M. Sc. Experimental Surgery.

EXPERIMENTAL STUDIES IN TRANSPLANTATION OF SMALL BOWEL MUCOSA TO THE RECTUM.

by

G. S. Brody, B. Sc., M.D.

Research Assistant in Experimental Surgery,
Demonstrator in Anatomy, McGill University.

Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the Degree of Master of Science.

June, 1959. McGill University.

PREFACE

Progress in surgical therapy is based upon the firm understanding of the anatomical, physiological and pathological processes involved in any specific disease entity. Each contribution must necessarily be only the addition of a brick, or at best a keystone, to the vast masonry work of scientific knowledge. Although a new fact may at first crown this tower, it soon assumes its role as part of the basework to support the addition of further progress. It is this fact which probably inspired Dr. S. C. Skoryna to define research as the "re-searching" for already elaborated truths upon which to build our hypotheses.

It is the appreciation of this concept that is probably the most significant thing I have learned from my present associations and investigations.

I have greatly enjoyed and, I hope, profited from my year at the Donner Building. Both the work itself and the atmosphere of friendliness, co-operation and stimulation provided by my associates and mentors have contributed to this. I hope that the future will provide many more similar opportunities.

ACKNOWLEDGMENTS

I wish to thank Dr. D. R. Webster for providing me with the opportunity to carry out this present work.

I am most grateful to Dr. J. R. McCorriston for his invaluable advice and supervision at all stages of my work. His constant interest and moral support throughout the year, and his aid in editing this thesis were most appreciated.

Dr. S. C. Skoryna was constantly available for advice and suggestions on many matters pertaining to this work,for which I am most grateful.

Dr. D. S. Kahn and Dr. Y. Clermont very kindly advised on and examined the histological sections. Mrs. G. Phillips, Mr. B. C. Thomlinson and Miss M. Kruchinen prepared and stained the sections.

Dr. F. M. Woolhouse provided very useful information on grafting techniques.

My gratitude also to Mr. Albert Nagy, without whose hard working and capable management of the operating rooms and dog pens, these investigations would have been impossible. He was ably assisted by Mr. N. Vermes and Mr. S. Hardy. Mr. T. Canko humanely cared for the animals involved.

I wish to thank my colleages, Drs. A. Makonnen, M. S. Varma, T. A. Richards, B. J. Perey, B. Thompson, G. E. Pablo, E. Jow, J. G. Susset and R. Iliescu-Constantine, many of whom assisted me in operating and in caring for the animals. My associations with this group was one of the most pleasant aspects of my year.

Thanks is also due Miss Anne Watkins for her most pleasantly proffered help in many aspects of this work.

My gratitude is due to Miss Helen MacArthur who so excellently diagrammed my operative procedures, and to Mr. Harold Coletta, who took most of the photographs in this thesis.

Mr. J. Woticky of All Canada Engineering transformed my crude diagrams into most of the specially designed equipment used in this study.

Dr. D. A. Murphy's advice on the care and management of dogs was most appreciated.

Mr. Carl Schneider and Dr. J. Luccioli very kindly translated German and Italian references.

The Montreal Children's Hospital very generously donated a pediatric sigmoidoscope.

I wish to thank Mrs. Beryl Parker, secretary of the Department, for doing the clerical work and for typing this thesis.

Finally, my deepest thanks to my wife, Sonia, for her translations of French and Spanish, for her constant encouragement and for her understanding of why so many evenings were interrupted by dogs requiring care.

G. S. Brody,
June, 1959.

TABLE OF CONTENTS

Page

TABLE OF ILLUSTRATIONS.

INTRODUCTION

At present, our surgical approach to diseases which are anatomically associated with the mechanism of bowel continence is unfortunately only a compromise. When dealing with a carcinoma of the terminal bowel, for example, we must, to observe the basic principles of cancer surgery, sacrifice a great deal of normal tissue in an attempt to completely excise the tumor. Thus, the nature of the disease dictates that life be preserved at the expense of function.

However, there are diseases of non-malignant character, notably Familial Polyposis and Idiopathic Ulcerative Colitis (in their uncomplicated phase) in which only the mucosa of the bowel is involved, and hence, only this layer need be removed to effect a cure. At present, we are unable to provide patients with these latter afflictions with an operation which will guarantee complete freedom from the unhappy sequelae of their disease and at the same time preserve normal continence.

This project was undertaken in an attempt to devise a method by which the diseased mucosa of the rectum could be removed and replaced by healthy small bowel mucosa. This would preserve the vital neuromuscular mechanisms upon which normal fecal continence depends.

CHAPTER I.

SURGICAL DISEASES INVOLVING THE ENTIRE COLONIC MUCOSA.

There are two conditions in which a disease process, in its uncomplicated phase, is confined to the entire mucosal layer of the large bowel with the muscularis remaining normal. These two, Idiopathic Ulcerative Colitis and Familial Polyposis, will be discussed briefly with emphasis on the surgical therapy.

Idiopathic Ulcerative Colitis: This is a disease of obscure origin, usually occurring in young adults and characterized by frequent, loose bowel movements containing blood and large amounts of mucus. In the more severe phases, the patient may literally be "wedded to the toilet." Generally, the disease runs a chronic course, punctuated by acute exacerbations, but occasionally, may be acute from the onset, resulting in rapid inanition and death if untreated.

Although on occasion only segments of the large bowel are involved, in the majority of cases the entire colon from the ano-rectal junction to the ileo-cecal valve is diseased. Dukes and Lockhart-Mummery in 1957 stated that the disease tends to spread cephalad leaving "burnt out" areas behind. The same authors report a 15% incidence of involvement of the terminal ileum.

Pathologically, (Boyd, 1955), the bowel shows many superficial areas of ulceration ranging in size from tiny erosions to several inches in diameter. These are most frequently related to the teniae. The mucosa is reddish-purple with many petechiae and in about 10% of cases is swollen to form "pseudo polypi." Any changes in the other layers of bowel are secondary, and inflammatory in nature.

In general, the treatment of ulcerative colitis is medical and will not be discussed here. Most authors (Wheelock and Warren, 1955; Cattell and Colcock, 1955) agree that 50 to 60% of patients eventually require surgery, which is indicated in most instances for the complications resulting from this disease.

The indications for surgery are:

1. Perforation: Acute perforation is fairly uncommon but "chronic perforation" producing abscesses and fistulae are quite frequent.

2. Hemorrhage: This may be due directly to ulceration or to poor vitamin K absorption producing hypoprothrombinemia.

3. Malignancy: Carcinoma of the colon occurs in 3 to 5% of patients with a history of ulcerative colitis (Rice-Oxley and Truelove, 1950, Dukes and Lockhart-Mummery, 1957). This is considerably higher than the general population incidence. It may be even higher (up to 12%) in patients with severe disease (Cattell, 1953). It usually occurs after the disease has been present for 10 to 20 years, (Dukes, 1952). Generally, the prognosis is poorer than that of carcinoma uncomplicated by colitis.

4. Obstruction or Stricture: This complication is fairly uncommon, but when it does occur is usually restricted to the rectum.

5. Segmental involvement: While unfortunately uncommon, this situation is of obvious surgical importance.

6. Acute severe exacerbation: When uncontrollable by medical means, this may become a surgical emergency.

7. Intractability: This is a difficult situation to define, but generally when the patient is chronically disabled after a long trial on a medical regime, with weight loss, anemia, etc., then surgery should be considered.

8. Multiple anal fistulae: These are frequent and difficult to eradicate. This process may eventually result in a rigid fibrosed sphincter.

9. Systemic disorders: These include arthritis which is rheumatoid in character, thrombophlebitis and liver disease, (Bargen, 1953; Dennis and Karlson, 1955).

The immediate surgical care of such catastrophes as acute perforation, hemorrhage and obstruction are obvious. In attacking the disease process itself several procedures have been advocated.

1. Ileostomy: This places the colon at rest in an attempt to obtain healing. It is useful as an initial procedure in severely ill patients who cannot tolerate more extensive surgery due to their debilitated condition. Cattell (1942) and Bacon and Trimpi (1950) convincingly showed that re-anastomosis to the large bowel was usually unsatisfactory.

2. Partial colectomy: This is useful when the disease is segmental.

3. Total colectomy and abdominal ileostomy: Most surgeons agree that the removal of the diseased bowel is preferable to mere diversion of the fecal stream. This may be done in one or more stages. Miller, Gardner and Ripstein (1949) showed that a one stage total colectomy, even in acute cases, resulted in a lower morbidity and rapid convalescence. This has gained wide support in other centers (Crile and Thomas, 1951; Goligher, 1953; Palumbo and Rugtiv, 1956).

Permanent abdominal ileostomy using modern surgical technique is no longer frought with the dangers and complications associated with this procedure two decades ago. These people gain weight, regain their health and with the aid of modern applicances and Ileostomy Associations made an acceptable social adjustment, including marriage, pregnancy and employment involving social contacts.

4. Ileo-rectal anastomosis: Despite the excellent

adjustments made by the "ileostomist," it is obvious that retention of fecal continence would be a far more acceptable situation. Because of this, many surgeons still feel that there is a place for preservation of the rectum. Aylett (1955 and 1957) is the foremost proponent of this approach and reports excellent results. Unfortunately, in the hands of others, (Corbett, 1953; Wangensteen, 1955), results are not as satisfactory. Also by leaving the rectum, one does not remove the threat of malignancy.

5. Other surgical procedures: Vagotomy (Eddy, 1951), pelvic autonomic neurectomy (Scott and Cantrell, 1949, Shafiroff and Hinton, 1950; Schlitt et al, 1951) and prefrontal lobotomy (Levy et al, 1956) have all been tried with indifferent results.

Familial Polyposis:

This is an hereditary disease transmitted by either parent as a dominant characteristic (McCorriston, 1957), affecting males and females alike and usually appearing at puberty or early adulthood. It may be silent or present with bloody diarrhea, abdominal pain and tenesmus and occasionally go on to malnutrition, anemia, etc.

Pathologically, the colon is studded with literally thousands of polypi which have an extremely great tendency to malignant degeneration. As this is a fairly rare disease, the incidence of malignancy varies greatly in the various small series reported. Hullsiek (1928) found carcinoma in only 36% of 127 cases he followed. Lewkowicz and Joseph (1956) state that almost 100% of the untreated patients over 30 years of age will develop carcinoma. It is unanimously agreed that because of the high rate of malignant degeneration, the presence of multiple polyposis is an absolute indication for surgery.

The methods of treatment are: Firstly, total colectomy with permanent abdominal ileostomy. This removes all potentially malignant tissue, but destroys the fecal continence mechanism. More popular today is colectomy with ileo-rectal anastomosis followed by periodic examination of the rectal stump and fulguration of any suspicious areas. Many authors report a high degree of success with this latter technique (Teicher and Abrahams, 1956; Schaffer, 1952, Everson and Allen, 1954; Colman and Eckert, 1956). However, success depends upon a high degree of patient co-operation.

Cole (1959) reports two patients in whom the rectal polyps gradually disappeared following colectomy with ileo-rectal anastomosis. Despite exhaustive testing, he could detect no biochemical changesin the regressing adenomata.

However, Ravitch and Sabiston (1947) and Lockhart-Mummery, et al (1956) report the occurrence of carcinoma in the rectal remnant despite careful periodic observation and fulguration.

One other method of treatment for ulcerative colitis and multiple polyposis should be discussed. Following experiments in dogs, Ravitch in 1948 reported a new surgical technique for anal ileostomy. With this procedure the entire diseased mucosa was removed and yet continence was maintained. Essentially it involved total colectomy followed by dissection of the rectal mucosa and submucosa away from the muscularis. The terminal ileum was then brought through the lumen of the muscular rectal wall to anastomose with the anus. Ravitch (1958) has done this in over 20 cases to date and reports excellent results in those patients with polyposis, but rather indifferent results in patients with ulcerative colitis. He attributes the latter to the emotional make-up of these patients, plus their debilitated condition at the time of surgery, combined frequently with a bowel which has suffered the many ravages of infection.

The early post-operative course of these patients is frequently most uncomfortable due to severe diarrhea, tenesmus and excoriation of the peri-anal skin. This, however, disappears in a few weeks after the terminal ileum dilates. The patients with good results have 3 to 4 bowel movements per day and lead a normal life.

Very little support has been given Ravitch's work in North America or in England. Best (1952); Goligher (1951); Shackelford (1955), among others, after trial, could not recommend this procedure. However, many surgeons writing in the South American and European Literature, report excellent results using this technique: (Roux et al, 1956; Devine and Webb, 1951; Mammoni, 1958; Lanzara, 1955; Cattan et al, 1952; Pesci and Sangiorgo, 1958).

CHAPTER II.

OBJECT OF THE EXPERIMENT.

In the preceding chapter, we have described two diseases, predominantly appearing in young people, requiring total colectomy and loss of the continence mechanism if the offending lesion is to be completely removed. Needless to say, a permanent ileostomy at any age is a crippling tragedy, but is especially so in a young individual.

The purpose of this experiment is to devise a method of eradicating all the diseased mucosa without interfering with continence. It is well known that an ileo-rectal anastomosis is compatible with normal bowel function. (Goligher, 1951; Best, 1952; Colman and Eckert, 1956). Thus we hoped to preserve the entire rectal musculature while denuding it of the diseased mucosal layer, which would then be replaced by a free graft of healthy small bowel mucosa. Then the rest of the diseased colon could be excised and the terminal ileum anastomosed to a functioning, relined rectum.

It has been demonstrated by other workers that bowel mucosa is transplantable (Maguire, et al, 1958), and will still retain its absorptive function (Neumann, 1959). We also know that the bowel musculature will support a graft of skin, (Keller, 1933), or bladder mucosa, (Martin, et al, 1959), and retain its motility. With this background knowledge, plus an understanding of the anatomical and physiological basis of the mechanism of fecal continence, it was hoped that this approach would be feasible.

CHAPTER III.

ANATOMY & HISTOLOGY

The anatomy of the human bowel is well-known and will not be discussed here. However, the intestine of the dog differs from that of man in certain important aspects which should be noted. (Miller, 1955).

Gross Anatomy:

In a medium sized animal (12 to 18 Kg.), the jejunum averages 3.5 meters in length, while the ileum is considered to be only 12 to 15 cm. long. Peyer's patches occur throughout the length of the small intestine, including duodenum, and are easily seen externally as flattened, ovoid projections to one or the other side of the anti-mesenteric border. They are more frequent at the distal end and, as in man, penetrate through the submucosal layer.

The colon is even more dissimilar. It is suspended from a mesentery throughout its entire length except for the rectum and cecum. The cecum is a kinked vermiform appendage marking the otherwise indistinct ileo-cecal junction. There is no appendix. Taeniae coli as such do not exist, for the outer longitudinal muscle layer completely encircles the colon as it does in the small bowel.

Miller describes a short ascending and transverse colon which may be reduced or absent. This writer's experience is that no ascending and only a very short transverse segment exists in most instances; therefore, almost all of the colon may be considered to be descending. This averages about 25 cm. in length. Because of the mesenteric attachments of the large bowel in the dog, the existence of a sigmoid region is purely arbitrary.

Miller considers the rectum to begin at the pelvic inlet; therefore, being about 10 cm. in length. However, if we use the anatomical criteria as applied to man, (Grant, 1958), the rectum begins where the large bowel loses its mesentery and is, therefore, only 3 to 4 cm. long in the dog.

Histology:

There is a marked paucity of literature on the histology of the dog's bowel. One can only assume a certain similarity to that of man except for the following known differences.

Dogs, as do all other carnivores, have three layers of muscularis in their small intestine. The third layer is a so-called oblique layer (Titkemeyer and Calhoun, 1955), lying between the circular muscle and the submucosa, and averaging 1/3 to 1/5 the thickness of the circular coat. It is demarcated by a pallisading layer of nuclei on its outer surface and is paler staining and more vacuolated than the other muscle coats. (Fig. 1).

Close inspection with high power microscopy (Fig. 2) reveals that the muscle fibres in this layer run in much the same direction as the circular fibres. The significant factor which differentiates this layer is the presence of many collagenous strands running from the submucosa, which interdigitate with the muscle fibres. This probably acts to anchor the muscle to the submucosa and suggests that the true nature of the so-called oblique layer may hitherto have been misunderstood by some authorities. (Trautmann and Fiebiger, 1949). However, for the sake of clarity, weshall continue to refer to this layer as oblique.

The muscularis mucosae in dogs is extremely

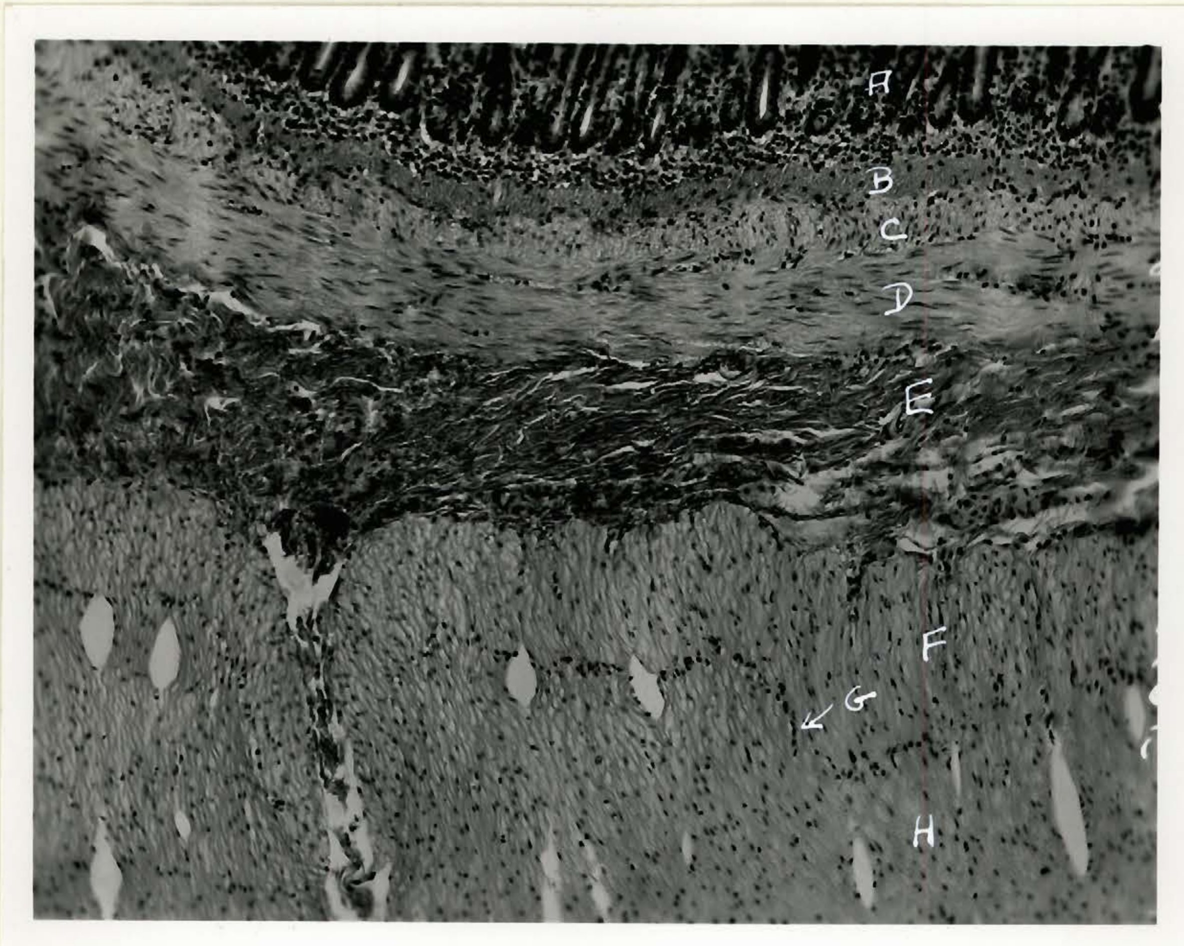

FIGURE 1.

Normal Dog Small Bowel (x 125)
A) Mucosa. B) Special connective tissue layer. C) Inner circular layer of muscularis mucosae. D) Outer longitudinal layer of muscularis mucosae. E) Submucosa. F) Oblique layer of muscularis. G) Pallisading layer of nuclei. H) Circular layer of muscularis.

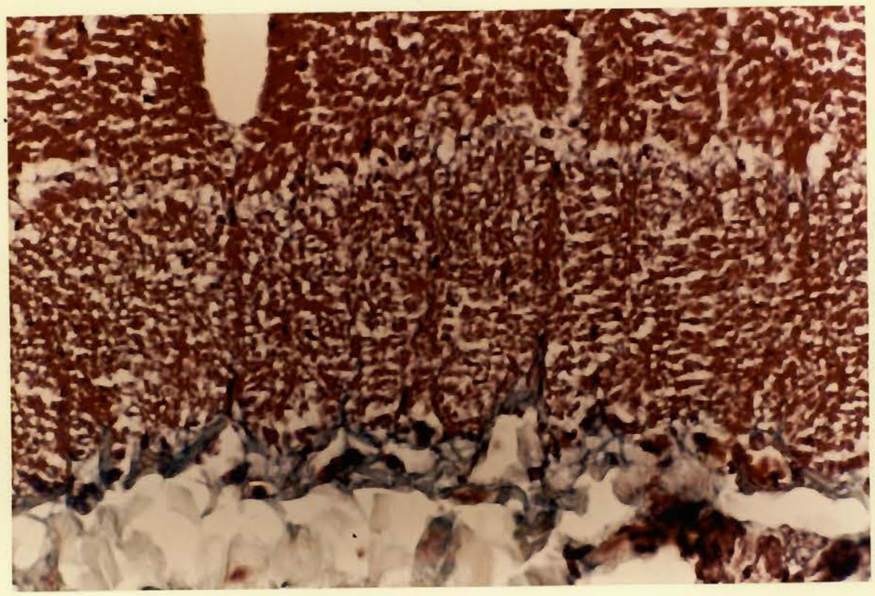

FIGURE 2.

"Oblique" layer of muscularis showing pallisading layer of nuclei and collagenous interdigitations. (Masson trichrome). (x 400).

thick, averaging 71 μ (Titkemeyer and Calhoun, 1955), and consisting of an inner circular and an outer longitudinal layer. The same authors demonstrated a special connective tissue layer, staining markedly eosinophilic, and averaging 30 μ in thickness, occurring between the muscularis mucosae and the crypts of Lieberkühn and parallel with the former. This layer does not occur in any other animal as far as is known and does not correspond to the ultra-thin basal membrane, described in man, which follows the convolutions of the mucosa.

Special staining techniques (Fig. 3) reveal that this layer is mainly collagenous, with only some elastic fibres. Under the high power microscope, connective tissue fibres are seen to run into the muscularis mucosae and out into the villi from this layer, suggesting that it functions as an anchoring mechanism. (Fig. 4).

Macrodissection:

Certain anatomical facts about the dissection of the layers of bowel wall should be considered: In the small bowel, the circular and longitudinal layers of muscle dissect off with ease. The so-called oblique layer is very

FIGURE 3.

Special connective layer - mainly collagenous; only small amounts of elastic tissue seen. (Elastic Van Gieson). (x 400).

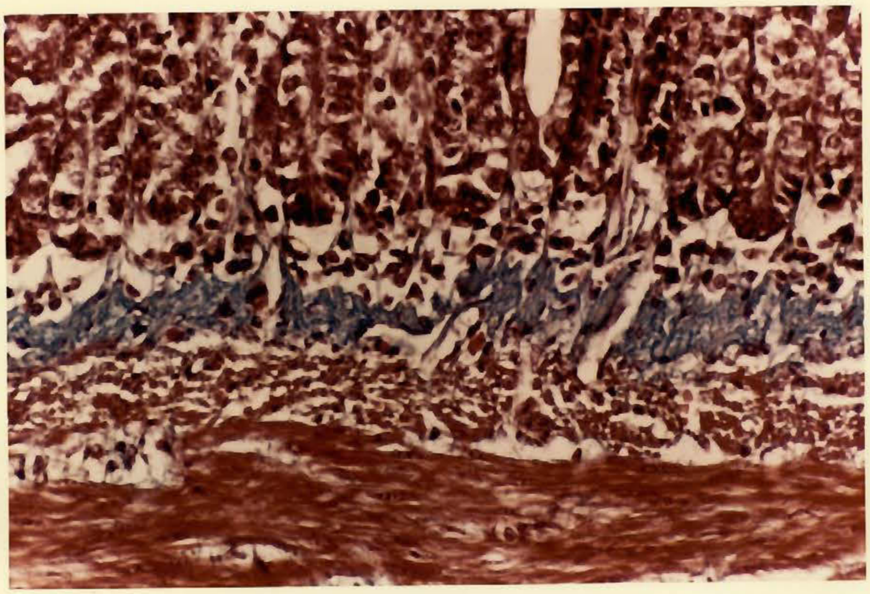

FIGURE 4.

Special connective tissue layer showing collagenous interdigitations. (Masson tri-chrome). (x 400).

difficult to separate from the submucosa except in small shreds (see Chapter VIII). It should be pointed out that, on dissection, this layer runs transversely to the long axis of the bowel in our specimens.

The submucosa, as in most other animals, provides almost all of the tensile strength of the bowel wall, a fact made use of by butchers in making sausage, and surgeons in suture material.

The muscularis mucosae, although relatively thick in the dog, does not dissect freely, but remains intimately attached to the submucosa. (Fig. 5).

The mucosa itself dissects away from the rest of the bowel quite cleanly, but it is extremely friable and has no more tensile strength than wet blotting paper. Therefore, removing this coat as a single sheet, without tears, requires a great deal of care. The cleavage plane is between the special connective tissue layer previously described and the muscularis mucosae, leaving a smooth shiny surface on the mucosal side. (Fig. 6).

Considerable individual variation was noted among various animals in the ease with which the mucosal

FIGURE 5.

Dissected Submucosal Layer (x 125)
A) Muscularis mucosae. B) Submucosa. C) Oblique layer of muscularis.

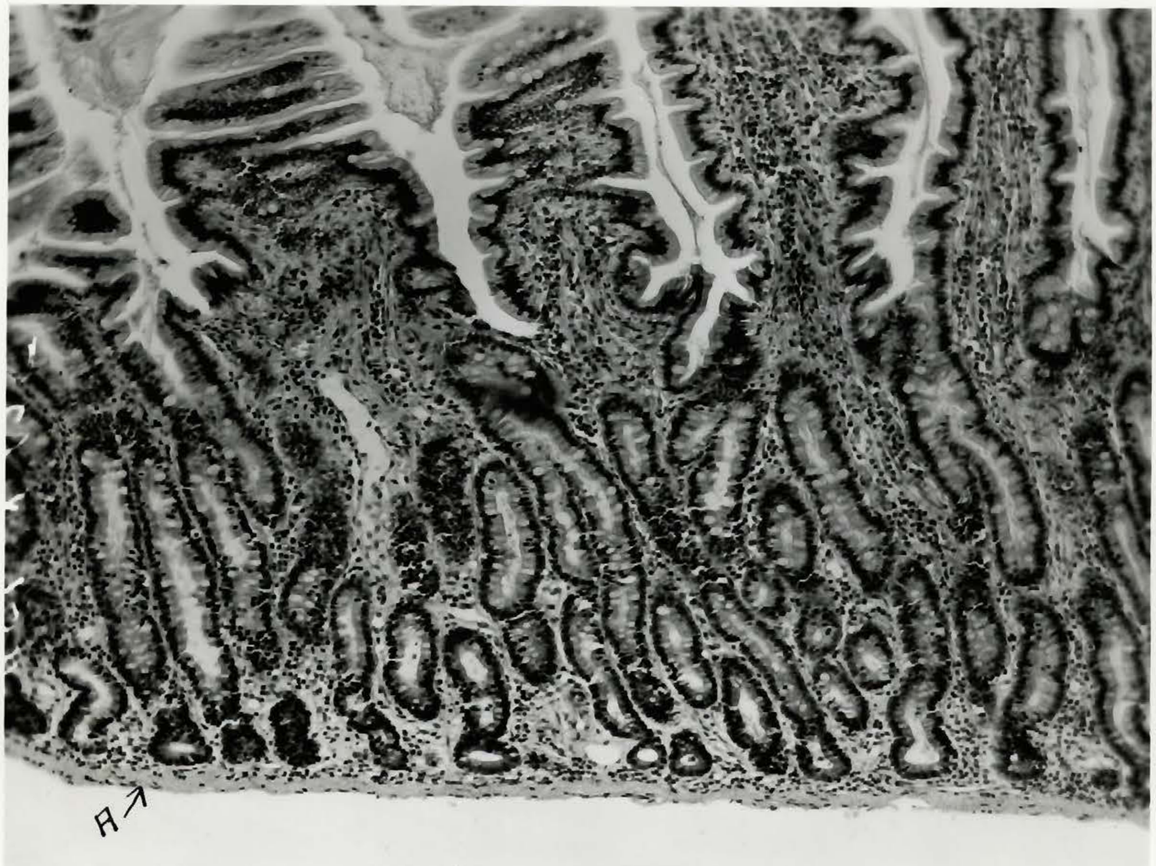

FIGURE 6.

Jejunal Mucosa (x 125)
A) Special connective tissue layer

layer was dissected. (See Chapter XI). The only dogs which we could predict with reasonable certainty to have easily dissectable bowel were those of predominately Spaniel characteristics. For this reason, we presume an individual or breed variation in the thickness or tensile strength of the connective tissue layer. A careful search of the literature and consultation with various authorities (Habel; Calhoun, 1959) reveals no information on this subject.

In the large bowel (Fig. 7) we are only concerned with eradication of the rectal mucosa; however, removing this layer alone without remnants is impractical. Ravitch and Sabiston in 1947 demonstrated that a cleavage plane could be developed between the submucosa and muscularis of the rectum with surprising ease, leaving no mucosal remnants. (Fig. 8). If care is taken, the muscularis, although friable, maintains its integrity.

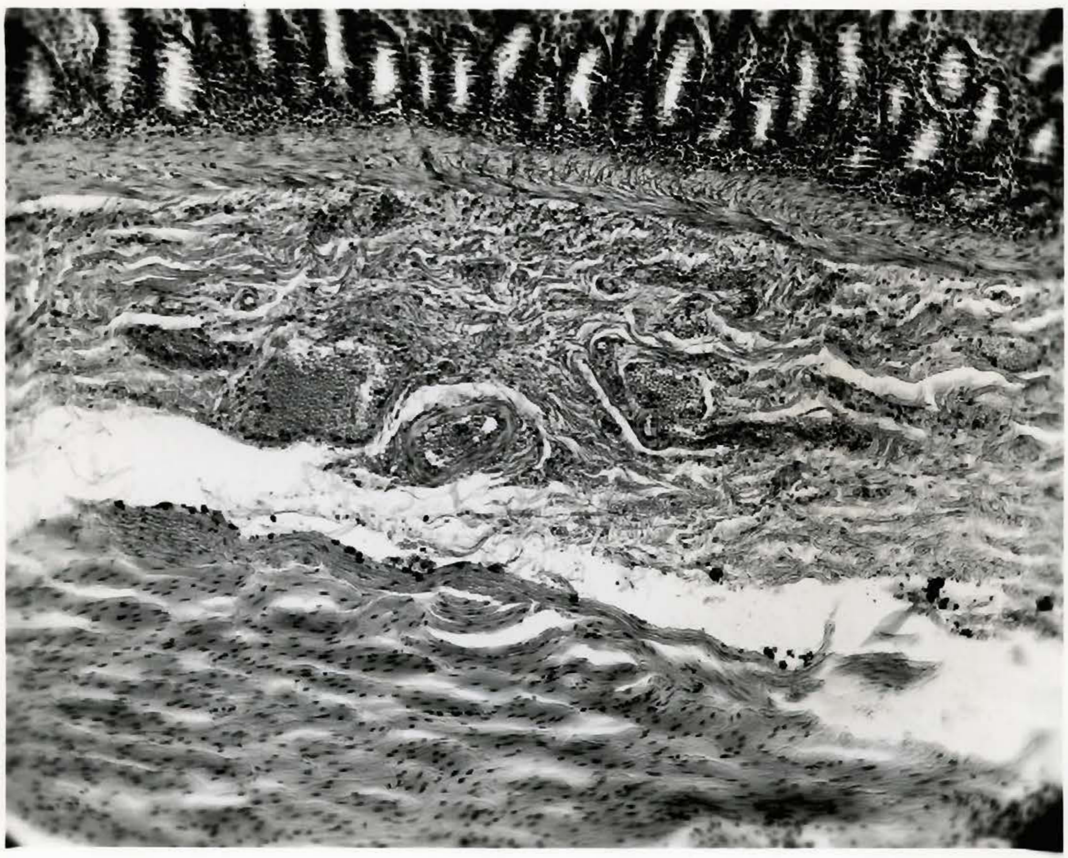

FIGURE 7.

Normal Dog. Colon (x90)

Although an artefact in this section, the space between the submucosa and muscularis demonstrates the cleavage plane of dissection.

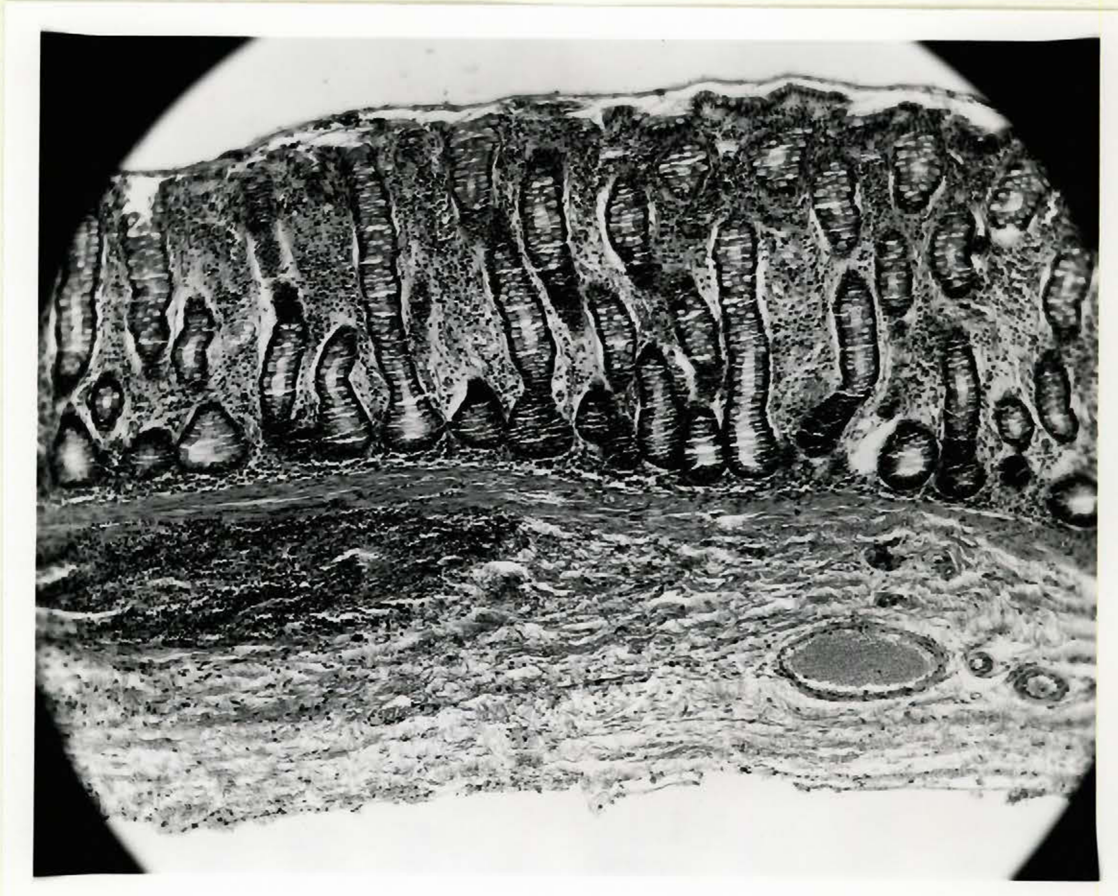

FIGURE 8.

Dissected Mucosal Submucosal Layer of Colon (x85).

CHAPTER IV.

PHYSIOLOGY OF THE FECAL CONTINENCE MECHANISM.

A great deal is as yet unknown about the mechanisms of fecal continence and much of our present evidence is contradictory. Most of the standard physiological and surgical textbooks, while otherwise complete, almost ignore this important function. However, certain basic facts are known.

Gaston in 1948 and 1951 reported his findings following experimental studies on the lower bowel. He concluded that fecal continence is subdivided into reservoir and sphincteric elements.

Reservoir continence:

The colon, especially the left half, acts as a storage organ for feces and adapts itself insensibly to the changing bulk of its contents. This is facilitated by the increased viscosity and reduced volume of the bowel contents after water absorption in the terminal ileum and right colon, (Perry, 1956), and growth of bacteria in the colon. Both of these functions depend on the relatively slow movement of the fecal stream. When sufficient mass is accumulated, or when stimulated by various reflexes (eg. gastro-colic) peristalsis propels the colonic contents into the rectum. This phase of continence is well demonstrated in a patient with a sigmoid colostomy, who with proper care, develops control of sorts for solid material.

Sphincteric Continence: This mechanism is not merely a purse-string phenomenon, but is a well co-ordinated ano-rectal reflex. By means of pressure measuring balloons, Gaston showed that the internal sphincter plays no part in continence as it relaxes as soon as the rectum is distended. This is a reflex mediated in the spinal cord, (Denny-Brown and Robertson, 1935). Indeed, the cutting of the internal sphincter produces no disability at all, (Goligher, 1951). The feces, on entering the rectum, produce the familiar lower pelvic sensation and stimulate an expulsive peristaltic movement. This initiates a reflex via the parasympathetic tracts, (Goligher, 1951), mediated in the brain and effected via the hemorrhoidal nerves to stimulate the external sphincter and resist the expulsive forces. The active contractions of the external sphincter soon diminish due to fatigue (usually in less than one minute) but as rectal peristalsis reaches its peak in a few seconds and quickly recedes, continence is preserved. Thus the external anal sphincter reflexly contracts beyond its normal tone in response to rectal peristalsis unless this reflex is consciously inhibited.

The receptor areas in the rectum were for many years considered to be in the mucosa as it was noted that local anesthetic agents blocked this sphincteric reflex, (Garry, 1933). However, the understanding that these agents have a direct paralytic effect on smooth muscle (Goodman and Gilman, 1955), plus subsequent proof by Karlan in 1959, confirmed by this present work, shows that the afferent impulses are initiated by "contraction" or "pressure" receptors (more accurate terms than "stretch" receptors) in the rectal muscularis. The activity of these receptors progressively decreases from the distal to the proximal rectum and is entirely absent in the sigmoid colon.

The mechanism of differentiation between solids and gases (liquids being poorly distinguished) is uncertain, but may be related to the different pressures exerted by each, (Goligher, 1951), or may be determined by the anal skin.

Clinical proof of these theories is offered by Gaston. He describes a patient who had a total resection of her rectum with a sigmoid-anal anastomosis. Although the woman could actively constrict her anus, she was unaware of the proper time to do so, and thus really had no more than a perineal colostomy with a functionally ineffective sphincter. Similarly, this explains how the elderly individual, whose measured voluntary anal contractile force is very low, retains his continence by a corresponding decrease of his rectal expulsive powers.

In contradiction to the above concept is the currently popular sphincter preserving operations for carcinoma of the rectum. Many authors, Waugh et al (1954); Best (1954); Hughes (1957); Mayo et al (1955) and Gerst and Seidenberg (1956), to name a few, report a high percentage of continence following removal of the entire rectum, and even anal skin, and anastomosis of the sigmoid to the anus. Up to 75% of these people, in large series, are claimed to retain the urge to defecate with functional sphincter control. (Waugh, et al, 1954).

Similarly, section of the pelvic parasympathetic fibres, as reported by various authors, (Scott and Cantrell, 1949; Shafiroff and Hinton, 1950; Schlitt, et al, 1951) for the treatment of ulcerative colitis, does not seem to interfere with the ano-rectal reflex. Indeed, in these series, continence is improved by the decrease in bowel motility.

Attempts have been made to determine the minimum amount of reservoir organ required to maintain function and the minimum amount of residual rectum necessary to preserve the ano-rectal reflex. Both of these probably vary with the segment involved, requiring necessarily greater length, the more proximally one chooses the segment to preserve.

Goligher in 1951 assessed 171 patients who had undergone various operative procedures and drew these conclusions: Seven to 8 cms. of retained distal rectum will give an invariably good reflex. His results with 6 cms. of rectum preserved were about 80% favorable and perhaps his failures were due to nerve damage caused by inversion of the rectal stump for a "pull-through" procedure. In proximal recto-sigmoidectomy for prolapse, however, only 1.75 cms. of rectal remnant gave 50% of the patients what was described as fair continence; the rest did poorly. These people, of course, originally had poor rectal tone.

Ileo-proctostomy gave invariably good results, but the ileo-anostomy described by Ravitch in 1948 gives variable results in different hands (See Chapter I).

Of course, in all procedures of the above type, a period of adjustment is required before continence returns.

Although it is fairly well established that the terminal 30 cms. of ileum will assume most of the water absorptive function of the excised colon (Wangensteen and Lillehei, 1955), it is a matter of conjecture whether the ileum will function as a reservoir organ in the absence of large bowel. Those who claim good results with Ravitch's procedure and its resultant dilatation of the small bowel must feel that ileum will substitute for colon. Swenson (1959) reports that in children with Hirschsprung's disease involving the entire colon and part of the terminal ileum, a total colectomy and rectosigmoidectomy followed by "...a pull-through type of anastomosis such as that used in the common form of congenital megacolon," is the treatment of choice. After a period of time in these patients "growth and development become normal." Swenson does not discuss the degree of continence achieved, but states only that his series of 5 patients "have done well." By this description these patients would be left with 2 to 3 cm. of rectum, (Swenson, et al, 1951), which would be a considerable length at the age when this procedure is usually done. The fact that these patients are children may also explain why results are better than might be expected in adults.

Various techniques have been devised to construct a reservoir from the small bowel. Valiente and Bacon in 1955 experimented on dogs with a "pantaloon technique" of side to side anastomosis of an 'S'-shaped segment of terminal ileum, interposed between the anus and small bowel, with equivocal results. Gilbertas and Blanchard in 1957 constructed a pouch from a loop of ileum with a side to side anastomosis and reported fair results in one case. Karlan, et al in 1959 repeated Valiente's experiment, but left a 4 cm. denuded segment of rectum. He found that this functioned poorly. A side to side pouch of iso-peristaltically aligned ileum gave excellent results in the dog, if the rectal cuff was preserved; however, when attempted on one patient, the results were equivocal.

It may be of interest to note here that all of Karlan's preparations which received a simple ileo-anostomy died of inanition after a few weeks, whereas if only 4 cm. of rectum, even denuded of mucosa, was left, the animals survived.

It is apparent from the contradictory evidence presented that complete understanding of the continence mechanism is yet to be worked out. Gaston's observations on continence appear to be valid for physiological situations, however, what is yet uncertain is the extent to which the body is able to adjust to or substitute for these mechanisms in pathological or abnormal situations.

CHAPTER V.

PHYSIOLOGY OF MUCOSAL REPAIR WITHIN THE BOWEL.

Most investigations into the regeneration of bowel mucosa have been confined to the stomach and duodenum. This is probably due to the interest stimulated in this region by the frequent occurrence of peptic ulceration in man. However, recently, more interest has been developed in the repair processes occurring in ulcerations of the small and large bowel. Of necessity, most of this work has been done on experimental animals and because of the anatomical and physiological differences among various species, great care should be taken before correlating these results with those of man.

For example, Longmire et al (1952) showed that cicatrization was far more marked in the stomach of a cat from which the mucosa had been stripped than in the stomach of a similarly treated dog. The cleavage plane developed for stripping the mucosa from the dog's stomach left the bases of many small crypts from which re-epithelialization occurred. In the cat, no epithelial remnants were left behind; therefore, all repair must have been from the periphery. This , of course, would be a much slower process and explains the high degree of scarring observed in the cat's stomach. In man, stripping the stomach's mucosa would leave a base similar to that demonstrated in the cat. Thus, any results obtained through experimentation on the dog in this particular situation, would be erroneous if applied to man.

Longmire et al (1952) working with cat and dog stomachs, Florey (1954) using cat duodenums and McMinn and Johnston (1958) experimenting with the rectums of cats, have reached similar conclusions about the basic patterns of mucosal repair. If the mucosa only is damaged and the muscularis mucosae remains intact (erosion), then repair is rapid via regeneration from the sides and from remnants of glandular epithelium that penetrate through the muscularis mucosae. This results in normal appearing bowel after healing is completed. However, if the m uscularis mucosae is destroyed (ulceration), then only the mucosa regenerates and the resultant healed area will be devoid of muscularis mucosae.

The healing mucosa grows over the granulating surface of the injured areas as a layer of flattened epithelial cells. Mitosis is evident several cells outward from the growing edge rather than in the marginal cells. Gradually, the cells become more cuboidal and the mucosa begins to form villi and crypts until a normal pattern is established. The muscular coat contracts in response to the initial trauma, thus greatly minimizing the area that requires bridging by epithelium.

Lumb and Protheroe (1955), who studied mucosal repair in the human colon, described a similar process of epithelialization. However, they were not able to follow their patients for sufficiently long periods to determine whether crypt and villi formation occurred.

Hightower (1958) and O'Conner (1956), using cats and rats respectively, as experimental animals, could not demonstrate complete re-epithelialization of large colonic or rectal ulcers. Both authors noted marked shrinkage of the ulcers secondary to muscular and later cicatricial contraction. Neither could demonstrate a full epithelial cover over these granulating areas. Hightower, however, described "flattened epithelial-like" surface cells which supported the fecal stream. It is most likely that this layer was true epithelium which had not yet thickened to cuboidal form. Fig. 35 demonstrates regenerating epithelium in the colon which shows these flattened cells becoming cuboidal and tending to form into villi.

The rate of repair is very difficult to assess. Leblond and Stevens (1948) and Leblond and Messier (1958) showed that the entire mucosal surface of the stomach and small bowel in both the albino rat and the mouse was replaced in 1 to 3 days. The actively dividing cells in the base of the crypts apparently slide less active cells towards the tips of the villi where they are shed into the bowel lumen. This suggests that under ideal circumstances epithelialization of a denuded surface could occur very rapidly. However, individual variations in intestinal flora and fauna, trauma from fecal contents, foreign material (sutures, etc.), and many systemic abnormalities (anemia, malnutrition, etc) could all act to delay and even prevent healing. This could well explain the results obtained by Hightower and O'Connor previously described.

CHAPTER VI.

HISTORICAL REVIEW OF FREE AUTOGRAFTING PROCEDURES.

Man's earliest experience with free autografting is generally attributed to the early Hindu Tilemaker caste. They used well beaten buttock skin and subcutaneous tissue in the reconstruction of noses, which were amputated as punishment for certain offenses. However, the dating of this procedure is somewhat confused. (Rogers, 1959). Freeman (1912) and Davis (1919) attribute this to Sushruta, the father of surgery in India, who described the reconstruction of lost noses by pedicle flaps in the famous Samhita (circa 750-800 B.C.). A careful search of the English translation of the Sushruta Samhita (1907-1916) reveals no reference to free skin grafting. The first documented case discovered to date of this practice was that of Leroux in 1817.

The earliest record in the medical literature of free autografting is an interesting report by Sancassini in 1731 about Gambacurta, a woman charlatan in Florence. She dramatically advertised the miraculous powers of her healing balm by cutting a piece of "flesh" from her thigh, passing it around to her audience on a plate and then replacing it, following which it "united so well that the following evening it needed no further medication."

Although preceeded by others, (Bünger, 1823), (Warren, 1840, and Pancoast, 1844), Reverdin in 1869 independently conceived of and popularized free skin transplants in the form of pinch grafts. Later, Ollier (1872) and Thiersch (1886) described the split thickness graft, and Wolfe (1875) and Krause (1893) popularized the full thickness graft. Many surgeons have since modified and improved upon skin grafting techniques until our present highly successful methods were evolved.

Thus, until relatively recent years, free auto-grafting procedures remained confined to the skin. Today, of course, the autogenous transplantation of many tissues and organs is a routine part of our surgical armamentarium.

The history of transplanting mucosal surfaces (excluding oral mucosa) is relatively brief. Huggins (1931) and Copher (1938) transplanted bladder mucosa experimentally to study its unusual osteogenic properties. Marshall and Spellman (1955) used autogenous bladder epithelium to line the reconstructed urethra in hypospadias. Rush and Cliffton (1956) experimented with the same material to reline tracheal defects.

Gilbert et al (1958) used uro-epithelium to resurface isolated segments of small and large bowel, which had been denuded of mucosa, to produce a non-absorptive lining for an artificial bladder. Martin et al (1959) used a similar technique to construct artificial ureters.

Free grafts of uro-epithelium present no special problems in handling because of the adequate tensile strength of this tissue. The relatively few cell layers and uncomplicated structure of this material allow ample tissue fluid perfusion so that a high graft survival rate is possible.

Only three reports exist of free autotransplantation of bowel mucosa. Lippman and Longmire (1954) used mucosa-submucosal autografts of jejunum to reline the stomachs of dogs from which the acid producing mucosa had been removed. They had very little success, although they did demonstrate some graft survival. The complexity of the graft tissue plus the unfortunate choice of experimental animal probably contributed greatly to their failure (See Chapter V).

Maguire et al (1958) free grafted ileal mucosa to the chest wall of dogs in an attempt to obtain metaplasia and produce a substitute for skin,to be used for extensive burns. They had a high percentage of graft survival even when the mucosa was emulsified in a waring blender and spread on like peanut butter. They observed the gross changes in this tissue over a three month period and described the following sequence of events:

At 1 week the graft had a slick grey cobbled appearance. At 1 month it was smooth and pink and had contracted to 1/3 its original size. At 2 months a deep red, velvety appearance was noted and mucus was secreted. At 3 months shrinkage stabilized at 1/5 the original size. No metaplasia was noted. The same authors successfully transplanted gastric mucosa to the inguinal region of a human volunteer. After 6 months a similar degree of shrinkage was noted.

Neumann (1959) also transplanted ileal mucosa to the chest wall of the dog. He was able to show that protein, fats, carbohydrates and water were all absorbed by this transplanted tissue.

Only two references were found that might be considered free grafting of skin within the lumen of the large bowel. Keller (1933) used buried tubular skin grafts which were later opened out to repair anal strictures. Hinze (1957) described a case in which split thickness skin was used to line the cavity formed by necrosis and slough of the terminal segment of sigmoid colon after a pull through procedure.

Skin is too susceptible to irritation by bowel contents to be seriously considered as a lining for any area of the bowel or stomach.

CHAPTER VII.

PROCEDURE NO. 1 - RATIONALE

Two major technical problems present themselves. These are: Firstly, complete removal of the rectal mucosa without leaving any remnants, and, secondly, preparation of a suitable graft of small bowel mucosa.

Ravitch and Sabiston, in 1947, showed that an almost bloodless cleavage plane may be easily developed between the submucosa and muscularis of the rectum. This leaves a minimally traumatized muscular rectal wall which retains its innervation through Auerbach's plexus. Removing the submucosa ensures complete eradication of all mucosa.

Because of the extreme friability of the small bowel mucosa, which is barely able to support its own continuity, the mechanical problem of handling the graft material and fixing it in its bed, presents considerably difficulties. By leaving the mucosa attached to its strong submucosal base and using this more complex tissue as a graft material, the technical aspects of the problem were considerably simplified.

CHAPTER VIII.

PROCEDURE NO. 1 - MATERIALS & METHODS

Materials:

The only special material used in this procedure was a semi-rigid polyethylene tube used to stent the graft against the rectal wall. This was constructed from polyethylene film using a method described by Grindlay in 1948. Essentially, this involves tightly wrapping a layer of polyethylene sheet, sandwiched between two layers of cellophane, onto a lubricated glass or metal tube. The whole is then heated over a bunsen burner (protected by an asbestos wire gauze on a tripod) until the polyethylene turns from milky white to transparent. The heat fuses the polyethylene sheet into a solid walled tube and the lubricant allows the withdrawal of the form. The cellophane, which has prevented bubbling and burning of the polyethylene, easily peels off leaving a smooth walled, semi-rigid tube of inert material. The cellophane used was graded as P. T. 300 and the polyethylene was 0.102 mm. film. Wrapping a 40 cm. length of plastic about a metal tube of 1.4 cm. in diameter resulted in a plastic tube of 1.6 cm. outside diameter with walls of 1 mm. thickness. This technique varies from Grindley's only in the use of vaseline as a lubricant instead of soap, and a strip of "Scotch" tape, in lieu of cotton ties, to keep the outer cellophane layer from unravelling.

Methods:

Pre-operative preparation: Male and female mongrel dogs of medium size were chosen at random from the kennel population. Routinely, combined anti-canine distemper, anti-infectious hepatitis and anti-leptospira conicola sera, plus canine distemper vaccine were administered.

Bowel preparation consisted of:

Food withheld	48 hrs. pre-op.
Phenolphthalein, 1 1/2 grains	36 " "
Neomycin, 1 gram	36 " "
Neomycin, 1 gram	18 " "

Bowel preparation, cont:

Water withheld	12 hrs. Pre-op.
"Fleet" enema	" " "

A point was made of letting the dogs out of their pens at least 1/2 hour before administration of the anesthetic to ensure bowel and bladder emptying.

Anesthesia was established by means of intravenous "Nembutal." An endotracheal tube was inserted and a respirator attached. The dogs were shaved and placed on the operating table in such a way as to provide access to the abdomen and rectum. Skin preparation consisted of "Pro-soap" and tincture of iodine.

Operative procedure: A mid-line, lower abdominal incision was used exclusively. In males, this necessitated carrying the skin incision laterally around the penis and returning to the midline in the subcutaneous layers.

The entire colon and cecum were mobilized by dividing their blood supply. Sufficient terminal ileum was mobilized to provide more than enough length to reach the perineum, great care being taken to preserve the anastomotic arcades (Fig. 9). One-half gram of streptomycin and 400,000 units of penicillin were dusted into the peritoneal cavity and the abdominal wall was closed in layers.

Approaching through the anus, an incision was made circularly about the muco-cutaneous junction (Fig. 10 A). By dissecting in the proper plane, a tube of mucosa and submucosa was developed. (Fig. 10 B). By applying traction on this tube, the muscularis of the rectum was prolapsed through the anus and the mucosa-submucosal layer was peeled off with surprising ease. Very little bleeding was encountered. After the desired length was dissected, the muscularis was cut through and the marginal vessels ligated, thus entering the peritoneal cavity. (Fig. 10C). The entire colon was then delivered, followed by the terminal ileum, and the colon was amputated. (Fig.10D). The procedure thus far described is similar to that devised by Ravitch and Sabiston in 1947 as part of the technique for perineal ileostomy.

After inserting the semi-rigid polyethylene stent into the lumen of the terminal ileum, taking care not to abrade the mucosa, the proximal end of the colonic musculature was anastomosed to the terminal ileum over the

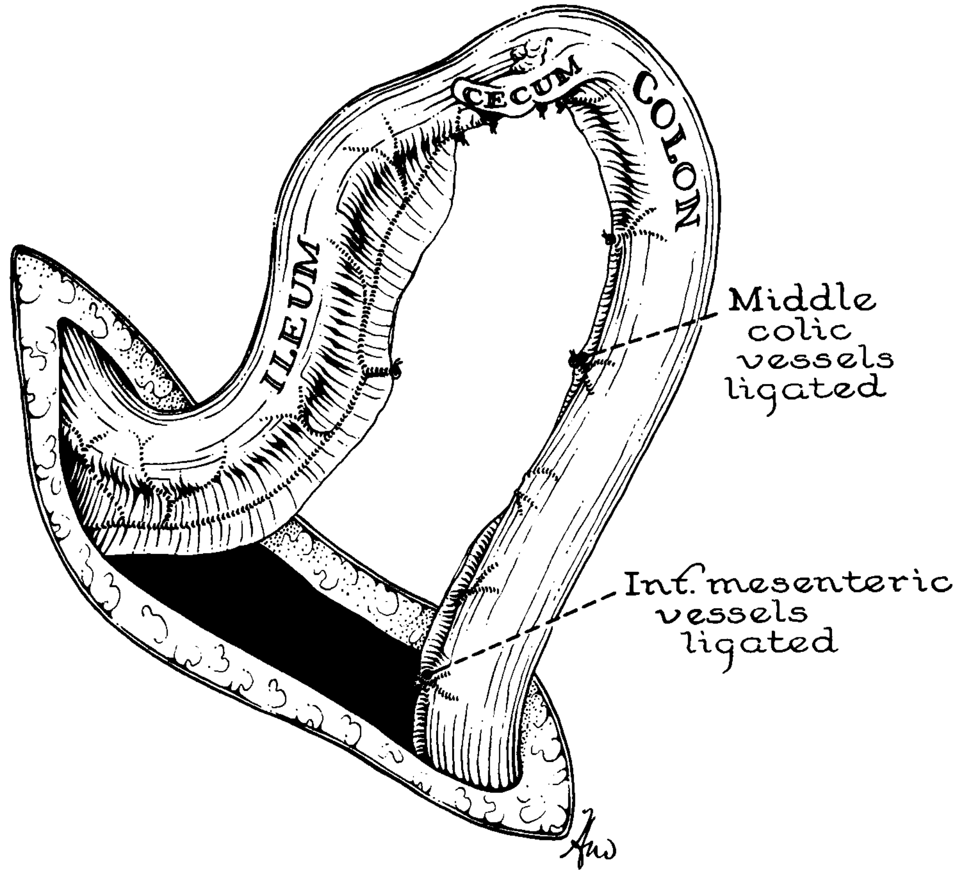

FIGURE 9.

Procedure No. 1.

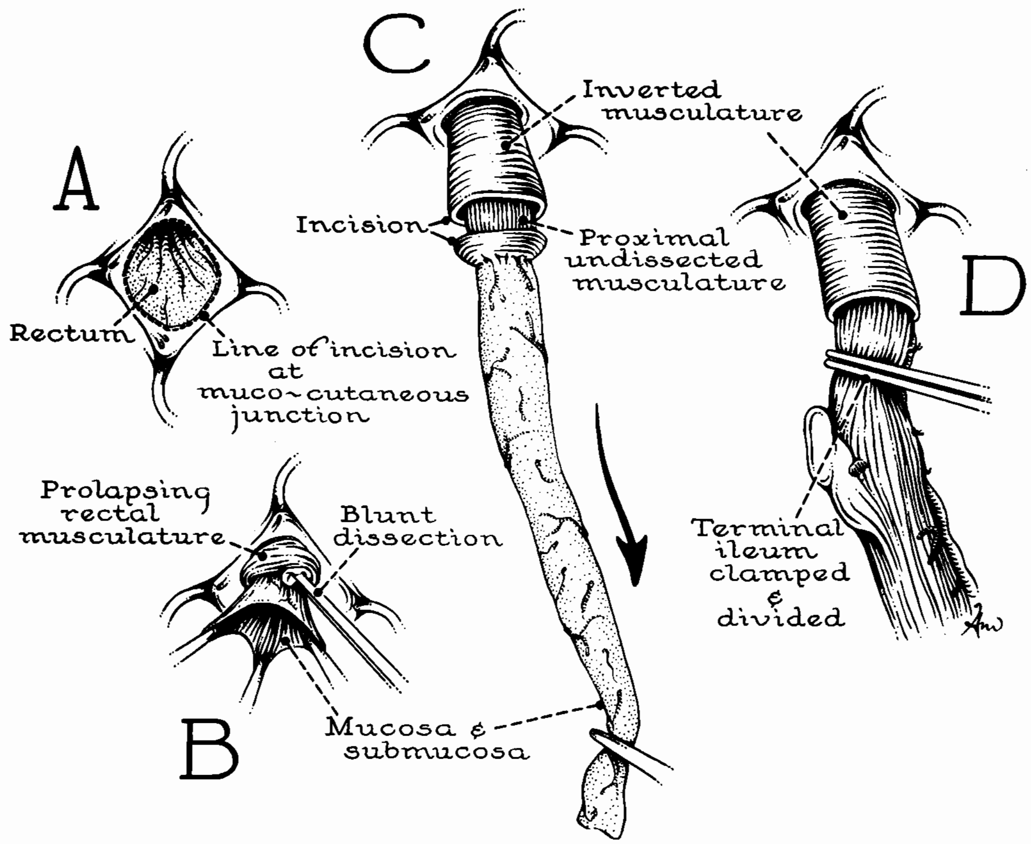

FIGURE 10.

Procedure No. 1 (cont)

proximal end of the tube. (Fig. 11 A). A sero-muscular layer of 4-0 atraumatic silk, followed by a through and through continuous layer of 2-0 chromic intestinal surgical gut was used as modified from Swenson et al (1951), (Fig. 11B). The ileum distal to the anastomosis was then denuded of serosa and muscularis, ligating the mesenteric vessels at the site of the anastomosis (Fig. 11C). The innermost layer of muscle fibres came away incompletely so that remnants required individual removal. A 2-0 gut stay suture was then placed through the proximal end of the stent and graft (mucosa and submucosa) to prevent the plastic tube from riding proximally.

The inverted rectum was then replaced and the graft and stent were amputated to proper length. The distal ends of the latter were sutured together with a continuous 2-0 chromic gut suture (Fig. 11 D). Three loose gut sutures were placed to approximate the end of the tube and anal skin, but still allow for drainage. (Fig. 11E).

Thus the dissection of the rectal mucosa has been accomplished and the musculature has been relined with ileal mucosa,backed by its supportive submucosal layer,

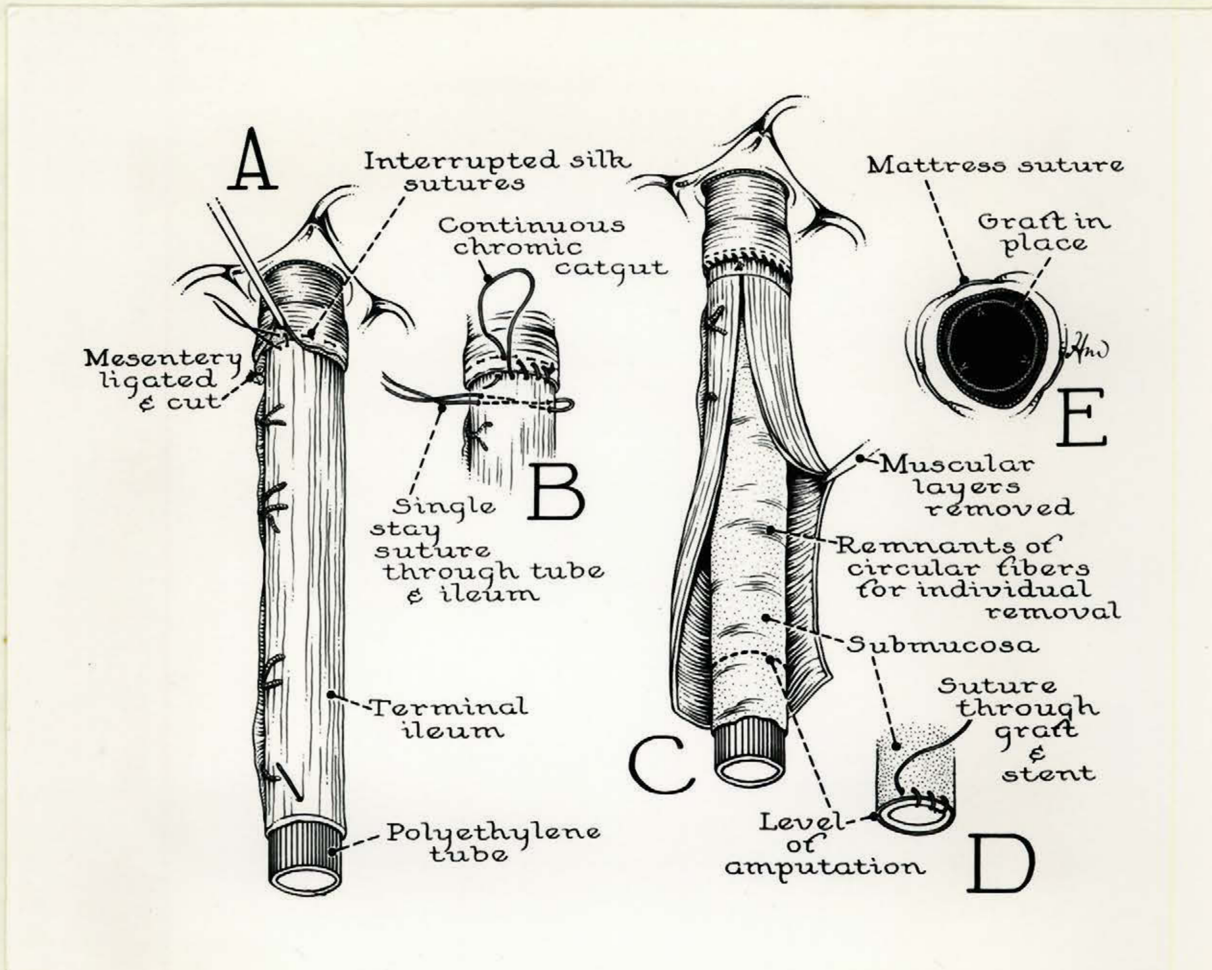

FIGURE 11.

Procedure No. 1 (Cont).

as a free graft. (Fig. 12). That is, the graft is devoid of blood supply despite its continuity with the normal small bowel.

Post-operative care: Skim milk was allowed on the first post-operative day, followed by progressively more solid foods until normal house diet was given on the fifth day. A penicillin (400,000 μ) streptomycin (1/2 gram) combination was given daily for a minimum of 3 days or until the plastic tube was spontaneously passed.

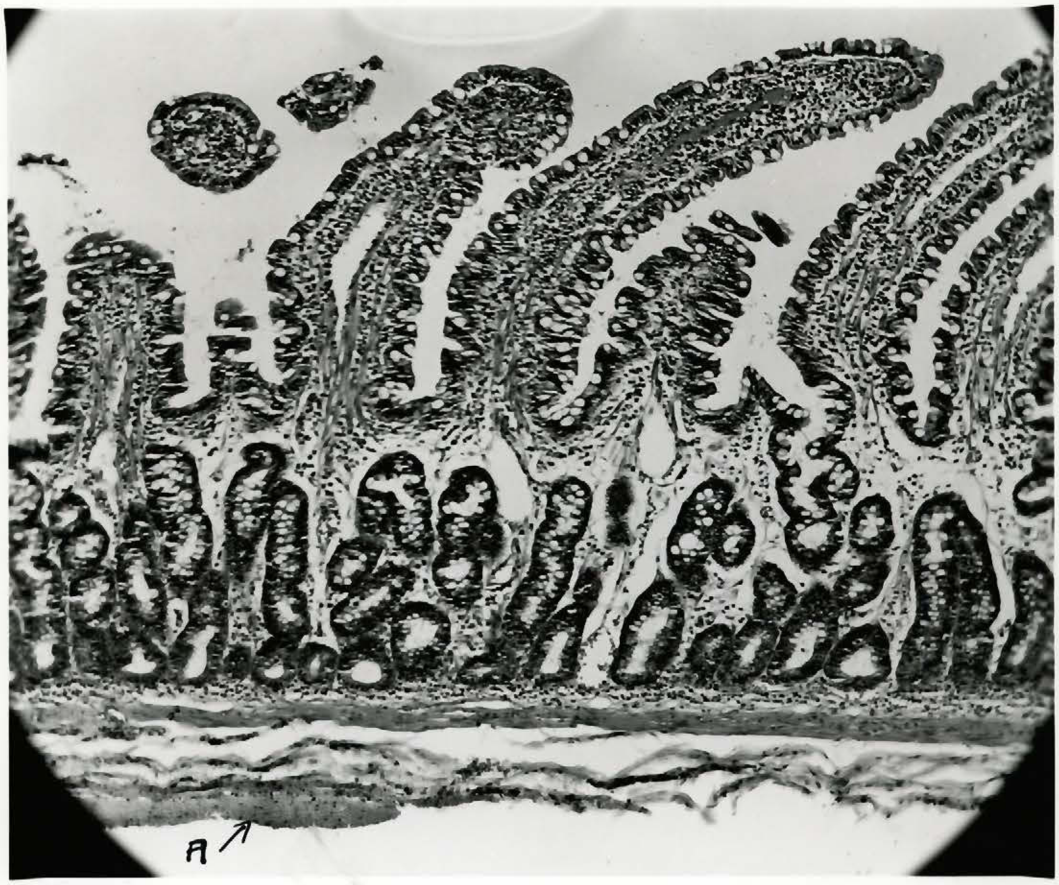

FIGURE 12.

Mucosal submucosal graft from terminal ileum. A) Remnant of oblique layer of muscularis. (x 100).

CHAPTER IX.

PROCEDURE NO. 1 - RESULTS

Fifteen animals were used in this series of which only 5 survived. The causes of death were as follows:

Peritonitis	3
Bowel obstruction	6
Due to: Pelvic abscess	2
Intessusception	2
Stricture	1
Peri-rectal hematoma	1
Grangrenous terminal ileum	1

The 5 survivors passed their plastic tubes spontaneously 1 to 4 days post-operatively. Two weeks after operation, sigmoidoscopy was done under light pentothal anesthesia. At the same time, X-rays were taken following barium enema.

In every case, a red-velvety rectal wall was observed which was seen to contract actively. Microscopic sections later proved that in some animals this normal appearing rectum represented true graft take, while in others, only granulation tissue could be demonstrated.

At first the stools were frequent, small and liquid. The animals were all aware of the defecatory urge as evidenced by their stance and active expulsive efforts. Indeed their actions in the early weeks were strongly suggestive of tenesmus.

Excoriation about the anus did occur, but was not severe. This was minimized by the animal keeping the area clean with his tongue. However, the frequent loose bowel movements soiled the animals' coats considerably in the early weeks. Although appetites remained good throughout the post-operative period, weight loss occurred over the first 6 weeks in every animal. This was partially regained before sacrifice. The dogs were highly susceptible to respiratory infections and episodes of bloody diarrhae both of which responded to antibiotic agents. Decubitus ulcers were common over the greater trochanters of the femura during the period of maximum weight loss. Lethargy in the early weeks was also noted.

Just before sacrifice, stools were semi-formed and copious, 8 to 10 being passed per day. Excoriation was much improved and the animals were generally cleaner and less lethargic.

The animals were sacrificed between 3 and 6 months post-operatively, after barium enema röntgenograms were repeated. Fig. 13 demonstrates the typical X-ray picture. No strictures or dilatation of the small bowel was noted.

Originally, the grafted segments varied from 7 to 11 cms. After sacrifice, the segment proximal to the peritoneal reflection of the pelvis was contracted so as to be unidentifiable. This left about 4 cms. of rectum in every case. Apparently shrinkage occurred only where the bowel was unsupported by surrounding tissue.

In 8 of the group which died of complications, no evidence of graft survival was noted. Two animals, which lived 1 and 2 weeks before succumbing to infection, showed 80% and 60% graft take, respectively.

FIGURE 13.

Barium X-ray Study of The Colon (6 months post-operatively).
A) Anus. B) Ileo-rectal anastomosis. Note no dilatation of small bowel.

Of the survivors, 2 showed full graft take and the other 3 demonstrated only small areas of surviving graft tissue. Although some epithelium regenerated from the ileum, these latter 3 dogs lived and thrived with their rectums lined mainly with granulation tissue. The rectums retained their sensory and motor functions as well. Figs. 14 and 15 demonstrate graft survival in these animals.

FIGURE 14.

Longitudinal section through ileo-rectal anastomosis showing loss of convolutions of mucosa in grafted region. (3 months post-operatively). A) Ileum. B) Grafted rectum. C) Suture. (x 4)

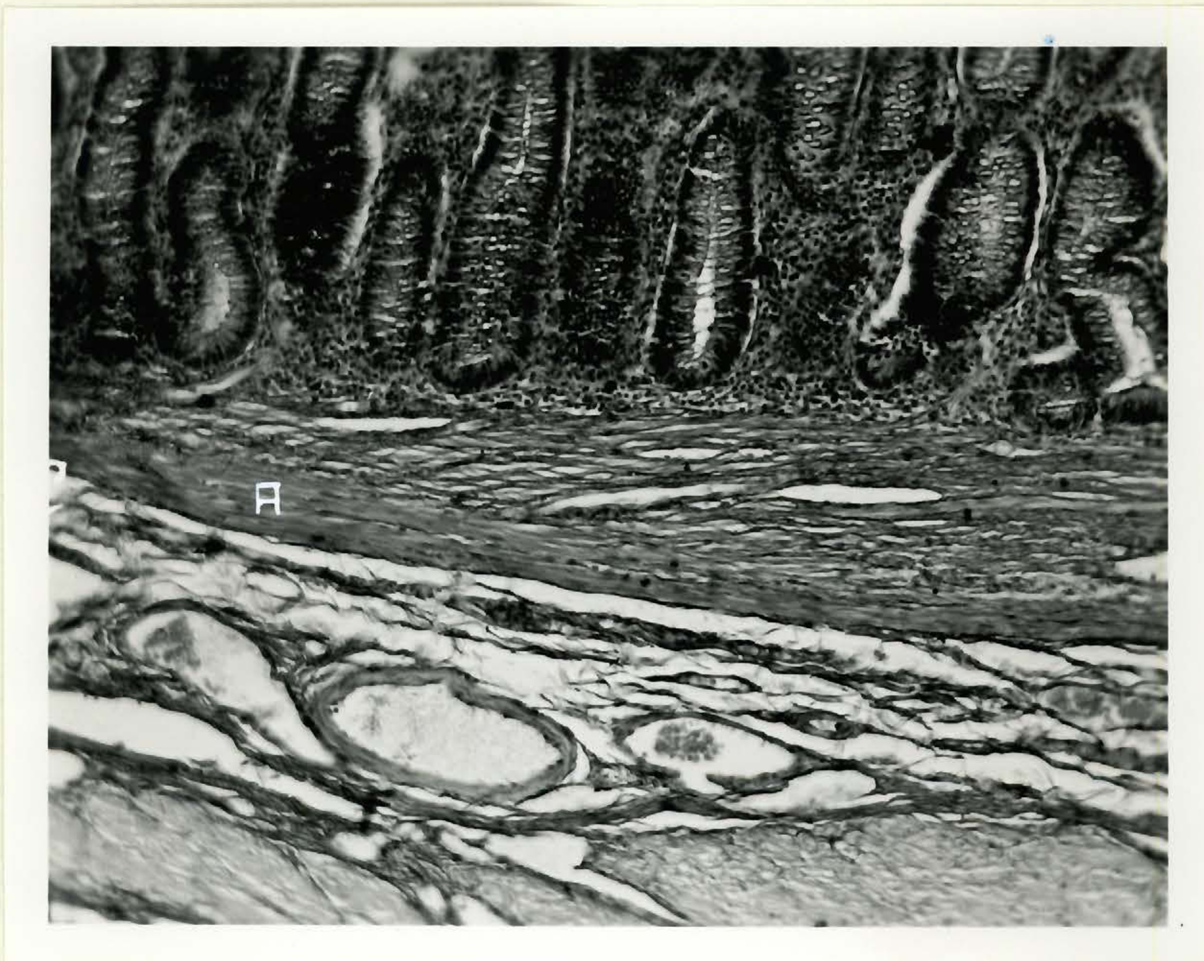

FIGURE 15.

View of grafted region. A) Surviving muscularis mucosae of graft. (x 125).

CHAPTER X.

PROCEDURE NO. 2 - RATIONALE

Because of the high mortality and relatively poor results of Procedure No. 1, an attempt was made to analyse and correct its faults. The following factors were considered to contribute to the poor results.

Dogs are known to tolerate ileostomy poorly (Ravitch, 1947). In the early post-operative period, before the graft has taken, these animals physiologically had an ileostomy. Only later, after the graft had taken and the mucosa began to function, could the dogs be considered to have an ileoproctostomy. Certainly, the poor nutrition and electrolyte disturbances attendant with an ileostomy did not favor graft survival, let alone the survival of the animal itself.

Secondly, the graft, with its precarious viability, was necessarily exposed to the fecal stream from the very beginning.

Thirdly, the complexity and thickness of the graft, consisting of mucosa and submucosa, considerably diminishes its chances of survival. One can well imagine how difficult it would be for the nutrient tissue fluids to penetrate the dense submucosa to support the surface epithelium.

To avoid these pitfalls, a technique was devised to handle the extremely friable mucosa and control it until well vascularized and firmly adherent to the muscle wall. To remove the hazards of ileostomy a defunctioning colostomy was done proximal to the grafted area. This also eliminated the problem of fecal contamination of the unrooted graft. After the graft had matured somewhat, the ileum was anastomosed to the relined large bowel.

CHAPTER XI.

PROCEDURE NO. 2 - MATERIALS & METHODS

Materials

Several pieces of equipment were specially designed to facilitate this procedure. A polyethylene tube of the same dimensions as described in Procedure No. 1 was similarly constructed. A set of 6 metal plates, each with 3 parallel rows of 4 needle pointed spikes were designed. The plates were 4 cm. long by 1 cm. wide and curved longitudinally to match the curvature of the polyethylene tube. These spikes were 3.5 mm. high and were placed parallel to each other; that is, only the center row was perpendicular to the curved plate, the outer rows being angled with the plate so as to parallel the center row. Figs. 17 and 23E). Thus the plates could be inserted in the polyethylene tube and using a specially fitted driver the spikes forced through the wall of the tube, facilitated by their parallel direction.

Two other polyethylene tubes were constructed in the manner previously described. One was of 1.2 cm. outside diameter to insert down the center of the first tube described, thus holding the spiked plates in place. (Figs. 20 and 23 E). This will be referred to as the central tube. The third tube was of 2.2 cm. inside diameter and was large enough to allow the first tube with its protruding spikes to pass easily through the lumen. (Figs. 20 and 28 B). This large tube will be referred to as the inserter tube.

Other apparatus used was an obturator for the inserter tube (Fig. 18), and for the middle sized polyethylene tube. A glass test tube (15 x 150 mm. lipless) was used for the latter (Fig. 16).

Originally, a blunt mucosal elevator was used for dissection, but subsequently, a specially designed dissector as shown in Fig. 21 was used. Fig. 21 also shows the wire loop stripper used. Photographs of all this equipment and their sequence of use are seen in Figs. 16 to 21.

Stage No. 1:

The animals were chosen, prepared and anesthetized as in Procedure No. 1. Using a similar mid-

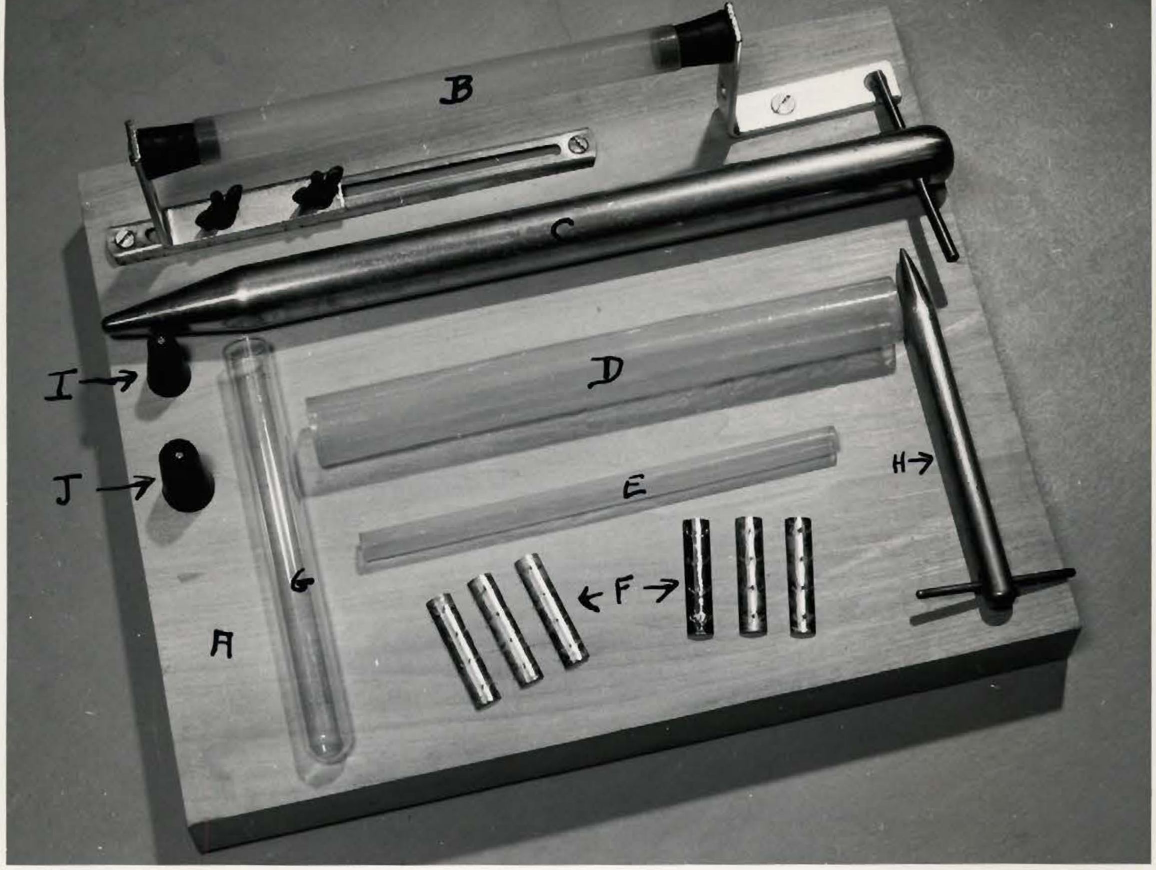

FIGURE 16.

A) Dissection stand. B) Polyethylene stent. C) Obturator for inserter tube. D) Inserter tube. E) Central tube. F) Spiked plates. G) Obturator for stent (test tube). H) Driver. I & J) Supporting corks to hold stent and graft while spiked plates are being driven.

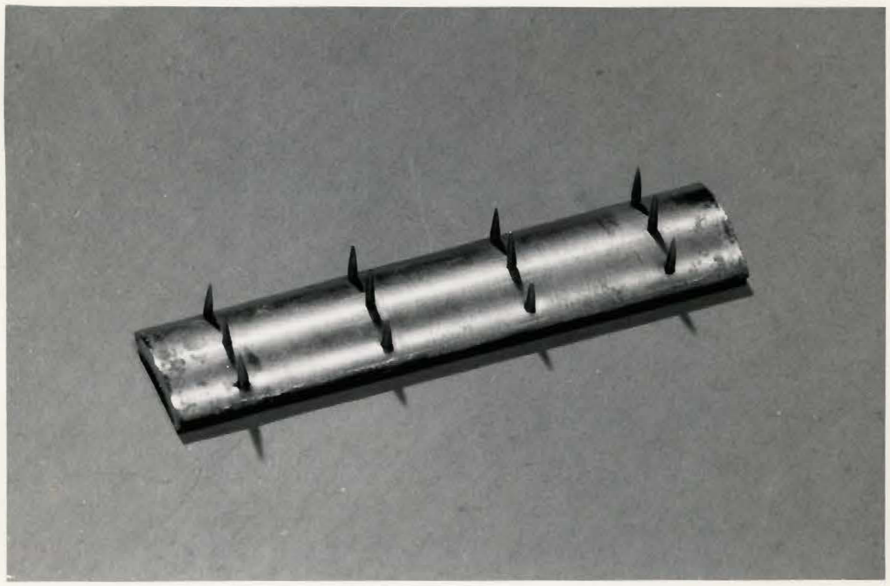

FIGURE 17.

Spiked Plate.

FIGURE 18.

A) Spiked plates in place at one end of tube.
B) Driver. C) Inserter tube with obturator.

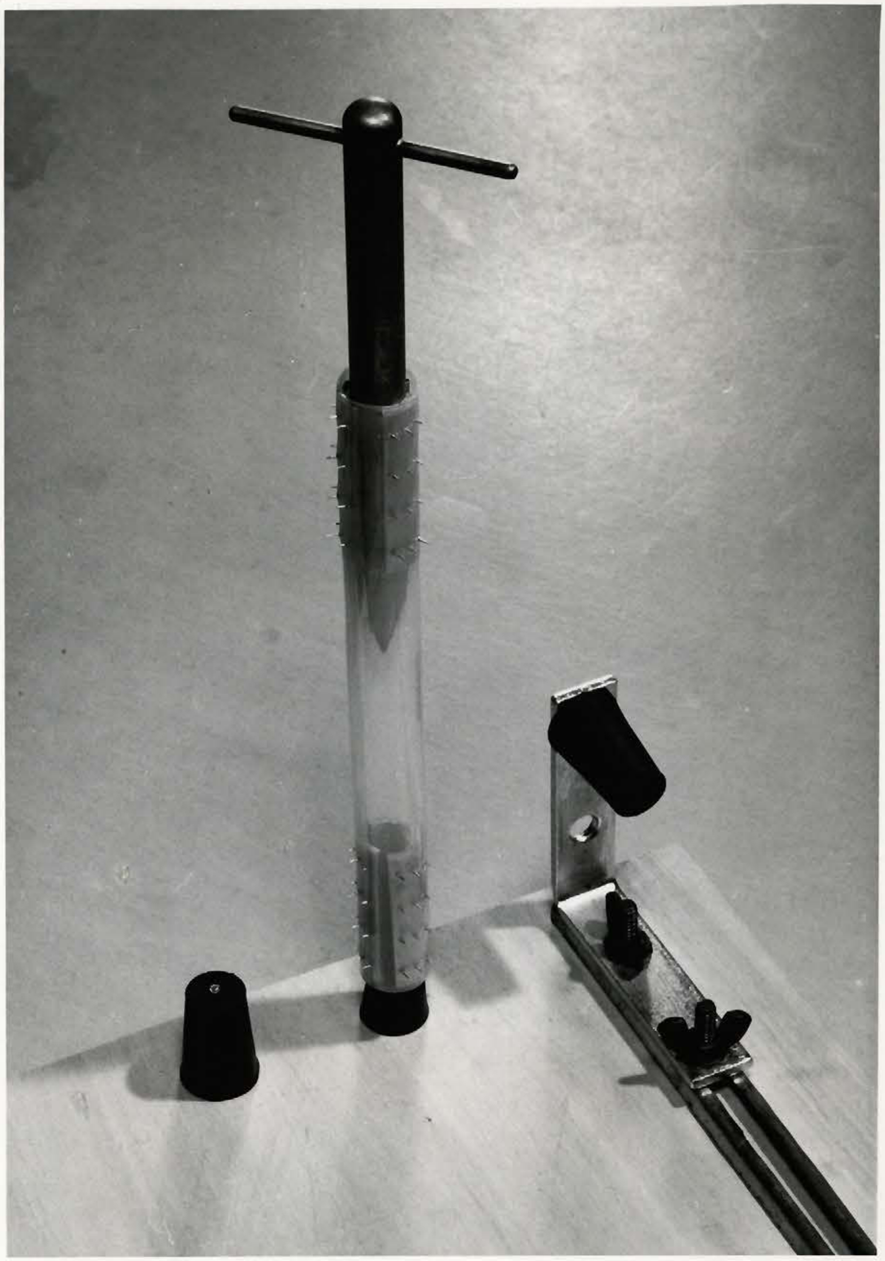

FIGURE 19.

Spiked plates and driver in place.

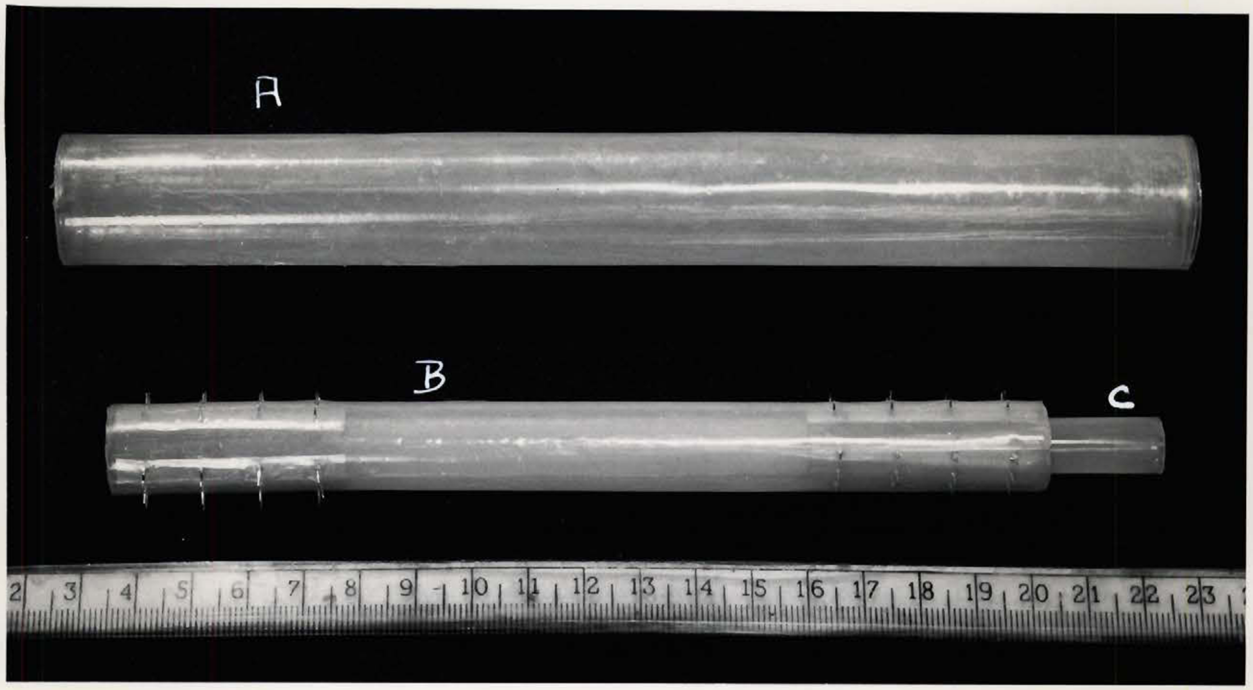

FIGURE 20.

A) Inserter tube. B) Polyethylelene stent with spikes in place. C) Central tube. (Scale in cms.)

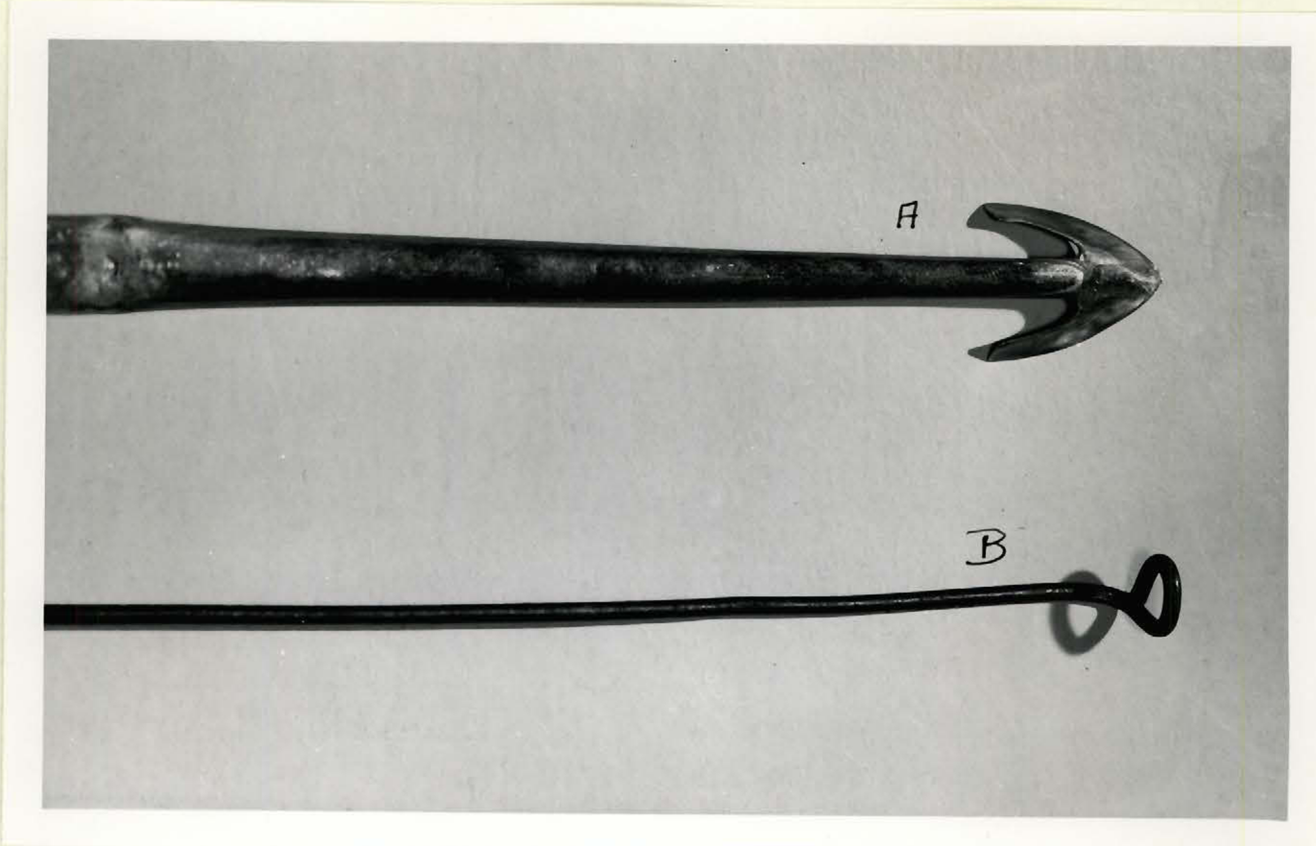

FIGURE 21.

A) Mucosal dissector. B) Stripper.

line incision, a segment of proximal jejunum was excised, (chosen for its large lumen and minimum number of Peyer's patches), taking care to preserve the vasculature until the very last moment. (Fig. 22). After the vessels were divided, the specimen was immediately drained of blood (to minimize petechiae formation) and placed in saline. Using the obturator (glass test tube) to prevent mucosal abrasion, the polyethylene tube was threaded through the lumen of the specimen. Keith needles were placed through the bowel and tube at each end to keep the intestine from contracting longitudinally. This was then mounted between the brackets of the dissecting stand. (Fig. 23 A).

A longitudinal incision was made with a scalpel, just through the muscle coats and these were peeled off, the attached mesentery being removed with this layer. (Fig. 23 A). This left the few inner fibres of muscle as described in Chapters III and VIII. Using a mucosal elevator, a cleavage plane was developed between the submucosa and mucosa and this was carried longitudinally

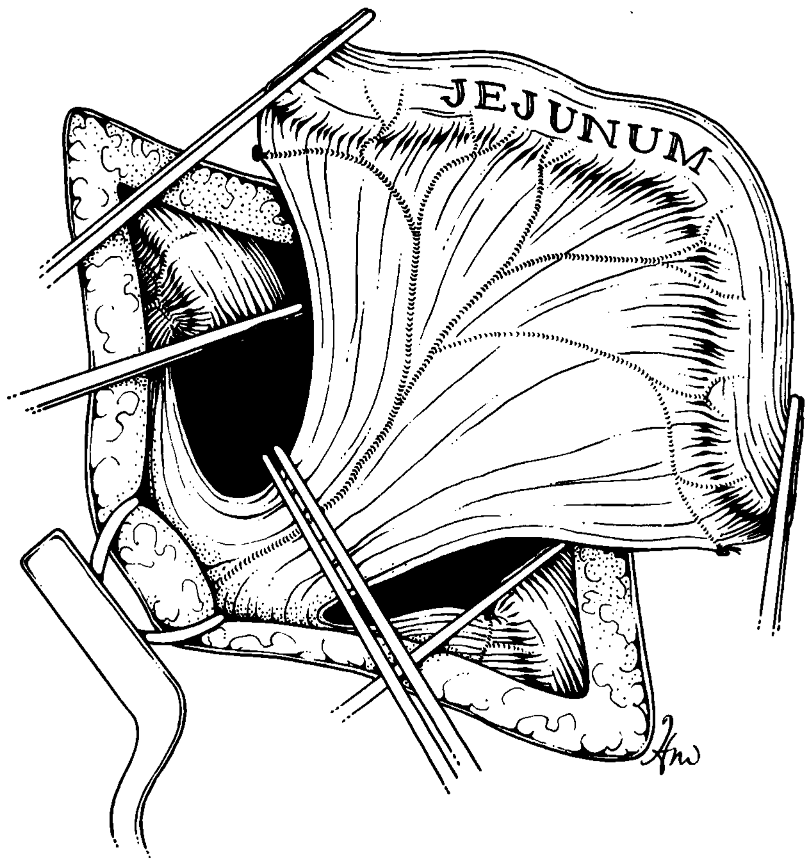

FIGURE 22.

Procedure No. 2.

along the specimen, incising the submucosa as it was dissected free (Fig. 23 B). Then, working laterally, the entire submucosa was dissected free, leaving a tube of mucosa; the smooth shiny outer surface of which was the special oonnective tissue layer described in Chapter III. (Fig. 23C, 24). The above dissection was at best painstaking, but in some animals, impossible. It was found that about 1/2 of the animals operated upon had insufficient tensile strength in their mucosa to support the dissection. This resulted in a graft too badly shredded to be usable. Because of this, the mucosal dissector pictured in Fig. 21 was developed. This, inserted in the correct cleavage plane, dissected one-half of the specimen at a time, two passes being required to completely separate the graft. (Fig. 25B). The submucosa was not incised until completely free of the mucosa; thus all the stress was carried by the submucosa and no tension was placed upon the graft itself. Using this technique, only in one animal were we unable to suitably dissect the mucosal layer.

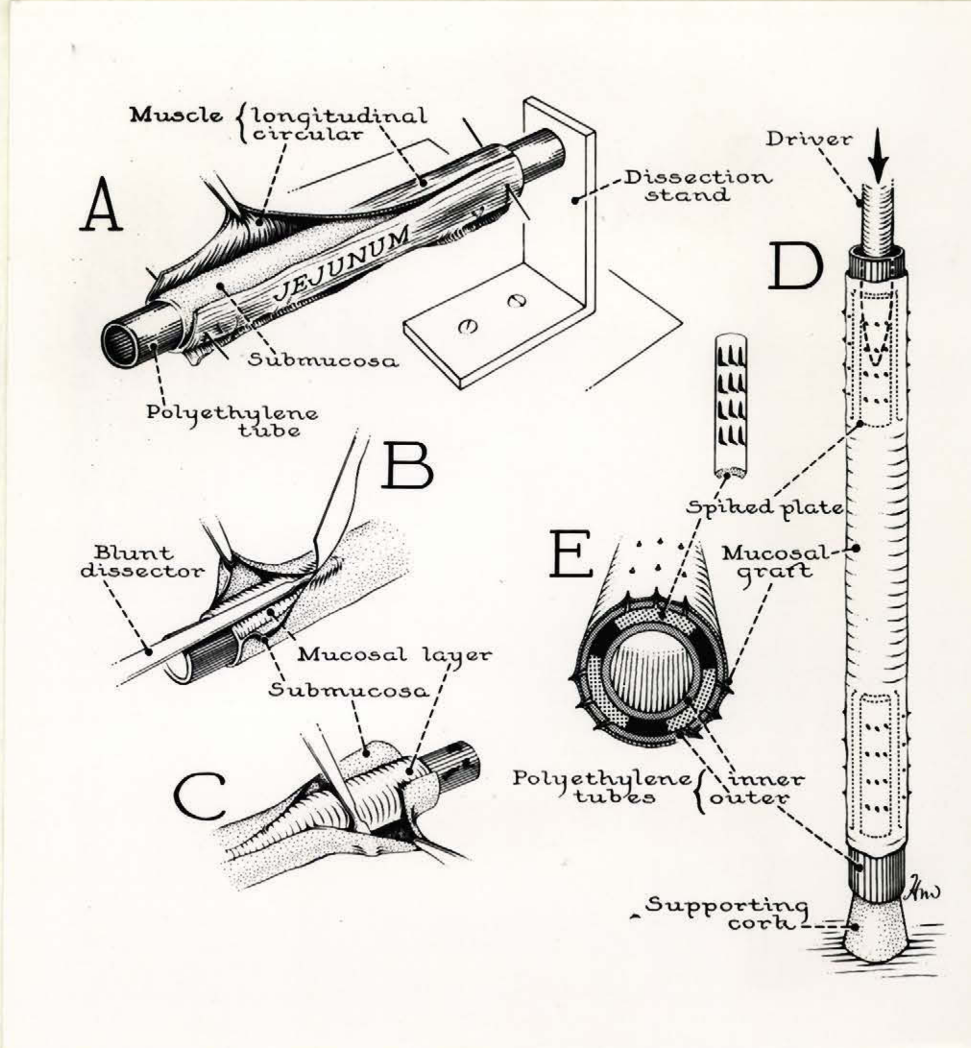

FIGURE 23.

Procedure No. 2 (Cont.)

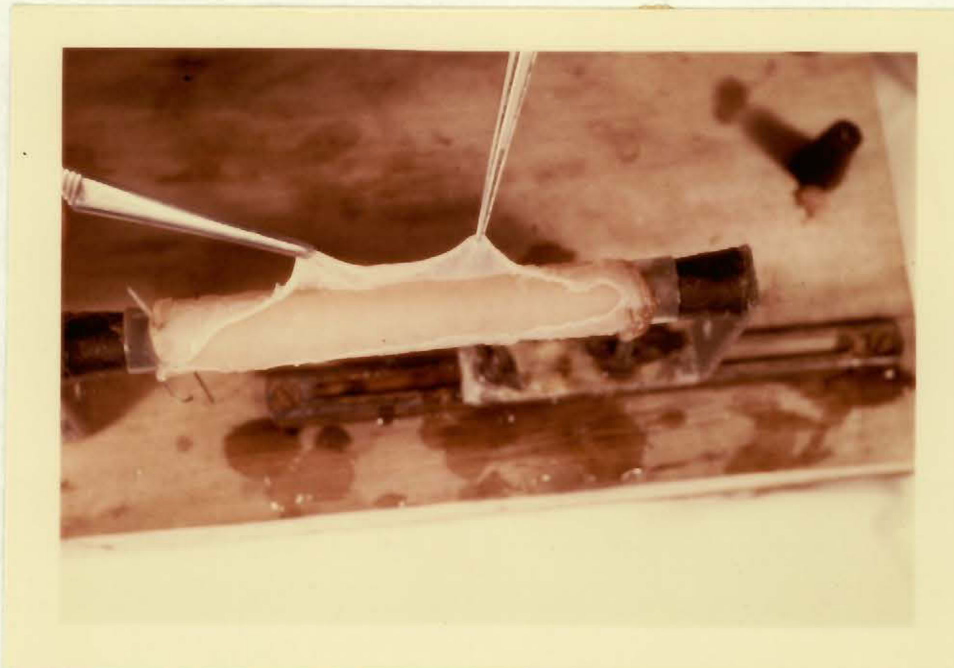

FIGURE 24.

Dissection of mucosal graft.

With the graft prepared in this manner, the ends of the tube and graft were trimmed to the required length. The spiked plates were then inserted and driven through the wall of the tube and graft with the driver. (Fig. 23 D). Three plates were placed at each end of the tube and the central tube was inserted to hold these in place. (Figs. 26, 23 E). The entire apparatus was then stored in saline soaked sponges until required.

The colon was then clamped and cut midway between the superior and inferior mesenteric arteries. The marginal vessels were tied on both sides and heavy cotton traction sutures were placed in the distal segment.

Approaching through the anus, a circular incision was made at the muco-cutaneous junction and a cleavage plane between the submucosa and muscularis was developed similar to that described in Chapter VIII. (Fig. 27A & B). It was found that if traction was placed on the mucosa-submucosal tube, intessuscepting the musculature, a tear would develop in the mesentery.

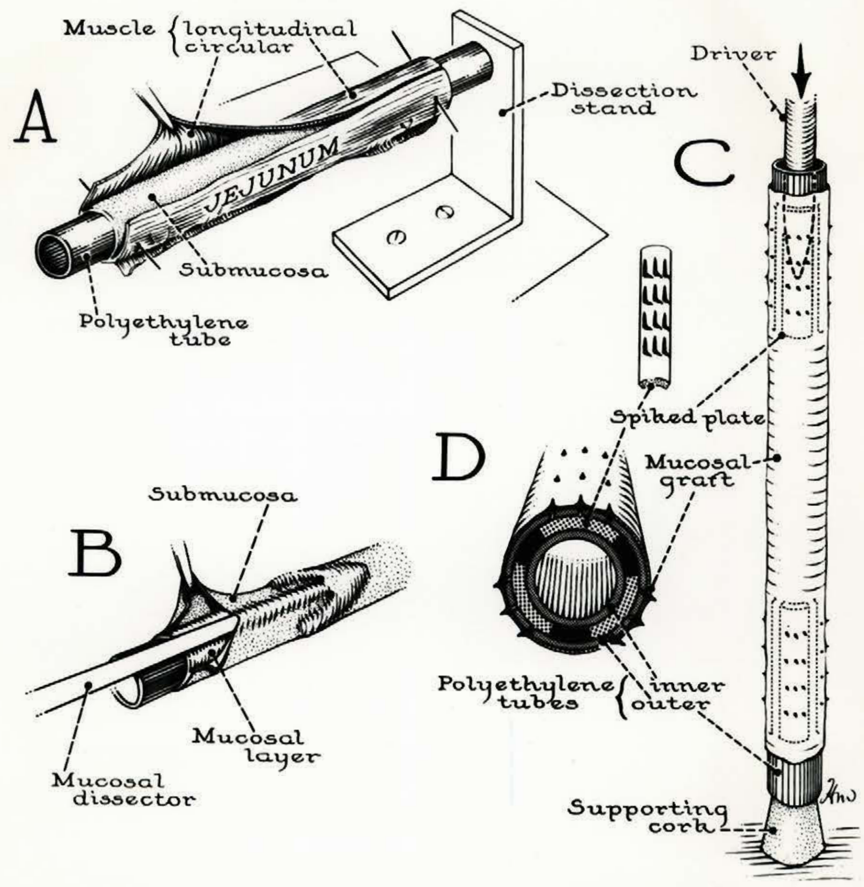

FIGURE 25.

Procedure No. 2 (Cont.)

FIGURE 26.

Mucosal graft with spiked plates in place and central tube being inserted.

Therefore, a wire loop stripper (Fig. 21) was constructed of 1 cm. loop diameter. The tube of mucosa and submucosa was then threaded through the lumen of the stripper, which was then passed cephalad. The stripper passed with ease almost to the point of division of the colon, cleanly dissecting in the proper layer. Guidance from above by an assistant was required to prevent perforation of the friable muscular tube. In this way, most of the descending colon could have been stripped of its inner layers (Fig. 27 C).

It should be pointed out here that although it was deemed necessary, from a physiological point of view, to graft only the rectum, the long narrow pelvis of the dog made any rectal anastomosis from above technically difficult. Therefore, a workable length of colon was also grafted to bring the operative site into the abdomen.

Because of the mesenteric hematomas formed as the severed vessels of the colonic wall retracted through the muscularis, and because even minimal bleeding in the lumen of the muscular tube would interfere with graft take, vascular clamps were placed on the inferior mesenteric artery, and on the aorta below it, before passing the stripper. These were removed after the graft was in place - usually 10 to 20 minutes later.

The trauma to the distal colon caused the muscle to go into spasm and hence the traction sutures plus constant push on the stripper from below was required to keep the distal bowel from contracting into the pelvis. A double layered purse-string suture of 2-0 chromic surgical gut was placed in the proximal end of the muscular tube. About 2 cm. proximal to this, the muscle wall was incised circularly (tying the marginal vessels) leaving a cap of normal colon approximately 1 inch long, trailing the long tube of dissected mucosa and submucosa (Fig.27D). The mucosa-submucosal tube was ligatured at its distal end to prevent spill

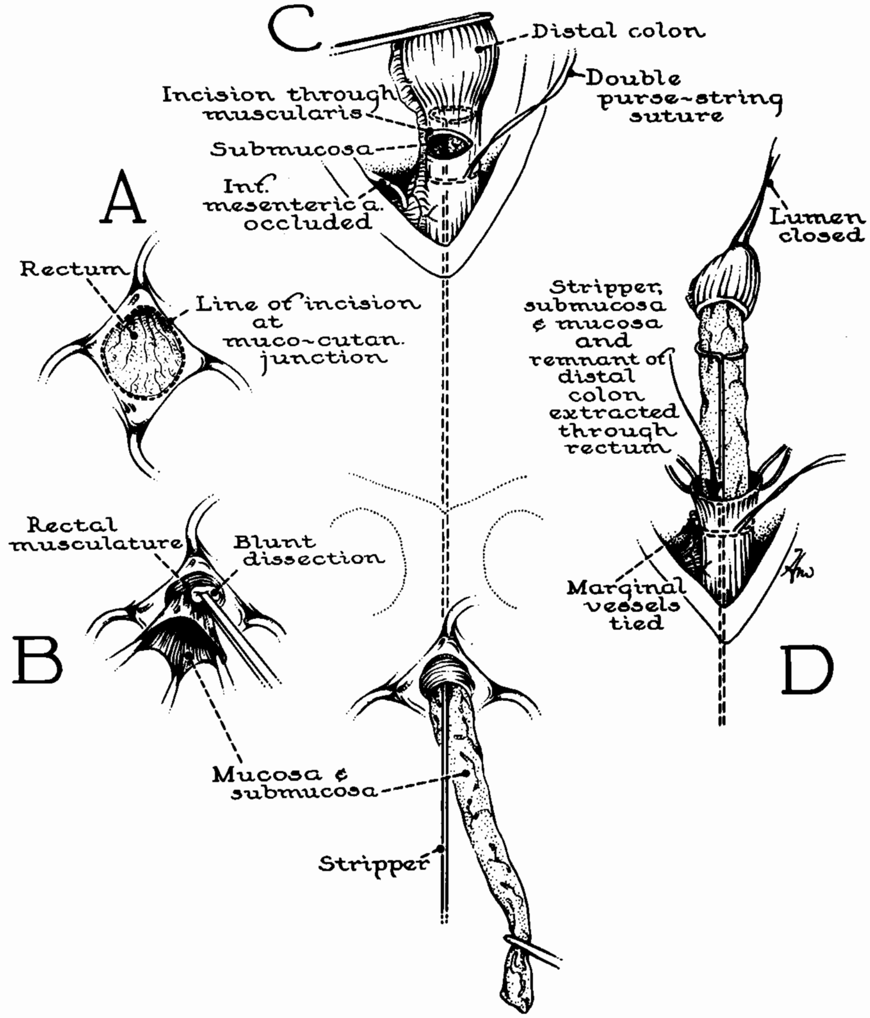

FIGURE 27.

Procedure No. 2. (Cont.

and the entire specimen was extracted proximally and discarded.

The large inserter tube with its obturator was then passed from below, again care being taken not to perforate the muscular wall. (Fig. 28A). After withdrawal of the obturator, the stented graft was inserted into the lumen of this large tube to the desired level and then the inserter tube was removed. (Fig.28B). This allowed the muscular wall to fall against the graft and impale itself upon the spikes. The purse-string was drawn tight; the proximal stump was oversewn.

The jejunal ends were anastomosed with a 2-layered open closure and the proximal colon brought through a stab wound in the left abdominal wall as a terminal colostomy. The protruding end of colon was inverted on itself and mucosa was sewn to skin with a continuous 2-0 surgical gut suture (Fig. 28D). Four hundred thousand units of penicillin and 1/2 gram of streptomycin were dusted into the peritoneal cavity,

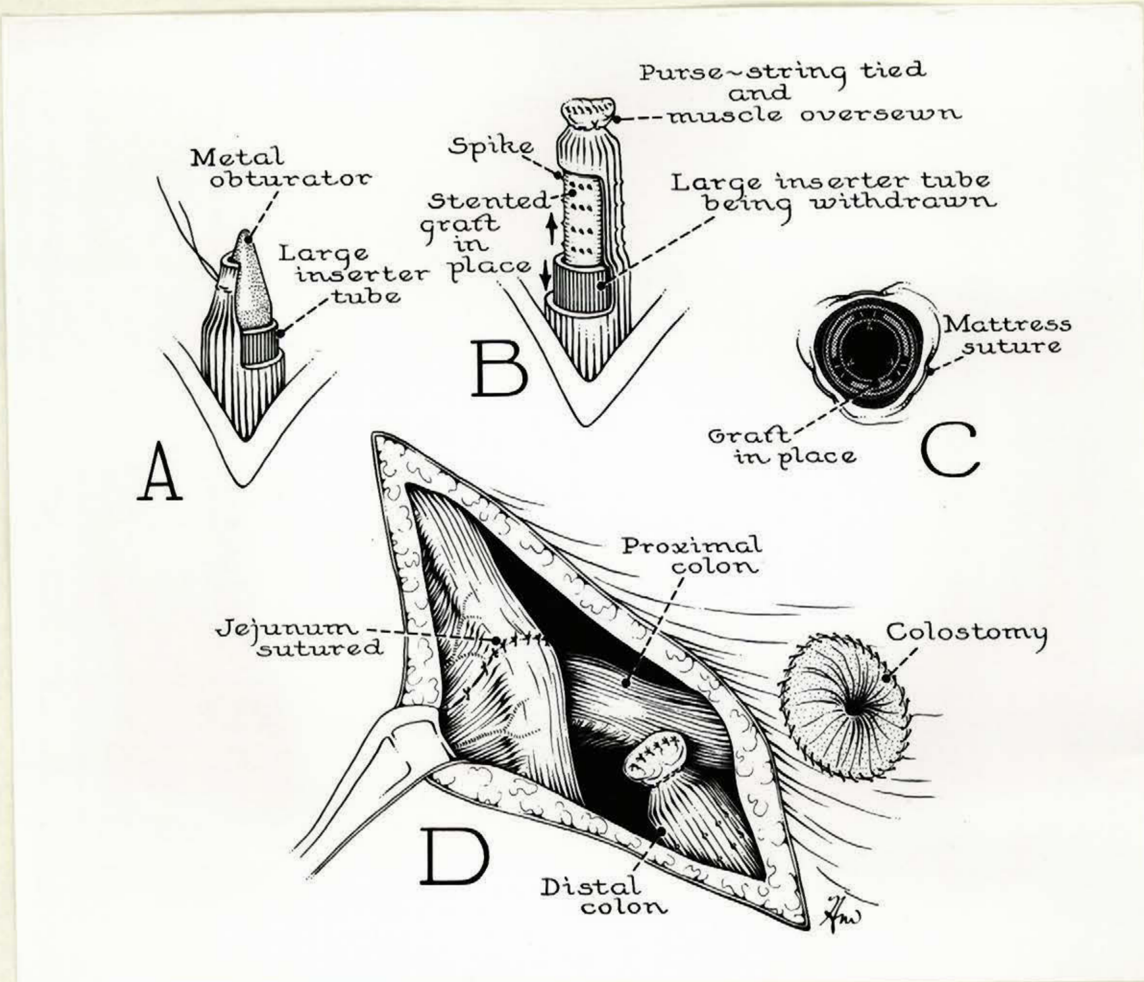

FIGURE 28.

Procedure No. 2 (Cont).

and the abdomen was closed in layers. Three cotton mattress sutures secured the peri-anal skin to both polyethylene tubes and yet allowed drainage. (Fig. 28C).

Thus, in effect, the stent had been firmly anchored to the graft bed with the friable mucosa between under no stress.

Post-operative antibiotics were administered for 5 days. On the fourth day, the spiked plates were removed under light pentothal anesthesia, but the plastic tube was left sutured in place until the dog worked it free. This usually took from 8 to 12 days. Otherwise, post-operative care closely paralleled that given for animals in Procedure No. 1.

Stage No. 2:

Two weeks, after this initial procedure, the dogs were prepared for operation as before. Opening through a midline incision, the terminal ileum was exposed and divided near the cecum. The cecal end was closed by a double row of 2-0 chromic gut inverting sutures and the proximal colon left as a mucous fistula. The proximal end of the severed ileum was mobilized, taking care to preserve its blood supply. The grafted colonic stump was then identified and a longitudinal incision made on the anti-mesenteric border near the proximal end. A biopsy was taken to determine if there was any graft take. Using urethral dilators and then the finger, the lumen was dilated. Experience showed that attempts to dilate from below usually resulted in perforation of the wall. The terminal ileum was then anastomosed, end to side, to the slit in the colon, using one continuous layer of 2-0 chromic intestinal gut suture. Edema and induration of the colon wall precluded the use of more than one layer of sutures. After dusting 400,000 units of penicillin and 1/2 gram of streptomycin into the peritoneal cavity, the abdomen was closed in layers. During the procedure, 250 ccs. of whole blood was administered to the animal.

CHAPTER XII.

PROCEDURE NO. 2 - RESULTS

Twenty animals were used in this series. Eight went on to Stage 2, of which, 3 survived. Six were sacrificed prior to the second stage because of obliteration of the rectal lumen. This occurred in an early group in which the plastic stent was removed at the same time as the spiked plates, rather than being left in for 8 to 12 days as was our subsequent practice.

The causes of death were as follows:

A. Death prior to Stage 2:

Sacrificed due to rectal stenosis	6
Peritonitis	1
Wound dehiscence	1
Intessusception	1
Undetermined	2

B. Death after or during Stage 2:

Anesthetic death	2
Respiratory infection	1
Peritonitis	1
Pelvic abscess	1
Kennel accident	1

After the first operation, the dogs were lively and thriving. The appliance stimulated frequent expulsive efforts which continued even after the rectum was completely emptied of apparatus.

During Stage 2, the rectal segment was found to be thickened, edematous and friable, with the lumen sufficiently narrowed to require dilatation. No muscular function could be elicited at this time.

Following Stage 2, the animals responded in a way similar to those of Procedure No. 1 with respect to appetite, weight loss, stool character, defecatory sensation, excoriation and soiling of their coat. Weight loss was a little more rapid and severe and recovery was slower than those of Group 1.

Similar susceptability to respiratory infections and diarrhea was also noted.

Just prior to sacrifice, the stools of the long term survivors were semi-solid and copious, each animal having 8 to 10 per day.

Under light pentothal anesthesia, barium enema rontgenograms were obtained. These showed an adequate length of rectum of slightly larger calibre than the undilated terminal ileum. (Fig. 29). Examination confirmed the absence of feces in the rectum, inferring that the rectal musculature was functioning. The animals were then sacrificed 12, 13 and 14 weeks after the initial operation.

Five of the 20 animals used in this procedure demonstrated 50% or more graft survival. Nine showed some evidence of take and in the remainder (6) only granulation tissue could be demonstrated.

All of the 3 long-term survivors showed over 50% take and 1 was fully epithelialized. In the partially covered specimens, (Fig. 30), the patches of graft epithelium were isolated from normal mucosa, indicating that these were true graft take rather than epithelial regeneration. Significantly also, was the fact that the mucosa stopped abruptly at the margins of the granulation tissue with no evidence of typical regenerating epithelium. The graft mucosa was lush, mature and showed mitotic activity. (Figs. 31 and 32).

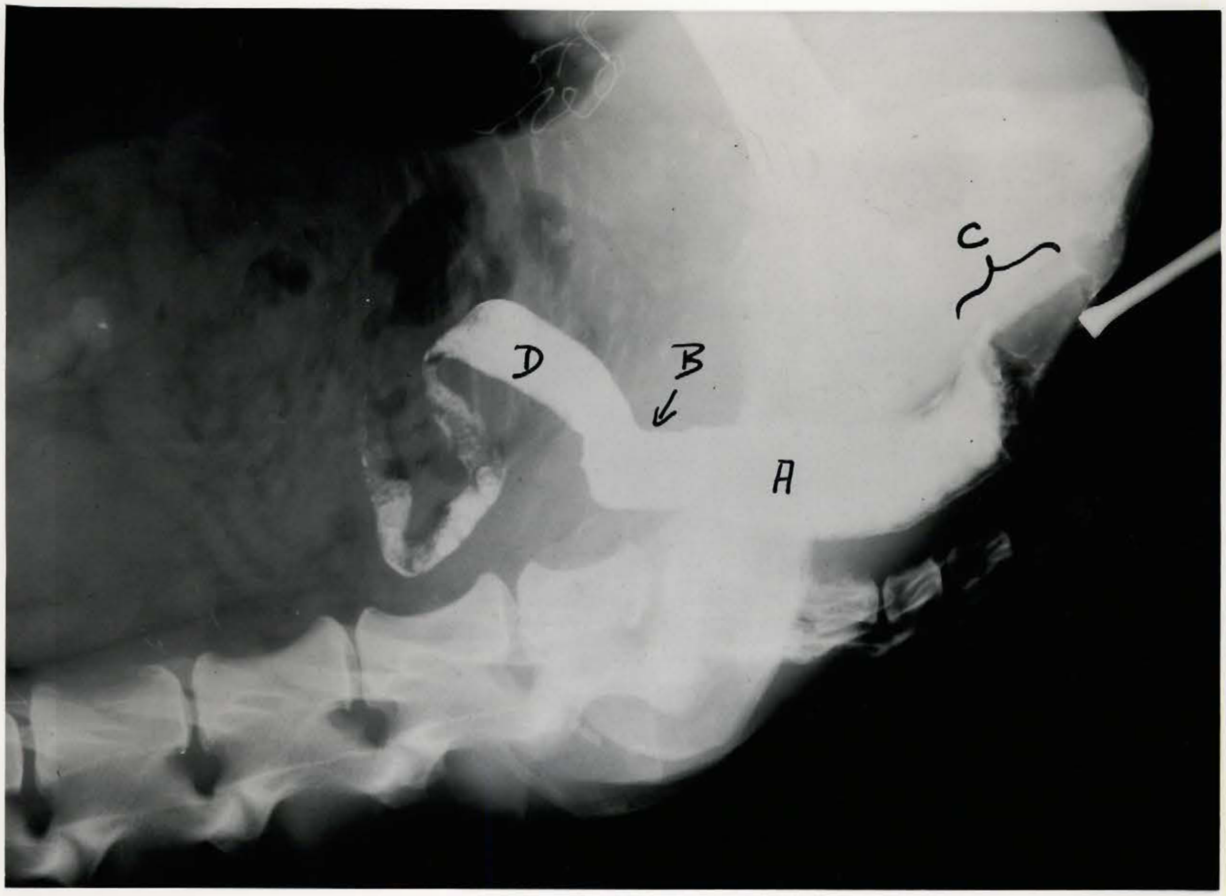

FIGURE 29.

X-ray of barium filled bowel. A) Rectum
B) Site of anastomosis. C) Anus.
D) Ileum.

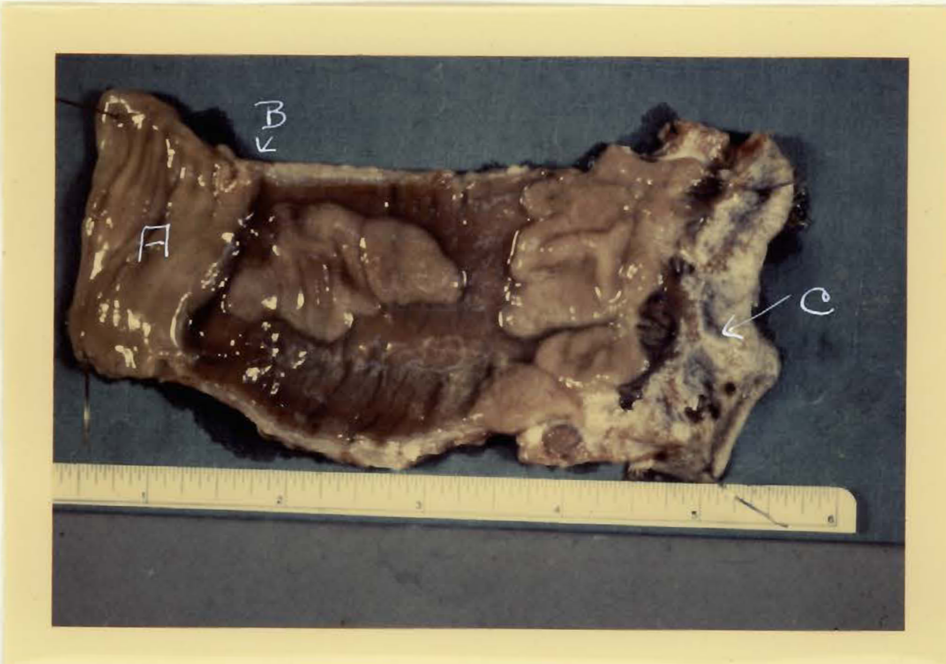

FIGURE 30.

Rectum showing islands of graft take on a muscular bed covered with a thin layer of granulation tissue. A. Ileum. B. Ileo-rectal anstomosis. C. Anal skin (Scale in inches).

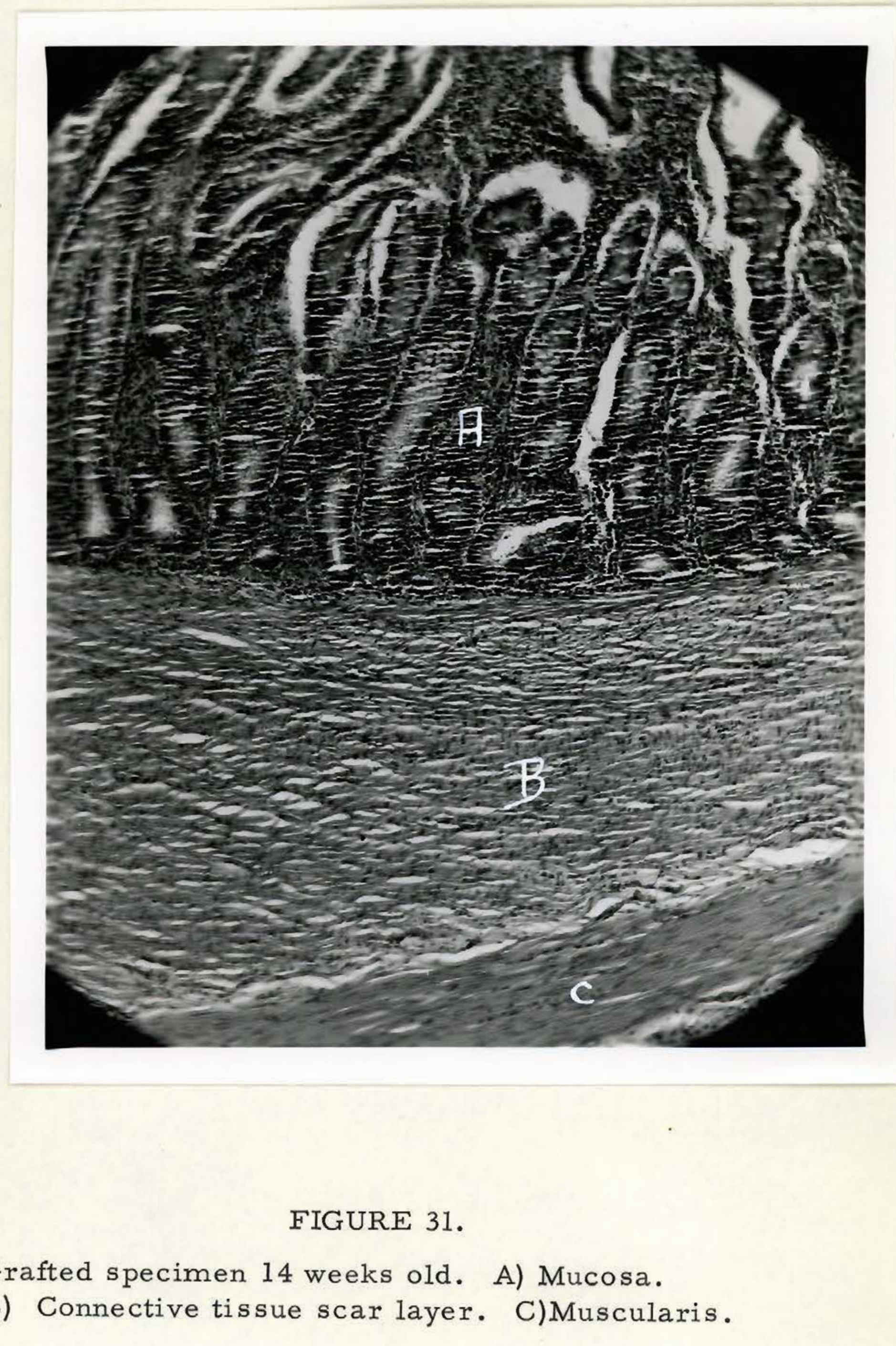

FIGURE 31.

Grafted specimen 14 weeks old. A) Mucosa.
B) Connective tissue scar layer. C)Muscularis.

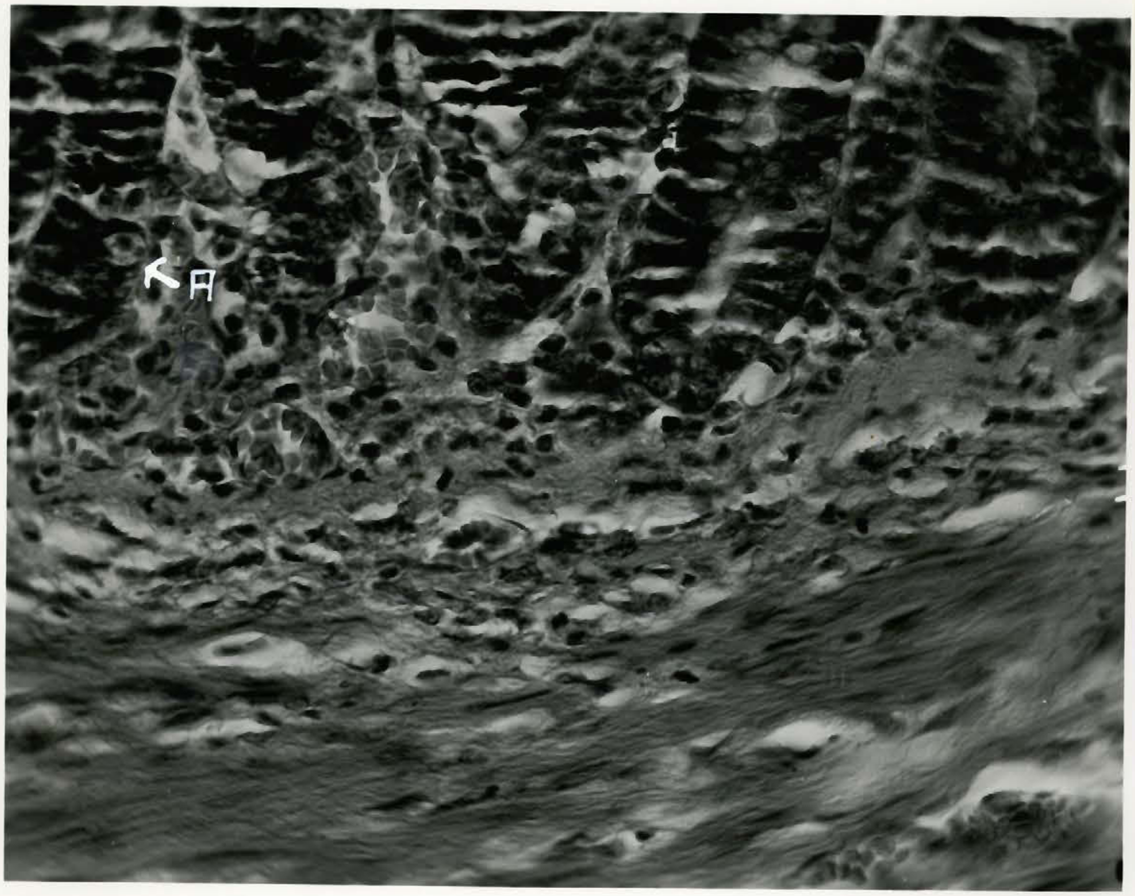

FIGURE 32.

Graft Survival using Procedure No. 2.
(A) Mitotic figure in base of crypt of
Lieberkühn. (x 400).

Further evidence that the epithelium covering the rectal segment represented true transplantation, rather than epithelialization, is seen in Figs. 33 and 34. The animals from which these speciments were obtained did not progress to Stage 2, so that the only source of mucosa was from graft tissue.

Some of the non-surviving animals which demonstrated partial graft take also showed evidence of epithelium regenerating outwards from the islands of graft tissue (Fig. 35); however, this was uncommon.

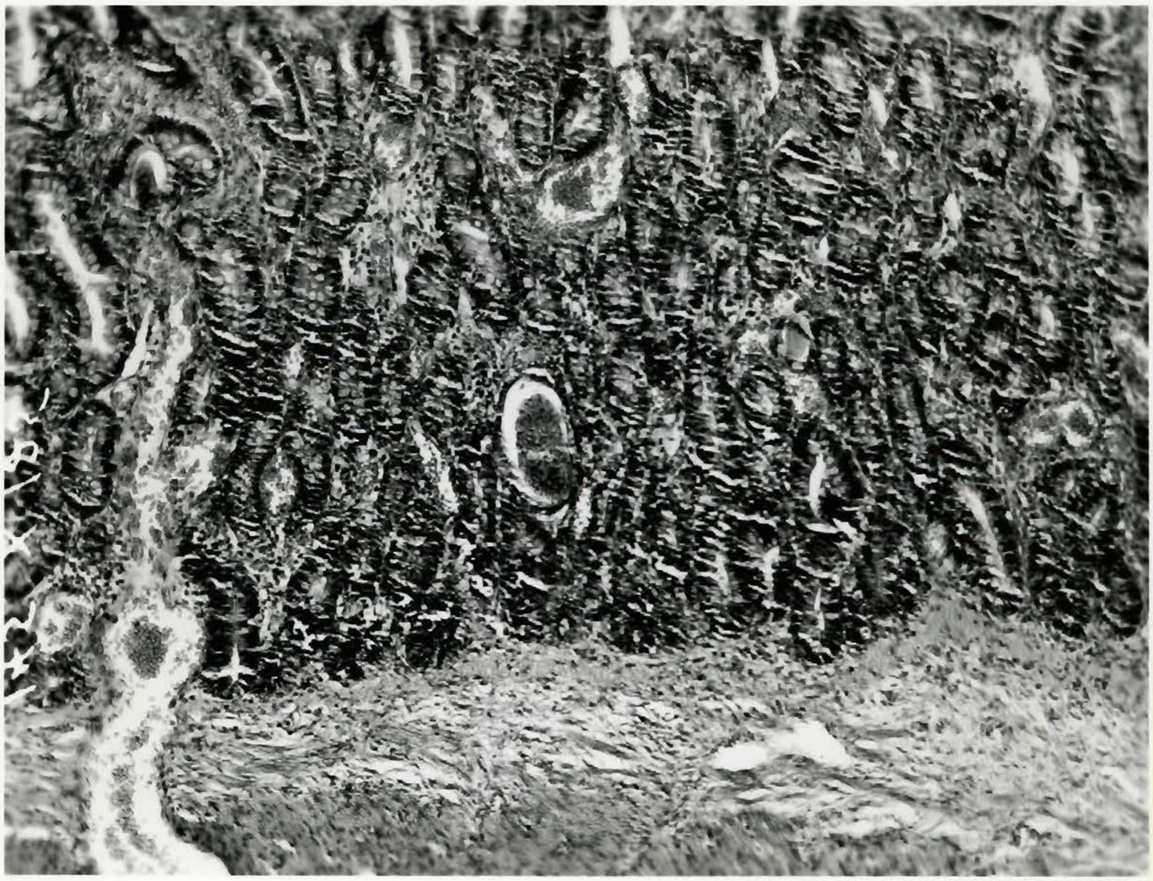

FIGURE 33.

Graft survival showing profuse vascularization of the epithelium, nineteen days after initial grafting procedure. (x 125).

FIGURE 34.

Biopsy of the most proximal portion of grafted rectum, taken during Stage 2. Vascularization of the graft is marked. (x 100).

FIGURE 35.

Flat cell layer of regenerating epithelium. Thickening to cuboidal form plus a tendency to villi formation is seen. (x 200).

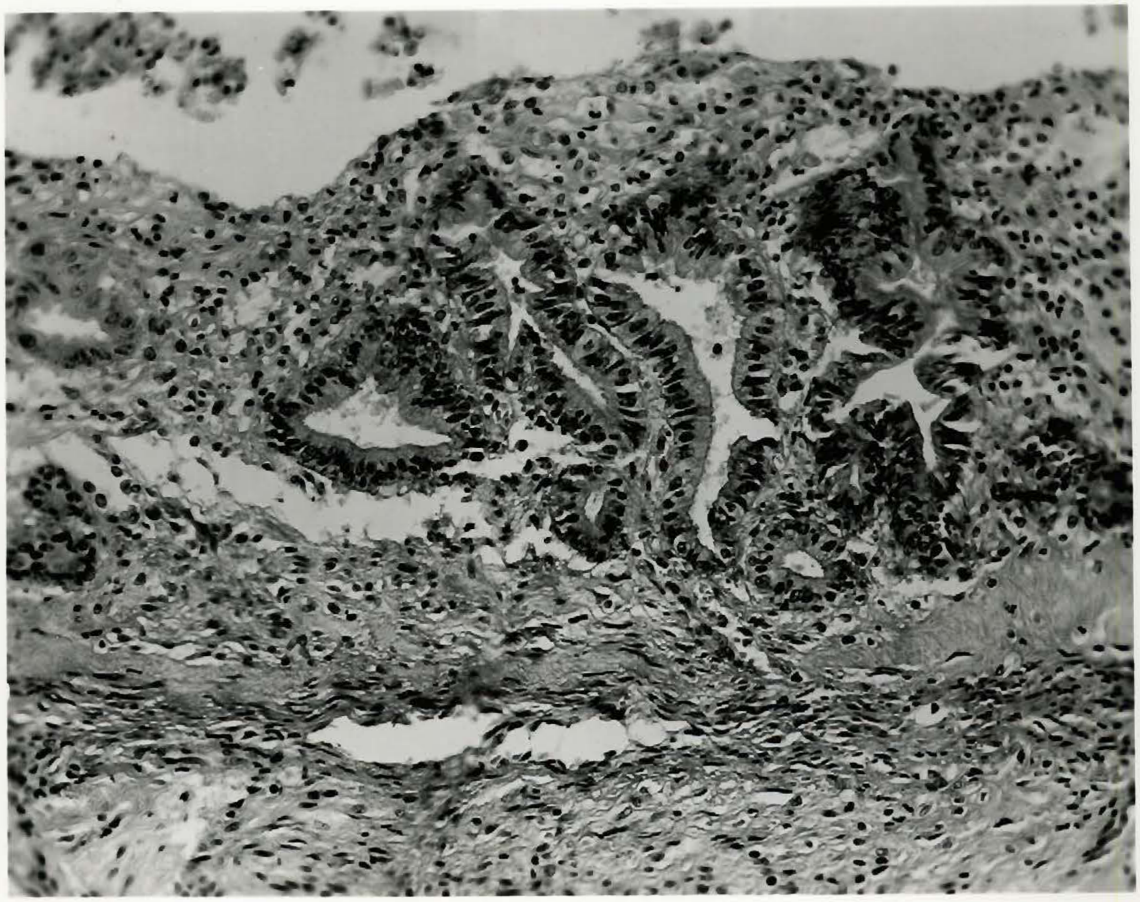

FIGURE 36.

Two week post-operative specimen showing epithelial remnants buried in granulation tissue. No mitosis is seen and the viability of these cells are uncertain. (x 200).

CHAPTER XIII.

DISCUSSION

Procedure No. 1 has the advantage of being a single stage, relatively simple operation. Furthermore, there is no break in continuity between the graft and small bowel mucosa and there is no need for an abdominal stoma. Despite these advantages, graft survival could be demonstrated in only 26.7% of animals, and only one-half of these proved to be full takes. Probably the greatest single factor contributing to the poor result is the thickness and complexity of the graft tissue. Perfusion by tissue fluids could hardly be expected to keep the surface mucosa alive until vascularization penetrated the thick submucosa and muscularis mucosae.

Procedure No. 2 provided a relatively thin graft with consequently greater chance for survival. This is evidenced by an overall 70 % of animals showing significant graft take. However, the need for 2 operative procedures, the abdominal stoma and the complex techniques and equipment required to handle the graft are all disadvantages.

One of the major problems in Procedure No. 2 was the high incidence of stenosis of the grafted segment. In every instance, the rectal wall was thickened, friable, edematous and non-contractile when examined during the second operation; yet the wall became pliable and contractile and was of ample calibre by the time the animal was sacrificed. It is generally accepted today that ano-rectal wounds heal best when the patient has normal bowel movements. (Turell, 1958). The peristaltic movements operate the lymphatic and venous "pumps," while the stool acts as an excellent dilator. In view of this, one must assume that it was the diversion of the fecal stream which permitted the deterioration of the rectal segment.

While it is true that the trauma of stripping was the stimulus for the edema and induration, the rectal wall was protected by the passage of the fecal stream in animals subjected to Procedure No. 1. The indurated and thickened rectum, seen after Procedure No. 2, also became soft and pliable after bowel continuity was restored.

Thus, it is difficult to protect the delicate graft from the trauma of fecal contents, and yet allow the beneficial effects of normal function to protect the rectal musculature.

An epithelial graft will not survive if a hematoma forms between it and its bed. Therefore, great care should be taken to prevent bleeding from the graft bed. Despite clamping of the aorta and inferior mesenteric artery, a certain amount of bleeding from the rectal wall must occur after release of these vessels. Similarly, in Procedure No. 1, the inversion of the rectum probably kinks the vessels sufficiently to stop hemorrhage, and these may well re-open when the bowel is replaced. Because the bleeding surface is so inaccessible, control by usual methods is impossible. While the stents used in these operations probably provide sufficient pressure for hemostasis, damaging bleeding may occur at the moment the stents are put in place.

Hematomas not only cause graft failure, but provide an excellent nidus for infection which may partially explain the high rate of sepsis.

The assessment of fecal continence in these animals is difficult. We know that they have sufficient sensation and sphincter action to control the feces, however, no estimate of reservoir function could be made. The latter would require conscious voluntary control. It is significant to note that patients who have undergone Ravitch's operation (See Chapter I) have a great deal of urgent diarrhea in the first few weeks which is quite distressing. By conscious resistance to the defecatory urge, the reservoir capacity of these patients increases so that in 12 to 14 weeks they are having only 3 or 4 bowel movements a day. This is associated with dilatation of the terminal ileum. In our animals, who made no conscious attempt at control, no small bowel dilatation was noted.

CHAPTER XIV.

CLINICAL CORRELATION

The results of the previously described techniques are not sufficiently encouraging to warrant trial on human patients. However, the development of improved methods may yet prove the soundness of the basic principles involved. Because of the anatomical differences between the intestine of man and dog, noted in Chapter III, an attempt was made to determine whether dissection of the bowel layers was technically feasible in man.

Ravitch in 1948 proved that the dissection of the mucosa-submucosal layers of the rectum was similar in man and dogs. Indeed, a case is reported by Catchpole (1952) of a severely disturbed patient, in a mental institution, whose aberrations were directed towards the rectum. She succeeded in stripping her own rectal and sigmoidal mucosa and submucosa as a complete tube, and only rapid surgery saved her life.

No literature was available on the macro-dissection of human small bowel. Therefore, fresh segments of human ileum were obtained at the time of operation from three different individuals. Two specimens were from patients undergoing surgery for construction of ileal bladders, and were obtained according to the technique described in Chapter XI to minimize vascular congestion. Dissection, immediately after removal, proved to be relatively easy but somewhat painstaking. On both occasions, a complete tube of mucosa was obtained using only forceps and small Metzenbaum scissors, although the tissue was as friable as that of the dog. In contrast to the smooth shiny surface of the mucosal graft seen in the dog (due to the special connective tissue layer), in man, the dissected surface was shaggy and dull. Fig. 37 is a section through human ileal mucosa.

The third specimen was obtained from a patient undergoing right hemi-colectomy for carcinoma.

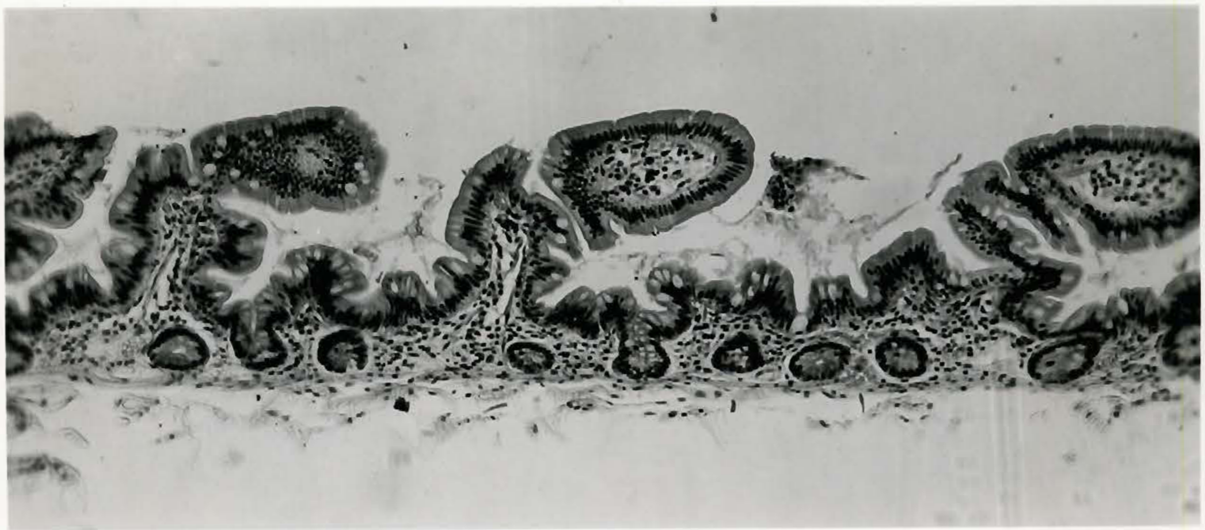

FIGURE 37.

Human ileal mucosa dissected free from
the other bowel layers.
(x 125).

Surgical considerations required that the vasculature to the segment of terminal ileum involved be occluded for about 20 minutes before removal. This specimen proved to be too congested and edematous to allow an unbroken dissection of the mucosa.

CHAPTER XV.

CONCLUSIONS

Small bowel mucosa has been successfully transplanted to the large bowel as an autogenous free graft in dogs. We have demonstrated that the extremely friable mucosal layer of the small intestine may be dissected and handled satisfactorily, and made to grow on the denuded rectal musculature.

The incidence of success is higher when the relatively simple mucosa alone is used as a graft rather than the more complex mucosa backed with submucosa. The former, however, requires more involved techniques of handling.

Regeneration over granulating areas from the islands of graft take were uncommon in our preparations, but did occur.

While proven theoretically possible, improvements in techniques are required before clinical application of our methods is justified.

We were unable to infer, from the results obtained in the dog, the degree of fecal continence to expect if these procedures were attempted in man. This is due to the lack of conscious attempts at continence in the dog, which in man appear to be so important to the return of fecal control following surgical intervention.

Our investigation disclosed some hitherto unrecorded facts about the histology of the small bowel in the dog. The nature of the special connective tissue layer of the lamina propria, and the so-called oblique layer of musculature were investigated. Our evidence suggests that both of these layers act as exaggerated mechanisms for anchoring one bowel layer to the other by means of interdigitating collagenous fibres.

We were also able to provide corroberation of certain facts elicited by other workers about the regeneration of bowel mucosa and the mechanisms of fecal continence. We have shown that, in the dog, mucosa will regenerate to cover a denuded area of large bowel, in a manner similar to that described by Sir Harold Florey (1954) in the duodenum of the cat. Also, we have added to the weight of evidence offered by Gaston (1951), Karlan (1959), etc. supporting the existance of the ano-rectal reflex as part of the fecal continence mechanism.

As so frequently occurs in research, our attempts to solve one problem led to an awareness of many other unanswered questions, often in fields relatively unrelated to the original situation.

CHAPTER XVI.

SUMMARY

1. Most currently acceptable surgical approaches to the treatment of Idopathic Ulcerative Colitis and Familial Polyposis do not combine both complete eradication of the diseased tissue and preservation of the fecal continence mechanism. One or the other of these desirable end results must be sacrificed depending on individual circumstances.

2. Removal of the diseased rectal mucosa and replacement by free grafts of healthy small bowel epithelium to the preserved rectal musculature was attempted.

3. The anatomical and histological differences between canine and human bowel were discussed. The results of macro-dissection of the layers of small and large canine bowel were described.

4. The physiology of fecal continence was discussed. Evidence in support of the existance of the

ano-rectal reflex was demonstrated.

5. Factors affecting mucosal repair within the bowel were considered. Mucosal regeneration in the rectum of the dog was shown to closely parallel the pattern described in most other species of experimental animal.

6. An historical review of free autografting of epithelium was presented.

7. Utilizing a free graft consisting of terminal ileal mucosa and submucosa in continuity with normal bowel epithelium, the denuded rectum was relined. In 26.7% of the animals operated upon, graft take could be demonstrated. Overall mortality was 66.7%.

8. Using specially devised techniques, jejunal mucosa, denuded of its submucosa, was used as graft material. This was done as a two stage procedure. Seventy percent of these animals showed evidence of graft take. Mortality was 78.5% excluding the animals which were intentionally sacrificed before Stage 2.

9. The resulting fecal continence was poor in these dogs, but this result is not comparable to what might occur if conscious attempts at control were possible, as in man.

10. The anatomical dissections required to perform these procedures were demonstrated to be feasible in man.

BIBLIOGRAPHY

1. Aylett, S. O.
Ulcerative colitis treated by total colectomy and ileo-rectal anastomosis.
Brit. Med. J. 1: 1060, 1955.

2. Aylett, S. O.
Total colectomy and ileo-rectal anastomosis in diffuse ulcerative colitis.
Brit. Med. J. 1: 489, 1957.

3. Bacon, H. E. and Trimpi, H. D.
The selection of an operative procedure for patients with medically intractable ulcerative colitis.
Surg., Gyne. & Obst. 91: 409, 1950.

4. Bargen, J. A.
Diseases of the liver associated with ulcerative colitis.
Ann. Intern. Med. 39: 285, 1953.

5. Best, R. R.
Evaluation of ileoproctostomy to avoid ileostomy in various colon lesions.
J. A. M. A. 150: 637, 1952.

6. Best, R. R.
Sphincter preservation operation for carcinoma of the rectum, rectosigmoid and lower sigmoid.
Amer. Surgeon. 20: 1264-72, 1954.

7. Boyd, W.
Path. for Surgeons.
7th Ed. W. B. Saunders Company, Philadelphia, 1955. pp. 205.

8. Bünger, C.
Gelungener verscuh einer Nasenbildung aus einem völlig Getrennten Hautsluck aus dem Beine.
J. d. Chir. u. Augenh. 4: 569, 1823.

9. Calhoun, M. L. (Prof. of Anatomy, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan, U. S. A.)
Personal Communication, 1959.

10. Catchpole, B. N.
Self-inflicted stripping of the rectal and colonic mucous membrane.
Brit. J. Surg. 39: 464, 1952.

11. Cattan, R., Champeau, R. and Bloch-Larocques, P.
Serious hemorrhagic colorectitis treated by total colectomy and anastomosis of the ileum to the anus.
Arch. des Malad. de l'Appareil Dig. et des Malad. de la Nutrition. 41: 523, 1952.

12. Cattell, R. B.
Closure of ileostomy in ulcerative colitis.
Ann. Surg. 115: 956, 1942.

13. Cattell, R. B.
Discussion on the surgery of ulcerative colitis.
Proc. Royal Soc. Med. 46: 1021, 1953.

14. Cattell, R. B. and Colcock, B. R.
The surgical treatment of ulcerative colitis.
Postgrad. Med. 17: 114, 1955.

15. Cole, J. and Holden, W.
Spontaneous regression of adenomatous polyps of the rectum following total colectomy and ileo-proctostomy.
Presented at the Central Surg. Assoc. 16th Annual Assembly, Montreal, 1959.

16. Coleman, S. T., Eckert, C.
Preservation of rectum in familial polyposis of the colon and rectum.
A. M. A. Arch. Surg. 73: 635-644, 1956.

17. Copher, G. H.
The effect of urinary bladder transplants and extracts on the formation of bone.
Ann. Surg. 108: 934, 1938.

18. Corbett, R. S.
Discussion on the surgery of ulcerative colitis.
Proc. Royal Soc. Med. 46: 1028, 1953.

19. Crile, G. Jr., Thomas, C. Y.
Treatment of acute toxic ulcerative colitis by ileostomy and simultaneous colectomy.
Gastroenter. 19: 58, 1951.

20. Davis, J. S.
Plastic Surgery: Its Principles & Practice.
P. Blakiston's Son & Co., Philadelphia, 1919.

21. Dennis, C. and Karlson, K. E.
Minimization of risk and disability in idiopathic ulcerative colitis.
Amer. J. Surg. 90: 761, 1955.

22. Denny-Brown, D., Robertson, E. G.
An investigation of the nervous control of defecation.
Brain. 58: 256, 1935.

23. Devine, J., and Webb, R.
Resection of rectal mucosa, colectomy and anal ileostomy with normal continence.
Surg. Gyne. & Obst. 92: 437, 1951.

24. Dukes, C. E.
Ann. Eugenics (Comb), 17: 1, 1952.

25. Dukes, C. E. and Lockhart-Mummery, H. E.
Practical points in the pathology and surgical treatment of ulcerative colitis.
Brit. J. Surg. 45: 25, 1957.

26. Eddy, F. D.
Late results of vagotomy in the treatment of idiopathic ulcerative colitis and regional enteritis.
Surgery. 29: 11, 1951.

27. Everson, T. and Allen, M.
Subtotal colectomy with ileosigmoidostomy and fulguration of polyps in retained colon.
A. M. A. Arch. Surg. 69: 806, 1954.

28. Florey, Sir H.
Lectures on general pathology. W. B. Saunder's Co., Philadelphia, 1954, pp. 515.

29. Freeman, L.
Skin grafting for surgeons and general practitioners.
C. V. Mosby Co., St. Louis, 1912.

30. Goligher, J. C. and Hughes, E.S.R.
Sensibility of rectum and colon: Its role in mechanism of anal continence.
Lancet. 260: 543, 1951.

31. Goligher, J. C.
The functional results after sphincter-saving resections of the rectum. (Hunterian Lecture, March, 1951).
Ann. Roy. Coll. of Surg. 8: 421, 1951.

32. Goligher, J. C.
Discussion on the surgery of ulcerative colitis.
Proc. Royl. Soc. Med. 46: 1025, 1953.

33. Garry, R. C.
The responses to stimulation of the caudal end of the large bowel in the cat.
J. Physiol. 78: 208, 1933.

34. Gaston, E.
Physiology of fecal incontinence.
Surg., Gyne. & Obst. 87: 280, 1948.

34a. Gaston, E.
Continence after rectal resection.
J. A. M. A. 146: 1486, 1951.

35. Gerst, G. R. and Seidenberg, B.
Experiences with a sphincter preserving operation.
A. M. A. Arch. Surg. 73: 63-65, 1956.

36. Gilbert, M. G., Pace, W. and Izant, R. G., Jr.
Uro-epithelial transplantation to intestinal segments: An experimental study.
J. Urology. 80: 237, 1958.

37. Gilbertas, A., Blanchard, H.
Rectalitis therapy with total colectomy and reconstruction of Neo-rectum with Ileoplasty.
Arch des Malad. de l'Appareil Digestif et des Malad. de la Nutrition. 46: 236, 1957.

38. Goodman, L. S. and Gilman, A.
The pharmacological basis of therapeutics. 2nd Ed.
The MacMillan Co., New York, 1955, pp. 360.

39. Grant, J. C. B.
A method of Anatomy. 6th Ed.
The Williams & Wilkins Company, Baltimore, 1958, pp. 352.

40. Grindlay, J. H.
Method of making tubes of polyethylene film for use in anastomosis of the common bile duct, trachea and pelvic colon.
Surg. 24: 22, 1948.

41. Habel, R. E. (Assoc. Prof. of Anatomy, New York State Vet. Coll. Ithaca, N. Y.)
Personal communication, 1959.

42. Hightower, F.
Regeneration of the mucosa of the colon following its removal in short segments of the bowel: An experimental study.
Ann. of Surg. 147: 775, 1958.

43. Hinze, R.
Thiersch's Patch technique in repair of rectal defects.
Zent. f. Chir. 82: 1153, 1957.

44. Huggins, C. B.
The formation of bone under the influence of epithelium of the urinary tract.
Arch. Surg. 22, 377, 1931.

45. Hughes, E.S.R.
Preservation of anal sphincter in treatment of carcinoma of the rectum.
Med. J. Aust. 44: 315-318, 1957.

46. Hullsiek, H. E.
Mulitple polyposis of the colon.
Surg., Gyne. & Obst. 47: 346, 1928.

47. Karlan, M., McPherson, R. C. and Watman, R. N.
An experimental evaluation of fecal continence - Sphincter and Reservoir - in the dog.
Surg., Gyne. & Obst. 108: 469, 1959.

48. Keller, W. L.
Annular strictures of the rectum and anus.
Amer. J. Surg. 20: 28, 1933.

49. Krause, F.
Uber die Transplantation grosser ungestielter Hautlappen.
Verhandl. Deutsch. Qes. F. Chir. p. 46, 1893.

50. Lanzara, G. C.
Anal ileostomy for polyposis.
Policlinico: Sezione Chir. 62: 361, 1955.

51. Leblond, C. P. and Stevens, C. E.
The constant renewal of the intestinal epithelium in the albino rat.
Anat. Rec. 100: 357, 1948.

52. Leblond, C. P. and Messier, B.
Renewal of chief cells and goblet cells in the small intestine as shown by radioautography after injection of thymidine-H^3 into mice.
Anat. Rec. 132: 247, 1958.

53. Leroux des Tillets, J. J.
Report of Mr. H. Dutrochet on Indian rhinoplasty,
J. Med. Chir. Pharm, etc. 44: 106, 1817.
(cited by Gnude, M. T. and Webster, J. P. 'The Life and Times of Gaspare Tagliacozzi,' New York, Herbert Reichner, 1950.)

54. Levy, R. W., Wilkins, H., Herrmann, J. D. Lisle, A. C., Jr., and Rix, A.
Experiances with prefrontal lobotomy for intractable ulcerative colitis.
J. A. M. A. 160: 1277, 1956.

55. Lewkowicz, F. C. and Joseph, E. G.
Familial polyposis of the colon.
A.M.A. Arch. Surg. 72: 346, 1956.

56. Lippmann, H. N. and Longmire, W. P.
Studies on the transplantation of free jejunal mucosa-submucosal autografts to the mucosa-stripped stomach of the dog.
Ann. of Surg. 140: 86, 1954.

57. Lockhart-Mummery, H. E. and Dukes, C. E. and Bussey, H. J. R.
The surgical treatment of familial polyposis of the colon.
Brit. J. Surg. 43: 476, 1956.

58. Longmire, W. P., Jr., Beal, J. M., Lippman, H. N. and Bishop, R. C.
Studies on the regeneration of gastric mucosa in the experimental animal.
Surgery. 32: 384, 1952.

59. Lumb, G. and Protheroe, R. H. B.
Biopsy of the rectum in ulcerative colitis.
Lancet. 269: 1208, 1955.

60. Maguire, C., Georgiade, N., McWhirt, J. and Pickrell, K.
The use of gastro-intestinal mucos membrane as a replacement for skin - an experimental study.
Plastic & Reconstructive Surg. 22: 139-49, 1958.

61. Mammoni, M.
Coloprotectomy andileo-anal anastomosis in ulcerative colitis - remote results.
Prensa mid Argentina. 45: 823, 1958.

62. Marshall, V. F. and Spellman, R.
Construction of urethra in hypospadius using vesicle mucosal grafts.
J. Urology. 73: 335, 1955.

63. Martin, L. S. J., Duxbury, J. H. and Leadbetter, W. F.
Uro-epithelial lined small bowel as a ureteral substitute.
Surg., Gyne. & Obst. 108, 439, 1959.

64. Mayo, C. W. and Fly, O. A.
Analysis of 5 year survival in carcinoma of the rectum and rectosigmoid.
Surg., Gyne. & Obst. 103, 94, 1955.

65. McCorriston, J. R.
Familial polyposis of the large intestine.
Canad. J. Surg. 1: 52, 1957.

66. McMinn, R. M. H. and Johnston, F. R.
Repair of wounds of the mucosa in the rectum of the cat.
J. Embryol. & Exper. Morphol. 6: 509, 1958.

67. Miller, G. G., Gardner, C. McG., Ripstein, C. B.
Primary resection of the colon in ulcerative colitis.
Canad. Med. Assoc. J. 60: 584, 1949.

68. Miller, M.
Guide to the Dissection of the Dog. 3rd Ed.
Edwards Bros., Inc., Ann Arbor, Michigan, 1955.

69. Neumann, A.
Personal communication, 1959.

70. O'Connor, R. J.
Mucosal repair in the rectum.
Brit. J. Surg. 44: 93, 1956.

71. Ollier, L. X.E.L.
Sur les griffes cutanees ou autoplastique.
Bull. Acad. Med. Paris, 1: 243, 1872.

72. Palumbo, L. T. and Rugtiv, G. M.
Ulcerative colitis and familial polyposis.
Postgrad. Med. 19: 543, 1956.

73. Pancoast, J.
A treatise on Operative Surgery: Comprising a description of the various process of the art, including all new operations.
Carey, Philadelphia, 1844.

74. Perry, B.
Water absorption in the ileum and colon.
Surgical Forum. 6: 347-51, 1956.

75. Pesci, A. and Sangiorgo, M.
Clinical and physiopathological notes on total colectomy with preservation of sphincter function.
Policlinico Sezione Chir. 65: 91, 1958.

76. Ravitch, M. M. and Sabiston, D. C.
Anal ileostomy with sphincter preservation.
Surg., Gyne. & Obst. 84: 1095-99, 1947.

77. Ravitch, M. M.
Anal ileostomy with sphincter preservation in patients requiring total colectomy for benign lesions.
Surgery. 24: 170, 1948.

78. Reverdin, J. L.
Greffe épidermique.
Bull. Soc. Imperiale Chir. Paris, 10: 483, 1869.

79. Ravitch, M. M.
Personal communication, 1958.

80. Rice-Oxley, J. M. and Truelove, S.
Ulcerative colitis - course and prognosis.
Lancet. 258: 663, 1950.

81. Rogers, B. O.
Historical development of free skin grafting.
Surg. Clin. of N. A. 39: 289, 1959.

82. Roux, G., Marchal, G and Baumel, H.
Ileo-Ano-Plastie.
Marseille Chirurgical 8: (4), 1956.

83. Rush, B. J. and Cliffton, E. E.
Experimental reconstruction of the trachea with bladder mucosa.
Surgery. 40: 1105, 1956.

84. Sancassini.
Letter to Mr. Sebatiano Rotari, included in the "Fisico mediche dilucidazioni" by Dr. Sancassini, Rome, 1731 (cited by Rogers, B. O. Historical development of free skin grafting. Surg. Clin. of North America. 39: 289, 1959.)

85. Schaffer, R. C.
Familial colonic polyposis.
Amer. J. Surg. 84: 477, 1952.

86. Schlitt, R. J., McNally, J. J., Shafiroff, B.G.P., Hinton, J. W.
Pelvic autonomic neurectomy for ulcerative colitis.
Gastroenter. 19: 812, 1951.

87. Scott, H.W.J. and Cantrell, J. R.
Colonmetrographic studies of the effects of section of the parasympathetic nerves of the colon.
Bull. Johns Hopkins Hospital. 85: 310, 1949.

88. Shackelford, R. T.
Surgery of the alimentary tract. Vol. II.
W. B. Saunders, Philadelphia, 1955, pp. 1610.

89. Shafiroff, B.G.P. and Hinton, J. W.
Denervation of the pelvic colon for ulcerative colitis.
Surg. Forum.p134, 1950.

90. Sushruta.
An English translation of the Sushruta Samhita based on original Sanskrit Text. 3 volumes. Edited and published by Kaviraj Kunja Lal Bhishagratha, Bose, Calcutta, 1907-1916.

91. Swenson, O., Segnitz, H. H. and Shed, R. H.
Hirschsprung's disease: New surgical treatment.
Amer. J. Surg. 81: 341-347, 1951.

92. Swenson, O.
Hirschsprung's Disease (Aganglionic Megacolon)
New Eng. J. Med. 260: 972, 1959.

93. Teicher, I. and Abrahams, J. I.
The treatment of selected cases of multiple polyps, Familial Polyposis and Diverticular disease of the colon by subtotal colectomy and ileoproctostomy.
Surg., Gyne. & Obst. 103: 136, 1956.

94. Thiersch, C.
Über Hautnerpflanzung.
Verhandl. deutsch. Ges. F. Chir. 15: 17, 1886.

95. Titkemeyer, C. W. and Calhoun, M. L.
A comparative study of the structure of the small intestines of domestic animals.
Amer. J. Vet. Res. 16: 152-159, 1955.

96. Trautmann, A. and Fiebiger, J.
Fundaments of the histology of the domestic animals.
Trans. by Habel, R. E. Comstock Publishing Assoc., Ithaca, New York, 1949.

97. Turell, R.
Healing of ano-rectal wounds.
J. A. M. A. 166: 780, 1958.

98. Valiente, M. A. and Bacon, H. E.
Construction of pouch using pantaloon technique for pull-through of ileum following total colectomy.
Amer. J. Surg. 90: 742, 1955.

99. Wangensteen, O. H.
Discussion of paper by Karlson, K. E. and Dennis, C.
Amer. J. Gastroenter. 24: 436, 1955.

100. Wagensteen, O. H. and Lillehei, R. C.
Bowel function after colectomy for carcinoma, polyposis and diverticuli.
J. A. M. A. 159: 163, 1955.

101. Warren, J. M.
Rhinoplastic Operations: With some remarks on the autoplastic methods usually adopted for the restoration of parts lost by accident or disease. Cited by Rogers, B. O. 'Historical development of free skin grafting.' Surg. Clin. of North America. 39: 289, 1959.
Clapp, Boston, 1840.

102. Waugh, J. M., Miller, E. M. and Kurzweg, F.T.
Abdomino-perineal resection with sphincter preservation for carcinoma of the mid-rectum.
A. M. A. Arch. Surg. 68: 469, 1954.

103. Wolfe, J. R.
A new method of performing plastic operations.
Brit. Med. J. 2: 360, 1875.

104. Wheelock, F. C. and Warren, R.
Ulcerative colitis.
New Eng. J. Med. 252: 421, 1955.