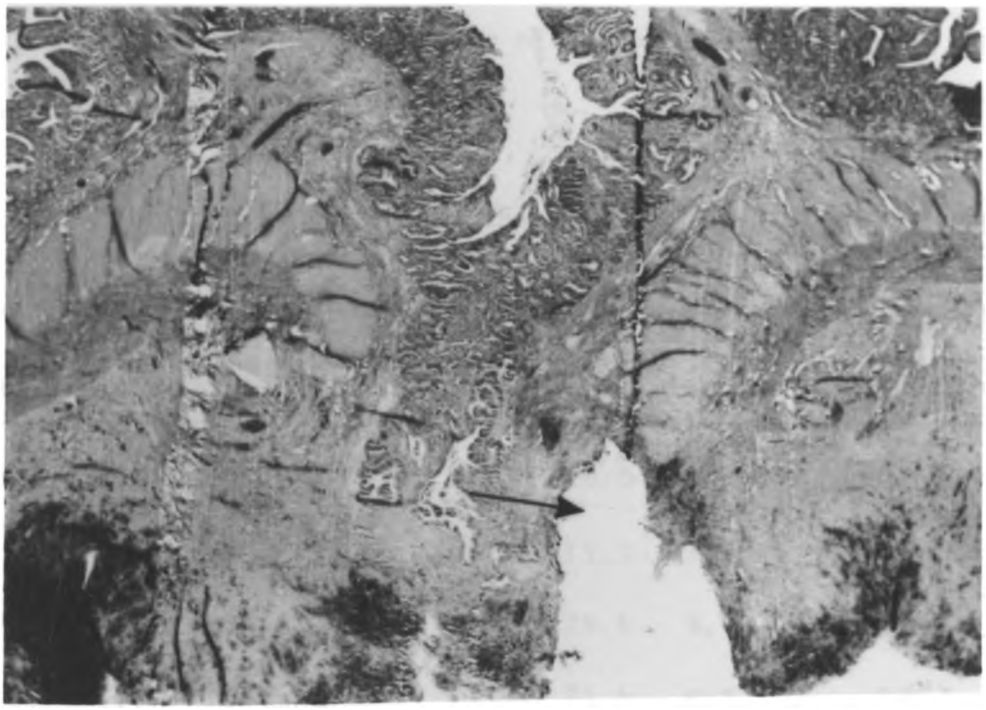


Figure 15. Type 3 approximation. Arrow shows space formerly occupied by suture material. H/E stain x 5.

Figure 16. Type 3a approximation. The epithelial island (arrow) contained some eosinophilic substance.



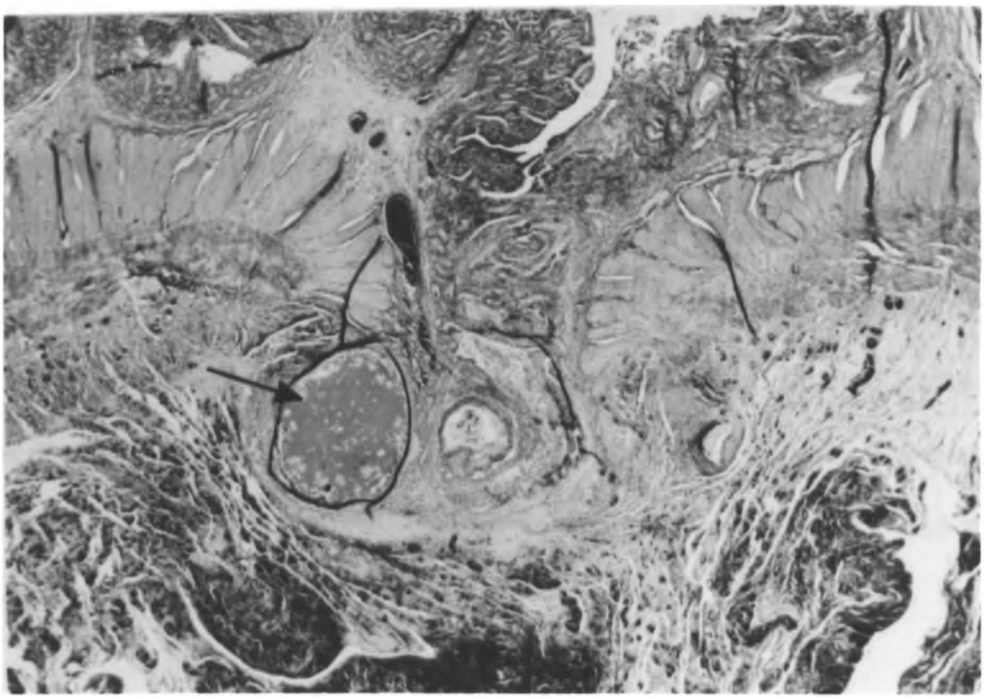


Table 13 shows the percentage of the various types based on the 3 suture materials and total.

Table 13. Histologic evaluation of the anastomoses, expressed as % of total observations.

Suture	1	1a	2	2a	3	3a	Total Observations
Catgut	12.5	29.2	4.2	25.0	25.0	4.2	24
Nylon	3.7	29.6	7.4	40.7	11.1	7.4	27
Vicryl	0	22.2	3.7	40.7	29.6	3.7	27
Average	5.4	27	5.1	35.5	21.6	5.1	78

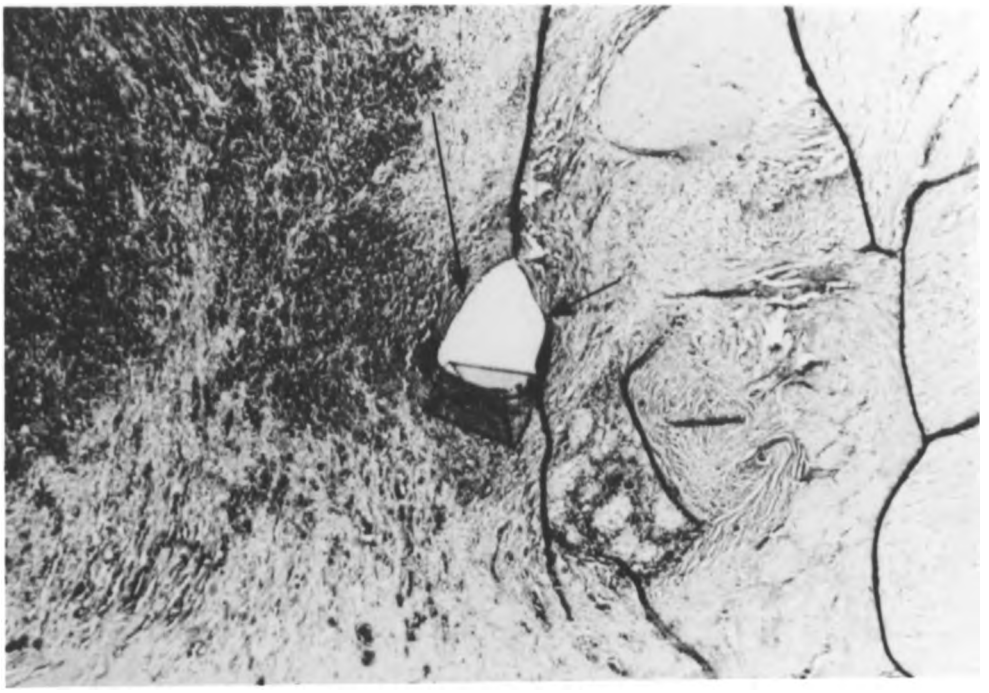
Cellular Reactions

Nylon. At 7 days, cells observed were mainly fibroblasts around the suture material. Few macrophages and eosinophils were present. At 21 days there was more collagenous tissue. Generally, the reaction was minimal (Figs. 17 and 18).

Polyglactin 910 (Vicryl). Cells observed were macrophages, giant cells, eosinophils, neutrophils, and a few fibroblasts and lymphocytes. There was cellular infiltration around suture material even at 7 days. The reaction at 21 days was more intense than at 7 days (Figs. 19 and 20). Compared to catgut macrophages and giant cells were predominant.

Figure 17. Nylon reaction at 7 days (arrows). Suture material was displaced during the cutting of the section. H/E stain x 31.25.

Figure 18. Nylon reaction at 21 days (arrows). Collagen fibers were relatively more mature. H/E stain x 80.



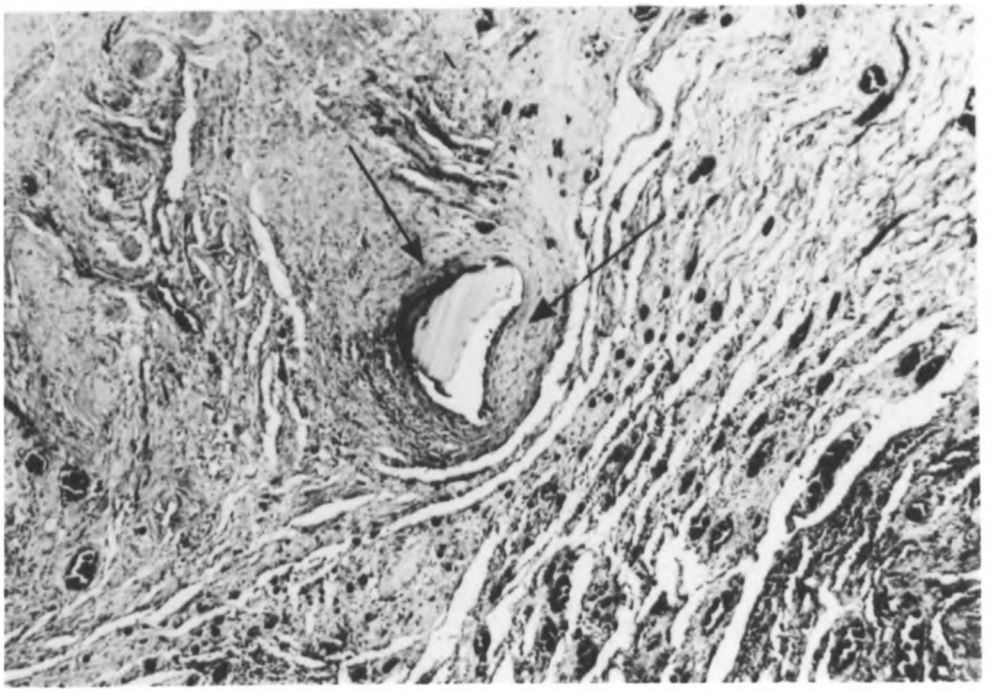
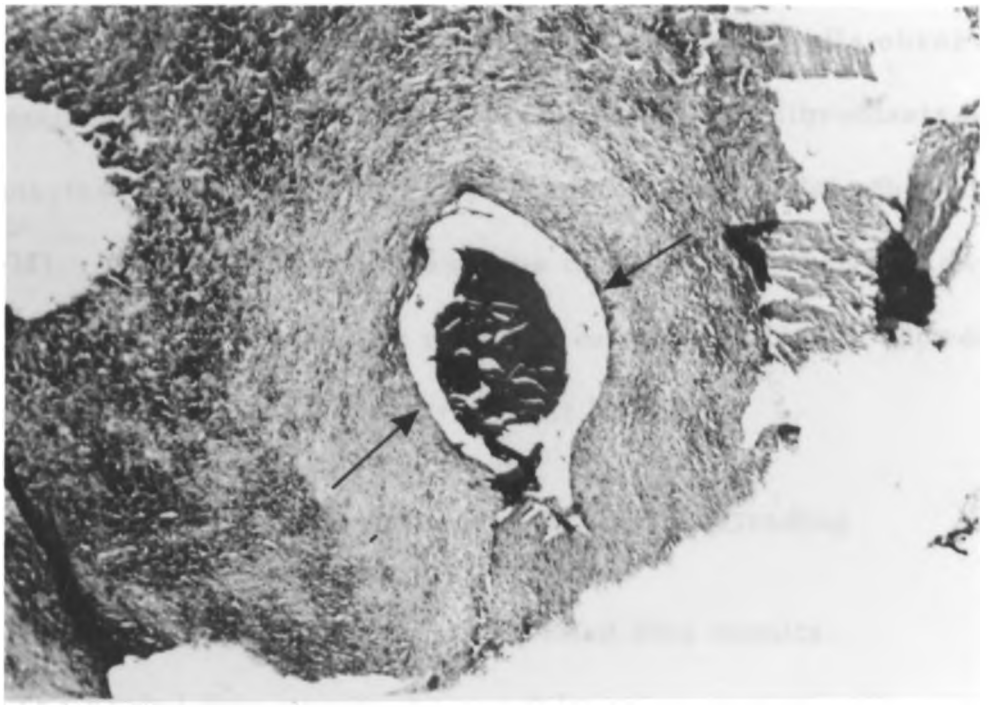
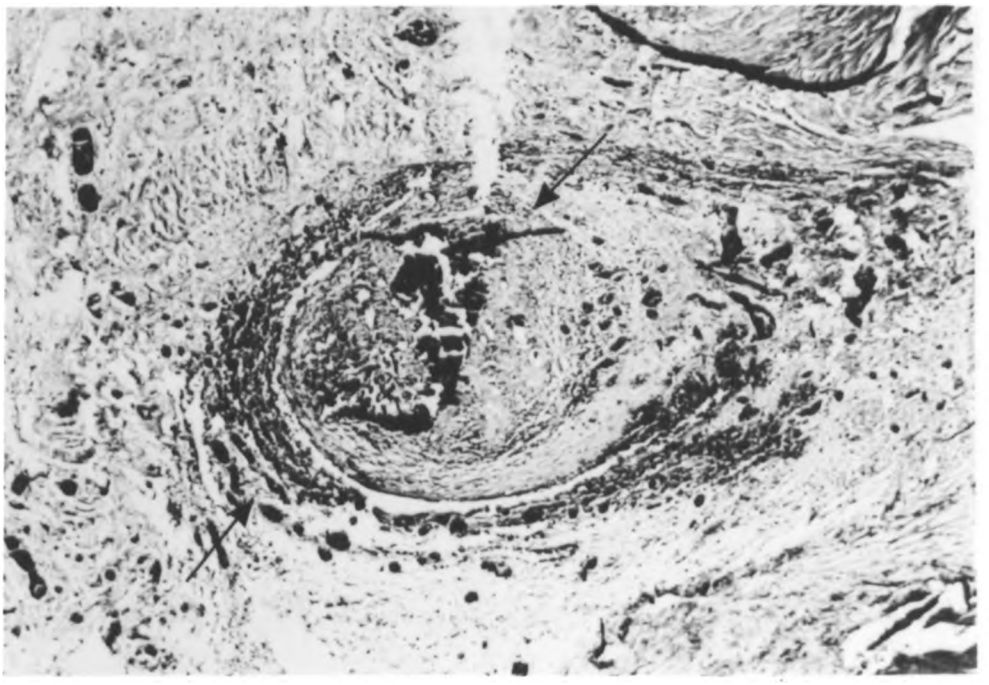


Figure 19. Catgut reaction at 7 days (arrows). Clear area around the suture material may be a shrinkage artifact. H/E stain x 50.

Figure 20. Catgut reaction at 21 days (arrows). The reaction is relatively more intense than at 7 days. Notice suture material fragmentation. H/E stain x 80.





Catgut. Neutrophils were predominant. Other cells observed were macrophages, giant cells, eosinophils and few fibroblasts and lymphocytes. Fibroblasts at 7 days tended to encapsulate the suture material. The reaction at 21 days was more intense than at 7 days (Figs. 21 and 22). The suture material in some instances showed fragmentation and cellular infiltration at 21 days.

Pooled Data Results and Comparative Grading

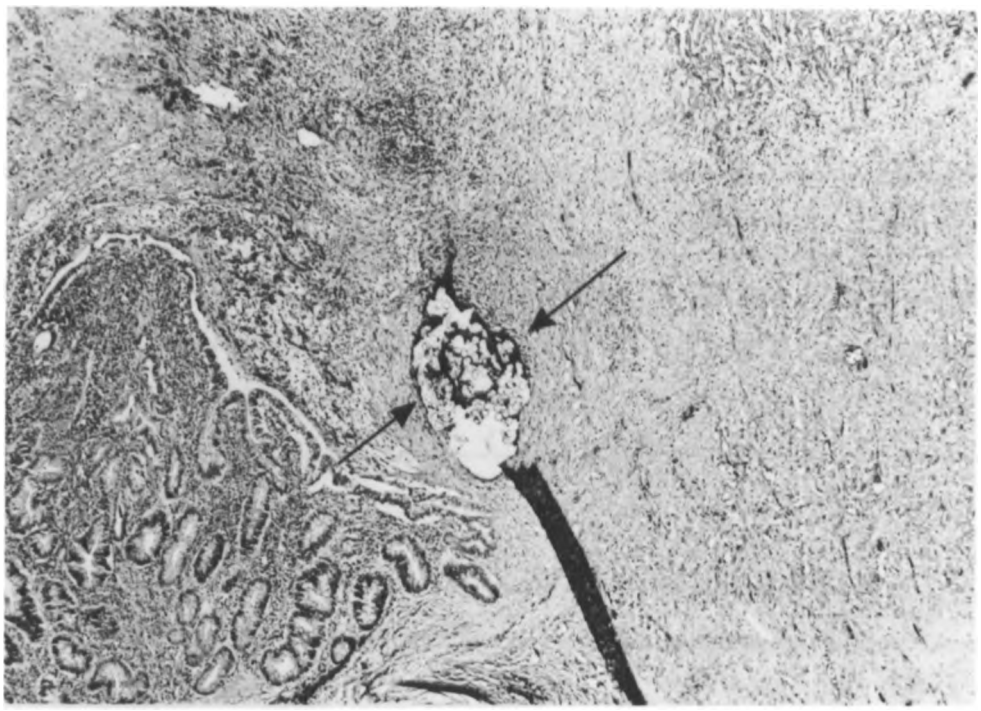
Tables 14, 15, 16 and 17 have pooled data results.

The pooled data results were not based on statistically significant levels but on apparent general trends favoring one suture material or the other.

Comparative grading was done to present the pooled data results numerically. Points were given to the 3 suture materials as follows: Best = 3 points; middle = 2 points; and last = 1 point. Table 16 contains the findings.

Figure 21. Vicryl reaction at 7 days (arrows). H/E stain x 31.25.

Figure 22. Vicryl reaction at 21 days (arrows). Reaction is relatively more intense than at 7 days. H/E stain x 31.25.



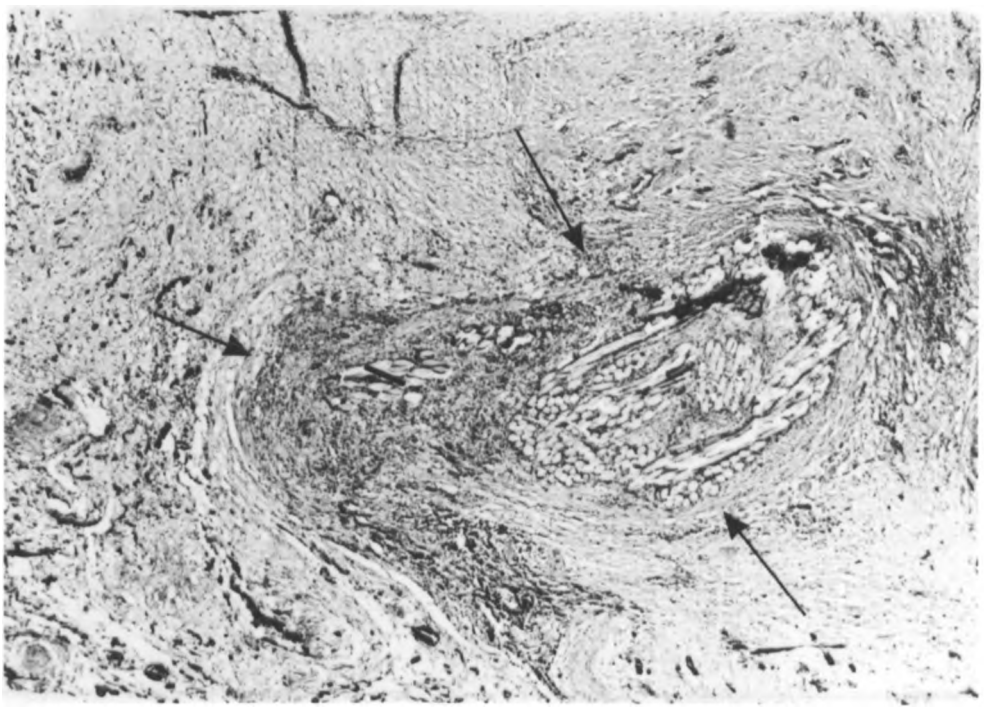


Table 14. Pooled results data. % normal mean value.

	Best	Middle	Last
Bursting Pressure (7 days)	Nylon	Catgut	Vicryl
Breaking Strength (7 days)	Nylon	Catgut	Vicryl
Breaking Strength (21 days)	Catgut	Vicryl	Nylon

Table 15. Pooled results data. % above normal median value.

	Best	Middle	Last
Bursting Pressure (7 days)	Nylon	Catgut	Vicryl
Breaking Strength (7 days)	Catgut	Vicryl	Nylon
Breaking Strength (21 days)	Catgut	Nylon	Vicryl

Table 16. Histologic evaluation.

Percentage Types 1 and 1a Anastomoses

Best	Middle	Last
Catgut	Nylon	Vicryl

Relative Cellular Reaction

Best	Middle	Last
Nylon	Vicryl	
	Catgut	

Table 17. Comparative grading.

	Nylon	Vicryl	Catgut
1) % Normal Mean			
Bursting Pressure (7 days)	3	1	2
Breaking Strength (7 days)	3	1	2
Breaking Strength (21 days)	1	2	3
2) % Above Normal Median			
Bursting Pressure (7 days)	3	1	2
Breaking Strength (7 days)	1	2	3
Breaking Strength (21 days)	2	1	3
3) % Types 1 and 1a Anastomoses	2	1	3
4) Relative Cellular Reaction	3	1.5	1.5
Total	18	10.5	19.5

DISCUSSION AND CONCLUSIONS

Methods of evaluating gain in tissue strength (bursting pressure and breaking strength) and morphologic changes in the healing wound were used to evaluate the effects of suture materials on healing of small intestinal anastomoses in cattle.

Bursting pressure seems to be a good indicator of the anastomotic integrity because it involves the entire circumference of the anastomosis. However, it is the most criticized method of evaluating the gain in strength of intestinal anastomosis due to the rigidity of the anastomotic site making it not distend proportionally with the uninvolved gut (Nelsen & Anders, 1966; Cronin et al., 1968; Peters, 1972; Hastings & VanWinkle, 1972; Hastings et al., 1975; Peacock & VanWinkle, 1976). Its weaknesses tend to limit its usefulness in evaluating biophysical properties of healing.

Breaking strength measurement has been recommended (Peacock & VanWinkle, 1976) but its clinical significance is doubtful because the test strips used do not represent the entire anastomosis. In our study test strips came from the same site on the gut wall, every test strip tested had two stitches included and the test strips were uniform in size. Considering this, it appears, the test strips were adequate.

Bursting Pressure

Bovine mesentery is in two separate layers within 2.5 cm from the intestinal wall and it attaches on either side of mesenteric side instead of attaching directly to the mesenteric border. The two layers of the mesentery had to be left intact because it was observed that if the mesentery was trimmed close to the intestinal wall, oxygen leakage invariably occurred in the area originally covered by the mesentery at a distance from the anastomosis. In order to overcome this problem, 2.5 cm of mesentery was left attached to the intestine. However, this caused the test segment to form a loop instead of being straight after it was attached to the clamp and also during the time it was inflated with oxygen.

The length of the test segment was dictated by the looping tendency. Segments shorter than 20 cm could not be attached to the system without tearing the attached mesentery. Thus, all the test segments were 20 cm long. In addition, not all the test segments had the same diameter. Although the size variations occurred, each animal had a control and 3 test segments, one for each suture material. Size variation might have influenced the variation in the results.

The rate of inflating the test segment with oxygen was not precisely controlled. The same person inflated all the test segments and as such, it was felt that the slow rate of inflation used was fairly

uniform. However, it would have been more accurate if the rate of inflation was constant.

Point of disruption may have been influenced by normal variations in tissue strength from animal to animal or by the removal of adhesions. It may be worth noting that no test segment disrupted exactly at the site held by the tie bands or directly at the edges of the cylindrical plexiglass to which the test segment was attached.

Majority of the normal test segments burst within 1 cm from the edges of cylindrical plexiglass holding the test segment. This could have been due to possible weakening of the tissues at the site during attachment.

Disruption at a point away from the anastomosis may have been due to the anastomosis being stronger, or the tissue being weakened by inflammation or tissue harvesting techniques. More nylon sutured anastomoses burst at a point away from the anastomosis than for the other two suture materials. In fact,the majority of vicryl and catgut suture anastomoses burst at the anastomosis. This implies some effect of sutures on the strength of the anastomosis. It seems the more reactive sutures have weaker anastomoses which results in bursting directly on the anastomosis.

Breaking Strength

The positions of the stitches were determined visually before fixing the plexiglass template in place for cutting. The mucosal surface was slippery and if the test strip was cut with a regular blade (Bard Parker), there was a tendency to slip and cut unevenly. A dermatome blade was used to reduce this problem.

The selection of a standard length for the test strip was based on the fact that breaking always occurred away from the anastomosis when 10 cm long test strips were used. With 6 cm long test strips, there was a higher tendency to break at or near the anastomosis. Due to this, 6 cm (3 cm on either side of the anastomosis) was chosen as the standard length.

There was a tendency for the test strip to slip out of the clamp when a distracting force was applied. In order to overcome this, the clamping surfaces were roughened and the amount of tightening required was a matter of feel (experience).

At times re-runs of a test strip were indicated and this was mainly in cases of slippage or where breaking occurred at or next to the clamp. Thus, one side of the test strip was shorter than the other after the re-attachment. Most of the test strips broke on the same side they broke during the first run. These re-runs might be expected to require less distracting force to break since the test strip was somewhat weakened, but most of the times they were stronger

than the first run. However, the re-runs added a variable to the results.

Results were based on the largest yield point reading of each test strip. There was marked variations between cows and even for the same cow and anastomosis. Adler et al., 1967 using strips from the colon of the dog made similar observations. In our study we thought that the variation in the point of breaking and breaking strength was due to the presence of adhesions, inflammation and suture material.

The point of breaking was variable although generally it occurred within 1 cm from the anastomosis on one side or the other. Adhesions may have contributed to the variation if the test strip came from an area where adhesions had been removed or if there was a considerable adhesion left attached.

At 7 days more of the nylon sutured anastomoses broke at or next to the anastomosis. Catgut had the lowest number breaking at the anastomosis. Thus, assuming that breaking at or next to the anastomosis was a sign of tissue weakness, then nylon sutured anastomoses were weaker. This may have been due to the nylon material cutting through the tissue weakened by the inflammatory reaction. Catgut, which swells when wet, had the least cutting effect during distraction and thus produced the strongest anastomosis. The technical quality of the anastomosis may have contributed to the difference

between catgut and vicryl sutures since catgut had the highest percentage with the best alignment of tissues. Catgut and vicryl anastomoses had the higher percentage breakage between the anastomoses and the clamp as compared to nylon. This may be attributed to the more extensive inflammation associated with absorbable sutures and supports partly the observation by Hastings et al.(1975) that absorbable suture material sutured anastomoses are weakened up to 6 cm from the anastomosis.

At 21 days, considering the number of test strips breaking at the anastomotic site and assuming that test strips breaking at or next to the anastomosis had weaker anastomoses, nylon had the lowest percentage breaking at these points. Therefore, nylon sutured anastomoses could be considered the strongest, catgut second and vicryl was a close third. The apparent weakness of the anastomoses sutured with absorbable material was possibly due to the delayed inflammatory reaction. The greater variation in alignment of the intestinal wall layers in vicryl anastomoses may explain the superior results of catgut sutured anastomoses. It appears that the stronger the anastomosis, the greater the tendency of the test strip to break between the anastomosis and the clamp (normal portion). This part of the test strip was not expected to have as much scar tissue as the anastomotic site and as such it might have been a weaker portion. The fact that nylon is a non-absorbable material may have contributed to

the greater strength of nylon sutured anastomoses in the face of a resolved inflammatory response.

Histologic Evaluation

Quality of Anastomosis

Catgut had the highest percentage with anastomoses close to the desired type of apposition, followed by nylon and vicryl. Looking at the results in retrospect, it was remembered that catgut suture material, due to its tendency to break when tied down too quickly, had the first throw tied down more cautiously. Vicryl which had the best handling quality, had the first throw tied down more quickly. In view of the fact that all the anastomoses were done by the same person, and the assistant was the same person except for 2 cows (12 anastomoses), the variation in the type of anastomosis, might be what usually happens in crushing suture pattern. This might be the cause of post-operative complications associated with crushing pattern.

Chromic catgut suture had the first throw tied down more cautiously to accomplish the crushing phase and this gave the best results. Thus, more care may be necessary with crushing technique in tying down the first throw. This may reduce the amount of eversion of the layers of intestinal wall. With crushing technique eversion may occur in the spaces between sutures since the suture brings together the tissues mainly at the site of their placement. Eversion of the mucosa and the muscular layer is probably enhanced by the fact that after the

intestine is transected, the muscular layer contracts and the mucosa curls over the muscular layer due to loose attachment of mucosa to the submucosa.

It is probably important to mention that all the anastomoses we performed were watertight at completion of suturing despite the high incidence of eversion.

The single histologic section studied for each anastomosis did not necessarily reflect the alignment for the entire anastomosis. Serial sections of the entire anastomosis may give a better picture of apposition.

Cellular Reaction

Nylon and chromic catgut suture materials reactions were similar to that described in literature.

Vicryl had some cellular infiltration between strands even at 7 days. Earlier report by Craig et al. (1975) said that there was minimal cellular infiltration at 5 days in subcutaneous tissue and muscles of rats. Generally the reaction to vicryl was relatively similar to that of chromic catgut. Information in literature about vicryl is rather confusing. Horton et al. (1974) said that the reaction was less than that of catgut and VanWinkle et al. (1975) said that vicryl was more reactive than catgut in dog's skin.

Finally, using a comparative grade on pooled data, we found that chromic catgut sutured anastomoses showed the best healing

properties followed by nylon and vicryl respectively. The results indicated that chromic catgut was a better absorbable suture material for jejunal anastomoses in cattle than vicryl.

More studies on crushing technique are desirable. Studies involving making serial sections of the entire anastomosis to determine the rate of eversion will provide information on the quality of the crushing technique for end-to-end appositional intestinal anastomoses.

SUMMARY

The purpose of this investigation was to study the influence of suture materials on healing of intestinal anastomosis in cattle. A total of 120 anastomoses were performed, 40 using each of monofilament nylon, chromic catgut and polyglactin 910 (vicryl) suture materials. Results were based on observations at 7 days and 21 days post-operatively. The findings are summarized below:

1) Chromic catgut sutured anastomoses were the best overall in healing properties. Nylon was the second, and vicryl was the last.
2) There was a marked variation within the results of both the bursting pressure and breaking strength. There was no statistical difference in the results of bursting pressure and breaking strength but generally there were trends favoring one suture material or the other. Breaking strength results were variable in the same animal and even the same anastomosis.
3) The point of bursting or breaking was variable. In other words, not every anastomosis disrupted at the anastomotic site.
4) Accurate apposition of the cut ends was present in 32.4% of the 78 histologic sections studied. The other anastomoses had one or more layers everting. Frequently there was one or more mucosal islands on the serosal side.

5) Chromic catgut had the highest percentage of the anastomoses with the expected apposition of the intestinal ends; nylon was the second and vicryl had the lowest.

6) Vicryl was no better than chromic catgut regarding intensity of cellular reaction at 7 and 21 days.

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