THE STUDY OF THE ETIOLOGY OF POST-SURGICAL OBSTRUCTION IN PATIENTS WITH HIRSCHSPRUNG'S DISEASE

SAMUEL WILLIAM MOORE

A THESIS SUBMITTED IN FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

UNIVERSITY OF CAPE TOWN

1993

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

DEDICATION

To Ann, Jenni, David, Samuel and Jonathan for their love, patience, support and understanding.

And to the patients, without whom this work would not have taken place.

HARALD HIRSCHSPRUNG

(1830 - 1916)

A DEFINITION OF HIRSCHSPRUNG'S DISEASE

Hirschsprung's disease is a functional obstruction of the gastrointestinal tract resulting from the congenital absence of ganglion cells in the myenteric plexus of varying lengths of distal bowel.

SUMMARY

The cause of obstructive symptoms following otherwise successful surgical correction of Hirschsprung's disease, is the subject of considerable debate. In addition to known causes such as technical factors or a retained aganglionic segment, the association with co-existing dysplastic conditions of the enteric nervous system has attracted considerable interest in recent years. The object of this research was to identify a patient group with obstructive symptoms and to explore etiological factors. A secondary aim was to assess the role of abnormal enteric nervous system development on the outcome of surgery for Hirschsprung's disease.

The aim of the preliminary studies was to evaluate patients with Hirschsprung's disease to assess the extent of the problem. An initial retrospective study was undertaken on 378 patients with Hirschsprung's disease who presented to the Red Cross War Memorial Children's Hospital between the years 1957-1991. Demographic features, familial associations, associated abnormalities, timing and clinical presentation, length of segment, management and outcome of this patient sample were among the factors explored. Results of this study confirmed the clinical impression that the majority of patients do well in terms of eventual anorectal control. In addition, the study failed to demonstrate any significant associations with postoperative obstructive symptoms and the clinical parameters studied. In a number of continent patients, however, ongoing symptoms of an obstructive nature were observed.

The true extent of the problem was difficult to assess on retrospective evaluation and this led to a second study which involved the recall of survivors for current evaluation.

Patients who were selected were those who could be expected to have a good result; those with a known cause for their obstructive symptoms were excluded. One hundred and seventy-eight patients responded to recall and interview. These represented 74.1% of traceable survivors. The patient sample was representative of the total patient population in terms of demographic features, length of aganglionic segment and clinical features. One hundred and fifteen of these patients were older than 4 years of age, an arbitrary level set for the evaluation of anorectal function. Ninety-four percent of these patients were continent. Sixteen (14%) of the 115 had obstructive symptoms not attributable to other factors. Specific investigation of this group showed a high incidence of complications and failure to thrive. Although enterocolitis

occurred in 31% of these patients, no specific association with enterocolitis could be identified.

These patients were further investigated both clinically, radiologically and manometrically to determine possible causes of their dysfunction. The internal anal sphincter did not appear to be the source of the problem on manometric assessment of anorectal function. No significant differences were detected in any of the measured manometric parameters of internal anal sphincter function in patients with obstructive symptoms, those without postoperative obstruction or controls. A restored postoperative rectosphincteric reflex was an uncommon finding and in the light of the satisfactory clinical result in the majority of patients, little significance was attributed to the presence or absence of the RSR in determining postoperative anorectal function. This pointed to a possible role of the abnormally developed enteric nervous system in producing these symptoms.

Examination of rectal suction biopsies in the 16 patients with obstructive symptoms showed histological evidence of residual abnormalities of the enteric nervous system in 14 (87.5%). Histological abnormalities included neuronal intestinal dysplasia (9 [56%]), aganglionosis in 4 and ganglioneuromatosis in 1. In two patients the biopsy was normal. The group of 9 patients with co-existing NID represented 7.8% of those 115 patients over the age of 4 years, who responded to long term follow-up which is lower than the reported incidence.

These findings appeared to support a hypothesis that enteric nervous system abnormalities may be a possible cause of obstructive symptoms following the surgical correction of Hirschsprung's disease. On the other hand, considerable debate surrounds the significance of co-existing features of NID in the residual bowel of patients undergoing Hirschsprung's pull-through surgery. In order to clarify some of these issues, the second part of the thesis attempted to explore aspects of the NID debate.

Biopsy specimens from 32 patients undergoing surgical treatment of Hirschsprung's disease were explored in a prospective study. In addition, control specimens were obtained from patients undergoing surgery for conditions other than Hirschsprung's disease as well as 5 patients with NID. A wide spectrum of morphological changes was observed in the enteric nervous system of the ganglionated bowel of patients with Hirschsprung's disease, NID and control specimens. Hyperganglionosis was shown to be present on full transverse sections of

the ganglionated bowel in 4 patients with Hirschsprung's disease. Enteric nervous system morphology in these 4 were similar to those observed in the NID group. In patients without hyperganglionosis, enteric nervous system morphology was comparable to that of controls. Methods of assessing hyperganglionosis were evaluated and found to be subject to considerable variation. The most reliable single parameter of assessing hyperganglionosis was the number of cells per ganglion in the submucous plexus. Ganglion cell counts varied considerably within the sections, suggesting that the diagnosis of hyperganglionosis remains an ongoing problem.

Although AChE staining patterns similar to those seen in NID were observed in the ganglionated bowel of 19% of patients with Hirschsprung's disease, it was observed in 38% of the control patients. Differences in morphology suggested a more severe pathological process in those with NID. Difficulties in assessing the significance of the histological observations led to the hypothesis that the clinical significance of the histological findings of NID are related to the overall picture of severity.

A histological scoring system was developed to test this hypothesis and correlated with the clinical outcome of the patients. Positive associations were observed between a score greater than 50% and functional postoperative obstruction in the NID group as well as in 80% of patients with co-existing NID and Hirschsprung's disease. This suggests that the scoring system is of value in predicting those patients who will develop clinical symptoms of obstruction postoperatively.

In order to investigate whether hyperganglionosis and other features of NID are an acquired feature, an animal model of non-strangulating lower intestinal obstruction was developed. The effect of prolonged obstruction on the enteric nervous system was ascertained. Two anaesthetic protocols were employed (Xylazine or Ether in association with Ketamine hydrochloride). Results demonstrated no morphological features of NID resulting from prolonged intestinal obstruction. An increase in AChE expression noted in certain animals was attributed to the effects on the alpha-2 adrenergic receptor by the anaesthetic agent Xylazine. This finding suggests a role for the alpha-2 adrenergic receptors of the sympathetic nervous system in the increase of AChE. The increase in AChE expression was not associated with an

increase in Tyrosine hydroxylase, the rate limiting enzyme in catecholamine production. The possible significance of these findings is discussed.

The reasons for an increase in AChE expression in Hirschsprung's disease and NID are unclear. Further biochemical studies into the molecular forms of AChE were undertaken to explore the cholinesterase role in the pathophysiology of Hirschsprung's disease. An increase in AChE was demonstrated in the aganglionic bowel and was associated with a disturbance in the AChE:BChE ratio, confirming previous reports. The increase was in the AChE fraction and not the total cholinesterase activity. In further sucrose velocity gradients, this increase in AChE was shown to consist mainly of an increase in the tetrameric form, AChE-G4. The majority of this increased enzyme isoform was found to be in the amphiphilic form on sequential extractions in low salt, high salt and detergent solutions. Because this form predominates in the embryological development, a possible role for AChE as a neuromodulator is suggested.

In keeping with the disturbance in the AChE:BChE ratio, levels of BChE activity were, in general, exceptionally low in aganglionic tissue. A significantly higher BChE activity level was noted in the aganglionic tissue of patients with co-existing NID. This could possibly account for the hyperganglionosis observed in NID. It is also suggested that the increased BChE in the aganglionic segment of patients with co-existing NID could form the basis of a possible diagnostic tool.

Further exploration of genetic associations failed to reveal any possible linkage with obstructive symptoms. This area requires more research and future studies are being planned.

STATEMENT OF CANDIDATE

I declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the work nor any part of it has been submitted for another degree to this or any other university.

ACKNOWLEDGEMENTS

The work presented in this thesis was undertaken at the Red Cross Children's Hospital in Cape Town, South Africa. I wish, in particular, to thank Professor Sid Cywes for his inspiration and continuing advice and especially his support and help during this project.

In addition, I would like to express my indebtedness to Professor R Kaschula, head of the department of Pathology at the Red Cross Hospital for his valuable insights and instruction in the difficult field of histopathology. In addition, I acknowledge his help in photographing the histopathological slides.

I am also indebted to many others for assistance given during the course of this research. In particular, I would like to thank the following people

- Professor Heinz Rode for his continuing encouragement and help
- Professor Alistair Millar for introducing me to the field of anorectal manometry
- Doctor Glynnis Johnson and Mr Peter de Wet for assistance in the biochemical assays. I wish to thank Dr Johnson especially for her enthusiasm and help in identifying the isomeric forms of AChE
- Doctor David Laing for sharing many hours with me over the microscope and for valuable insights. Doctor Martha Cohen for specific help in assessing the histochemistry of patients with acquired aganglionosis
- Colleen Jackson, Di Blake and the team in the histopathology laboratory who did many hours of extra work in processing the specimens
- Ms Jeanette Melis and Mr Matthew Bok for assistance with the animal study
- Ms Rene Albertyn, Sr Ferreira and the staff of the Anorectal Clinic for assistance and support during the patient interviews
- Dr Bob Stunden, for help with computerization in the initial stages of this project
- Professor Eric Bateman for his friendship, help and valuable advice
- Professor P Beighton for the photograph of Harald Hirschsprung
- My special thanks to Mrs Lynore Heuer for asistance in setting out this manuscript and to Ms M Moss and Mrs M van Niekerk for assistance with the art work and final preparation

To others who helped at one stage or another, as well as my family and friends and especially my wife, Ann, for her considerable help and support, I would like to express my sincere thanks.

ABBREVIATIONS

AG	Aganglionosis
AChE	Acetylcholinesterase
ACP	Anal Canal Pressure
ACPD	Anal Canal Pressure difference
ASPB	Anal sphincter pressure barrier index
BChE	Butyrylcholinesterase
ChE	Cholinesterase (Total)
ENS	Enteric Nervous System
E	Embryonal day
GI	Gastrointestinal
GIT	Gastrointestinal tract
HD	Hirschsprung's Disease
HNK	Human Natural Killer cells
MHC	Major Histocompatability Antigens
MTV	Maximal tolerated volume
NSE	Neuron Specific Enolase
NID	Neuronal Intestinal Dysplasia
RRP	Resting rectal pressure
RSR	Rectosphincteric relaxation reflex
SERP	Soave endorectal pull-through
TCA	Total colonic aganglionosis
TOH	Tyrosine hydroxylase
VIP	Vasoactive Intestinal polypeptide

CONTENTS

LIST OF TABLES

LIST OF FIGURES

SECTION A

INTRODUCTION

CHAPTER 1

A PERSPECTIVE OF HIRSCHSPRUNG'S DISEASE

1.1 A DEFINITION OF HIRSCHSPRUNG'S DISEASE

Hirschsprung's disease is a functional obstruction of the gastrointestinal tract resulting from the congenital absence of ganglion cells in the myenteric plexus of varying lengths of distal bowel.

1.2. INTRODUCTION

Hirschsprung's disease (aganglionic megacolon) is the commonest cause of neonatal intestinal obstruction and is a fairly frequent cause of functional low intestinal obstruction in older children. It is currently understood to be a neurodevelopmental disorder, the absence of ganglion cells in the submucosal and Auerbach's plexus believed to be caused by a lack of caudal migration or differentiation during intrauterine neurogenesis (Okamoto 1967). As such, it may possibly represent one end of a spectrum of neurodevelopmental disorders resulting from a defective maturation of myenteric ganglion cells in addition to migration and differentiation defects (Gershon 1980; Le Douarin 1974; Meijers 1987).

Hirschsprung's disease occurs in 0.2% of the population, 17.6 per 100 000 population or 1 in 5000 live births (Ehrenpreiss 1970; Holschneider 1982; Fadda 1987). The male to female ratio is 4:1 and has been reported in all racial groups. It been reported to be uncommon in blacks in the USA (Swenson 1973), but the true incidence in Africa is as yet unclear.

A variety of surgical procedures have been developed for the surgical management of Hirschsprung's disease since the original operation of Swenson and Bill (Swenson 1948). Satisfactory results have been reported (Kleinhaus 1979; Holschneider 1983; Morikawa 1989) and the postoperative results appear to be similar for the various operations.

Despite the good overall outcome following surgical correction, the majority of series include some patients who have a less than favorable outcome (Lawson 1972; Rehbein 1960, 1966; Kleinhaus 1979; Holschneider 1983; Nixon 1985; Mishalany 1989; Molenaar 1989). Long term outcome is largely independent of the type of surgical procedure and very similar complications occur following all the most frequently used procedures (Holschneider 1983; Kleinhaus 1979). In a review of the published results of over 5000 patients reported in the literature, Holschneider (1982) identified a recurring postoperative complication of obstipation in approximately 10% of patients. Obstructive symptoms occur after all types of surgical pull-through procedures (Lawson 1972; Kleinhaus 1979). An incidence of 6-18% has been reported in children treated with the Swenson, Duhamel and Soave procedures (Lawson 1972; Soto 1977; Kleinhaus 1979; Holschneider 1982; Cass 1990). Lawson (Lawson 1972) reported an incidence of postoperative obstruction in 17% of patients. Some more recent series (Mishalany 1987; Keiley 1990; Kluck 1986) although incomplete, drew attention to an even higher incidence of functional obstruction despite a good anatomic result. It is thus possible that the importance and incidence of obstructive symptoms has been underreported in certain series, particularly where reports concentrate on anatomic results and continence only.

Obstructive symptoms had been attributed to inadequate resection of **aganglionic bowel** (Lawson 1972; Keiley 1990) **or mechanical causes** such as postoperative stricture formation (Keiley 1990; Holschneider 1982). **Technical and mechanical causes** have been most frequently implicated as the factors responsible for the post surgical problem of obstructive symptoms which include pelvic infection and anastomotic leak resulting in **scarring and stricture formation** (Fonkalsrud 1986). As a result, a number of technical modifications to existing operations have been introduced (Swenson 1957; Duhamel 1960; Grob 1959; Denda 1966; Ikeda 1967). These modifications have failed to prevent the development of postoperative obstructive symptoms and these still occur in a significant proportion of patients who underwent modified pull-through procedures (De Lorimier 1986).

Increased activity or **achalasia** of the internal anal sphincter (Fadda 1987), **motility disorders** (Morikawa 1989) or **residual segments of the dysplastic** enteric nervous system (Fadda 1987; Kluck 1986), are other possibilities which require further evaluation.

Internal anal sphincter spasm was implicated as a possible cause of Hirschsprung's disease as early as 1900 (Fenwick 1900). Hurst (1925) recognized the importance of the internal anal sphincter in producing a megarectum. Initially, it was thought that sphincter spasm caused the obstruction. A comparison with achalasia of the lower oesophageal sphincter has suggested that failure of the internal anal sphincter to relax in response to the correct stimuli is the more likely mechanism (Hurst 1934). Both mechanisms may be operative in different patients with an obstructive clinical picture.

The internal anal sphincter has been identified by Rehbein (Rehbein 1960, 1966) and others (Holschneider 1982; Hecker and Holschneider 1973; Holschneider 1983; Holschneider and Fendel 1974) as the main cause of obstructive symptoms. Rehbein (Rehbein 1966) reported a 20-30% incidence of sphincter achalasia in patients following the Rehbein and other procedures.

The natural growth in the length of the anal canal length from 1.9 cm in the newborn, to 2.5 cm in a 10 year old child and 4 cm in an adult (Golligher 1975) may magnify a small technical problem occurring at the time of initial surgery and may result in postoperative outlet obstruction.

The length and function of the residual internal anal sphincter remains one of the constant technical problems associated with surgery for Hirschsprung's disease. None of the procedures in current use is designed to remove the whole of the internal anal sphincter despite numerous technical modifications being made to the four most commonly performed procedures (Swenson 1957; Grob 1959; Boley 1964; Denda 1966). Rehbein (Rehbein 1966) stressed the importance of overcoming "**sphincter achalasia**" in the postoperative period (Rehbein 1982; Fadda 1987). The technical challenge for the surgeon remains the creation of a sufficiently low posterior anastomosis without bringing about faecal incontinence.

In a number of series (Rehbein 1966; Holschneider 1982; De Lorimier 1986), obstruction was not adequately prevented by the forceful dilatation of the anal canal. This suggests that although certain patients may have some form of outlet obstruction, the sphincter is not the only possible mechanism causing obstructive symptoms following an otherwise successful surgical correction of Hirschsprung's disease.

Abnormal histologic features have been reported in 10-15% of patients with persisting postoperative symptoms (Cass 1990; Kluck 1986; Molenaar 1989). Rehbein (Rehbein 1966) and Kluck (Kluck 1986) have both suggested that residual co-existing neuronal dysplasias may contribute to the persistence of obstructive symptoms into the postoperative period. These include histologically diagnosed neurodysplastic disorders, such as immaturity of ganglion cells (Bughaighis 1974), hypoganglionosis (Dajani 1986, Rintala 1992), ganglioneuromatosis (Khan 1987, Staple 1964, Ternberg 1965) as well as neuronal intestinal dysplasia (NID) (Puri 1977; Schärli 1981).

Although the role played by dysplastic histological features is as yet unclear, subtle alterations in proximal ganglionated bowel may not be recognized in the residual bowel at the time of surgery. These may account for certain incomplete responses to apparently adequate surgical management. In support of this view, Kluck (Kluck 1986) reported abnormal immunocytochemical staining to a neurofilament monoclonal antibody in 18 (82%) of the 22 patients who had postoperative obstipation. These 22 represented 20.3% of the 108 patients operated on for Hirschsprung's disease over a 9 year period. This suggests that co-existing disease contributed to persisting symptoms in the majority of his patients with postoperative obstructive symptoms.

Clarification of the functional significance of atypical histologic features in the proximal ganglionated bowel in Hirschsprung's disease may influence decisions taken during the operation. These include the resection level at the time of pull-through, as well the determination of the length of bowel to be resected. It may, in addition, serve as a means of predicting the functional outcome of surgical management.

1.3 AIMS OF STUDY

This project was commenced with the following aims:

1. To develop a database from which the incidence, demographic features and associated conditions of Hirschsprung's disease could be evaluated.
2. To evaluate the long term outcome of Hirschsprung's disease in a defined population (patients treated at Red Cross War Memorial Children's Hospital 1957-1990) was evaluated and to assess functional outcome.
3. To determine the relative incidence and causes of obstructive symptoms to test the hypothesis that obstructive symptoms are a significant postoperative complication and are related to sphincter overactivity.
4. To explore the debate surrounding the incidence and significance of Neuronal intestinal dysplasia (NID) which included attempts to define NID and to improve the capability of diagnosing it as it co-exists with Hirschsprung's disease.
5. To examine by experimental methods the hypothesis that other factors may lead to secondary alterations of the enteric nervous system which may influence results.

We limited our research mainly in these areas of investigation but where additional aspects of the pathophysiology were encountered, they were studied to establish their relationship in the etiology of Hirschsprung's disease.

1.4. A LITERATURE PERSPECTIVE OF HIRSCHSPRUNG'S DISEASE

The first report of congenital megacolon is believed to have been made by the Dutch anatomist Frederick Ruysch in 1691 (Leenders and Sieber 1970). Other early case reports were submitted by Parry (1825) and Billard (1829) (Leenders 1970). Early descriptions were mostly of adults with a relatively short history of intestinal obstruction (Finney 1908; Schneiderhöhn 1915; Ehrenpreiss 1946).

Some early reports described cases could have been due to other causes of megacolon (eg. inflammatory) rather than congenital aganglionosis. This problem of definition was very much a part of the history of the description of this disease for many years, even after publication of the first scientific description by Harald Hirschsprung (Hirschsprung 1888; Corman 1981).

Parry (Parry 1825) first described the critically important narrow distal segment occurring in association with the megacolon. He published the autopsy findings of an adult who died from intestinal obstruction following a long history of flatulence and constipation. His description was that of a megacolon which terminated at the beginning of the sigmoid flexure at which point it returned to almost its "normal" size. Subsequent reports by Barth (1870) and Gee (1884) also demonstrated this feature in a 10 year old and in a 4 year old respectively.

Jacobi (1869) reported two male infants with intestinal obstruction occurring within the first few days of life, one of whom died of sepsis following a colostomy which had successfully relieved the obstruction. The other responded to repeated washouts. Other reports of similar findings in young adults also appeared in the literature (Ebers 1836; Peacock 1872)

The report by Harald Hirschsprung in 1886 was the first detailed description of congenital aganglionosis. His lecture entitled "Stuhlträgheit zigheit neugeborener infolge von dilatation und hypertophie des colons", was delivered to the Gesellschaft fur Kinderheilkunde in Berlin in 1886. In this lecture, Hirschsprung described observations on what he thought to be a new and rare disease based on the autopsy findings in 2 children. His initial paper on the subject was published somewhat later (Hirschsprung 1888; Corman 1981).

In his meticulous analysis of the two cases, who had survived for 7 and 11 months respectively, Hirschsprung stressed the congenital nature of the disease and in 1904 named it "congenital dilatation of the colon" (Hirschsprung 1904). He offered no definitive opinion about the possible etiology and pathogenesis of this condition but believed that the colon was congenitally defective. Lack of appreciation of his second important observation, namely the narrowed rectum, led to a fifty year delay in the adequate management of this disease. As a result, the most commonly held theory before 1945 was that the primary site of the disease was in the hypertrophied dilated segment. In the period of time between Hirschsprung's original description until 1948, the emphasis placed on the dilated proximal segment led to considerable confusion between acquired and congenital causes of megacolon.

1.4.1 Early Theories on Pathogenesis

1.4.1.1 *The role of the internal anal sphincter*

An early opinion by Marfan (1895) was that a mechanical obstruction, caused by the elongation and kinking of the bowel at the rectosigmoid junction, played an important role in the disease. This opinion was widely accepted at the time.

A skeptical Treves (1898) appreciated the obstructive nature of the changes in the proximal dilated colon. He believed that the anal sphincter was involved in the pathogenesis of the disease and as a result he excised the distal bowel as well as the anal sphincters of a female patient to create a perineal anal stoma. Although this opinion was not appreciated by other surgeons of his era, Treves' action was supported by the long term survival of the patient. Johnson, reporting on the patient 60 years later, following a laparotomy for adhesive bowel obstruction, described her as having led an "unusually active life" (Johnson 1957).

The concept of anal achalasia originated from the work of Fenwick (Fenwick 1900) who attributed idiopathic megacolon to a spasm of the internal sphincter muscle. Hurst (Hurst 1925) postulated that the sphincter was not in spasm but that a functional disturbance existed resulting from the inability of the sphincter to open. This was considered to be similar to the achalasia occurring at the lower oesophageal sphincter which at the time had already been reported (Hurst 1925).

1.4.1.2 *A neurogenic hypothesis for megacolon*

Hawkins (1907) suggested neuropathic dilatation and hypertrophy as the basis for the paralysis of the bowel. Cameron (1928) demonstrated inflammatory replacement of ganglion cells similar to that encountered in cardiospasm.

Fraser (1926) suggested that a delay in the acquisition of inhibition was a cause of the dilatation and hypertrophy of the muscle. Hurst (1925) noted similarities to achalasia on the basis of decreased parasympathetic activity of the anal sphincter in Hirschsprung's disease.

Supported by the experimental work of Adamson and Aird (1932), the concept of autonomic imbalance became widely accepted. A report by Adson (1937) led to a spate of lumbar sympathectomies being performed on these patients (Wade and Royle 1927) with largely poor results (Ross 1935; Whitehouse 1943). In addition, it also led to various forms of medical therapy being attempted (Klingman 1938; Law 1940) with varying degrees of short term relief of symptoms. Noting the poor results of medical and surgical treatment, Whitehouse (1943) suggested segmental resection. Grimson (1945) proposed that the resection be extended to a colectomy because of failure of segmental resection in a number of cases.

In his classic monograph, Ehrenpreiss (1946) compared the radiological findings of 10 affected infants with 100 normal newborn infants. He noted that the colonic distension which developed after birth resulted from motility disturbances existing from birth in all 10 patients. Although the exact cause of the motility disturbance could not be determined, he postulated "a dysfunction of the evacuation of the colon of as yet unknown origin, occurring without morphological and mechanical causations and giving rise secondarily to a characteristic dilatation of the colon" (Ehrenpreiss 1946). At this stage Ehrenpreiss believed that the megacolon in Hirschsprung's disease developed as a result of the obstruction assumed to be of neurogenic origin (Ehrenpreiss 1946, 1966).

1.4.1.3 *Morphological characteristics - the aganglionosis saga*

Even before 1948, histologic changes in patients with Hirschsprung's disease had been reported. Aganglionosis of the distal segment had first been reported by Tittel in 1901 (1901) and confirmed by other isolated reports (Brentano 1904; Dalla Valla 1920, 1924; Cameron 1928; Robertson 1938; Tiffin 1940). Aganglionosis was originally thought to be a secondary phenomenon caused by intestinal obstruction (Adamson 1932). In addition, difficulties in interpreting the abnormal histologic findings were experienced and normally ganglionated bowel was reported by other workers (Schmidt 1909; Adamson 1932; Hurst 1934). Adamson and Bird (1932) believed that the rare absence of ganglion cells was a secondary phenomenon produced by the pathologically significant distension and chronic stasis in the megacolon. On reviewing the literature, Tiffin et al (1940) suggested that cases of absence of the myenteric plexus may form a separate group of "idiopathic megacolon". Whitehouse (1943) also assumed that the aganglionosis was a secondary phenomenon and advocated the resection of the dilated colon leaving the proximal and distal portions in situ.

1.4.1.4 *Emphasis on the "Narrowed" Distal Intestinal Segment*

Although the narrowed distal segment was described early on, the significance was not appreciated. Credit for the first description has been given to Alvarez (1922) although this was mentioned in some early reports (Parry 1825; Barth 1870; Gee 1884). Robertson and Kernohan (Robertson 1938) and Tiffin (Tiffin 1940) suggested that the disease was due to abnormal peristalsis across the aganglionic segment.

Subsequent to the demonstration of distal aganglionosis in 1948 (Zuelzer 1948; Whitehouse 1948), the emphasis shifted at last to the importance of this distal segment in the management of Hirschsprung's disease.

In January 1948, Swenson and Bill presented their work to the Society of University Surgeons describing "a narrowing of the distal colon on barium enema and the absence of peristalsis in the distal colon" (Swenson and Bill 1948). They suggested that the removal of the colonic segment with preservation of the sphincter ani cured these patients . Despite the claim by Swenson that this report "was the first evidence that the ganglionic defect was part of the disease" (Swenson 1989), his observation was based on manometric, not histologic, data.

Swenson's claim that they were the first to publish the cause of the obstruction as well as the first successful treatment for this condition (Swenson 1989), is supported by the fact that the reports identifying the localized absence of ganglion cells in the distal segment as the cause of the functional obstruction were published later in that eventful year (Zuelzer and Wilson 1948; Kernohan and Whitehouse 1948).

On the other hand, in view of the omission by Swenson to mention the aganglionic segment in his initial report as well as the fact that his conclusions were based on the absence of peristalsis in the narrow segment, Zuelzer and Wilson must be given the credit for being the first workers to correctly interpret the significance of the distal aganglionic segment (Zuelzer and Wilson 1948). Their study, published in February 1948, correlated the clinical, pathological and radiological data of 11 patients with Hirschsprung's disease and reported ganglion cells in the proximal portion of the intestinal tract, whereas "distally none could be found" on study of the 5 of the 11 cases. They concluded that the functional disturbance of intestinal motility in such cases was related to the congenital absence of nerve cells in the enteric nervous system. This concept is still held today and appears to explain the clinical presentation of Hirschsprung's disease.

The work of Whitehouse and Kernohan published in June 1948 confirmed the distal aganglionosis in Hirschsprung's disease. They observed an additional increase in the size of the peripheral nerves in the distal colon. The transitional zone was noted to be variable in extent and contained occasional isolated ganglion cells with some abnormal peripheral nerves. Whitehouse, in particular, noted a very long transitional zone with a variable number of ganglion cells. This included hyperganglionosis in the enteric nervous system in some cases. The significance of this observation is currently being appreciated in patients where there is co-existing neuronal intestinal dysplasia (NID).

Bodian and Stephens (1949) reviewed 73 patients and demonstrated the aganglionosis in 15 stored autopsy specimens. They noted 2 major groups of patients with megacolon. Thirty - nine of the 73 patients reviewed were noted to have characteristic Hirschsprung's disease. There was also a second group of patients with a milder clinical course among the remainder who responded to

conservative treatment. These could possibly have had a very short segment aganglionosis or other subtle neuropathic lesions causing their megacolon (Cass 1986).

1.4.1.5 *Interest in the Proximal Megacolon of Hirschsprung's disease*

The first 50 years following the scientific description of the disease by Harold Hirschsprung in 1886 were mostly spent ignoring the distal aganglionic segment of bowel and thus preventing the development of an effective treatment of Hirschsprung's disease. Difficulty in interpreting the histological findings in the proximal megacolon still exist and the confusing picture of the possible causes of megacolon which emerged, still requires clarification despite recent advances in classification (Kristamurthy and Schuffler 1979; Rode et al 1992).

Functional obstruction of the colon without aganglionosis has been attributed to several histologic features. These include hypoganglionosis (Munakata 1992), immaturity of the ganglion cells (Bughaighis 1974), neuronal colonic or intestinal dysplasia (Puri 1977; Schärli 1981), ganglioneuromatosis of the nerves of the bowel wall (Staple 1964; Ternberg 1965), or other conditions of a congenital or acquired nature, including chronic idiopathic intestinal obstruction (CIIP) (Schuffler 1982). Histologic features are often difficult to interpret and so specialized histochemical or immunocytochemical techniques are employed. The interpretation of histologic findings is complicated by the possible coexistence of other neuronal dysplasias with Hirschsprung's disease and variations in the length of the transitional zone.

Well documented reports of skip lesions of aganglionosis exist in the literature (Tiffin 1940; Bodian 1949; Sprinz 1961; Lawrence 1961; Leenders 1970; MacIver 1972). If these exist they are considered to be a rare occurrence

(Ehrenpreiss 1965; Weinberg 1970). Neuronal intestinal dysplasia is also known to be patchy on occasions (Rintala 1989).

Architectural and neurotransmitter marker abnormalities have been reported in the proximal dilated bowel of patients with continued obstructive symptoms after apparently successful surgery for Hirschsprung's disease (Tam 1990). The intention of this study is to explore possible causes of these obstructive symptoms.

1.4.2 A brief history of surgery for Hirschsprung's disease at the University of Cape Town

Under the pioneering leadership of Professor JH Louw, a small paediatric surgical unit was established at the Groote Schuur Hospital in Cape Town in 1952. The first successful long term survivors of surgery for Hirschsprung's disease had their surgical pull-through procedures in 1953 (Cywes 1976).

With the opening of the Red Cross War Memorial Children's Hospital on the 18th June, 1956, paediatric surgery in the Western Cape took a major step forward. This series commenced in 1957, when the work of the hospital became established. The first 2 survivors of surgery for Hirschsprung's disease, 23 yrs old at the time, were present at the inaugural lecture of Professor S Cywes, the first incumbent of the newly created Charles FM Saint Chair in Paediatric surgery in 1975 (Cywes 1976).

The initial surgical procedure for Hirschsprung's disease was the Swenson rectosigmoidectomy. A relatively high incidence of complications prompted a change to the Duhamel procedure. Forty consecutive patients were compared in a report by Professors Louw and Cywes (Cywes 1967; Louw 1981), the results being comparable. Certain theoretical and practical benefits prompted a change

to the Soave procedure in 1967. With the addition of the Boley modification since 1970, it has become the standard procedure for short segment disease in our unit. More than 200 of these procedures have been performed to date. A technical modification involving a low posterior anastomosis (Swenson 1957) was introduced in the early 1970's and splitting of the muscle cuff (Kasai 1975) was introduced in 1977. Long term follow-up has confirmed satisfactory results in the majority of patients, as far as continence is concerned.

CHAPTER 2

EMBRYOLOGICAL DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM AND CURRENT THEORIES OF HIRSCHSPRUNG'S DISEASE ETIOLOGY

2.1. EMBRYOLOGICAL DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM

The embryological development of the neural, myogenic and gastrointestinal hormonal systems may affect the gastrointestinal function of the child. The developmental changes in these systems continue after birth and have only been estimated as being fully mature by the age of 2 years (Smith 1969).

2.1.1 The origin of enteric neuroblasts

At approximately 18-21 days gestation the roof of the primitive gut separates from the neural plate. It then becomes attached to the notocord. The exact site of origin of the enteric nervous system (ENS) has been a subject of debate (Andrew 1971; Meijers 1989). Theories include a mesodermal origin (Remak 1847), the sympathetic nervous system (Kuntz 1910; Jones 1942) and neural tube (Abel 1912) as sites of origin.

The current understanding of the origin of the ENS is based on the avian embryo experiments of Yntema and Hammond (1954) and Le Douarin (1974). These theories suggest that nerve cell precursors arise from the craniocervical portion of the embryonic neural tube. These neuroblasts then follow the vagal fibres down the primitive gut (van Capenhout 1946; Yntema 1945; Okamoto 1967).

2.1.2 Migration of enteric neuroblasts

In the human foetus, the migration of neuroblasts in the gut is completed by the 12th week of gestation. The landmark article by Okamoto and Ueda (1967) demonstrated large vagal trunks reaching the pharynx at the 5th week of

gestation. Simultaneously, branches of the pelvic and pre-aortic plexuses reached the rectum. The sympathetic ganglia had been formed by this stage but intramural ganglia were absent. By the 6th week neuroblasts appeared as far as the cardia of the stomach. By the 7th week this had progressed and neuroblasts were observed in the proximal portion of the midgut. In the 8th week the neuroblasts were present in most of the intestine except the distal colon and rectum.

These findings suggest that a surge of neuroblasts progresses down the primitive gut in a craniocaudal direction following the wave of de-epithelialization of the splanchno-pleural wall. The developing gut is also elongating at this time. Consequently, the observed wave of migration may result from a passive displacement of neuroblasts as well as from migration of neuroblasts (Meijers 1989).

By the 12th week, neuroblasts were present in almost the entire length of the gut down to the distal rectum. In the chicken this has been shown to be most active at the 13 somite stage and is completed by the 14 somite stage (Allan 1980; Meijers 1989). In rats, neuroblast migration is completed by the 12th day of a 21 day gestation (Ito 1977). By way of contrast, it is completed by the 9th day of a 19 day gestation in the mouse (Rothman 1982).

The aggregation of neuroblasts to form ganglion cells then occurs. Those neuroblasts which are the first to migrate form the myenteric plexus. Neuroblasts differentiate to form the Auerbach's and Meissner's plexuses. The submucous plexus is formed from neuroblasts of the myenteric plexus migrating through the circular muscle. Evidence of a myenteric plexus is present in the stomach by the 12th week. This development continues in a caudocranial direction during the third and fourth months. Any interruption of enteric nervous

system development could conceivably give rise to congenital aganglionosis. An interruption at a different time of development may give rise to a neuronal dysplasia.

Neurones of the sensory and autonomic systems derive from the neural crest (Weston 1970; Le Douarin 1980). The maturation and cytodifferentiation of these cells occurs after their migration from the neural crest to their final destination (Le Douarin 1974; Noden 1975; Gershon 1980; Meijers 1989; Vaos 1989). The process of maturation may occur in the pre-migration phase, during migration or after migration is completed (Patterson 1978). This proliferation of neuroblasts, which may continue after migration has occurred, may also contribute to the impression of a caudad migration of neuroblasts (Rothman 1984). The elongation of the gut may also play a role in creating the impression of a progression down the bowel.

The almost simultaneous appearance of precursor cells at both ends of the gut reported in avian experimental studies (Le Douarin 1974; Keller 1976; Gershon 1980; Tam 1986; Vaos 1989), piglets (Hirobe 1988) as well as in humans (Vaos 1989) suggests a dual gradient of neuroblast development.

The theory that a second source of enteric neuroblasts exists in the sacral area is at variance with the work of Okamoto and Ueda (Okamoto 1967). Tam (1986) pointed out that although his experimental findings suggest a second source of sacral neuroblasts, it does not contradict that of Okamoto and Ueda. His observations were made between the 9th and 21st weeks and theirs between the 5th and 12th week of embryonal development. Should the congenital defect be already present prior to his observations, a single craniocaudal gradient could still be valid.

Meijers (1989), on the other hand, found no enteric nerves in the hindgut following transection of the bowel distal to the vanguard of advancing neuroblasts in the chick model. This suggests that the neural crest on its own does not give rise to normal enervation of the hindgut (Meijers 1989). Webster (Webster 1973) also suggested that the lumbosacral neural crest is not a source of migration of nerve cells migrating from the sacral level. His experiments demonstrated normal pelvic parasympathetic plexuses in mutant mice despite a failure in the colonization of the distal bowel with neuroblasts. The issue of a second sacral source of enteric neuroblasts has not yet been resolved and further evidence is required before a final conclusion can be reached.

2.1.3 Neuroblast differentiation

The differentiation and migration of neuroblasts are closely linked. The neuroblasts from the vagus which have migrated to the ventrolateral portion of the pharynx have the potential to differentiate into carotid body cells, nerve sheath Schwann cells, calcitonin producing cells and tissue deriving from the mesenchyme as well as the ENS (Hammond 1964; Pearse 1973; Le Douarin 1974; Ciment 1983; Kirby 1983; Bockman 1985).

The processes involved in the differentiation of these multipotential cells into region-specific phenotypes are poorly understood. There is some evidence that local embryonic matrices are important in the determination of region-specific phenotypes of neural crest cells (Perris 1988). A suitable microenvironment also appears essential for further neuronal development (Tam 1986; Vaos 1989). Cellular differentiation may precede migration and cholinesterase activity has been demonstrated in neural crest neuroblasts before migration takes place (Meijers 1987). The interaction between migrating enteric neuroblasts and the local microenvironment may be influenced by the genetically predetermined characteristics in these cells (Rothman 1984).

Morphoregulatory proteins or triggers are thus necessary for the initiation as well as the maintenance of a particular differentiated state. In keeping with the transitory totipotential nature of the neuroblasts, adrenergic properties are transiently expressed before cells differentiate into mature cholinergic neurones. This step is mediated by a specific morphoregulatory substance (Patterson 1978).

Local embryonic matrices have been reported as determining the region-specific phenotypes in neural crest cells in the neuronal processing pathway in the early stage of embryonal development (Perris 1988). Extracellular matrix proteins (hyaluronidate, glycosaminoglycans and fibronectin), form a network into which the neuroblasts migrate. These are important factors in cell adhesion and movement in the development of precursor cells at the early stage of embryonal development (Tibboel 1987; Perris 1988). There is some evidence that fibronectin is the most important of these substances (Erickson 1984). Fibronectin has been shown to increase the activity of neuroblasts in vitro (Sieber-Blum 1981). It also provokes adrenergic neuron development (Sieber-Blum 1981) and may act as an intermediary for nerve growth factor (Kamagata 1985).

On the other hand, studies with monoclonal antibodies in the chick model have shown no relationship between the relative locations of fibronectin and the migrating neuroblasts, suggesting that other factors may also be involved in this process (Tucker 1986).

Meijers et al (1989) have demonstrated neural crest colonization up to day 14 of embryonic life. Demonstration of HNK-1 immunoreactive mesenchymal cells at the site of the normal submucous plexus in the aneural bowel of chicken

embryos, has led Meijers and his co-workers to conclude that the HNK-1 immunoreactive mesenchymal cells are the targets or welcoming committee of migrating neuroblasts. Further studies suggest the expression of a 42 kD and 44 kD glycoprotein by these cells may be of significance in ENS development in the chicken model (Meijers 1989).

Morphoregulatory molecules have been identified in the development of the ENS. Examples of these are N-CAM, E-cadherin, N-cadherin and fibronectin. The HNK-1 regulatory molecules on the migrating neuroblasts have yet to be defined (Meijers 1989). Layer (1991) has reported a clear cut sequence of BChE, AChE and HNK-1 expression of cells in neurodevelopment and has suggested that the cholinesterases may have a neuromodulator role in the process of neurodevelopment.

Cholinesterase and peptidergic activity have been regarded as markers of mature phenotypic expression in the ENS (Rothman 1984). A report that antifibronectin-antibody inhibits cholinergic differentiation in the distal colon of E14-15 rat embryos suggests that fibronectin is an important factor in the in vitro maturation of the cholinergic phenotype following migration (Kamagata 1985). Jacobs-Cohen (1987) has presented some evidence that the hindgut and foregut are colonized with neuroblasts almost simultaneously. Rothman (1984) reported on the phenotypic expression by precursors of peptidergic neurones. He suggested that the appearance of a wave of mature neuroblasts may represent a maturation of precursor cells rather than an actual migration of neuroblasts.

2.2 CURRENT THEORIES OF HIRSCHSPRUNG'S DISEASE ETIOLOGY

The etiology of Hirschsprung's disease remains unclear. Essentially, aganglionosis may result from defective neuroblast migration (Okamoto 1967), proliferation (Bolande 1974) or a hostile local microenvironment (Rothman 1984; Jacobs-Cohen 1987; Vaos 1989), resulting in poor maturation. All three of these mechanisms possibly play a part in the development of Hirschsprung's disease.

2.2.1 Abnormalities of neuroblast development

2.2.1.1. *Defective distal migration of neuroblasts*

Okamoto and Ueda have suggested that terminal gut aganglionosis results from failure of caudal migration of ganglion cells in early embryonic life (Okamoto 1967). The reason why some neuroblasts fail to complete their migration is unknown.

Defects in the number of neuroblasts undergoing division have been suggested as a possible cause of Hirschsprung's disease (Bolande 1974). This hypothesis suggests that a defect occurs in the wave of neuroblasts progressing down the developing gut in a craniocaudal direction. It has been demonstrated that the front layer of the migrating neuroblasts proliferate during migration and even after aggregation (Meijers 1989). It is therefore possible that malformation of neural networks that control the function of the enteric nervous system or the loss of inhibitory neurones, may be involved in the etiology of Hirschsprung's disease and other neuronal dysplasias.

The influence of a hostile microenvironment appears to only affect the intrinsic but not extrinsic nerve development (Rothman 1982). Jacobs-Cohen (1987) has shown that in vitro, neuroblasts colonized control hindgut in co-culture experiments whereas the terminal 2 mm of distal bowel was resistant to the

development of neuroblasts in the lethal spotted mouse. Reasons for this may include the absence or abnormality of morphoregulatory substances. Deficiency of fibronectin has been reported as causing abnormal cholinergic and adrenergic development in the fetal colon (Kamagata 1985).

The important morphoregulatory molecules involved in neuroblast migration, proliferation and differentiation, are still unknown. Once identified, they may provide the clues to the real cause of Hirschsprung's disease. More recently, Payette (1987) has suggested that neuroblast migration may be prematurely arrested by the **thickened reduplicated enteric basal laminae** of the aganglionic segment of bowel. Further hypotheses include early stimulation of **premature neurite extension** which results in the premature cessation of neuroblast migration. The stimulus to premature neurite extension or cessation of the craniocaudal migration of neuroblasts might be located in the proximal segment and transitional zone rather in the aganglionic zone.

A further hypothesis suggests a possible **absence of an anti-trophic factor** as a cause of the excessive extrinsic neurofibrils of the lamina propria and muscularis mucosa in Hirschsprung's disease (Gannon 1969). The exact nature of the affected tropic factor remains unknown but further investigation of patients with Hirschsprung's disease may yield further clues.

2.2.1.2 *Defects in neuroblast differentiation and maturation*

An alternative explanation derives from defective differentiation and maturation of neuroblasts during development. The mechanisms by which this may occur include disordered proliferation of the enteric neural crest cells (Bolande 1974), disorders of enteric mesenchyme or a hostile local microenvironment in the developing intestine (Le Douarin 1974; Gershon 1980; Jacobs-Cohen 1987; Meijers 1987; Fujimoto 1989).

Extracellular matrix proteins are important factors in cell adhesion and movement in the development of precursor cells at the early stage of embryonal development (Meijers 1987). Recent evidence shows that local embryonic matrices determine region specific phenotypes of neural crest cells (Perris 1988). A suitable microenvironment is essential for neuronal development (Tam 1986; Vaos 1989). The possibility therefore exists that a failure of maturation or death of neuroblasts may occur after migration. This hypothesis is supported by animal experiments as well as clinical evidence suggesting the loss and malfunction of ganglion cells in oesophageal achalasia (Adams 1976), acquired aganglionosis (Touloukian 1975, 1988) and zonal type aganglionosis (Kadair 1977). If true, this could possibly explain the low incidence of Hirschsprung's disease in premature babies.

The theory of a dual gradient of neuroblast development favours a neuroenvironmental hypothesis. Even if this were proven, the arrest of the craniocaudal gradient of neuroblast migration could still be valid.

Both migration and functional maturation of neuroblasts could possibly be inhibited by common factors in the pathogenesis. Absence of these factors may result in Hirschsprung's disease or dysplastic changes depending on the timing and degree of interference with normal development.

2.2.1.3 *A vascular theory of Hirschsprung's disease*

Theories of a possible vascular cause of Hirschsprung's disease have been advanced (Earlam 1972, 1985). Taguchi (1985) has identified vascular abnormalities in the affected bowel in support of this hypothesis.

Further support for this hypothesis comes from the infrequent association of intestinal atresia and Hirschsprung's disease. Because atresia of the small intestine has been shown to be caused by an acute intrauterine vascular accident (Louw 1955), its association with congenital aganglionosis could possibly support an ischaemic hypothesis for Hirschsprung's disease (Haffner 1969; Earlam 1985). Possible causes of Hirschsprung's disease associated with intestinal atresia include an early first trimester vascular accident with loss of bowel. This will result in failure of the caudal migration of the myenteric plexus (Okamoto 1967).

It has also been suggested that excessive rotation of the intestine could be responsible for temporary ischaemia resulting in anoxic damage to the nerve cells (Earlam 1985). The presence of malrotation and an abnormal blood supply would possibly support this hypothesis, but early development of ganglion cells in the gut and the lack of histological evidence of ischaemia in the colon in most cases do not support this view (de Villiers 1966; Meijers 1987). In addition, most atresias have been shown to occur much later in embryological development (Hyde 1968; Rafferscheid 1982). Aganglionosis caused by an intra-uterine vascular accident occurring later in pregnancy has been shown to be associated with muscle fibrosis (de Villiers 1966).

The hypothesis of a vascular cause for Hirschsprung's disease would appear, at this stage, to be largely based on circumstantial evidence. Little experimental data is available demonstrating aganglionosis resulting from a vascular insult in the early stage of gestation when the neuroblast migration occurs. On the other hand, there is some experimental data suggesting that aganglionosis possibly results from a vascular accident occurring late in gestation (Earlam 1985). Abnormal arteries have been identified in affected bowel of patients undergoing surgical correction for Hirschsprung's disease (Lister 1966; Taguchi 1985).

Earlam (1985) has suggested that temporary ischaemia of 4 hour's duration is the cause of selective destruction of ganglion cells without permanent damage to other bowel wall components.

The main body of evidence against a possible vascular theory includes the failure of experimental selective ischaemia to result in aganglionosis (de Villiers 1966; Meijers 1987). In experiments with dogs, ganglion cells were identifiable after the hypoxic event in spite of features of hypoxia being produced in mucosa and muscle (de Villiers 1966). Furthermore, the experimental ligation of the main branch of the omphalomesenteric artery in chicken embryos to produce temporary and permanent intestinal ischaemia at an early stage of development (Meijers 1987) caused stenosis or intestinal atresia without selective loss of enteric neurones. Sympathetic neurones have been shown to be affected in animals after one hour's ischaemia (Tyohara 1986).

Our own findings in 3 cases where intestinal atresia was associated with Hirschsprung's disease (Moore 1990) suggests that the most plausible explanation for this association is the tendency of a malrotated, loaded proximal bowel to undergo volvulus in the presence of pre-existing Hirschsprung's disease. This hypothesis would explain the reported high incidence of long segment congenital aganglionosis in distal ileal and colonic atresia. The distended mobile small bowel or right side of the colon would be more likely to result in a volvulus in this situation. There are marked regional differences in the sensitivity of the neuromuscular system to hypoxia in experiments on the large intestine of animal models (Wood 1986). This is a possible reason for the divergent experimental results in reported series (Earlam 1972, 1985 ; Cogbill 1982; West 1990).

Further evidence of vascular involvement originates from reports of acquired aganglionosis. Touloukian and Duncan (Touloukian 1975) reported acquired aganglionosis in a stressed premature baby with enterocolitis. This could have resulted from a redistribution of the capillary circulation away from the gut to protect vital organs during a state of shock (Touloukian 1975).

Acquired aganglionosis following surgery for Hirschsprung's disease has been reported. These include reports of cases following the Swenson (Ehrenpreiss 1965, 1966; Daudet 1970; Cohen 1993), Duhamel (Daudet 1970; Rafferscheid 1982; West 1990) and Soave (Cogbill 1982; West 1990; Cohen 1993) procedures. In the initial report of acquired aganglionosis, Ehrenpreiss (1965, 1966) observed hyaline fibrosis in some of the vascular walls, together with an increase in fibrous tissue in the submucosa. This lent support to the view that an ischaemic or other hypoxic event may have contributed to the development of aganglionosis in these patients. This has not been a feature of other studies, however (West 1990), and the number of reports of acquired aganglionosis not associated with a surgical procedure (Touloukian 1975; Chow 1977; Towne 1979; Dimler 1981; Weinberg 1982) suggests that other factors may also lead to the development of aganglionosis.

2.2.1.4 *Genetic aspects of Hirschsprung's disease*

There is a considerable amount of conflicting evidence about the nature of the genetic association in Hirschsprung's disease. The tendency for more than one member of a family to be affected (Bodian 1963; Emmanuel 1965; Gordon 1966; Passarge 1967) and reports of concordance in monozygotic and dizygotic twins (Cohen 1982; Siplovich 1983) suggests a genetic component in the pathogenesis of Hirschsprung's disease.

An incidence of familial occurrence in 2.4% to 9% of affected patients has been reported (Passarge 1967; Boley 1978; Garver 1985; Moore 1991). No clear pattern of inheritance has as yet been identified (Bodian 1963; Passarge 1967; Moore 1991). Associations with other conditions such as dominant sensorineural deafness (Weinberg 1977), Waardenburgh syndrome (Branski 1979; Omenn 1979; Farndon 1983), neurofibromatosis (Schocket 1957), neuroblastoma (Passarge 1967; Gaisie 1979), phaeochromocytoma (Schocket 1957; Bolande 1974; Passarge 1967), the MEN Type II b syndrome (Bolande 1974; Khan 1987) and other abnormalities (Passarge 1967), suggests some common inherited factor. The genetic defect giving rise to syndromes such as the Waardenburgh syndrome is probably one of several mutations which carry an increased risk of Hirschsprung's disease (Branski 1979).

The incidence of Hirschsprung's disease in Down's syndrome is 1 in 300 cases (Bodian 1963; Emmanuel 1965; Gordon 1966; Passarge 1967; Omenn 1979; Cohen 1982; Spouge 1985). The relatively constant association with Down's syndrome suggests a possible genetic component in the etiology which may be related to the 21st chromosome (Swenson 1953). In humans there are additional reported associations with the short arm of chromosome 2 (Webb 1988), Chromosome 10 (Martucciello 1992), the long arm of chromosome 13 (Sparkes 1984; Lamont 1989) and the long arm of chromosome 22 (Beedgen 1986). The recently reported association with a deletion on the long arm of the 10th chromosome is of particular interest because of its association of MEN type 2a (Matthew 1987; Simpson 1987). Several genetic loci may be involved in the normal and consequently abnormal development within the enteric nervous system and may be defective in conditions such as Hirschsprung's disease as well as in other neuronal dysplasias. Genes involved in the production of Hirschsprung's disease and other associated anomalies may affect similar regions of the neural crest.

Additional evidence in favour of a genetic basis for Hirschsprung's disease is to be found in animal experiments. Congenital aganglionic megacolon has been ascribed to a single gene mutation in mice (Lane 1966) and horses (Hultgren 1982; McCabe 1990). It is associated with the autosomally recessive inherited piebald trait (Lane 1966; Hultgren 1982; McCabe 1990). Three possible genetic loci have been located in animal models which affect myenteric ganglion cells and pigment cells arising from the neural crest (Hultgren 1982; McCabe 1990). There is some evidence that a single dominant gene has a role to play in man in the more extensive forms of Hirschsprung's disease (Gordon 1966; Badner 1990).

In humans, simple Mendelian inheritance could not be demonstrated by Bodian and Carter (Bodian 1963) and a sex-linked multifactorial inheritance pattern has been suggested.

By way of contrast, the infrequency of associated anomalies in patients with familial disease and the low overall incidence would suggest an independence from genetic mechanisms of inheritance in most cases. In support of this view, a lesion of the neural crest would need to be extremely localized to affect only a short segment of the large bowel.

An alternative explanation is that the influence of the genetic defect is indirect or that it lies in a regulating genetic mechanism such as the Hox genes (Wolgemuth 1989). This latter hypothesis is of particular interest in those patients where other dysplastic features co-exist with Hirschsprung's disease. Multigene families have recently been shown to play a role in the regulation of embryogenesis in vertebrates (Dressler 1988; Wolgemuth 1989; Chisaka 1991; Wright 1991). Current evidence suggests that a common set of genes, the

homeobox genes, encode transcription factors during development and play a role in the migration of nerve cell precursors from the neural crest (Dressler 1988).

Over-expression of the Hox 1.4 transgene on day 12.5 in the developing intestine of the transgenic mice embryos has been shown to produce a megacolon in their offspring (Wolgemuth 1989). The features of the resultant megacolon suggests a possible neuronal dysplasia but the observation of aganglionosis in the most terminal portion of the bowel opens up new avenues of investigation of a possible genetic link. Other homeobox genes which have been identified as having a potential role in the migration of nerve cell precursors from the neural crest include the Hox 2.1 "homeo-gene" transcripts detected in autonomic ganglia arising from the neural crest (Holland 1988) and the Hox 7.1 gene (Robert 1989). These controlling genetic mechanisms have been shown to function in vertebrates as well as in invertebrates (Gehring 1987; Chisaka 1991; Wright 1991). They open new possibilities in the study of developmental disorders of the nervous system.

2.2.1.5 *Influence of other Environmental Factors on the Etiology of Hirschsprung's Disease*

Reports of discordance in monozygotic twins (Harmon 1988), the low incidence in premature babies (Ehrenpreiss 1970; Swenson 1973), a lack of concordance in the length of segment (Goldberg 1984; Lipson 1987) as well as acquired aganglionosis have raised the question whether environmental factors influence the etiology of this condition (Moore 1979; Lipson 1987).

Degeneration and destruction of colonic ganglia have been produced experimentally in animals by injection or administration of various toxins

(Ehrenpreiss 1966). More recently, an animal model of aganglionosis has been developed from experiments using tetrodotoxin (Fujimoto 1989).

Chow et al (Chow 1977) speculated that a **viral infection**, probably acquired in utero, could have been the cause of acquired aganglionosis. The patient in question had degenerate ganglion cells and a mononuclear infiltrate in the submucosa of the rectum at the age of 5 days and subsequent aganglionosis at 7 months. A cytomegalovirus infection has been suggested as a possible contributing factor (Herschlag 1984; Sonsino 1984). Other possible environmental factors such as **maternal hyperthermia** have been suggested (Lipson 1987) but definitive proof is lacking.

The cause of Hirschsprung's disease remains unclear. There is as yet no definitive evidence that any of the different theories are the chief cause and current evidence suggests a multifactorial origin. A report demonstrating a marked elevation in class 11 major histocompatability antigens (MHA) in the aganglionic bowel (Hirobe 1992), raises the possibility of a role of an **autoimmune mechanism** for the maldevelopment of the ENS. The observed increase in MHC was not present in the normally ganglionated bowel of these patients and may lead to a reappraisal of the current understanding of the etiology of Hirschsprung's disease.

CHAPTER 3

THE PRINCIPLES AND OUTCOME OF THE SURGICAL MANAGEMENT OF HIRSCHSPRUNG'S DISEASE

3.1. PRINCIPLES OF SURGICAL MANAGEMENT AND RESULTS

The standard surgical management of Hirschsprung's disease varies between centres. Traditionally, an initial colostomy is performed at the time of diagnosis, followed by a definitive pull-through procedure 3 to 9 months later (Foster 1990). More recently, the management by bowel irrigation techniques and early one-stage surgery as advocated by Ehrenpreiss (1970) has been re-examined as an alternative means of management (Carcassonne 1984; Cass 1990). This does away with the need for a covering colostomy (Ghinelli 1993) and in turn leads to definitive surgery being performed at a much earlier stage. Performance of this technique within the neonatal period has proven successful in small series of patients. (So 1980; Carcassonne 1984,1989; Cass 1990). On the other hand, in a collective review of 880 patients treated with the Swenson procedure, Sherman (1989) reported a 21% incidence of persistent enterocolitis in those operated on under the age of 4 months.

Debate about the necessity for surgical decompression with a definitive pull-through continues. Many surgeons feel that such procedures are unnecessary and perform one stage pull-through procedures without a covering colostomy or caecostomy (So 1980). Nixon (1990) expressed the need for caution and recommended reverting to a covering colostomy or caecostomy because of the high incidence of infections. In his series, infections following procedures for Hirschsprung's disease were second only to enterocolitis as a cause of death. Foster et al (1990) suggested a covering colostomy in patients under the age of

10 months. In older patients he suggested an interval of 3-6 months between colostomy and pull-through procedure.

Definitive pull-through surgical procedures have undergone numerous modifications since 1948 (Duhamel 1960, 1964; Grob 1959, 1962; Louw 1961; Martin 1962, 1967; Soave 1964; Boley 1964; Denda 1966; Ikeda 1967; Steigen 1968; Kasai 1975). Most of these modifications are based on the original concept described by Swenson and Bill (1948) and adhere to the principle of removing the functionally obstructive segment of aganglionic bowel and reanastomosis. Exceptions to this include ultra-short aganglionic segments which can be relieved by myectomy (Lynn 1975) and the anterior resections described by State and Rehbein. In these latter procedures removal of the entire aganglionic segment is not a primary aim.

3.1.1 The Swenson rectosigmoidectomy

This operation is based on the original concept of Swenson and Bill (1948). During laparotomy, the extent of the aganglionic bowel is identified and a resection 10 cm proximal to the transitional zone performed. Following further resection at the rectosigmoid junction, careful dissection of the rectum is necessary keeping close to the rectal wall to preserve bladder and pelvic nerve function. The distal stump is closed and everted through the anus to allow anastomosis to the anal cuff outside the abdominal cavity.

Swenson subsequently introduced a partial sphincterotomy by placing the posterior line of anastomosis at the dentate line (Swenson 1951). He reasoned that the high rate of postoperative enterocolitis occurred on the basis of persistent sphincteric obstruction. This latter modification has also been incorporated into other pull-through procedures.

Successful Swenson pull-through procedures have been reported in neonates (Carcassonne 1984, 1989). Sherman (1989) reported a satisfactory outcome in 89.9% of 880 patients in a collective review of Swenson procedures.

3.1.2 The Duhamel procedure

This popular procedure is widely used for the management of all forms of Hirschsprung's disease. It has also been noted to have some definite advantages in older patients (Louw 1966) and in total colonic aganglionosis (TCA) where further modification includes a long ileal anastomosis (Martin 1967; Cywes 1967).

The technique consists of surgical identification of the ganglionated bowel as in other operations, mobilization and pull-through posterior to the distal segment of aganglionic bowel which is retained as a pouch. Following an incision to the posterior anal canal just above the dentate line, the bowel is pulled through and anastomosed. The common wall is then divided and the pouch created by closing the upper border of the retained aganglionic segment (Martin 1962,1967; Soper 1968).

A tendency towards constipation occurs in the postoperative period. Anastomoses were therefore placed lower to overcome the resistance of the internal anal sphincter. If the anastomosis was performed too low, problems with continence occurred and Grob (1959) suggested making the anastomosis higher (ie. 1,5-2 cm from dentate line).

Of interest to this study is the fact that Duhamel also modified the low anastomosis to obtain a partial sphincterotomy (Duhamel 1964). He finally recommended that the anastomosis be performed at the level of 1 cm above the dentate line thus reaching an almost identical conclusion to that of Swenson

(1951). Problems still exist even with these modifications and Davies and Cywes (Davies 1983) reported pouch stasis with inadequate pouch emptying following the Duhamel procedure.

In the early years, Kocher or Lloyd-Davies crushing clamps were utilized to crush the spur (Duhamel 1960; Steichen 1968) but frequent reapplication was necessary. The more recent introduction of the GIA stapling device has allowed a simplification in performing the anastomosis (Canty 1982; Nixon 1985). Longer stapling devices now available make the procedure even easier (Steichen 1987; Rescorla 1992). More recent modifications include a stapling technique which avoids the use of mechanical sutures (Steichen 1987). A recent comparison of mechanical sutures versus stapling techniques has reported a lower incidence of pouch problems in those with a stapled anastomosis (Buyukunal 1991).

3.1.3 The endorectal pull-through (Soave)

The endorectal pull-through procedure was first described by Yancey (Yancey 1952) following experiments on dogs and cadavers. This early technique was a sutured endorectal pull-through procedure but the demise of the only patient resulted in the unpopularity of this procedure.

Although not the first to describe the use of the endorectal pull-through procedure, Soave popularized the non-suture technique of endorectal pull-through (Soave 1960). This procedure avoided pelvic dissection with the potential for nerve damage and preserved the sensory nerves within the muscle cuff. Soave's concept of "dynamic adhesions" resulted in to the use of seromuscular sutures to stabilize the lower end plus an in situ flatus tube at the time of surgery (Soave 1960). Subsequent trimming of the protruding bowel took place at 14 days.

Primary suturing of the anastomosis introduced by Boley in the USA (1964,1968) and Denda in Japan (1966) avoids this step. Both of these techniques were similar to that previously described by Yancey (Yancey 1952).

Further modifications have included the partial sphincterotomy achieved by an oblique anastomosis similar to that recommended by Swenson (Swenson 1951). Splitting of the muscular cuff was shown to aid dissection and to prevent cuff stenosis (Kasai 1977; Nixon 1985).

At present, the endorectal pull-through procedure is probably the most frequently performed pull-through procedure for Hirschsprung's Disease (Kleinhaus 1979) and has been performed in more than 200 patients in our unit since 1967 as the routine procedure for short segment aganglionosis.

The benefits of endorectal pull-through procedures include the removal of all rectal disease, preserving enervation to the rectum, bladder and genital organs and the retention of the normal pathway for defecation. This has led to its increasing use in benign disease (Coran 1985). Reported long term results are largely satisfactory (Soave 1985; Polley 1986; Morikawa 1989) but there is a reported tendency to stricture and diarrhoea (Soave 1985).

3.1.4 Anterior resection - The State and Rehbein procedures

In contrast to the principle of the removal of all aganglionic bowel as recommended by Swenson, State recommended a low anterior resection, leaving a residual six to ten centimeters of aganglionic bowel (State 1952, 1963). Perhaps his most significant contribution was his suggestion that an extended resection of the left colon be performed at the time of surgery. Of interest to

the current study is that he based this recommendation on the observation that the proximal bowel did not always function normally in the postoperative period.

The technique of low anterior resection was further developed by Rehbein (Rehbein 1960) who paid particular attention to the outlet obstruction caused by the internal anal sphincter. Although the length of the residual aganglionic segment was initially longer, this was subsequently shortened to 3-7 cm in a child (Rehbein 1966). Forceful dilatation of the internal sphincter is a routine part of the Rehbein procedure by means of retractors as well as postoperative dilatations.

3.1.5 Anorectal myectomy (Lynn)

This procedure, based largely on the technique of Lynn and van Heerden (Lynn 1975), avoids the need for intra-abdominal procedures. The removal of a strip of internal sphincter muscle weakens the internal sphincter sufficiently to provide adequate treatment for short aganglionic segments. Although obviously inadequate for longer segments of aganglionosis, it is of some value in very short segment aganglionosis (Nixon 1990) or in cases of anal outlet obstruction (Scobie 1977) particularly in the postoperative patient.

3.1.6 The adequacy of surgical correction of Hirschsprung's disease

Postoperative results may be influenced largely by the type of procedure performed and the experience of the surgeon. Good long term results are a tribute to the correctness of the original concept of Swenson. As a result a certain similarity of results is evident for the various procedures.

The overall long term results of most published series show good results following all four of the standard pull-through procedures currently performed (**Table 3.1**). Some degree of minor soiling may persist beyond 4 years of age,

but this generally corrects itself by the time the child undergoes puberty. Major soiling is uncommon and may arise as a result of infectious complications and scarring. In some patients, overflow incontinence results from constipation. Subtle alterations in the proximal ganglionated bowel may also account for poor postoperative function as well as some of the incomplete responses of otherwise apparently adequate operations.

TABLE 3.1

REVIEW OF OUTCOME OF SURGERY FOR HIRSCHSPRUNG'S DISEASE - REPORTED RESULTS MAJOR SERIES

Author	Year	No.	Procedure	Good outcome	Mortality
Swenson	1957	200	Swenson #	79.5%	6.5%
Wyllie	1957	152	Swenson #	73.1%	5.9%
Kostia	1962	89	Swenson/Duhamel	84.3%	6.7%
Dorman	1967	813	Swenson/Duhamel #	89.3%	7.3%
Louw	1967	80	Swenson/ Duhamel	93%	7.5%
Ehrenpreis	1970	1553	Sw/Duh/Soave #	90%	7.1%
Puri	1972	89	Swenson	98.8%	5%
Neilsen	1972	157	Swenson	98.8%	8%
Soto	1977	301	Sw/Duh/Soave #	93%	2.8% *
Soave	1977	357	Soave #	82.6%	3.9%
Grosfeld	1978	89	Duhamel	95.5%	7.8% *
Kleinhaus	1979	1196	Sw/Soave/Duh #	98.4%	1.7% *
Holschneider	1982	439	Reh/Sw/Soave/Duh	86.4%	5.2%
Ikeda	1984	1628	Duhamel #	64.9%	1.9% *
Martin	1985	108	Duhamel	95%	1%
Polley	1985	92	Soave	100%	1.5% *
Nixon	1986	155	Sw/Duh/Soave	97.5%	3.2% *
Sieber	1986	151	Sw/Duh/Soave	81%	1.7% *
Vane	1986	214	Duhamel	91.1%	1.8% *
Weitzman	1986	80	Swenson	89.6%	1.2% *
Harrison	1988	139	Sw/Duh/Soave	87%	7%
Joseph	1988	121	Soave	85.6%	4.8%
Sherman	1989	880	Swenson #	89.9%	2.4% *
Foster	1990	63	Sw/Duh/ Soave	87.9%	11.1%

* = Late mortality only
\# = Review series (includes smaller series)

3.2 CHOICE OF PULL-THROUGH PROCEDURE

The choice of procedure depends on the training, resources and individual preference of the surgeon. Those procedures most commonly performed are the Duhamel, Soave and Swenson procedures although the Rehbein procedure still has many advocates.

A survey of the Surgical section of the Pediatric section of the American Association of Surgeons in 1979 showed that nearly half of American surgeons prefer the Soave endorectal pull-through . Approximately one third preferred the Duhamel procedure and the remainder chose the Swenson procedure (Kleinhaus 1979). Good long term results were achieved by competent surgeons in 4 out of 5 patients. In the remainder, the type of complication tended to vary with the type of procedure performed. Rehbein's procedure has many advocates. It is often avoided on theoretical grounds although good results have been reported in long term follow-up studies (Holschneider 1982).

It is interesting to note the conclusions which have been drawn as to the role of the internal anal sphincter in the etiology of postoperative enterocolitis and outlet obstruction. Swenson developed his modification of a partial sphincterotomy when faced with a problem enterocolitis which he attributed to outlet obstruction. On the other hand, Duhamel (Duhamel 1960) and Grob (Grob 1959), addressed the problem of soiling because the anastomosis had been performed too low. The ensuing modifications to their procedures resulted in the preservation of a similar amount of sphincter for the preservation of postoperative function as well as a partial sphincterotomy created by a low anastomosis. This would suggest that a postoperative dysfunction of the sphincter may be a possible cause of persistent postoperative outlet obstruction.

Characteristic problems associated with particular types of procedures are anastomotic leaks in the Swenson procedure, constipation and dilatation of the rectal pouch in the Duhamel procedure, cuff stenosis, diarrhoea and incontinence reported with the Soave endorectal pull-through and the residual segment obstruction following the Rehbein procedure. In 15-20%, symptoms such as constipation, diarrhoea, enterocolitis or other major problems are experienced and occasionally minimal symptoms will persist (Lawson 1972; Sieber 1986). Many of these problems are of an obstructive nature (Lawson 1972) and it is my intention in this thesis to explore these factors to establish possible causative factors. It is not clear why some patients respond to operations designed to leave a residual segment and others with an adequate resection on frozen section at the time of operation develop postoperative obstructive symptoms.

3.3 COMPLICATIONS OF HIRSCHSPRUNG'S SURGERY

3.3.1 Early complications

Early postoperative complications include anastomotic insufficiency, stenosis, prolonged ileus, adhesive obstruction, postoperative strangulation of bowel and retraction of neorectum (**Table 3.2**). A survey of early postoperative complications reported in the literature following the Soave endorectal pull-through procedure is listed in **Table 3.3**. Wound sepsis may be present to varying degrees and other complications of sepsis or pelvic or presacral abscesses may be evident. Acidosis may be associated with excessive fluid and electrolyte losses in long segment disease and enterocolitis associated with Hirschsprung's disease may be present. Incontinence may also occur if too low an anastomosis has been performed. Early complications following the Soave procedure are a reported higher rate of anastomotic leakage (4-7.7%) and stenosis (9.4-23.7%) compared with other procedures.

TABLE 3.2

POSTOPERATIVE COMPLICATIONS

A. Early Complications

1. Anastomotic insufficiency
2. Stenosis
3. Prolonged ileus
   adhesive obstruction
   strangulation of bowel
4. Retraction of neorectum
5. Wound sepsis major
   minor
6. Complications of sepsis
7. Metabolic - Associated with long segment disease
8. Incontinence following a low anastomosis
9. Pelvic or presacral abscess
10. Hirschsprung's associated enterocolitis

B. Late complications

a. Constipation Incomplete resection
   Sphincter achalasia
   Stricture
b. Incontinence
   Encopresis
c. Faeculoma
d. Enuresis / urinary incontinence / dysuria
e. Impotence
f. Fistulas
g. Ileus
h. Death

TABLE 3.3

LITERATURE REVIEW - EARLY RESULTS SERP

AUTHOR	YEAR	NO	LEAK /CUFF ABSCESS	NEORECTAL RETRACTION	STENOSIS	WOUND INFECT	
CHARLES	1967	13	1	1	1	-	
LILLY	1967	10	1	1	0	1	#
BOLEY	1968	13	0	1	1	-	*
SAN FILIPPO	1972	18	3	-	-	1	*
SOPER	1972	15	3	-	-	-	
DEODHAR	1973	20	6	4	1	3	~
KLOTZ	1973	19	0	-	3	1	*
SATOMURA	1974	20	-	1	1	-	
DAVIES	1975	69	2	0	3	-	#
KRASNA	1976	28	1	-	1	-	
BARANOWICZ	1977	15	2	1	2	-	@
SOTO	1977	28	1	-	1	-	
SOAVE	1977	357	22	-	45	13	
	1977	147	5	4	-	2	
JORDAN	1979	60	1	-	-	3	*
KLEINHAUS	1979	93	1	-	14	-	#
	1979	187	11	-	18	-	*
SO	1980	20	0	0	0	0	#
JORDAN	1981	10	-	-	-	2	*
CRAM	1982	5	1	-	-	-	
HOLSCHNEIDER	1982	105	8	7	35	8	#
CARCASSONNE	1984	3	-	-	3	-	@
POLLEY	1986	55	1	-	1	6	*
*referred cases	4	2	-	1	-		
JOSEPH	1988	97	8	-	18	10	
MOLANDER	1989	26	3	1	9	-	
TOTAL		1437	73 (5%)	21 (1.5%)	158 (11%)	49 (3.4%)	

* = 2 Stage procedure
\# = 'True' Soave procedure
\- = No record
@ = 1 Stage procedure
~ = 3 Stage procedure

The traditional management by initial colostomy of the pull-through procedure (ie a 3-stage procedure) has been challenged. A tube caecostomy is a safe alternative to a covering colostomy (Moore 1992) and no increase in anastomotic leaks were noted in the 61 patients in this series who underwent Swenson procedures. The current trend towards performing the pull-through

procedure much earlier without a defunctioning complementary procedure (So 1980; Carcassonne 1984; Cass 1990) has a historical foundation in the practice of Ehrenpreiss. Initial results of this early management protocol have shown considerable promise in small series (So 1980; Carcassonne 1984; Cass 1990).

3.3.2 Late complications

The major long-term complication in the postoperative period following Hirschsprung's surgery is that of **constipation.** The incidence of postoperative constipation is therefore one of the most practical methods of measuring successful therapy (Joppich 1982). Although a procedure-related incidence of postoperative constipation has been observed (Joppich 1982), the long-term incidence approaches 9% for almost all procedures (Kleinhaus 1979; Joppich 1982). The incidence of postoperative constipation in the large review series by Holschneider was as high as 35.8% following the Rehbein procedure and 41.5% following the Duhamel procedure (Holschneider 1982), while Kluck reported 22 out of 108 patients (20.3%) with postoperative obstipation (Kluck 1986).

Reported routine clinical and pathologic investigations have usually failed to identify a cause (Kleinhaus 1979; Kluck 1986), although sphincter achalasia has been implicated (Holschneider 1982; Joppich 1982). The incidence of incomplete resection of the aganglionic segment has been estimated as being 2.2% for the Soave, 3.6% and 3.8% for the Swenson and Rehbein techniques and 1.2% following the Duhamel procedure (Soave 1977). Lawson (Lawson 1972) estimated a 17% incidence of residual segment disease.

Although 3-7 cm of residual aganglionic distal bowel remains in patients treated with the Rehbein technique, those with postoperative obstructive symptoms were dilated down onto the pelvic floor on abdominal X-ray, suggesting that the obstructive element was related to achalasia of the sphincter (Rehbein 1966).

Other possible causes such as **stenosis** are well documented (Ikeda 1975; Baranowicz 1977; Holschneider 1977; Grosfeld 1978) and some may occur secondary to vigorous anal dilatation (Eisenhammer 1974).

Although **incontinence** has been reported in as many as 19.6% following the Swenson procedure and 7.2% following the Duhamel technique (Soave 1977), the incidence following the Soave technique appears to be low (Ehrenpreiss 1970; Klotz 1973; Soave 1977). Joppich (Joppich 1982), on reviewing 6158 reported cases noted an overall incidence of 8.3% and 13.9% within their own 412 patients. The majority of patients do well following the modified surgical techniques currently employed (Moore 1990).

Intermittent soiling has been associated with constipation, diarrhoea and a **faeculoma** and appears to be related to overflow (Clausen 1963).

Diarrhoea and enterocolitis may persist into the postoperative period despite faecal diversion (Fujimoto 1988) and subsequent pull-through (Soave 1977; Grosfeld 1978; Joppich 1982), although some appeared to be related to TCA (Vane 1986). In a 40 year multinational retrospective analysis of 880 Swenson procedures, Sherman et al (1989) noted that 67.2% of the 58 postoperative sphincterotomies were performed for enterocolitis (an observation already made by Swenson in 1964).

Other late complications include genitourinary symptoms such as **enuresis** (Ehrenpreiss 1970; Soave 1977; Neilsen 1972), **incontinence** of urine (Puri 1977), **dysuria** and **impotence** (Puri 1977). The reported incidence varies from 0-9.7% (Joppich 1982). The suggestion that genitourinary symptoms result from damage to pelvic nerves is considered unlikely by some workers because

the incidence is only slightly greater than the normal range of urinary problems in this age group. It has been suggested that these symptoms may be related to psychological disturbances associated with long periods of hospitalization and trauma (Joppich 1982).

The persistence of some early septic complications in the form of **perianal fistulae** appears to be uncommon (Joppich 1982), but has been reported in isolated cases following the Swenson (Puri 1977), Duhamel (Grosfeld 1978), and Soave (Deodhar 1973) procedures. A relationship with perirectal abscess formation has been reported (Baranowicz 1977).

Death, although uncommon, has been reported as a late complication of surgery for Hirschsprung's disease. Joppich (1982), reviewing 293 deaths in 5646 reported cases, reported a 2.2% risk of late death resulting from Hirschsprung's disease. A mortality rate of 26% in patients with Down's syndrome has been reported (Rescorla 1992). Sequelae of enterocolitis and infection have been shown to be the major causes of both the early and late deaths (Nixon 1990) accounting for more than 50% of deaths overall.

SECTION B

A CLINICAL STUDY OF HIRSCHSPRUNG'S DISEASE

CHAPTER 4

THE BACKGROUND - A RETROSPECTIVE STUDY OF HIRSCHSPRUNG'S DISEASE 1957-1990 RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, CAPE TOWN

4.1 AIM OF STUDY

The aim of this project was to assess retrospectively:

- The presentation, clinical features and outcome of patients treated surgically for Hirschsprung's disease at the Red Cross War Memorial Children's Hospital in Cape Town over a 34 year period (1957-1990).
- To assess the outcome and the incidence of long term problems following the surgical correction of Hirschsprung's disease.
- To assess the incidence of postoperative obstructive symptoms.
- To gain insight into the possible causative factors of postoperative obstructive symptoms.

4.2 MATERIALS AND METHODS

4.2.1 Patient study population

All patients treated for Hirschsprung's disease at the Red Cross Children's Hospital during the period July 1957 through December 1990 were entered into the study.

4.2.2 Patient selection criteria

Patients seen with Hirschsprung's disease during the 34 year period under review (1957 to 1990), were included in the study.

Additional patients who had either been operated on before the opening of the Red Cross Hospital or in whom no definitive diagnosis of Hirschsprung's disease could be established were excluded. Among the latter were some patients excluded due to lack of data to establish the diagnosis of Hirschsprung's disease.

Epidemiological data obtained from the location of the patient's birth enabled an estimation to be made of the prevalence of Hirschsprung's disease within greater Cape Town. The reliability of this estimation of incidence was limited by the problems normally associated with the careful delineation of the population at risk especially in hospital based studies.

4.2.3 Selection criteria of patient sample

Clinical and pathological records of all children with histologically proven Hirschsprung's disease were reviewed from hospital, research and pathology records. The hospital records used as the source material included the hospital folders, the departmental records and copies of summaries of admissions. Also included were the departmental research records, the pathological record books, the operating theater records as well as any correspondence.

These records were individually scrutinized to ensure that the diagnosis had been confirmed. The completeness of the database (vis-a-vis the completeness of the series, and the collation of information) was checked against all available information.

The patient's place of birth was recorded to enable the determination of the prevalence of Hirschsprung's disease within Cape Town. Current addresses and whereabouts were noted in order to recall patients for a long term follow-up study.

4.2.4 Patients excluded

Out of a total of 391 potentially selectable patients, 8 were excluded on the basis of insufficient data or a different final diagnosis. Four of these had a diagnosis of neuronal intestinal dysplasia (NID).

Out of the 383 possible patients who remained, a further five patients in the series had undergone successful surgical treatment for established Hirschsprung's disease prior to the opening of the Red Cross Hospital. These were also excluded as no records could be found at the Red Cross Hospital. These two groups represented 3.3% of those patients who were potentially selectable. The remaining 378 patients with histologically confirmed Hirschsprung's disease were included in the study.

4.2.5 Demographic data

Data collected included race, sex, age, address, general and physical characteristics. Other demographic data collated included geographical distribution, perinatal and family history and associated anomalies. In addition, the age at presentation and symptoms were also recorded.

4.2.6 Clinical data collection

Details of diagnosis on all patients as well as details of antenatal and perinatal histories, presenting symptoms, the length of aganglionic segment and delay in presentation were recorded. Details of the diagnosis as well as type of

procedure performed and the presence or absence of postoperative complications were also investigated.

Possible etiological factors in the antenatal and family history as well as associated anomalies were specifically looked at. Special attention was paid to the presence of any factors common to patients with obstructive symptoms.

4.2.7 Ascertainment of data

This questionnaire was then completed as far as possible for each patient from data available from the records analyzed as above.

The accuracy of the data was assessed by cross checking available information for consistency.

When the data sheets (**Appendix A**) were considered complete, they were entered into a database programme based on a personal computer version of DBASE III Plus.

4.2.8 Methods of determining prevalence

The addresses of all patients were computerized and those living in greater Cape Town selected. Birth rates were obtained from the Medical Officer of Health of the City of Cape Town as well as the Regional Services Council of the Western Cape. These were collated to yield a birth rate for greater Cape Town (**Appendix B**). Serious underreporting of birth rates in some population groups especially in earlier years was noted. The resulting figure is regarded as an estimation of prevalence only. The true incidence still requires to be determined.

4.2.9 Assessment of postoperative function

Patients from the above study who had definitive procedures performed were included in the assessment of function. The records of the patients included were indexed into a separate file from the database. These were then analyzed with particular attention being paid to the early postoperative function and complications as well as the late function and complications. Particular reference was made to the age of follow-up.

4.2.10 Collation of radiological data

Radiological data were obtained from retained records within the Department of Radiology. Radiological review of available films was carried out with the cooperation of the Radiology Department of the Red Cross Children's Hospital. Where films were no longer available, the radiologist's report was accepted as evidence of the presence or absence of radiological features.

4.2.11 Histological evaluation

Confirmation of the histological diagnosis of Hirschsprung's disease depended on locating the pathologist's report. Where this was not immediately available, the individual histological records were scrutinized and a record made of the diagnostic features. In cases where doubt existed, the individual pathological slides and blocks were located and reviewed by experienced histopathologists.

4.2.12 Evaluation of outcome

The functional outcome of patients in whom definitive surgical procedures had been performed was then analyzed separately to assess post-operative results. Details of length and nature of follow-up were analyzed.

Long-term functional results of the Swenson, Duhamel and Soave procedures were investigated in this retrospective survey as far as available records permit.

Groups of patients with specific postoperative symptomatology (eg obstructive symptoms) were identified and investigated. Those symptoms attributable to mechanical or technical causative factors were noted.

4.2.13 Completeness of data

Incomplete records in 58 patients resulted from the destruction by fire of hospital records for the early period of this study. A few early patients may have been omitted due to the inability to verify the diagnosis.

4.2.14 Statistical analysis

Statistical analysis between groups was by the Chi-squared test or by the Fishers exact test where comparison was between two groups of patients with small numbers.

Small number non parametric methods, one way Anova and the Kruskal Wallace one way testing by Ranks were methods employed for comparison between unpaired groups. Results were expressed as 95% confidence intervals of means or a p value.

4.3 RESULTS

4.3.1 Patient sample

Three hundred and seventy-eight patients with biopsy proven Hirschsprung's disease were treated over a 34 year period between 1957-1990. Definitive surgical procedures were performed on 330 of these patients (**Figure 4.1**).

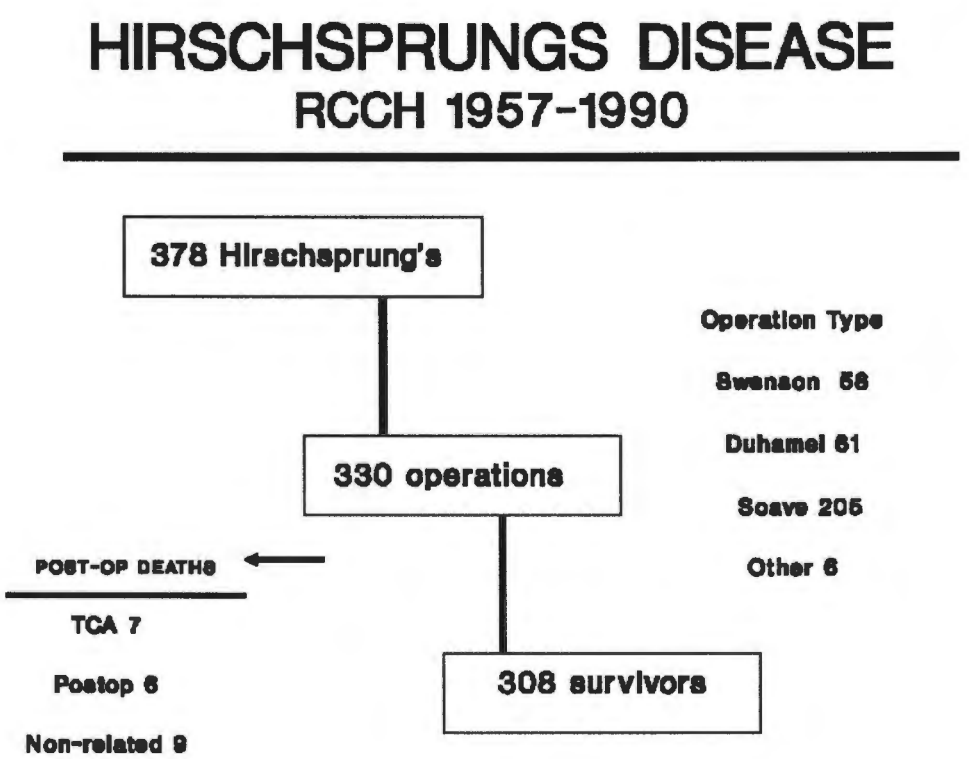


Figure 4.1 Flow chart demonstrating patient groups

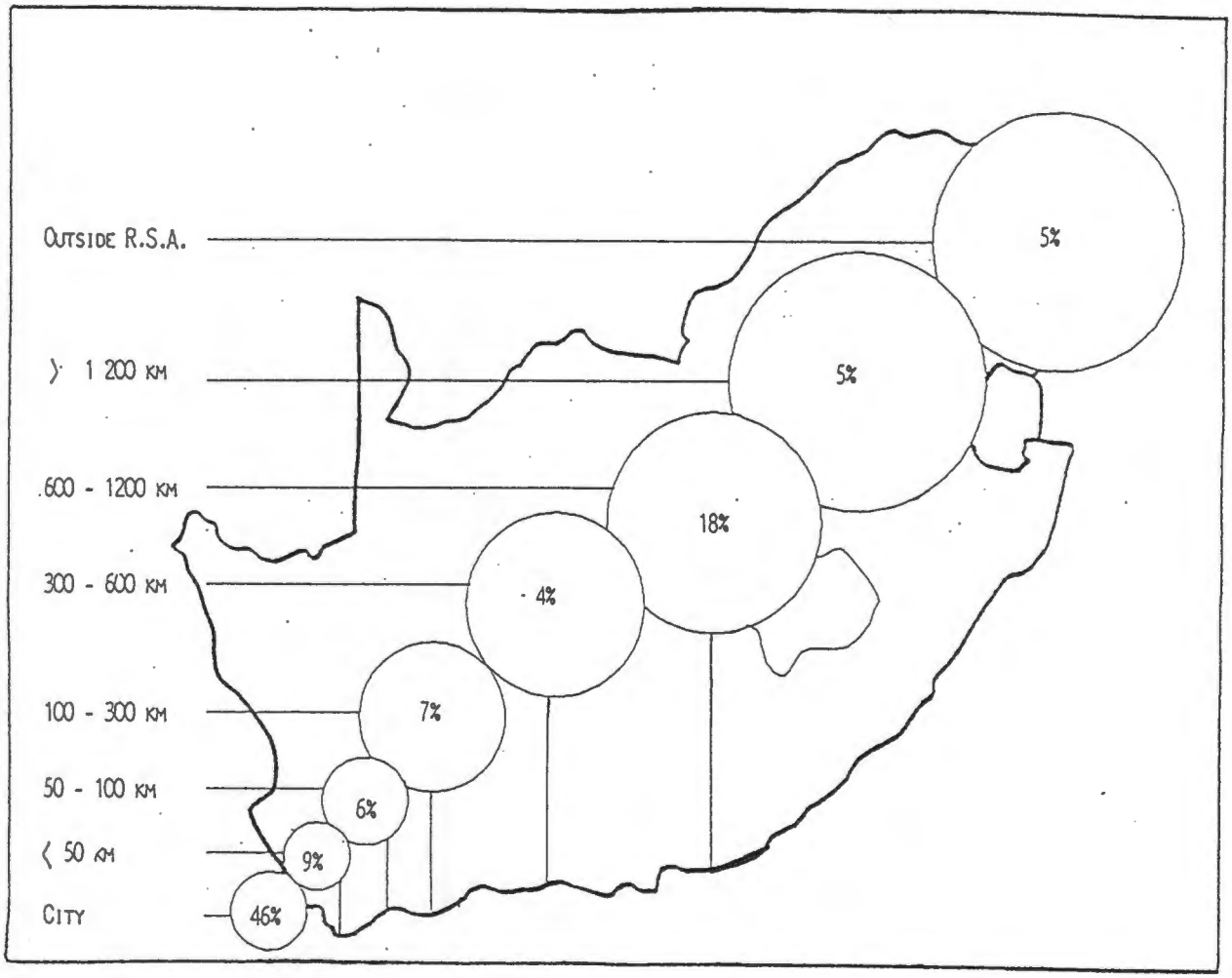
Fifty-eight of the 378 patients did not have a definitive surgical procedure performed at the Red Cross Children's hospital. Nine of these were excluded on the basis of definitive procedures having been performed elsewhere. A further 4 were excluded because of permanent stomas due to associated anomalies. A further 8 patients were still awaiting surgery at the time of closing this study, but have subsequently been operated on. Four patients could not be traced and were lost to follow-up following colostomy having been performed. These patients may have died but no evidence of this could be found. There were 33 known deaths prior to definitive surgery for Hirschsprung's disease. In 2 of these the diagnosis was made at postmortem.

4.3.2 Prevalence of Hirschsprung's disease in Cape Town

The geographical origin of these patients is shown in **Figure 4.2.** In 46% the place of birth was within the greater Cape Town metropolitan area. The remainder came from centres outside of Cape Town and 5% of these were from areas further than 1200 km away but still within the Republic of South Africa. A further 5% came from beyond the borders of the Republic.

One patient with Hirschsprung's disease occurred in the greater Cape Town area for every 5726 thousand live births where birth figures were available (**Appendix B**). The uneven distribution of cases and the probable serious under-reporting of birth rates for one sector of the population at certain stages of history are noted as possible factors which may influence the reliability the data. This therefore precluded a true estimation of incidence but gives some information on the prevalence of the condition in this geographic area. It does, however, correspond with the reported incidence of the disease in other series (Holschneider 1982).

Three babies were scored at less than 37 weeks gestation. Two of these were born in the Cape Town Metropolitan area and weighed 1200 and 1900 grams at birth respectively. This resulted in a prevalence of approximately 1 patient with Hirschsprung's disease in 12 000 premature births.



City	46%	< 50 km	9%
500-100 km	6%	100-300 km	7%
300-600 km	4%	600-1200 km	18%
> 1200 km	5%	Outside RSA	5%

Figure 4.2 Geographic distribution of patient sample.

4.3.3 Race and sex incidence

The series compared 285 males to 93 females a ratio of 3.0 males to every female. This ratio varied slightly in different population groups and was 2.5 males to every female in those patients of mixed descent. This difference was not found to be statistically significant on testing (**Table 4.1**).

TABLE 4.1

RACE SEX INCIDENCE HIRSCHSPRUNG'S DISEASE

	n	Male	Female	M/F Ratio
Caucasian	119	95	24	3.6
Mixed descent	210	151	59	2.5
African	49	39	10	3.9
Total	378	285	93	3.0

4.3.4 Antenatal history

Out of 226 evaluated cases, the antenatal history was reported as normal by the parents in 208 (92%).

One hundred and eighty births were normal vaginal deliveries, 11 had a forceps delivery and 23 Caesarian sections were performed. Thirteen of these were for cephalopelvic disproportion. The incidence of surgical intervention was within the accepted norms for delivery in Cape Town. Postnatal asphyxia was seldom recorded and good APGAR scores were recorded in the majority.

4.3.5 Birth weight and gestational age

The average birth weight of patients was 3129 gm for the 156 patients where this information was available. Nineteen patients (12.2%) weighed less than 2.5 kg at birth, but in only 3 of these (1.9%) was the baby scored as being below 37 weeks of gestation. One infant was born at 28 weeks gestation and the other two were born at 32 and 33 weeks gestation respectively.

4.3.6 Associated anomalies

The associated anomalies noted in this group of patients are listed in **Table 4.2**. A family history of Hirschsprung's disease was noted in only 3 out of 61 patients with associated anomalies. None of the ten patients with Down's syndrome had familial transmission of Hirschsprung's disease. The only abnormalities associated with familial occurrence were ichthyosis, a skeletal abnormality and mental retardation (**Table 4.2**).

4.3.7 Familial aspects of Hirschsprung's disease

In the 34 year period under review, 32 of 378 patients (16 families) had a further family member with histologically proven Hirschsprung's disease (**Table 4.3**). These 16 represented 4.5% of the 351 families in the series. There was

TABLE 4.2

ASSOCIATED ANOMALIES HIRSCHSPRUNG'S DISEASE
(61 out of 378 patients)

	No.	Total
GASTROINTESTINAL		
MALROTATION	4	
INTESTINAL ATRESIA	3	
MECKEL'S DIVERTICULUM	3	
DUODENAL ATRESIA	2	12
DOWNS SYNDROME		10
OTHER CHROMOSOMAL ANOMALIES		1
OPHTHALMIC ANOMALIES		
- Micro/Anophthalmia	2	
- Congenital Ptosis	2	
- Squint	3	
- Congenital cataract	1	8
CARDIAC ANOMALIES		8
GENITOURINARY		7
MUSCULOSKELETAL		6
NEUROLOGIC ANOMALIES		
- Microcephaly	1	
- Myelomeningocoele	2	
- Neurofibromatosis	1	4
MENTAL RETARDATION		2
MISCELLANEOUS		3

TABLE 4.3

FAMILIAL HIRSCHSPRUNG'S DISEASE

FAMILY NUMBER	INDEX CASE	INDEX LENGTH	FAMILIAL OCCURRENCE	SEGMENT LENGTH
1	female	desc.colon	male offspring	splenic flex
2	female	total colon	male sib mother NID	total colon desc.colon
3	male	rectosigmoid	male sib male sib 2 female niece	total colon total colon total bowel
4	male	rectosigmoid	1st cousin	rectosigmoid*
5	male	rectosigmoid	1st cousin	rectosigmoid*
6	female	jejunum	female sib male cousin	jejunum* rectosigmoid*
7	male	rectosigmoid	female cousin(1) female cousin(2)	jejunum* jejunum*
8	male	rectal	female sib male sib	rectosigmoid total colon
9	male	total colon	male sib	total colon
10	male	rectosigmoid	male sib	rectosigmoid
11	female	rectosigmoid	male sib	total colon
12	male	rectosigmoid	male sib	rectosigmoid
13	female	rectosigmoid	male sib	splenic flex
14	male	splenic flex	female twin	total colon~
15	female	rectosigmoid	male nephew	rectosigmoid
16	male	rectosigmoid	male offspring	total colon~

(~ = Treated elsewhere / * = Reflected > once)

more than one affected child per family in 10 families (2.8%) and both parent and child in 3 families (**Table 4.3**). One parent known to have neuronal intestinal dysplasia (NID) had two children with total colonic aganglionosis (TCA).

In one case, an affected twin with total colonic aganglionosis died in another hospital prior to the surviving twin being transferred, but was included for completeness of the series.

Although no significant difference was noted between male and female probands, fifty percent of males with TCA had a family history, but in only 2 cases was this transmitted through a female sibling (**Table 4.3**).

Aganglionosis extended beyond the rectosigmoid in 59% of the familial group as opposed to 26% of the non-familial group. A significantly higher incidence of TCA was noted in those with a family history; 11 of 32 (34.3%) compared with 22 out of 346 (6.4%) without a family history (Fisher's, $p<0.01$) (**Table 4.4**). Progression of length of segment in succeeding generations was noted in two families.

Associated anomalies occurred in 58 (16.7%) of those without familial occurrence and 3 (9.4%) of the familial group. No specific etiological factors were identified in the familial group, the antenatal and perinatal history being unremarkable.

TABLE 4.4

FAMILIAL INCIDENCE AND EXTENT OF DISEASE

	FAMILIAL		NON FAMILIAL	
	Male	Female	Male	Female
RECTOSIGMOID	9	4	200	57
COLONIC	7	1	53	14
TCA	6	5	10	12
TOTAL	22	10	263	83

4.3.8 Presenting features of Hirschsprung's disease

4.3.8.1 *Age of diagnosis*

The average age of presentation of Hirschsprung's disease in this patient sample was 13 months. Associations between the time of diagnosis, the length of the aganglionic segment and the chief clinical features are noted in **Table 4.5**. One hundred and ninety-two (50.7%) presented in the neonatal period. In a further 3 patients, symptoms began in the neonatal period with some delay in the appreciation of symptoms and a late diagnosis a few weeks later.

Of the 186 patients who presented after the neonatal period, 96 presented between 1 and 12 months of age. Twenty-three presented in the second year of life and 67 presented later than 24 months. Twenty-six of these patients were more than 5 years of age at diagnosis. Four of these were older than 10 years, the oldest patient being 20 years of age at diagnosis. The average age at diagnosis in patients presenting after the first year of life was 51 months.

TABLE 4.5

DIAGNOSIS, PRESENTING SYMPTOMS AND LENGTH OF SEGMENT

	Neonatal		Older than 1 month		
	R/Sigm (n = 118)	L/seg (n = 74)	R/sigm (n = 153)	L/seg (n = 33)	
Abd.Distention	78	52	118	23	
Delay meconium	53	41	28	5	
Constipation	29	18	111	21	*
Intestinal obstruction	69	53	44	12	*
Enterocolitis	16	11	26	10	
Perforation	8	4	4	2	
Failure to thrive	2	5	2	3	
Septicaemia	1	4	3	0	
Other	30	16	37	8	

* Chi-square, $p < 0.05$

4.3.8.2 **Length of aganglionic segment**

The majority of patients (71.5%) had a short segment of aganglionosis confined to the rectosigmoid region and 33 (8.7%) had total colonic aganglionosis (TCA) (**Figure 4.3**).

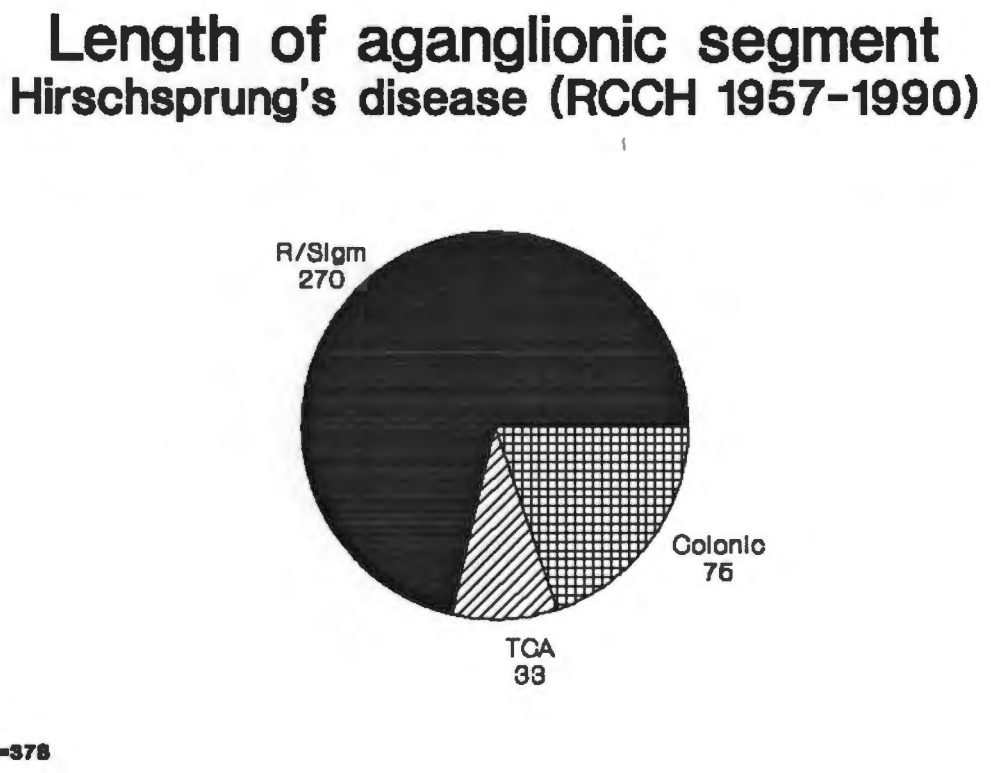


Figure 4.3. Pie chart of length of aganglionic segment in patients with Hirschsprung's disease

In 9 (45%) of these the aganglionosis extended into the proximal small bowel and even up to the stomach in 1 instance. One third (3 patients) of these cases with extensive proximal extension of the aganglionic segment had a family history. This has also been noted in 1 further case in 1991 following the closure of this series. A more detailed representation of the distribution of the level of aganglionosis in the colon is depicted in **Figure 4.4.**

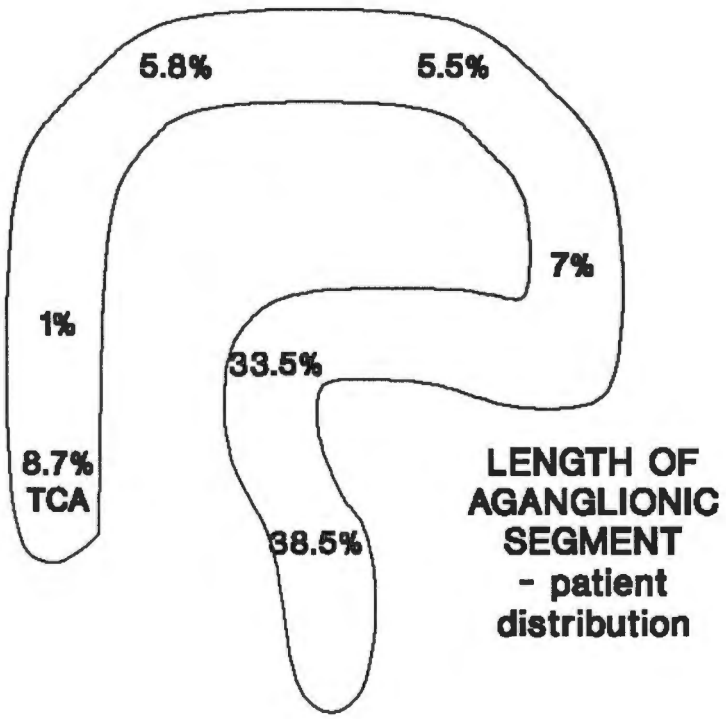


Figure 4.4 Diagram of colon showing level of aganglionosis in patient sample.

4.3.8.3 *Age of presentation and length of aganglionic segment*

The length of the aganglionic segment in the 192 patients presenting in the neonatal period is compared with the length of aganglionic segment in those patients presenting outside of the neonatal period in **Figure 4.5**.

Twenty-four (72,7%) of the patients with TCA presented in the neonatal period. The remaining 9 patients with TCA presented outside of the neonatal period, 2 of them presenting later than 12 months of age. Significant differences between those presenting in the neonatal period and those presenting later, were found in those with long segment colonic disease and in those patients with TCA, particularly in those presenting beyond the first year of life. Two patients who

presented later than the first year of life had TCA. These represented 2.2% of those patients presenting beyond the first year of life.

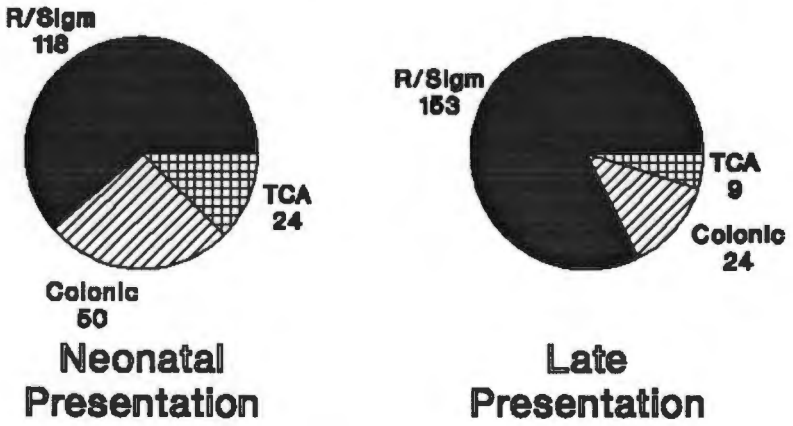


Figure 4.5. Pie chart depicting length of aganglionic segment and timing of presentation.

4.3.8.4 *Symptoms and timing of clinical presentation*

The presenting symptoms of patients presenting within and beyond the neonatal period are reflected in **Table 4.5.**

The chief presenting feature in 271 patients (72%) with Hirschsprung's disease was abdominal distension. This was seen with almost equal frequency in patients presenting both in and outside the neonatal period.

Constipation was the most prominent presenting symptom in patients with rectosigmoid disease presenting beyond the neonatal period. This difference

was found to reach significance when compared with the neonatal period (Chi-square, $p<0.01$). On the other hand, patients presenting in the neonatal period had a significantly higher incidence of neonatal intestinal obstruction (Chi-square, $p<0.01$). The incidence of intestinal obstruction with bile stained vomiting was also significantly higher (Chi-square, $p<0.05$) in patients with long segment colonic disease or total colonic aganglionosis.

Enterocolitis was noted on presentation in 63 cases (17%) and was identified as the primary cause of death in 25 (40%) of them **(Table 4.5)**. Nineteen of these 25 patients demised in the early period soon after diagnosis. The incidence of enterocolitis is compared with the length of the aganglionic segment and age at diagnosis in **Table 4.5**. Thirty-six (57%) of those with enterocolitis presented initially after the neonatal period. In 23 (36.5%) the diagnosis was made later than 3 months of age (i.e. with delayed diagnosis). Nine (14.2%) of these were actually older than 12 months. No significant difference in the incidence of enterocolitis in those with delayed presentation could be demonstrated in this series. In addition, no influence of the length of aganglionic segment on the incidence of enterocolitis was observed.

Intestinal perforation, although uncommon, was twice as frequently encountered in the neonates and nearly 3 times as many patients presented with failure to thrive in the older group (25/186 versus 7/192) (Fishers, $p<0.01$).

4.3.9 Special investigations in the diagnosis of Hirschsprung's disease

4.3.9.1 *Radiological features*

The contributory value of the plain abdominal X-ray in the 310 available films or reports, is summarized in **Table 4.6.** Abnormal findings were noted in 241 of the abdominal series available (77%). In 163 (52%), the plain abdominal films themselves suggested the diagnosis and in a further 78 patients (32%) abnormal features were detected. The most useful views for detecting abnormalities were the prone film to outline the colon and the inverted lateral view to demonstrate the pelvic colon.

The subsequent barium enema was of diagnostic use in more than two thirds of the patients investigated and was diagnostic in 174 of these cases. Abnormal features were noted in a further 57 cases giving an overall value of the barium enema in 231 cases (88%) (**Table 4.6**). Identification of a possible cone level was made in 81% of these.

TABLE 4.6

RADIOLOGICAL DIAGNOSIS OF HIRSCHSPRUNG'S DISEASE

	Diagnostic Features	Abnormal Features	Cone Level
Plain AXR (n = 310)	163 (52%)	78 (32%)	-
Barium Enema (n = 263)	174 (66%)	57 (22%)	213 (81%)

4.3.9.2 *Histological evaluation and diagnosis*

All patients had histological confirmation of aganglionosis. The biopsy showed all the diagnostic histological and histochemical features in 282 (93%) of the 301 rectal biopsy reports where full details were available for review. In 16 of the remainder, problems with the evaluation of specimens resulted from technical difficulties. False negative biopsies were obtained in a further 3 patients in whom the diagnosis was later confirmed on repeat biopsy. In the remaining 77 early cases, aganglionosis was the only feature recorded.

4.3.10 The management of Hirschsprung's disease

4.3.10.1 *Defunctioning colostomy*

A preliminary colostomy was performed on the majority of patients. Three hundred and one of these stomas were performed at the Red Cross Hospital. The remainder had a defunctioning colostomy (usually in transverse colon), performed prior to referral. One hundred and twenty-five were divided colostomies in the proximal colon and 154 were situated in the transverse colon. Frozen section identification of ganglionated colon was performed prior to the formation of the proximal colostomy. A further 22 patients had a defunctioning ileostomy, the majority of these having TCA.

4.3.10.2 *Complications of colostomy*

Complications occurred in 78 (26%) of patients with stomas and there were 3 unrelated deaths. The nature of these complications are listed in **Table 4.7.** Prolapses recorded in the table were those requiring surgical intervention. Retraction of the stoma occurred in 7 of the colostomies performed elsewhere prior to referral.

Twenty-three patients (7.6%) required a repeat laparotomy following colostomy, 14 for intestinal obstruction, 7 for colostomy retraction (colostomy performed elsewhere), 1 for intussusception and 1 for a perforated biopsy site.

4.3.10.3 *Patients without definitive pull-through surgery following colostomy*

Out of the 378 patients in this series (1957 through December, 1990), 2 cases were diagnosed at postmortem examination and were thus eliminated from further study by the exclusion criteria. Thirty-one patients died following colostomy but prior to planned definitive surgery. Diagnosis was made at postmortem in 2 cases. These patients will be discussed later in this chapter.

4.3.11 Results of definitive Hirschsprung's surgery and complications

4.3.11.1 *Types of surgical procedures performed*

A total of 330 definitive surgical procedures were performed for Hirschsprung's disease (**Table 4.8**). Historically, the initial procedure performed in this series was the Swenson procedure (n = 58). This was then followed by the Duhamel procedure (n = 61). This group included 16 patients with TCA in whom an extended Duhamel procedure was performed. Soave procedures have been the primary operation of choice for short segment aganglionosis since 1967. Following an initial 20 cases of the "true" Soave procedure, the practice was modified to perform the Boley sutured anastomosis (n = 185). Six other initial surgical procedures were performed for specific indications including 2 State

TABLE 4.7

COMPLICATIONS OF DEFUNCTIONING COLOSTOMY (N = 78)

	n	Transverse Colostomy	Near Colostomy	Other
Prolapse	30	18	11	1
Adhesive Obstruction	14	6	4	4
Excoriation of skin	10	3	6	1
Stomal retraction	7	7	0	0
Enterocolitis	5	3	1	1
Wound Infection	4	2	2	0
Faeculoma	4	2	2	0
Pneumonia	2	1	1	0
Intestinal Perforation	1	0	1	0
Intussusception	1	1	0	0

TABLE 4.8

PULL-THROUGH PROCEDURES FOR HIRSCHSPRUNG'S DISEASE

n = 330

	TOTAL	R/SIGM	L/SEG	TCA
Swenson (1957-1961)	58	42	12	4
Duhamel (1960-1990)	61	33	12	16*
Soave True Soave (1967-1970)	20	17	3	0
Boley modification (1970-1990)	185	143	40	2
Other	6	5	0	1

* Extended Duhamel for TCA

Other procedures

Myectomy (Lynn)	2
Anterior Resection (State)	2
Stevens (with ARM)	1
Ileo-anal anastomosis	1

procedures, 2 myectomies (Lynn 1975), 1 Stevens pull-through where an anorectal malformation co-existed and 1 patient who had an ileoanal anastomosis for aganglionosis of the entire colon.

4.3.11.2 *Early complications of definitive surgical procedures*

Early postoperative complications are listed by operation in **Table 4.9**. In addition to those listed, 55 patients (17%) had persistent buttock excoriation. In 21 (41%) of these the Soave procedure had been performed.

4.3.11.3 *Specific procedure-related complications* were relatively uncommon. The incidence of anastomotic leak, early stricture formation and postoperative micturition disturbance was significantly higher following the Swenson procedure (Fisher's, $p<0.05$). Wound sepsis was noted particularly in the early part of this series and a wound infection requiring further management was reported in 30 (9%) of the 330 procedures performed. A cuff abscess was also reported in 11 (3.3%).

4.3.11.4 *Caecostomy related complications*

Skin excoriation and local inflammation requiring either local or systemic therapy was rarely observed. Premature dislodgement of the tube occurring in 2 patients was due to inadequate fixation but no adverse sequelae were noted. One persistent faecal fistula required surgical closure following caecostomy tube removal.

Procedure-related complications appeared to occur in fewer patients in those with a caecostomy than in 87 patients with a 3-stage procedure (**Table 4.10**). Anastomotic leak and cuff abscess occurred in 2 (1.8%) patients with a caecostomy. In a further 3 patients (3%) distal colo-anal stenosis was noted. All of these patients responded to anal dilatation.

TABLE 4.9

EARLY COMPLICATIONS FOLLOWING PULL-THROUGH PROCEDURES

	Swenson n=19	Duhamel n=6	Soave (Boley) n=38	Soave (True) n=11	Other n=3
Wound Sepsis	5	2	18	2	3
Cuff abscess	4	1	4	2	0
Leak	4	1	1	1	1
Stricture	7	0	5	1	0
Micturition Disturbance	4	0	1	0	0
Neorectal Retraction	1	1	1	1	0
Pneumonia	0	0	6	2	0
Ileus	0	0	2	0	0
Haemorrhage	0	1	0	0	0

TABLE 4.10

EARLY COMPLICATIONS FOLLOWING SOAVE ENDORECTAL PULL-THROUGH PROCEDURES (SERP) : 1967 - 1990 (205 PATIENTS)

	LEAK /STRICTURE	NEORECTAL RETRACTION	CUFF ABSCESS
2 STAGE SERP (with caecostomy) [n = 122]	2 (1,6%)	3 (2.5%)	0
3 STAGE SERP (no caecostomy) [n = 87]	8 (9,1%)	3 (3.4%)	1 (1,1%)
Total	10 (4.9%)	6 (2.9%)	1(0,5%)

4.3.11.5 *Long-term procedure-related complications*

No long term postoperative complications occurred in 127 patients (39%). In the remainder, certain of the complications were procedure related although others were not related to the procedures performed.

Abdominal distension persisted postoperatively in 64 patients. This was a statistically higher in those undergoing the Swenson and Duhamel procedures than in those undergoing the Soave procedure (Chi-square, $p<0.05$) (**Table 4.11**).

Persisting **constipation** (in patients followed up a mean of 11 years) was significantly higher in those with the Duhamel than in those in whom the Soave procedure had been performed (Chi-square, $p<0.01$) (**Table 4.11**).

Other significant long-term differences were a significantly higher incidence of cuff stricture, micturition disturbance and persisting enterocolitis in those with the Swenson procedure (Fishers, $p<0.05$).

The incidence of postoperative enterocolitis was also much higher in those with TCA (Fishers, $p<0.001$). Other observed differences in long term outcome and complications were not significant on testing (**Table 4.11**).

Preoperative enterocolitis persisted in 11 patients. In 9 of these there was evidence of postoperative obstruction. In 7 the histological picture was that of neuronal intestinal dysplasia (NID). A further 2 patients had alternating constipation and diarrhoea suggesting an overflow element.

TABLE 4.11

LATE COMPLICATIONS FOLLOWING PULL-THROUGH SURGERY FOR HIRSCHSPRUNG'S DISEASE

Type	Sve	T/Sve	Duh	TCA	Sw	Other
Diarrhoea	21 (11%)	1 (5%)	2 (6%)	7 (31%)	9 (15%)	2 (33%)
Enterocolitis	13 (7%)	3 (15%)	3 (6%)	5* (31%)	11* (19%)	1 (16%)
Abdominal Distension	27 (14%)	7 (35%)	11 (24%)	1 (6%)	17* (29%)	1 (16%)
Cuff Stricture	12 (6%)	4 (20%)	1 (2%)	0 -	8* (13%)	0 -
Buttock Excoriation	10 (5%)	0	2 (4%)	3 (18%)	0 -	1 (16%)
Soiling	7 (3%)	0 -	0 -	0 -	1 (1%)	0 -
Constipation	17 (9%)	2 (10%)	12* (26%)	0 -	10 (17%)	2 (33%)
Intestinal Obstruction	14 (7%)	4 (20%)	4 (8%)	0 -	4 (7%)	0 -
Rectal Prolapse	7 (3%)	0 -	1 (4%)	0 -	0 -	0 -
Micturition Disturbance	3 (1%)	0 -	2 (4%)	0 -	7* (12%)	0 -
Sexual Dysfunction	3 (1%)	0 -	4 (9%)	0 -	6 (10%)	1 (16%)

* = Fishers, $p<0.05$

Postoperative sexual problems were experienced by 14 patients. These made up 21.5% of the 65 patients older than puberty at follow-up and thus represented a significant complication (**Table 4.11**). There were 5 female and 9 male patients with sexual complications. In females the major complaints were dyspareunia and infertility. In the male patients inadequate erections were reported in 5. A significantly higher incidence of sexual dysfunction was observed following the Swenson procedure (Fisher's, $p<0,01$) and Duhamel (Fisher's, $p<0.05$) than in those undergoing the Soave procedure suggesting that this may be a procedure related complication.

In addition, **a residual segment** of aganglionic bowel was present in 14 patients. In these patients the cause was inadequate surgery during the early stages of performing the procedure. Long term micturition dysfunction was significantly higher following the Swenson procedure and was lowest following the Soave operation.

4.3.12 Procedures for postoperative complications

A total of 35 patients (10,7%) had required a repeat pull-through procedure during the period under review. This included 4 patients whose primary procedure had been performed elsewhere. A Duhamel procedure was performed in 10 which was for TCA in 2. In a further 12, a Swenson procedure was performed and in 9 a Soave endorectal pull-through had been carried out. Of the latter, 6 followed the classical or true Soave and 3 followed the Boley modification.

The reason for repeat surgery was stricture formation in 18 and residual aganglionic segment in 14. In 1 further patient with TCA, an inaccurate frozen section report resulted in a repeat procedure. The remaining 2 were due to other technical problems.

Secondary procedures performed were true Soave to a modified Soave procedure in 7, Duhamel to Duhamel in 5, Swenson to Duhamel in 4, Swenson to Soave in 2, a Duhamel to Soave in 1, and a Swenson to Swenson in 1 patient. Other procedures performed included 2 sphincterotomies. In 1 further patient a State procedure was converted to a Duhamel at the second operation. Ileal pouch reservoirs were created in a further 2 patients with TCA who had persisting symptoms.

4.3.13 Mortality of Hirschsprung's disease

There were 33 deaths prior to definitive pull-through surgery in this series. In 2 cases, the diagnosis was made at postmortem. In 1 of these cases there was a family history. The remaining 31 deaths were mainly due to enterocolitis (22 patients), associated anomalies (7 patients) and other septic complications (2 patients). In 17 (77%) of the deaths from enterocolitis, the entire colon was aganglionic with extension into small bowel.

A further 22 of the 330 undergoing definitive surgery died postoperatively. The cause of death in these patients was unrelated to Hirschsprung's disease in 9 cases. In 3 of these cases the family brought to light previously unrecorded deaths during the follow up study. The remaining 13 deaths included 7 which were related to TCA with extensive small bowel involvement and 6 (1.8%) were related to septic complications following surgery. There were 308 survivors of the 378 cases of Hirschsprung's disease. Seven (12%) of the deaths occurred in those subjected to the Swenson procedure, 10 (18%) the Duhamel procedure and in 5 (9%), death occurred in patients following the Soave endorectal pull-through (SERP).

In total 53 (14%) of the 378 patients with Hirschsprung's disease died. Adjustment of this figure for those 9 in whom the cause of death was unrelated (eg Motor vehicle accident etc), resulted in an adjusted mortality of 11.9%. Thirteen (3.9%) of the deaths followed the 330 definitive pull-through procedures. Of these, 7 (54%) were in patients with TCA.

4.4 DISCUSSION

4.4.1 Demographic factors

The majority of patients had a good functional outcome following the surgical management of Hirschsprung's disease. The demographic features of this general population of patients with Hirschsprung's disease reveal several interesting features. Careful study of the place of birth shows a prevalence of 1 patient for every 5726 live births within the greater Cape Town Metropolitan area (**Appendix B**). The number of cases referred from other major centres was in keeping with the population densities of those areas. This was approximately the same proportion of the birth rate as the Cape Town sample.

Despite the likelihood of serious under-reporting from one sector of the population, particularly during the earlier years of this study, this series has a similar incidence to that previously reported in other studies (Swenson 1973; Kleinhaus 1979; Holschneider 1982; Ikeda 1984). In a survey of the surgical section of the American Academy of Pediatrics Kleinhaus (1979) reported one case in every 5257 live births. Ikeda (1984), in a review of 1628 cases occurring in Japan, reported an incidence of one patient with Hirschsprung's disease for every 4697 births.

The racial distribution in this series is similar to the normal spectrum of children seen at the Red Cross Children's Hospital. The condition occurred in all race groups and the lower numbers of African patients can be partly explained on the

basis of referral patterns. The prevalence in black patients is, however, higher than that reported by Swenson (Swenson 1973). The male to female ratio is similar to that reported by Ikeda (1984) and Ryan (1992). Although there appeared to be an increase in the number of female patients of mixed race resulting in a lower male to female ratio, this difference did not reach statistical significance on testing. In the absence of other factors, it is difficult to explain as it is not related to length of the affected segment or any other feature.

The perinatal course did not appear to contribute to an unfavorable outcome and was uneventful in the majority of patients. This study does not support the hypothesis of Bughaighis that perinatal asphyxia causes postoperative dysfunction resulting from an immaturity of nerve cells (Bughaighis 1971).

The high average weight and the high incidence in full term babies confirmed the clinical impression that Hirschsprung's disease is largely a disease of large full term babies. The incidence in premature infants was less than half that of full term infants (**Appendix B**). Twelve percent of 156 patients where the birth weight was recorded were below 2.5 Kg birth weight. This is higher than the 5.5% reported by Ikeda (1984) and the 7% incidence reported by Ryan (1992). This may possibly reflect nutritional differences between the populations studied. In this study, only 3 (1.9%) babies were scored at less than 37 weeks gestational age. An analysis of the patient population reported by Ryan (1992), showed 6 babies (3.3%) scored at less than 37 weeks gestation in his series. Only 2 of the premature babies were born in the Cape Town Metropolitan area giving an approximate incidence of 1 in 12 000 premature births. It must be recognized, however, that this is a rough estimate as all parameters regarding premature births have not yet been clarified (**Appendix B**).

These observations raise the question as to why a difference between premature and mature babies should exist in a neurodevelopmental condition. It may indicate that aganglionosis arises from a relatively late disturbance of neuroblast development rather than an event occurring during the migratory phase.

4.4.2 Familial and genetic associations

Familial and genetic mechanisms of transmission of Hirschsprung's disease were selected out for subgroup analysis in our study. In keeping with previous studies (Emmanuel 1965; Passarge 1967; Gordon 1966; Kleinhaus 1979; Ikeda 1984; Spouge 1985; Ikeda 1986; Badner 1990; Ryan 1992) the incidence of associated congenital anomalies has been noted to be low. The lack of other reported associations such as dominant sensorineural deafness (Weinberg 1977), Waardenburgh syndrome (Omenn 1979; Branski 1979), neurofibromatosis (Schocket 1957), neuroblastoma (Passarge 1967), phaeochromocytoma (Schocket 1957; Passarge 1967), the MEN Type II(b) syndrome (Khan 1987) and other abnormalities (Passarge 1967), is in keeping with other reports (Ikeda 1984; Ryan 1992). Analysis of the incidence reported by Ikeda in 1628 cases gives a 14.3% incidence of associated abnormalities which compares favourably with the 16.1% noted in this study.

The presence of a meningomyelocoele in two of our patients as well as in two further relatives (Gordon 1966) might point to a common neurocristopathy as suggested by Bodian (1963). A similar mechanism of origin might also apply in a further 8 cases who had congenital ophthalmological problems. The development of the eye precedes the migration of nerve cell precursors from the craniocervical portion of the embryonic neural tube.

Such associated anomalies were not significantly more frequent in patients with familial occurrence than in isolated cases (9.6% and 16.7% respectively). This

observation suggests that in this series, a different pattern of genetic inheritance existed for associated anomalies than that of familial occurrence.

The incidence of familial incidence was 8.46% (32/378) but only 4.5% of the 351 families represented had a familial association with Hirschsprung's disease. The entire colon was involved in a significantly higher number of patients with a familial occurrence [11/32 (34.3%)] (Chi-square, $p<0,01$) **(Figure 4.6)**. Fifty percent of males with TCA had a family history of Hirschsprung's disease. In only 2 of these was the condition transmitted through a female sibling.

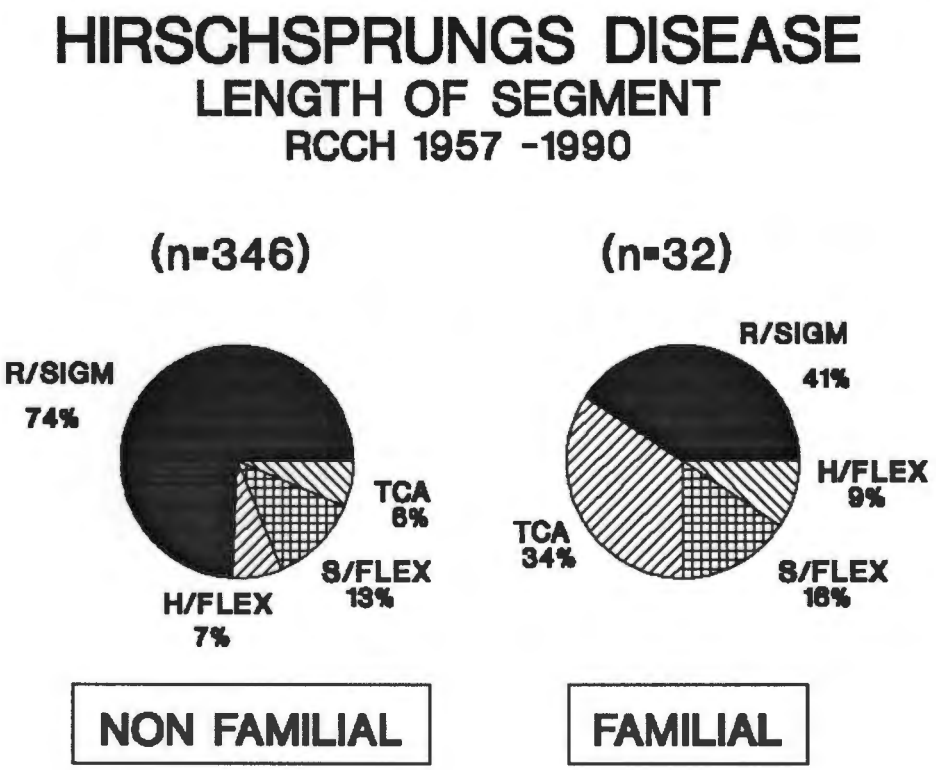


Figure 4.6: Pie charts demonstrating differences between length of aganglionic segments in familial and non-familial cases (R/Sigm = Rectosigmoid; H/Flex = Hepatic flexure;

S/Flex = Splenic flexure; TCA = Total Colonic Aganglionosis)

Progression of length of segment in succeeding generations was noted in two families. This transmission was via an affected mother in 2 cases and a father in 1 case (**Table 4.3**). The affected offspring was a male in 2 of the cases. The female offspring was the second born female infant of a mother with 3 affected step brothers. In two of these cases the intestinal aganglionosis affected the entire colon and extended proximally to the upper small bowel. In 1 instance almost the entire GIT in the female child was affected. Ganglion cells were only detected in the stomach in this patient. The association between a familial occurrence and a longer segment suggests an etiological factor which occurs at an earlier stage of embryogenesis of the enteric nervous system.

Family histories were scrutinized for evidence of genomic imprinting. A lack of consistent transmission patterns led us to the conclusion that although certain features of genomic imprinting do exist, many of the patterns of transmission do not fit in with this explanation.

There was no higher incidence of co-existing neuronal intestinal dysplasia in the familial cases despite a reported possible genetic link between the two conditions (Schärli 1981; Fadda 1987; Pistor 1987; Rintala 1989; Moore 1992).

4.4.3 Timing of presentation, presenting symptoms and length of aganglionic segment

The time of presentation and length of segment demonstrated a similar pattern of length of aganglionosis in the percentage of patients presenting at any one given time (**Figure 4.5**). This suggests that length is not the only criterion for time of presentation and consequently the severity of the presenting symptoms.

One hundred and ninety-two (50.8%) patients were diagnosed within the neonatal period with a further 6.1% diagnosed at 1 month. A further 90 patients

(24%) were diagnosed as having Hirschsprung's disease after one year of life. The number of neonatal diagnoses improved over the 34 year period under review. In the first 4 years of the hospital's experience, prior to 1961, only 24.2% (8/33) of patients were diagnosed in the neonatal period. In the next ten years this improved to 40.2% (35/87). In the decades preceding and succeeding 1980, the percentage of neonatal diagnoses further improved to 56,7% (59/104) and 53% (51/98) respectively. The number of neonatal diagnoses is lower than some other reported series (Polley 1986) but this probably reflects the referral patterns of the peripheral areas of our health service area and our position as a tertiary referral center in a developing country. It is also similar to the reported experience of Great Ormond Street over a similar period of time (Risdon 1989). This is of interest as the Great Ormond Street is also a tertiary hospital with a number of referrals from distant centres. By way of further comparison, a survey of 1628 patients treated in Japan between the years 1978 -1982, 49% were diagnosed in the first month of life (Ikeda 1986). Klein (1984) reported 26 cases of Hirschsprung's disease out of 137 patients with neonatal obstruction.

The overall experience of the length of the affected segment is in keeping with reports from other large published series (Kleinhaus 1979; Holschneider 1982). Patients presenting after 12 months of age had a higher incidence of rectosigmoid aganglionosis than the overall group. Those presenting early (in the neonatal period) had a greater tendency towards a longer segment of aganglionosis (**Figure 4.5**).

Despite these trends, the length of aganglionic segment did not appear to be the only determining factor of the time of diagnosis in these patients. Patients with obstructive symptoms did not appear to have longer segments of affected bowel. Eight patients with TCA in this series presented later than 6 months.

Patients with long segment aganglionosis have been reported to present at beyond 6 months of age and there have even been reports of survival of patients with TCA into adult life (Rafferscheid 1982). The late presentation of patients with a long aganglionic segment is not easily explained but is in keeping with other series where the severity of the clinical presentation was not always found to depend on the length of the aganglionic segment (Wyllie 1957; Fairgrieve 1963; Stone 1965; Nixon 1976; Rafferscheid 1982). The reasons for a severe onset of early symptoms in certain patients in contrast to a less severe clinical picture in others with the same or an even longer segment, is as yet unexplained.

Intestinal obstruction was a common feature of patients presenting in the neonatal period and constipation was a prominent complaint of those presenting late. Differences in symptomatology at presentation between those in the neonatal period and those presenting later are summarized in **Table 4.5.** In 94 (49%) neonates a delayed passage of meconium was noted whereas this only occurred in 33 late presenters (17.8%). In patients with obstructive symptoms , abdominal distension remained a prominent symptom. On the other hand, its absence in 25-33% of patients could result from the retrospective nature of this study. Distension remained a fairly constant feature in patients with postoperative obstructive symptoms.

Intestinal obstruction occurred more frequently in those patients presenting within the neonatal period (Chi-square, $p < 0.01$). This higher incidence of intestinal obstruction with bile stained vomiting was not entirely confined to those with longer aganglionic segments. In patients with rectosigmoid disease, the incidence of intestinal obstruction in the neonatal period (53/118 or 46.4%) was higher than in those presenting later (44/155 or 28.7%) (Chi-square, $p = <0.05$).

The overall incidence of symptoms was limited by the lack of recall of those patients presenting later (ie outside of the neonatal period). This was particularly true of symptoms such as a delay in passage of meconium after birth. This results in a lower overall incidence for this presenting symptom. In new patients seen during the study period it was almost always present.

It is still unknown why some patients with short segment disease present with acute symptoms, while others with longer segments have such mild symptoms. Ehrenpreis (Ehrenpreis 1970) suggested that the relatively benign course of congenital megacolon in adult patients may be due to a low degree of spasm in the narrow segment and anal sphincter. It has also been attributed to an abnormal adrenergic nerve supply (Touloukian 1975) and peptidergic abnormalities in the non-adrenergic, non-cholinergic nervous system (Nirasawa 1986).

4.4.4 Areas of diagnostic difficulty

There were two groups of patients whose abdominal X-rays were reported as being normal. These were mostly neonates and those with total colonic aganglionosis. The development of colonic distension over the first few days of life (Ehrenpreiss 1946) may be the reason for the initial normal appearance of the X-ray film in neonates. It is well known that the X-ray appearances are often difficult to interpret in patients with total colonic aganglionosis (Cremin 1982) which may add further diagnostic difficulty.

The findings of this study suggest that the plain abdominal X-ray alone is often of value in suggesting the diagnosis, given the clinical history. Abnormal radiological features were observed in a total of 77% whereas the diagnosis was suggested on abdominal films in 52% of patients.

In the early stages of this study the Barium enema was the major means of diagnosis. Swenson (1973) estimated that the barium enema was diagnostically misleading in as many as 23% of neonates, but that this rate steadily dropped to 12.7% at 1-12 months and was 6.5% after 12 months of age. It provides valuable additional help in establishing the diagnosis before the rectal biopsy results are available but is presently used to determine the site of the transitional zone. A Barium enema may be normal in patients who have been decompressed prior to radiological investigation as well as those with total colonic aganglionosis (Davies 1981). The routine use of barium enema has been questioned (Smith 1991), although it is still widely used.

Discrepancies were noted between the radiological cone and the aganglionic level identified histologically. In the patients of this series, this discrepancy was most striking in those with a long segment or total colonic aganglionosis. These findings are in keeping with other reports (Davies 1981). The addition of a 24 hour delayed film (Louw 1978; Cremin 1982) helped to clarify the diagnosis in cases where this was employed.

Apart from the difficulty in radiologically identifying the cone level, particularly in certain forms of long segment aganglionosis, no correlation could be drawn between the radiological features and eventual outcome. In particular, no evidence of co-existing neuronal dysplasias was detected by the initial X-Ray features in those cases where this diagnosis was established postoperatively.

4.4.5 Late complications of pull-through surgery

Certain of the complications were related to specific types of surgical procedures performed. Enuresis and sexual dysfunction was more common with the

Swenson procedure whereas constipation was more frequent following the Duhamel procedure (**Table 4.11**).

Patients with diarrhoea and enterocolitis in the postoperative period were difficult to separate on clinical grounds. The reason for the higher incidence of diarrhoea following an extended Duhamel procedure for total colonic aganglionosis was probably on the basis of the absent colon. Obstructive symptoms were present in 17 of the remainder suggesting a possible etiological relationship.

The major sexual problem in males was a poor erection, and in females dyspareunia or infertility. These were identified in 14 patients and were associated with a micturition disturbance in 2. They occurred following the Soave, Duhamel and Swenson procedures and following a sphincterotomy in 1 patient. There was a higher incidence following the Swenson procedure.

Late postoperative complications included enterocolitis, constipation, cuff strictures and subsequent continence problems. These are compared to reported results from large collective series in **Table 4.12** (Joppich 1982; Holschneider 1982). A higher incidence of constipation and enterocolitis may partly reflect the historical nature of this series. The lower incidence of soiling, enuresis and strictures is more in keeping with some more recent series (Kleinhaus 1979; Polley 1986; Molander 1989).

An overall mortality of 11.9% compares favorably with the 12% mortality reported by the Great Ormond Street group over a similar period of time (Nixon 1985). An adjusted 3.9% mortality for those submitted to pull-through procedure, compares with other series (**Table 3.1**). Of these, 7 deaths occurred in patients with TCA.

4.4.5.**1** *Residual aganglionic segment*

In 14 patients with some form of postoperative obstruction, a residual aganglionic segment was incriminated. A repeat pull-through procedure was successfully performed in these patients. In 8 of these patients the cause could be attributed to an inadequate initial resection of the aganglionic segment or an inadequate initial pull-through procedure (eg State procedure, Myectomy) performed elsewhere.

4.4.5.**2** *Cuff strictures*

18 of the remaining patients had developed a severe postoperative stricture in the anal canal presumably caused by postoperative infection and possible anastomotic leak.

Of those with stricture formation, 8 followed the Swenson procedure, 1 the Duhamel procedure and 5 the Soave procedure. The remainder were mostly from those undergoing a true Soave procedure but in one patient a stricture was noted 10 years after a successful Soave operation. In 11 of these patients a second pull-through procedure was required to alleviate symptoms.

TABLE 4.12

COMPARISON OF LATE COMPLICATIONS WITH INCIDENCE REPORTED IN THE LITERATURE

	SOAVE	DUHAMEL	SWENSON
Diarrhoea	10.7% (6%)	14.7% (5.9%)	16% (11.6%)
Enterocolitis	7.8% (13.2%)	13.1% (4.7%)	16% (3.7%)
Stricture	7.8% (7.8%)	1.6% (13%)	13% (8,3%)
Constipation	9.2% (8.3%)	19.6% (6.5%)	33% (10.4%)
Micturition disturbance	1.5% (15.3%)	6.5% (14.3%)	16% (10.4%)
Soiling	3.4% (3%)	0 (6.7%)	1% (12.5%)

(This series without brackets, reported incidence in brackets).

4.4.5.3 *Other functional causes of obstructive symptoms*

In other patients with symptoms of rectal obstruction the cause appeared to be unexplained on clinical grounds or on the basis of complications of surgery. There was no evidence of an anastomotic leak or any other complications which may have resulted in the development of postoperative outlet obstruction. It is this group that may be considered to have potential residual abnormalities of sphincter or bowel function. This identified a target group for further investigation in this study.

4.4.5.4 *Hirschsprung's associated enterocolitis*

Enterocolitis (EC) remains a significant cause of pre- and postoperative morbidity and mortality and accounted for at least 53% of the deaths in this series. It was a presenting feature in less than 20% of the patients in this series but in certain cases occurred in the postoperative period without a previous history. In this study there was no demonstrable connection between delay in diagnosis and the incidence of enterocolitis as can be seen in the following graph **(Figure 4.7)**

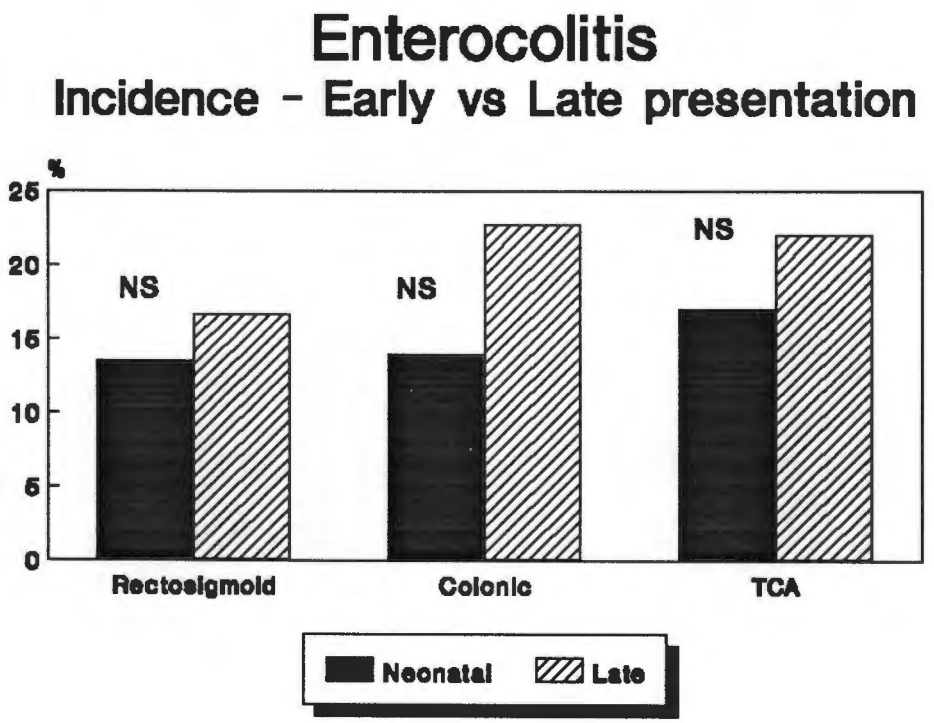


Figure 4.7: Bar graph showing the incidence of enterocolitis related to time of presentation.

The link between enterocolitis and postoperative obstruction is not clear but Swenson modified his operation in the belief that it contributed to postoperative enterocolitis (Swenson 1953). This will be discussed at a later stage in this thesis.

4.4.6 Postoperative continence

Although some delay in attaining continence occurred in the majority, continence was satisfactory by the age of 8 years in all but a few isolated cases. Eight patients had long term difficulty controlling defecation. In one patient normal control was reported at 25 years of age having improved significantly at 18 years. In 4 of the remainder, incontinence was often associated with constipation and some degree of outlet obstruction. The delay in achieving complete continence may, in many, be related to surgical or psychological factors.

4.4.7 Limitations of the clinical study

The major limitation of the clinical study lies in the retrospective nature of the data. Assessment of function therefore rests on the available data which is incomplete in some instances.

On the basis of this retrospective study with its inherent limitations of completeness of data it became clear that there was a need for a current evaluation of the adequacy of function and an assessment of the nature of the complications.

Based on the available data, I concluded that, over the mean 5 year follow-up of patients following surgery, 93% had a satisfactory outcome. However to test this finding I set out to reassess all patients of the larger group who could be recruited. This would permit more complete evaluation of postoperative problems and provide a longer mean time of postoperative observation. This study forms the subject of the next chapter.

CHAPTER 5

THE LONG TERM OUTCOME OF HIRSCHSPRUNG'S DISEASE - A CURRENT EVALUATION OF PATIENTS

5.1 AIM

The aim of this study was to examine more closely the view that the overall long term status of patients who have undergone surgery for Hirschsprung's disease is good. In addition, this study identifies the nature and prevalence of symptoms like that of obstruction, occurring in the postoperative period, which require further investigation.

5.2 MATERIALS AND METHODS

5.2.1 Patient study population

An attempt was made to recruit all surviving patients who had undergone surgical correction of Hirschsprung's disease between 1957 and 1990. These survivors numbered 308. Ethical approval was obtained from the Ethical Research Review Committee of the University of Cape Town to conduct the study. Patients excluded from recall were those known to have died and those declining to participate in the study.

5.2.2 Recruitment of sample

The addresses and present whereabouts of all known surviving patients were traced from hospital folders, computerized hospital records, last known address, research records, correspondence, regional telephone directories and in certain cases from other patients. Those older than 4 years of age were included in a clinical long term evaluation.

Patients were initially contacted by questionnaire or telephonically and interviews arranged with those responding to recall. The study was conducted between

the years 1986-1990. It consisted of a personal interview, full clinical examination with the particular emphasis on the anorectal function and final outcome.

5.2.3 Data collection

Interviews were carried out by the author in the presence of either a research social worker or a stomatherapist. They were conducted according to a standardized format (Appendix A) and information required for long term evaluation was obtained. Where interview was not possible due to patients living at a distance a telephonic interview was conducted using the same questionnaire format.

5.2.4 Demographic data

Background information on patients was obtained from the records studied retrospectively in the previous chapter and verified during the interview. At the time of interview, details of family history, parental age, consanguinity and a family tree going back at least 2 generations were obtained where possible. Additional information recorded included age at follow-up, physical development as well as the weight and height for age.

Blood samples were taken from as many family members as possible where familial involvement was identified for detailed chromosomal study and DNA analysis as part of an allied study beyond the scope of this thesis.

5.2.5 Examination and investigation

A full physical and digital rectal examination was performed. Rectal examination assessed the presence or absence of rectal prolapse, the tone of the anal sphincter, the ampulla capacity (ie. degree of distension) and the amount of faeces present. Rectal manometry and suction biopsy were then performed in

patients with residual symptoms. Manometric assessment was performed on obstructed patients as well as a randomly selected sample of those without obstructive symptoms. The results are discussed in the in following section.

5.2.6 Physical development

Anthropomorphic indices were calculated from the recorded age in months, sex, weight and height. Height for age (HA) and weight for age (WA) indices were calculated up to 18 years of age (Waterlow 1977). Weight for height (WH) indices were calculated according to the recommendations of an international WHO working party (1986). The recommended limitations on the calculation of weight for height are a height less than 145 cm in male children up to 11,5 years (138 months) and a height of less than 137 cm in females less than 10 years (120 months) (Gorstein 1989).

WHO reference populations were used for weight and height for age comparisons (Dibley 1987). Comparison was by Standard deviation units (Z-Scores) which give a score of 1 for the 75th centile weight for age and 2 for the 97th centile (Dibley 1987). Scores of -1 and -2 represent the 25th and 3rd centiles respectively. Patients falling outside of these parameters are given a score of minus 3 or plus 3. Low anthropometry levels were regarded as a score of less than minus 2 SD units (WHO working party 1986; Dibley 1987).

5.2.7 Developmental milestones, personality adjustment and educational achievement

Milestone achievement, psycho-social adjustment and school performance were investigated and the results recorded. During the interview the relationship to peers, participation in extramural activities and attitude to socializing with the opposite sex were specifically examined. The patient's personality adjustment was assessed during the interview with specific reference to the attitude to medical staff and hospitals.

5.2.8 Assessment of postoperative function

Assessment of anorectal function was established by recording stool frequency, stool consistency, stool control and sensory appreciation. Specific attention was paid to the fine discrimination of rectal gas versus liquid or solid stool. An assessment of reservoir function of the rectum was also performed. Long term medication use and dependency was also assessed.

Patients older than 4 years were assessed for long term functional outcome and those with obstructive symptoms identified. Special attention was paid to patients with obstructive symptoms not responding to repeated anal dilatation or other therapeutic measures. These findings were correlated to the age of the child and the type of definitive operation performed.

Methods of assessment of postoperative anorectal function included the Kelly scoring system (Kelly 1969, 1972), the modified Wingspread score (Stephens 1984) and the Holschneider scoring system (Holschneider 1983, 1988) in keeping with other studies on postoperative functional outcome (deVries 1988; Stephens 1984; Holschneider 1983,1988; Kelly 1969, 1972) (See appendix D for details of scoring systems).

5.2.9 Statistical analysis

Statistical analysis between large groups was by the Chi-squared test. Where the comparison involved two groups of patients with small numbers, Fishers exact test was utilized.

Small number non parametric methods, one way Anova and the Kruskal Wallace one way testing by Ranks, were methods employed for comparison between unpaired groups. Results were expressed as 95% confidence intervals of means or a p value.

5.3 RESULTS

5.3.1 Patient Sample

Three hundred and thirty of the 378 potential patients had a definitive surgical procedure. Twenty-two of these died. Of the remainder, 308 were eligible for follow-up assessment.

Seven (12%) of the deaths followed the Swenson procedure, 10 (16%) the Duhamel procedure and in 5 (2.4%) death followed the Soave endorectal pull-through (SERP). The cause of death was unrelated to Hirschsprung's disease in 9 of the 22 patients. In 3 of these cases the family brought to light previously unrecorded deaths during the follow up study.

Of the remaining 13 deaths, 7 were related to TCA with extensive small bowel involvement. In only 6 patients (1.8%), was death directly attributable to complications of surgery. The most common cause of death being sepsis in these patients. One patient died of an unrelated cause 2 years following assessment but was left in the study group as he was well at the time of interview.

Of the 308 survivors eligible for recall, 32 (14 with residual aganglionic segments and 18 postoperative strictures) were excluded on the basis of having a known cause for a poor result. This left a possible 276 patients available for recall. In theory, this group should represent those patients who could be expected to have a favorable outcome.

5.3.2 Response to recall

Of these 276 patients, 27 were known to be lost to follow-up despite all efforts to obtain their current addresses. This left a potential 249 patients to respond to recall. One hundred and seventy-eight of these, (ranging from 1-36 years), were interviewed. This gave a response rate of 71.4% in the study.

Interviews were conducted personally by the author in 175 cases and in the remaining 3 cases the patient was seen by a colleague. More than one consultation was conducted in 60% of patients interviewed.

Of these 178, 8 were still awaiting definitive surgery at the time of this study but have subsequently been operated on. In 3 of those responding to recall, stomas were required due to complications of previous surgery. This left 167 patients following the definitive surgical procedure who could be assessed as far as anorectal function is concerned.

An arbitrary level of 4 years was set for functional evaluation as being the age at which the majority of children will have achieved day and night continence. One hundred and fifteen (65%) were older than 4 years at interview (**Figure 5.1**). The mean long term follow-up age in this group was 10.0 yrs. The long term evaluation of anorectal function will concentrate on this group.

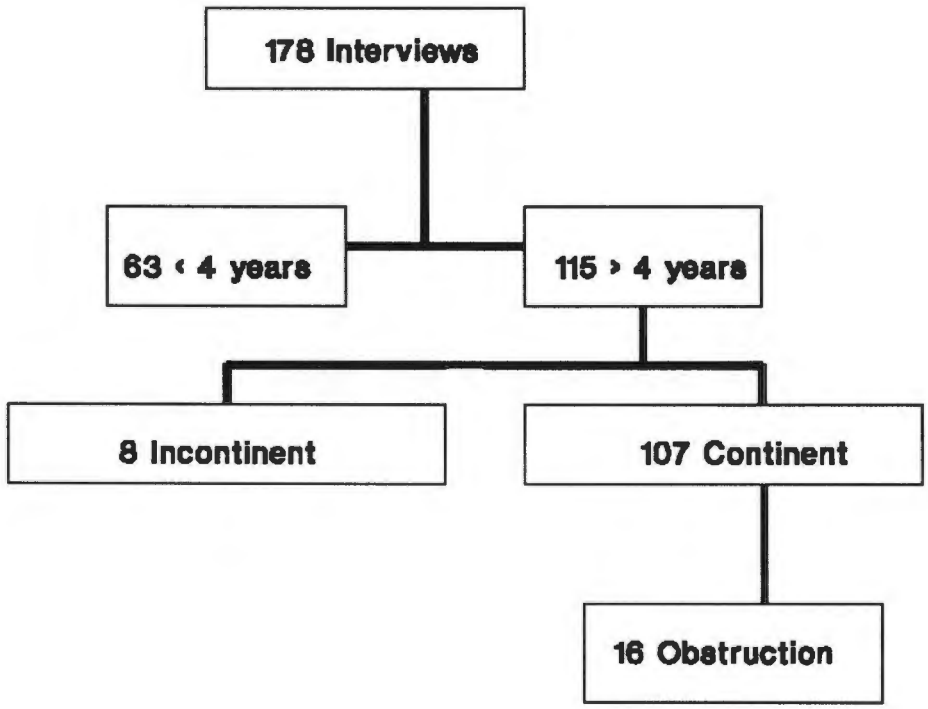


Figure 5.1: Flow chart showing distribution of follow-up patient sample

5.3.3 Representative nature of sample

5.3.3.1 *Demographic features and comparison of sample population:*

A comparison of the demographic data between those responding to recall and those not followed-up is shown in **Tables 5.1-5.2.** One hundred and forty-one of these patients were male and 37 were female, a ratio of 3.8 to 1 **(Table 5.1).**

Demographic features such as race, sex incidence and geographical origin were not significantly different from those not responding to interview as well as in the older patient group. Many of the non-responding patients were referred from distant health centres or rural tribal areas where follow-up is difficult.

Observed differences between this study and those patients not responding to

TABLE 5.1

COMPARISON OF RACE AND SEX OF PATIENTS RESPONDING TO INTERVIEW

	Interviewed		> 4 years		No Interview	
	Male	Female	Male	Female	Male	Female
	n = 141	n = 37	n = 89	n = 26	n = 145	n = 55
Caucasian	44	16	33	12	51	8
Mixed Race	83	21	53	14	69	37
Black	14	0	3	0	25	10
Male : Female Ratio	3.82		3.42		3.06	

TABLE 5.2

TIMING OF PRESENTATION

	Interviews n = 178	> 4 years n = 115	No Interview n = 200
Neonatal	98 (55%)	65 (56,5%)	94 (47%)
1-3 months	23 (13%)	11 (9,6%)	29 (14.5%)
3-12 months	24 (13.4%)	14 (12,2%)	24 (12%)
13-60 months	26 (14.6%)	18 (15,7%)	36 (18%)
> 60 months	7 (4%)	7 (6%)	17 (8.5%)

recall were not significant as far as the length of aganglionic segment, timing of presentation or type of procedure performed were concerned (**Tables 5.2 - 5.4**).

The surgical procedures performed in the total patient sample were comparable to the spectrum of procedures performed in the long term follow-up group (**Table 5.4**).

Seventy-seven Soave procedures, 21 Duhamel and 13 Swenson procedures were represented in the patient group older than 4 years of age. Other procedures were performed in a further 4 patients. These included 2 patients in whom a myectomy had been performed, 1 ileoanal anastomosis and 1 Stevens pull-through for an associated anorectal malformation.

The proportion of Swenson procedures was lower (11.3%) in those followed up long term as opposed to the total patient sample (21.8%). This can be partly explained by the early nature of the Swenson procedures in this series as well as the relatively high mortality in the early stages. The difference in the representation of surviving patients is less marked (11.3 vs 18.5%) when this increased mortality is taken into account. Similarly, there was a higher representation of patients with the Soave procedure in those interviewed (**Table 5.4**). The sample groups appeared to be largely compatible in other respects demonstrating that the long term follow-up study was probably representative of the whole. Differences in representation will be taken into account in any conclusions drawn from this series.

5.3.3.2 *Short-term results and early complications:*

The short-term results and early complications appeared largely comparable in the three groups giving a further indication as to the validity of the comparison with the follow-up study (**Table 5.5**). A slightly higher proportion of patients had

TABLE 5.3

LENGTH OF AGANGLIONIC SEGMENT

	Interview n = 178	> 4 years n = 115	No Interview n = 200
Rectosigmoid	123 (69%)	81 (70,4%)	147 (73.5%)
Colonic long segment	41 (23%)	25 (21,8%)	34 (17%)
TCA	14 (8%)	9 (7,8%)	19 (9.5%)

p = Not significant

TABLE 5.4

TYPE OF SURGICAL PROCEDURE

Type of procedure	Interviews n = 178	> 4 Years n = 115	No interviews n = 200
SWENSON	14 (7,8%)	13 (11,3%)	43 (21,5%)
DUHAMEL	26 (14,7%)	21 (18,2%)	34 (17%)
SOAVE	124 (69,7%)	77 (67%)	82 (41%)
OTHER	3 (1,7%)	3 (2,6%)	3 (1,5%)
STOMA/MISC	11 (6,1%)	1 (0,9%)	38 (19%)

TABLE 5.5

EARLY COMPLICATIONS

	Soave n = 77		Duhamel n = 21		Swenson n = 13 ˜	
Anastomotic leak /Cuff abscess	2	(2.5%)	1	(4.8%)	4	(31%) *
Stricture	2	(2,5%)	-		-	
Wound sepsis	8	(10.3%)	3	(14%)	2	(15%)
Buttock excoriation	24	(31%)	8	(38%)	-	
Micturition disturbance	-	-	1	(7.7%)		
No complications	39	(51%)	9	(42.8%)	5	(38%)

* $p<0.01$

˜ plus 3 other procedures and 1 stoma

no complications following the Soave procedure (51%) but this difference was not significant on testing. The incidence of severe buttock excoriation was highest in the group post Soave ERP, being noted in 24 of the 77 patients in the long term follow-up group (31%). Although this was a fairly representative figure (Buttock excoriation was noted in 23% of all patients with Soave procedures responding to recall [29/124]), the difference was not significant on testing.

Soave procedures had a significantly lower number of anastomotic leaks than patients undergoing the Swenson procedure (Fishers, $p<0.01$). The high incidence of anastomotic leak or cuff abscess following the Swenson procedure can be partially explained on patient selection but the overall incidence still remains higher than that of the Soave procedure. Superficial wound sepsis was noted in a similar proportion of patients following the Soave, Duhamel or Swenson procedures (**Table 5.5**). The 2 strictures observed in patients undergoing the Soave procedure represented those identified during the study and need to be seen in the light of the exclusion of 18 other patients with strictures from the study population. One patient had a micturition disturbance following a Swenson procedure.

5.3.3.**3** *Late complications:*

Complications occurring on long term follow-up in this study group are analyzed according to the original surgical procedure in **Table 5.6**. The exclusion of 30 patients with known long term complications (stricture / residual segment) from the study population must be borne in mind when interpreting these results.

A significantly lower ($p<0.01$) incidence of constipation, sexual dysfunction and micturition disturbance was noted following the Soave procedure when compared to the Duhamel and Swenson procedure in keeping with our previous observations. The low numbers of patients in the sample group must be borne

TABLE 5.6

LATE COMPLICATIONS FOLLOWING 167 PROCEDURES

Symptom n = 124	Soave n = 26	Duhamel n = 14	Swenson n = 3	Other
Diarrhoea (33%)	18 (10.7%)	7 (26.9%)	1 (7.1%)	1
Enterocolitis	10 (6%)	5 (19.2%)	2 (14.2%)	0
Buttock Excoriation	9 (5.4%)	4 (15.3%)	0	0
Soiling	4 (2.4%)	4 (15.3%)	5 (35.7%)	0
Constipation	9 (5.4%)	5 (19.2%)	6 (42.8%)*	0
Abdominal distention (66%)	21 (12.6%)	4 (15.3%)	5 (35.7%)	2
Adhesive Intestinal Obstruction	9 (5.4%)	0	2 (14.2%)	0
Obstructive Symptoms	15 (9%)	0	1 (7.1%)	0
Residual Segment	7 (4.2%)	5 (19.2%)	2 (14.2%)	0
Micturition Disturbance (33%)	2 (1.2%)	0	3 (21.4%)*	1
Sexual Dysfunction	2 (1.2%)	1 (3.8%)	5 (35.7%)*	0

* $p < 0.01$

in mind in drawing conclusions from these findings. Persistent diarrhoea following the Duhamel procedure was related to a long aganglionic segment extending beyond the splenic flexure in all 7 patients where this was recorded. There were 16 patients with obstructive symptoms in whom there was no lasting therapeutic effect of repeated anal dilatations.

5.3.4 Physical development of the child

5.3.4.1 *Weight for age:*

Because of incomplete questionnaire information of patients investigated through the post or telephonically, a reliable weight for age could only be obtained in 153 of the follow-up group. The overall distribution of centiles of weight for age and height for age is shown in the accompanying bar graph **(Figure 5.2)**.

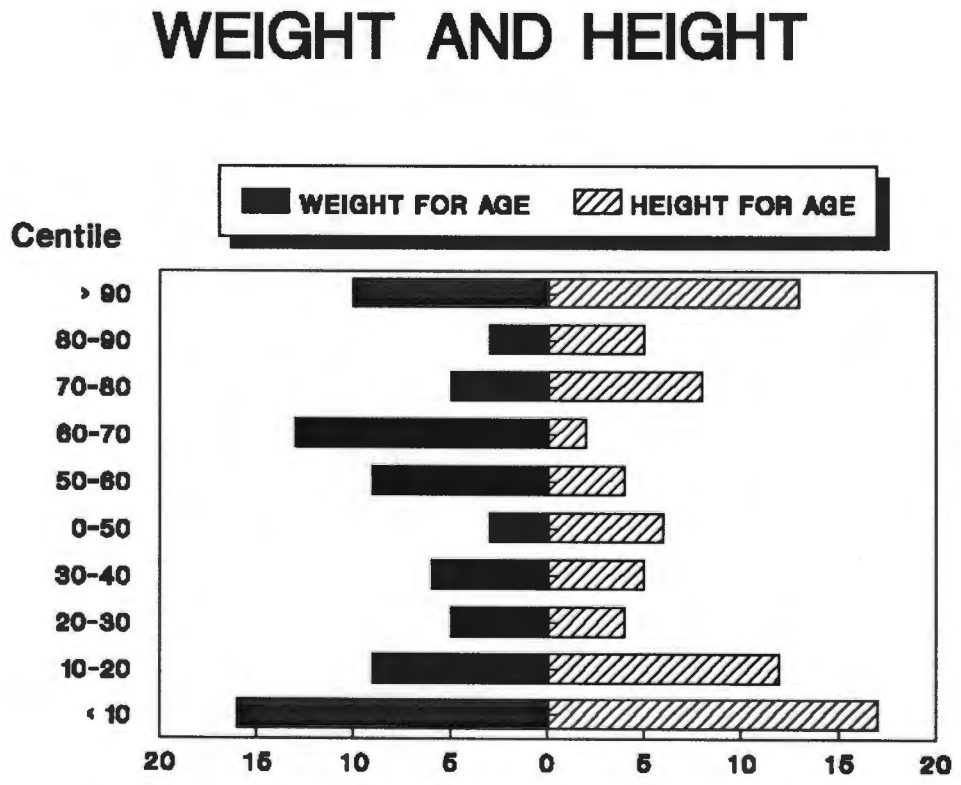


Figure 5.2: Bar chart showing comparative weight and height distribution

A reliable weight for age could be obtained in 110 of the 115 patients over the age of 4 years at follow-up. Of these, 43 were over the 50th percentile, 39 were between the 10th and 50th centile, 21 between the 3rd and the tenth centiles. Six patients were below the third centile weight for age (**Table 5.7**).

Only 5 of the 27 patients (18.5%) diagnosed later than 12 months of age had a weight for age below the 25th percentile at the time of interview. This suggests that normal weight for age is regained with time following the surgical correction of Hirschsprung's disease. Comparison of weight for age with a WHO reference group shows a similar Z-Score distribution in support of this assumption (**Figure 5.3**).

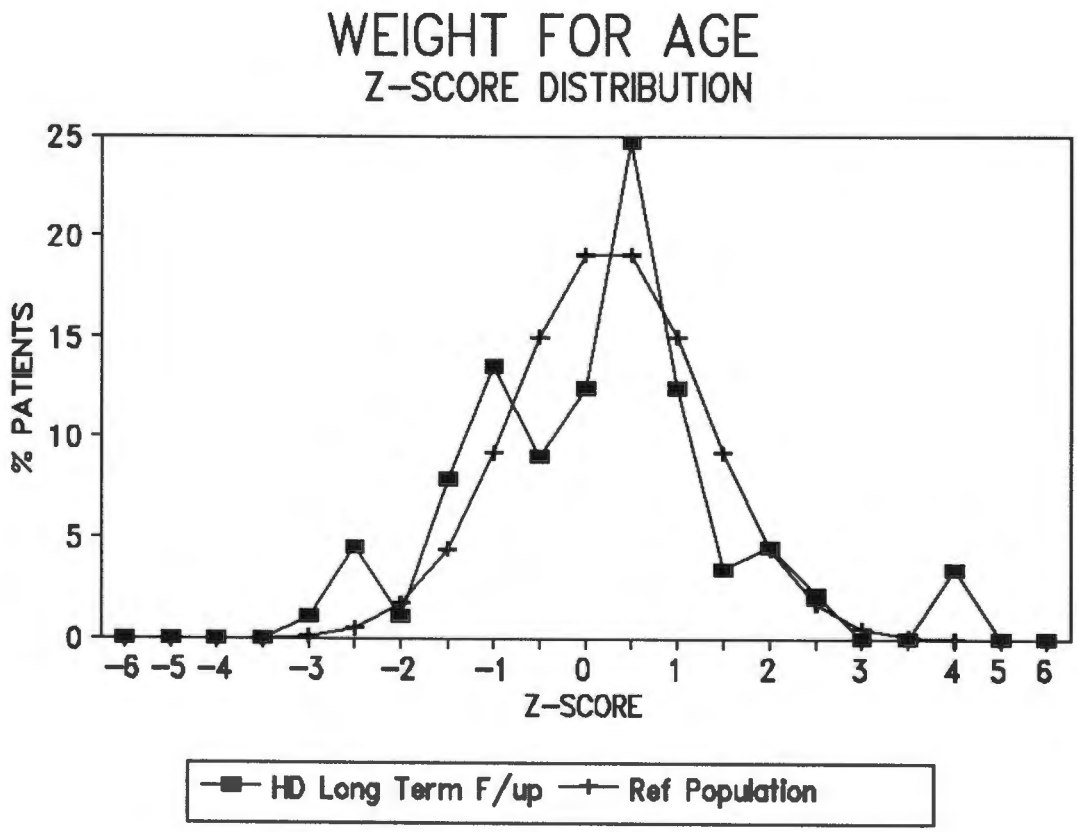


Figure 5.3: Graphic comparison of weight for age with WHO reference group

5.3.4.2 *Height for age:*

Difficulties in obtaining an accurate height measurement was experienced in patients assessed by telephone or through the post. The height for age distribution is tabled in **Table 5.7**. Of the 100 patients over the age of 4 years at follow-up, 48 were over the 50th percentile, 38 were between the 10th and 50th centile, 8 between the 3rd and the tenth centiles and 6 were below the third centile weight for age.

TABLE 5.7

WEIGHT AND HEIGHT - HIRSCHSPRUNG'S DISEASE

FOLLOW-UP STUDY

	Weight for age		Height for age	
Centile	**All ages** n=153	**> 4 years** n=110	**All ages** n=131	**> 4 years** n=100
>50	54	43	60	48
10-15	56	39	48	38
3-10	31	21	10	8
<3	11	6	13	6

5.3.4.3 *Standard deviation scores (Z-Score):*

The weight for age Z-score distribution (**Figure 5.3**) for patients assessed post Hirschsprung's disease is comparable to that of a World Health Organization

reference sample (1986). Although the previous percentile chart (**Figure 5.2**) shows a paucity of patients with a normal height distribution, the Weight / Height Z-Score distribution were, however, almost normally distributed in comparison to the reference group (**Figure 5.4**).

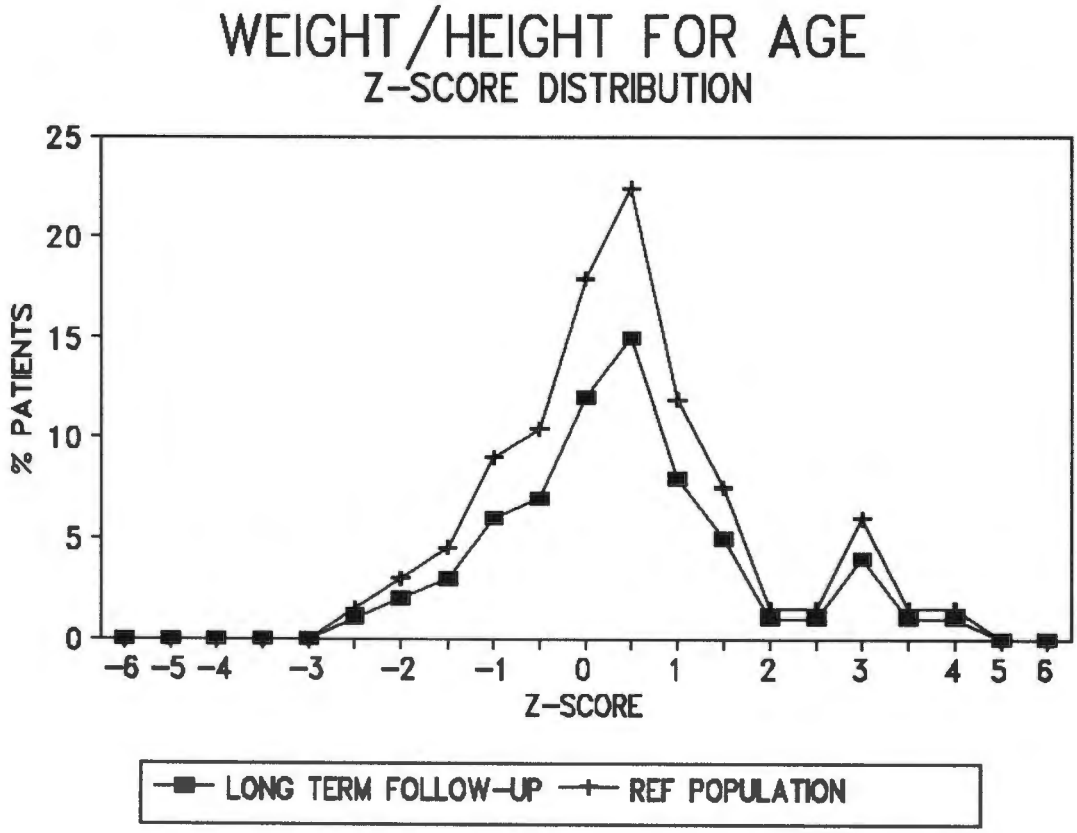


Figure 5.4: Comparison of Height/Weight Z-Scores of Hirschsprung's disease patients with a reference population

5.3.5 Influence of age on physical development

On analysis of anthropomorphic parameters in those patients over as well as under the age of 4 years there appeared to be a link between age and physical development. The average age of those in the well nourished group (ie. > 50th centile weight for age) was 13 yrs. Those between the 10 and 50th centile had an average age of 10 yrs and in those between 3 and 10 centiles it was 7 yrs. In patients with a recorded weight for age less than the 3rd centile the average age

was 5 yrs. This data suggests a trend towards an increased incidence of patients over the 50th centile of weight or height for age in those assessed later. These observed differences between the groups failed to reach significance on testing. Although physical development may initially be affected by the disease, it would appear that it returns to normal following successful treatment in the majority of cases.

5.3.6 The influence of obstructive symptoms on physical development

Severe obstructive symptoms were present in 16 of the 115 patients followed-up long term. In 5 there was a failure to thrive. Although the number of patients under the 10th centile weight for age was somewhat higher in the obstructed group, this was not found to be significant on testing. The influence of obstructive symptoms on physical development is discussed in the following chapter.

5.3.7 Developmental Milestones, personality adjustment and educational achievement

5.3.7.1 *Developmental milestones:*

Of the 115 patients followed-up long term, 9 had delayed milestones. Three were mentally defective, one of whom had microcephaly. One further child was assessed as having cerebral palsy but had a Coxsackie myositis diagnosed by muscle biopsy following definitive pull-through. His milestones were delayed as well as his shape formation and visual perception on testing. No specific causes were identified in the remaining 5 cases.

5.3.7.2 *Personality adjustment:*

The majority of patients over the age of 4 years at follow-up were well adjusted members of society. Behavioral disturbances identified in 5 patients were severe enough to warrant interventional therapy. Attitudes to medical and nursing

personnel and hospital were mostly positive but 12 of the older patients demonstrated signs of considerable stress during interview.

Extramural activities were affected in 11 patients by persistent soiling (7), congenital abnormalities (2) and other problems (2). Difficulties in relationships with peers were noted in 10 of these patients.

5.3.7.3 *Educational achievement:*

Forty two patients were not yet at school and could thus not be assessed. Satisfactory school performance was achieved in all but 19 (26%) of the remaining 73 patients over the age of 4 years. Three of these were mentally retarded and a history of encephalitis or meningitis with residual problems was noted in 4. In a further 12 patients there were problems related to schooling. This group included the 7 patients with a poor functional outcome.

5.3.8 Evaluation of anorectal function

5.3.8.1 *Stool frequency:*

A comparison between the early postoperative stool frequency and the stool frequency attained on later follow-up assessment is demonstrated in **Figure 5.5**.

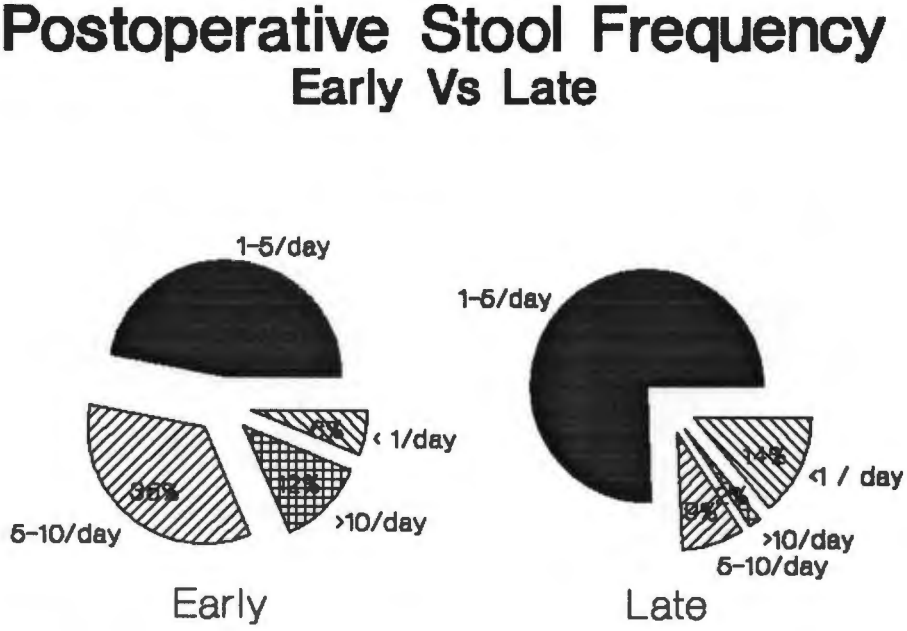


Figure 5.5: Pie charts showing differences in stool frequency at early and at long-term follow-up.

Increased stool frequency (> 5 stools per day) was recorded in 42 cases (36.5%) in the early postoperative phase. Twenty-eight of these (66%) followed the Soave endorectal pull-through procedure. By way of contrast, the incidence of patients with increased stool frequency had decreased to 12 patients (10%) at the time of long term follow-up. Of these, 7 had long segment disease with extension of the aganglionic segment beyond the splenic flexure. The total colon was affected in 5. There was further evidence of enterocolitis in 9 (75%) of these 12 patients on long term follow-up which may also help to explain the increased stool frequency. Six of these 12 patients (50%) followed the Soave procedure.

In addition to a decrease in the number of patients with frequent stools on late assessment, there was a significantly higher ($p<0.01$) incidence of patients having between 1 and 5 stools per day in the 115 patients over the age of 4 yrs (87/115).

The improvement in the stool frequency probably reflects the results of an increase in neorectal capacity and an increased reservoir function which occurs with time. In 16 patients less than 1 stool per day was recorded and in 5 of these the frequency was less than 1 stool every 2 days.

5.3.8.2 *Stool consistency:*

In the 115 patients interviewed, 65 patients (58%) had a normal stool consistency. In the remaining patients, stools were pasty in 28, liquid in 11 and hard in 11. Liquid stools occurred as the result of long aganglionic segments in 4 of the 11 cases (36%). In 2 of those with hard stools, these alternated with episodes of diarrhoea.

5.3.8.3 *Stool control:*

89 patients (77%) had full confident continence which was considered normal by the patient and the parents. In a further 18 (22%) patients minor degrees of mucus leak resulted in slight soiling or "skid marks" on the underclothing particularly at night.

In 7 patients there were episodes of soiling, smearing and incontinence during the day and night. A history of urge incontinence was obtained in 4 of these patients, 3 of whom had a history of repeated surgery with infection and scarring, resulting in a small reservoir capacity. In 1 patient a colostomy prevented assessment of stool control.

5.3.8.4 *Sensory appreciation and discrimination between flatus and faeces*

Seventy one patients had normal sensory appreciation, 22 were unsure and sensation was absent in 8. Sixty-four of those with normal sensation could confidently discriminate between rectal flatus and faeces. In 7 incontinent patients difficulties in the discrimination between flatus and faeces as well as impairment of sensory perception was reported. In the remainder, patients were unable to distinguish flatus from faeces or were uncertain. Rectal sensation was specifically tested for in the manometric assessment and will be dealt with in the following section.

5.3.9 Medication Requirements

Of the 115 patients aged 4 years and over, 37 (32%) continue to require some postoperative medication. Fifteen of these (13%) remain therapy dependent. In the remaining 22 patients (19%) occasional intermittent therapy is required for acute episodes of diarrhoea or constipation only.

5.3.10 Functional outcome

5.3.10.1 *Scoring systems in the assessment of long-term functional outcome*

Adequate information was available for functional assessment by scoring in 109 patients over the age of 4 years. Assessment by the Kelly scoring system was further hampered by the inability to perform rectal examinations on 12 patients followed up telephonically or by questionnaire.

All the 97 patients who were able to be assessed by the Kelly scoring system had a good to fair result (**Figure 5.6**). In 60 of these (55%), the score was greater than 4 indicating an excellent result. The outcome was fair in 38 (35%) and unknown in the 12 (10%) unable to be assessed.

By way of comparison, a satisfactory result was noted by means of the Wingspread scoring system in 103 of the 109 patients (94%). 76 of these (70%) had excellent postoperative function. Evaluation by means of the Holschneider scoring system gave a good to fair outcome in 106 (97%). A score between 10 and 14 was obtained in 60% indicating an excellent result. In 5 a the score was between 0 and 4 indicating poor functional outcome. A comparison between the results obtained by these scoring systems is shown in the accompanying bar graph (**Figure 5.6**).

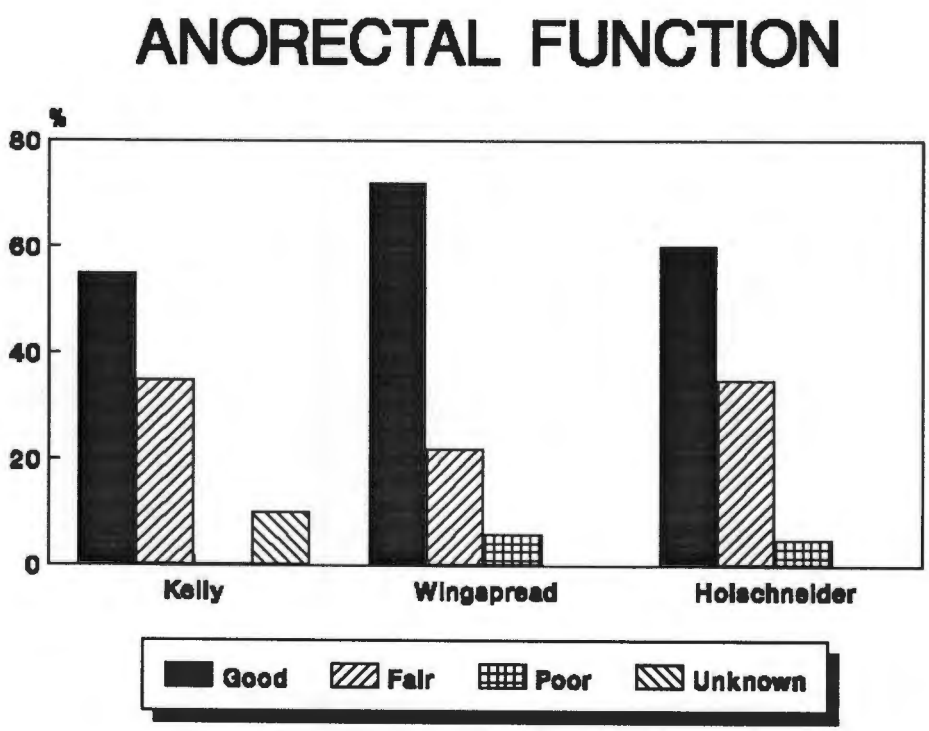


Figure 5.6: Bar Graph showing comparison of functional results following surgery for Hirschsprung's disease as assessed by 3 different scoring systems.

5.3.10.2 *The influence of age on functional outcome:*

The influence of age on the functional outcome is demonstrated in **Table 5.8.** In more than 26.9% of the 167 patients available for review, assessment was

hampered by the presence of patients who would not normally be expected to be continent because of age. The degree of stool control increased with increasing age and was considerably better in those over the age of 4 years. Only 52% were considered to be fully continent below the age of 4 years. This is in contrast to the 87.5% with full control in the 9-12 age group. Only 8.3% over the age of 9 years had a mucus leak and 4.1% had poor functional control. An age of 4 years was therefore considered as an arbitrary level to assess continence in the patients followed up in this section of the study.

TABLE 5.8.

ANORECTAL FUNCTION AND AGE

(167 OF 178 PATIENT INTERVIEWS)

Age (yrs)	No.	Functional outcome			
		Continent	Skid marks	Incont	Uncertain
0-3	52	27 (52%)	6 (11.5%)	5 (9.6%)	14 (26.9%)
4-8	41	27 (65.8%)	11 (26.8%)	3 (7.4%)	0 -
9-12	24	21 (87.5%)	1 (8.3%)	2 (4.1%)	0 -
>12	50	38 (76%)	10 (20%)	2 (4%)	0 -

5.3.10.3 *Definitive pull-through procedure type and outcome:*

77 patients (67%) of those over the age of 4 years responding to recall had the Soave Endorectal pull-through, 13 (11.3%) with the Swenson and 21 (18.3%) following the Duhamel procedure. Other procedures performed in 4 patients

(2.6%) and included ileoanal anastomosis in a patient with TCA, 2 myectomies for very short segment aganglionosis and a Stevens pull-through procedure in one patient with an anorectal malformation.

The functional outcome relating to procedure performed is seen in **Table 5.9.** There were patients with a good outcome as well as those with a poor outcome following the different procedures. Whereas the results of the Soave and Duhamel procedures were more or less comparable, less favorable results were noted following the Swenson procedure.

5.3.10.4 *The effect of management on outcome:*

Timing between colostomy and pull-through procedure:

Of the 115 patients interviewed over the age of 4 years, details of the stoma were available in 105. In 8 patients a one-stage procedure had been performed. In the remainder where records had been lost, the details could not be recalled by the patient.

Out of these 105 where the details of the stoma were available, colostomy was performed in 99 and ileostomy in 6. Near colostomies had been performed in 43 and transverse colostomies in 49. The type of stoma performed was determined by the prevailing policy at the time as well as the length of the aganglionic segment.

TABLE 5.9

FUNCTIONAL OUTCOME AND SURGICAL PROCEDURE

Functional Outcome	SERP n=77	Duhamel n=21	Swenson n=13	Other n=4
Good	56 (72%)	16 (76%)	7 (54%)	2 (50%)
Fair	18 (23%)	4 (19%)	4 (31%)	1 (25%)
Poor	3 (5%)	1 (5%)	2 (15%)	1 (25%)

A definitive pull-through procedure followed at an average of 9 months later. In 72 the interval between the stoma formation and the definitive pull-through procedure exceeded 6 months but was less than 6 months in 33. In those patients with procedure related complications, 6 (8.3%) were from the group with a longer interval between colostomy and definitive procedure and 9 (27%) of those operated on with a less than 6 month interval (Fisher's, $p<0.5$).

5.4 DISCUSSION

5.4.1 Representative nature of patient sample

The response rate was 71.4% of patients recalled for interview. Demographic features in these patients appeared to be comparable to the total patient sample. In addition, the length of aganglionic segment, the timing of presentation and clinical features were similar in the two groups. A lower representation of those procedures performed early on in the series (eg. Swenson, Duhamel) was observed in the interview group. This did not, however, reach statistical

significance but these differences were taken into account in interpreting the results.

5.4.2 Final patient status

Evaluation of as many parameters as possible showed that 86 (74.7%) of the 115 patients followed up long term had excellent anorectal function and appeared to be well adjusted members of society. A further 22 patients (19.2%) had relatively minor problems which emerged on systematic questioning. Seven patients (6.1%) had long term incontinence problems with resulting psychosocial maladjustment.

Where affected, physical growth appeared to return to normal limits in the majority with time. Emotional and psychosocial development appeared to be within normal limits in the absence of severe symptoms. Given good family support structures the outcome of those with minor problems was no less favorable than those with an excellent outcome. In the absence of a stable emotional basis, problems were magnified. Where incontinence resulted, the child appeared to be affected and poor schooling was observed in the majority.

Continence is not always easily quantified on clinical assessment. It would appear that a number of different factors have a bearing on the eventual functional stool control achieved by a postoperative patient. These factors include in principle the involuntary control of the anal sphincter complex, certain anatomical arrangements related to the pelvic musculature and terminal colon, rectal capacity, sensation, the ability to discriminate between flatus and faeces and certain reflex muscle actions. In addition, it would appear that the balance of forces pertaining in the anorectal region at any given moment of time is of the greatest importance in maintaining continence. In this regard, the consistency of the stool, the maintenance of a normal anorectal pressure barrier, the strength

and co-ordination of the gastrointestinal peristaltic waves as well as the amount of anorectal reserve all appear to have a role. Postoperative changes in the anorectal reserve may account for certain patients experiencing a temporary loss of continence if one of these factors is changed (eg diarrhoea). Functional postoperative outlet obstruction may have its origin in subtle alterations in this balance of forces.

The majority of scoring systems have been developed for assessment of anorectal function following anorectal malformations. Their suitability for a full assessment of patients who are mostly continent is open to question. This is particularly true in conditions where the problems are of a more subtle nature. In the absence of a suitable alternative, these scoring methods were used in this study. The choice of the Wingspread, Kelly and Holschneider scoring methods was because they offered a means of combining the functional, clinical and manometric observations. Although giving a good idea of the patient's continence status, their use as a means of addressing problems encountered by these patients is limited. As a result a possible area for further research would be to adapt the existing scoring procedures to increase the sensitivity of the assessment.

5.4.3 The effect of management on outcome

A significantly higher incidence of postoperative complications was noted in patients in whom less than 6 months had elapsed between colostomy and definitive surgery (Fisher's, $p<0.5$). This observation should probably be interpreted with care in the light of reports of successful neonatal surgery in other series (Carcassonne 1982; Cass 1990). On the other hand, there appears to be some support for the concept of a waiting period until the large bowel has returned to a more normal calibre in patients presenting with a grossly distended

colon. In these cases the evidence appears to justify a wait of at least 6 months between colostomy and definitive pull-through procedure.

5.4.4 The problem of obstructive symptoms

In 16 patients who were continent, there were recurrent problems related to postoperative outlet obstruction and they were repeatedly seen with acute clinical symptoms. These 16 patients with obstructive symptoms will be studied in detail in the following chapter to investigate the cause.

CHAPTER 6

CLINICAL EVALUATION OF PATIENTS WITH OBSTRUCTIVE SYMPTOMS FOLLOWING THE SURGICAL MANAGEMENT OF HIRSCHSPRUNG'S DISEASE

6.1 OBSTRUCTIVE SYMPTOMS - A DEFINITION

Symptoms which result from obstruction to the evacuation of intestinal or rectal contents in postoperative patients have been termed obstructive symptoms. These obstructive symptoms include acute or subacute gaseous abdominal distension plus a combination of any of the following symptoms : viz. vomiting, constipation, diarrhoea, infrequent stool, intermittent loose stools and chronic symptoms of faecal retention (ie. less than 1 stool/24 hrs). In addition, these may occur in association with episodes of diarrhoea or enterocolitis and gaseous distension. Postoperative obstruction was only considered to be of significance if the severity of the symptoms resulted in repeated outpatient visits and admissions.

6.2 INTRODUCTION

A group of patients were identified at interview as having persistent obstructive symptoms following surgically corrected Hirschsprung's disease. The methods of identifying these patients are outlined in the previous chapters. These patients became the target group for this study.

6.3 AIM

The aim was to assess the clinical features, possible causes and response to management of patients with persistent obstructive symptoms following Hirschsprung's surgery.

6.4 MATERIAL AND METHODS

6.4.1 Study population

Functional results were evaluated in the 178 patients responding to recall who had definitive surgery for Hirschsprung's disease (HD) at the Red Cross Children's Hospital (1957-1990). As outlined in the previous section, patients excluded from recall were those known to have died and those declining to participate in the study.

6.4.2 Recruitment of patient sample

From the total of 178 patients interviewed, 115 were more than 4 years of age and thus suitable for long term follow-up. Out of this group, 16 patients were identified as having a clinical picture of persistent obstructive symptoms. In these patients the symptoms did not respond to anal dilatations and other forms of minor treatment suggesting a long standing problem.

6.4.3 Demographic data

This group of 16 patients with obstructive symptoms were subjected to clinical review, examination, manometric evaluation, and rectal biopsy plus dynamic video proctography where possible.

Demographic data such as age, sex, race, symptomatology, or length of aganglionic segment were investigated. In addition, the prenatal and familial history was obtained. The demographic data between those patients with obstructive symptoms and those with normal anorectal function was compared.

6.4.4 Ascertainment of Data

The interview was based on the questionnaire employed in the preceding chapters. Additional information was obtained from the computerized database created during these studies to fill in historical details. A complete physical

examination was then performed and the results recorded. Manometric evaluation was then performed as outlined in the following section.

6.4.5 Radiological Methods

Standard plain abdominal radiographs included erect, supine and prone views. These films were read by the author in consultation with radiological colleagues. Patients for defaecography or videoproctography were selected on the basis of patient availability, consent and suitability for the procedure.

6.4.5.1 *Method of videoproctography:*

The technique involved the injection of a barium/water mixture (2:1) into the rectum and marking of the external orifice with a radio-opaque marker. Following control decubitus exposures, the table was moved into the upright position and lateral views taken by video camera with control X-Ray films being taken on a Philips Diagnost 73 apparatus with the patient sitting on a radiolucent commode (Mahieu 1984). The patient was then asked to strain whilst at the same time contracting the external sphincter muscles. Defecation was then permitted with video screening of the physiological response including internal anal sphincter relaxation. The anal canal length and anorectal angle were measured along guidelines established by a working party (Keighley 1989) as well as the method of Shorvon (1989). The amount of pelvic floor descent proved difficult to evaluate due to the inability of children to comply fully with instructions.

6.4.6 Rectal biopsy and histochemistry

Following parental consent, submucosal biopsy specimens were taken at 3 cm and 5 cm above the anocutaneous line in older patients or at 2 cm and 4 cm in younger patients. Haematoxilin and eosin stained slides and frozen section preparations for acetylcholinesterase were prepared according to the Meier Rüge

modification of the staining technique of Karnowsky and Roots (Karnowsky 1964; Meier Rüge 1972). Masson trichrome stains were used to demonstrate fibrous tissue where aganglionosis was present (Lillie 1965).

6.4.7 Statistical analysis

Data was compared by means, medians and 95% confidence intervals for means. Groups were compared by chi-square or Fishers exact test. The latter method was employed to compare groups too small for chi-squared testing. One way Anova testing by Ranks for unpaired groups (Kruskal Wallace) was used where required.

6.5 RESULTS

6.5.1 Patient sample

Sixteen out of the 115 patients over the age of 4 years had no evident cause of obstructive symptoms. Possible causes for this phenomenon were the subject of this investigation.

6.5.2. Demographic factors

No specific demographic factors was noted in patients from the different racial groups, there being 2 Caucasian patients, 14 patients of mixed race and 1 black patient in the study population.

A history of antenatal complications was present in only 3 patients (17%). A hypertensive crisis resulted in a premature birth at 26 weeks gestation in one baby who weighed 1200 grams at birth. Two further patients had a birth weight less than 2500 grams but were born at 36 weeks gestational age and at term respectively. Two of these patients were delivered by caesarean section with no postnatal asphyxia.

Nearly all patients presented with a delay in the passage of meconium, abdominal distension, constipation, and intestinal obstruction. Enterocolitis was a presenting feature in 4. The mean age of the diagnosis of Hirschsprung's disease was 12 months of age, ranging from shortly after birth to as late as 59 months. In 8 patients (50%) the diagnosis had been made after 6 months.

All of these patients had been treated for short segment aganglionosis and no other specific features that might influence outcome were detected.

6.5.3. Clinical assessment

Fifty percent of these patients presented at follow-up with complications which included failure to thrive, and episodes of enterocolitis, septicemia and shock (**Table 6.1**). In addition to abdominal distension, other presenting symptoms varied from intermittent attacks of watery stool to infrequency of defecation. In 12 of the 16 patients, constipation was the chief presenting complaint. Assessment of the internal anal sphincter revealed a normal rectal anatomy with no clinical increase in sphincter tone. Little long term improvement had been achieved following forceful anal dilatation in these cases.

Table 6.1

Complications in patients with obstructive symptoms
n = 8

Symptom	no of patients
Failure to thrive	5
Enterocolitis	3
Septicaemia	2
Shock	2

The height and weight for age showed a trend towards a higher incidence of patients underweight for age in the obstructed group when compared to the rest of the follow-up group (**Figure 6.1**). These differences did not, however, reach significant levels on testing (**Table 6.2**).

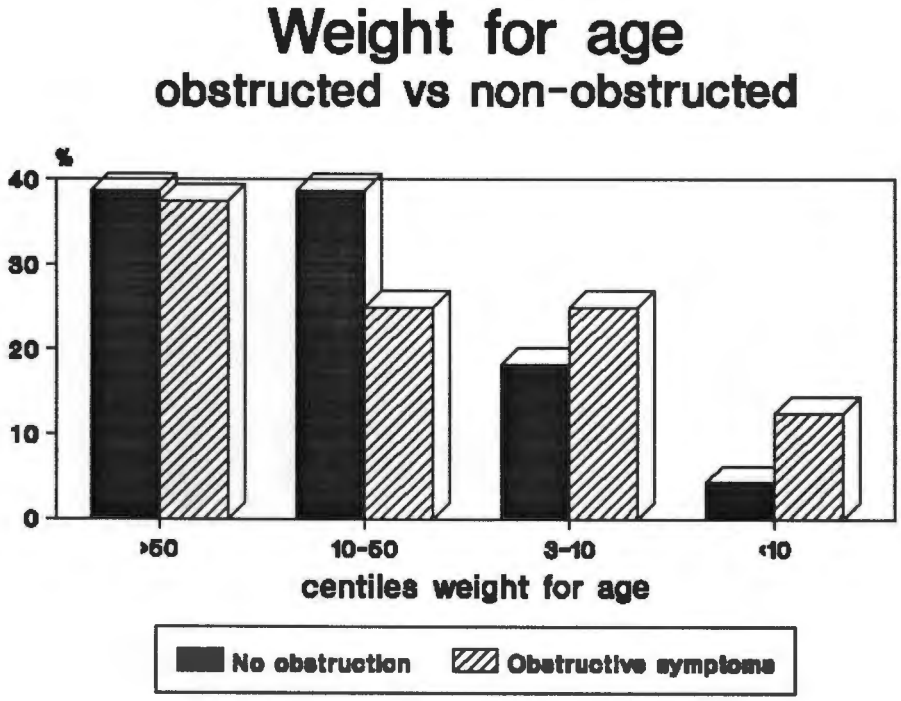


Figure 6.1. Bar graph comparing weight for age in a group of Hirschsprung's long-term follow-up patients with obstructive symptoms and the non-obstructive patients on long-term follow-up.

TABLE 6.2.

THE INFLUENCE OF OBSTRUCTIVE SYMPTOMS ON PHYSICAL GROWTH - WEIGHT AND HEIGHT

Centile	Weight for age		Height for age	
	Obstn n = 16	Non-obst n = 129	Obstn n = 16	Non-Obst n = 107
>50	5 (31%)	48 (37%)	7 (44%)	50 (47%)
10 - 50	5 (31%)	50 (39%)	6 (38%)	42 (39%)
3 - 10	4 (25%)	24 (19%)	1 (6%)	8 (8%)
< 3	2 (13%)	7 (5%)	2 (12%)	7 (6%)
Fisher's, p = non significant				

6.5.4 Surgical procedures implicated

Patients with obstructive symptoms included 1 (6%) following Swenson's procedure, 14 (88%) following the Soave endorectal pull-through (SERP) and 1 (6%) following the Duhamel procedure. These represented 7.6% of the Swenson procedures interviewed, 7.1% of Duhamel procedures not performed for TCA and 18% of Soave procedures performed. A further patient with obstructive symptoms following a Duhamel procedure was excluded on the basis of having had the primary surgical pull-through procedure performed at another institution.

The low number of patients responding from those groups operated on early in the series (eg the Swenson procedure) may have resulted in an underestimation

of the incidence of obstructive symptoms following the Swenson and Duhamel procedures.

Although a higher incidence was observed in patients post Soave endorectal pull-through, this may have been based on the greater number of patients from this group who responded to recall. This difference was found to be non significant on testing. On the other hand, it may reflect a higher tendency to develop postoperative obstructive symptoms following the Soave procedure.

6.5.5 Radiological Features

Radiological evidence of megacolon was present with gas filled dilated colon on abdominal X-Ray in all 16 (**Figure 6.2**). The anorectal angle ranged between 53 and 92 degrees with a median value of 80 degrees. These were within the normal range of reported normal values for young patients (Mahieu 1984; Shorvon 1989). Anal canal length ranged between 4 and 13 mm with a median value of 10.5 mm.

Dynamic videoproctography was performed on 5 of these patients and defaecography was performed without video control in a further 5. In 2 patients there were irregularities of the rectal mucosa. Continence and the ability to support a column of barium was present in all of those tested. The anal canal opened widely during evacuation in 2 of these patients and no evidence of sphincteric obstruction was noted in the remainder. One patient had non-evacuation of barium on 2 separate occasions. This failure to evacuate was attributed to be most likely due to the patient's inability to comply with instructions rather than sphincter achalasia, although this is not excluded.

The Radiological features of these patients did not conform to the features of sphincter achalasia as noted by Holschneider (1982) (**Figure 6.3**). The flattened

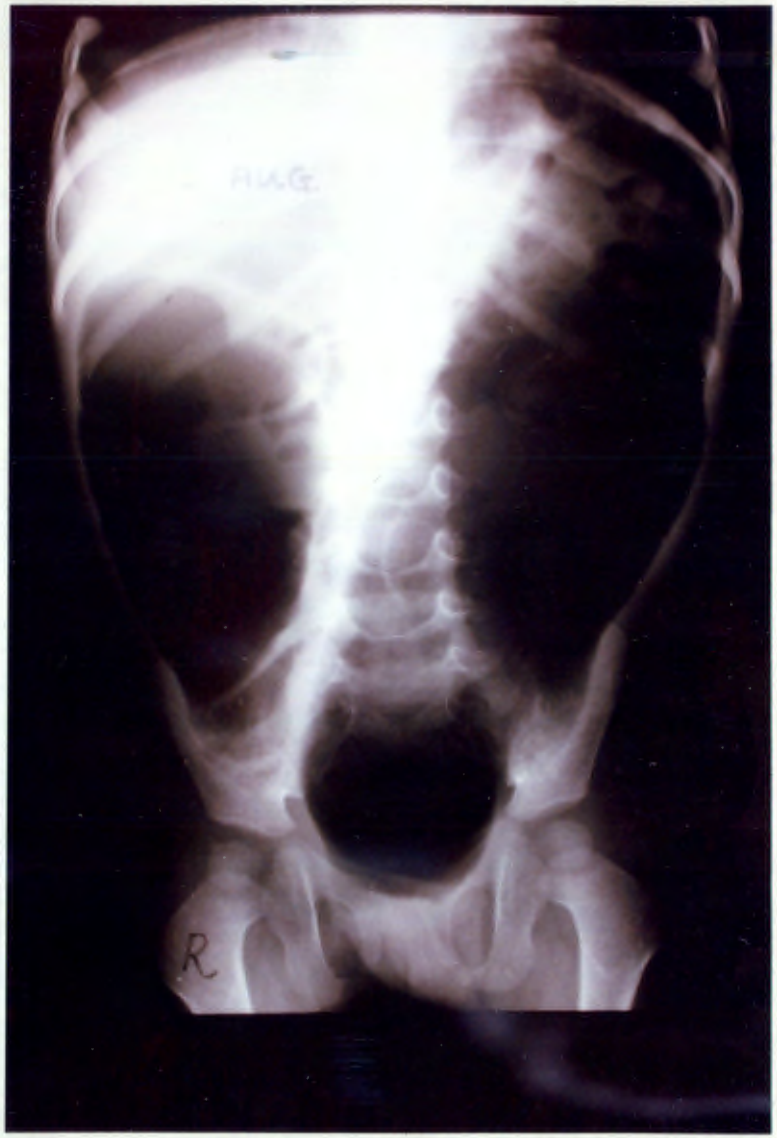


Figure 6.2: Abdominal radiograph showing dilated bowel in patient with Hirschprung's disease and obstructive symptoms

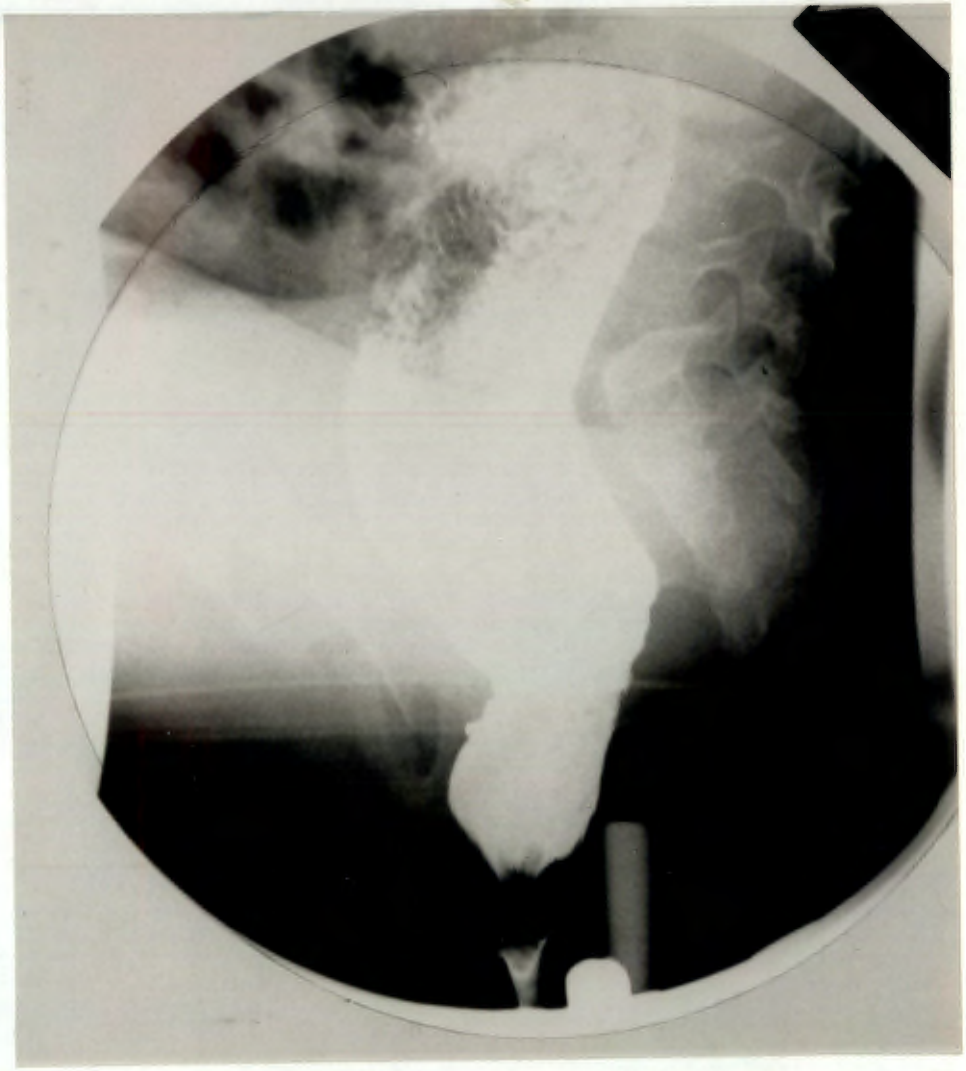


Figure 6.3: Radiograph taken during video-proctotraphy and evacuation of rectum by patient with obstructive symptoms post Hirschsprung's surgery (note lack of features of achalasia)

part of the posterior rectal wall just behind the main muscular impression was noted to be well defined in 2 patients. The diverticular type protrusion of the posterior rectal pole described in sphincter achalasia was not observed in any of the patients tested although it was observed in one patient with NID who had a SERP procedure for NID without associated Hirschsprung's disease.

6.5.6. Histological Features

The histological findings of the submucosal rectal suction biopsies are summarized in **Figure 6.4.** In 9 of the 16 obstructed patients (56%), hyperganglionosis was noted in association with an increased acetylcholinesterase (AChE) staining pattern of the neurofibrils. This was in keeping with the description of neuronal intestinal dysplasia (NID) (Schärli 1981; Fadda 1987). These 9 constituted 7.8% of the 115 patients over the age of 4 years responding to interview.

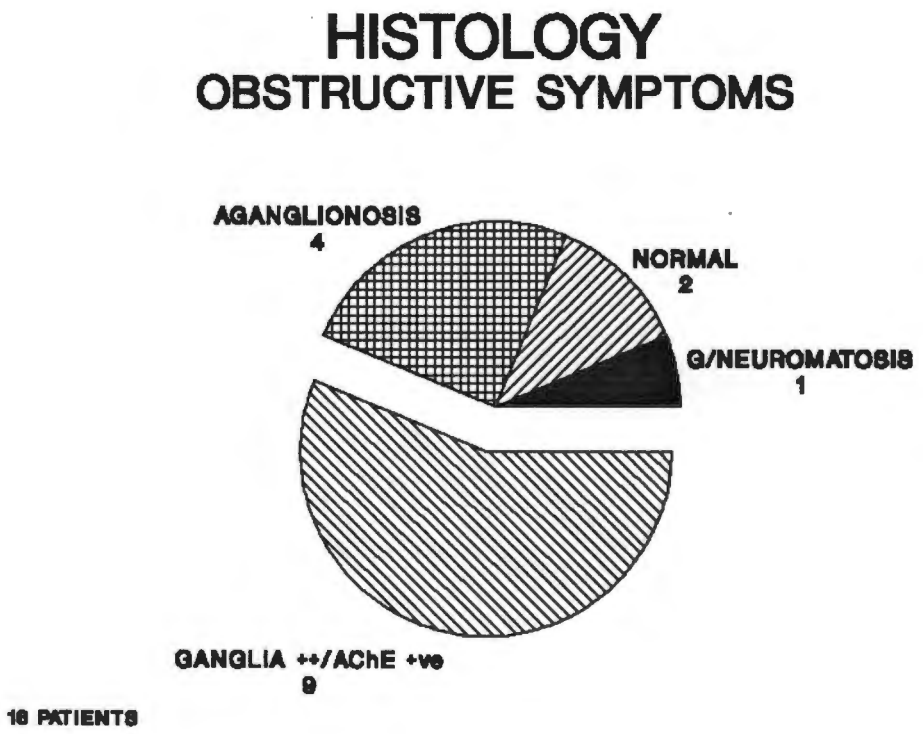


Figure 6.4. Pie graph demonstrating the incidence of histological observations on rectal suction biopsy specimens in patients with obstructive symptoms following Hirschsprung's surgery.

Four patients had aganglionosis on repeated rectal biopsy specimens. This was confirmed on repeated biopsy specimens in which every effort was made to accurately site the biopsy sites in ganglionated bowel. Review of the pull-through specimens showed that the bowel had been normally innervated at the surgical pull-through procedure. The aganglionosis extended as high as 6 cm from the anal verge in one patient in whom colonoscopic biopsies were taken every 2 cm below 20 cm. Myofibrosis was a feature in 2 of these patients, with the increased fibrous tissue being confirmed on Masson trichrome stain [**Figure 6.5 (a and b)**]. In the light of these findings, it was concluded that the aganglionosis had most likely been acquired postoperatively.

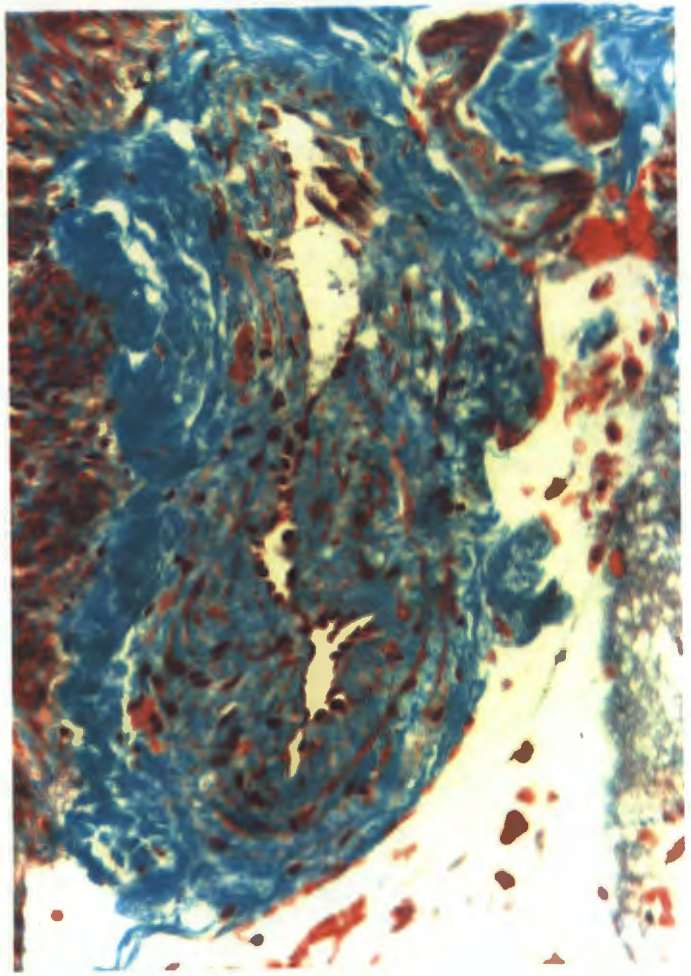


Figure 6.5(a): Section of colonic wall from patient with 'acquired' aganglionosis showing fibrosis of media with subendothelial thickening in a submucosal blood vessel (Masson trichrome x150).

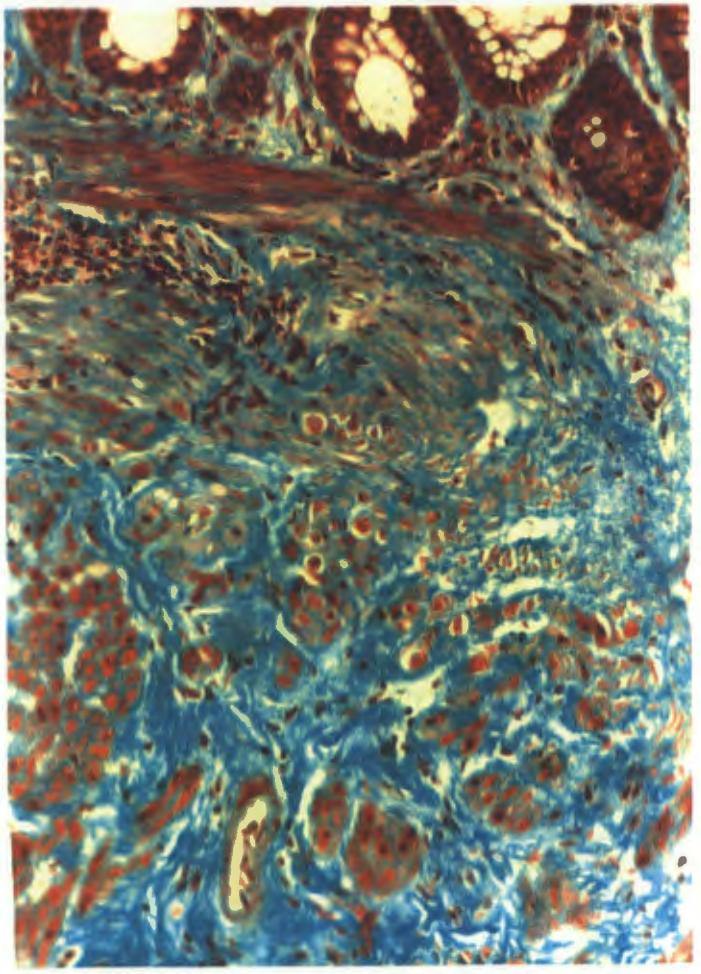


Figure 6.5(b); Section of colonic wall from a patient with 'acquired' aganglionosis showing atrophy of muscle of muscularis mucosa with fibrous replacement and increased dense collagen in submucosa (Masson trichrome x100).

In 2 patients normal rectal mucosa was noted and in 1 further patient grossly thickened peripheral nerves was in keeping with of ganglioneuromatosis. There were no clinical features of MEN IIb or Neurofibromatosis in this patient. The presence of sufficient numbers of ganglion cells in the myenteric plexuses as well as the degree of peripheral nerve thickening did not suggest transitional zone.

6.6 DISCUSSION

It has been noted that there is a similar complication rate of between 18 and 20% (Holschneider 1982) for the four pull-through procedures most commonly used in the definitive surgery for Hirschsprung's disease. Some of these complications are the result of technical deficiencies of the original surgical procedure, anastomotic insufficiency or may result from postoperative sepsis. In others, the obstruction continues despite treatment and functional causes are a possible cause. The possible reasons for these functional derangements are a subject of considerable debate but can be divided into causes of outlet obstruction and those of colonic inertia. Outlet obstruction results from the failure of the pelvic floor to relax during attempted defecation. Colonic inertia may be caused by motility disturbances of the GIT resulting in slow transit. It is possible to have both outlet obstruction and colonic inertia present in the same patient (Yoshioka 1987).

In an extensive literature review of more than 5000 patients by Holschneider (Holschneider 1983), which included 400 patients from his own series, 9-10% of patients were noted with persistent postoperative obstipation. The incidence of these postoperative symptoms remained fairly constant throughout most series regardless of the surgical procedure that had been performed. The overall

incidence of postoperative obstructive symptoms is also comparable to other reports (Lawson 1972; Holschneider 1983).

Variations in the surgical technique during the period under review included lower anastomosis (Swenson 1953, 1964; Grob 1959; Duhamel 1964), partial sphincterotomy (Swenson 1964), the sutured Soave procedure (Boley 1964) and posterior splitting of muscle cuff (Kasai 1977). The similarity of results from various different types of surgical procedures suggests the presence of common etiological mechanisms. In addition, the constant nature of obstructive symptoms despite the introduction of the various technical innovations suggests an alternative etiology to technical or mechanical causes.

Mechanical factors resulting from causes such as postoperative stricture formation, scarring, acute angulation of the descending colon above the anastomosis, residual aganglionosis (Holschneider 1982; Keiley 1990), overactivity of the anal sphincter (Fadda 1987), motility disorders (Morikawa 1989) or residual segment disease in ganglionated bowel (Fadda 1987) are possible explanations currently advanced for postoperative obstructive symptoms. Postoperative stricture formation and scarring as well as known cases of residual aganglionic segment were excluded from the above series by the patient selection criteria. The role of the internal anal sphincter, motility disorders and as yet unidentified residual segment disease remain possible etiological factors and will be explored further in later sections of this study.

The problem of postoperative obstructive symptoms have been receiving more attention of late. This is partly due to the possible coexistence of other neuronal abnormalities in the proximal normally ganglionated bowel of patients with Hirschsprung's disease (Schärli 1981). Tam (1991) reported abnormal peptidergic innervation of the proximal ganglionated bowel in Hirschsprung's

disease. Yamataka (1992) has reported a decrease in numbers of neuromuscular junctions in the proximal ganglionated bowel. These findings suggest that obstructive symptoms post Hirschsprung's surgery may be on the basis of abnormal ENS development and thus abnormal function. The implications of co-existing neuronal intestinal dysplasia as a possible etiological factor remains a controversial issue at this stage (Kluck 1986; Molenaar 1989; Schofield 1991; Yunis 1992). It is the intention of this study to explore the significance of this finding in a later section. Management protocols for these co-existing conditions are not as yet clear. Kimura (1992) has suggested rectal myotomy as a method of treatment of postoperative obstructive symptoms.

The 16 patients identified in this study had no clinical correlation with other possible contributing factors such as perinatal factors, hereditary factors, delay in presentation or a history of enterocolitis. Delay in presentation more than 6 months was noted in 10 out of 16 (62%) patients and in 6 out of the 9 (66%) patients with features of NID. This suggests that although the incidence of a delayed presentation was higher in those with obstructive symptoms than in the overall group, it is probably not a significant factor in producing the histological changes associated with NID.

There was no evident cause of the obstructive symptoms on interview or on physical examination. In particular, there was no increase in anal tone on rectal examination in the obstructed patient group. Defaecography failed to demonstrate sphincter achalasia and the results were similar to those reported following the endorectal pull-through for conditions not related to Hirschsprung's disease.

The amount of residual sphincter left in situ at the time of surgery is difficult to quantify and poses a practical problem for the surgeon. If this segment is left

too long obstruction may ensue and if the anastomosis is brought down too far incontinence will occur.

The internal anal sphincter must be seriously considered as a possible cause of obstructive symptoms. The anal sphincter has been shown to be involved in Hirschsprung's disease (Holschneider 1982; Fadda 1987). None of the currently employed surgical procedures aim at removing the entire sphincter. Sphincter achalasia has been blamed as the cause of the postoperative outlet obstruction (Holschneider 1982). Rehbein (1966) identified achalasia of the anal sphincter as one of the major causes of recurrent constipation and the resultant megarectum.

On the other hand, even when a partial sphincterotomy is performed by a low posterior suture line at the pull-through procedure (Dentate line), a number of children still require readmission to resolve continued outlet obstruction (Mishalany 1987; de Lorimier 1990). Lawson (1972) attributes success to vigorous anal dilatation. He did note that symptoms persisted in 2 of the 8 patients in his series with residual segment disease treated with forceful anal dilatation. Forceful anal dilatation is not entirely without its own complications and Eisenhammer (1974) has pointed out that scarring may occur from repeated anal dilatations. This damage resulting from anal dilatations has been confirmed manometrically by Holschneider (Holschneider 1983). Forceful anal dilatation or partial sphincterotomy, although of value in some patients, did not cure the obstructive symptoms in the patients included in this study of older patients. This suggests that the problem does not lie entirely in the abnormal sphincter in those patients without a residual aganglionic segment.

These findings suggest that sphincter achalasia is not the only factor in producing postoperative obstructive symptoms. This is supported by the

radiological studies performed in this study and neither the dynamic videoproctographic studies nor routine defaecography demonstrated features suggestive of achalasia of the anal sphincter. This was further investigated by the study of manometric parameters as outlined in the following section.

Other possible factors to be borne in mind in the light of current information include residual abnormal innervation of the rectal wall. Although all of these patients had a rectosigmoid aganglionic segment without other distinguishing features, the postoperative functional problem appeared to be caused by poor colonic wall motility. The association with hyperganglionosis and the ACHE positive neurofibrils in the lamina propria in 9 of the 16 patients is in keeping with the histological features of NID. The role of co-existing diseases of the enteric nervous system has not yet been clarified and wide variation in the incidence of NID has been reported (Fadda 1987; Pistor 1987; Lake 1989; Schofield 1991; Yunis 1992). Co-existing NID then suggests a possible explanation of these postoperative problems which requires verification by further studies. It is not the only possible explanation, however, and enterocolitis is a second possible etiological factor to be considered.

6.6.1 The role of postoperative enterocolitis in patients with obstructive symptoms

The association of abdominal distension with diarrhoea (Nixon 1990) raises the question as to the role of Hirschsprung's associated enterocolitis in these patients. Enterocolitis usually presents with fever, abdominal pain and diarrhoea (Bagwell 1992) which differ from the symptoms of obstruction, but milder forms may be present. The diarrhoea associated with Hirschsprung's disease has previously been suggested to be a mild form of enterocolitis (Sieber 1986). Paradoxical diarrhoea may be related to obstruction and has been labelled "masked constipation" in the past (Holschneider 1982). Many of the patients interviewed described symptoms of meteorism which is probably the result of

secondary liquefaction of stagnant stool due to bacterial decomposition. This may in turn alter the mucosa resulting in certain observed histological changes (Teitelbaum 1989). It is therefore possible that an association exists between postoperative obstruction and enterocolitis. Symptoms appeared to be more severe in those with obstructive symptoms suggesting that enterocolitis may have a part to play in the development of colonic distension in these patients.

Although the precise etiological mechanism of enterocolitis is unknown, obstruction has been thought to play a part (Bill 1962; Holschneider 1982; Teitelbaum 1988). Persistent sphincteric obstruction was regarded by Swenson to be the reason for the high rate of postoperative enterocolitis in his series (Swenson 1964). As a result he modified his procedure to include a partial sphincterotomy by placing the posterior line of anastomosis at the dentate line. Alternative suggestions as to possible ways in which obstruction can influence the etiology of enterocolitis include mucosal ischaemia resulting from mechanical distension of the bowel (Bill 1962) or a mucosal ischaemic effect caused by over distension. The latter may result in the invasion of bacteria into the bowel wall (Bill 1962; Teich 1986). These findings suggest that a case can be made for associating obstruction with the increased incidence of enterocolitis in this patient group. On the other hand, the histological features of enterocolitis which include crypt abscesses, ulceration, leukocyte aggregation, Paneth cell metaplasia and a marked immunocyte response were not detected in the rectal biopsies (Fujimoto 1988) of this series. This suggests that enterocolitis probably does not cause the post-operative obstruction, but may result from it.

By way of contrast, patients who present with enterocolitis, are prone to recurrence under varying stresses even after surgical excision of the aganglionic segment and the pull-through of normal bowel (Fujimoto 1988). This hypothesis also fails to fully explain the reported persistence of enterocolitis after colostomy

and pull-through in the absence of postoperative obstructive symptoms (Fujimoto 1988). In addition, enterocolitis did not appear to play a major role in the group under study and was a presenting feature in only 2 of the 16 (12.5%) patients. Neither of these patients were associated with the histological features of NID. Postoperative Hirschsprung's associated enterocolitis was noted in 3 of the obstructed patients. There was no significant difference in the incidence of enterocolitis occurring in the long term follow-up group.

Other possible causes of Hirschsprung's associated enterocolitis include a hypersensitivity to bacterial antigens as in pseudomembranous colitis (Berry and Fraser 1968). Pseudomembranous colitis, although rare may occur in association with Hirschsprung's disease (Bagwell 1992). Recent work on Clostridium difficule infection (Rietra 1978; Holst 1981; Thomas 1986) further suggests a possible bacterial etiological agent. Enterocyte adherence of Clostridium difficule in association with a mucin abnormality (Fujimoto 1988; Wilson -Storey 1989) and a defective immunological defence mechanism (Wilson-Storey 1988) has been suggested as a possible mechanism for the development of enterocolitis (Teitelbaum 1989). A further suggestion that an increase in prostaglandin E1 activity (Teitelbaum 1988) is the possible cause of enterocolitis, has met little enthusiasm. Enterocolitis remains a major management problem in Hirschsprung's disease although the mortality has shown a decline in recent years (Teitelbaum 1988). The association with obstruction remains unclear and further studies are required to clarify this association.

6.6.2 A management protocol for postoperative outlet obstruction

One important reason for identifying the exact nature of the etiological mechanism of postoperative obstructive symptoms is to allow an appropriate management protocol to be developed. The suggested management protocol which was developed during the course of this study is demonstrated in **Figure 6.6.**

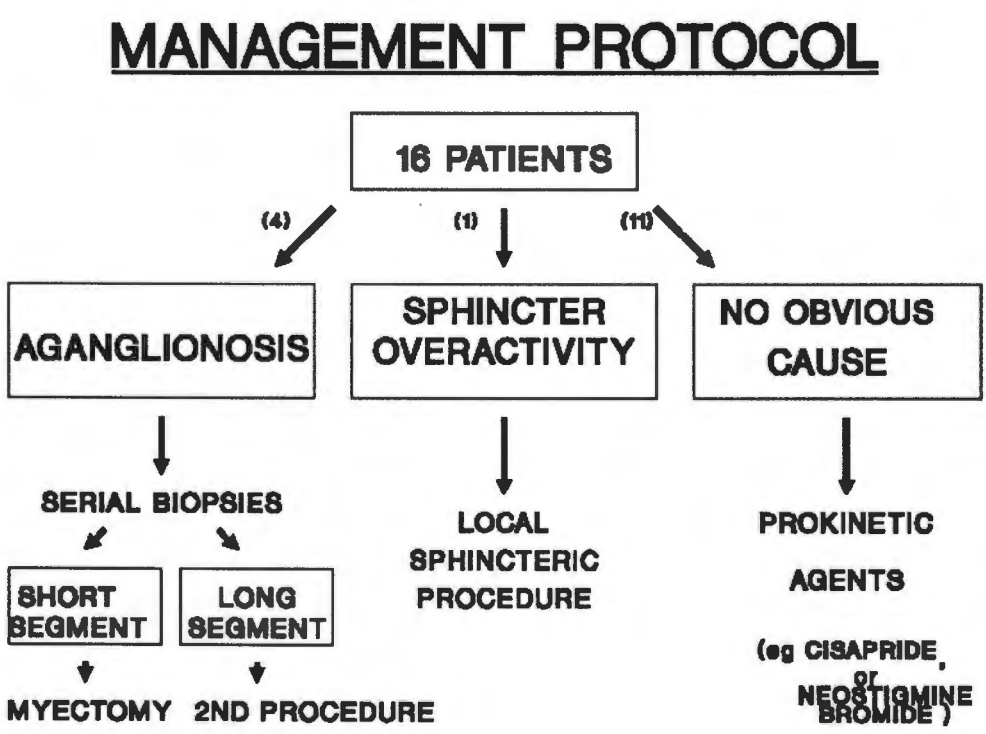


Figure 6.6. A suggested management protocol based on the cause of obstructive symptoms following Hirschsprung's surgery

6.7 CONCLUSION

Although normal postoperative function of the pull-through bowel occurs in the majority of patients following surgery for Hirschsprung's disease, obstructive symptoms were identified in 7.8% (16 patients) of a long term follow-up study group. The presence of myenteric ganglia in the proximal bowel does not appear to always guarantee a normal postoperative functioning bowel. A number of

pathological variations have been shown to co-exist in this study and appear to be the probable cause of these symptoms. Clinical and radiological evidence suggests that the sphincter is not the chief problem. Described features of sphincter achalasia were absent on videoproctography.

The number and degree of AChE staining neurofilament hypertrophy appeared to be related to the more severe symptoms in these patients. In addition, a prospective histochemical assessment of two patients noted a positive acetylcholinesterase stain and hyperganglionosis in all specimens up to the caecum in this series. Both developed subsequent postoperative obstructive symptoms and were shown to have delayed transit times. Histological mapping of the colon at the time of initial surgery may therefore be necessary in order to obtain a full assessment of the proximal bowel prior to definitive surgery.

SECTION C

CHAPTER 7

THE MANOMETRIC ASSESSMENT OF ANORECTAL FUNCTION

7.1 INTRODUCTION TO ANORECTAL MANOMETRY

The anorectum depends on normal intestinal smooth muscle as well as the integrity of the internal anal sphincter, the striated muscle sphincters of the external sphincter complex and other muscles of the perineal muscle diaphragm to function normally.

The internal anal sphincter has long been the focus of manometric evaluation because of its role in the maintenance of continence and its control of normal defecation. The absence of the normal rectosphincteric reflex in patients with suspected Hirschsprung's disease forms the basis of a well established diagnostic test. Abnormal sphincteric function and persistent impaired peristalsis in Hirschsprung's disease are possible causes of anorectal dysfunction following pull-through surgery. The length and function of the residual internal anal sphincter remains one of the constant technical problems associated with Hirschsprung's surgery.

7.2 AN HISTORICAL PERSPECTIVE

The early formulation of the necessary theoretical and mathematical principles for the construction of an accurate pressure device, and the early development of cardiovascular manometry permitted the measurement of intraluminal pressure as a means of assessing function (Brody 1951). Other pioneering work into gastrointestinal physiology by Bayliss and Starling (1899), Cannon (Cannon 1939) and others (Templeton 1932) assisted in the further understanding of gastrointestinal function.

Nevertheless, it was not until Swenson (1948) and Hiatt (1951) demonstrated physiological changes in intraluminal pressure as measured by small balloons placed at various sites in the bowel that the value of manometry as a tool in the investigation of gastrointestinal physiology was appreciated. Swenson and Bill (1948) noted the functional obstruction of the aganglionic bowel in Hirschsprung's disease as well as the normal peristaltic activity of the proximal ganglionated bowel. They then used this observation to develop a surgical procedure for the treatment of Hirschsprung's disease viz. excision of the aganglionic segment and a pull-through rectal anastomosis. Hiatt (1951) demonstrated a lack of ongoing peristaltic activity in the spastic distal colon of patients with Hirschsprung's disease which was capable of mass contraction only. These observations led him to conclude that after an often initial encouraging result, inadequate resection is followed by the onset of obstruction with time.

The first observation that rectal distension produced internal anal sphincter relaxation was by Gowers (1877). Denny Brown and Robertson (1934) noted a relative relationship between the motor activity of the rectum and relaxation of the anal sphincter and concluded that the rectal contraction waves initiated the resulting intramural reflex. Nixon and Gallagher (1964) concluded that normally functioning ganglion cells are necessary in the myenteric plexus for the reflex to occur.

Hiatt (1951) observed that the normal rectoanal response of relaxation of the anal canal to appropriate rectal distension was absent in patients with Hirschsprung's disease but he failed to recognize the importance of this observation. The clinical significance of the absent internal sphincter relaxation reflex in children with Hirschsprung's disease has been reported by Schuster

(1963) and has been confirmed in a large number of other reports (Schnaufer 1967; Lawson 1967; Tobon 1968; Howard 1972; White 1973; Verder 1974; Meunier 1976; Holschneider 1976; Imamura 1977; Ito 1977; Meunier 1978 among others). This led to the recognition of a manometric means of diagnosis of Hirschsprung's disease in the older child based on the absent rectosphincteric reflex.

Aaronson and Nixon (1972) reported an 85% accuracy in manometrically diagnosing Hirschsprung's disease in 100 consecutive patients using modified equipment. In addition to a lack of the rectosphincteric reflex in patients with Hirschsprung's disease, a number of other features have been noted in work on open tipped catheters (v Issendorf 1983; Krogh Pedersen 1989). These features include a lack of adaptation reaction to increased stool bulk, a lack of spontaneous relaxation in the affected segment , irregular anorectal fluctuations in the presence of an essentially normal anorectal pressure profile and normal voluntary muscle reactions.

Further advances have occurred in the use of anorectal manometry as a physiological tool in the assessment of gastrointestinal function. These include a better understanding of reservoir function by means of the adaptation reaction (Schärli and Kiesewetter 1969), factors involved in maintaining continence which include the continence reaction (Schärli 1969, 1970) and the pressure barrier produced by the internal anal sphincter. In addition, the reflex initiation of the propulsive mass contraction in defecation appears to be of importance (Schärli 1970, 1989). An appreciation of the balance of forces involved in the maintenance of continence (Schärli and Kiesewetter 1969) is an important step forward in identifying the pathophysiology of abnormal anorectal function. Five maturational stages have been reported after surgical pull-through procedures

(Schärli 1989) depending on the adaptation reaction, the identification of the continence reaction and other factors involved in defecation.

Sphincter achalasia has been identified as a causative factor in postoperative obstruction in 20-30% of patients following the Rehbein and other procedures (Rehbein 1982). The importance of overcoming **"sphincter achalasia"** in the postoperative period has been stressed by some authors (Rehbein 1982; Fadda 1987).

The use of anorectal manometry as a diagnostic tool has increased over the past few decades and numerous reports have appeared in the adult and paediatric literature.

The pressure profile allows a comparison of the rectal resting pressure with that of the sphincter zone (Henry 1985). Several methods of assessing anorectal pressures have been reported and range from strain gauges to balloon probes. The most reliable results have been shown to be from a perfused tube system (van Issendorf 1983) or advanced microcircuitry (Varma 1984). The opinion of the tripartite working party on manometry was that a water filled balloon system fulfills all the requirements (Keighley 1989). The quantitative assessment of pressure data has provided interesting but conflicting information, probably due to a lack of standardization of methodology and the type of probes utilized for measurement (Schärli 1989).

Electro-physiological activity of smooth muscle and the sphincter complexes has been widely reported (Holschneider 1982, 1983; Frenckner 1983) and would appear to have relevance in the assessment of the causes of incontinence.

7.3 A HISTORY OF ANORECTAL MANOMETRY IN CAPE TOWN

The use of the anometer was replaced by Aaronson in the mid-1960's using a probe similar to that reported by Lawson and Nixon (Lawson 1972). The introduction of the "Newcastle" probe by Boston in 1976 (Boston 1976) and its subsequent modifications led to a reliable method of determining the presence or absence of an anorectal relaxation reflex in young children. Davies (Davies 1981) highlighted some difficulties experienced in the diagnosis of certain forms of Hirschsprung's disease , especially those with total colonic aganglionosis. A multiple balloon system in series based on the design of Ustach (1969) and Lloyd (Personal communication) was introduced in the 1980's.

During the course of this study the technique was further developed and expanded to include a mechanically controlled perfused tube method. In addition, a new parameter, the anal sphincteric pressure barrier was developed and will be described in the following section.

7.4 THE DIAGNOSTIC ACCURACY OF MANOMETRIC DIAGNOSIS OF HIRSCHSPRUNG'S DISEASE

Anorectal manometry has been an important diagnostic procedure in Hirschsprung's disease due to the absence of the normal internal sphincter relaxation reflex to rectal balloon distension in these patients. This reflex activity of the internal anal sphincter has been reported as being "regained" in the postoperative phase due to the ingrowth of proliferating presynaptic neurones (Schärli 1970, 1989; Ikeda 1975; Holschneider 1982, 1983).

The accuracy of assessing the presence or absence of a recto-sphincteric relaxation reflex has been debated in the literature (Schnaufer 1967; Tobon 1968; Arhan 1976; Aaronson and Nixon 1972; Frenckner 1975; Meunier 1976,

1978; Von Issendorf 1979; Boston 1976, 1977) and there is some evidence that technical factors may play an important role (Boston 1976, 1977; Iwai 1988). Verder (Verder 1973, 1974) recorded a rectoanal sphincteric reflex as early as 2 hours after birth in mature and immature infants. Boston (Boston 1976, 1977) noted an abnormal reflex in only 5 out of 101 normal neonates examined on the first day of life returning to a normal response in all of the patients by the age of 28 hours. When these patients were tested on the 3rd day, the incidence of a positive reflex was significantly increased when compared with the first day. Tamate (Tamate 1984) also demonstrated that the anorectal manometry could be a reliable diagnostic tool in the neonatal period and Iwai (Iwai 1988) reported a definitive diagnosis of Hirschsprung's disease on manometric criteria in 17 out of 21 neonates (81%). Manometry does not replace histological and radiological methods of diagnosis and its major value would appear to be in the assessment of the unusual or difficult case. Davies (Davies 1981) sounded a note of caution in his report on patients with total colonic aganglionosis where the response may be variable.

Certain patients still appear to demonstrate a marked delay in the appearance of the rectoanal relaxation reflex despite technical modifications. Reflex relaxation of the internal anal sphincter to balloon rectal distension is said to be fairly reliably elicited in infants more than 12 days old (Boston 1977). In addition to being used as a diagnostic tool, anorectal manometry can be used to assess the postoperative function of the neorectum and provide information which may be used to identify possible causes of persistent postoperative symptoms (Schärli 1989).

7.5 MANOMETRIC MEASUREMENT OF ANORECTAL FUNCTION - THE ANORECTAL PRESSURE PROFILE

The pressure profile of the human anal canal reflects the effects of several muscle complexes including the internal and external anal sphincters and the puborectalis. The pressures recorded on anorectal pressure profile are the net result of muscle tone , elastic resilience and tissue turgor of the bowel wall and represent the resistance or opening pressure when measured by a perfused tube probe. The resting rectal pressure proximal to the pelvic diaphragm is low and the maximal pressure is noted just proximal to the anocutaneous line and the anal verge.

Radial variations in pressure have been reported. There is a significant decrease in anterior segment pressures during a voluntary squeeze in the upper rectum and a decrease in the posterior segment of the lower rectum with mid canal pressures being equilibrated on four quadrant measurement (v. Issendorf 1983; Taylor 1984; Miller 1988).

7.6 MANOMETRIC ASSESSMENT OF POSTOPERATIVE PROBLEMS AND ASSESSMENT OF ANORECTAL FUNCTION

In the majority of reports dealing with the long term functional results of definitive pull-through surgery for Hirschsprung's disease, the emphasis has been on the continence of the majority. Results have been expressed as being good, satisfactory or unsatisfactory. Although this is probably an adequate means to discuss outcome, many subtle problems may possibly go under-reported. In depth assessment brings to light a number of previously unappreciated problems (Frenckner 1983; Mishalany 1987), one of the most prominent of these being symptoms of an obstructive nature.

Although much work has been done on the manometric diagnosis of Hirschsprung's disease, there are not many postoperative anorectal manometric studies (Holschneider 1980; Frenckner 1983). This manometric study has thus been undertaken to evaluate the role of the internal anal sphincter and the pathophysiological changes in the postoperative patient which may contribute to the successful outcome of surgery.

CHAPTER 8

MANOMETRIC INVESTIGATION OF POSTOPERATIVE FUNCTION

8.1 INTRODUCTION

It is widely believed that the majority of patients continue to have a very tight anal sphincter after the surgical correction of Hirschsprung's disease despite the partial sphincterotomy achieved by a pull-through anastomosis being placed 0.5 cm from the dentate line (DeLorimier 1990). This may result in multiple readmissions for obstructive symptoms. Investigation of anal sphincteric function therefore appeared to be an important area for further study.

The tonic activity of the internal anal sphincter is responsible for most of the anorectal pressure barrier at rest. Anorectal manometry measures the pressures influencing the anorectal pressure barrier and may help to identify physiological and pathological changes. Technical variations lead to disparate results and difficulties in comparing individual series. On the other hand, there are also physiological variations affecting results. The intensity of sphincter tone may differ between patients and can conceivably vary as much as 20% between observations due to slow wave contractions (Krogh Pedersen 1989).

8.2 AIM OF STUDY

The purpose of the manometric study was three fold :

Firstly, to ascertain the role of the anal sphincter in the postoperative continence of the surgically treated patient with Hirschsprung's disease.

The **second objective** was to assess neorectal function and its influence on long term postoperative outcome.

Thirdly, to investigate whether a difference existed in patients with obstructive symptoms where histological investigation had demonstrated a dysplastic enteric nervous system.

8.3 DEFINITION OF TERMS

The terms used in the manometric study were defined as outlined below.

The **resting rectal pressure (RRP)** was defined as the pressure difference between the intrarectal pressure and the baseline as measured on removal of the catheter and averaged over 3 separate readings.

The **maximal anal canal pressure (ACP)** was defined as the maximal pressure obtained on withdrawal of the probe through the anal canal area and was also averaged over 3 separate readings.

The **anal canal pressure difference (ACPD)** was calculated as the difference between the anal canal and resting rectal pressures.

The **resting length of the anal sphincter** corresponded to the length of the high pressure zone, calculated as that length of rise in pressure above the resting rectal pressure as the catheter was drawn through the anal canal at a given speed and a paper speed of 25mm per second.

The **anal sphincteric pressure barrier (ASPB) index** is a measurement of the anal sphincteric pressure barrier and is calculated by measuring the area under the high pressure zone of the anorectal pressure profile tracing in square millimeters. It combines the pressure measurement with the length over which such a pressure is exerted to manometrically express the sphincteric resistance.

Rectal sensitivity was measured in terms of the sensation threshold volume as well as the appreciation of incremental increases in rectal balloon volume coupled with an increasing desire by the patient to defecate. The finer sensory appreciation and function of the patient was assessed on the basis of systematic questioning about the presence or absence of an increasing sensation of fullness on progressive rectal distension. Results were graded as good (sensory perception coupled with a desire to defecate), fair (an increasing sensation of rectal fullness without desire to defecate) or poor (absence of sensation and absence of desire to defecate).

The **sensation threshold** was defined as the level at which the patient first felt the increase in balloon volume.

Neorectal capacity was defined as the volume of air in the intrarectal latex balloon on maximal sensation (ie maximal tolerated volume) (Stryker 1985).

Rectal compliance represents the quotient of volume difference and pressure difference in the postoperative rectum and is measured in milliliters per millimeter of mercury.

The **maximal squeeze pressure** was defined as the maximal pressure obtained anywhere in the anal canal during maximal pelvic floor contraction (Keighley 1989) and was measured on three separate readings as demonstrated by the intrasphincteric balloon pressure changes.

Anorectal fluctuations were slow fluctuating waves noted on correct positioning of the balloon in the internal sphincter.

The **rectosphincteric inhibitory reflex (RSR)** was defined as any reduction in the resting anal canal pressure after inflation of air into a rectal balloon observed consistently over at least three readings (Keighley 1989).

Variations in the length of the anal canal with various ages was taken into consideration. Measurement was made at a distance from the anal verge corresponding to the area of maximal pressure as measured on the anorectal pressure profile.

The **adaptation reaction** was defined as the manometric representation of the rapid pressure increase followed by a slow pressure decrease occurring in the rectum in response to stimulation by water or air and is occasioned by the flexibility of the walls of the ampulla of the rectum.

Mass contractions are irregular incoordinated contractions of the rectum occurring in affected bowel in Hirschsprung's disease.

8.4 MATERIAL AND METHODS

8.4.1 Clinical study group

The clinical and pathological records used were those children with histologically proven Hirschsprung's disease identified in previous chapters.

8.4.2 Recruitment of patient sample

Patients responding to recall were requested to undergo manometric evaluation. Control groups were randomly selected from cohorts of approximately the same age. There was an arbitrary cut off at 4 years, below which manometric results were considered suspect unless sedation was been used.

8.4.3 Demographic features

Those patients, identified in section B of this thesis as having obstructive symptoms which had not responded to treatment measures such as repeated anal dilatation, formed a special group of obstructed patients for investigation. Control groups consisted of a randomly selected group of patients from those without obstruction who responded to recall post surgical management of Hirschsprung's disease, a group of patients with a normal rectal suction biopsy referred for investigation of chronic constipation and a group of patients without any gastrointestinal complaints and normal stooling patterns. These normal volunteers were mostly patients admitted for elective minor surgery. The children were psychologically prepared in collaboration with the parents prior to carrying out the procedure and parental consent obtained. Parents were usually present at the manometric procedure.

8.4.4 Ascertainment of data

The studies were performed by a single investigator. A digital rectal examination preceded the insertion of the rectal probes. Sphincter tone was clinically assessed as being normal, increased or decreased on the basis of a rectal examination prior to manometric assessment in the patients studied.

Manometric data was collected on a continuous 8 channel recording system (Hewlett Packard 7758A) with an external transducer (HP1280) which had been standardized to a scale of 2mm Hg for every millimeter in the vertical axis. A hard copy tracing was displayed on heat sensitive paper. The position of the probe, paper speed and puller speed were indicated on the tracing along with the patient's demographic details and date of examination. The tracing was annotated for volume and timing of rectal balloon insufflation and reflex relaxations indicated. Manometric parameters were calculated during and

immediately following the procedure and recorded on the tracing and on the form developed for keeping records (**Appendix A**).

8.4.5 Patient preparation

Manometry was performed on unprepared bowel with the patient at rest in the left lateral decubitus position with hips and knees flexed. Where sedation was necessary, non hypnotic drugs were used if possible. In difficult children, ketamine hydrochloride at a dose of 2 mg per kg was given intramuscularly.

8.4.6 Manometric technique

8.4.6.1 *Standardization of equipment*

The measuring device was initially calibrated against a column of mercury to 100mm Hg corresponding to a scale of 0-100 with each vertical 1mm of paper representing 2mm Hg. Sensitivity could be adjusted to 50 or 25mm Hg from an electronic selection panel, should this be required.

The length and calibre of probes used were identical and the same connecting system of tubes was used throughout the study. The connecting tubes and probes were checked for the absence of air bubbles which might have influenced results.

8.4.6.2 *Technique of manometric measurement*

Anorectal manometry was performed, using a combination of a perfused catheter pull-through technique and a multiple balloon series. This technique was followed by the separate insertion of a probe containing 4 balloons in series (**Figure 8.1**) as a closed system method of evaluating postoperative rectal physiology.

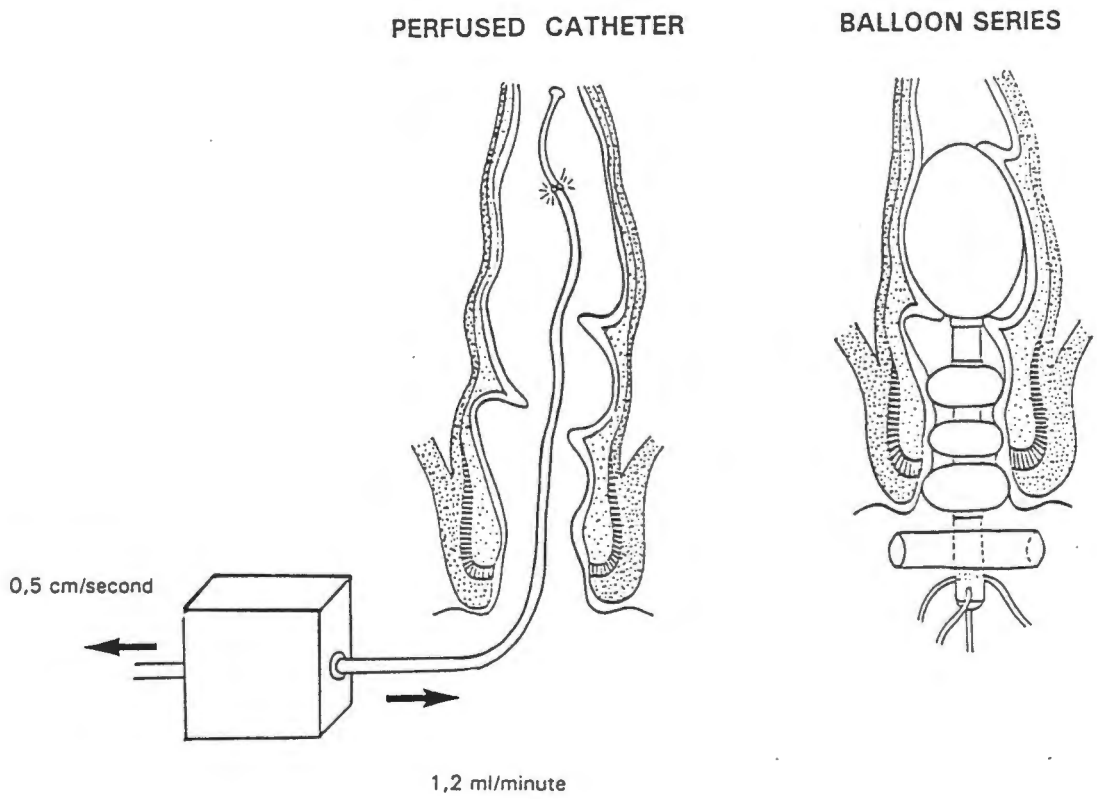


Figure 8.1 Methods of manometric assessment used in this experiment. Pressure readings were taken from the perfused tube system and reflex activity from the 3 balloon series.

A single lumen adapted polyethylene catheter probe (38cm long) with an internal diameter of 2mm, perfused at a rate of 1.25 ml per minute was inserted into the lower rectum to a depth of 10-12 cm. Recording points were holes situated radially at 120 degree intervals, 2.5 cm from the blunted tip.

The probe was left in situ until the patient relaxed and intestinal function normalized. The amplitude, type and regularity of the propulsive wave response to fluid infusion were recorded and the resting rectal activity calculated as the number of contractions per minute.

Pressure measurements began 8cm from the anal verge as represented by a mark on the catheter. The catheter orifices were sufficiently proximal so that withdrawal of the catheter, for anorectal pressure measurement, did not result in its complete removal. The catheter was withdrawn at a constant speed of 0.48cm per second by a motorized withdrawal system attached to the catheter and positioned so that the axes of traction followed the longitudinal axes of the anal canal. The withdrawal continued until the recording vents were exposed but the tip of the catheter remained within the rectum. Reinsertion of the catheter and repeat anorectal profilometry was performed until at least three comparable tracings were obtained.

The **anorectal pressure profile** was measured from the means and consisted of resting rectal pressure (RRP), maximal anal canal pressure (ACP), the anal canal pressure difference (ACPD) and the anal sphincteric pressure barrier (ASPB). The **resting length of the anal sphincter** was measured by the motorized withdrawal of a perfused tube at a known speed, calculated at 0.48mm/sec.

Following removal of the probe a second 4 balloon probe similar to that suggested by Ustach (1969) was inserted to a predetermined depth so as to place the anal sphincter balloon at the depth of the maximal anal canal pressure as noted on anorectal profilometry. This required adjustment in some cases due to the variability in anal canal length.

Using the balloon system in series, measurements were recorded of **neorectal capacity**, estimation of the **sensation threshold** and the **maximal tolerated volume**, as well as the adaptability of the distal bowel. The rectal balloon which was used consisted of an oval latex balloon approximately 1.5 x3 cm in size. This balloon was inflated in 5 to 20 ml increases in volume depending on the age and size of the child. The **sensation threshold** and **maximal tolerated volume**

were recorded on questioning the child and observing their response to an increase in rectal distension. To quantitate the patient's ability to sense the inflation of an intrarectal balloon, the volume of the balloon was increased using 10 - 20 ml increments of air into the intrarectal balloon via a three way tap. The volume of the increments varied with the size of the child and an attempt was made to inject the air over a standard time period. The test was terminated when the patient complained of an uncontrollable urge to defecate or severe discomfort.

The **reflex response of the internal sphincter** to rectal distension was recorded via a separate transducer pre-amplifier onto a different recording channel. Where sufficient co-operation was obtained, between 3 and 5 **squeeze pressures** were measured at the end of the study and averaged to calculate a squeeze pressure.

Recording was by way of a three way tap and a plastic tube with an internal diameter of 2 mm and 1.2 meters in length, connected via a pressure transducer (HP1280) to a carrier preamp (HP 8805A) and recorded on an 8 channel recorder (Hewlett Packard 7758A) calibrated to 100mm Hg and run at a paper speed of 25mm per second. Measurements were then averaged over three separate comparable tracings and the results plotted.

The quotient of volume difference and pressure difference in the postoperative rectum was calculated from the trace by the method suggested by Ihre (1974). The result obtained was the **rectal compliance**.

Motility studies were measured indirectly from the anorectal pressures at rest. They included the observed rhythmical activity of the anorectum and the response to maximal stimulus.

8.4.7 Manometric parameters measured

8.4.7.1 *The anorectal sphincteric pressure barrier*

The anorectal pressure profile represented the pressure barrier of the sphincteric complex required to prevent the leaking of intrarectal contents. The resting rectal pressure, maximum anal canal pressure and anal canal pressure difference were calculated from the anorectal pressure profile tracing.

The length of the anal canal also influenced the functioning of the anal sphincteric mechanism. This could be calculated from the tracing because of the mechanical withdrawal of the probe at a constant speed. Other factors such as the neorectal capacity, sensation, rhythmic activity and reflex function were considered important parameters to measure as they form part of the "balance of forces" occurring in postoperative anorectal function.

8.4.7.2 *Calculation of anal canal length*

The length of the high pressure zone (HPZ) of the anal canal is calculated as a product of the following formula.

HPZ Length (cm)	=	measured length (cm)	x	puller speed (0.48cm/sec)	x	paper speed (25mm/sec)

8.4.7.3 *Calculation of anal sphincteric pressure barrier index (ASPB index)*

The area under the curve of the high pressure zone of the anorectal pressure profile appears to be the manometric representation of the anal sphincteric pressure barrier. This includes both the pressure difference as well as the length over which this pressure is exerted.

The anal sphincteric pressure barrier was calculated as the area in square millimeters under the curve of the high pressure zone of the anorectal pressure profile tracing (**cf 8.5.4.5, Figure 8.5**).

8.4.7.4 *Correlation of parameters of anorectal function*

Anal sphincter function was assessed by the amplitude and regularity of anorectal fluctuating waves, the anal sphincteric pressure barrier calculated from the anorectal resting pressure profile and the presence and morphology of the internal sphincter relaxations.

The presence or absence of mass contractions, alterations in the adaptation reaction and an estimation of rectal capacity and compliance also appeared to be important parameters. Sensory perception was measured by the threshold level of sensory perception and the presence or absence of an increasing sensation of fullness and the presence or absence of a desire to defaecate.

8.4.7.5 *Manometric evaluation and clinical outcome scores*

Clinical outcome of long term evaluation of anorectal function was expressed in terms of the Kelly, Wingspread and Holschneider scoring system (**Appendix D**). In patients post Hirschsprung's surgery these were correlated with the manometric parameters.

8.4.8 Units of measurement

Anorectal pressure in this study was measured in mmHg. Results are also shown in kPa to allow comparison with other studies in keeping with the recommendations of a tripartite working party (Keighley 1989). Measured values are tabulated for both sets of values.

Length was calculated in centimeters and the anal canal sphincteric pressure difference index calculated in square millimeters. Compliance was measured in milliliters volume per millimeter Mercury (ml/mmHg).

8.4.9 Statistical methods

Statistical methods employed in this study were based on non-parametric tests for small numbers calculated by means of a statistical package (Statgraphics V) on a microcomputer. Due to the lack of normal distribution, results were expressed as medians as well as means and comparative data displayed by means of 95% confidence interval of means. Testing of unbalanced or asymmetric data was carried out using the Kruskal-Wallis one-way analysis by Ranks. Results of both testing methods are expressed where appropriate.

8.5 RESULTS

8.5.1 Patient sample

Anorectal manometry was performed on 67 patients and was divided into 4 groups.

Group 1 (Obstructed) n = 16

Patients with obstructive symptoms post surgery

for Hirschsprung's disease

Group 11 (Non-obstructed) n = 21

No obstructive symptoms post surgery for

Hirschsprung's disease

Group 111 (Constipation) n = 20

Patients with chronic constipation but a normal

rectal biopsy

Group 1V (Normal Controls) n = 10

Patients without gastrointestinal symptoms

Manometric results were compared between these groups to ascertain the parameters of anorectal function in normal as well as postoperative patients.

8.5.2 Patient sedation

Sedation was required to perform the study successfully in 2 patients in the obstructed group and 4 within those with chronic constipation in whom the manometric procedure was considered to be of diagnostic importance. No patients in the other groups were sedated and the procedure was abandoned in 4 control patients who were omitted from the study when the need for sedation became apparent.

8.5.3 Demographic factors

The age of those tested ranged from 3 to 36 years with a median of 5 years in the obstructed group and 7.5 years in normal controls (**Figure 8.2**).

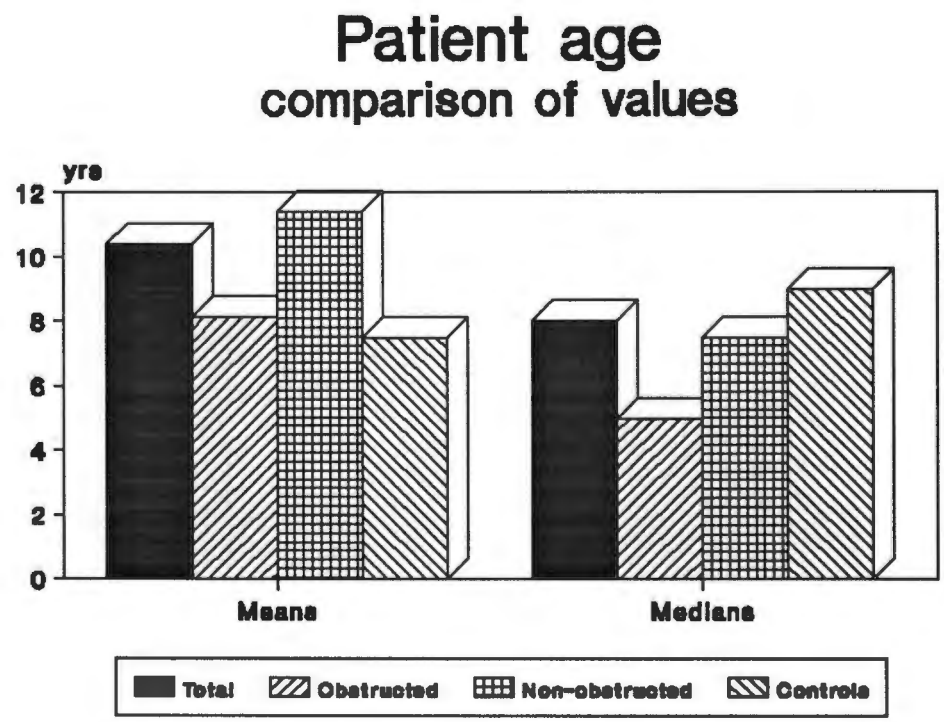


Figure 8.2. Comparison of age of manometric subjects

Median values were somewhat lower in the obstructed group than in the non-obstructed patients post pull-through surgery and those with chronic constipation. These differences in age were found to not reach significance on statistical analysis.

8.5.4 Anorectal pressure profile

8.5.4.1 *Resting rectal pressure*

The range and interquartile ranges as well as the median values of measured resting rectal pressures are reflected in **Table 8.1.** The median values of resting rectal pressures showed little variation and no overall differences could be detected between the groups on statistical testing.

8.5.4.2 *Maximal anal canal pressure*

Although considerable differences were observed in the maximal anal canal pressures measured in individual patients (**Table 8.1**), the variation in medians and the interquartile ranges were not striking. In addition, the 95% confidence intervals of the means were comparable.

8.5.4.3 *Anal canal pressure difference*

The anal canal pressure differences (maximal anal canal pressure less the resting rectal pressure) are reflected in **Table 8.1.** The lowest anal canal pressure difference median value is seen in the obstructed group.

TABLE 8.1

MEDIAN VALUES ANORECTAL MANOMETRY

	RRP mmHg (kPa)	ACP mmHg (kPa)	ACPD mmHg (kPa)	LENGTH cm	ASPB mm2	AGE (yrs)	RECTAL MOTILITY (freq/min)	COMPLIANCE ml/mmHg
Postoperative Obstructive Symptoms n = 16	8 (1,06)	21,85 (2,91)	14 (1,86)	2,375	116	5	20	4,5
No Obstructive symptoms n = 21	6 (0,79)	26,3 (3,50)	16,6 (2,21)	3,36	169,5	9	19	6
Chronic Constipation (Normal rectal Biopsy) n = 21	6 (0,79)	27,7 (3,69)	23,3 (3,10)	2,88	128,5	11	20	7,05
Normal controls n = 10	8,8 (1,17)	31,8 (4,23)	22,1 (2,94)	2,84	162	7,5	22	5
p Value	0,942	0,490	0,273	0,122	0,224	0,695	0,110	0,560

RRP = Resting Rectal Pressure

ACP = Maximal Anal Canal Pressure

ACPD = Anal Canal Pressure Difference

ASPB = Anal Sphincteric Pressure Barrier

A comparison of the resting rectal pressures, maximal anal canal pressure and anal canal pressure differences is reflected in **Figure 8.3**. The median anorectal pressure profiles were somewhat higher in the constipated and normal control groups than in those post surgical pull-through for Hirschsprung's disease. No statistical differences were found between these groups on calculation.

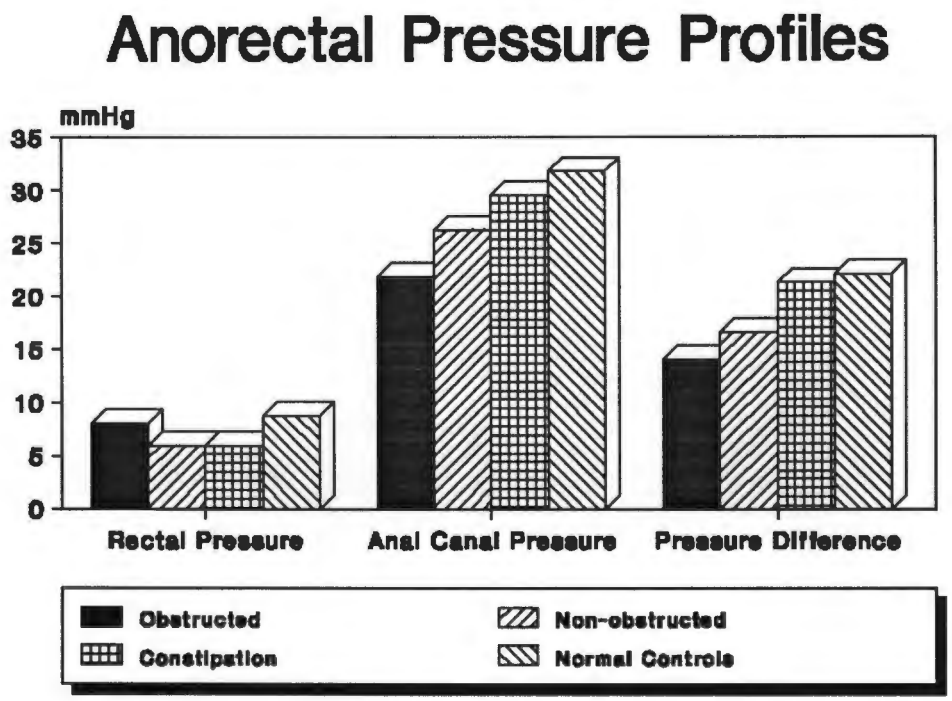


Figure 8.3. Bar graph showing comparison of anorectal pressure profiles between patients with postoperative obstruction, those with no obstruction, chronic constipation and the normal controls.

A possible explanation for the lower pressure observed in the postoperative patients is the partial sphincterotomy achieved by the low posterior anastomosis performed during the surgical procedure.

8.5.4.4 *Length of the anal sphincter complex*

The upper quartile value of the normal control patient group (3.64 cm) was taken as the upper limit of normal. There appeared to be an increase in anal canal length as well as the sphincteric pressure barrier in two obstructed patients with a high pressure zone much longer than the upper confidence of 3.64 cm indicating some outlet obstruction. Age also appeared to influence the anorectal sphincteric complex length. In this sample, there was a lack of a significant difference between the ages of patients in the different groups on testing.

The length of the sphincteric high pressure zones did not differ significantly in the different groups of patients tested. Nevertheless, the median value of the high pressure zone length appeared to be shorter in the obstructed postoperative patients than in the controls (**Figure 8.4**).

8.5.4.5 *Anal sphincteric pressure barrier (ASPB) index*

The anal sphincteric pressure barrier is demonstrated in **Figure 8.5.** Comparison of the anal sphincteric pressure barriers (ASPBs), also showed no significant differences between the four groups (**Figure 8.6**). Nevertheless, the ASPB in obstructed patients was somewhat higher than that of non-obstructed patients and the normal controls. In the obstructed group the ASPB approached the mean value calculated in the group with chronic constipation.

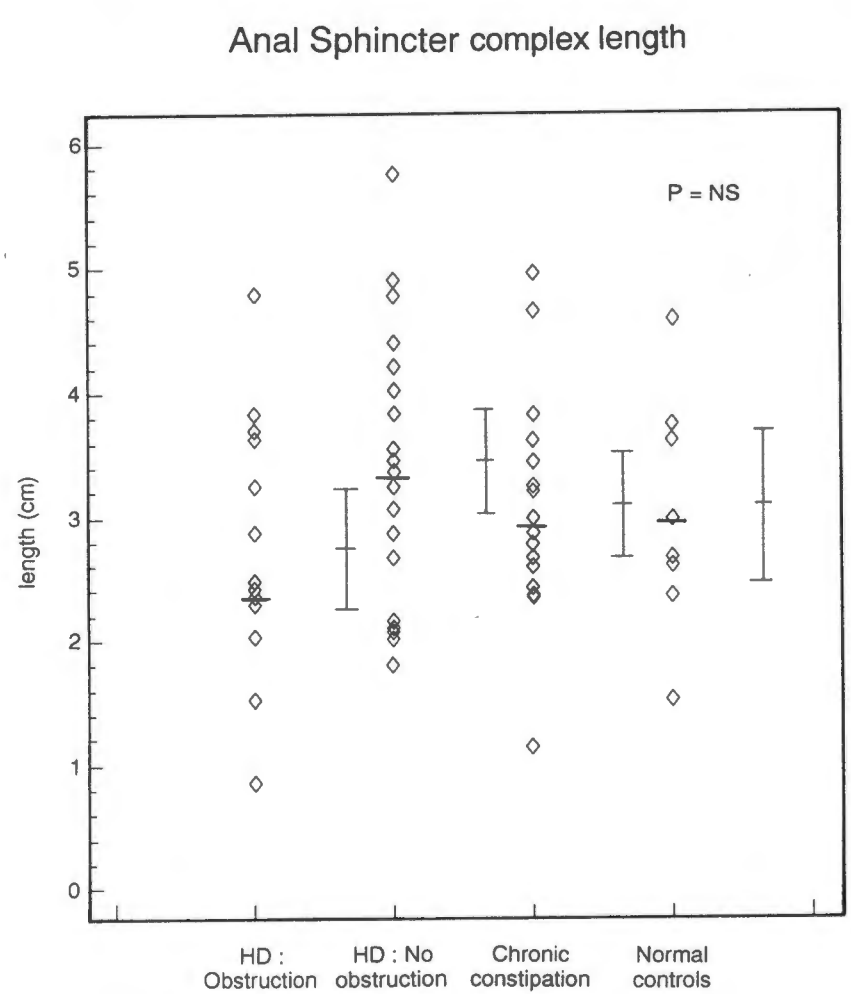


Figure 8.4: **Scatter plot of anal sphincter length, showing median values (broad line in data), means and 95% confidence intervals in patients with Hirschsprung's disease (with or without obstruction) and controls (with or without chronic constipation)**

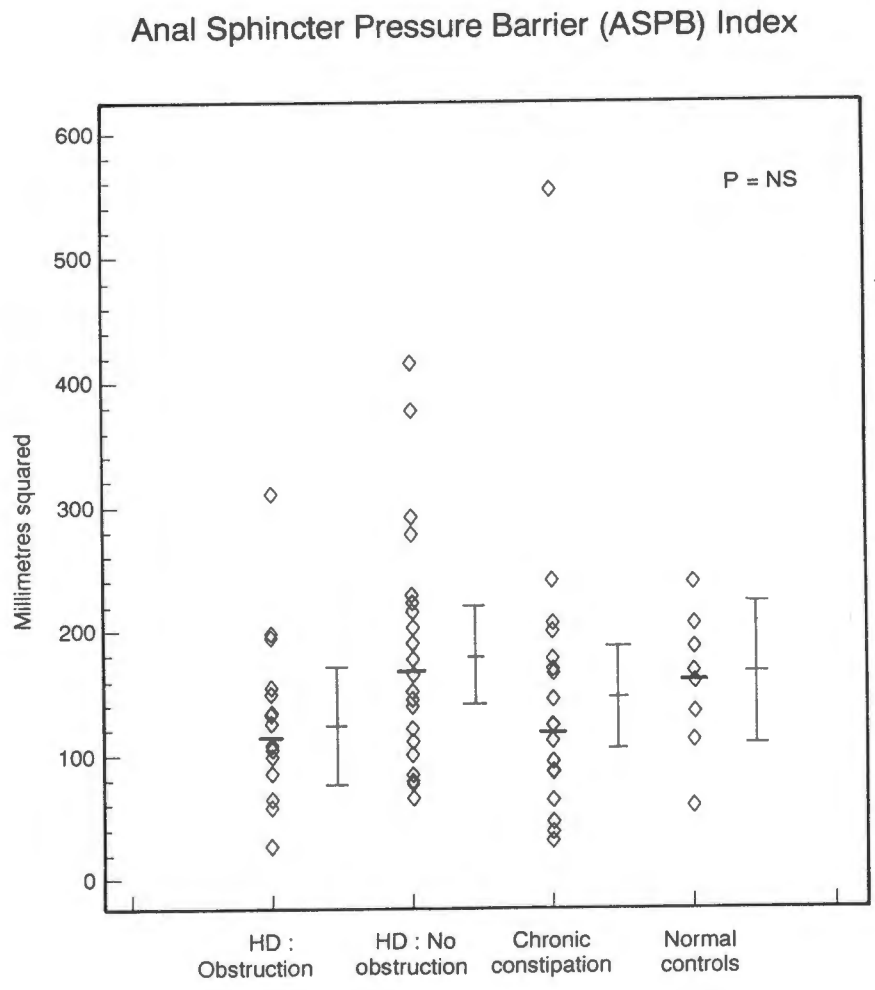


Figure 8.6: **Scatter plot of anal sphincter pressure barrier index, showing median values (broad line in data), means and 95% confidence intervals in patients with Hirschsprung's disease (with or without obstruction) and controls (with or without chronic constipation).**

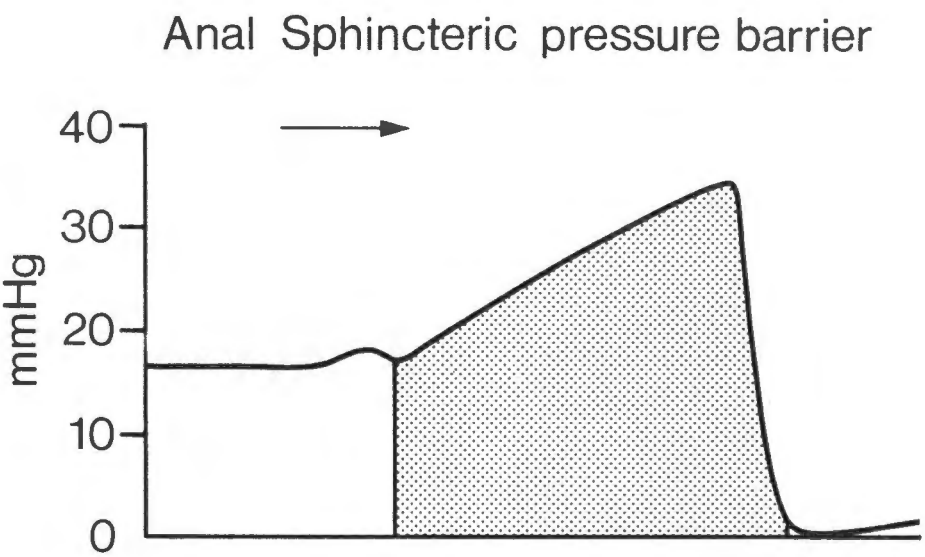
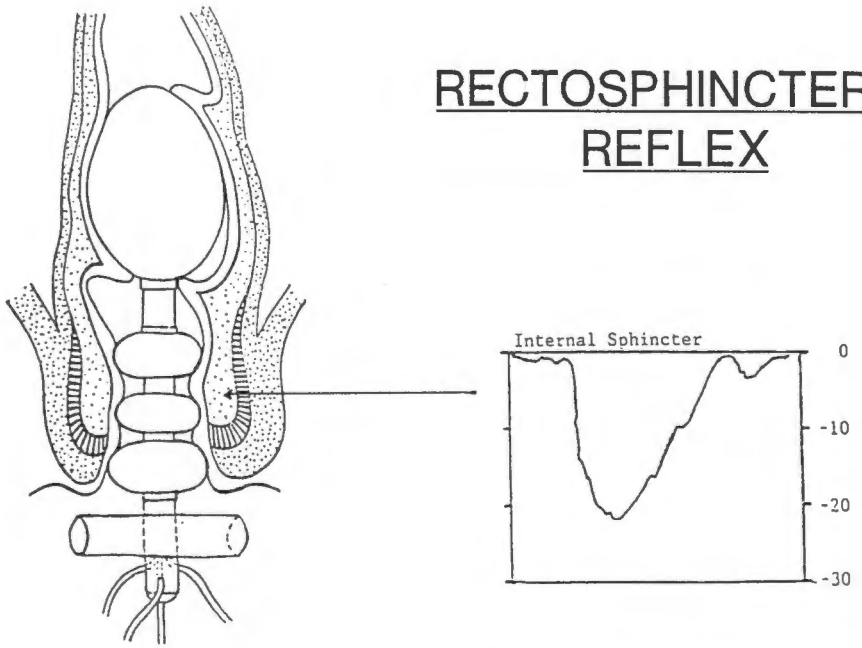


Figure 8.5 Anorectal pressure profile tracing demonstrating the anal sphincteric pressure barrier as the shaded area under the high pressure zone (mm2). (The arrow shows the direction of the catheter withdrawal)

8.5.5 Anorectal sphincteric function

8.5.5.1 *Rectosphincteric relaxation reflex*

The normal internal sphincteric relaxation reflex was present on rectal balloon distension in all controls but was only identified in 6 of the 37 patients (16.2%) tested post Hirschsprung's surgery. There were 3 positive reflexes in patients with obstructive symptoms and a further 3 within the group without obstruction (Figure 8.7).



	POSITIVE	NEGATIVE
OBSTRUCTED SYMPTOMS	3	12
NON OBSTRUCTED	3	25

Figure 8.7 Diagrammatic representation of rectosphincteric reflex obtained on manometry.

8.5.5.2 *Squeeze pressures*

Sphincteric squeeze pressures were obtained in 23 of the patients, 12 being post Hirschsprung's pull-through surgery and 11 controls. In this subgroup, the mean age of the patients with Hirschsprung's disease was 10 yrs which correlated with 11 yrs for the control patients, suggesting that these were comparable samples.

Although age, and thus size, definitely have an important role to play in these results, the observed differences may also be affected by the degree of cooperation achieved. Patients with obstructive symptoms did not have higher squeeze pressures than those without obstructive symptoms.

8.5.6 Neorectal function

8.5.6.1 *Rectal capacity*

Rectal capacity as measured by the maximal tolerated volume of the rectal balloon, was comparable in all three groups (**Table 8.1**). Correlation of rectal capacity with compliance demonstrates an increase in compliance with a high rectal capacity.

8.5.6.2 *Postoperative sensation*

Distension of the balloon was generally appreciated on the first injection of air in the majority of patients. The mean sensation threshold of 36 mls of rectal balloon air reflects those in whom appreciation was at a much higher volume. A manometrically induced sensation of increasing fullness and a desire to defaecate was present in 27 of the patients tested (70%). Sensory appreciation was diminished in those of the chronic constipation group with incontinence.

8.5.6.3 *Rectal compliance*

The measurement of rectal compliance ranged from 4-10 ml per mmHg in the normal controls with a median value of 6 ml per mmHg. The highest values recorded in this series were noted in patients with chronic constipation where the mean was 11.8 ml per mmHg (median 8 ml per mmHg).

8.5.7 Rectal motility

8.5.7.1 *Resting rectal motility*

No statistically significant differences in the resting rectal activity levels were recorded between normal controls, chronically constipated, and patients following pull-through surgery for Hirschsprung's disease (**Table 8.1**). An average rate of 14 contractions per minute was noted in obstructed patients with the histological features of NID in contrast to 22 contractions per minute of the overall postoperative group. Those with acquired aganglionosis had a median 24 contractions per minute.

The amplitude of rectal contractions varied between 2 and 8 mmHg in all four patient groups and no other significant differences were noted between groups.

8.5.7.2 *Mass contractions*

Mass contractions did not occur in any of the manometric studies performed on normal or constipated controls or in patients with the modified Soave endorectal pull-through. Mass contractions were noted in 4 of the 5 patients tested following a Duhamel procedure. They thus appeared to be related to the type of procedure.

8.5.7.3 *Propulsive rectal waves*

Balloon stimulation of the rectum resulted in the initiation of further propulsive rectal activity in 7 of the 10 normal controls, 11 of the constipated group and in 9 patients following Hirschsprung's surgery. In the majority of cases this was a slow wave response of 1 or 2 contractions per minute.

8.5.7.4 *Adaptation reaction*

Adaptation reactions were present in all the normal controls and in 13 of the constipated group. In the postoperative group 9 of the 37 patients were noted

to have adaptation reaction. Two of these had obstructed symptoms without aganglionosis. No distinct differences in the pattern or amplitude of adaptation responses were noted between the patients belonging to the different groups.

8.5.7.5 *Anorectal pressure fluctuations*

Fluctuating anorectal pressure waves were helpful in identifying the correct placement of the balloon probe at the site of the anal sphincter. Although identified in normal controls, no pressure fluctuations could be detected in 14 of those with chronic constipation. In the Hirschsprung's group, 25 patients had anorectal fluctuations including 8 with obstructive symptoms. Little difference was noted in those patients in whom the fluctuations appeared regular and those where an irregularity was detected.

8.5.8 Correlation of manometric findings with clinical outcome

Correlation between clinical outcome and manometric observations is shown in **Table 8.2**. Using the Wingspread criteria (**Appendix D**) clinical outcome was assessed as being in the good category in 17, fair in a further 17 and poor in 3 patients who had persistent soiling problems. Similar results were obtained for the Kelly and Holschneider scoring systems.

8.5.9 Correlation of manometric pressures with histological observations

Histological features of NID were noted in 9 of the 16 patients with obstruction. Manometric values from this subgroup of patients did not differ significantly from those without obstructive symptoms (**Table 8.3**). There was a decrease in the number of rectal fluctuations recorded per minute as opposed to a reported anorectal hyperexcitability in patients with NID (Krebs 1990). No increase in anorectal fluctuations were noted in patients with NID.

TABLE 8.2

POSTOPERATIVE HIRSCHSPRUNG'S DISEASE MANOMETRIC EVALUATION BASED ON OUTCOME

	RRP mmHg	ACP mmHg	ACPD mmHg	MEDIAN VALUES SPHINCTER LENGTH (cm)	MTV (ml)	ASPB mm^2
Good n = 17	6	21.7	115	11.36	1180	131
Fair N = 17	8	26.3	18	11.28	150	154
Poor N = 3	11.5	37	25.5	2.83	150	378

TABLE 8.3

MANOMETRIC OBSERVATIONS POSTOPERATIVE PATIENTS WITH NID

	NID	OBST	NO OBST	CH CONST	NORMAL
Resting rectal pressure (mmHg)	4.6	8.6	6	6	8.8
Anal canal pressure (mmHg)	21.7	25	26.3	27.7	31.8
Anal canal pressure difference (mmHg)	13	15	16.6	23.3	22.1
Sphincter length (cm)	2.4	2.3	3.4	2.9	2.8
Anal sphincter pressure barrier (square mm)	105	131	169	128.5	162
Rectal motility (freq/min)	12	20	19	20	22

8.6 DISCUSSION

The internal anal sphincter has been shown to be involved in Hirschsprung's disease (Lawson and Nixon 1967; Schnaufer 1967) and the rectosphincteric reflex is absent (Schuster 1965). This is the basis of the manometric test used to diagnose Hirschsprung's disease. Inadequate resections for Hirschsprung's disease have been reported to result in persistent obstructive symptoms (Swenson 1964; Lawson 1972). Although this is generally true, procedures such as the State and Rehbein apparently successfully leave some aganglionic bowel in situ (State 1952; Rehbein 1958).

Significantly higher resting rectal pressures and length of the anal canal have been reported in patients with Hirschsprung's disease than in normal controls (Ohi 1989). As none of the operations currently performed for Hirschsprung's disease aims to remove the action of the internal sphincter, sphincter achalasia is a further possible cause of postoperative obstructive symptoms (Holschneider 1982; Fadda 1987). Holschneider (1980) noted a significantly higher incidence in the postoperative anorectal pressure profile following procedures where the internal sphincter is left intact. These included patients who had the original Soave, Rehbein or the original Swenson and Duhamel techniques. Decreased anorectal pressure profiles were noted following the Swenson or Grob's modification. This may be due to the partial sphincterotomy resulting from a low posterior anastomosis.

Postoperative function following surgical pull-through of ganglionated bowel in patients with Hirschsprung's disease has been shown to depend largely on a balance of the forces at the anorectal region (Schärli and Kiesewetter 1969). Factors which are of importance to determine postoperative function include the length of bowel involved, the operative technique, postoperative rectal capacity

and efficiency, rectal sensation and an objective measurement of anal sphincter function (Holschneider 1980, 1982; Frenckner 1983).

8.6.1 Demographic factors of manometric sample

The selection of patients was on a random basis and although this was not a stratified sample, there appeared to be no significant group differences in the major characteristics which would affect the observed results. The obstructed patients in this study all had short segment Hirschsprung's disease and there were no other specific discriminating features to separate them from other postoperative Hirschsprung's patients (**cf Section B**).

As far as possible, controls were selected from approximate age matched groups on a random first come basis. Differences in age distribution were not found to be significant on testing. Similarly, other demographic factors did not appear to play a significant role.

8.6.2 The effects of patient sedation

The influence of low dose Ketamine sedation is uncertain. Reductions in rhythmical activity and pressure measurements have been reported under general anaesthesia (Frenckner 1983). On the other hand, Paskins et al (1984) reported no significant differences in resting rectal pressures, rectal inhibition or rhythmical activity of the rectum in 142 children investigated for chronic constipation with Ketamine sedation when compared with those of 225 children of similar age examined without Ketamine anaesthesia.

In this study, sedation was only used in 2 patients in the obstructed group and in 4 in the constipated group where the procedure was considered to be of diagnostic significance. The small number of the sample given sedation and a

lack of clarity in the reported effects suggest that this is not a major factor in this particular study.

8.6.3 Comparison of the anorectal pressure profiles

Anorectal manometry can be divided into measurements taken in the resting phase before rectal distension, the early inflation responses following the injection of 20-50ml air into the rectal balloon and the physiological functions of the rectum (Holschneider 1983). Manometric studies in patients with postoperative obstructive symptoms have been reported as showing a raised anorectal pressure profile and an absence of a rectosphincteric relaxation reflex in the absence of aganglionosis (Mahour 1990). By way of contrast, there were no significant differences observed in the median values of resting rectal pressure, maximal anal canal pressure and the anal canal pressure difference in the obstructed group when compared with control groups of non-obstructed postoperative patients. These results were also similar to those of patients with chronic constipation and the normal controls.

The normal control group mean and median pressures were comparable to previous reports (Schärli and Kiesewetter 1969). Resting rectal pressures were comparable in all four groups (**Table 8.1**). On the other hand, the maximal anal canal pressure and anal canal pressure difference had somewhat lower median values in the postoperative group. These lower pressures may represent the effect of the partial sphincterotomy resulting from the low posterior anastomotic line during surgery.

The role of the anal sphincter as the cause of postoperative obstructive symptoms is therefore open to question. There was no increase in the anal sphincteric pressure barrier (either by a rise in resting rectal pressure, maximal anal canal pressure, anal canal pressure difference or anal sphincteric pressure

barrier index) in patients with postoperative obstruction when compared to controls. These findings are consistent with other reports (Schärli 1969; Holschneider 1980; Iwai 1989), although higher resting anal canal pressures have been reported in some series (Mishalany 1987). Others (Frenckner 1983, Ohi 1989) have reported little difference between pre - and postoperative anorectal pressure profiles and Ohi et al (1989) reported a return to normal rectal pressures after 3 years. This is in keeping with the findings in the older patients of this study. By way of contrast, the resting rectal pressure was increased in patients with obstructive symptoms and aganglionosis. Observed mean values approached the reported preoperative level (Ohi 1989). This suggests that in these patients, the sphincteric obstructive picture persists, presumably because of the aganglionosis.

A reasonable conclusion which may be drawn from this data, is that the internal sphincter function is not increased in the majority of patients with an obstructive clinical picture. This means that the obstructive symptoms are probably not caused by a supercontinent anal sphincter. On the other hand, the resting rectal pressure was increased in patients where an aganglionic segment persists. This suggests that obstruction occurs in this subgroup and may form a basis for future studies.

Certain difficulties still exist in the interpretation of this data and other possible reasons for these observations include sphincter achalasia. Although the internal anal sphincter may still have an part to play in abnormal postoperative anorectal function, there was no evidence of sphincter achalasia on clinical, radiological or manometric data in this series. In addition, the patients with postoperative obstructive symptoms did not respond to anal dilatation. Further correlation of manometric and clinical findings would appear to be an essential step in the assessment of postoperative anorectal function.

8.6.4 Differences in anal sphincter complex length

Under resting conditions, the high pressure zone of the anorectal pressure profile represents the functional resistance area in the anal canal. This high pressure region largely reflects the tonic contraction of the internal anal sphincter (Bennett 1964; Frenckner 1983) which exerts its influence on the upper two thirds of the anal canal (Kerremans 1968).

The length of the anal canal appears to be an important factor in determining the resistance offered by the anal sphincteric mechanism. We noted that the same amount of anal resistance may be achieved by a lower pressure difference over a longer length of anal canal as that of a higher pressure over a shorter length.

Lengths of the anal canal obtained in this study are within the reported normal limits (Nivatvongs 1981; Iwai 1989; Schärli 1989). In the 4 obstructed patients with acquired aganglionosis the anal canal was significantly longer ($p<0.05$) when compared with 10 obstructed patients with NID on rectal biopsy. There appeared to be some degree of anal sphincter outlet obstruction in a further 2 patients and a favorable clinical response resulted from a myectomy procedure. These findings led to an investigation of the role of the internal anal sphincter resistance in producing postoperative obstructive symptoms. To combine both the length and pressure profile, a new parameter, the anal sphincteric pressure barrier index, was calculated from the anorectal pressure profile tracing.

8.6.5 Measuring the anal sphincteric pressure barrier (ASPB) Index

8.6.5.1 *The ASPB Index*

The concept of an anorectal resistance force maintaining continence has been addressed in other studies (Iwai 1987; Shandling 1987). These previous measurements of resistance (Iwai 1987) or anorectal force (Shandling 1987)

have the disadvantage of being difficult to conceptualize both manometrically and as a dynamic in the changing physiological mechanisms involved in anorectal function. The resistance offered by the tonic action of the anal sphincters is expressed manometrically by means of several variables.

From our study of the sphincteric action and the anorectal pressure profile tracing, it was clear that the area of the high pressure zone on the anorectal pressure profile (**Figure 8.5**) appears to represent both the amount of pressure exerted by the tonic contraction of the pelvic musculature as well as the length over which such a pressure gradient exists. Thus the area under the curve of the anorectal pressure profile would appear to be a measurement of the functional resistance of the sphincters at the time of testing and partially reflects vector profilimetry. In addition, it represents the resistance of the pelvic musculature to the leakage of rectal contents. This maintains the continence in a particular patient at rest.

In order to facilitate interpretation and comparison by giving an index value to this anal sphincter pressure difference, I expressed the area under the high pressure zone in square millimeters. I have termed this the Anal Sphincteric Pressure Barrier (ASPB) index as it manometrically represents the tonic muscular resistance to the leakage of contents from the rectum. It has further application in the assessment of anorectal function following surgery for other anorectal conditions.

8.6.6 Postoperative sphincteric function

Only 3 patients out of the study group of 67 patients reported soiling. The anorectal pressure profile was not able to predict a lack of control in these patients. Functional control appeared to be the nett result of a balance of the forces operative in the anorectal region as previously reported (Schärli 1969,

1972, 1989). Continence did not appear to be entirely dependent on the anorectal pressure profile or anal sphincter pressure barrier and other factors appeared to play a significant role.

8.6.6.1 *Response to stimulation - postoperative recovery of the internal anal sphincter relaxation reflex*

Previous reports suggest that the absent rectosphincteric reflex can be regained postoperatively in 39-66% of patients (Holschneider 1983; Nagasaki 1989). The relaxation reflex does appear to recover following Hirschsprung's pull-through surgery in certain patients, but in the experience of this study these negative manometric deflections do not generally conform to the normal configuration (ie. it may be an abnormal reflex).

Holschneider (1980, 1983) noted various stages of possibly maturing relaxation reflex in 66.2% of 423 patients studied between 1 and 6 years following surgery. The highest incidence of a present postoperative relaxation reflex in Holschneider's series was in those with the Duhamel and Rehbein procedures as well as other procedures where the internal sphincter was intact. A normal reflex in amplitude and duration was reported in 20.6% of these children. In the remainder, the initial mass contraction observed during the rectal distension phase, was followed by a rudimentary sphincteric relaxation reflex which had no relationship to the distending volume. There was also an increase in the depth of the negative deflection with an increased volume of the insufflated balloon.

The lowest incidence of demonstrable reflex activity followed the Swenson procedure in Holschneider's series (1983). A particularly high incidence of recovery of the rectoanal sphincteric reflex has been reported by other workers in postoperative patients who have had the Soave procedure (Carcasonne 1984; Soave 1985). In Holschneider's series the incidence of a positive reflex in

patients following the Soave procedure was 75.2%. There are similar reported findings following myectomy procedures (Suzuki 1970; Lynn 1975; Nagasaki 1989). This is in contrast to the findings of this study and that of other workers (Frenckner 1983; Mishalany 1987; Ohi 1989). This study showed a much lower incidence of a demonstrable rectosphincteric reflex in patients following the modified Soave procedure.

Despite suggestions that postoperative function is related to the regaining of a rectosphincteric reflex (Holschneider 1983; Nagasaki 1989), no clear correlation has been established to date. These suggestions are attractive because the recovery of the rectosphincteric reflex provides a reasonable means of identifying those who will eventually achieve full continence. By way of contrast, a number of patients in Holschneider's series (1983) were fully continent despite the presence of very rudimentary internal sphincteric relaxation reflexes. Other reported series (as well as this study) show excellent function in the presence of a relatively low incidence of reflex response (Mishalany 1987; Ohi 1989) .

A negative deflection in response to inflation of the rectal balloon was noted in only 6 out of the 43 postoperative patients (14%) in this study. Three of these were within each group. This is supported by the excellent results in 94% of patients interviewed and is in keeping with other reported findings (Frenckner 1983; Mishalany 1987). One possible explanation for the discrepancy between these series is the low posterior anastomosis (as performed in our series) which results in a partial sphincterotomy of the internal anal sphincter.

Technical difficulties in obtaining a reflex as well as the fact that false negative tests for the relaxation reflex are well described, particularly in the patient with gross distension of the rectal ampulla. Error rates of between 8 and 16% as

well as false positives and false negatives have been reported (Meunier 1978; Morikawa 1979). Murray (1990) noted an absent rectosphincteric reflex using a microtransducer system up to 120 mls in the rectal balloon in 7 out of 10 patients with chronic constipation but without aganglionosis.

It is possible that artifacts may include features which resemble relaxation of the internal anal sphincter. Loenig-Baucke (1983) reported in patients with Hirschsprung's disease that a 40 ml inflated balloon placed 6 to 7 cm above the anal verge produced some recordings similar to internal sphincter relaxation, whereas in controls and in children with chronic constipation, reflexes could be obtained independent of the rectal position of the balloon for distances of 6 cm or 11 cm from the anal verge. This suggests that the position of the rectal balloon is important in certain cases where false positives are obtained. In addition, adult studies (Horgan 1989) have shown an absence of the rectoanal inhibition reflex in some patients with a very low anal canal resting pressure and in patients with a low rectal excision, suggesting that other factors can influence the incidence of postoperative rectosphincteric reflex obtained on testing. This did not appear to be the case in the current study, as fluctuations of pressure arising from the anal sphincter were looked for and assisted in the correct positioning of the balloon probe. In patients where such activity could not be identified, some degree of technical difficulty was experienced (Holschneider 1982). On the other hand, the partial sphincterotomy resulting from the low posterior anastomosis may have resulted in a decrease of this activity in certain patients.

8.6.6.2 *Squeeze pressures*

The external anal sphincter contributes only 15-30% of anal canal pressure at rest (Duthie 1965) and the levator ani exerts its maximal effect on the upper third of the anal canal. It can therefore be argued that the assessment of the

external anal sphincter function is of lesser significance following surgery for Hirschsprung's disease as none of the current procedures involve the external sphincters. It appears that the internal anal sphincter is affected in patients with a low anastomosis during Hirschsprung's surgery. Its role in postoperative anorectal function needs to be defined. On the other hand, some decrease in external sphincter function has been demonstrated in patients treated for Hirschsprung's disease (Springhall 1990).

Technical difficulties were encountered in obtaining consistent squeeze pressures especially in the younger patients where communication obstacles existed. In addition, squeeze pressures could not be obtained in those requiring sedation. In the postoperative group mean values were slightly lower (23 mmHg versus 29 mmHg), possibly as a result of the surgical interference. Mean values of squeeze pressures were highest in subjects over the age of 10 years, in keeping with other reported findings (Iwai 1989).

8.6.7 Neorectal function

Anorectal function is related to the balance of a number of factors such as an adequate length and degree of anorectal resistance (Schärli 1969; Taylor 1973; Arhen 1976; Iwai 1979). These factors have already been discussed in the earlier discourse.

Other factors such as rectal capacity, postoperative anorectal sensation (Read 1982) and the force of the rectal expulsive waves (Schärli 1969) play a part in postoperative neorectal function and must be evaluated.

8.6.7.1 *Rectal capacity*

The measurement of neorectal volume is important in assessing postoperative anorectal physiological function. Stool frequency is directly related to the length

of remaining bowel, the procedure performed and the rectal capacitance. An argument advanced against the endorectal pull-through operation is that the reduction in rectal capacity and compliance causes postoperative diarrhoea (Holschneider 1980). The high incidence of buttock excoriation noted in the early complications reported in this series may be partly explained on the basis of this finding. Stoller (1987) has shown little clinical or physiological advantage to adding a reservoir to endorectal pull-through procedures, suggesting that reservoir function returns to normal in the majority. Similarly, Frenckner (1983) demonstrated a similar rectal capacity post Soave procedure to that of normal individuals. In this study, neorectal capacity differed very little from normal controls. The higher rectal capacity observed in those with chronic constipation is expected, due to rectal dilatation in these patients.

8.6.7.2 *The role of sensation in neorectal function*

The appreciation of rectal balloon inflation somewhere between 10 and 40 ml is considered to be within normal limits (Murray 1990). This was present in more than 70 % of those patients tested. No clear difference in sensation and rectal capacity were noted between those with obstructive symptoms and controls.

Poor appreciation of sensation was observed in the 6 patients with incontinence in this series suggesting that sensory appreciation is an important factor in the maintenance of continence. The sensation loss appeared to be mostly the loss of awareness of incremental volumes in the rectal balloon and the lack of a desire to defaecate even at high rectal balloon volumes. This observation is supported by other studies in adult patients (Read 1982) and similar findings have been reported for patients with soiling and constipation which were resistant to treatment (Murray 1990). Recent reports (Sun 1990; Nagashima 1992) have shown a statistically significant ($p<0.0001$) relationship between rectal sensation and the electrical activity of the external anal sphincter. This

was associated with rectal contraction but not with internal anal sphincter activity. These findings have led to asking the question whether a lack of continence may result from no external anal sphincter contraction when the rectosphincteric reflex results in internal sphincter relaxation.

8.6.7.3 *Neorectal function*

In this series a high incidence of persisting mass contractions were noted following the Duhamel procedure. The cause of mass contractions is not fully understood and the reported association with an aganglionic bowel segment was noted by Hiatt (1951). A high incidence of mass contractions in patients undergoing the Swenson and Duhamel procedures was thought by Holschneider (1982) to be due to operative damage to the sacral nerve fibres. On the other hand, it's association with the Duhamel procedure may possibly be explained by the residual aganglionic portion left in situ (Wood 1973). This would also be a valid reason in those undergoing the Rehbein procedure.

Holschneider attributed the cause of a low compliance and an abnormal adaptive reaction of the rectum to the abnormal muscle cuff following the Soave procedure (Holschneider 1982, 1983). The values for rectal compliance in this series were, however, comparable to those obtained in normal controls (Holschneider 1982). This indicates that the postoperative rectal elasticity returns to normal limits with time.

These findings are substantiated by other reports in the literature. In the series reported by Iwai (Iwai 1987) rectal compliance increased as bowel function improved. The high pressures initially noted in the postoperative period in patients with total colonic aganglionosis gradually returned to normal 1 year after a Lester Martin procedure.

In other respects, neorectal performance appeared to be comparable between groups and no specific features were identified to indicate a difference in physiological function in the neorectum.

8.6.8. Rectal motility

Propulsive waves were noted to be mostly of the slow variety following the Swenson and Duhamel procedures. Ohi (1989) reported 25-27.3 basal rhythmic waves per minute in the rectum of normal children depending on age. Observed differences in this study did not reach significant levels on testing and slight alterations in the basic rhythmical activity of patients after corrective operations may have little significance in terms of anorectal function (Ohi 1989).

8.6.9 Correlation with histological features of NID

The manometric observations suggested a possible motility disturbance in patients with obstructive symptoms. There was no increase in the anorectal pressure profile nor an increase in anorectal fluctuations in these patients, which was in keeping with a report on the manometric findings in patients with NID (Krebs 1990). The 14 pressure fluctuations per minute recorded in the obstructed group was lower than the 22 per minute of the total manometric study group and 24 per minute in those with acquired aganglionosis. The decreased rectal motility of the obstructed group was largely the result of a significantly lower number of rectal fluctuations in those patients with features of NID as opposed to those without (Kruskal Wallace, $p<0.05$). This is in contrast to the anorectal hyperexcitability previously reported in patients with NID (Krebs 1990).

It is important to note that the series reported by Krebs (1990) did not include patients with co-existing NID and Hirschsprung's disease. The postoperative nature of our sample may be a major limiting factor in drawing any conclusions

about the manometric findings of NID. In addition, only a few reflexes were obtained in the tests carried out in this study. This resulted in difficulty in determining whether the internal sphincter reflex action was or was not proportional to the volume of the distension by the rectal balloon.

8.7 SUMMARY OF MAJOR MANOMETRIC FINDINGS

- No significant differences in anorectal pressure profile could be demonstrated in all but 2 patients with postoperative obstructive symptoms. Manometric assessment was of value in recognizing those patients with super continence based on an increase in sphincteric resistance.
- The anal sphincteric pressure barrier index is a useful new parameter of manometric measurement. No differences in anal sphincteric pressure barrier were noted in postoperative patients with obstruction.
- Neorectal function returns to normal in the majority of patients.
- Rectosphincteric reflexes were only observed in 6 (16.2%) of the postoperative Hirschsprung's cases and did not appear to correlate with functional outcome.
- The frequency of resting rectal activity was decreased in obstructed patients suggesting a motility problem. This is due to a lower number of rectal fluctuations in patients with NID.
- Patients with NID and postoperative obstruction did not significantly differ from other patients apart from a decreased rectal motility.
- Sensory defects appeared to contribute to poor anorectal control in 3 patients.
- A suggested management protocol is presented based on these observations.

8.8 TECHNICAL CONSIDERATIONS

Certain technical considerations must be taken into account in interpreting manometric data. The first relates to the limitations of the probes themselves and the second to the factors affecting anorectal manometric measurements.

8.8.1 Limitations of manometric probes

Although balloon systems have been widely used to measure intraluminal pressure changes, they are subject to certain technical problems which may influence the results. These include the standardizing of balloon compliance and the fact that the balloon obstructs the bowel thus causing the bowel to be stimulated. The resultant contractile waves may influence results considerably and have limited the usefulness of balloons in measuring anorectal pressures in the past. This problem has been overcome by the development of microballoon systems (Holschneider 1982).

However, balloon probes are still widely used to elicit the relaxation reflex and other physiological responses (Ustach 1969; Holschneider 1982).

Open tipped perfused catheters, developed in the 1960's for oesophageal manometry, are reported to yield more consistent pressure measurements (Brody 1951; v Issendorf 1983). This technique uses a low compliance pressure system which is perfused by normal saline at an extremely low flow rate to minimize the degree of gastrointestinal distension.

Ultraminiature silicone force transducers have been used as internal force transducers and radiotelemetry capsules are not without problems in interpretation of results, certain types giving more consistent readings than others (Millar 1988).

A working group into anorectal functional assessment has stated that a water-filled balloon system fulfills all the necessary criteria for anorectal manometry (Keighley 1987). The accuracy of the manometric system is subject to a number of influences which are beyond the scope of total control in a clinical setting. In addition to the radial and individual response, an intrasubject variation of up to 20% can result from the influence of physiological influences such as slow and ultraslow contractile waves at the time of measurement (Krogh Pedersen 1989). As a result, stimulation of the rectum was kept to a minimum, no bowel preparation being used and the anorectal pressure profile was performed first. In addition, the catheter probe design included the creation of radial side holes situated 5 cm from the catheter tip, thus allowing re-testing with minimal repeat stimulation.

8.8.2 Technical problems associated with manometry

A certain amount of methodological variation may contribute to variations in the observed values when measuring intraluminal pressure.

Technical factors such as voltage fluctuations or electronic noise are possible factors which may affect the function of the measuring instrument. These are largely unavoidable under normal hospital circumstances. Preventable technical factors such as the presence of air bubbles in the line, the length and calibre of the probes and the conducting tubes, the standardization of the instrument and uniformity of investigator technique were accommodated in the methodology of this study.

The measurement of the pressure generated by the anal canal and internal anal sphincter is related to the law of La Place. Thus the intraluminal pressure is equal to the stress characteristic of the muscle multiplied by the muscle thickness and divided by the radius. Variations in balloon measurements of

pressure are well described (Lemen 1974; Beardsmore 1980; Asher 1982). Corrections of pressure and temperature were not generally necessary in this series as the perfused catheter was used for measuring the anorectal pressure profile. Thin perfused tube probes have been identified as being a reasonably accurate probe for the measurement of the anorectal pressure profile (v.Issendorf 1983; Krogh Pedersen 1989). No significant differences have been demonstrated in perfusion rates varying between 0.5 and 1.2 ml per minute per orifice over a range of catheter withdrawal 0.19, 0.33 and 0.48 cm per second (McHugh 1987). Our catheter was perfused at a controlled 1.2 ml per minute and withdrawn at a constant 0.48cm per second.

The net pressure recorded by the manometric system is a function of the tone of several separate muscle groups, but in addition it reflects the tissue turgor and resilience of soft tissues around the rectum and anal canal. As a result, marked longitudinal and radial variations of anorectal pressure profiles have been reported (Taylor 1984). This variation was taken into consideration and radial side holes were added to the perfused catheter probe.

Where corrections for pressure were required, the principles of Boyle's law (V1 x P1 = V2 x P2) were used. Temperature corrections are usually not possible due to the difficulty of ascertaining the variations of temperature inside the balloon during its inflation and deflation (Ihre 1974). We also noted difficulty in the interpretation of pressures taken from balloon probes caused by the distorting effect of the rectal walls and secondary responses within the rectal wall itself were more likely to occur with rectal balloon stimulation.

Two tests where balloon measurements were used, were the degree of the rectosphincteric relaxation reflex and the measurement of compliance. The relaxation reflex was assessed as being present or absent. There was little

difference in repeated compliance readings. This suggests that this was a reliable means of assessment. Squeeze pressures were subject to difficulties in obtaining sufficient patient co-operation, particularly in the younger patients tested.

The standardized nature of the study, the single investigator and the adjustments made for any observed contributing factors on an ongoing basis during the course of the study, helped to keep these influences at a minimum.
Difficulties in interpreting the results of manometric findings arise partly from a lack of standardization of the reporting of results. In this regard, the efforts of two recent working parties are noteworthy in bringing about consensus (Keighley 1987; Mishalany 1989). Results were thus also expressed in kPa to facilitate comparison with other reported series.

8.9 CONCLUSION

Manometric investigation of the existing balance of forces allows an objective evaluation of anorectal function. Symptoms may result from a disturbance of any of these forces and the identification of possible areas of abnormal function may influence the management of the patient.

The hypothesis that obstructive symptoms post Hirschsprung's surgery are caused by sphincter overactivity in most cases has not been supported by the manometric findings of this study. Manometry was able to identify the 2 patients with sphincter overactivity and predict the success of an anorectal myomectomy.

This study shows that the resistance offered by the anal canal is not significantly increased in the majority of patients with postoperative obstruction. In patients where aganglionosis was not the cause, the sphincter, in fact, appeared shorter. Residual disease of the intestinal wall resulting in a motility disturbance is a

possible explanation of the prolonged transit and obstructive symptoms. Obstructive symptoms resulting from co-existing NID in patients with Hirschsprung's disease is a rather controversial subject and will be addressed in the following section.

SECTION D

CHAPTER 9

THE NID DEBATE

Proximal residual abnormalities of the ENS are a possible cause of postoperative dysfunction in otherwise successfully surgically managed patients (Smith 1967; Lawson 1972; Touloukian 1975; Chow 1977; Meier-Rüge 1986; Kluck 1986; Larsson 1988; Mishalany 1989; Cass 1990; Tam 1991). Inadequate resection of abnormally functioning bowel may lead to postoperative dysfunction (Howard 1984). Retention of a residual hypoganglionic transitional zone (Howard 1968; Meier Rüge 1970), a lack of neuromuscular junctions (Yamataka 1992) or co-existing neuronal dysplasias (Puri 1977; Meier Rüge 1974; Fadda 1987; Pistor 1987) are amoung the possible reasons for less than optimal function in the patient following otherwise successful surgically managed Hirschsprung's disease. Despite these numerous reports, the significance of NID in terms of postoperative gastrointestinal dysfunction remains a subject of contention (Lake 1989; Schofield 1991; Yunis 1992).

Anatomical abnormalities demonstrated by silver staining (Smith 1967) or other immunocytochemical techniques (Tam 1991) suggest that additional functional abnormalities of the myenteric plexus co-exist with Hirschsprung's disease. Abnormal patterns of neurotransmitter staining have been reported in the **proximal normally ganglionated bowel** of patients with Hirschsprung's disease at the time of surgical pull-through (Touloukian 1975; Chow 1977; Meier-Rüge 1986; Larsson 1988; Tam 1991). This is also true of patients with postoperative obstructive symptoms which were attributed to a residual dysplastic Enteric Nervous System (Kluck 1986; Mishalany 1989).

The incidence of co-existing NID with Hirschsprung's disease varies between 0 and 75% of patients in reported series (Briner 1986; Polley 1986; Fadda 1987; Lake 1989; Schofield 1990;

Simpser 1991; Meier-Rüge 1992; Schärli 1992). Although in certain reports, the high incidence of co-existing NID with Hirschsprung's disease is based on small patient numbers (Briner 1986), the essential features have been confirmed in a number of studies (Munakata 1985; Briner 1986; Fadda 1987; Heiming 1990; Meier-Rüge 1990, 1992; Kunde 1991; Borchard 1991). The mandatory histological features for a diagnosis of NID have been agreed on following the consensus convention of German pathologists (Borchard 1991).

By way of contrast, an incidence of 20-27% of co-existing NID in patients with Hirschsprung's disease has received wider acceptance (Rintala 1989; Schärli 1992). Despite this, a 50% incidence of co-existing NID has been recently reported in a large series of patients with Hirschsprung's disease (Meier-Rüge 1992).

On the other hand, the lack of uniformity in incidence and the high incidence in certain reported series has led some authors to take the view that the increase in AChE staining which conforms to the classical description of NID is a "descriptive histological appearance rather than a unique clinicopathological entity" (Lake 1989; Schofield 1991). A regional or ethnic predilection for NID or a lack of uniform diagnostic criteria are cited as possible explanations for the observed discrepancies (Schofield 1991; Yunis 1992). The consequent relationship between NID and persisting obstructive symptoms has been questioned.

Manometric and electromyographic studies in postoperative patients report motility disturbances which persist in certain patients following Hirschsprung's surgery (Howard 1968; Meier Rüge 1970; Mishalany 1987; Springhall 1990). These lend further weight to the argument that residual neuronal dysplastic conditions of the bowel may influence the success of the surgical procedure.

The wide range of dysplastic features described in NID may result in variations of interpretation as to what constitutes NID. This wide range of histological features has been grouped into at least 6 different distinctive patterns (Meier-Rüge 1992; Schärli 1992) based on the original

histological classification of Meier-Rüge (Meier-Rüge 1972). A recent consensus of the mandatory features necessary for the diagnosis of NID includes hyperplasia and giant ganglia in Meissner's plexus, as well as nerve cell buds on parasympathetic nerves and an increase in AChE activity in mucosal nerves and submucosal arterial walls (Borchard 1991; Schärli 1992; Meier-Rüge 1992).

The clinical significance of an increase in AChE staining neurofibrils as well as its role in producing postoperative gastrointestinal dysfunction remains open to debate. Reports of false positive (vd Staak 1981; Hamoudi 1982; Huntley 1982; Barr 1985) or false negative (Ariel 1983; Barr 1985; vd Staak 1981) AChE staining patterns have been reported in Hirschsprung's disease . Abnormal AChE staining patterns have also been described in patients with structural or anatomical gastrointestinal abnormalities (eg anorectal malformations) (Rintala 1989), chronic inflammatory disease of the gastrointestinal tract (Storsteen 1953) in addition to co-existing neurodysplastic conditions such as neuronal intestinal dysplasia (NID) (Meier Rüge 1974; Schofield 1991). This influence of other factors on AChE staining patterns should lead to a cautious approach to the interpretation of AChE staining patterns alone. Functional outcome is most likely to be determined by a combination of ganglion cell status taken in conjunction with the abnormal AChE findings in the affected bowel.

The use of special histochemical and immunohistochemical stains in patients with Hirschsprung's disease has led to a great deal of additional information which has assisted in the evaluation of the ENS status. Not all investigators use the same methodology, resulting in possible further reasons for the variation in reported results. Dehydrogenase stains, Neuron Specific enolase (NSE), S100 and PGP9.5 staining methods have recently been reported as being of considerable value in identifying hyperganglionosis (Borchard 1991; Schärli 1992). Much of the interpretation of these stains remains experimental at this stage (Borchard 1991). The intra-operative identification of the pull-through segment relies mostly on the detection of ganglion cells in frozen sections of serial seromuscular biopsies. The frozen section identification of ganglion cells is mostly reliable but the identification of ganglion cells alone may not be

sufficient to ensure normal postoperative function (Schärli 1992). On occasion, sections are difficult to interpret and further histochemical and immunocytochemical staining may be required (Lake 1989). The possible contributory effect of red blood cell AChE contamination (Ito 1977; Blisard 1986) is important to clarify due to increasing emphasis on increased AChE staining in the arterial walls in the diagnosis of NID (Borchard 1991) .

Further questions which may arise as to the nature of the pathophysiological process which results in the observed histological features of NID, include disorders of parasympathetic or sympathetic nerve function or a possible secondary response to intestinal obstruction (Yunis 1992). Some support for the latter hypothesis can be obtained from the observation that the anorectal dysfunction improves with time (Schärli 1992) in a number of children with NID. This observation could just as easily be explained if the less severe cases improve due to alterations in the existing balance of forces involved in anorectal function.

Despite recent advances, the minimum histological criteria required to diagnose NID, which will result in long term postoperative functional disturbance, are unclear. The clinical, manometric and histochemical findings of this study (Sections B and C) have provided some evidence that in our series the presence of co-existing features of NID in the pulled through segment of bowel in Hirschsprung's disease may be of long term functional significance.

The lack of clarity and definition surrounding the diagnosis of NID, particularly as it relates to co-existence with Hirschsprung's disease and postoperative obstructive symptoms, prompted further study.

This section of the project commenced in 1988, which was prior to the availability of agreed diagnostic criteria for the diagnosis of NID (Borchard 1991). The aim was to assess the incidence of histological abnormalities in the proximal ganglionated surgical pull-through bowel and to establish morphometric parameters for the assessment of co-existing NID and other neurodevelopmental dysplastic conditions. A further aim was to correlate biochemical and

histological findings with postoperative function to obtain some clarity as to the specific role of AChE in these conditions.

CHAPTER 10

GANGLION CELL MORPHOLOGY IN THE GANGLIONATED PROXIMAL BOWEL OF PATIENTS WITH HIRSCHSPRUNG'S DISEASE

10.1 INTRODUCTION

The accurate assessment of the pulled-through segment remains one of the most important factors in the successful outcome of the surgical management of Hirschsprung's disease. Surgical pull-through of aganglionic or transitional bowel is a potential cause of obstructive symptoms following otherwise successful surgical management (Lawson 1972; Howard 1984). The postoperative function of ganglionated bowel in Hirschsprung's disease may be related to ganglion cell morphology in the residual bowel. On the other hand, there is not always a clear correlation between the histochemical findings and postoperative function (Schärli 1992).

A number of neurodysplastic conditions may "co-exist" with Hirschsprung's disease and thus affect the residual colonic segment in Hirschsprung's surgery. These include hyperganglionosis, ganglion cell immaturity, hypoperistalsis syndrome and colonic intestinal dysplasia.

The **hyperganglionosis** associated with NID represents a significant increase in the number of ganglion cells in the submucosal plexus (Schärli 1981; Fadda 1987; Simpser 1991, Schärli 1992). Hyperganglionosis, when marked, is a diagnosis not easily missed by an experienced histopathologist. Some difficulties in the diagnosis have been reported (Lake 1989), however, due to a lack of clarity as to which parameters are most reliable in identifying less obvious cases. Certain previous studies have largely concentrated on ganglion cell number and size (Munakata 1978). Aldridge and Campbell (Aldridge 1968)

quantified the number of ganglion cells per cluster as the most reliable method of assessing ganglion cell numbers and accepted up to 5 per cluster as normal in Meissner's plexus. In addition, more than 5 submucosal ganglion cell clusters per high power field or large clusters of ganglion cells containing more than 10 ganglia per high power field have been suggested as possible diagnostic parameters to denote hyperganglionosis (Schofield 1991). In a recent series by Schärli (Schärli 1992), up to 7 ganglion cells per cluster have been accepted as being within the normal range. In this recently published series (Schärli 1992), the density of ganglion cells in the sub-mucous plexus in patients with NID were "4 times as large and contained 6 times more cells than normal".

The identification of **immature ganglion cells** may pose a practical problem for the histopathologist. Meier-Rüge (1982) has stressed the importance of other staining methods such as the dehydrogenase reaction or NSE in the evaluation of ganglion cell morphology. The exact number of ganglion cells per given area of bowel wall may be influenced by variations related to age and the level of tissue section as well as the degree of distension of the bowel wall.

A number of reports of co-existing **hypoganglionosis** have been reported both in patients with Hirschsprung's disease (Meier-Rüge 1970; Ehrenpreiss 1970; Munakata 1978) as well as in those without Hirschsprung's disease (Bentley 1964, 1966). Acquired hypoganglionosis has been reported after surgical pull-through for Hirschsprung's disease (Rafferscheid 1978; Dajani 1986). Hypoganglionosis is an extremely uncommon condition and is infrequently diagnosed. The diagnosis of hypoganglionosis is made when there is a minimum of ten times fewer ganglion cells in the affected segment than in normal controls. Local experience of hypoganglionosis has mostly represented an extended hypoganglionic **transitional zone**, especially in patients with extensive total colonic aganglionosis (Kaschula - personal communication).

The **morphology** of ganglion cells may vary considerably in the ENS (Munakata 1978). Small monopolar ganglion cells which have been labelled **"hypogenetic"** have been reported (Munakata 1985). Hypogenesis of the ganglion cells is defined as a condition where ganglion cells number one third of normal bowel and also have a 20% decrease in surface area on average. Hypogenesis has been reported to occur in the myenteric plexus (Munakata 1978) and the submucous plexus (Schärli 1992) but is difficult to diagnose.

Immaturity of ganglion cells has been reported to influence the function of the intestine (Bughaighis 1971; Emery 1973; Munakata 1978). Immaturity must be interpreted in the light of the gestational age, postnatal age and a knowledge of the variations of normal postnatal development. In addition, the recognition of immature cells is not always easy as other cells such as hypertrophied glial cells and fibroblasts may lead to misinterpretation (Ariel 1983). These immature ganglion cells have a smaller, darker nucleus without a recognizable nucleolus (Ariel 1983). Special staining methods may be necessary to clarify the ganglion cell morphology and identify immature cells (Schärli 1992; Meier-Rüge 1992).

The morphology of ganglion cells in the submucosal plexus may differ from that of the myenteric plexus (Ariel 1983). This is an important difference to document as morphological changes in NID may be confined to the submucosal plexus (Borchard 1991; Meier Rüge 1992). In addition, both plexuses are used in the evaluation of bowel in Hirschsprung's disease. Aganglionosis of the submucosal plexus has been established on rectal biopsy. Additional intraoperative investigation of the intermyenteric plexus by means of seromuscular biopsy specimens establishes the normal ganglionation of the pull-through bowel.

Other morphological features associated with neuronal intestinal dysplasia are heterotopia of ganglion cells in the lamina propria with close proximity to peripheral nerves as well as in the circular muscle layer (Borchard 1991; Schärli 1992).

This study was undertaken to try to clarify the spectrum of normal and abnormal findings. The ganglionated segment of bowel pulled-through in the surgical treatment of Hirschsprung's disease was studied and compared with the histological features observed in controls.

10.2 AIM OF STUDY

The aim of this study was to

- Evaluate ganglion cell morphology in the ganglionated bowel pulled through in the surgical management of Hirschsprung's disease.

- Identify abnormal patterns of ganglion cell distribution and morphology in the pulled-through segment.

- Establish the presence or absence of co-existing hyperganglionosis in the ganglionated bowel.

- Evaluate other features of NID co-existing with Hirschsprung's disease.

- Analyze current methods of assessment of co-existing hyperganglionosis in the ganglionated bowel of patients with Hirschsprung's disease.

- Identify and clarify the current histological criteria for co-existing neuronal dysplasia of the ganglion cell population of the proximal bowel pulled-through at surgery for Hirschsprung's disease.

10.3 MATERIALS AND METHODS

10.3.1 Patient study group

The morphological features of the ganglion cell morphology in the proximal colon were evaluated in patients undergoing surgical pull-through for Hirschsprung's disease. The bowel was identified as ganglionated on frozen section prior to pull-through in these cases. Control tissue preparations were obtained from 3 further groups for comparison.

10.3.2 Ethical permission

Ethical permission to perform this study was obtained from the University of Cape Town Ethical and Research Committee.

10.3.3 Recruitment of patient sample

Patients presenting for surgery for Hirschsprung's disease between January 1988 and January 1991 were randomly entered into the prospective study.

10.3.4 Recruitment of control patient group

Control specimens were obtained from age matched patients being submitted to surgical procedures during this period.

An additional normal control group consisted of comparable tissue sections from postmortem specimens where bowel pathology was absent. Enzymatic stains were considered as unreliable due to possible postmortem changes (Testylier 1991). On the other hand, the ganglion cell morphology ought not to be affected. Conclusions were therefore drawn from the ganglion cell morphology only in this group of patients.

A further 5 full thickness specimens were obtained from patients with **neuronal intestinal dysplasia** (NID) in the absence of Hirschsprung's disease. These patients had symptoms severe enough to warrant colostomy and a histological picture conforming to the classical description of NID was present (Schärli 1981).

10.3.5 Demographic data

Clinical information recorded included the sex, age, race, age at diagnosis, associated anomalies and family history of the patients. This data was taken into consideration in the evaluation of the histochemical findings.

10.3.6 Ascertainment of data

The ascertainment of clinical and demographic data was obtained by personal interview with the patient and parents as well as the hospital records. The same questionnaire format and recording system was employed as in section 1 of this thesis.

10.3.7 Specimen collection

Fresh specimens of histologically aganglionic and ganglionated bowel was prospectively obtained at surgery from children undergoing colonic pull-through surgery for Hirschsprung's disease. These were personally collected by the author from the operating theater and transported directly to the laboratory. Aganglionic and transitional bowel samples were also obtained from the same specimens.

Control specimens of fresh tissue were obtained from patients with non-colonic disease undergoing colonic surgery during the same period being of similar age and sex. Fresh resected tissue was obtained at the time of colostomy, colonic interposition or closure of colostomy.

10.3.8 Handling and storage of specimens

Fresh surgically resected tissue was transported to the histochemical laboratory for processing. Specimens were collected from the operating theater and personally delivered to the laboratory minutes following surgical resection. In the laboratory, the specimens were orientated by the author and suitable representative biopsy specimens taken and snap frozen in liquid nitrogen. Sections taken from bowel obtained from a closure of colostomy procedure were taken as far away from the colostomy site as possible. Immediate processing of specimens requiring fresh tissue for histochemical techniques was performed.

10.3.9 Biopsy size

The minimum adequate biopsy size was assessed as being approximately a 1 cm x 1 cm full thickness of bowel wall. A full transverse section of bowel was preferred if technically possible as it allowed a more accurate ganglion cell count. Efforts were made to obtain clean cross sections and avoid oblique sections as the orientation of the specimen was considered an important factor in interpretation.

10.3.10 Tissue preparation

Routine Haematoxylin and Eosin preparations followed an initial frozen section assessment. Complete transverse sections of bowel were sectioned to make possible a comparison between the total ganglion cell count in a transverse section and other means of testing hyperganglionosis. This eliminates the potential differences in the mesenteric and antimesenteric ganglion cell counts previously reported (Gabella 1971; Ogawa 1987). Transverse sections of bowel specimens were obtained from 5 patients with established NID to act as

additional controls.

Histological methods which were used included a frozen section, AChE staining, paraffin sections and other special immunocytochemical staining techniques where indicated. Frozen sections were cut at 15 μ thickness, paraffin embedded tissues at 3 μ thickness, and AChE staining was performed according to the standard modified Karnowsky and Roots technique on 20μ tissue sections (Meier Rüge 1972).

10.3.11 Histological evaluation

Histological observations were recorded in joint sessions by the author with staff of the pathology department, under the supervision of senior anatomical pathologists. On histological evaluation, the number, size, position and morphological appearance of the ganglion cells in the resected surgical margins of the bowel segments were studied. These segments were pulled through at the time of surgery for Hirschsprung's disease. Techniques used for assessing numbers of ganglion cells included counting the number of clusters of ganglion cells per high power field, the number of ganglion cells per 5 mm of slide and the number of ganglion cells per cluster in both the submucosal and intermyenteric plexuses. These results were then compared for both the submucosal and myenteric plexuses.

Each count represented the mean of a minimum of 10 individual readings and were repeated in both the Meissner's and Auerbach's plexuses on Haematoxylin and Eosin as well as histochemical stains for AChE (Meier Rüge 1972) on 20 μ sections.

Evaluation of the degree of maturity of a ganglion cell was based on the size and appearance of the ganglion cell, the presence of cytoplasmic vacuolation and the

presence of mature nucleoli within the cell body. Maturity must be related to gestational and chronological age. As none of these patients were born before term, the gestational age was not considered to be a major factor.

10.3.12 Ganglion cell volume and size

The size of submucous and myenteric plexus ganglion cells was initially assessed as being large, normal or small on a visual analog scale of 3 based on the observer's impression. This was then correlated to the patient's age and other variables such as the orientation of the specimen and nature of the section.

Further more exact measurement of ganglion size was by means of the mean cell surface area (μ). This was performed by measuring the area of the cut surface of ganglion cells by means of a computerized image analysis system (IBAS Contron system). For each patient the mean values of 20 consecutive readings were recorded commencing at the cut edge of the specimen. The statistical values calculated by means of dedicated computerized software (IBAS system).

10.3.13 Peripheral nerves

The presence or absence of thickened peripheral nerves in the intermyenteric zone was recorded. When combined with large foamy ganglion cells or hypoganglionosis this was taken to suggest that the specimen originated from the transition zone.

10.4 RESULTS

10.4.1 Patient evaluation

A total of 69 fresh full thickness colonic specimens were evaluated. These consisted of 32 patients with Hirschsprung's disease and 32 controls (which consisted of 22 patients undergoing surgery for unrelated conditions and 10 postmortem specimens in patients who died from unrelated disease). In

addition, a separate control group of 5 patients with NID in the absence of Hirschsprung's disease were studied.

10.4.2 Demographic features

10.4.2.1 *Age*

There was little difference in the median ages (10.5 months) of the 2 groups as well as in the interquartile ranges (**Table 10.1**). There were also no significant differences noted on statistical analysis. These observations suggest that for practical purposes the ages of patients within the 2 groups were comparable.

10.4.2.2 *Race and Sex*

The race and sex distribution of these two groups were also comparable (**Table 10.2**) and there was no bias towards male or female or race in any particular group.

10.4.3 Hirschsprung's disease study population

The pull-through specimens of bowel were taken from patients with typical changes of Hirschsprung's disease in the aganglionic segment. In 2, there was an extensive aganglionic segment extending into the small bowel.

TABLE 10.1

AGE OF PATIENTS IN PROSPECTIVE STUDY

	Median age (mths)	Range (mths)	I/Q Range (mths)
Hirschsprung's disease	10.5	0-96	16
Controls	10.5	0-251	15.5

p = NS

TABLE 10.2

RACE AND SEX DISTRIBUTION: HISTOLOGICAL STUDY

	Hirschsprung's disease		Controls	
	Male	Female	Male	Female
Caucasian	4	1	4	0
Mixed descent	21	1	20	3
African	5	0	5	0

10.4.4 Control study population

The **control specimens of bowel** were obtained from children without Hirschsprung's disease undergoing a variety of unrelated surgical procedures (**Table 10.3**). An additional 10 **postmortem specimens** were obtained from routine postmortems on children who had died from unrelated causes.

Five patients who were diagnosed as **NID** without Hirschsprung's disease were investigated to establish a norm for the assessment of ganglion cell morphology in **NID**.

The diagnosis of NID was made by experienced histopathologists on the basis of hyperganglionosis in Meissner's plexus plus a typical AChE staining pattern.

10.4.5 Morphological features of ganglion cells and the transition zone.

Ganglion cells appeared attenuated and spread out in the group with NID particularly where intestinal dilatation had occurred. There were 6 cases (18%) where the morphology of the ganglion cells was considered to deviate from the normal pattern. These morphological features are listed in **Table 10.4.** In 3 of these patients (9%) the histological features were those of transitional zone and included hypoganglionosis, big foamy ganglion cells, the presence of peripheral nerves and a positive AChE staining neurofibrils. In 2 of these patients, there was extensive aganglionosis extending into the small bowel.

TABLE 10.3

SOURCE OF CONTROL SPECIMENS

Group A (n = 20)		
	Anorectal malformation	12
	Colonic interposition	4
	Enterocolitis	2
	Other	2
Group B (n = 10)		
	Postmortem	10

TABLE 10.4

GANGLION CELL MORPHOLOGY

	NUMBER OF PATIENTS					
	Hirschsprung's		Controls		NID	
Hyperganglionosis	5	(19%)	4	(18%)	5	(100%)
Immature ganglion cells	2	(7%)	2	(9%)	0	
Ectopic position	3	(11%)	0		3	(60%)
Hypoganglionosis	2	(7%)	0		0	
Large Ganglia	6	(23%)	6	(27%)	4	(80%)
"Hypogenetic" ganglion cells	2	(7%)	0		0	

Small ganglion cells were noted in the other 3 specimens. In one, the presence of small dark round cells with deeply staining cytoplasm was in keeping with the description of a "hypogenetic" type of ganglion cell by Munakata (1985).

These 6 patients were excluded from future assessment of ganglion cell numbers.

10.4.6 Co-existing hyperganglionosis in Hirschsprung's disease - evaluation of ganglion cell counts

In 26 cases the histological sections were suitable for ganglion cell evaluation. A comparison of the median ganglion cell counts (median of 10 readings) in Meissner's and Auerbach's plexuses of these patients is shown in **Table 10.5.** There were few demonstrable differences in the numbers of ganglion cells per high power field observed in those with Hirschsprung's disease and controls as well as those counted per 5 mm of tissue on the microscopic slide .

A comparison of the Meissner's and Auerbach's plexuses showed considerable variation in the 95% confidence intervals of the 10 readings taken in individual patients. In 5 of these patients a mean of more than 5 ganglion cells per cluster in Meissner's plexus was recorded (**Figure 10.1**).

In Auerbach's plexus 12 patients had more than 5 cells per cluster (**Figure 10.2**). In only 3 patients were both the mean number of ganglion cells in Meissner's and Auerbach's plexus greater than 5. By increasing the cut off level from 5 to 7 cells per cluster, in keeping with other reports (Schärli 1992), individual readings exceeded 7 per cluster in the Meissner's plexus in 4 of the 5 patients. The mean ganglion cell value was only greater than 7 per cluster in one of these cases.

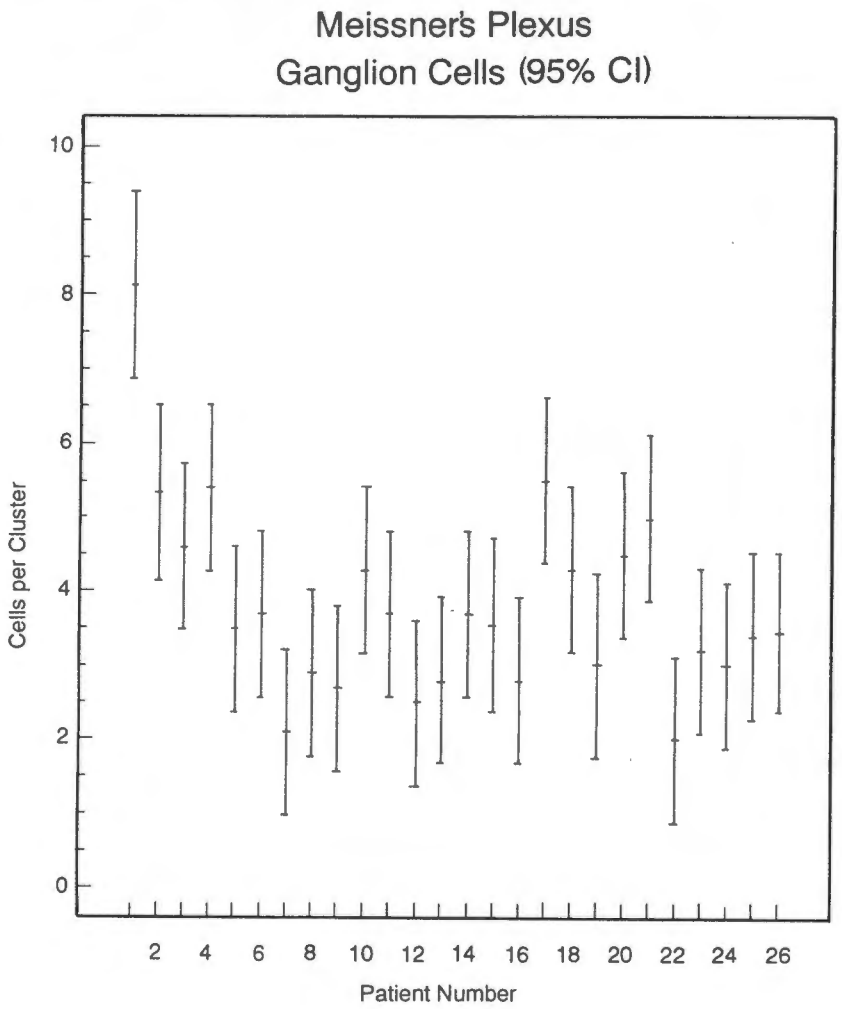


Figure 10.1: **Mean values and 95% confidence intervals of ganglion cell counts of Meissner's plexus (a minimum of 10 observations)**

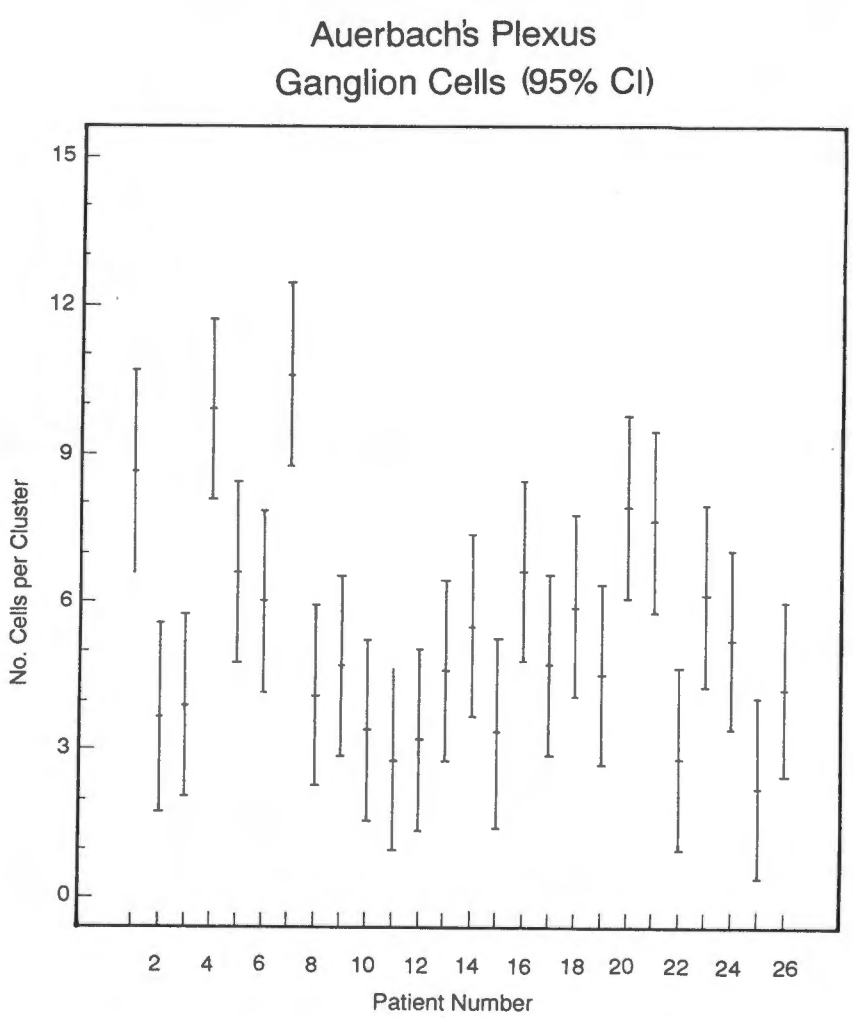


Figure 10.2: **Mean values and 95% confidence intervals of ganglion cell counts of Auerbach's plexus (a minimum of 10 observations)**

TABLE 10.5

HIRSCHSPRUNG'S DISEASE : MEDIAN GANGLION CELL COUNTS

	Total ganglion cell count > 200					
	Meissner's Plexus			Auerbach's Plexus		
	No/CI	No/HPF	No/LPF	No/CI	No/HPF	No/LPF
Hirschsprung's disease (n = 4)	3.5	2.5	14	6.5	3.5	6
NID (n = 3)	4	1	7	26	3	7

	Total ganglion cell count <200					
	Meissner's Plexus			Auerbach's Plexus		
	No/CI	No/HPF	No/LPF	No/CI	No/HPF	No/LPF
Hirschsprung's disease (n = 6)	3.5	4.5	18.5	5	4	13
Controls (n = 10)	3.8	1.5	8	11	3	13.5

By way of contrast, more than 7 ganglion cells per cluster were recorded in Auerbach's plexus in 5 patients. Three of these corresponded to individual readings greater than 7 per cluster in Meissner's plexus but the mean ganglion cell count exceeded 7 cells per cluster in the submucosal plexus in only 1 case.

On the other hand, there were more than double the number of ganglion cells per cluster in patients with **NID (Table 10.5)**. This increase was more than 5 times that of the mean or median values of the total Hirschsprung's disease sample. The number of ganglion cells per cluster exceeded 7 in Meissner's plexus in only 1 of the controls studied. This was a patient with an anorectal malformation.

10.4.7 Parameters of assessment of hyperganglionosis - evaluation by total assessment of the colonic ganglion cell population in a transverse section of bowel.

To evaluate the methods used to identify hyperganglionosis, the total ganglion cell counts obtained on complete transverse sections of the bowel were compared. A satisfactory complete transverse section was possible in 10 patients with Hirschsprung's disease and in 10 postmortem controls. These were then compared with transverse sections from 3 patients with NID. The results are recorded in **Table 10.6**.

TABLE 10.6

MEDIAN VALUES GANGLION CELLS

	Meissner's Plexus			Auerbach's Plexus			Total ganglion	Total No ganglia
	No/Clust	No/HPF	No/LPF	No/Clust	No/HPF	No/LPF		
Hirschsprung's disease n - 10	3.5	3.5	12	5	4	15.5	146	40
Controls n = 10	3.8	1.5	8	11	3	13.5	135	37
NID N = 3	4	1	7	36	3	7	393	90

Total ganglion cell counts clearly demonstrated the hyperganglionosis occurring in the bowel wall in those patients with NID (**Table 10.5**). The number of ganglia counted on transverse section were higher in NID (90 per circumference) than in the Hirschsprung's group (median value 45) or the controls (median value 37). These differences were also shown to be of statistical significance on testing (Kruskal Wallace, $p<0.001$).

The median values of ganglion cells counted per circumference of bowel in the Hirschsprung's disease sample (159 cells) were higher than those recorded in the control group (137 cells). This difference was not significant on testing.

Although the median values of the total ganglion cell counts were lower in patients from the Hirschsprung's group, more than 200 ganglion cells per circumference were noted in four cases. A comparison between the ganglion cell counts in these four patients shows a marked similarity to the ganglion cell counts of patients with NID (**Table 10.6**). In addition, a comparison of the ganglion cell counts obtained in the remainder approximated those of controls. This confirms the co-existing hyperganglionosis in these patients and suggests a similar histological picture to that of NID.

10.4.8 Influence of age

There did not appear to be significant differences in the number of cells per high power field, the number per 5 mm slide or the number per cluster in the various age groupings studied in control patients (**Table 10.7**). The decreased values noted in the Auerbach's plexus in those older than 24 months, probably represents an increase in bowel lumen with age.

TABLE 10.7

THE EFFECT OF AGE ON GANGLION CELLS IN CONTROL PATIENTS

	Meissner's			Auerbach's		
	no/cl	no/HPF	no/LPF	no/c	no/HPF	no/LPF
Neonatal	3.3	1	6	8	4	14
1-6months	3.6	2	8	13	4	13
7-12 months	4.3	2	10	8	3	11
12-24 months	4.4	2	8.5	9.5	3	12.5
>24 months	5.3	1	7	7	3	9*

* Kruskal Wallace $p < 0.05$

10.4.9 Ganglion cell size

There were 7 patients with small ganglia (ie. score of 1) in the Hirschsprung's disease group when measured by a visual analog scale. There were no controls with a similar score. Large ganglia (score of 3), on the other hand, were observed in 6 patients from both groups. The remainder had normal sized ganglia.

Computerized measurement showed smaller mean values for ganglion cells in the Hirschsprung's group than in the controls (**Table 10.8**). There was little variation in ganglion size in the ganglionated bowel of Hirschsprung's disease or in the controls in patients over the age of 6 months. In the neonate with Hirschsprung's disease, a significantly smaller mean ganglion size (Kruskal Wallace, $p<0.05$) was observed (**Figure 10.3**). Although a similar trend towards smaller ganglion cells was noted in patients between one and 6 months of age, this did not quite reach significance on testing by ranks (Kruskal Wallace, $p=0.06$).

The lower mean value of ganglion size was probably due to an overall smaller size of ganglia in the proximal bowel of patients with Hirschsprung's disease.

By way of contrast, the mean cut surface area of the ganglia was noted to be large on computerized measurement in only 2 patients from the Hirschsprung's disease group. Large ganglia were not, however, confined to the Hirschsprung's disease group and larger ganglion cell cut surface area were also noted in 4 controls. These included 2 patients with an anorectal malformation. In one patient the increase in ganglion volume appeared to be due to glial cell hyperplasia. Poor functional outcome was not consistently present in those with large ganglia in either the Hirschsprung's group or controls.

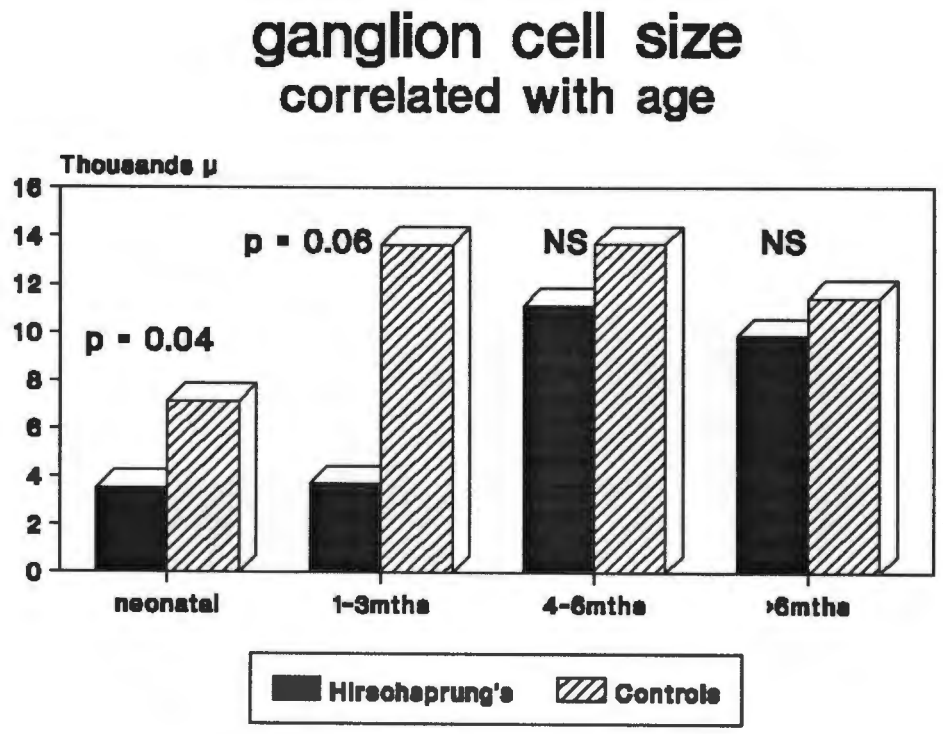


Figure 10.3 Comparison of mean ganglion cell size related to age

TABLE 10.8

COMPARISON OF GANGLIA SIZE

(computerized measurement)

	Median (μ)	Range (μ)	I/Q Range (μ)
Hirschsprung's Disease	5408	682-50546	2454-7807
Controls	10439	3050-28916	7031-13612

The presence of large ganglia was noted to depend on a number of factors including the obliquity of the section and proximity to a colostomy. For these reasons, it was considered an unreliable parameter to indicate neuronal dysplasia.

Small ganglia were noted on light microscopy as well as on computerized measurement in 7 cases in the Hirschsprung's group. These were significantly smaller than those of the remaining patients (Kruskal Wallace, $p<0.05$). The ganglion cells in 2 of these patients were small monopolar cells suggesting a hypogenetic cell type. In a further 2 patients, small immature cells were related to extensive total colonic aganglionosis with small bowel extension. The remaining 3 young patients appeared to have smaller collections of ganglion cells. These may have represented immature ganglion cells but lacked the other features of immature cells, however. Poor functional outcome was noted in the 2 patients with the small monopolar cells.

10.4.10 Ganglion cell maturity

Immature ganglion cells were noted in pull-through bowel of a further 2 patients with Hirschsprung's disease. In one, they were observed to be in association with abnormally thickened nerve fibres in a patient with an extensive long segment aganglionosis who also had scanty ganglion cells in the pull-through segment of bowel. This indicated a possible area of transition from the aganglionic to normally ganglionated bowel. Immature cells were not a feature of the control sections on Haematoxylin and Eosin (H and E) stained sections.

10.4.11 Ectopic ganglion cell sites

Heterotopic ganglion cells within the lamina propria was observed in only 1 specimen in the Hirschsprung's group and were associated with immature cells and functional postoperative disturbance. No ectopic ganglion cells were noted in the control group.

10.4.12 Ganglion cells in peripheral nerves

Ganglion cells were observed as occurring in proximity to as well as being included within the peripheral nerves in a further 3 patients (**Figure 10.4**) as opposed to their absence in normal bowel. Ganglion cells in association with peripheral nerves was associated with large ganglia in 2 of these instances. But in only 1 of these was a moderate increase in the number of ganglia noted.

10.4.13 Correlation with postoperative functional disturbance

Abnormalities in ganglion cell morphology was detected in 7 of the 32 surgical resection margins in the patients with Hirschsprung's disease. Abnormal postoperative functional outcome was detected in all patients with abnormal ganglion cell morphology although some improvement was noted in 2. Hyperganglionosis was noted in 2 patients. Ganglion cell counts of greater than

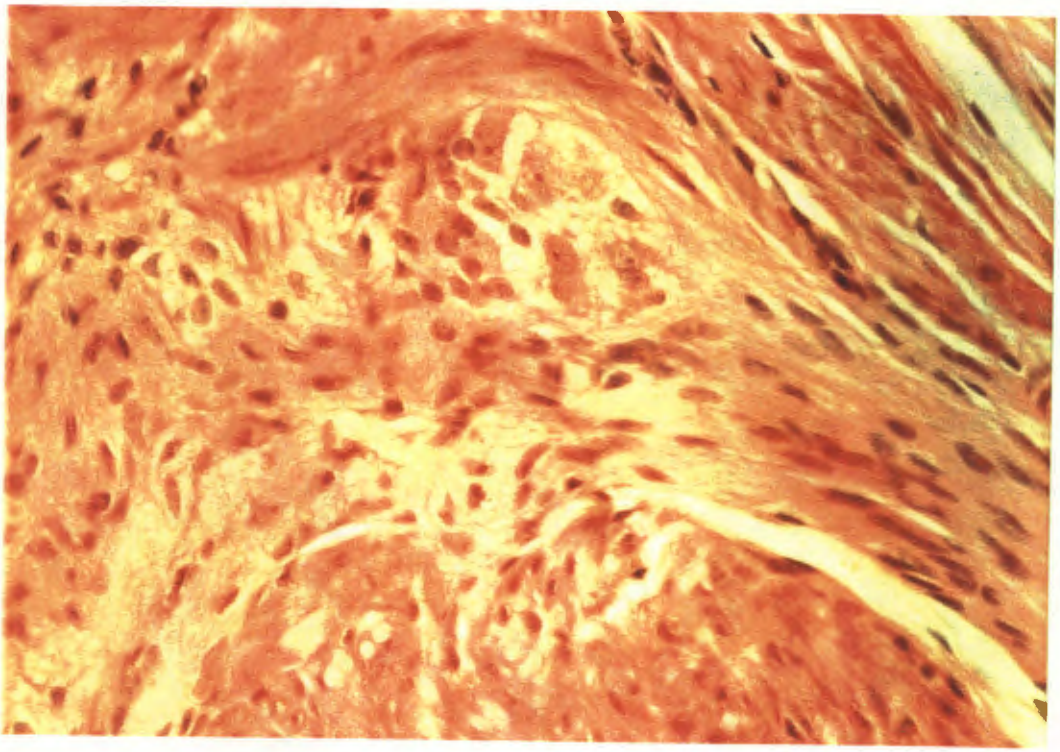


Figure 10.4 (a) H and E section of colonic wall showing ganglion cells in association with a peripheral nerve in the ganglionated proximal bowel of a patient with Hirschsprung's disease

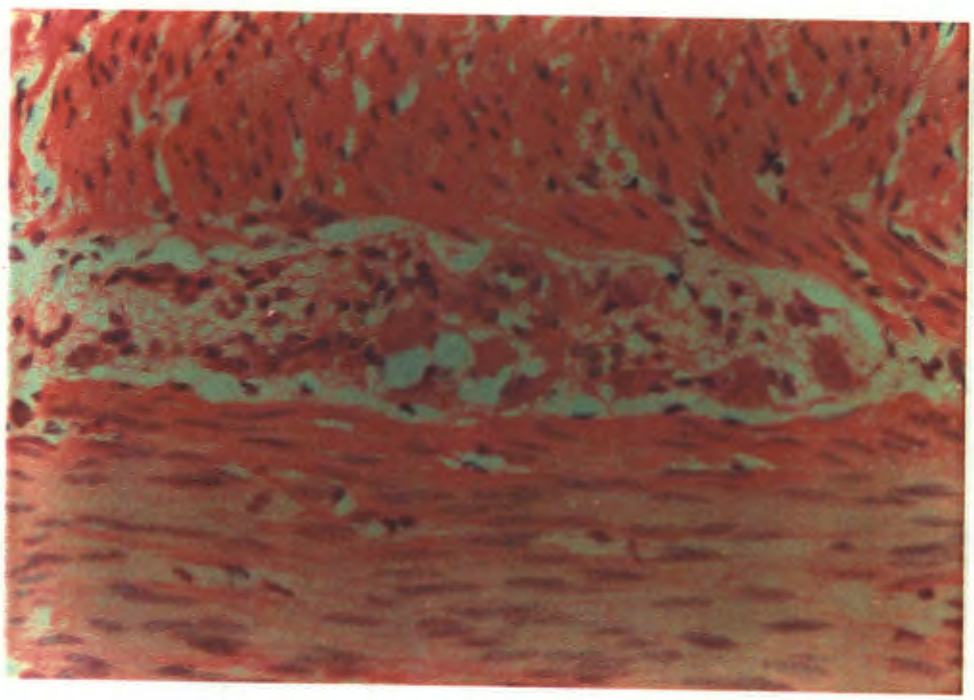


Figure 10.4 (b) H and E section of colonic wall showing normal ganglion cells in the ganglionated proximal bowel of a patient with Hirschsprung's disease.

7 cells per cluster in Meissner's plexus were noted in 4. The clinical significance of these findings will be discussed further in **Chapter 12.**

10.5 DISCUSSION

10.5.1 Normal and abnormal structure and function of the enteric nervous system

Residual abnormalities within the ENS in the ganglionated proximal bowel in Hirschsprung's disease suggests a possible cause for the postoperative obstructive symptoms observed in the clinical section of this study **(Section B)**. In this prospective evaluation of patients undergoing Hirschsprung's surgery, histological features covered a wide range of different features in the proximal ganglionated bowel.

A number of morphological irregularities were noted in 6 of the patients. In 3 of these the pull-through level was in the transitional zone. Much of the histological debate as to whether NID coexists with Hirschsprung's disease centres on the differences between NID and the transitional zone. The essence of this discussion rests on a proper definition of the transitional zone in Hirschsprung's disease.

The transition from the proximal ganglionated bowel to the aganglionic distal bowel in piebald lethal mice includes a gradual decrease in the number of ganglion cells and fewer postganglionic intermuscular cholinergic nerves (Webster 1974). Thus, just prior to the ganglionated segment, an area of scanty ganglion cells may be present (Garrett 1969; Howard 1968; Munakata 1978). There is considerable variation in the length of the transitional zone in individual patients. There is also some degree of overlap where an occasional peripheral nerve may be present and the AChE staining neurofibrils become fewer until eventually normally innervated bowel is reached. Proliferation of acetylcholinesterase neurofibrils is generally regarded as being uncommon in the

transitional bowel (Munakata 1978). In addition, some degree of ganglion cell immaturity may also be present.

Much of the debate surrounding the diagnosis of NID revolves around the methods used to quantify ganglion cell dysplasia and secondly, the identification of increased AChE positive neurofibril activity on histochemical staining. As the latter is the subject of the following chapter, this discussion will confine itself to the evaluation of ganglion cell distribution and morphology.

The first task was therefore to evaluate the current methods of comparing ganglion cells in 4 groups. These groups consisted firstly of the proximal ganglionated segment of patients with Hirschsprung's disease and secondly of a group of controls with miscellaneous bowel conditions not related to Hirschsprung's disease or to NID. As only 3 of these control patients came from patients with normal bowel (specimens taken at colonic interposition), a further 10 postmortem sections where bowel pathology was not expected were randomly selected to act as normal controls. Because this debate revolves around the co-existance of NID with Hirschsprung's disease, an additional reference group of 5 patients diagnosed as having NID by experienced pathologists without features of Hirschsprung's disease, were also studied.

10.5.2 Difficulties in quantifying hyperganglionosis

The term hyperganglionosis refers to an increase in the number of ganglion cells and has been reported as occurring in association with severe symptoms of bowel dysfunction (Howard 1984). It has not been clear in the past whether this increase refers only to the number of ganglia, number of ganglion cells per cluster, the total ganglion count or to the number of ganglia per given area of bowel wall. More recently, a consensus of opinion by European pathologists indicates hyperplasia of the ganglia with many little ganglion cells (Borchard

1991). In addition to an increase in the number of ganglion cells, an associated increase in the size of the ganglia (clusters of ganglion cells) also appears to be of importance in establishing the presence of NID (Fadda 1987).

The upper limit of normal has been accepted as between 5 and 10 ganglion cells per cluster (Aldridge 1969; Schofield 1990; Schärli 1992) but the exact cut off point is not yet clear, although 7 per cluster in Meissner's plexus has recently been advocated as the most reliable indicator of hyperganglionosis (Borchard 1991). The contrast between normal ganglion cells and hyperganglionosis is demonstrated in **Figure 10.5** (a and b)

In order to attempt to answer questions with regard to the diagnosis of hyperganglionosis, we counted the number of ganglion cells per cluster, the number per high power field (20 times magnification), the number of ganglion cell clusters per 5 mm bowel wall, as well as the total count where a transverse specimen of good quality could be obtained from the same area (**Table 10.6**). These parameters were compared with the "impression test" on medium magnification as well as the total number of counted ganglion cells and ganglion cell clusters in selected specimens to assess the more reliable indicator of hyperganglionosis. This study demonstrated that, although individual counts of more than 7 cells per cluster were recorded, a considerable amount of variation occurred. Mean counts were only greater than 7 cells per cluster in 1 patient, although the total ganglion cell counts showed hyperganglionosis in a further 2 cases.

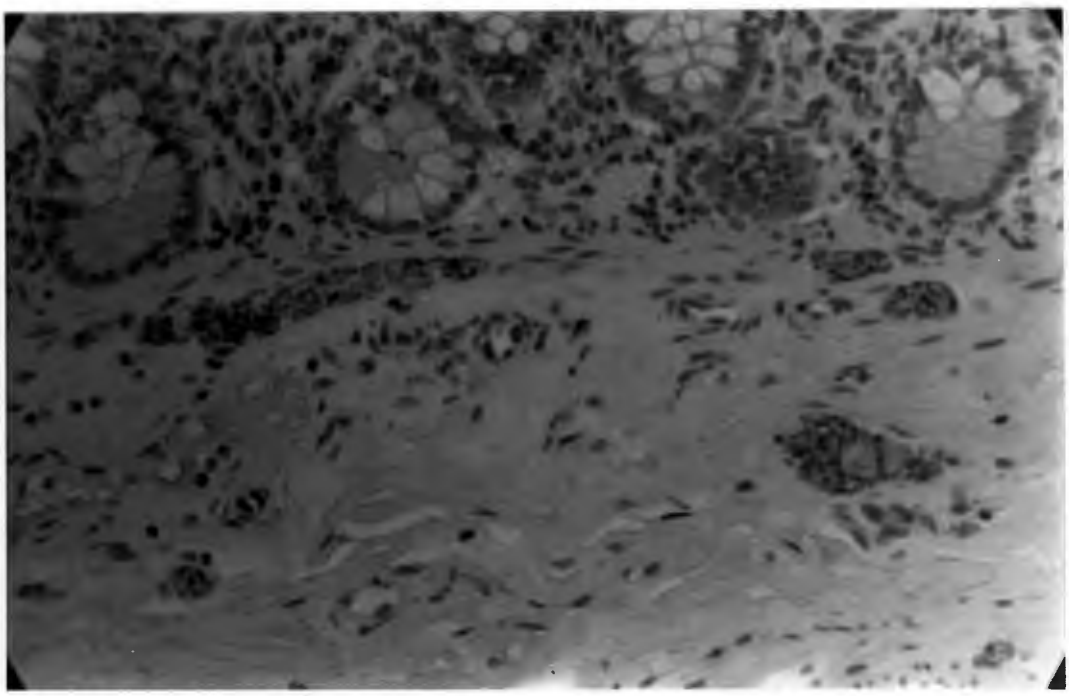


Figure 10.5a. Paraffin section of colonic mucosa stained for neurone-specific enolase (NSE) by immunoperoxidase method: Neuronal intestinal dysplasia: large ganglia with numerous neurones staining for NSE (x160)

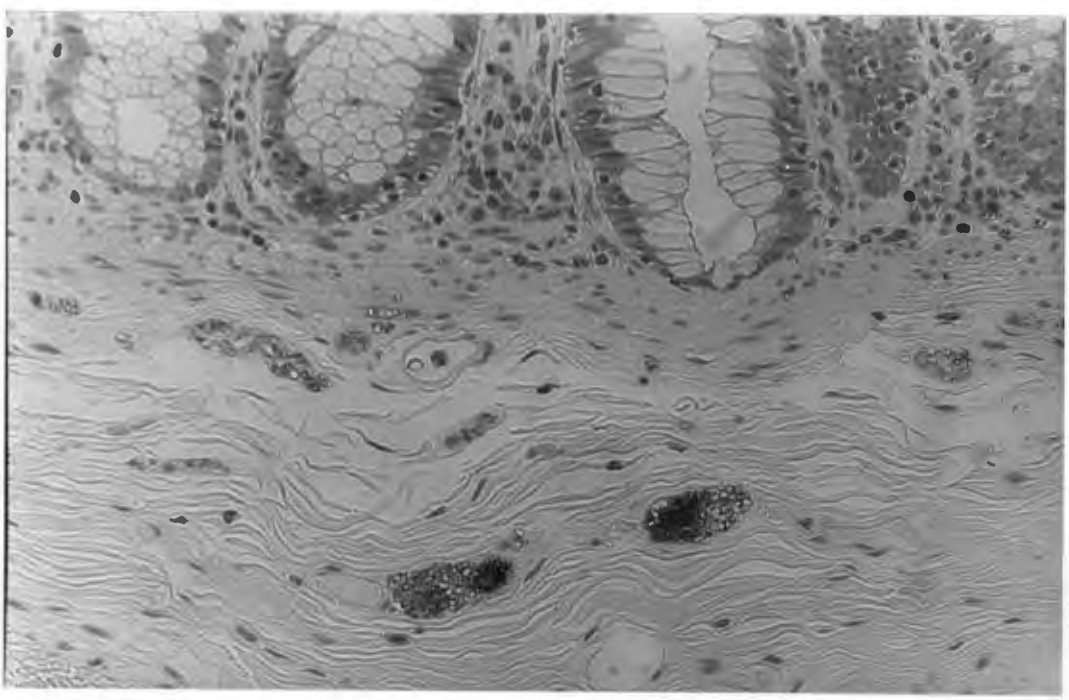


Figure 10.5b. Paraffin section of colonic mucosa stained for neurone-specific enolase (NSE) by immunoperoxidase method: Normal control shows smaller ganglia with fewer neurones staining for NSE (x160).

This finding of features similar to those of NID in the ganglionated bowel of patients with Hirschsprung's disease confirms the co-existance of NID. Discrepancies were observed between the submucosal and intermyenteric plexuses and increased numbers of ganglion cells per cluster were observed in Auerbach's plexus of the control group. In addition, the number of cells counted per high power field as well as per 5 mm also appeared to be affected by the degree of distension of the bowel lumen. This complicated the diagnosis of hyperganglionosis and led to the conclusion that caution should be shown in diagnosing hyperganglionosis on 1 parameter only. It is likely that the number of cells per cluster in Meissner's plexus is the more reliable parameter than Auerbach's plexus.

On the other hand, the variations in the ganglion cell counts in the same section, could possibly be explained on the basis of immature cells which did not stain on the H and E preparations. Further studies are being carried out using immunocytochemical stains, to clarify this point. A comparison was made between the hyperganglionosis observed on the total count of the number of ganglion cells in transverse sections of a circle of bowel wall and the histological "impression". Results suggested that the latter is a fairly reliable indicator of hyperganglionosis when allowances for the dilatation of the specimen and section are made.

The median value of ganglion cells per cluster in the submucous plexus was 3.6 cells per cluster in the control group, 1 patient with an anorectal malformation had more than 7 cells per cluster in the submucous plexus. This is in keeping with other reported findings in anorectal malformations (Schärli 1992), but has also been reported in normal controls (Schofield 1990).

These findings suggest that although the number of ganglion cells (ie. >7 per cluster) is probably the most reliable indicator of hyperganglionosis it may be subject to certain variables and on its own does not diagnose hyperganglionosis. The total count of ganglion cells per circumference of bowel is the best indicator of all, although labour intensive. Reasonable correlation with total ganglion cell counts was achieved by the "impression test".

10.5.3 Coexistence of hyperganglionosis in the ganglionated bowel in Hirschsprung's disease

The hyperganglionosis occurring in NID is clearly shown in the total ganglion cell counts of transverse sections of a complete circle of bowel (**Table 10.6**). None of the Meissner's plexuses were normal in the NID group and the increase in ganglion cells in these patients was increased in both Meissner's and Auerbach's plexuses in 2 of the 3 patients studied. The mean ganglion cell count per cluster in the submucosal plexus in this group may have been artificially depressed by one surprisingly low value. This occurred in a patient in whom large ganglia and hyperganglionosis were noted on examination of a total transverse section of bowel. In addition, individual ganglia showed more than 7 cells per cluster in Meissner's plexus in sections taken from this patient. In this instance, the increase in the size of the ganglia resulted from glial cell hyperplasia.

These results suggest that the hyperganglionosis seen in NID affects both the Auerbach's and Meissner's plexuses as well as the total count of ganglion cells and the number of ganglion cell clusters. The small numbers of patients within this series must be borne in mind in interpreting this data. Glial cell hyperplasia has been previously described as a feature of patients with chronic intestinal pseudo-obstruction in other studies (Navarro 1990).

Comparison of the ganglion cell counts in the transverse sections in 10 patients with Hirschsprung's disease, 10 controls and 3 of the 5 patients with NID demonstrated a group of 3 out of the 10 Hirschsprung's patients with ganglion cell counts approximating the NID group. This increase was both in the total number of ganglion cells counted as well as the number of cell clusters. These observations were consistent in both the Meissner's and Auerbach's plexus. Patients with less than 200 ganglion cells on transverse section had ganglion cell counts similar to those of the control group. This suggests a similar histological pattern between those patients with co-existing hyperganglionosis and those with NID, whereas other patients had similar ganglion cells to the controls.

10.5.4 Morphological features of ganglion cells

10.5.4.1 *Ganglion size*

There was a statistically significant difference in the sizes of ganglia in neonates with and without Hirschsprung's disease (Kruskal Wallace, $p<0.05$). Large ganglia, on the other hand, are a distinctive feature noted in the majority of patients with NID (Puri 1977; Borchard 1991; Schärli 1992). Even after allowing for the possible influences of gestational and chronological age, there was a considerable variation in the size of the cut surface of the ganglia in the patients tested (**Figure 10.3**). This was also noted in the group with NID where the cells were attenuated and spread out possibly due to the dilatation of the bowel. This suggests that the ganglion cells are non-compressable but elongate and became attenuated as intestinal dilatation occurred.

A number of technical problems were encountered in the measurement of the ganglion cell size. Previous suggestions that the diameter of ganglion cells is a reliable measurement of size (Munakata 1978), proved technically difficult due to the attenuation and elongation of ganglion cells in a number of specimens and the obliqueness of section in a few instances. Total cell volume, a three

dimensional measurement would therefore be preferred as a measurement of ganglion cell size but is technically difficult to perform. Although technical aspects such as the nature of the section and individual variation must be borne in mind, the mean cellular area of ganglion cell sections was not found to be significantly different in the ganglionated bowel of patients with Hirschsprung's disease when compared to controls and patients with NID. Because of these variations, we concluded that the area of the cut surface of the ganglion cells was the most reliable parameter associated with NID. Measurement of this cut surface area was performed more easily in the Auerbach's plexus although the increased volume was also present in Meissner's plexus. Further studies are required to clarify the further significance of ganglion size as a reliable indicator of NID. Dysfunction was later noted in those with small hypogenetic cells as well as those with large ganglion volumes and hyperganglionosis.

10.5.4.2 *Abnormal ganglion cell distribution*

The presence of heterotopic ganglion cells in the lamina propria has been cited as a dysplastic feature but ganglion cells in this position may merely constitute a variant of normal (Lake 1989).

The distribution of ganglion cells within the peripheral nerves was present in some patients with NID but also was noted in some patients without features of NID in keeping with other reported series (Schofield 1990). The presence of ganglion cells in other abnormal sites appeared to be associated with abnormal innervation, although the observation of single cells carries little significance (Borchard 1991). In the one specimen where this finding was striking, many other abnormal features were present.

10.5.4.3 *Ganglion cell maturity*

Ganglion cell maturity was a possible other factor noted in both control and Hirschsprung's patients which may influence postoperative function. Mature cells have been described as being large and multipolar with a sharp nuclear membrane as well as the presence of a nucleolus (Smith 1969). Small ganglion cells were considered as being immature and in certain patients were difficult to identify as they did not stain with the AChE stain. They were, on occasion, difficult to distinguish from fibroblasts and hypertrophied glial supporting cells.

Bughaighis (1971) has suggested anoxia as a contributing factor to immaturity of ganglion cells. However, no history of anoxia at birth was elicited in these patients. Smith (1969) has shown that the duration of the pregnancy and not the birth weight is the deciding factor in ganglion cell maturity at birth. Evidence from animal experiments supports this theory and guinea-pig neurones have been shown to arrange themselves into discrete ganglia and have a maturing appearance towards the end of gestation (Puri 1980). Immature ganglion cells have been shown to undergo a gradual and steady progress in maturation from early embryonic life through to early childhood (Smith 1969). Symptoms caused by poorly functioning immature ganglion cells may therefore improve with time. This may explain the improvement in symptomatology in some patients.

10.5.5 **The influence of the transitional zone on postoperative outcome**

The pull-through of transitional zone has been implicated in postoperative constipation (Becker 1985). There is considerable variation in length and the transitional zone has been reported to extend 7-12 cm proximal to the macroscopic site of transition (Ogawa 1987). Ganglion cells have also been reported to be present 4 cm lower on the antimesenteric than on the mesenteric side of the bowel in the transitional zone (Ogawa 1987). The identification of the features of a transitional zone at the time of surgery and the avoidance of

this area has prevented the high incidence of hypoganglionic bowel reported in certain other series (Howard 1984). The variation in the length of the transitional zone and the ability to distinguish NID from an occasionally long transitional zone is important. Possible non-resection of a segment of the transitional zone of Hirschsprung's disease may lead to postoperative obstructive symptoms.

In 2 of the 3 patients with features of the transitional zone in the pull-through margin of ganglionated bowel, extensive aganglionosis had been diagnosed with extension high into the small bowel. Pull-through of transitional zone was considered unavoidable in these patients due to the long proximal extent of the transitional zone and the amount of bowel already lost at surgery. In the one remaining patient, features of transitional zone were detected on full assessment of the bowel wall following surgery.

10.5.6 Other factors influencing ganglion cell counts

Other factors which may influence the number of ganglion cell clusters per unit area include age, position of the specimen taken, the obliquity of the section, bowel size and distension. In addition, the type of staining method used may be of importance.

Subjective variations in the density of ganglion cells were observed with the position on the bowel wall (ie mesenteric or antimesenteric), the degree of dilatation of the bowel and the orientation of the specimen. These influenced the number of cells per high power field and the number per 5 mm slide.

Age did not appear to contribute significantly to the number of cells per high power field, the number per 5 mm slide or the number per cluster for the age groupings studied. A higher concentration of the ganglion cells can be expected

in newborns than in the older child. This probably represents the higher concentration of ganglion cells per unit area present prior to the spreading out of the ganglia in the growing bowel (Aldridge 1968). Our study demonstrated a decrease in the number of ganglion cells per 5 mm in the child over the age of 24 months.

In some patients it was clear that technical factors such as obliquity of the section or difficulty in the visualization of immature ganglion cells may influence the result. Technical difficulties in the visualization of ganglion cells may be directly related to the nature of the section and the quality of the stain (Lake 1989) but special staining techniques should be employed to overcome this difficulty.

Immunocytochemical stains included Tyrosine Hydroxylase, VIP, NSE and S100. The interpretation of the findings of these special staining methods are still considered experimental (Borchard 1991) and were considered to be beyond the scope of this study. An ongoing study which has arisen out of this work on ganglion cell morphology is still continuing at present. Immunocytochemical stains may show further light on the parameters used to define hyperganglionosis in the future.

10.5.7 Correlation of histological findings with postoperative functional disturbance

Of patients with abnormal ganglion cell morphology in the proximal ganglionated segment in Hirschsprung's disease, six had hyperganglionosis. The hyperganglionosis was confirmed on total ganglion cell counts in 4. In the remaining two, complete circles of bowel could not be obtained for evaluation due to technical reasons. There were 2 hypoganglionic proximal bowel segments with features suggestive of transitional zone, and the ganglion cells appeared immature in a further 2.

Small hypogenetic cells without features of immaturity were also noted in 2 patients. Heterotopic ganglion cells in the lamina propria and muscularis mucosa were only noted in 1 patient of the Hirschsprung's group and ganglion cells were noted within peripheral nerves in 3. Hyperganglionosis was also present in 2 out of these cases.

There was no constant relationship between ganglion cell morphology and obstructive postoperative symptoms. Postoperative dysfunction improved with time in the 2 patients with immature ganglion cell morphology. In those patients with a hypogenetic ganglion cell morphology, postoperative obstructive symptoms persisted. The presence of ganglion cells in the segment of bowel pulled through at surgery for Hirschsprung's disease did not guarantee normal postoperative function.

10.6 CONCLUSIONS

The conclusions drawn from this study were as follows:

- A wide spectrum of dysplastic features occurs in the pulled-through bowel in Hirschsprung's disease and this includes hyperganglionosis, hypoganglionosis, ganglion cell immaturity and other morphological ganglion cell abnormalities.
- Hypoganglionosis may be associated with a long transitional zone in patients with TCA and extensive small bowel involvement. It may result in functional impairment.
- Hyperganglionosis does coexist in the proximal ganglionated segment in patients with Hirschsprung's disease and does not appear to be a feature of the transitional zone, but is associated with NID.

- Hyperganglionosis is best reflected in the number of ganglion cells per cluster and may affect both the Meissner's and Auerbach's plexuses.
- Meissner's plexuses were abnormal in NID and glial cell hyperplasia may account for an increase in the size of the ganglia in the enteric nervous plexuses in certain cases.
- Total ganglion cell and ganglia counts of transverse sections of bowel may be used to identify hyperganglionosis.
- The number of ganglion cells in the Meissner's plexus usually exceeds 7 per cluster in patients with NID. This feature did not appear to be specific to NID and was noted in 1 control plus patients with Hirschsprung's disease.
- Correlation with postoperative obstructive symptoms was not always constant in the presence of hyperganglionosis in the absence of other features of dysplasia.
- Tiny "hypogenetic" forms of ganglion cell morphology may result in postoperative dysfunction and an obstructive picture.
- Other features such as immaturity and ectopic ganglion cells may indicate NID but were not specific diagnostic features of this condition. They may, however, influence the postoperative outcome if they occur in association with other dysplastic features. They should therefore be regarded as a possible cause of postoperative obstructive symptoms.

10.7 LIMITATIONS AND VALIDITY OF THIS STUDY

10.7.1 Normal values - the validity of conclusions drawn from control specimens

The range of findings within the control group of patients covered a wide spectrum of ganglion cell findings. Criticism can be levelled at the control group due to the inclusion of patients with anorectal malformations and other patients with potential abnormalities of the enteric nervous system, because NID may have co-existed in some of them. This is particularly true of bowel taken from

patients with associated anorectal malformations, as reports of histological abnormalities suggestive of NID have been reported in them (Rintala 1989; Schärli 1992).

On the other hand, differences in the ganglion cell morphology of patients with anorectal malformations, those obtained from age matched controls at postmortem, or other control sections were not marked. These differences did not achieve significance on testing. There were also no significant differences in age, the number of ganglion cells per high power field , the number of ganglia per 5 millimeters of the slide and the number of ganglion cells per cluster in the myenteric plexus between controls taken at postmortem in patients without gastrointestinal disease and those obtained at surgery. Although isolated dysplastic findings were noted in certain control specimens (eg. hyperganglionosis in 1), the overall histological findings were not consistent with features of NID.

10.7.2 Ganglion cell number and morphology

Ganglion cell counts were expressed as the median of a minimum of ten observations using light microscopy on H and E stained preparations. Examination of H and E stained preparations is advocated in the literature for the identification of ganglion cells (Borchard 1991). Small immature ganglion cells may be difficult to differentiate from small glial cells on H and E sections (Borchard 1991). Certain immunocytochemical stains (eg. NSE, S-100, PGp9.5) assist by identifying the immature cells on the section. This was confirmed in the NSE and VIP stains used in this series. A comparison in readings between immunocytochemistry and light microscopy was considered to be beyond the scope of this study and is the subject of an ongoing project. As such, this omission is a limitation to the findings of this study and may explain certain of the lower numbers of ganglion cell counts in the submucous plexus. Reasons for

this included initial technical difficulties with certain staining techniques (eg.NSE). In addition, consensus as to which stains were of the greatest value (Borchard 1991; Schärli 1992; Meier-Rüge 1992) were only published after the main body of this work had been completed.

The main aim of this study was to establish the presence of hyperganglionosis by means of objective criteria in patients with Hirschsprung's disease. Hyperganglionosis demonstrated on H and E sections is unlikely to be falsely positive as the tendency of light microscopy is towards lower readings. This was clearly demonstrated on the counts of the entire number of ganglion cells in a transverse section of bowel.

10.7.3 Ganglion cell count and position

There was a greater number of ganglia on the mesenteric aspect of the bowel ring specimens than on the antimesenteric aspect on transverse sections (ie denser on the mesenteric side). This was allowed for where possible in the experimental technique. This is an alternative explanation for some of the discrepancies observed between the mean ganglion cell counts and higher values obtained in patients with hyperganglionosis on transverse sections of bowel.

10.8 COMMENT

In view of the extremely wide spectrum of dysplastic features in residual bowel in Hirschsprung's disease, the prediction of function remains problematic. Further evaluation of dysplastic features and the role of an increase in AChE staining is necessary and will be addressed in the following section.

CHAPTER 11

HISTOLOGICAL AND HISTOCHEMICAL CORRELATES OF ACETYLCHOLINESTERASE ACTIVITY IN HIRSCHSPRUNG'S DISEASE

11.1 INTRODUCTION

The diagnosis of Hirschsprung's disease is based on the clinical presentation, anorectal manometric findings, radiological and histological features. The definitive diagnosis of intestinal aganglionosis is based on the histochemical demonstration of the presence or absence of ganglion cells in the myenteric plexus of the affected segment of bowel.

An increase in AChE staining neurofibrils in the lamina propria and muscularis mucosa is noted on histochemical staining in aganglionic bowel segments.

This increased AChE activity is demonstrated by means of the AChE staining technique (Karnowsky 1964) adapted for intestinal use by Meier-Rüge (Meier-Rüge 1972) (**Appendix C**). AChE staining is considered to be the most valuable investigation to demonstrate the abnormal nervous tissue within the bowel wall (Meier-Rüge 1972, 1992).

The principle of the staining method for AChE is based on the Ellman Reaction (Ellman 1961). Thiocholine is produced as acetylthiocholine is hydrolyzed by the enzyme in the tissue and reaction with a copper ion demonstrates AChE positive neurofibrils by a brown stain. Iso-Ompa is used as the selective butyrylcholinesterase (BChE) inhibitor in the histochemical staining method.

Proliferation of hypertrophic autonomic nerve fibres with increased acetylcholinesterase (AChE) activity in the lamina propria and muscularis mucosa

is reported in the aganglionic bowel. The density of these fibres varies in extent between patients but the fibres are reported to be coarse and refractile in Hirschsprung's disease (Meier-Rüge 1972; Wakely 1984).

The accuracy of the AChE stain in diagnosing Hirschsprung's disease has been reported to be as high as 99-100% (Elema 1973; Kreiner 1976; Lake 1978; Ikawa 1986; Kurer 1986; Hinkel 1989). The suggestion that abnormal AChE histochemistry alone is diagnostic of Hirschsprung's disease (Elema 1973; Meier Rüge 1974) has been shown to be unreliable. There are reports of false positive (vd Staak 1981; Hamoudi 1982; Huntley 1982; Barr 1985; Borchard 1991) and false negative (vd Staak 1981; Ariel 1983; Barr 1985) AChE staining patterns.

AChE staining patterns in the aganglionic segment of bowel are well described, but very few studies examine the proximal ganglionated segment at the time of surgical correction. Possible variations in AChE staining patterns occurring with age (de Brito 1987) include AChE neurofibrils within the lamina propria and muscularis mucosa which decrease with increasing age and may only remain detectable within the circular muscle of the bowel wall. Chow (1977) described this as a Type B AChE staining pattern of Hirschsprung's disease. Meier-Rüge (1992) has reported similar AChE staining patterns in NID.

11.2 AIM

The aim of this study was therefore to

- Evaluate the extent of AChE positive staining patterns in the proximal pull-through segment of bowel at the time of Hirschsprung's surgery.
- Evaluate the relationship between AChE staining in Hirschsprung's disease and Neuronal Intestinal Dysplasia (NID) and that of controls.

- Specifically compare AChE activity in patients identified as having co-existing NID with that of the ganglionated bowel in Hirschsprung's disease and that of controls.

11.3 MATERIALS AND METHODS

11.3.1 Patient study group

This study evaluated the AChE activity in the proximal colon shown on frozen section to contain ganglion cells in patients undergoing surgical pull-through for Hirschsprung's disease. Control specimens were obtained from patients without Hirschsprung's disease as well as patients with established NID.

11.3.2 Recruitment of patient sample

Patients with Hirschsprung's disease presenting for surgery between January 1988 and January 1991 were included in the study. Controls were patients with conditions not related to Hirschsprung's disease undergoing elective surgery during the same period. Controls also included a group with the histological picture of NID in the absence of Hirschsprung's disease. The same patient sample was used as in the ganglion cell study of the previous chapter with the exception of postmortem samples.

11.3.3 Demographic data and ascertainment of data

Similarly, the method of recording clinical information and the ascertainment of data was the same as described in the previous chapter.

11.3.4 Diagnostic criteria and limitations of study

Although the aganglionic segment was fully assessed in these patients, this study confined itself to the staining patterns of the nerves and neurofibrils in ganglionated bowel.

Because of controversy regarding methods of assessing AChE staining patterns, the following limitations were applied during this study.

- AChE positive neurofibrils must extend deep into the mucosa to reach the mucosal surface.
- One fine wispy AChE positive neurofibril per crypt was accepted as being within the normal range. An increase in the number of AChE positive neurofibrils per crypt was considered a significant deviation from the accepted normal pattern. Severity was determined by the presence of more than 2 neurofibrils per crypt.
- The extent of neurofilament branching and the inclusion of cell bodies were noted and appeared to represent a more severe type of reaction.
- An increase in AChE staining neurofibrils within the muscularis and adjacent submucosa, the so called type B AChE staining pattern of Hirschsprung's disease (Chow 1977; Meier-Rüge 1992), was recorded if present.
- The length of extension of abnormal histological features proximal to the pull-through level was evaluated where possible. (This may indicate the extent of the affected segment and facilitate surgical resection in a localized segment of abnormal bowel.)

11.3.5 Histochemical assessment

Fresh surgically resected full thickness specimens were prospectively collected from 26 carefully documented patients undergoing Hirschsprung's surgery. Aganglionic, transitional zone and ganglionated bowel were identified by frozen section histology and representative samples selected in the pathology laboratory by the author. Specimens were snap frozen in liquid Nitrogen at minus 160 degrees Celsius in preparation for AChE staining. The AChE staining technique of Meier Rüge (1972) **(Appendix C)** was used to stain the frozen tissue sections.

The sections were examined by light microscopy. AChE staining demonstrated the AChE enriched neurofibrils in the lamina propria and muscularis mucosa of affected segments.

The same procedure was employed to evaluate the bowel of 22 controls undergoing elective colonic surgery for conditions other than Hirschsprung's disease. We also evaluated 5 patients with NID in the absence of Hirschsprung's disease.

In addition to AChE staining, a full histological evaluation was performed which included the assessment of ganglion cell morphology as described in the previous chapter. The diagnostic features of NID included hyperganglionosis with giant submucosal ganglia containing between 7 and 15 ganglion cells per cluster (Meier-Rüge 1992; Schärli 1992). In addition, other histological features were noted (eg. the presence of nerve cell buds along the large afferent parasympathetic nerve fibres, nerve cells located within the afferent fibres or the presence of heterotopic submucous nerve cells in the lamina propria or muscularis mucosa (Borchard 1991; Meier-Rüge 1992)).

Specimens were selected some distance from a colostomy to minimize the influence of previous surgery. This was in keeping with the recommendations of a working group of pathologists (Borchard 1991). Furthermore, those specimens taken from patients with colostomy and those taken from patients without colostomy were separately assessed to establish whether a link between prior surgery and the presence of AChE staining neurofibrils existed.

11.4 RESULTS

11.4.1 AChE staining in the proximal pull-through bowel in Hirschsprung's disease

Typical ACHE staining neurofibrils were noted in the aganglionic segment of all patients with Hirschsprung's disease (**Figure 11.1**). Increased numbers of thin cholinergic nerve fibrils were also noted in the muscularis propria and lying between the gland crypts in the lamina propria of patients with NID which were absent in normal bowel (**Figure 11.2**).

The patterns of AChE staining in the proximal pull-through bowel in the 26 patients in the Hirschsprung's disease patient group are listed in **Table 11.1.**

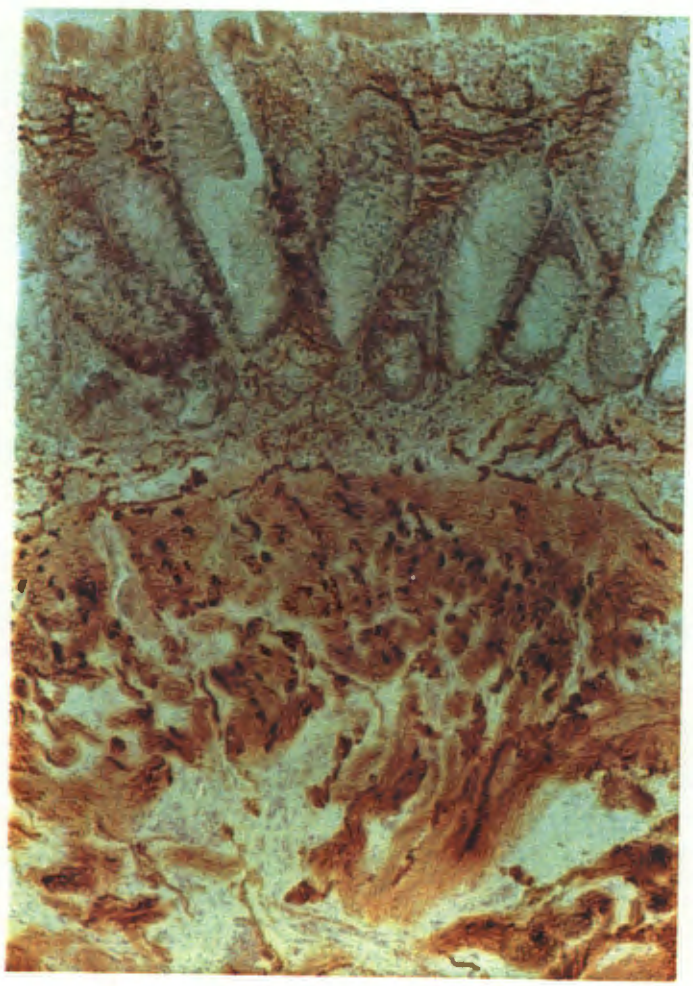


Figure 11.1 Section of aganglionic segment in Hirschsprung's disease showing the typical increase in AChE staining neurofibrils in the lamina propria and muscularis mucosa (x40).

Figure 11.2 (a) Frozen section of colonic mucosa stained for acetylcholinesterase. Neuronal intestinal dysplasia: increased numbers of thin cholinergic nerve fibrils in the muscularis mucosae and lying between gland crypts of lamina propria (x40)

Figure 11.2 (b) Frozen section of colonic mucosa stained for acetylcholinesterase. Normal control showing staining of ganglia in submucosa with virtually no cholinergic nerve fibrils in muscularis mucosae and lamina propria. (x40)

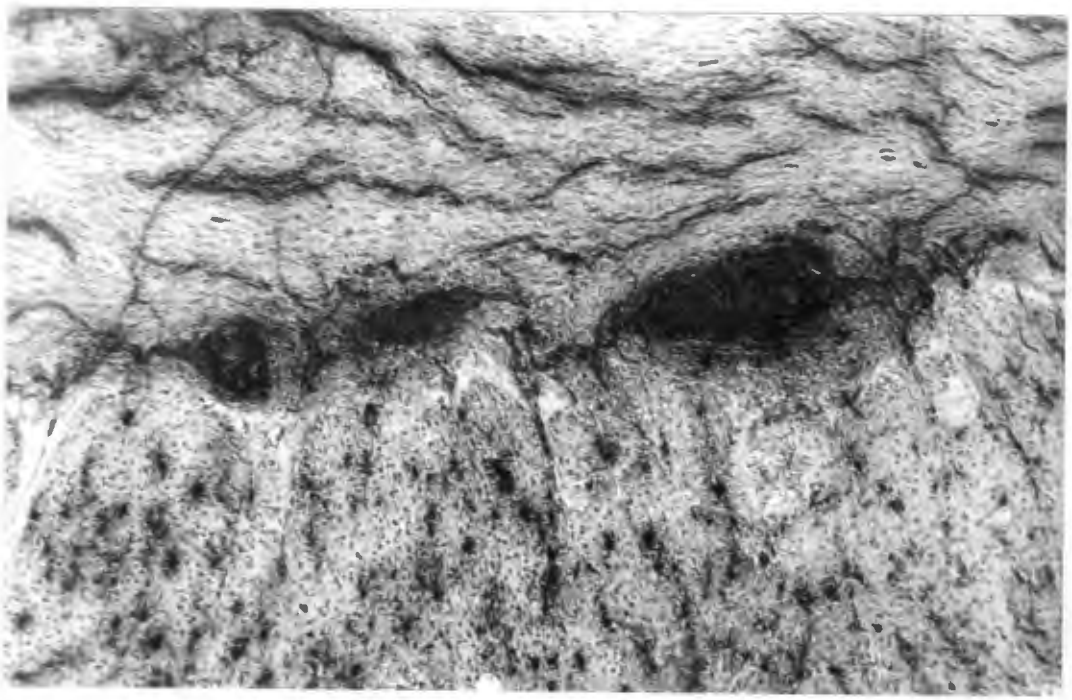


Figure 11.3 (a) Frozen section of muscularis propria stained for acetylcholinesterase: Neuronal intestinal dysplasia: heavy staining for enzyme in Auerbach's plexus with the presence of numerous cholinergic nerve fibrils in both longitudinal and circular muscle layers (x63).

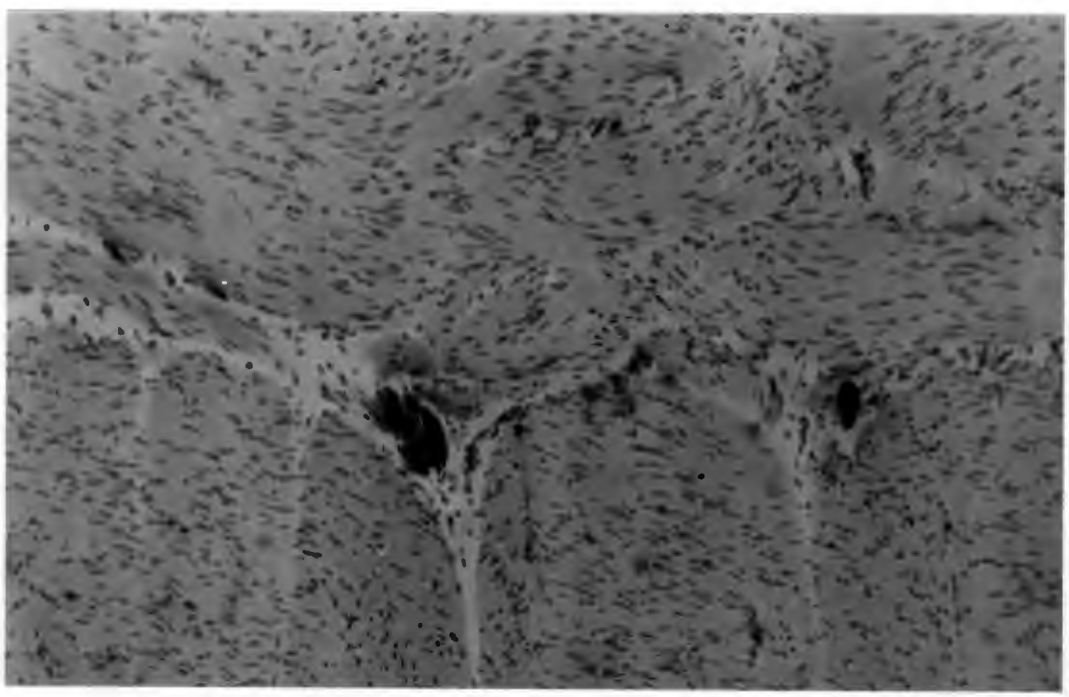


Figure 11.3 (b) Frozen section of muscularis propria stained for acetylcholinesterase: Normal control shows some staining for enzyme ganglia in Auerbach's plexus with sparse cholinergic nerve fibrils in longitudinal and circular muscle layers (x63).

TABLE 11.1

ACHE STAINING NEUROFIBRILS

	No of	% patients
Ganglionated bowel Hirschsprung's disease n = 26	5	19%
Controls n = 22	8	36%

Two or more AChE neurofibrils per crypt was noted in the lamina propria of the ganglionated segment in 6 of the 26 (23%) patients with Hirschsprung's disease (**Table 11.2**). In 2 of these there was associated branching of AChE neurofibrils with a "budding" pattern or inclusion of association of cell bodies. In 3 patients (11%), there was a striking number of positive nerve fibrils in the submucosa and muscularis mucosa which was considered to be a type B AChE staining pattern (Chow 1977). These all had other features of increased AChE staining in the lamina propria and muscularis mucosa. In 9 patients, thin, wispy AChE neurofibrils were noted in the lamina propria but these were not increased and were considered to be within the spectrum of normal. AChE staining neurofibrils were observed in the muscularis propria in NID (**Figure 11.3**). This feature was also observed in the proximal bowel of 15 (58%) of the 26 (23%) patients with Hirschsprung's disease. Thus, in only 11 patients (42%) was there a complete absence of AChE activity in the bowel wall.

In 4 of the 6 patients with increased AChE activity, AChE staining neurofibrils were noted in the adventitia of the submucosal arteries (**Table 11.2**).

The proximal extent of ACHE staining also varied and did not extend more than 7 cm proximal to the aganglionic zone in 21 of the 26 patients. Two of the 5 with proximal ACHE staining patterns had TCA with extensive involvement of proximal bowel, but in a further 2 the AChE staining pattern extended proximally as far as the appendix. In the remaining patient the extension was longer than 7 cm proximal to the aganglionic zone but returned to normal at the level of the transverse colon.

The depth of AChE staining varied between patients and although it was deeply staining in the more severe cases it may have been influenced by technical factors in others. Other variations in AChE staining were difficult to evaluate as they were without any clear cut pattern. In 2 specimens this appeared to be related to local tissue hemorrhage.

11.4.2 AChE staining in control patients

More than 2 AChE neurofibrils per crypt was noted in 8 out of the 22 (36%) control specimens investigated (**Tables 11.2 and 11.3**). None of these patients had associated branching, budding or associated cell bodies. These positive AChE staining patterns were observed in 5 (38.5%) of the 13 patients with anorectal malformation and in 3 (33%) out of the 9 remaining controls. In one patient with otherwise normal colon, a positive AChE staining pattern was noted in a patient undergoing a colonic interposition for long gap oesophageal atresia. The other 2 positive stains were in patients with a history of previous necrotizing enterocolitis.

TABLE 11.2

HISTOLOGICAL OBSERVATIONS OF AChE HISTOCHEMISTRY

	Hirschsprung's disease n = 26		Controls n = 22		NID n = 5	
AChE Neurofibrils						
> 2 + budding/branching	2	(7%)	-		2	(40%)
> 2 per crypt	4	(15%)	8	(36%)	2	(40%)
1 per crypt	9	(34%)	5	(23%)	1	(20%
Absent AChE	11	(42%)	10	(45%)	0	
AChE Type B	3	(11%)	3	(14%)	3	(60%)
Increased AChE in muscularis propria	15	(58%)	13	(59%)	2	(40%)
Increased AChE in submucosal arteries	6	(23%)	-		4	(80%)

A type B AChE pattern was observed in 3 although some AChE staining nerves were noted in the muscularis propria of 13 of the control patients (59%). This was similar to the findings of the Hirschsprung's disease group. None of the control patients had AChE staining of the adventitious layer of the submucosal arteries in the absence of NID.

TABLE 11.3

ACHE STAINING NEUROFIBRILS IN CONTROL PATIENTS

(>2 neurofibrils per crypt)

	No of Pts	%
Anorectal malformation	5	46.6%
Enterocolitis	2	100%
Normal bowel	1	25%

Patients with NID had marked AChE activity in the lamina propria and muscularis propria. There were more than 2 neurofibrils per crypt in 4 of the 5 patients and a branching and budding pattern in 2. AChE staining neurofibrils were noted in blood vessel adventitia in 80%. There was one exception where the staining of the neurofibrils was increased but the number per crypt was not always greater than 2. Although hyperganglionosis was present in Auerbach's plexus in this patient, the giant ganglia noted in the submucosal plexus contained many glial cells and few ganglion cells in the majority. There were, however, isolated clusters containing more than 7 ganglion cells in the submucosal plexus.

A link between prior surgery and the presence of AChE staining neurofibrils in the lamina propria and muscularis mucosa could not be demonstrated.

11.5 DISCUSSION

11.5.1 The significance and extent of abnormal AChE staining neurofibrils in normally ganglionated bowel in patients with Hirschsprung's disease.

The diagnosis of Hirschsprung's disease remains a histologically based decision although clinical features, radiological findings and anorectal manometry may contribute to confirming the diagnosis. The AChE stain has been shown to be of diagnostic value in Hirschsprung's disease (Meier-Rüge 1972). It demonstrates the presence or absence of ganglion cells as well as the AChE mucosal nerve proliferation and abnormal nerve trunks of peripheral nerves (Meier-Rüge 1972; Trigg 1974; Lake 1978; Hamoudi 1982; Wakely 1984). AChE staining is also the preferred method to demonstrate the mucosal nerve proliferation that exists in affected bowel (Meier-Rüge 1972; Robey 1988). False positive tests in the diagnosis of Hirschsprung's disease are rare (Wakely 1984; Barr 1985) due to the characteristic staining pattern. By way of contrast, a certain amount of controversy surrounds the interpretation of finer AChE positive neurofibrils in the lamina propria and muscularis mucosa of ganglionated bowel (Lake 1989; Schofield 1990). In addition, AChE has been noted to stain non-cholinergic as well as cholinergic nerves adding to the difficulties in interpretation (Small 1990).

11.5.2 AChE staining in the Hirschsprung's disease patient group.

The diagnostic features of Hirschsprung's disease are multiple, coarse, interdigitating AChE staining nerves branching in the lamina propria and muscularis mucosa in association with aganglionosis (Meier-Rüge 1972; Wakely 1984). Typical findings were present in the aganglionic segment of all the patients with Hirschsprung's disease. The AChE staining patterns in this series mostly conformed to type A described by Chow (1977) but a type B pattern was noted in 3 patients. Although an intermediate type of staining pattern has

been reported (Chow 1977; de Brito 1987) this was not identified in any of the patients included in this study.

In addition to the abnormalities in ganglion cell morphology observed earlier in this section, variation in the number and thickness of nerve fibres in the lamina propria or muscularis mucosa was noted in the proximal ganglionated colon in keeping with other reported results (Robey 1988).

The positive AChE stain in 23% of the proximal ganglionated segments in Hirschsprung's disease, appeared to represent residual bowel pathology in the pull-through segment.

11.5.3 AChE staining patterns in controls

Normal bowel is reported as having no AChE staining nerve fibres (Ariel 1983; Wakely 1984; Barr 1985; Becker 1985) but a few fine, wispy nerve fibres may be present within the lamina propria (Wakely 1984; Lake 1989). These normal nerve fibres are distinguished by their fine wispy character. They do not go through to the mucosal surface and there is a lack of neurofibrils in the lamina propria in normal bowel. The diagnostic pattern of interdigitating, thick, coarsely staining neurofibrils in the submucosa as well as the lamina propria suggests Hirschsprung's disease rather than NID.

On the other hand, the presence of neurofibrils in the lamina propria alone is not sufficient for the diagnosis of Hirschsprung's disease (Wakely 1984) and too sensitive a test will result in erroneous results and over reporting. This is further complicated by the wide variation in reported results of co-existing NID. NID has been shown to co-exist in 20-27% of patients with Hirschsprung's disease (Schärli 1992). The nature of the neurofibril AChE staining patterns is less marked in NID and the definition as to what constitutes a severe reaction is not

as clearly defined (Schärli 1981, Fadda 1987; Robey 1988). This was in contrast to the 36% incidence of positive AChE neurofibrils in tissue sections from control patients. The significance of the positive AChE histochemistry in these patients is therefore open to question. The control tissue specimens were obtained from available surgically resected tissue during the period of the study. Postmortem tissue used for the ganglion cell study was excluded due to uncertainty of possible neurotransmitter changes (Testylier 1991). Although the control group in this study was subject to certain limitations and was not taken from a homogeneous group of patients, the findings in these 22 patients appears to represent a wide variation in AChE staining neurofibrils in patients without neurodevelopmental disease.

Positive AChE stains have previously been described in patients with anorectal malformations, necrotizing enterocolitis, allergic colitis and chronic inflammatory processes (Rintala 1989). These conditions were present in certain patients in the control group and this provides some explanation of the high incidence of positive AChE staining patterns in these patients as almost all the positive staining AChE stains were identified in these patients (**Table 11.3**). The one exception was in a patient with a normal colon undergoing colonic interposition for a long gap oesophageal atresia. This therefore raises the possibility that the increase in AChE neurofibrils is acquired. The role of surgical intervention and possibly intestinal obstruction on AChE staining remains unclear and will be further evaluated in a later chapter.

11.5.4 Other factors affecting AChE staining patterns

Factors such as patient **age of presentation** and **sex** did not appear to significantly influence the histological outcome. A small percentage of patients with false negative staining for AChE has been reported in patients with total colonic aganglionosis (TCA) (Elema 1973; v d Staak 1981). The one exception

in this series was a patient with TCA in whom the AChE stain was initially negative but who developed a strongly positive staining pattern a month later. This suggests that TCA may differ in its histological pattern from the usual features of AChE staining nerve tissue in Hirschsprung's disease.

The uncharacteristic pattern of AChE staining and the similarity of this case to other reports (vd Staak 1981; Ariel 1983; Barr 1985) suggests that the atypical disease pattern may be a possible reason for this feature (vd Staak 1981; Ariel 1983; Barr 1985).

The site of the biopsy material has been reported as being of importance. AChE staining has been reportedly unreliable in specimens taken proximal to the splenic flexure (vd Staak 1981; Meier Rüge 1984; Lake 1989) or at colostomy sites (vd Staak 1981). In three patients with short segment disease, AChE staining extended proximal to the splenic flexure. The proximal extension of AChE fibres has been reported to be related to poor function (Munakata 1985) but the nature and significance of the relationship has yet to be appreciated. The proximal extension of AChE in some cases appeared to be associated with postoperative dysfunction.

Factors that affected the histochemical staining for AChE the control group included the influence of previous inflammatory conditions of the bowel wall, the influence of previous surgery and the influence of other pathological conditions such as anorectal malformations and NID.

The influence of previous inflammatory conditions involving the intestinal wall showed features of positive AChE staining in 2 out of 3 patients with previous enterocolitis. In one patient with severe obstruction due to a late presenting anorectal malformation and resultant necrotizing enterocolitis, no evidence of

such changes was detected. This is in keeping with the overall findings of Barr (1985) who reported no morphological changes to the ENS and a negative AChE stain in 4 patients with NEC.

11.5.5 The influence of technical factors in positive AChE stains

Technical factors which possibly play a role in positive AChE staining include variations in technique resulting in differences in the depth of the stain, temperature variations or the possible influence of red blood cell AChE in haemorrhagic specimens.

The depth of AChE staining was not significantly different in the patients in this sample and the overall increase in AChE staining was confined to the nervous tissue. The specimens were processed under standard laboratory conditions and temperature variations did not appear to influence the result.

Toorman (1977) showed local accumulations of structureless AChE staining material which held no diagnostic significance. These have been suggested as being on the basis of local hemorrhage causing the release of red blood cell AChE (Ariel 1983; Wakely 1984; Blisard 1986). The influence of red blood cell AChE remains a possible factor in AChE staining particularly in haemorrhagic specimens (Blisard 1986; Borchard 1991). This could possibly explain local accumulations of structureless AChE staining but does not explain the specific increase in the number of AChE staining neurofibrils in Hirschsprung's disease and NID.

Investigation of these possible technical factors failed to reveal any conclusive evidence that they had affected the overall result.

11.5.6 The significance of AChE staining neurofibrils in histochemical sections

The observation that AChE staining neurofibrils are present in certain patients with postoperative obstructive symptoms in this study, raises several important questions as to the significance of co-existing NID in patients with Hirschsprung's disease. It has been suggested that the histological diagnosis of co-existing NID in ganglionated bowel in patients with Hirschsprung's disease may result in poor postoperative bowel function (Meier-Rüge 1992).

By way of contrast, the increase in AChE positive neurofibrils in controls raises the question of the specificity of such findings. Barr (Barr 1985) found no correlation to constipation or obstructive symptoms in 19 patients who had fine, wispy AChE neurofibrils in the lamina propria and muscularis mucosa at 18 months follow-up. This suggests that AChE neurofibrils occurring in isolation may be of little prognostic value.

The problem of interpretation exists due to the variation of the degree of functional impairment between patients with similar histological findings. Some patients improve symptomatically given sufficient time (Schärli 1992). In a much smaller group, postoperative obstructive symptoms may continue and be present on long term follow-up. The extent of the increased AChE activity in the mucosa of the bowel has been shown to not always accord with the level of the aganglionosis in patients with Hirschsprung's disease. A variable length of proximal extension has been reported (Goto 1985). In this study, there appeared to exist some relationship between those 5 patients with proximal extension of AChE neurofibrils in proximal bowel and postoperative obstructive symptoms.

These findings underline the need to fully evaluate the pull-through segment of bowel for the presence or absence of dysmorphic features. The diagnostic

interpretation of the length of the diseased bowel is a vital part of the successful surgical management of Hirschsprung's disease. Surgical decisions are based on frozen section findings at the time of surgery. Special staining techniques are infrequently employed to confirm these findings.

Histochemical staining techniques include an assessment of the AChE positive neurofibrils in the lamina propria and muscularis mucosa. Careful histological mapping of the colon at the time of colostomy plus a full evaluation of the ganglionated surgical margin at the time of pull-through will help to clarify these issues.

11.6 CONCLUSION

From this study we deduced that although abnormalities of ENS morphology do exist in proximal ganglionated bowel in patients with Hirschsprung's disease, they are of uncertain significance if fine and wispy and number less than 1 per crypt. The significance of an increased number of AChE neurofibrils remains uncertain. The type B pattern of AChE staining appeared to be of less significance than a high level of dysplastic features in the lamina propria. Patients with NID appeared to have a high incidence of AChE positive neurofibrils observed in the adventitious layer of the submucosal blood vessels. This was also noted in the ganglionated segment of 6 patients with Hirschsprung's disease and probable co-existing NID.

Positive AChE stains were observed in a high percentage (38%) of control patients with a past history of anorectal malformations, necrotizing enterocolitis or oesophageal atresia. It is also clear that the histochemical findings which denote a degree of co-existing NID resulting in long term obstructive symptoms need to be clarified and further research into the variations of normal is still required.

CHAPTER 12

A PROPOSED HISTOLOGICAL GRADING SYSTEM FOR THE EVALUATION OF NID CO-EXISTING WITH HIRSCHSPRUNG'S DISEASE

12.1 INTRODUCTION

Ensuring a good outcome of surgery for Hirschsprung's disease does not rest solely on the identification of ganglion cells in the pull-through segment of bowel (Schärli 1992). Co-existing neuronal dysplastic disease is thought to influence the postoperative outcome of the surgical treatment of Hirschsprung's disease (Lassman 1973; Gulotta 1977; Puri 1977; Kessler 1985; Fadda 1987; Meier Rüge 1990). Persisting obstructive symptoms which follow otherwise successful pull-through procedures may be due to co-existing dysplastic conditions of the ENS. The use of special histochemical and immunocytochemical stains appears essential to fully evaluate the ENS and has led to a great deal of additional information about the extent of the abnormal features within the ENS in Hirschsprung's disease and NID (Meier-Rüge 1992; Schärli 1992).

An historical lack of exact histochemical and immunocytochemical parameters defining NID as well as a wide variety of observed histological abnormalities in the pull-through segments of ganglionated bowel, has led to a certain amount of debate about the existence and significance of histological features of NID (Schofield 1990; Yunis 1992). Furthermore, the significance of particular histological observations in terms of their influence on postoperative function is not well established and appears to be variable in determining the severity of symptoms.

The degree of dysplasia therefore probably denotes clinical significance and recognizable patterns of histological features in NID have been reported (Schärli 1992). It is conceivable that NID may co-exist in mild forms without clinical significance or in relatively severe forms which result in obstructive symptoms.

A wide variety of abnormal histological and histochemical findings contribute to the diagnosis of NID (**Table 12.1**). Essentially, the histological picture represents an overall impression depending on the experience of the observer as well as the interpretation of normal and abnormal features. From this background it is clear that a scoring system based on the various histological features would allow some degree of comparison and evaluation of the overall histological picture.

12.2 AIM

The aim of this study was to test the predictability of postoperative dysfunction (obstructive symptoms) based on histological findings of NID as assessed by a histological scoring system.

12.3 MATERIAL AND METHODS

12.3.1 Patient study group

Twenty-six patients with Hirschsprung's disease and 22 controls, prospectively studied between January 1988 and January 1991, were evaluated. The histological observations were quantified according to the guidelines laid down in previous chapters in this section. Controls were full thickness specimens of colon obtained from patients without Hirschsprung's disease in a similar age spectrum.

Table 12.1

Histological Features of NID

A.	**Ganglion Cells:**
	Hyperganglionosis - Meissner's plexus - Auerbach's plexus
	Giant ganglia
	Heterotopia of ganglion cells (in lamina propria or muscularis mucosae)
	Ganglion cells in peripheral nerves
	Dysgenetic ganglion cells
B.	**AChE staining neurofibrils:**
	Lamina Propria
	Muscularis mucosa
	Circular muscle
	Blood vessel adventitia
	Proliferating nerve buds on AChE neurofibrils
C.	**Other**
	Smooth muscle in lamina propria (?)
	Hypoplastic sympathetic nervous system (Type A)

Histological observations on full thickness surgical pull-through specimens were entered into a computerized database. Special mention of the histological features of NID were made including hyperganglionosis, hypoganglionosis, giant ganglion cells, immaturity of ganglion cells, associated nerve cell bodies related to neurofibrils, AChE activity in nervous tissue, the presence of large nerve fibres, the presence of scattered ectopic ganglion cells within the muscularis mucosa and the submucosa and sympathetic hypoplasia (Schärli 1992). Arbitrary values were then attached to these observations based on their importance in the diagnosis of NID (**Table 12.2**). The maximal possible score was 12 points. The values were subsequently revised according to the observations of the patterns of abnormal histological features described in NID (Schärli 1992).

Correlation with functional outcome was used to assess the possible significance of these findings. The methods of clinical assessment used in this study were those developed in the clinical sample outlined in Section B.

TABLE 12.2

CRITERIA FOR THE ASSESSMENT OF NID HISTOCYTOCHEMISTRY

A. GANGLION CELL MORPHOLOGY		
1. Hyperganglionosis (increased number of ganglion cells)		
Mild	1	
Moderate	2	
Severe	3	3
2. Large Ganglia		1
3. Maturity of ganglion cells		
Immature	1	
Mature	0	1
3. Heterotopic ganglion cells (Ganglion cells within lamina propria or associated with peripheral nerves)		1
B. AChE STAINING NEUROFIBRILS		
1. Lamina Propria AChE neurofibrils (NF)		
- No Staining	0	
- Fine wispy NF not reaching mucosal surface	0	
- > 1 AChE staining NF per crypt Reaching mucosal surface)	1	
- Patchy increase in AChE NF stain (> 1 per crypt) with branching and associated nerve buds	2	
- marked widespread increase of AChE NF (>2 per crypt) with NF branching and associated nerve buds	3	3
2. Abnormal increase in AChE NF in Muscularis mucosa and adjacent submucosa / or AChE in blood vessel adventitia		2
3. Marked NF staining in muscularis propria		1
TOTAL		**12**

12.3.2 Method of AChE evaluation

Because of current controversy about the best methods of assessing AChE staining patterns, the following limitations were applied during this study.

- Only AChE positive neurofibrils which extended deep into the mucosa to reach the mucosal surface were considered as being significant (ie. a positive AChE reaction).
- One fine wispy AChE positive neurofibril per crypt was accepted as being within the normal range. An increase in the number of AChE positive neurofibrils per crypt was considered a significant deviation from the accepted normal pattern. If more than 2 neurofibrils per crypt were present an appropriate score was allocated (**Table 12.2**).
- The extent of neurofilament branching and the association of nerve cell bodies were noted and appeared to represent a more severe type of reaction and were therefore given a high score value.
- An increase in AChE staining neurofibrils within the muscularis mucosa and adjacent submucosa, the so called type B AChE staining pattern of Hirschsprung's disease (Chow 1977; Meier-Rüge 1992) was awarded a value if present.
- The length of extension of abnormal histological features proximal to the pull-through level was evaluated where possible. This may indicate the extent of the affected segment and facilitate surgical resection in a localized segment of abnormal bowel.

12.3.3 Histopathological scoring system

A scoring system was developed for the purposes of this study to provide a method of comparison and evaluation (**Table 12.2**). The histopathology score values were evenly divided between observations relating to the ganglion cells and those relating to the AChE staining neurofibrils. Arbitrary values were given to the observations on the basis of the weight given to each observation.

Results were expressed as the median values of at least 10 observations.

12.3.4 Statistical analysis

Results were expressed as medians with interquartile ranges except where an arbitrary score of 3 was used for outcome. In this situation, results were expressed as the mode.

Statistical analysis was by non parametric tests by ranks (Kruskal-Wallace) for non paired samples and the Wilcoxon signs test for two tailed probability for paired samples.

12.4 RESULTS

12.4.1 The spectrum of dysplastic features within the ENS

Results showed a wide spectrum of dysplastic features occurring in the NID group. A similar spectrum of histopathological features was also observed in the ganglionated bowel proximal to the aganglionic segment in certain patients with Hirschsprung's disease. Certain individual features were also noted in the colon of control patients without Hirschsprung's disease or NID. In this latter group abnormal findings were often individual observations which did not necessarily always correlate with the postoperative outcome of the patient. An overall pattern of dysplasia in the ganglionated bowel of the Hirschsprung's group, improved the correlation with a motility disturbance noted postoperatively.

Ganglion cell morphology observations in the Hirschsprung's patients included hyperganglionosis, immature ganglion cells and ganglion cells occurring in ectopic positions. The presence of large ganglia in the submucous plexus, hypoganglionosis or the presence of small, darkly staining (hypogenetic) ganglion cells was also noted (**Chapter 10**).

A negative AChE staining pattern with a ganglion cell morphology showing small darkly staining hypogenetic ganglion cells was observed in 2 patients who subsequently developed postoperative obstructive symptoms.

A wide range of cross-sectional cut area of ganglion cells was observed on computerized measurement with little overall significant difference between the Hirschsprung's and control groups. A certain degree of variability was also shown depending on the age of the patient and orientation of the specimen.

Study of the AChE stained sections showed a surprizing incidence of increased AChE staining neurofibrils in the lamina propria and muscularis mucosa in the ganglionated bowel segments from patients with Hirschsprung's Disease as well as in controls (HD 19%; controls 36% (**Chapter 11**). Those controls with an associated anorectal malformation had a 47% positive AChE staining pattern. Positive AChE stains also occurred in patients with a history of necrotizing enterocolitis and in 1 out of the 4 controls with normal bowel. In this latter case, the specimen was taken at the time of colonic interposition in a child with a long gap oesophageal atresia. Those control patients with negative AChE stains were obtained from patients undergoing colonic interposition for strictures following caustic ingestion. Despite the presence of positive AChE staining patterns in control specimens, these were seldom associated with a wide spectrum of dysplastic ENS features.

12.4.2 Histopathology scores

Median histopathology scores are listed in **Table 12.3.** Scores obtained in patients with NID were significantly higher (Kruskal Wallace, $p<0.01$). Although the mean and median scores of the Hirschsprung's patients were significantly lower than in patients with NID, certain individual scores approximated the same degree of dysplasia (**Figure 12.1**).

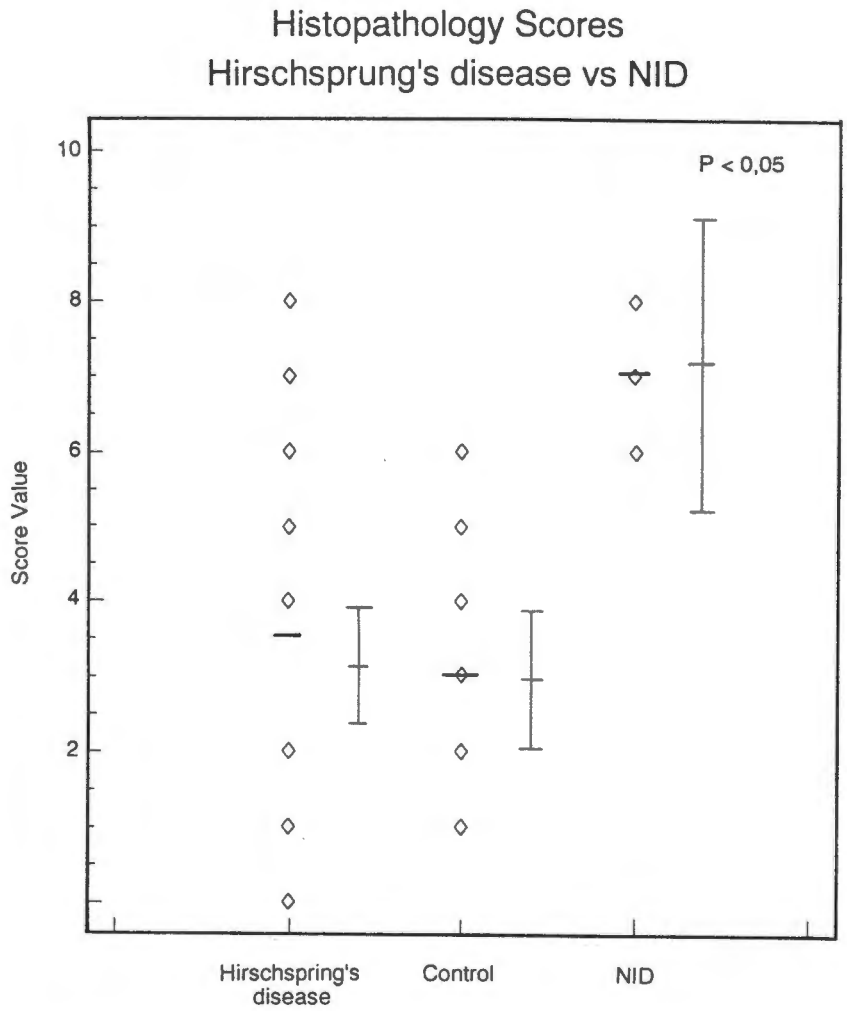


Figure 12.1. Scatter plot showing distribution of histopathology score values. Graph shows median values (broad line in data), means and 95% confidence intervals

12.4.3 The outcome of the histopathology scoring system

As expected, the median histopathology scores from the 3 groups of patients showed a higher score in the NID group. Median scores from both the Hirschsprung's and the control groups were significantly lower (**Table 12.3**). Although the overall score from patients with Hirschsprung's disease was lower, an almost equivalent degree of dysplasia was noted in 5 patients as in those patients with NID (**Table 12.4**). These 5 had a histopathological score greater than 6 out of a possible 12 points.

TABLE 12.3

MEDIAN HISTOPATHOLOGY SCORES (Max 12)

Patient Group	Median Score
Hirschsprung's Disease	4
Control	3
NID	7*

(*Kruskal Wallace, p = 0.003)

TABLE 12.4

OUTCOME HIRSCHSPRUNG'S DISEASE - BASED ON HISTOPATHOLOGY SCORE

	Score > 6	Mode	Score < 6	Mode
Hirschsprung's disease	5	obst	21	good
Controls	0	-	22	good
NID	5	obst	0	-

Obstructive symptoms developed in 4 of these but improved to some degree with time in 2 of them.

The high scores in patients with NID and the combination of more than 1 dysplastic feature in these patients argues for the status of a significant level of dysplasia being necessary to influence postoperative function. On the other hand, the lower score in patients with Hirschsprung's disease explains the

overall good result observed following the surgical correction of Hirschsprung's disease.

12.5 DISCUSSION

The capacity of ganglionated bowel to acquire normal postoperative function is of considerable importance in the surgery of Hirschsprung's disease. It is possible that potentially resectable segments of dysplastic bowel may be retained due to reliance on the presence or absence of ganglion cells to determine the resection level at the time of surgery. Because of the difficulty experienced by histopathologists in the diagnosis and interpretation of NID in the intestinal wall, a scoring system was developed (**Table 12.2**) to standardize interpretation of specimens in this study.

The range of scores obtained in the ganglionated bowel of patients with surgically treated Hirschsprung's disease was between 0 and 8 with a median score of 3.26 out of a maximum possible score of 12 points . In the control patients, the scores varied between 0 and 6 with a median score of 2.72. Only 1 patient with an anorectal malformation achieved a score of 50% (ie 6/12) whereas this was noted in 3 patients with Hirschsprung's disease.

Evaluation of functional outcome in these patients confirmed the good outcome in the majority in keeping with our previous study (Section 2). This is also in keeping with reported results from units with a high incidence of co-existing NID (Hanimann 1992). Those patients with known NID (Type B) in the absence of aganglionosis had a significantly higher score (Kruskal Wallace, $p < 0.002$) and a significantly poorer outcome in terms of obstructive symptoms (Kruskal Wallace, $p<0.0001$) than those with Hirschsprung's disease or controls. By way of contrast, those patients with Hirschsprung's disease and scores of less

than 6 did not significantly differ in outcome from controls as far as the overall score or outcome were concerned.

Although the overall outcome following pull-through procedures for aganglionosis was comparable to that of controls, in those 5 patients with a histological score of 6 or greater, there was a highly significant increase in the incidence of obstructive symptoms (Kruskal Wallace, $p = 0.0001$). The incidence of obstructive symptoms in this group was comparable to those with NID.

This finding led us to the conclusion that a significant degree of dysplasia was probably of more significance in terms of functional outcome. In addition, the degree of dysplasia is probably of more significance than individual observations. The cutoff level for a significant histopathological score appeared to be approximately 6 out of a possible 12 points as scores above this level resulted in an 80% risk for postoperative obstructive symptoms. Scores in the intermediate range (5-6) demonstrated a degree of dysfunction in some, but these were less severe and probably represent a milder condition. Although symptoms may be minimal initially, they may become more significant later on, depending on the prevailing balance of forces in the anorectal region. On the other hand, there was some clinical improvement in 2 of the affected patients of this series suggesting that some form of adaptation may occur.

Histological scores did not significantly differ above or below 6 months of age or in those presenting outside of the neonatal period. In addition, the outcome did not appear to be affected by the length of the aganglionic segment in the Hirschsprung's patients, in patients initially presenting with intestinal obstruction or by the site from which the specimen was taken.

A dysgenetic type of ganglion cell morphology was observed in association with low scores in 2 patients. This was in keeping with previous reports (Munakata 1985; Schärli 1992). Insufficient emphasis was therefore put on to the finding of a small hypogenetic ganglion cell type in the scoring system which leaves room for future improvement.

Although the overall outcome following pull-through procedures for aganglionosis was comparable to controls, a significantly higher incidence of obstructive symptoms was observed in those 5 patients with a histological score of 6 or greater. The incidence of obstructive symptoms in this group was comparable to those with NID, which suggests that the histological abnormalities identified by this score are most probably of significance. This appears to indicate that NID co-existing with Hirschsprung's disease is an important factor in the etiology of postoperative obstruction. Those patients with a high level of dysplastic factors identified on histology are those most likely to result in postoperative dysfunction. Above a 50% score level of abnormal features (ie 6 out of a possible 12 points), the clinical correlation with postoperative dysfunction exceeds 80%. Thus, postoperative dysfunction can be predicted with a fairly high degree of correlation on the basis of the degree of abnormal histological features identified in the bowel wall.

Reliance on ganglion cell or AChE staining patterns alone has been shown to lead to erroneous results (Lake 1989; Schärli 1992) and a complete assessment of the entire histochemical range of features is essential. As this is practically difficult to perform at the time of pull-through surgery due to time constraints and the special staining techniques required, it is probably best to fully map the colon at the time of initial colostomy. The functional outcome in patients with postoperative motility disturbances requires careful follow-up, as spontaneous

improvement may occur probably due to alterations in the existing balance of forces.

12.6 CONCLUSION

From these studies it became clear that the following conclusions can be drawn from the available data.

- A number of abnormal features may exist within proximal ganglionated bowel in patients with Hirschsprung's disease.
- Parameters for the measurement of these abnormalities have been developed which allow some measure of definition.
- We propose a modified scoring system as a possible means of distinguishing significant abnormal morphology from the range observed in controls without Hirschsprung's disease or NID.

12.7 LIMITATIONS OF THE HISTOPATHOLOGICAL SCORING SYSTEM

It must be emphasized that this scoring system was devised for use as a standardized method of assessing full thickness specimens for NID. Like other scoring systems, it remains a guide rather than a fixed set of rules. Adaptations of the scoring system for use in doubtful cases of Hirschsprung's disease or in the evaluation of rectal biopsies were also attempted but were of less clinical use. Diagnosis in these cases usually rests on the demonstration of aganglionosis in the affected bowel. The scoring system may also be used in patients without Hirschsprung's disease as an indication for a full-thickness biopsy where rectal biopsies indicate a significant deviation from the accepted norm. This is clearly demonstrated in the 5 patients studied who had NID in the absence of Hirschsprung's disease.

The value attached to any observation in the scoring system depends on the significance of that particular feature. Consensus still needs to be reached as to

the extent of deviation from the normal that each observation represents and this is a point of possible future investigation.

Little is known about the influence of certain other parameters such as obstruction, age or previous surgery and certain features may be of an acquired nature secondary to intestinal obstruction or other influences. This will be addressed in the following chapter.

CHAPTER 13

SECONDARY EFFECTS OF CHRONIC INTESTINAL OBSTRUCTION ON ENTERIC NERVOUS SYSTEM MORPHOLOGY IN AN ANIMAL MODEL

13.1 INTRODUCTION

The functional obstruction which results from aganglionosis of the terminal portion of the bowel (Hirschsprung's disease) has been shown to continue into the postoperative period in a certain proportion of patients. Possible reasons for the persistence of symptoms include a residual aganglionic segment, achalasia of the internal anal sphincter or the presence of other dysplastic disease of the enteric nervous system in the pulled through bowel.

In certain cases the aganglionosis has occurred in bowel which was shown to be ganglionated at the time of pull-through (acquired aganglionosis), suggesting that a certain amount of plasticity remains within the enteric nervous system. This concept is of particular importance in assessing whether reported pathological alterations in enteric nervous system architecture could not possibly arise from a secondary effect (Schofield 1991).

Because the majority of these patients present with symptoms suggestive of postoperative obstruction, it becomes imperative that the influence of such obstruction on the proximal bowel be identified. This is especially relevant because of the inflammatory process which may result and lead to enterocolitis which in turn can result in significant morbidity and mortality.

The association with enterocolitis is also present in the murine model of ls/ls mice which have been shown to have consistent aganglionosis of the terminal 2-3 mm of bowel (Webster 1973). Gershon and co-workers (Gershon 1980;

Jacobs - Cohen 1987; Rothman 1984) have shown a resistance to colonization of the distal portion of the bowel to the precursors of the ENS in ls/ls mice. Meijers (1989) and Le Douarin (1974) have shown similar findings in avian models.

13.1.1 Review of the role of mechanical obstruction in producing alterations in the ENS

The consequences of mechanical obstruction on the proximal 'normal' bowel have been the subject of a number of studies and are important in terms of the histological evaluation of disease patterns. Rintala (1989) attributes the findings of NID in a 50 year old female with clinically diagnosed chronic idiopathic intestinal obstruction (CIIP) to "secondary changes caused by recurrent attacks of intestinal obstruction". Similar findings have been reported by Munakata in 2 children with chronic idiopathic intestinal pseudo-obstruction (CIIP) (Munakata 1985). Although the diagnosis of CIIP may be questioned in these patients and the degenerative disease may possibly be explained as a secondary phenomenon to other neurodysplastic conditions, the role of obstructive episodes in producing secondary histological features remains important for the evaluation of NID and other dysplastic conditions of the ENS.

Animal studies demonstrated an increase in small intestinal diameter following obstruction (Gabella 1971). Difference in hydrodynamic effects of intestinal obstruction has been shown to increase vascular resistance and may contribute to the effects on the intestinal wall (Ohmann 1986). A slow intestinal distension, as seen in an incomplete obstruction or a stenosis, does not increase vascular resistance since the ability of the intestinal wall to distend is increased (Ohmann 1986).

Recent work on congenitally aganglionic Ls/Ls mice has shown some increase in muscle cell length and increased contraction in the hypertrophied proximal large bowel (Hillemeier 1990). No increase in acetylcholine (ACh) potency could be shown for the aganglionic muscle cells in this murine model.

Pickard (1981) demonstrated in a fetal lamb model that obstruction results in hyperplasia of ganglion cells in the dilated proximal segment, a decrease in ATP-ase production proximal to the obstruction and no involutional changes in the area of maximal distension. Gabella (1971, 1975, 1979) has reported hyperganglionosis in the small bowel proximal to subacute intestinal obstruction.

Studies on the bowel proximal to an atresia in a fetal lamb model (Tepas 1979) suggest that cholinergic anomalies may be the cause of persistent intestinal dysfunction in these animals.

These observations raise the question as to whether the observed patterns of changes noted to co-exist with Hirschsprung's disease may not be result from obstruction rather than co-existing neuronal intestinal dysplasia (NID) as suggested by some workers (Schofield 1991).

13.1.2 Choice of animal model

Experiments in a rat model (Gabella 1975, 1979) have shown increase in muscle cell number and size as well as hyperganglionosis in the small bowel in response to a chronic obstruction, including a marked increase in the smooth and rough sarcoplasmic reticulum. These findings differ from the reported degenerative changes which result from acute obstruction (Gabella 1975).

The distribution of the ganglion cells in the rat have been shown to be similar to that of the human (Ito 1984) and observations of the changes produced by mechanical obstruction probably have some substance when compared to the human gastrointestinal tract. Some work on the alterations in small intestinal muscle function following an experimentally produced stenosis has been performed in the rat (Gabella 1975). For these reasons a rat model was selected.

Although murine and rat models of total colonic aganglionosis exist (Kubota 1989), study of the ENS of the proximal bowel in these animals is open to criticism as the proximal bowel may also be abnormal or part of a transitional zone. Other models of experimentally produced aganglionosis (Okamoto 1967) have the same disadvantage as the effect of the toxin on the normal bowel is not yet established.

A study of the effects of prolonged mechanical obstruction on the ENS would therefore best be performed on otherwise normal colon. The aims of this study were limited to establishing whether a prolonged intestinal obstruction affects ENS morphology. Because of possible influences of development on the ganglion cells of the colon, the study was performed on freshly weaned rats.

13.1.3 Neurotransmitter identification in the rat colon

Light and electron microscopic studies of AChE in Auerbach's plexus of the developing rat colon have been reported (Ito 1984). Ito (1987) has reported a similar localization of AChE in rat colon on electron microscopy. Dale (Dale 1979) has pointed out differences in AChE activity in the large bowel of rats depending on the site of biopsy. The technique of Karnowsky and Roots (Karnowsky 1964) was used to stain for AChE.

Identification of the sympathetic nervous system in the ENS was achieved by staining for Tyrosine hydroxylase, the rate limiting enzyme for catecholamine production. Previous studies in Tyrosine hydroxylase have been performed on rat tissue (Strong 1990).

13.2 AIMS

The aim of this study was to examine the effects of prolonged intestinal obstruction on the ENS of rat colon in an animal model.

13.3 MATERIALS AND METHODS.

13.3.1 Ethical permission

Ethical permission to perform the study was obtained from the Animal Research Review Committee of the University of Cape Town.

13.3.2 Animal Model

Forty-three freshly weaned Long Evans (LE) rats were randomized into 3 groups.

Group 1: Control group (including sham operation) (n = 16)

Group 2: Experimentally produced incomplete obstruction with biopsy at 6 weeks post surgery (n = 18)

Group 3: Experimentally produced incomplete obstruction with biopsy at initial surgery and at 6 weeks post surgery (a paired study) (n = 9).

Number of animals per group

The final number of animals in each group was determined by the relatively high mortality experienced, particularly in the Xylazine group, due to difficulties in anaesthetic administration.

13.3.3 Anaesthetic protocols

Animals were randomly selected for inclusion in 2 groups using Ether or Xylazine, an alpha-2 adrenergic agonist. Both anaesthetic protocols used a combination of the anaesthetic agent with Ketamine HCL as a Xylazine/Ketamine or Ether/Ketamine anaesthesia. The pathophysiological basis of the established effect of Xylazine in decreasing gastrointestinal transit (Gross 1989) was thus investigated.

Technique of anaesthesia

The animals were weighed and anaesthetized by intramuscular injection of Ketamine 90mg/kg plus Xylazine 10mg/kg or Ether sedation by face mask plus Ketamine anaesthesia. The intramuscular route was preferred to the intra-peritoneal route as it avoided any chemical substances coming into contact with the bowel which may produce changes per se.

13.3.4 Surgical technique

On the induction of surgical anaesthesia as assessed by the foot pinch reaction, the abdominal cavity was opened and the distal bowel identified and the individual group protocol performed as follows.

A sham procedure was performed in Group 3 by opening the abdominal cavity and mobilizing the distal colon and opening the mesocolon over a distance of 1.5 cm.

a mosquito artery forceps to achieve partial obstruction only (Figure 13.1). The abdominal cavity was closed in 2 layers and the animals returned to their cages and allowed their usual water and food and given analgesia (Buprenorphrine 4-8 μg/kg). They were examined daily and their weight monitored.

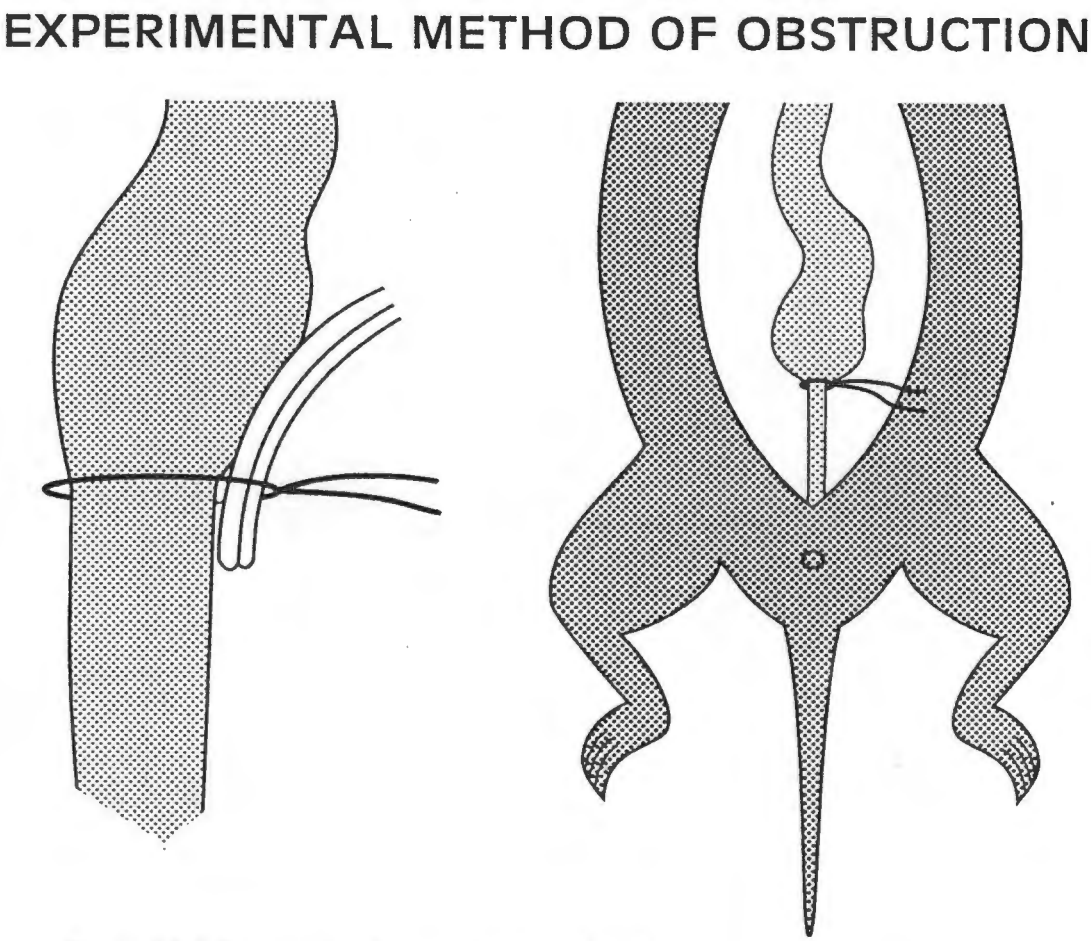


Figure 13.1 Experimental technique animal model

(Note the forceps tip preventing strangulation on application of the ligature)

Technique in group 3 was similar but allowed for the biopsy of the colon distal to the encircling ligature prior to creating subacute obstruction, the biopsy serving as an individual control.

13.3.5 End points of the study

The end point of the study was determined by the death or survival of the animals beyond 6 weeks. Alternatively loss of more than 20% of body mass in an animal who looked ill and had clinical features suggestive of septicaemia. The

13.3.5 End points of the study

The end point of the study was determined by the death or survival of the animals beyond 6 weeks. Alternatively loss of more than 20% of body mass in an animal who looked ill and had clinical features suggestive of septicaemia. The presence of severe enterocolitis was also regarded as an indication for termination. Euthanasia was performed in these animals by drug overdose at periods varying up to 110 days and a laparotomy performed during the initial anaesthetic phase. A segment of colon was removed 1 cm proximal to the site of obstruction.

13.3.6 Histochemical evaluation technique

Specimens taken from obstructed rats were compared with those of control animals undergoing a sham procedure. In those from the paired group (Group 3) the normal biopsies taken from the same rat prior to obstruction acted as individual controls. All specimens were stained for Tyrosine hydroxylase and Acetylcholinesterase activity to investigate the adrenergic and cholinergic systems. Staining patterns were investigated on light microscopy and recorded.

A visual analog scale varying between 3 and zero was used to assess the degree of AChE staining. This was performed separately for lamina propria, submucosa, muscle, nerves and ganglia and a total score calculated (Maximum 12).

13.3.7 Statistical analysis

Because of low numbers, statistical functions were mainly drawn from the paired group although observations were made for the obstructed and sham groups. Statistical analysis was by non-parametric tests for small numbers and in the case of the paired group analysis was performed by the Wilcoxon signed ranks test for two tailed possibilities.

13.4 RESULTS

13.4.1 Animal model of obstruction

Forty-three freshly weaned rats survived for a median of 27 days (**Figure 13.2**). Animals were divided into 3 groups. In the control group there were 25 specimens but only 16 animals due to the 9 initial biopsies in the paired animals acting as individual controls. These did not add to the total number in the series. Of the remaining 16, there were 8 sham procedures and the remainder were from normal animals. The obstructed group consisted of 9 matched pairs biopsied before and after obstruction and an additional group of 18 animals with prolonged intestinal obstruction.

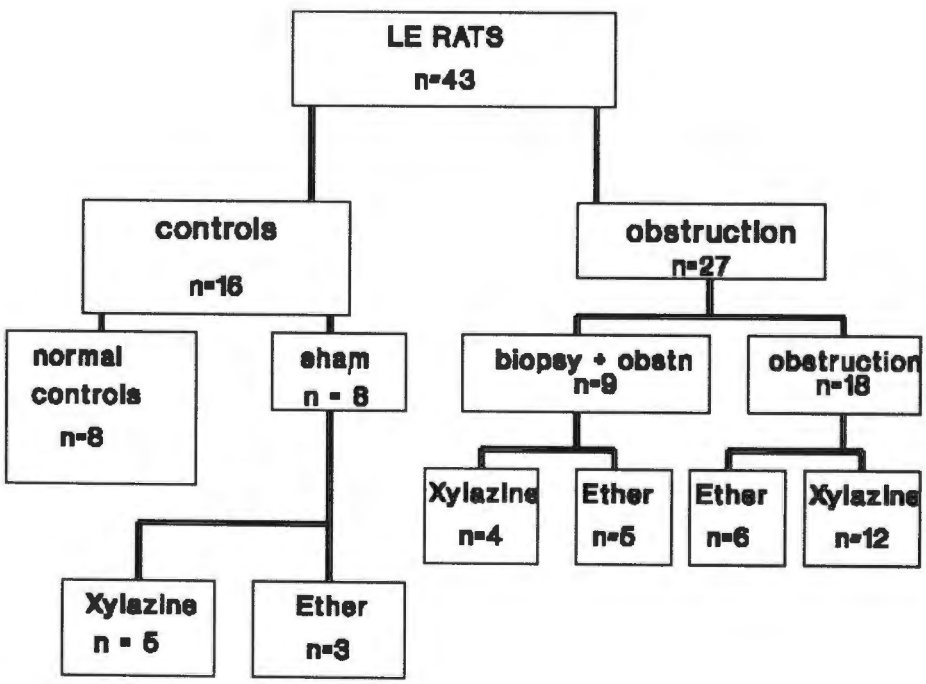


Figure 13.2 Flow diagram showing animal groups and anaesthetic technique employed.

The mean survival time of animals in the obstructed group was 30 days although 15 animals survived more than 35 days. The mean survival time of the sham group was 40 days.

The average weight at the time of the initial surgical procedure was 56 grams. The median weight of the sham animals was 231 gm which is considerably higher than that of the obstructed animals (177 grams) at the time of euthanasia. This observed difference in weights was partly due to the shorter survival of some rats but animals in the obstructed group did not gain weight as well as controls. In addition, the overall weight was influenced by the fact that the study was terminated in a number of animals who were euthanased because of a weight loss greater than 20% of body mass.

13.4.2 The effect of obstruction on enteric nervous system morphology

13.4.2.1 *Ganglion cell number, size and shape*

Evaluation of light microscopy of ganglion cells is summarized in **Table 13.1.** The mean number of ganglion cells per cluster was 7.5 and 8 in the sham and control groups respectively. This compared favorably with the mean values of 7.5 and 8 in the obstructed bowel of groups 2 and 3. There was little difference in the mean number of ganglia recorded per high power field (ie. 5.5 and 5 in the obstructed groups and 5 in the sham animals). The number of ganglia per 5 mm measured segment of the slide was significantly higher in the control group than in those with prolonged obstruction (Kruskal-Wallace, $p<0.001$). This can probably be explained on the basis of intestinal dilatation resulting from obstruction and a resultant spreading out of the ganglion cell population.

These relationships are demonstrated in the accompanying graph **(Figure 13.3).**

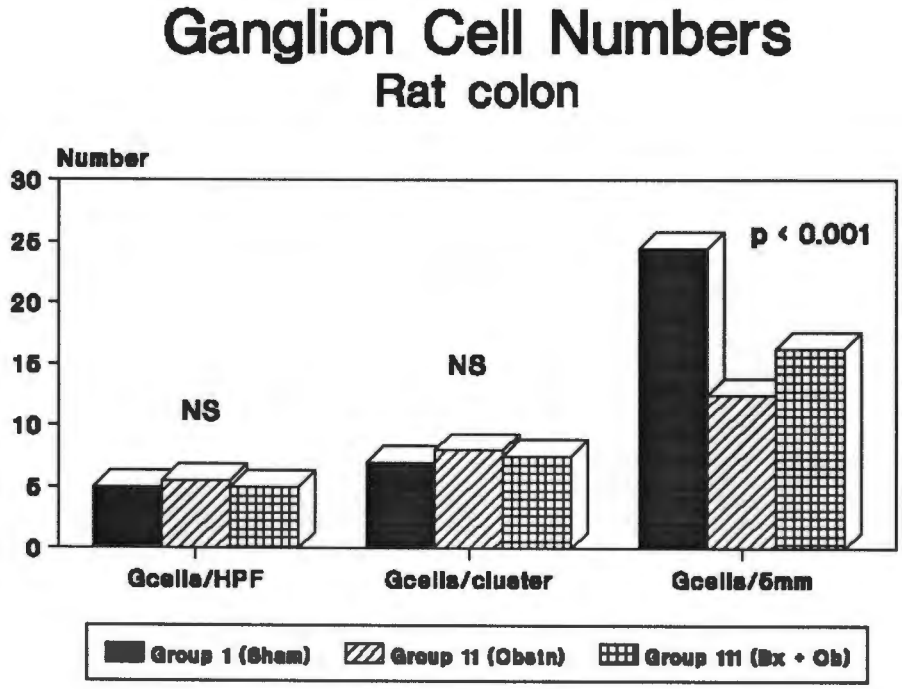


Figure 13.3 Bar graph showing ganglion cell counts per high power field (HPF), per cluster and per 5 mm of histological section of rat colon.

TABLE 13.1

GANGLION CELL NUMBERS IN RAT COLON

	no/cluster	no/HPF	No/5mm
Group1 (n = 16)			
a) controls	8	5	23
b) sham	7.5	7	24.5
Group 2 (obstruction) (n = 18)	7.5	5	12.5
Group 3 (biopsy + obstn) (n = 9)	8	6	17

Ganglion cell size as measured by the cut surface of the ganglion cells demonstrated no difference between those with obstruction and those without.

The ganglia appeared more attenuated and spread out following obstruction but had undergone no significant size alteration. The attenuated shape of the ganglia probably resulted from the non compressability of the cellular organelles and the spreading out of the ganglion cells with intestinal dilatation.

13.4.2.2 *The results of AChE histochemical staining*

The results of histochemical AChE staining as well as immunocytochemistry of the sympathetic nervous system are listed in **Table 13.2.**

No significant differences could be detected between those with and those without prolonged intestinal obstruction. Similarly, observed differences in the visual analogue scores of AChE activity did not reach significant levels.

TABLE 13.2

HISTOCHEMICAL AND IMMUNOCYTOCHEMICAL ASSESSMENT OF RAT COLON

	AChE +	AChE -	TOH +	TOH -
Group 1 Controls + Sham (n = 16)	5	8	2	3
Group 2 Obstruction (n = 18)	10	8	2	2
Group 3 Biopsy + obstn (n = 9)	4	5	2	3

13.4.3 The effect of different anaesthetic regimes

By way of contrast, increased AChE staining was noted in 14 out of 16 obstructed animals subjected to the Ketamine/Xylazine anaesthetic protocol and in none of those anaesthetized by a Ketamine/Ether protocol (Kruskal-Wallace, $p < 0.001$). A slight increase in muscle AChE was noted in the latter group but nerves did not appear to be affected. A comparison of AChE staining patterns (**Table 13.3**) demonstrated the significance of this difference (Kruskal-Wallace, $p < 0.001$). In those subjected to Xylazine anaesthesia, the main increase in activity was noted within the muscularis propria itself but existing nerves within the muscularis mucosa and lamina propria were also affected. Increased AChE activity was observed in Meissner's plexus and at the submucosal-muscularis propria interface in 10 rats, suggesting increased activity in this region (**Figure 13.4**). Similar features were noted in controls (**Table 13.3**). In 3 specimens

TABLE 13.3

INFLUENCE OF ANAESTHETIC AGENT ON ACETYLCHOLINESTERASE

	Xylazine			Ether		
	n	AChE +	Mode	n	AChE +	Mode
Group 1 (Sham)	5 *	5	+	5	1	Nil
Group 2 (Obstruction)	12	10	+ +	6	0	Nil
Group 3 (biopsy + obstruction)	4	4	+ +	5	0	Nil

(* 3 excluded for technical reasons)

from Xylazine anaesthetized rats, the biopsy was too small to evaluate the muscular layer. These 3 were excluded from this part of the study.

Submucosal AChE staining appeared to be mostly related to blood vessels and no new nerves appeared to have developed. An increase in the staining of neurofibrils in the lamina propria did occur in 10 animals with obstruction following Xylazine anaesthesia. These neurofibrils were not marked but fine and wispy and did not exceed 1 per crypt in number.

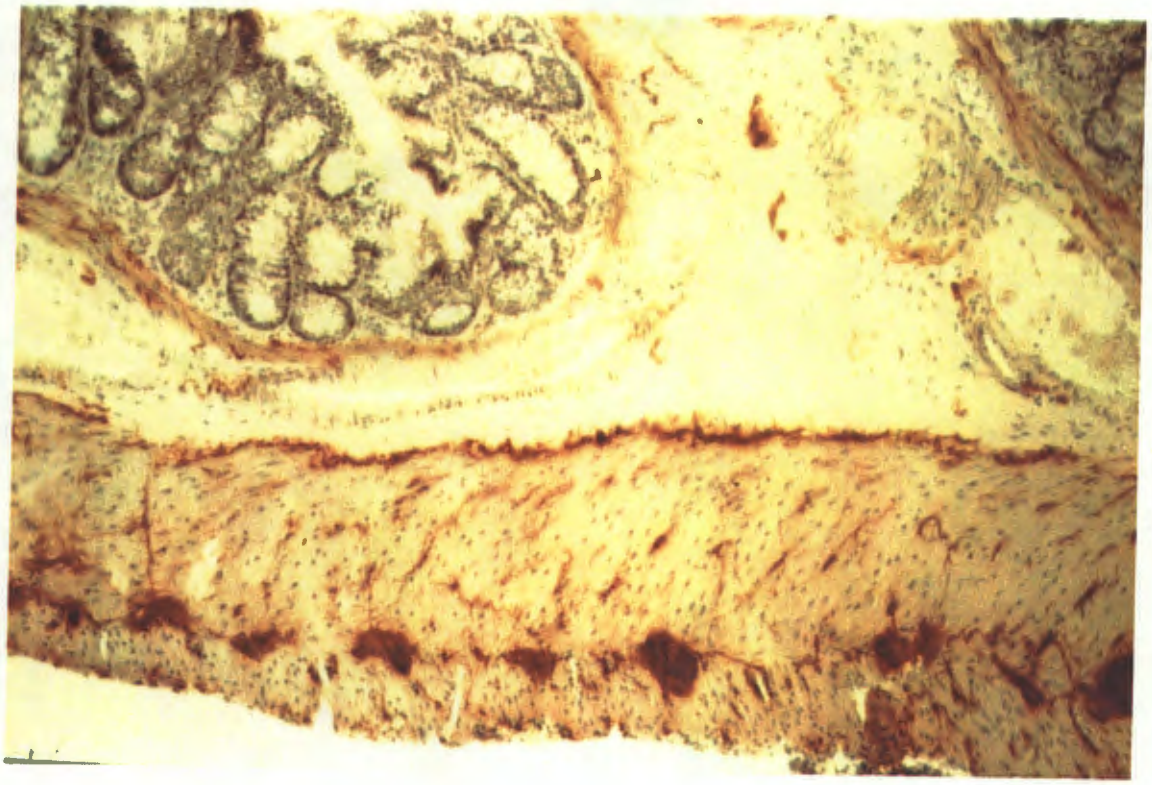


Figure 13.4 (a): Section of colonic wall from rat anaesthetized with Xylazine showing increased AChE staining in muscularis mucosa, submucosa, muscularis propria and ganglia (x 40).

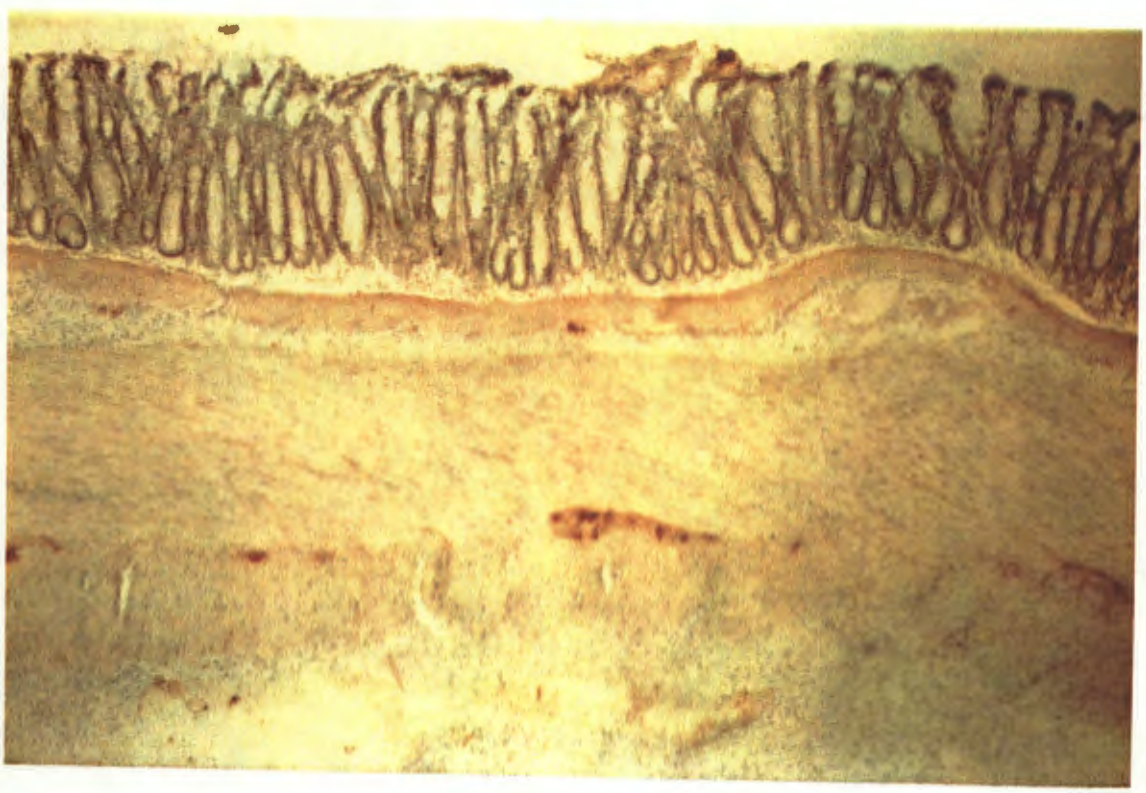


Figure 13.4 (b): Section of colonic wall from rat anaesthetized with Ether showing absence of AChE activity in the muscularis propria, submucosa and muscularis mucosa (x 40)

13.4.4 Tyrosine hydroxylase (TOH) staining patterns

Sympathetic activity was assessed by monoclonal antibody staining for Tyrosine Hydroxylase, the rate limiting enzyme of noradrenaline and adrenaline production. Staining patterns were not increased in 15 animals with subacute obstruction and failed to show any significant differences from the staining patterns observed in controls (**Table 13.2**). There was also no significant difference noted in TOH activity using various anaesthetic agents. Ganglia did however stain more prominently for TOH in young rats, suggesting that the sympathetic nervous system is more active in the younger animal.

13.4.5 The effect of surgical procedures on ENS morphology

Despite generous lower colonic biopsies with transverse intestinal repair in rats from Group 3, no differences were noted which could be attributed to surgery per se. This was also true in one rat where the bowel had been completely transected and an end to end anastomosis performed.

13.5 DISCUSSION

13.5.1 Evaluation of the animal model

AChE activity in the myenteric plexus of the rat, rabbit, chick and mouse have been extensively studied by light microscopy (Cantino 1970; Keller 1976; Webster 1973). Histochemical AChE staining has been shown to be detectable in the fetal rat at 18 days gestation and becomes a progressively stronger staining pattern throughout the rest of the gestational period (Cantino 1970; Ito 1984). A similar pattern of localization of AChE nerve fibres has been demonstrated in fetal, newborn and adult rats. Electron microscopic localization of intracellular AChE appeared to be mainly in the nuclear membranes and rough endoplasmic reticulum and this was used to identify the ganglion cell structures

(Ito 1984). From these studies, the inference has been drawn that the degree of AChE staining pattern reflects the degree of maturation of the myenteric plexus.

A postnatal increase in the number of neurones is also well established in the central nervous system of the rat (Altman 1966) as well as in the enteric nervous system (Gabella 1971). Experimental studies of the alterations in guinea-pig ileum resulting from surgically induced stenosis has shown an increase in the number and volume of neurones and ganglion cells (Gabella 1971). Alterations with age included a decrease in the number of cells in the myenteric plexus and a decrease in the cholinesterase activity in skeletal muscle and in heart muscle. These features were not found in the gastrointestinal tract levels of AChE activity. Studies in rat enteric nervous systems have shown an increase in cell numbers within 10 days of the experimental production of a subacute obstruction but that increase in cell size occurs only after 25-40 days (Filagamo 1954). Gabella (1971) has postulated that the increased numbers of ganglion cells in the small bowel accompanies the growth of the gut. This increase in nerve cells is followed by an increase in the size of small neurones which is then followed by a further increase in the neurone size (Gabella 1971).

The choice of an animal model of freshly weaned rats in this study allows for a certain amount of development to take place as the bowel matures and develops. This model restricts observations to postnatal influences only and does not account for prenatal developmental influences which may occur (Pickard 1981). A rider on animal studies in which the rat is the experimental animal is that the rat is noted for the immaturity of its enteric nervous system at birth. Any observations require validation in the human ENS before conclusions can be drawn (Gabella 1971).

13.5.2 The influence of prolonged obstruction on the number of neurones in distal rat colon

A count of the number of neural cells in rat tissue has previously been found to be technically difficult (Irwin 1931). An increase in ganglion cell numbers has been reported after birth. By way of example, a total of 420,000 neural cells has been estimated in the small bowel of a newborn rat whereas this number increases to 1,850,000 in the adult rat (Gabella 1971). Gabella (Gabella 1971) has shown that in the newborn rat small intestine, the neurones are more crowded than in the adult rat and he reported a total of 9405 +-977 nerve cells per cm in the small bowel. Variations in the degree of distension did not affect the counts in these animals (Gabella 1971).

Of particular interest to this study is an increase in the number of neurones in the hypertrophic ileum just above an experimentally induced stenosis (Gabella 1975). In a similar study on fetal lambs, Pickard (1981) reported hyperplasia of the ganglion cells in the dilated segment just proximal to the experimentally induced atresia. What makes this study of particular interest to the investigator interested in secondary effects of obstruction is the decrease in AChE staining noted in these specimens. Based on these experiments, Gabella (1971) hypothesized that the development of nerve cells takes place in three phases. Following the initial increase in cell number, there is an increase in size of nerve cells which is followed by a further neuroplasm increase once the reserve pool has been exhausted (Gabella 1971). In keeping with this hypothesis, there was an increase in nerve cell numbers within the first 10 days of an experimentally produced obstruction and this was followed by an increase in neuronal size between 25 and 40 days (Filagamo 1954). One disadvantage of these particular studies was that in the experimental model, ischaemia of the bowel resulted. In order to study the effects of obstruction in this study, an animal model was created in which the ischaemic element was minimized. Some similarity exists

between this experimental model and that of an encircling wire around a growing tree, in that the obstruction increased as the animal grew.

By way of contrast to these observations, this study demonstrated a decrease in the number of ganglion cells per 5 mm of histological section in the obstructed group. The probable explanation for this observation is the increase in bowel luminal diameter in the absence of an increase in the ganglion cell number. In the light of this finding, a reasonable conclusion is that there was probably no significant increase in ganglion cell numbers. No other specific morphological changes were noted in those with obstruction.

13.5.3 The pharmacological influence of anaesthetic agents and the importance of the alpha-2 adrenergic receptor in AChE increase

The CNS contains alpha-2 adrenergic receptors in both pre and post synaptic sites (Shipton 1991) and it is assumed that the same is true for the ENS. The known inhibitory effect of the adrenergic nervous system on intestinal motility is thought to be partially due to the action of the alpha-2 adrenergic receptor on the presynaptic nerve terminals of the cholinergic system (Allert 1987).

The current interest in the use of alpha adrenergic agonists in anaesthesia and analgesia has been largely due to their desirable central physiological effects which include anxiolysis, sedation, decrease in salivary secretion, bradycardia and a moderate drop in blood pressure. A side effect of these drugs is the inhibitory effect of the adrenergic nervous system on intestinal motility which is thought to be partly due to the action of the presynaptic alpha 2 adrenergic receptors on cholinergic nerve terminals.

The widespread use of alpha-2 agonist, Xylazine, in veterinary medicine is well established, particularly for its sedative role in the "darting" of wild animals. The

use of Xylazine in small laboratory animals is less clear. The influence of Xylazine on the gastrointestinal tract is well documented and results in a rapid decrease in smooth muscle tone even at low dosage (product information Rompun,Bayer). This action on the GIT is a probable result of an alpha-2 mediated decrease in sympathetic outflow via the vagus (Allert 1987). An alternative explanation is that Xylazine acts directly on the presynaptic alpha 2 adrenergic receptor as an alpha-2 receptor agonist (Gross 1989), inhibiting ACh release and thus decreasing intestinal motility. Although the sympathetic nervous system has been shown to modulate ACh release (Beani 1969), the increase in AChE is an unusual observation. Tyrosine hydroxylase activity was not increased which suggests that the increase in AChE production which followed stimulation of the alpha-2-adrenergic receptors may be caused by an as yet unknown synapse between the two circuits. It is possible that this connection may be at an axonal level thus avoiding the usual forms of neurotransmission. There are recent reports of alternative pathways which are ATP related which if applicable may explain this observation (Edwards 1992).

In a recent study by Visi et al (1990), the use of the alpha 2 receptor antagonist, Yohimbine, did not appear to enhance acetylcholine release in surgically resected colonic specimens from patients with Hirschsprung's disease. In addition, when noradrenaline was added to these specimens there was a reduction in the stimulation evoked release of acetylcholine in the affected aganglionic segments. These findings led Visi et al (Visi 1990) to postulate that an increase in acetylcholine release plus the lack of alpha-2-adrenergic receptor mediated noradrenaline modulation may partly explain the abnormal function of the affected distal aganglionic segment in patients with Hirschsprung's disease.

Once again, this demonstrates the extreme complexity of the enteric nervous system as well as the neural control of neurotransmitter function. A relationship

between the sympathetic nervous system via the alpha-2-adrenergic receptor and increased AChE is of importance in Hirschsprung's disease (Ehrenpreiss 1966) and NID. The sympathetic nervous system has been implicated in both of these conditions (Ehrenpreis 1966; Touloukian 1973). Aplasia of the sympathetic nervous system has been identified in NID type A (Fadda 1987). The relationship of the sympathetic nervous system to NID Type B is as yet unclear. Should such a relationship exist, further studies in the exact role of the alpha-2-adrenergic receptor may help to clarify the issue.

13.6 CONCLUSION

The number of neurofibrils and distribution of the cholinergic nervous system were not affected by prolonged intestinal obstruction in experimental rats. No hyperganglionosis or other histological features of NID developed. The results of the pharmacological stimulation of the cholinergic system via the alpha-2 adrenergic receptors suggests that this may be related to an increase in AChE expression. This may provide a possible explanation for the pathophysiological process causing increased AChE activity in the aganglionic segment of patients with Hirschsprung's disease or NID. Although an interesting speculation, definitive proof is lacking. The increased number of AChE staining neurofibrils noted in the intestinal wall in patients with neurodevelopmental conditions is not explained by this hypothesis.

The ability of an alpha-2 adrenergic agonist to pharmacologically stimulate the production of AChE within the nervous system raises new possibilities as to the possible mechanism of the increased AChE within the aganglionic segment. It is in all probability not an isolated factor in producing the histological features associated with Hirschsprung's disease. It is conceivable that the alpha-2 adrenergic receptor may have some role to play in maintaining the obstructive element postoperatively in certain patients with surgically treated Hirschsprung's disease.

SECTION E

CHAPTER 14

CHOLINESTERASE ACTIVITY AND MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN HIRSCHSPRUNG'S DISEASE

14.1 INTRODUCTION

14.1.1 The nature and function of AChE in Hirschsprung's disease

Increased levels of AChE activity have been identified in the aganglionic segment of Hirschsprung's bowel by both histochemical (Meier Rüge 1972) and biochemical methods (Boston 1975; Dale 1977, 1979; Bajgar 1979; de Wet 1980; Rakonczay 1984; Bonham 1985, 1988). The etiology and role of the increased AChE activity in the affected tissue of Hirschsprung's disease is as yet unclear.

Quantitative AChE assay has been shown to correspond with a histological increase in AChE staining (Boston 1975; Dale 1979; Patrick 1980; de Wet 1980; Bonham 1985). It has been suggested that this could form the basis of an additional investigation confirming the diagnosis of Hirschsprung's disease (Boston 1975; Dale 1979; de Wet 1980; Bonham 1987).

An increase in the total cholinesterase activity, comprising both specific acetylcholinesterase (AChE, Acetylcholine hydrolase, 3.1.1.7) and butyrylcholinesterase (pseudo-cholinesterase; BChE; Acylcholine acyl hydrolase, 3.1.1.8) was first reported in rectal suction biopsies by Boston (1975) from Newcastle. A threefold increase in the total cholinesterase levels in aganglionic tissue as compared to controls was reported in the rectal biopsies of 19 patients with Hirschsprung's disease. These findings were confirmed in a follow-up

study at Red Cross Children's Hospital by De Wet and Boston (de Wet 1980) and in other reports from the Newcastle unit (Dale 1977, 1979; Bonham 1985,1987). The use of the biochemical activity of AChE was suggested as being of possible diagnostic value. Levels of total cholinesterase activity (ChE) ranged between 0,3-5,8 units (mean 2.6 ± 1.0) in controls and 2.1-7.9 units (mean 3.8 ± 2.0) in aganglionic tissue in these studies. Reported AChE levels ranged between 0.3-7.9 units (mean 2.7 SD 1.4) for normal tissue and 5.3-30.2 (mean 15.2 SD 9.0) in the aganglionic bowel (Dale 1979). The reported percentage of AChE (the percentage of total AChE and non specific ChE activity) in ganglionated bowel was 27-73% (mean 50.8 ± 7.7%) compared with 70-84 % (mean 78.5 ± 6.8%) in aganglionic bowel (Dale 1977, 1979). An overlap of AChE activity levels between aganglionic bowel and ganglionated bowel in certain cases led Dale et al (1979) to suggest that the actual activity level was a more reliable unit of measurement for diagnostic purposes (Dale 1979). The reason for this step was the "grey zone" of intermediate results which was also reported in other studies (de Wet 1980). A possible reason for these lower values could be that the AChE:BChE ratio differs in patients with co-existing NID. If this difference were to be confirmed then biochemical assay may be of value to differentiate normal from abnormal bowel.

Additional reports of raised serum AChE activity levels in patients with Hirschsprung's disease (Boston 1978; She 1984) have been questioned in recent studies (Okasora 1983; Atias 1991) and remain a matter for further investigation outside of the scope of this study.

14.1.2 AChE molecular forms in Hirschsprung's disease

AChE intracellular activity has been shown to consist of four molecular AChE isoforms based on sucrose gradient analysis results (Vediere 1982; Swerts 1984). The active site of the AChE molecule has been recently demonstrated

by X-Ray crystallography to be at the bottom of a narrow gorge deep within the protein (Sussman 1991). Relatively little is known about the function, cellular location and control of the secretion of AChE in the autonomic nervous system. Because of the pathophysiological importance of AChE, its role in neurotransmission, structure and involvement in neurological disease as well as its possible functions are of considerable interest at present (Brimijoin 1991).

Isomeric forms of AChE are evident throughout a wide range of vertebrates suggesting that these molecular forms fulfil specific physiological functions (Massoulie 1982; Fernandez 1984). A broad differentiation can be made into globular and asymmetric forms of AChE. The six molecular forms which have been identified to date include 3 asymmetric (A12, A8, A4) and three globular forms (G4, G2, G1) (Bon 1979). The structure of these isomers have been shown to consist of globular monomers, dimers, tetramers and asymmetric forms and may be distinguished by sucrose gradient sedimentation analysis (Fernandez 1984; Bonham 1985). Globular isomeric forms may be further subdivided into those that do and those that do not interact with non-ionic detergents (Rakonczay 1981). Those molecular forms of AChE interacting with non-ionic detergents consist mainly of the AChE G4 isoform and are presumed to represent integral membrane proteins.

The tetrameric membrane-bound form of AChE appears to be the predominant form of the enzyme found within the central nervous system and it has been found in bovine (Chan 1972), rodent (Wenthold 1974; Rakonczay 1981) as well as in human brain (Sorenson 1982; Grassi 1982) and nerve tissue (Hall 1973; Kasa 1982). Most of the AChE in peripheral nerves is the 10S tetrameric form which is primarily associated with the external surface of the axolemma (Hall 1973; Rakonczay 1984).

Alterations in AChE isomeric forms in the aganglionic segment of Hirschsprung's disease have been reported (Bajgar 1979; Rakonczay 1984; Bonham 1985, 1988). Bajgar and Hak (1979) reported a second electrophoretic band of AChE activity in patients with Hirschsprung's disease which was not present in the control group. Bonham (Bonham 1985) reported a specific raise in the AChE tetramer G4 (270kDa) in the aganglionic segment on ultracentrifugation sedimentation criteria in 3 patients with Hirschsprung's disease. The difference in G4 AChE levels between normal and aganglionic zones were reportedly more marked in his series than was the total AChE activity. AChE types G2 and A12 were largely unchanged in the aganglionic zone but an additional moderate increase in the monomer G1 was also noted. Rakonczay (Rakonczay 1984) has suggested that this rise in AChE tetrameric form is merely related to the pathologically abnormal innervation of the aganglionic bowel. From the above findings it appears to be important to establish whether the entire cholinesterase fraction or merely a specific subfraction is affected in Hirschsprung's disease. Further study into the function and role of AChE may, in turn, lead to a better understanding of the molecular function of the AChE molecule and to provide some clues regarding its role in neurodevelopment.

14.1.3 Structure and function of AChE molecular forms

The structure of these isomeric forms of AChE varies. Dimers consist of 2 monomers linked by a disulphide bridge and tetramers consist of 2 dimers linked by quaternary interaction (Massoulie 1982). Those molecular forms of AChE interacting with non-ionic detergents consist mainly of the AChE G4 isoform and are presumed to represent complete membrane proteins. The A12 AChE isoform has a collagen tail and appears to be mainly anchored to the basal laminae in the synaptic clefts. It is thought to control the amount of acetylcholine reaching the AChE receptors on the muscle surface. The function of the G1 and G2 intracellular forms is also unknown but they are thought to probably represent

precursors of the more complex forms. Although the function is unknown, the membrane bound form is thought to possibly be responsible for the hydrolysis of acetylcholine following interaction with postsynaptic receptors. On the other hand, there is an increasing appreciation of alternative roles played by AChE. These include the hydrolyzation of other neurotransmitters such as substance P and met-enkephalin (Chubb 1980,1982) and a neuromodulator role in neurogenesis (Layer 1991).

14.2 BIOCHEMICAL METHODS OF EVALUATING ACHE

14.2.1 Biochemical assay of AChE activity

Biochemical assay of AChE activity is based on the Ellman Reaction (Ellman 1961) which measures the rate of production of thiocholine during the hydrolysation of acetylcholine by the enzyme in the homogenate. As part of this process, the continuous reaction of the liberated thiol group with the colour reagent produces the yellow anion of 5 - thio -2- nitrobenzoic acid. The rate of this colour production is measured in a recording spectrophotometer at 412 NM (peak absorbance of the yellow anion).

This action of AChE on thiocholine is the same principle utilized for the Meier-Rüge histochemical stain which demonstrates AChE positive neurofibrils by a brown stain (**Appendix C**). The selective inhibitor utilized in the histochemical stain is Iso-Ompa, an organophosphate.

Standard procedures for evaluating molecular forms of AChE include differential solubilization, velocity sedimentation in a sucrose gradient, techniques to reveal mode and degree of attachment to cellular structures or reflecting differences in quaternary structure (Hall 1973; Inestrosa 1981; Younkin 1982; Massoullie 1982; Rotundo 1984).

14.2.2 External factors affecting AChE activity

AChE activity may be affected by a number of external factors which include changes in temperature, pH and substrate concentration.

In mammalian tissue, AChE acts optimally at a temperature of between 37-40 degrees centigrade (Augustinsson 1948). Wu et al (Wu 1987) have demonstrated that there is loss of AChE activity due to denaturation at moderate pH and moderate temperature. pH changes below a pH of 7 may influence AChE activity but little alteration of activity occurs above this level (Bergman 1958). AChE is inhibited when an excess of substrate is present (Ellman 1961; Garry 1965; Hall 1973).

Other **physical factors** which affect AChE activity include **age** (de Brito 1987) and **stress** (Pryor 1966; Ebel 1963; Waller 1983). **Maternal undernutrition** has been shown to affect CNS AChE in progeny (Sereni 1966; Adlard 1971; Eckert 1979). By way of contrast there do not appear to be any differences attributable to race or sex (Woolley 1963; Pryor 1966).

14.3 AIMS

The aims of this study were as follows

- Evaluate the relationship between AChE levels and histochemical staining patterns in full thickness tissue sections.
- Authenticate the nature of the link between AChE levels and affected colonic tissue in Hirschsprung's disease
- Explore and identify the distribution of molecular AChE forms in histologically evaluated tissue.
- Specifically evaluate AChE activity in patients identified with Neuronal Intestinal Dysplasia co-existing with Hirschsprung's disease.

14.4 MATERIALS AND METHODS

14.4.1 Patient study group

The AChE activity in the ganglionated proximal colon of patients undergoing surgical pull-through for Hirschsprung's disease was evaluated. Control tissue was obtained from 3 further groups for reasons of comparison with AChE staining in normal bowel.

14.4.2 Recruitment of patient sample

The recruitment of patient sample was from the group of patients assessed in the previous chapter. Hirschsprung's disease patients presented for surgery between January 1988 and January 1991. The control patients group included those with a histological picture of NID in the absence of Hirschsprung's disease diagnosed by an experienced pathologist with a special interest in this field. Further control tissue specimens originated from surgical specimens in patients with conditions not related to Hirschsprung's disease or NID.

The group of patients with sections taken at postmortem which were used for the ganglion cell studies were not included in this study due to uncertainty about the effect of neurotransmitter alterations following death (Testylier 1991).

14.4.3 Demographic data and ascertainment of data

The methods of recording clinical information as well as the ascertainment of data were identical to those described in the previous chapter.

14.4.4 Collection, storage and preparation of specimens

The intestinal specimens were collected as part of the prospective study outlined in chapters 10 and 11. Specimens were taken adjacent to those submitted for histological evaluation and transported to the laboratory and snap frozen in liquid

nitrogen. These specimens were then stored at minus 60 degrees centigrade until assay was performed.

To eliminate the possible effects of erythrocyte and serum AChE, randomly selected specimens were subjected to a carefully controlled washing technique (10 times in normal saline). Comparison with results from unwashed samples was performed to identify the effect of red blood cell AChE on results.

14.4.5 Acetylcholinesterase extraction and assay

Full thickness intestinal and mucosal samples of the stored surgically resected tissue were thawed and homogenized in 0.1 phosphate buffer (pH 7.0), using a Potter homogenizer in 10 volumes of a 50 mM Tris HCl buffer at a pH of 7.4 (containing 5 mM of PMSF [phenylmethylsulphonylfluoride], 1% Triton, 1M NaCl and 3 mM EDTA).

Biochemical assays of AChE activity were performed by the Ellman Method (Ellman 1961) using 10 μM Lysivane (10-(2-diethyl-aminopropyl) phenothiazine; Ethopropazine; Sigma) as a specific BChE inhibitor. The level of specific acetylcholinesterase was calculated by subtracting the inhibited AChE level from total cholinesterase level following the method of Dale et al (1977).

[ie $R = 0.00055(\blacktriangle A - 0.001)/Co$]

[R = mol substrate hydrolysed min^{-1} g^{-1} wet weight tissue.

Co = tissue concentration in mg/ml

$\blacktriangle A$ = change in absorbance per min]

Enzyme activity was measured in units per litre (per gram of wet tissue). One unit, by definition, is the amount of enzyme that will ensure the conversion of 1 μmol of the substrate under standard conditions.

The percentage AChE was obtained by expressing the activity levels as a percentage of total cholinesterase activity [ie. (AChE activity/total cholinesterase activity) X 100] (Dale 1977).

14.4.6 The use of markers for AChE isoform identification in density gradients

The sedimentation coefficients of AChE molecular forms are well known for both animal and human tissue (Bon 1979). To locate the position of AChE isoforms in sucrose gradients, markers of molecules of known S-value were used (**Figure 14.1**). The most valuable markers for this experiment were bovine serum albumen (4.65 S) and catalase (11.2 S) to mark the 4S and 10S positions respectively. These markers were spun on a gradient which contained no other sample. The gradient was then fractionalized by standard laboratory techniques and the position of the marker proteins located by their absorbance of ultraviolet light at 280 nM.

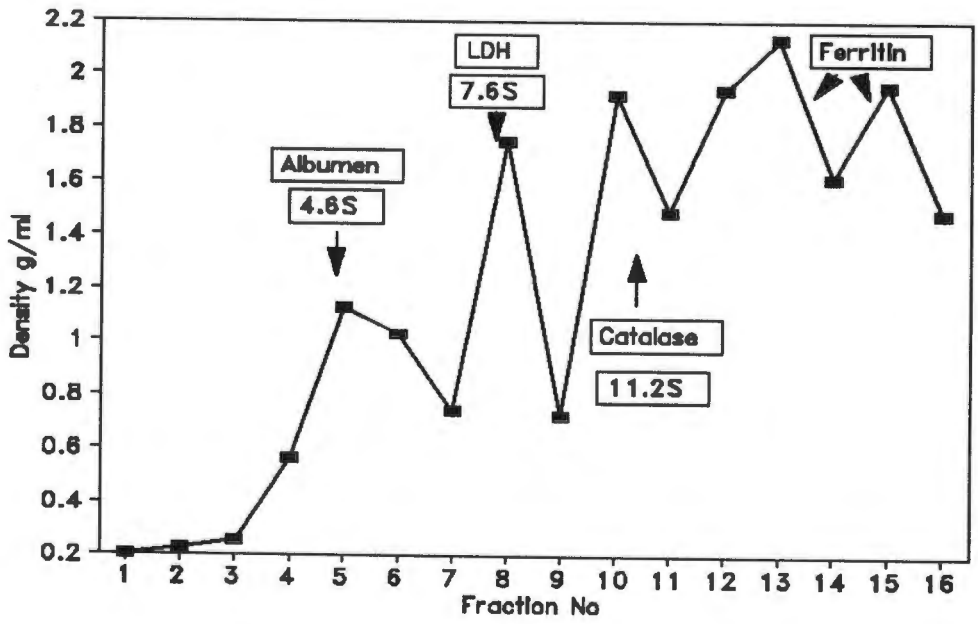


Figure 14.1 Graph showing marker proteins located by absorbance to ultraviolet light at 280 nM

Exact sedimentation coefficients of Ferritin were difficult to obtain because of variability depending on the Fe content in the molecule.

14.4.7 Determination of molecular forms of AChE in homogenized human tissue from patients with Hirschsprung's disease

Following clarification of homogenates at 14000 rpm (Beckman J5 refrigerated centrifuge), sucrose density gradients of the molecular weights of AChE isomers were then prepared by layering 300 μl of the homogenate in a 5-20% (w/v) linear sucrose gradient containing a buffer solution (viz. 1M NaCl, 5 mM EDTA, 50 mM Tris-HCL (pH6.8) buffer and 0.1% Triton X-100). Ultracentrifuge centrifugation was at 30 000 rpm (Beckman L8 refrigerated ultra centrifuge; SW 50.1 rotor arm) for 20 hours at 4 degrees centigrade. Fractionation of the gradients was by upward displacement and 0.26 ml fractions were collected. The AChE activity of the individual gradients was assayed by the Ellman method (Ellman 1961) using Ethopropazine (Lysivane; Sigma) as a specific BChE inhibitor.

Density was directly determined by weighing of the samples. The collecting tubes were pre-weighed to 0.1 mg. The gradient was fractionated into 18-19 fractions of 0.26 ml per tube. Reweighing of the fractions permitted calculation of the fractions by the following formula.

(Tube + fraction weight) - (empty tube weight)

= weight of fraction per 0.26 ml in grams.

Density was then expressed as grams per millilitre and plotted against the fraction number.

14.4.8 Identification of AChE molecular forms

The AChE velocity sedimentation coefficients for the molecular forms were identified by peaks in activity levels on using the Ellman reaction and further identification using markers of known molecular weight (**Figure 14.1**). In practice the G1 and G2 activities were found to be very close together on velocity sedimentation gradients making separation difficult. In addition, the A4 and A8 asymmetric forms had very low activity levels. The method of Fernandez (1984) and Gregory (1989) to demonstrate the three main activity peaks was therefore adopted to interpret the data. According to this method, sedimentation values between 3.5-6S were taken to represent the G1 and G2 molecular forms, values from 9-11S to represent the G4 and those between 15-17S to represent the A12 molecular forms.

14.4.9 Sequential extraction of AChE isoforms

Following the extraction procedure previously outlined, homogenates were centrifuged at 14 000 rpm for 20 minutes. The supernatant was regarded as enriched with soluble hydrophilic AChE isoforms [low salt soluble extract (LSS)]. The pellets were then washed a further two times in the same buffer and rehomogenised with the same volume of buffer to which Triton X-100 had been added. Following further centrifugation the supernatant was identified as being enriched with amphiphilic AChE [detergent soluble fraction (DS)]. The pellets were once again washed and homogenized in the original buffer volume with the addition of 1 M NaCl and 3 mM EDTA. The centrifugation process was once more repeated and the supernatant was regarded as being rich in asymmetric isoforms [the high salt soluble extract (HSS)]. Density gradients performed on these samples were fractionated and AChE activity levels determined.

14.4.10 Specific reagents used in experimental procedure

Acetylthiocholine iodide (Cat No. A 5751), Eserine sulphate (Cat. No. E 8625) Ethopropazine (10-[2-Diethylaminopropyl]-phenothiazine or Lysivane) (Cat No. E2880) and DTNB (5,5'-Dithiobis - (2 Nitrobenzoic acid) 3 Carboxy-4-Nitrophenyl Disulphide) (Cat. No. D8130) were obtained from the Sigma Chemical Co. USA.

14.4.11 Statistical methods

Statistical methods included the use of means, standard deviations, median values and 95% confidence intervals to compare data. Non-parametric methods used for analysis of data included the signed Wilcoxon test for two tailed probabilities and the Kruskal Wallace non parametric ranks test for asymmetric data.

14.5 RESULTS

14.5.1 Cholinesterase activity

The median values of Total Cholinesterase (ChE) activity, are listed in **Table 14.1**. Levels of ChE ranged between 1.2 and 22.7 units in ganglionated tissue (median 5.6 units) and between 1.4 and 19.2 (median 7.9 units) in aganglionic bowel in Hirschsprung's disease. Differences in tissue cholinesterase (ChE) activity between specimens obtained from the aganglionic, transitional and normally ganglionated bowel of patients with Hirschsprung's disease lacked significance when compared to controls (**Figure 14.2**).

Levels of AChE activity (measured by Lysivane inhibition), ranged between 0.94 and 12.52 units per gram tissue in ganglionated tissue (median 2.3 units), and 2.48 and 16.9 units per gram in aganglionic bowel (median 5.6 units) (**Table 14.2**). There were significantly higher AChE activity levels (Kruskal-Wallace, $p<0.05$) in aganglionic bowel (**Figure 14.3**).

TABLE 14.1

TOTAL TISSUE CHOLINESTERASE (ChE) ACTIVITY (units/gram)

	MEDIAN	MEAN	S/DEV	RANGE	I/Q RANGE
Group 1 Aganglionic bowel (Hirschsprung's disease) (n = 16)	6.9	7.8	4.3	1.4	5.9
Group 2 Ganglionated bowel (Hirschsprung's disease) (n-16)	5.6	8.2	6.5	1.2 -22.7	4.0 -9.6
Group 3 Controls (n = 16)	8.0	9.8	4.6	4.9 -.24	7.7 -10.6

TABLE 14.2

TISSUE ACETYLCHOLINESTERASE (AChE) ACTIVITY (units/gram)

	MEDIAN	MEAN	S/DEV	RANGE	I/Q RANGE
Aganglionic Bowel (n = 16)	5.6	6.5	3.6	2.6-16.9	4.0-8.0
Ganglionated Bowel (n = 16)	2.3	4.1	3.7	0.9-12.5	1.5-4.4
Controls (n = 16)	4.4	4.8	2.6	1.2-13.5	3.2-5.3

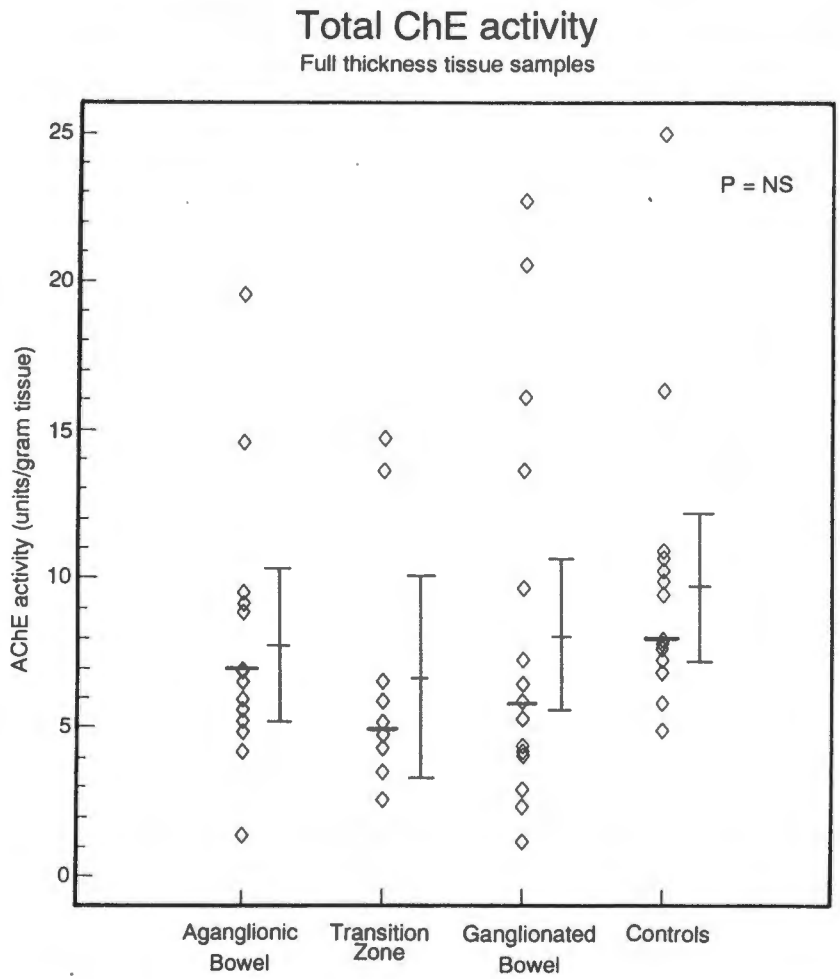


Figure 14.2: **Scatter plot showing distribution of ChE activity in aganglionic bowel in patients with Hirschsprung's disease and controls. Graph shows median values (broad line within data), mean values and 95% confidence intervals.**

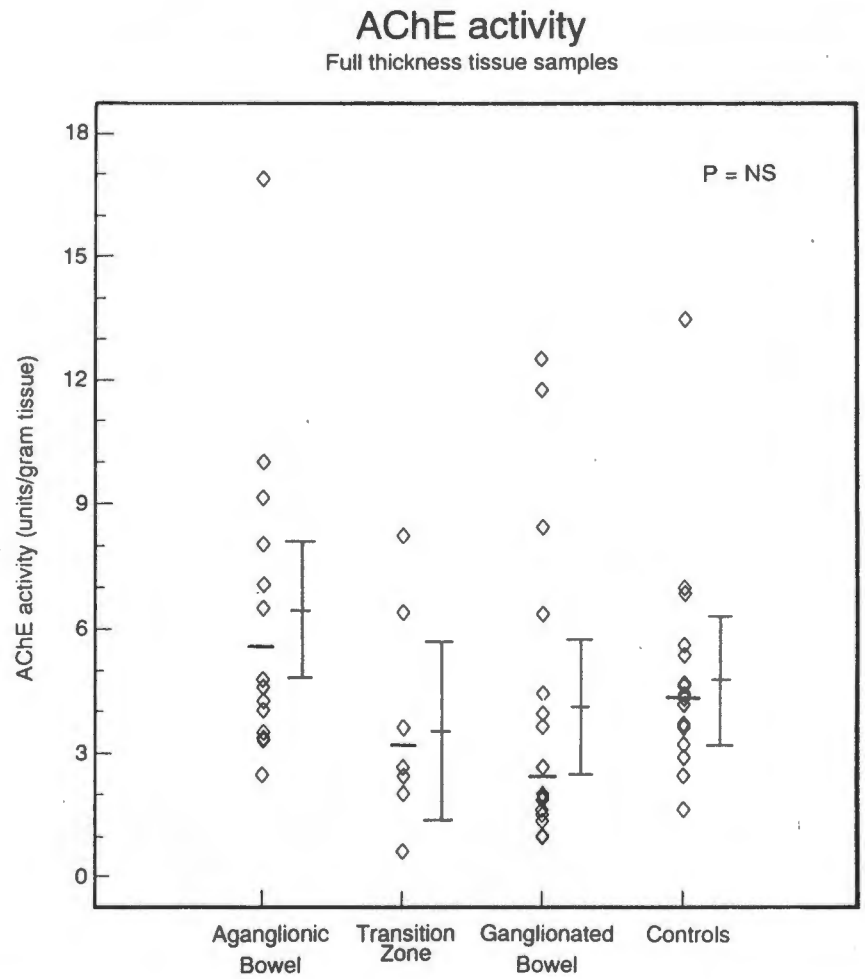


Figure 14.3: **Scatter plot showing distribution of AChE activity in aganglionic bowel in patients with Hirschsprung's disease (with or without obstructive symptoms) ganglionated bowel from Hirschsprung's disease and controls. Graph shows median values (broad line within data), mean values and 95% confidence intervals.**

The percentage AChE was also significantly higher in the aganglionic bowel (**Figure 14.4**). The median percentage AChE in ganglionated intestinal wall was 47.15% in contrast with the 78.75% median of the aganglionic segment (Kruskal Wallace, $p < 0.0001$).

Although lower AChE activity levels were detected in washed specimens, the differences lacked significance (**Table 14.3**). Storage did not appear to affect the results and no significant differences were noted in the AChE:BChE ratio in tests performed on fresh and stored specimens.

14.5.2 AChE molecular forms

When the tissue from affected and unaffected intestinal segments was fractionated on sucrose density gradients and analyzed by AChE enzyme assay, three major AChE containing peaks were found. The first at density 3 - 5 S corresponded to the monomer G1 and the dimer G2, the second at density 10 - 11 S corresponded to the tetramer G4 and the high density peak at approximately 17S to the collagen tailed form A12.

A significant increase in the percentage of the tetrameric AChE-G4 was observed in the affected aganglionic segment when compared to normally ganglionated bowel. This difference was not reflected in the AChE G1, G2 and A12 molecular forms. This is very clearly demonstrated in 6 patients where the paired samples had normal histological features without neuronal dysplasia or a transitional zone (**Table 14.4**). The difference between AChE G4 levels in aganglionic and ganglionated tissue was significantly different in these samples (Kruskal Wallace, $p < 0.01$). Although less marked, the increase in AChE G4 was also observed in bowel with histological features suggesting that the section came from the transitional zone (**Figure 14.5**).

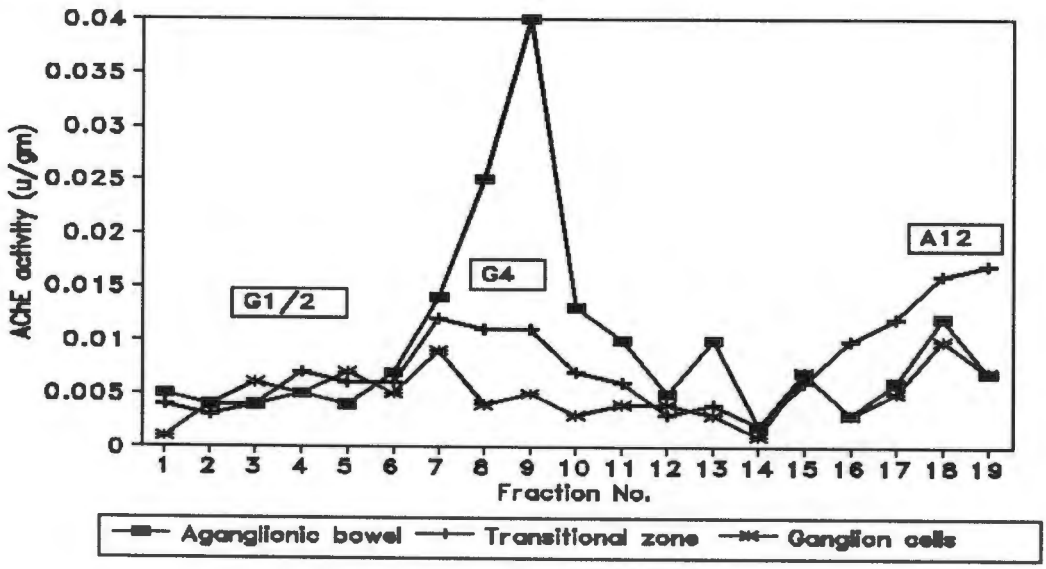


Figure 14.5: Density gradients from a patient (LA) with Hirschsprung's disease, demonstrating the differences between AChE isoforms in the aganglionic, transitional and ganglionated segments of bowel

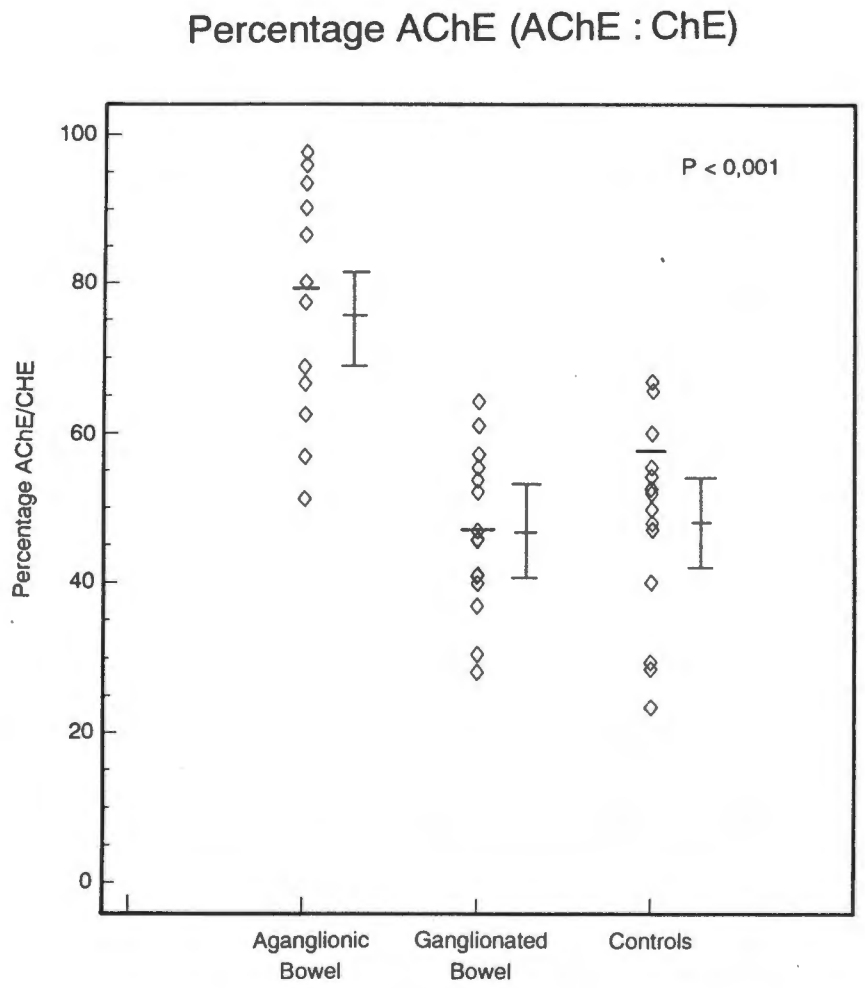


Figure 14.4: **Scatter plot of percentage AChE in full thickness samples of aganglionic and ganglionated bowel in patients with Hirschsprung's disease as well as controls. Graph shows median values (broad line in data), means and 95% confidence intervals.**

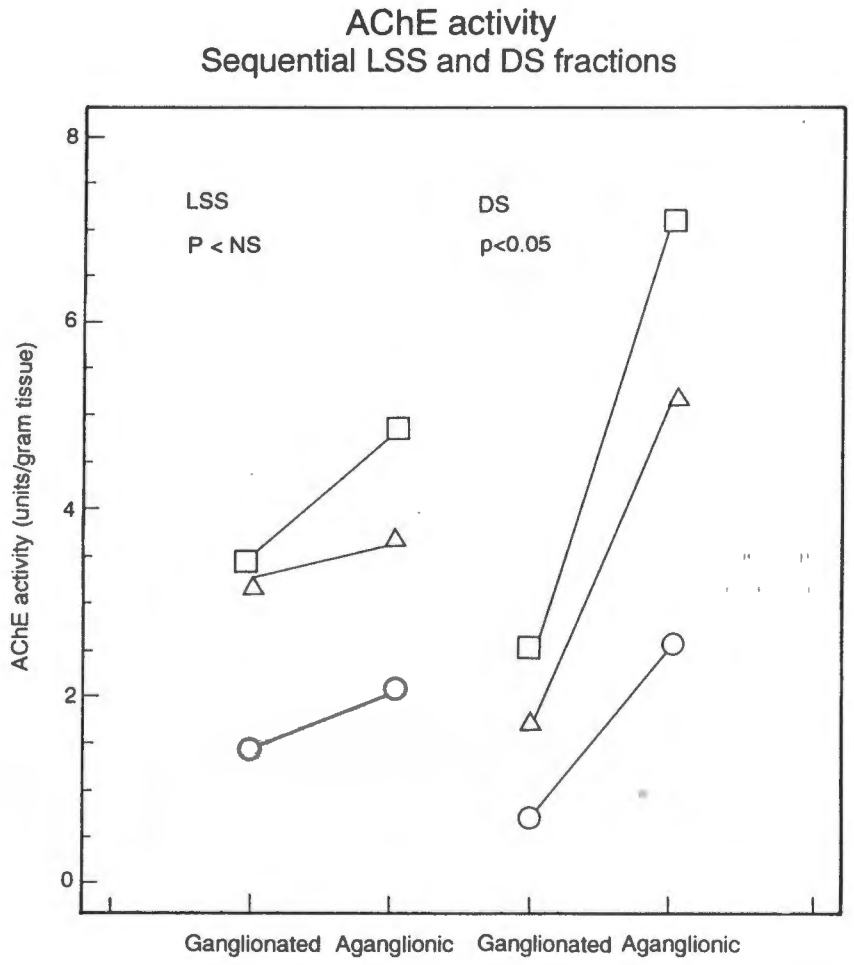


Figure 14.6: **Graph showing sequential extractions of AChE in low salt and in detergent-rich solutions in paired samples of aganglionic and ganglionated bowel.**

TABLE 14.3

THE EFFECT OF WASHING ON ACETYLCHOLINESTERASE ACTIVITY

	Percentage AChE activity				
	Mean	**S/Dev**	**Median**	**Range**	**I/Q range**
Washed specimens (n = 23)	61.2	17.0	59.9	30.8-93.4	45.9-77.4
Non-washed specimens (n = 23)	52.7	19.2	50	23.6-97.6	40.8-64.2
Wilcoxon Signs, p = 0.6					

TABLE 14.4

COMPARISON OF TISSUE AChE MOLECULAR FORMS - PAIRED SAMPLES

[Hirschsprung's disease (n = 6)]

	AChE G1/2	**AChE G4**	**AChE A12**
Aganglionic tissue	1.8 [±0.6] (20%)	4.5 [±0.9] (50%)	2.7 [±0.5] (30%)
Ganglionated tissue	1.9 [±0.7] (26%)	2.7 [±0.5] (37%)	2.7 [±1.6] (37%)
p value (Kruskal Wallis)	NS	<0.01	NS

TABLE 14.5

TISSUE BUTYRYLCHOLINESTERASE (BChE) ACTIVITY (units/gram)

	MEDIAN	MEAN	S/DEV	RANGE	I/Q RANGE
Group 1 Aganglionic bowel (Hirschsprung's disease) (n = 16)	0.27	0.47	0.61	1.4	5.9.9.4*
Percentage BChE	11.6%	15.7%	15.1%	0.26	3.01-26.8*
Group 2 Ganglionated bowel Hirschsprung's disease (n = 16)	0.77	1.32	1.89	0.13	0.29-1.28*
Percentage BChE	38.4%	34%	19.3%	4.7	13.2-46.9%*

*** Wilcoxon Signs, $p < 0.01$**

Further differences in AChE activity in the sequential extracts of the LSS and DS fractions show a difference in the detergent soluble extract in the aganglionic bowel (Kruskal Wallace, $p<0.05$) (**Figure 14.6**). This difference was not evident in the low salt or high salt extracts but was present in the detergent soluble (DS) sample. This suggested that the increase in AChE-G4 is mainly in the membrane bound amphiphilic subtype. On velocity sedimentation of the sequential extracts, the LSS extract was chiefly made up of the G1, G2 and some soluble G4 isomeric forms. The detergent soluble fraction mostly contained the G4 isomer but slight activity was noted in the G1 and A12 positions. The HSS fraction consisted mainly of the A12 isomer, as expected.

By way of contrast, the AChE levels appeared to be lower in the aganglionic zone in patients with residual dysplastic features of the enteric nervous system.

14.5.3 BChE levels in Hirschsprung's disease

A corresponding decrease in the percentage of BChE activity was observed in the aganglionic bowel when compared with controls and patients with normally ganglionated bowel (**Figure 14.7**). This decrease was of statistical significance on testing (Wilcoxon, $p<0.01$). In those patients with residual dysplastic histological abnormalities in the proximal bowel, a higher BChE activity level was noted in the aganglionic segment (**Figure 14.8**). This increase in BChE was not present in bowel specimens from those patients with Hirschsprung's disease without co-existing NID nor in controls. This would appear to be a significant difference in the samples tested and requires further investigation.

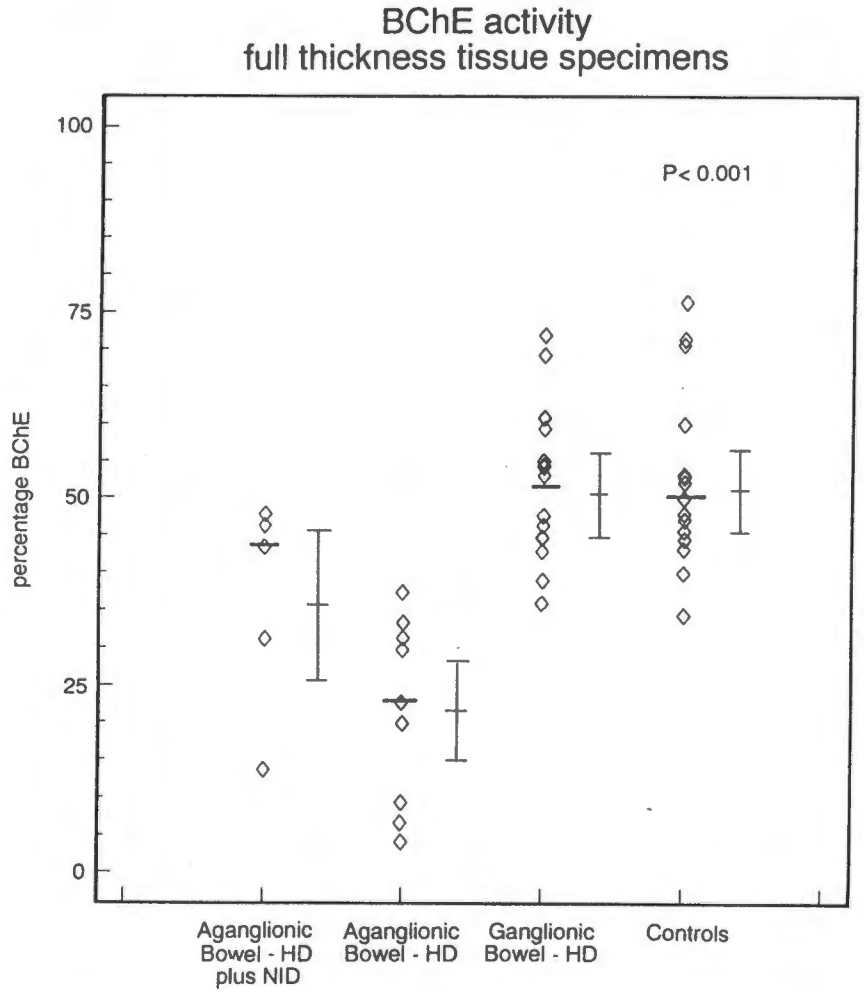


Figure 14.7: **Scatter plot showing distribution of BChE activity in aganglionic bowel in patients with Hirschsprung's disease (with or without obstructive symptoms), ganglionated bowel from Hirschsprung's disease and controls. Graph shows median values (broad line within data), mean values and 95% confidence intervals.**

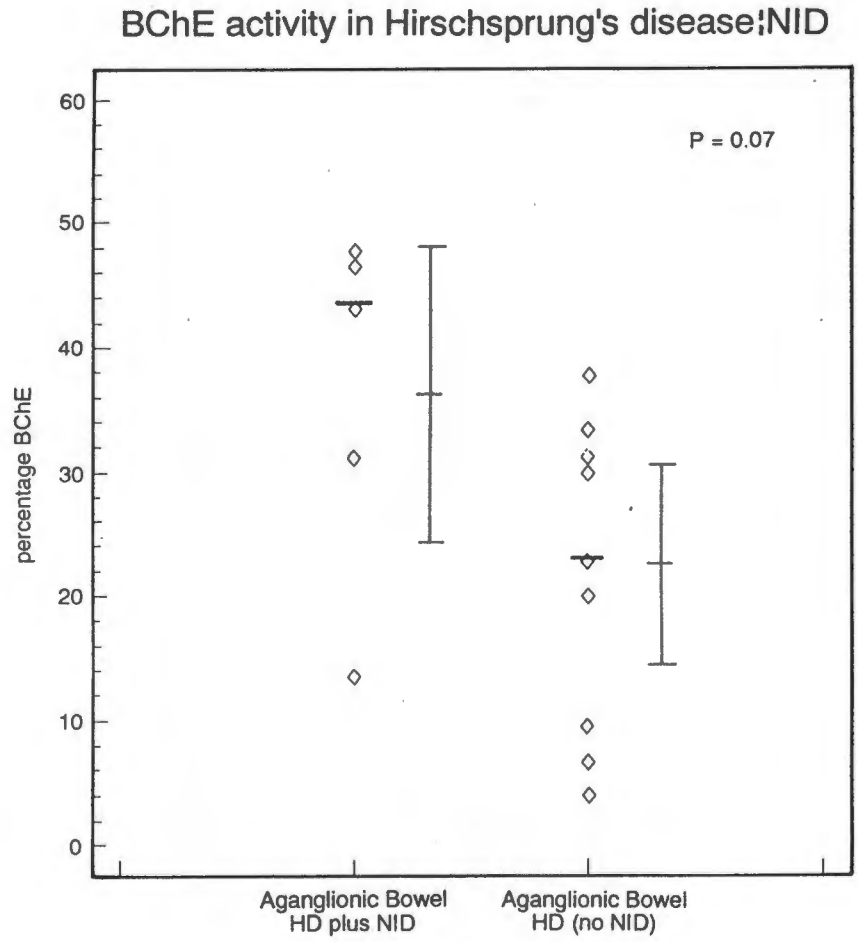


Figure 14.8: **Scatter plot showing distribution of BChE activity in aganglionic bowel (with or without NID in the proximal ganglionated bowel). Graph shows median values, mean values and 95% confidence intervals.**

14.6 DISCUSSION

14.6.1 Biochemical assay of AChE activity

Assessment of enteric AChE activity by quantitative biochemical analysis may be of some value in identifying residual disease in patients with Hirschsprung's disease. In addition, it may be of value in confirming the abnormal histological findings where debate exists.

The 95% confidence intervals of cholinesterase activity did not significantly differ between the aganglionic, transitional and normal segments of bowel from Hirschsprung's disease patients or from controls. The threefold increase in the total cholinesterase levels reported on rectal biopsy specimens (Boston 1975, Dale 1977) was not confirmed by this study of full thickness samples. A possible explanation of this discrepancy may be due to a high concentration of AChE G4 in the lamina propria (Bonham 1985).

In spite of this, the values obtained for ACHE activity in this study were similar to those reported by Dale et al for ganglionated and aganglionic tissue (Dale 1977).

By expressing the specific AChE activity as a percentage of the non specific ChE activity, the percentage of AChE was 47.15% and 78.75% in normally innervated and aganglionic tissue respectively. This is similar to the reported mean value of 54,6% for ganglionated and 78.9% for aganglionic tissue reported by Dale et al (1979).

Density gradient fractionation of whole homogenates of ganglionic and aganglionic tissue revealed a significant (Kruskal Wallace, $p<0.05$) difference in AChE-G4 as demonstrated by its sedimentation behaviour. This increased AChE-

G4 was identified chiefly in the detergent soluble extract, indicating that the isoform is amphiphilic.

From these results it may be concluded that the disturbance in cholinesterase expression in the aganglionic bowel of patients with Hirschsprung's disease lies chiefly in that proportion of AChE which is significantly increased (ie. AChE-G4). The increase in AChE-G4 was not mirrored by matching increases in the G1, G2, and A12 isoforms. It is also contrary to the anticipated response as the A12 molecular form is the one which has been shown to be "endplate specific" and has been shown to be the isoform induced by neural elements (Koenig 1978). The further possibility that the increase in the detergent soluble fraction represented more than one isoform (eg. AChE-A12 as well as AChE-G4) was eliminated by identifying the AChE-G4 content on sucrose velocity sedimentation.

Not only is the AChE activity increased in the aganglionic tissue, but there appears to be a further increase in the AChE:BChE ratio. This study confirmed the findings of Causse et al (1987) and Dale et al (1979), that the AChE:BChE ratio is increased. In keeping with this observation, the proportion of BChE was noted to be significantly lower in the aganglionic segment (Kruskal-Wallace, $p<0.05$) in Hirschsprung's disease. The group of patients with equivocal histochemical features and AChE activity within normal range is in keeping with previous reports (Dale 1977; de Wet 1980) where similar patients were identified. Of considerable interest were the differences between the AChE:BChE ratio noted in this group and those with more classical Hirschsprung's disease. This difference was noted in the aganglionic segment of bowel and the most striking feature of this particular subgroup is an increase in the BChE activity in patients with co-existing NID. This suggests a different pathophysiological process in patients with NID in the ganglionated bowel.

Possible alternative explanations for this are the small patient numbers and the variation in absolute values of AChE activity obtained in different patients. On the other hand the demonstration of a grey area in the biochemical measurement of AChE activity in previous studies (Dale 1979; de Wet 1980) tends to suggest that a difference exists in certain patients. If this finding is substantiated in future studies, it may be a useful adjunct to the diagnosis and evaluation of NID which co-exists with Hirschsprung's disease.

From this data it is clear that the proportion of AChE was increased relative to the BChE activity in affected intestinal segments. It is therefore possible that the decrease in BChE activity may be of considerable significance in pathophysiology of abnormal neurogenesis in Hirschsprung's disease and NID. The differences in BChE activity in the affected bowel in patients with a dysplastic ENS could be significant due to its reported neuromodulator role in neural development. The chief area of influence of BChE is in the proliferative phase of ganglion cell embryogenesis (Layer 1983,1991). Disturbance of cholinesterase function may therefore be a possible embryological basis for increases in cell numbers and hyperganglionosis in patients with NID.

14.6.2 The influence of physical factors on AChE activity

These experiments were performed under controlled laboratory conditions to eliminate the effect of major temperature or pH variations. Regulation of substrate concentration was more difficult because of the nature of the whole tissue homogenates. Hall (1973) reported inhibition of the 10S and 16S molecular forms at substrate concentrations in excess of 1.25 mM. Acetylcholine activity has been reported at much lower levels in the intestinal wall in aganglionic bowel than the 1.25 mM inhibition level reported by Halli

(1973). There was a significant increase in the 10S isomeric form in affected tissue suggesting that substrate concentration was not a major factor influencing the determination of AChE levels.

Investigation of the effect of red blood cell AChE by randomly washing certain specimens of tissue did not appear to influence AChE G4 activity. AChE A12 levels, on the other hand, appeared to be lower in washed specimens.

Other possible factors affecting AChE activity in the enteric nervous system included previous surgery, inflammatory conditions or other congenitally acquired conditions.

Surgery per se did not appear to affect the level of AChE activity in the bowel. Patients with inflammatory bowel conditions also did not appear to have significantly higher AChE activity in the tissue despite AChE staining neurofibrils in 2 of these patients.

Despite this increase in AChE staining, little overall change of AChE activity was noted on biochemical assay in those with positive AChE neurofibrils in the lamina propria and muscularis mucosa.

14.6.3 The influence of residual Hirschsprung's disease and NID on the biochemical assay of AChE

The difference in the AChE : BChE ratio in the aganglionic segments of patients with Hirschsprung's disease and controls appears to be specific to Hirschsprung's disease. The AChE G4 levels remained high in the proximal ganglionated bowel in patients with TCA or an extended transitional zone. Other patients with positive AChE staining patterns resulting from congenitally

acquired conditions of bowel such as anorectal malformations did not demonstrate the same increase in AChE G4.

In patients who had co-existing NID in the proximal ganglionated pull-through segment of bowel, the AChE levels were significantly lower and those of BChE higher (Kruskal-Wallace, $p<0.05$). The pattern of molecular isoforms of AChE also differed and patients with a poor outcome and postoperative obstructive symptoms had a significantly lower level of AChE G4 (Kruskal-Wallace, $p<0.01$) and a higher level of AChE G1 and G2 (Kruskal-Wallace, $p=0.05$) in the aganglionic segment.

Although these observations were contrary to what was expected and are based on a relatively small patient sample, it suggests that the increase in AChE G4 is limited to aganglionic bowel in Hirschsprung's disease and is not as marked in patients with NID in the absence of Hirschsprung's disease. This observation offers a possible explanation for the lower AChE activity or "grey zone" area previously reported in certain patients (Dale 1979; de Wet 1980). Increased AChE G4 levels may therefore be a marker for Hirschsprung's disease and can be of use in identifying the presence of transitional bowel in the pull-through segment.

14.6.4 Molecular forms of AChE in Hirschsprung's disease

Study of the density gradients confirmed the differing AChE molecular form distribution in aganglionic tissue reported by other workers (Bajgar 1979; Rakonczay 1984; Bonham 1985,1988). The increase in AChE activity in the aganglionic tissue appeared to be caused mainly by a selective increase in the AChE-G4 activity in aganglionic tissue. This increase of a specific molecular form of AChE alludes to a possible specific etiological factor or pathophysiological role. Further detergent solubility fraction studies have shown

that this AChE-G4 increase chiefly consists of an increase in the amphiphilic form of AChE G4. All of this suggests that the increase in AChE in Hirschsprung's disease is chiefly of the amphiphilic form of the tetrameric AChE-G4.

In addition to the increased AChE activity in Hirschsprung's disease, derangements of AChE expression have also been reported in other congenital conditions. Soluble forms of AChE have also been reported to be increased in the liquor in fetuses with neural tube defects (Chubb 1979). These findings suggest an association between AChE and developmental disorders of the nervous system.

The reason for this increase in a specific molecular AChE isoform is unclear. An early report (Rakonczay 1984) suggested that the alteration in AChE molecular forms may result from the pathological increase of nerve tissue in Hirschsprung's disease. Although this explanation has some merit, it fails to explain why normal nerves do not stain for AChE in contrast to the coarse refractile staining nerve tissue in Hirschsprung's disease.

There is increasing evidence that AChE plays a role in development of the CNS and ENS (Layer 1991; Luo 1992; de Gandarias 1992). Embryological studies have demonstrated high levels of AChE being expressed in developing neuroblasts immediately prior to the growth of the spinal motor axons in avian models (Layer 1983, 1991; Weikert 1990). A significant and selective decrease in AChE G4 forms have also been shown in the brains of developing rat fetuses (Meneguz 1992). The expression of AChE has been shown to follow closely on an increase in the BChE activity of the migrating cells (Layer 1983, 1991) and has more recently been shown to be related to the expression of the HNK-1 epitope (Layer 1991).

Current evidence points towards amphiphilic AChE-G4 as the chief molecular form of AChE expressed by the central nervous system (Gennari 1985). This AChE molecular form is anchored to the membrane via a 20 kDa hydrophobic domain (Heider 1992). The mechanism by which AChE acts is not yet clear but in addition to a neuromodulator function, a protease function has been suggested (Small 1990).

Proteases attached to the cell membrane have been shown to be part of the mechanisms controlling the migrating neural cells during development (Unkeless 1973). These form part of the intricate balance that exists between the proteases from the cell itself and the protease inhibitors which then modulates neurite elongation and development (Monard 1988). As neurite outgrowth is an early cellular event which results in neuronal cell differentiation, the timing of this event and rate of neural outgrowth is of critical importance in the establishment of synapses in the nervous system (Changeaux 1976). This forms part of an extremely complex set of mechanisms involving multiple interactions between the neurite growth cone, the developing cell membrane and certain components of the extracellular matrix. The role of AChE in the growth of long projecting axons is not yet clear (Weikert 1990). Experimental evidence supports its role in cellular migration and neurogenesis (Layer 1983; Mulholland 1992) and it is possible that AChE may act as a regulator of neurite outgrowth. Other proteases such as plasminogen activator also have a role to play and another calcium dependent protease mechanism has been identified at the level of the neurite growth cone (Monard 1988).

There is further evidence that AChE may itself mediate cell to cell contact and may possibly be involved at a local level of differentiation (Layer 1991). AChE is known to be located near the presynaptic calcium channels (Brimijoin 1991). The reported increase in extracellular matrix deposit as well as an increase in AChE

production which occurs around myotubule cultures in response to hypercalcaemia (Bursztajn 1991) are of considerable interest. The increased AChE activity in these experiments was shown to be in the monomeric and dimeric AChE molecular forms. Tetrameric and asymmetric forms were not affected. These findings suggest a possible further function of certain molecular forms of AChE at a matrix level. In addition, they raise further questions as to the exact role of the various molecular forms of AChE in neurodevelopment.

From this evidence it would appear that the disturbance in AChE in Hirschsprung's disease and other neuronal dysplasias possibly results from the persistence or expression of embryological forms of the enzyme. On the other hand, significant differences in tissue concentrations of acetylcholine (ACh) have been identified between aganglionic and ganglionic tissue in Hirschsprung's disease (Visi 1990). This increase in ACh may explain the increased AChE in Hirschsprung's disease, but does not explain why this increase should be in the tetrameric isoform. The assymmetric forms are associated with the synaptic cleft and should increase in response to increased ACh secretion.

The observed differences in the AChE:BChE ratio in the bowel wall of patients with features of NID suggest that the pathophysiological processes in these patients may differ. To strengthen the argument for an embryological function for the cholinesterases, there is some evidence that BChE expression occurs predominantly in Schwann cells (Friede 1965; Dubovy 1987) and is related to the division of nerve cells. Thus a higher level of BChE activity in the aganglionic zone of patients with co-existing NID, offers a possible explanation for the hyperganglionosis which occurs. This observation warrants further investigation and future studies are planned to investigate the developmental role of BChE in order to better define its role in neurogenesis (Layer 1983, 1991).

Cholinesterases, therefore, appear to play an important role in neural development and warrant further study. The pathophysiology of Hirschsprung's disease and other neuronal dysplasias may result from defects in the modulators of neuroblast development. These studies open new possibilities with regard to the influence of AChE on neurogenesis and raise further questions about the exact role of the various molecular forms of AChE in neurodevelopment.

The findings of this study relate to the specific function of these isoenzymes of AChE with particular reference to developmental roles for AChE in Hirschprung's disease. The identification of AChE-G4 in the nervous tissue of the enteric nervous system with further anatomical localization to the nerve membranes, raises interesting possibilities for further research into the molecular structure and properties of the enzyme.

14.6.5 Genetic implications

Altered expression of AChE in certain neurological and neuromuscular disorders such as Alzheimer's disease, Hirschsprung's disease, meningomyelocoele and certain muscle dystrophies (Atack 1983, Brimijoin 1991), suggests that it is important to explore the site of the cholinesterase genes for a possible linkage. It has been shown that a single allele with 4 coding exons (1, 2, 3A, 3H) code for AChE (Sikorav 1987; Rotundo 1988). The structural diversity of the molecular forms are then generated by alternative splicing of the exons 3A or 3H (Soreq 1984) and by post-translational modification as well as by covalent association with other molecules. The AChE gene has recently been located on the long arm of chromosome 7 (Getman 1992; Erlich 1992). The AChE gene maps to a position where there is an unusually high incidence of chromosomal abnormalities associated with haemopoietic tumours (Soreq 1991). This reinforces the hypothesis that it acts as a neurotropic agent. Of further interest is the localization of the Hox 1 gene to the 7th chromosome (Bucan gene has,

however, been located on the short arm and not the long arm of the 7th chromosome. By way of contrast, the BChE gene (EC 3.1.1.8) has been mapped to the 3q26-ter position (Erlich 1992). It is possible that the disturbance of the AChE:BChE ratio identified in this study is genetically based and the link to the genetic location therefore requires further study.

14.7 CONCLUSION

This study has confirmed by biochemical assay that AChE activity is significantly increased in the aganglionic bowel. This increase is in the AChE fraction and disturbs the AChE:BChE ratio. Increased AChE has been shown to largely consist of an increase in the AChE-G4 molecular form. Further investigation has shown a significant increase in the detergent soluble fraction which is not reflected in the low salt or high salt extractions. This suggests that the increase in AChE-G4 consists mainly of the amphiphilic subtype which has an extra 20 kDa domain. This molecular form is linked to ENS development and a possible embryological role is postulated.

AChE-G4 is raised in affected tissue and can be regarded as a potential marker for Hirschsprung's disease. It is still raised in the transitional zone which may assist in differentiating this region from areas of co-existing neuronal dysplasia. Patients with a proximal dysplastic ENS, on the other hand, appeared to have a much lower AChE and a higher BChE in aganglionic tissue. This high level of BChE in the aganglionic bowel of these patients, suggests that BChE may have an equally significant role to play in the abnormal development of the ENS. The role of cholinesterases in abnormal neural development, requires additional investigation and further studies are being carried out.

CHAPTER 15

CONCLUSION

15.1 SUMMARY OF MAJOR FINDINGS AND THE NID DEBATE

Considerable debate still surrounds the causes of persisting obstruction in the patient who has had a surgical correction of Hirschsprung's disease. In the retrospective study, a good outcome was observed in the majority following the surgical correction of Hirschsprung's disease. Familial studies suggested a similar etiology for Hirschsprung's disease and NID.

Long term follow-up recorded a good functional result in 94% of the 115 patients older than 4 years of age, (an arbitrary level set for the evaluation of anorectal function), who responded to interview. Despite these good results, obstructive symptoms persisted in 16 of these patients. Fifty percent (ie. 8 out of 16 patients) had complications such as failure to thrive, persistent enterocolitis and some had episodes of septicaemia and shock. There was no obvious cause identified on clinical examination to account for this clinical presentation. This group of patients was therefore further investigated to determine the cause of their dysfunction.

Rectal examination failed to identify the cause although abdominal radiographs demonstrated the megacolon and megarectum in these patients. Similarly, defaecography and videoproctography failed to identify any definite cause. More specifically, the described radiological features of achalasia of the internal anal sphincter were absent.

On manometric testing, the internal anal sphincter did not appear to be the obstacle in 15 of these patients. One patient was shown to have an increase in

the anal sphincter pressure barrier suggesting an outlet obstruction. The patient was successfully treated with a subsequent rectal myectomy. Manometric investigation of the remaining patients with obstructive symptoms failed to demonstrate any significant differences of any of the measured manometric parameters of internal anal sphincter function. There were also no clear differences between those postoperative patients with obstruction and those with normal anorectal function. Further comparison with groups of patients with chronic constipation or controls without gastrointestinal disease also showed no significant differences.

In the course of the manometric studies, a determination of the manometrically expressed anal sphincter pressure barrier (ASPB) was found to be of considerable value in identifying those patients with sphincter overactivity. A restored postoperative rectosphincteric reflex (RSR) was an uncommon finding and occurred in only 3 patients with and 3 patients without obstructive symptoms. In the light of the satisfactory clinical result in the majority of patients, little significance was attributed to the presence or absence of the RSR in terms of promoting postoperative continence. The reason for few positive reflexes in the postoperative patient is probably attributable to the partial sphincterotomy created by the performance of a low posterior anastomosis at the time of pull-through surgery.

Other manometric parameters which appeared to be of value included a difference in sensory perception in patients with incontinence. This finding suggested that an intact anorectal sensory mechanism is a possible factor in the maintenance of continence.

Causes other than achalasia of the internal anal sphincter with resultant outlet obstruction which were considered included technical faults and the coexistence

of neurodevelopmental dysplasias within the enteric nervous system. As far as technical problems were concerned, known problems were excluded in the selection criteria and the patient sample was drawn from those patients who could be expected to do well. In addition, no further evidence of technical causes were detected on clinical examination or manometric testing.

Histological evidence of residual abnormalities of the enteric nervous system (ENS) was present in the rectal suction biopsies of 14 of these 16 patients. Aganglionosis was observed in 4 of these patients and appeared from all available clinical data to be an acquired problem. If this assumption is proven to be correct, it points towards a certain amount of postnatal plasticity within the ENS. In 9 of the 16 patients (56%), typical histological features of NID were noted on rectal suction biopsy. These 9 patients represented 7.8% of the 115 over the age of 4 years responding to recall. Ganglioneuromatosis was recorded in 1 further patient and in 2 the rectal suction biopsies were within normal limits. These findings suggested that abnormalities of the enteric nervous system may well account for a number of the obstructive symptoms occurring in patients with otherwise successful treatment of Hirschsprung's disease.

Considerable debate surrounds the significance of the presence of features of NID in the residual bowel of patients undergoing Hirschsprung's pull-through surgery. The main points of argument in this debate may be partly explained by a lack of well defined histological criteria of NID in the past. Despite recent advances in this field, current histological methods still often fail to predict long term functional disorders on the basis of the observed abnormal morphological features in the ENS. In particular, the significance of the observed histological findings in terms of the predictability of long term clinical outcome requires clarification. It is also possible that certain histological features are of greater importance in this regard than others.

In order to test the hypothesis that dysplastic morphological features of the ENS result in postoperative obstructive symptoms, a prospective study was undertaken. This study set out to objectively assess full thickness biopsies of the ganglionated pulled through segment of bowel in a group of 32 patients undergoing Hirschsprung's surgery. These findings were then compared to control patients and a group of patients with NID presenting during the same time period.

Histological observations included a relatively high incidence of abnormal findings in the proximal ganglionated bowel in Hirschsprung's disease. These dysplastic features of the ENS were not confined to patients with Hirschsprung's disease but were also noted in patients in the control group. These included patients with anorectal malformations, enterocolitis as well as in one patient with a normal colon and oesophageal atresia. A comparison with the histological observations in the 5 patients with NID (in the absence of Hirschsprung's disease), demonstrated similar features in 5 patients with Hirschsprung's disease. Similar histological features in certain controls were isolated findings and only reflected an overall degree of dysplasia in 1 patient with an anorectal malformation. This in turn suggested that an overall degree of dysplasia is required for postoperative functional impairment to occur.

This led to the concept that the significance of dysplasia, in terms of postoperative function, can be quantified by the degree of dysplasia of the ENS. To allow quantitative assessment of the degree of dysplasia, a hypothetical grading system was developed based on the histological findings of NID. Testing this hypothesis against the observed histological features gave an 80% correlation with postoperative obstructive symptoms in those 5 patients who had a histopathological score equalling more than 50% of the total (ie.6/12).

This further suggested that the degree of ENS dysplasia rather than the presence of individual abnormal histological signs is of the greatest significance in predicting the functional outcome.

These studies called into question the best standardized method for the assessment of hyperganglionosis. Although low power field impression and the number of cells per cluster of ganglion cells demonstrated some correlation to the predictability of hyperganglionosis, the diagnosis of hyperganglionosis was found to be most reliable on evaluating a full circle of bowel lumen. Some indication of the extent of the hyperganglionosis was achieved by the number of ganglion cells per cluster in the submucous plexus.

The identification of positive AChE staining neurofibrils in 37% of the control group can be partially explained on the basis of the origin of the specimens from abnormal bowel in 7 of the 8 patients. In 1 patient a positive staining pattern was obtained in otherwise normal colon taken during a colonic interposition for long gap oesophageal atresia. This finding suggests that false positive AChE staining patterns may occur and is a possible explanation for a certain number of false diagnoses of co-existing NID in patients with Hirschsprung's disease.

Other contributing factors such as inflammation, previous surgery and the presence of other congenital abnormalities (including anorectal malformations) appeared to affect the histological picture in control patients. They did not, with one exception, appear to produce the overall picture of NID nor attain a significant level in the histopathological score. The dysplastic picture was in a patient with an anorectal malformation, a known association.

Maturation of immature ganglion cells may result in improved function. The wide discrepancy of functional results in the literature and an observed

improvement in postoperative function in many of the cases of NID (Schärli 1992), supports this assumption. There were, however, only 2 cases of immature ganglion cells identified in the ganglionated segments of Hirschsprung's disease patients. In addition, immature cells were not identified in the group with postoperative obstructive symptoms.

In addition to immaturity, certain patients with NID have been reported to regain normal histology with time (Schärli 1992; Meier-Rüge 1992). If true, this suggests that certain of the morphologic features attributed to NID may be of an acquired nature. This may then revert to normal once the causative factor is removed. It is possible that the features of NID observed in 9 (7.6%) of the 115 older patients followed up long term could have been of an acquired nature in the absence of evidence to the contrary.

In order to test this hypothesis, an animal model of non-strangulating obstruction was created in developing rats. Instead of resulting in hyperganglionosis, there was a significant decrease in the number of ganglion cells per 5mm in the obstructed animal. The number of ganglion cells per high power field or per cluster were relatively unchanged, following obstruction. The failure of obstruction per se to produce either hyperganglionosis or positive AChE neurofibrils in this developing animal model, does not support an acquired etiology for the histological features. The increased AChE staining in response to alpha-2-adrenergic stimulation by Xylazine would appear to identify the sympathetic nervous system as playing a potential role in an increased expression of AChE. This area of research requires further study to clarify the nature of this influence and understand the pathophysiology of an increase in AChE.

The findings of these experiments suggest that co-existing dysplasia is probably a developmental disorder of the enteric nervous system. As such, co-existing NID does have a significant role to play in the functional outcome of patients undergoing corrective surgery for Hirschsprung's disease. The long term follow-up of these patients suggests that although early symptomatology may improve in some, in a much smaller group this leads to long term clinical problems. In this series this occurred in 7.8% of patients evaluated.

Although the present sample numbers are small, these findings suggest that the histopathological scoring system may be of future value to identify patients with a significant degree of dysplasia most likely to develop postoperative functional problems. Clinical symptoms of obstruction appeared unlikely to improve with time in this patient group. This may, in addition, be of considerable value to the planning of the resection level if this is based on colonic mapping at the time of initial surgery.

In order to study the nature and role of AChE in aganglionic tissue, a further study was carried out into AChE activity in full thickness tissue samples obtained from the patients included in the prospective histological study. Representative samples of aganglionic and ganglionated tissue were homogenized and the AChE extracted. Biochemical AChE assay showed little differences in the total ChE activity in the full thickness specimens of aganglionic and ganglionated bowel. On the other hand, the level of AChE was significantly raised in the aganglionic bowel. There also appeared to be an alteration in the AChE:BChE ratio in aganglionic tissue which was not reflected in the ganglionated bowel. In addition, AChE activity was increased in the transitional zone of those tested. This may assist in the identification of transitional zone at the time of surgery.

The increase in AChE was shown, on sucrose sedimentation velocity, to be mainly in the 10.5S position which corresponded to the marker protein catalase and AChE G4. Serial extractions in low salt, high salt and high detergent containing solutions confirmed that this AChE was mainly in the amphiphilic form. There was a significant rise in the proportion of AChE in relation to BChE in the affected tissue. In certain patients, the histological features of co-existing neuronal dysplasia correlated with "grey zone" AChE tissue activity levels between the aganglionic and the normal levels. Those patients with histological features of NID had a significantly higher BChE level in the aganglionic zone. Exceptions to this rule were those patients who did not demonstrate a marked rise in AChE-G4 activity. The two patients in this category were those with small dysgenetic ganglion cells. These appeared to differ from those patients with co-existing NID. The increase in AChE G4, therefore, would appear to be specific for Hirschsprung's disease and can also be used to differentiate the transitional zone from patients with co-existing NID. BChE activity appears to be much higher in the aganglionic zone in this group.

Although the exact meaning of these findings is not yet clear, current evidence points towards a neuromodulator function for AChE during neurogenesis. Differences in the cholinesterase composition in affected tissue may provide clues about the timing and pathophysiology of abnormal development of the ENS and yield insights into the etiological factors of disturbances in ENS development.

15.2 IMPLICATIONS ON CLINICAL MANAGEMENT

Implications of these findings on clinical management includes the recognition and removal of dysplastic segments in the surgically pulled through bowel at the time of surgical correction of Hirschsprung's disease. To achieve this goal, a thorough mapping of the colon at the time of colostomy is required. Histological evaluation of surgically resected material should include specific histochemical

stains for complete evaluation. Further investigation and management of patients with postoperative obstructive symptoms should commence with an identification of the causative factor. Clinical management depends on the causative factor and includes correction of surgically correctable causes and the use of prokinetic agents in those cases where neuronal dysplasias appear to be the chief cause. A suggested protocol for the management of obstructive symptoms has been outlined.

15.3 FURTHER RESEARCH ON THE NORMAL AND ABNORMAL DEVELOPMENT OF THE ENS

15.3.1 The significance of co-existing NID with Hirschsprung's disease

The above studies have highlighted the need for more research into the nature and importance of co-existing NID with regard to its effect on postoperative outcome in the surgery of Hirschsprung's disease.

The finding of features normally associated with neuronal dysplasia in patients without Hirschsprung's disease underlines the need for a clear definition as to which histological findings constitute a significant enough degree of dysplastic elements within the ENS to result in postoperative symptoms. Although this study has proposed certain tools such as the histological grading system, larger trials on more normal bowel specimens are required to evaluate the extent of the normal spectrum of dysplastic features.

15.3.2 The role of ACHE in the embryological development of the ENS

Current "in vivo" and "in vitro" data suggests roles for both butyryl and acetyl cholinesterases in the regulation of cell proliferation and neurite growth in the development of the avian enteric nervous system (Layer 1991). The neuromodulator action may possibly take place at a molecular level as the "in vitro" addition of a selective BChE inhibitor has been shown to result in a

reduction of AChE gene expression (Layer 1991). A shift from the low molecular weight forms to the tetrameric forms of both AChE and BChE in these patients suggests that these molecular forms have important roles in development. The chief function of the cholinesterases during embryogenesis may well be a protease function as suggested by Small (1990). This action may be at the growth cone of the developing neurite. This suggestion could possibly explain the reason for the increase in AChE in affected bowel in Hirschsprung's disease. Developmental studies of the role of the AChE-G4 tetramer in the development of the ENS are being planned to test this hypothesis.

The timing of these influences may be of critical importance in determining the nature of the resulting ENS abnormality. By way of example, increased BChE activity noted in this study may explain the hyperganglionosis reported in patients with NID. The embryological function of BChE appears to be more to do with the division of cells. On the other hand, AChE would appear to be related to the maturation and differentiation processes (Layer 1991). Further study of these embryological roles may in turn lead to a better understanding of the pathophysiological mechanisms involved in the etiology of Hirschsprung's disease.

15.3.3 The genetic basis of Hirschsprung's disease and neuronal intestinal dysplasia

As has been noted earlier in this study, the reported familial incidence for Hirschsprung's disease is between 2.4 and 9% (Passarge 1967; Boley 1978; Cohen 1982; Garver 1985; Moore 1991). This suggests a genetic basis for at least some forms of this disease. Further support arises from a higher familial incidence reported in patients with very long segment aganglionosis (Gordon 1966; Kleinhaus 1979) and a significantly higher number of patients ($p<0,001$) with total colonic aganglionosis was noted within the familial group in this series (Moore 1991).

Encouraged by recent successes in genetic analysis, molecular biologists have sought to identify a candidate gene for Hirschsprung's disease. The nature of genetic influences in the development of the enteric nervous system remain unclear. From animal experiments it has been deduced that congenital aganglionic megacolon appears to be caused by a single gene mutation in mice (Lane 1966) and horses (Hultgren 1982; McCabe 1990), and is associated with the autosomally recessive inherited piebald trait (Lane 1966; Hultgren 1982; McCabe 1990). Three genetic loci have been located in animal models which affect myenteric ganglion cells and pigment cells arising from the neural crest (Lane 1966; McCabe 1990).

Simple Mendelian inheritance could not be demonstrated by Bodian and Carter (Bodian 1963) who suggested a sex linked multifactorial inheritance pattern. Evidence for a single dominant gene for the more extensive forms of Hirschsprung's disease has been reported in man (Badner 1990). In humans, a reported association exists with chromosome 21 (Swenson 1973), the short arm of chromosome 2 (Webb 1988), chromosome 10 (Martucciello 1992), the long arm of chromosome 13 (Sparkes 1984; Lamont 1989) and the long arm of chromosome 22 (Beedgen 1986). The possibility therefore emerges that several genetic loci may be involved in the normal and abnormal development of the enteric nervous system. The answer must lie in the mechanics of embryogenesis of the ENS.

There is some evidence that Homeobox genes have a potential role in the migration of nerve cell precursors from the neural crest (Gehring 1987). The role of multigene families in the regulation of embryogenesis in vertebrates has been receiving some recent attention (Dressler 1988; Wolgemuth 1989). Homeobox containing genes which have been identified as having a potential

role in the migration of nerve cell precursors from the neural crest are the Hox 2.1 "homeo-gene" transcripts detected in autonomic ganglia arising from the neural crest (Holland 1988) and the Hox 7.1 gene (Robert 1989; Hill 1989). Recent work on the mouse homeobox Hox 1.4 , situated on mouse chromosome 6 (Wolgemuth 1989) has suggested that in transgenic mice embryos the addition of an extra Hox 1.4 gene resulted in megacolon in the resulting mouse embryos. Aganglionosis has only been demonstrated in the most terminal portion of the bowel in this animal model but the production of increased levels of Hox 1.4 mRNA resulted in an overexpression of the Hox 1.4 transgene on day 12.5 in the developing intestine of the transgenic mice. Based on these findings, it becomes clear that the defects in neurodevelopment may occur within the migrating neuroblasts themselves. The cells that provide clues to the migrating neuroblasts during differentiation and maturational phases may themselves be defective. This area of research would appear to be of special interest in the pathogenesis of neuronal dysplasias of the enteric nervous system.

The similarity of presentation of Hirschsprung's disease and its association with Neuronal intestinal dysplasia and the possible role of genetic factors, lends importance to further understanding of the relationship between these two pathological entities. The co-existence of NID and Hirschsprung's disease alludes to a theoretical possibility of similar pathogenesis of both defects (Fadda 1987). It is therefore not surprising that, in addition to these conditions co-existing, they may recur in members of the same family with familial Hirschsprung's disease as observed in one family described in this series.

Given the likelihood of a genetic component to these conditions, the set of genes would need to be extremely large to individually determine each synapse. Whereas the responsible genes may dictate general principles of development, the activity of the immature synapse or the integrating function of postsynaptic

neurones also play a significant role in neurogenesis (Changeux 1976). At the local microenvironmental level, the process appears to be extremely complex. The selective recognition of cell surfaces, activity of the growth cone and developing nerve terminals, selective regressive phenomena and the influence of the target cell on synapse stabilization may all have a part to play. Neuromodulators active in these areas may be defective and result in variations in connectivity which result in dysplastic features in the ENS. The mechanism by which AChE acts is not yet clear but its increase in abnormal development of the nervous system suggests a neuromodulator function. This action may possibly take place at a molecular level as the "in vitro" addition of a selective BChE inhibitor has been shown to result in a reduction of AChE gene expression (Layer 1991). An unusually high frequency of chromosomal abnormalities occur near the map position of the AChE and BChE genes in haemopoietic tumours. As a result of these findings, Soreq (Soreq 1991) has suggested a role for cholinesterases in tumorigenesis. A shift from the low molecular weight forms to the tetrameric forms of both AChE and BChE in these patients suggests that these molecular forms have important roles in development. Further study of the mRNAs involved in affected tissue may shed further light on this aspect.

15.4 CONCLUSION

Neural development appears to be an extremely complex inter-relationship between the cell membrane, neuromodulators and neural growth inhibitors. The timing and nature of any interference may be of critical importance in determining the expression of any defect as aganglionosis or neuronal dysplasia. A number of secondary mechanisms are possible at this level which may inhibit neuromodulator function and thus affect eventual outcome. Further study is indicated to understand these pathophysiological mechanisms and possibly devise interventional therapy which restores normal development.

REFERENCES

Aaronson I, Nixon H. A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung's disease. Gut; 1972; 15: 138-146.

Abel W. Further observations on the development of the sympathetic nervous system in the chick. J Anat Physiol; 1912; 47: 35-71.

Adams CWM, Brain RHF, Trounce JR. Ganglion cells in achalasia of the cardia. Virchows Arch A; 1976; 372: 75-79.

Adamson WAD, Aird I. Megacolon: evidence in favour of a neurogenic origin. Br J Surg; 1932; 20: 220-233.

Adlard DB, Dobbing J. Elevated acetylcholinesterase activity in adult rat brain after undernutrition in early life. Brain Res; 1971; 30: 198-199.

Adson AW. Hirschsprung's disease: indications for and results obtained by sympathectomy. Surgery; 1937; 1: 859-877.

Aldridge RT, Campbell PE. Ganglion cell distribution in the normal rectum and anal canal. J Pediatr Surg; 1968; 3(4):475-490.

Allan IJ, Newgreen DF. The origin and differentiation of enteric neurons of the intestine of the chick embryo. Am J Anat; 1980; 157: 137-154.

Allert JA, Adams HR. Pharmacologic considerations in selection of tranquilizers, sedatives and muscle relaxant drugs used in inducing animal restraint. J Am Vet Med Ass; 1987; 191(10):1241-1244.

Altman J, Das GD. Autoradiographic radiological studies of postnatal neurogenesis 1: a longditudinal investigation of the kinetics, migration and transformation of cells incorporating titrated thymidine in neonate rats with special reference to postnatal neurogenesis in some brain regions. J Comp Neurol; 1966; 126: 337-390.

Alvarez WC. The mechanisms of the digestive tract. New York; PB Hoeler Inc, Harper and Row; 1922.

Andrew A. The origin of the intramural ganglia (1V). The origin of enteric ganglia a critical review and discussion of the present state of the problem. J Anat; 1971; 108: 169-184.

Arhan P, Faverin C, Devroede G, Dubois F, Coupris L, Pellerin D. Manometric assessment of continence after surgery for imperforate anus. J Pediatr Surg; 1976; 11: 157-166.

Ariel I, Vinograd I, Lernau OZ, Nissan S, Rosenmann E. Rectal mucosal biopsy in aganglionosis and allied conditions. Hum Pathol; 1983; 14(11): 991-995.

Asher MI, Coates AL, Collinge JM, Milic-Emile J. Measurement of pleural pressure in neonates. J Appl Physiol; 1982; 52(2):491-494.

Atack JR, Perry EK, Bonham JR, Perry RH, Tomlinson BE, Candy J, Blessed G, Fairburn A. Molecular forms of acetylcholinesterase in senile dementiia of Alzheimer type: selective loss of the intermediate (10S) form. Neurosci Lett;1983; 40: 199-204.

Atias O, Finaly R, Meyerstein N, Mares AJ. Erythrocyte acetylcholinesterase activity in Hirschsprung's disease in Israel. J Pediatr Surg; 1991; 26(2): 190-191.

Augustinsson KB. Cholinesterases. A study in comparative enzymology. Acta Physiologica Scand; 1948; 52(Suppl): 1-182.

Badner JA, Sieber WK, Garver KL, Chakravarti A. A genetic study of Hirschsprung's disease. Am J Hum Genet; 1990; 46: 568-580.

Bajgar J, Hak J. Acetylcholinesterase activity and its molecular forms in rectal tissue in the diagnosis of Hirschsprung's disease. Clin Chim Acta; 1979; 93: 93-95.

Baranowicz B. The results of Soave's operation for Hirschsprung's disease. Z Kinderchir; 1977; 20(1): 49-56.

Barr LC, Booth J, Filipe MI, Lawson JON. Clinical evaluation of the histochemical diagnosisis of Hirschsprung's disease. Gut;1985; 26: 393-399.

Barth O. Hochgradige kotstauung ingevolger einer durch zu langes mesocolon zustandgekommen darmverlagerung. Wagners Arch d Heilk; 1870;11:119.

Bayliss WM, Starling EH. The movements and innervation of the small intestine. J Physiol (Lond); 1899; 24: 99-143.

Beani L, Bianchi C, Crema A. The effect of catecholagnines and sympathetic stimulation on release of ACH from the guinea-pig colon. Br J Pharmacol; 1969; 36: 1-17.

Beardsmore CS, Helms P, Stocks J, HatchDJ, Silverman M. Improved oesophageal balloon technique for use in infants. J Appl Physiol; 1980; 49: 735-742.

Becker J, Oosthuizen A, Grobbelaar NJ, Welsh RIH, Venter ID. The transition zone in colonic aganglionosis. S Afr Med J; 1985;23: 135.

Beedgen B, Nutzenadel W, Querfeld U, Weiss-Wichert P. Partial trisomy 21 and 11 due to paternal 11;22 translocation associated with Hirschsprung's disease. Eur J Pediatr; 1986;145: 229-232.

Bennett RC, Duthie HL. The functional importance of the internal anal sphincter. Br J Surg; 1964; 51: 355-357.

Bentley J. Some new observations on megacolon in infancy and childhood with special reference to the management of megasigmoid and megarectum. Dis Colon Rectum; 1964; 7: 462-469.

Bergman F, Rimon S, Segal R. Effect of pH on the activity of eel esterase towards different substrates. Biochem J; 1958; 68:493-499.

Berry CL, Fraser CC. The experimental production of colitis in the rabbit with particular reference to Hirschsprung's disease. J Pediatr Surg; 1968; 3: 36-42.

Bill AH, Chapman ND. The enterocolitis of Hirschsprung's disease its natural history and treatment. Am J Surg; 1962; 103: 70-74.

Billard CM. Maladies des infants. Paris.1828

Blisard K, Kleinman R. Hirschsprung's disease: a clinical and pathologic overview. Hum Pathol; 1986; 17(12): 1189-1191.

Bockman DE, Kirby ML. Neural crest interactions in the development of the immune system. J Immunol; 1985; 135: 766s-768s.

Bodian M, Stephens FD, Ward BCH. Hirschsprung's disease and idiopathic megacolon. Lancet; 1949; i: 6-11.

Bodian M, Carter CO. Family study of Hirschsprung's disease. Ann Hum Genet; 1963; 26: 261-271.

Bolande R. The Neurocristopathies, a unifying concept. Hum Path; 1974; 5: 407-429.

Boley SJ. New modification of the surgical treatment of Hirschsprung's disease. Surgery; 1964; 56: 1015-1917.

Boley SJ, Dinari G, Cohen MI. Hirschsprung's disease in the newborn. Clin Perinatol; 1978; 5: 41-60.

Bon S, Vigny M, Massoulie J. Asymmetric and globular forms of Acetylcholinesterase in mammals and birds. Proc Natl Acad Sci USA; 1979; 76(6): 2546-2550.

Bonham JR, Dale G, Scott DJ, Wagget J. Molecular forms of acetylcholinesterase in Hirschsprung's disease. Clin Chim Acta; 1985; 145: 297-305.

Bonham JR, Dale G, Scott DJ, Wagget J. A 7-year study of the diagnostic value of rectal mucosal acetylcholinesterase measurement in Hirschsprung's disease. J Pediatr Surg; 1987; 22: 150-152.

Borchard F, Meier-Ruge W, Wiebecke B, Briner J, Müntefering H, Fodisch HF, Holschneider AM, Schmidt A, Enck P, Stolte M. Innervation abnormalities of the large bowel: Classification and diagnosis. Pathologe; 1991; 12: 171-174.

Boston V, Cywes S , Davies M. Qualitative and quantitative evaluation of internal anal sphincter. Gut; 1977; 18: 1036-1044.

Boston VE, Cywes S, Davies MRQ. Serum and erythrocyte acetylcholinesterase assay in Hirschsprung's disease. J Pediatr Surg; 1978; 13: 407-410.

Boston VE, Dale G, Riley KWA. Diagnosis of Hirschsprung's disease by quantitative biochemical assay of AChE in rectal tissue. Lancet; 1975; 2: 951-953.

Branski D, Denn NR, Neale JM, Brooks LJ. Hirschsprung's disease and Waardenburgh's syndrome. Pediatrics; 1979; 63: 803-806.

Brentano A. Uber Einen Fall von Hirschsprunscher Krankheit. Verh Dtsch Ges Chir ; 1904; I: 265-268.

Brimijoin S, Lennon VA. Selective destruction of preganglionic sympathetic nerves by antibodies to acetylcholinesterase. J Neural Transm; 1991; 34: 139-145.

Briner J, Oswald H, Hirsig J, Lehner M. Neuronal Intestinal Dysplasia - clinical and histochemical findings and its association with Hirschsprung's disease. Z Kinderchir; 1986; 41(5): 282-286.

Bucan M, Yang-Feng T, Colberg-Poley A, Wolgemuth D, Guenet JL,Francke U, Lerach H. Genetic and cytogenetic localization of the homeobox containing genes on mouse chromosome 6 and human chromosome 7. EMBO J; 1986; 5(11): 2899-2905.

Bughaighis A, Emery J. Functional obstruction of the intestine due to neurological immaturity. Prog Pediatr Surg; 1974; 3:37-52.

Bursztajn S, Schneider LW, Jong Y, Berman SA. Calcium and ionophore A 23187 stimulates deposition of extracellular matrix and acetylcholinesterase release in cultured myotubes. Cell Tiss Res; 1991; 265: 95-103.

Büyükünal C, Sarimurat N, Erdogan E, Danismend N, Senyüz OF, Yeker D, Büyükyhiz G, Adali Y. The early and late results of the Duhamel-Martin operation in the treatment of Hirschsprung's disease. Czechoslovak Congress of Paediatric Surgery; 1991; Prague, Czechoslovakia; 1991: 24.

Cameron JAM. On the etiology of Hirschsprung's disease. Arch Dis Child; 1928; 3:210-211

Cannon WB. The law of denervation. Am J Med Sci; 1939; 198: 737.

Cantino D. An Histochemical study of the nerve supply to the developing alimentary tract. Experimentia; 1970; 26: 766-767.

Canty TG. Modified Duhamel procedure for treatment of Hirschsprung's disease in infancy and childhood: review of 41 consecutive cases. J Pediatr Surg; 1982; 17: 773-778.

Carcassonne M, Morrison-Lacomb G, Le Tourneau JN. Primary corrective operation without decompression in infants less than three months of age with Hirschsprung's disease. J Pediatr Surg; 1982; 17: 241-243.

Carcassonne M, Delarue A. Management of Hirschsprung's disease: the definitive operation: which, when, why and how. Aust NZ J Surg; 1984; 54: 435-438.

Cass D. Hirschsprung's disease - an historical review. Prog Pediatr Surg; 1986; 20: 199-214.

Cass D. Hirschsprung's Disease. Whats new in Gastroenterology?;1990; 35: 1-5.

Cass DT. Neonatal one-stage repair of Hirschsprung's disease. Pediatr Surg Int; 1990; 5: 341-346.

Causse E, Vaysse P, Fabre J, Valdiguie P, Thouvenot JP. Cholinesterase activities in resected bowel specimens from children with Hirschsprung's disease. Clin Chim Acta; 1987; 167: 51-57.

Causse E, Vaysse P, Fabre J, Valdiguie P, Thouvenot JP. The diagnostic value of Acetylcholinesterase Butyrylcholinesterase ratio in Hirschsprung's disease. Am J Clin Pathol; 1987; 88(4): 477-480.

Chan SL, Shirachi DY, Bhargava HN, Gardner E, Trevor AJ. Purification and properties of multiple forms of brain acetylcholinesterase (EC 3.1.1.7.). J Neurochem; 1972; 19:2747-2758.

Changeaux JP, Danchin A. Selective stabilization oof developing synapses as a mechanism for the specification of neuronal networks. Nature; 1976; 264: 705-712.

Chisaka O, Cappechi MR. Regionally restricted developmental defects resulting from targeted disruption of the mouse homeobox gene hox - 1.5. Nature; 1991; 350: 473-481.

Chow CW, Chan WC, Yue PCK. Histochemical criteria for the diagnosis of Hirschsprungs disease in Rectal suction biopsies by Acetylcholinesterase activity. J Pediatr Surg; 1977;12(5): 675-680.

Chubb IW, Hodgson AJ, White GH. Acetylcholinesterase hydrolyses Substance P. Neuroscience; 1980; 5: 2065-2072.

Chubb IW, Pilowsky PM, Springell HJ, Pollard AC. Acetylcholinesterase in human amniotic fluid: an index of fetal neural development? Lancet; 1979: 688-690.

Ciment G, Weston JA. Enteric neurogenesis by neural crest-derived branchial arch mesenchymal cells. Nature; 1983; 305: 424-427.

Clausen EG, Davies OG. Early and late complications of the Swenson pullthrough operation. Am J Surg; 1963: 372-380.

Cogbill TH, Lilly JR. Acquired aganglionosis after Soave's procedure for Hirschsprung's disease. Arch Surg; 1982; 117:1346-1347.

Cohen I, Gadd M. Hirschsprung's disease in a kindred : A possible clue to the genetics of the disease. J Pediatr Surg; 1982;17: 632-634.

Coran AG. New surgical approaches to ulcerative colitis in children and adults. World J Surg; 1985; 9: 203-213.

Corman ML. Classic articles in colorectal surgery - Harald Hirschsprung. Dis Colon Rect; 1981; 24(3): 408-410.

Cremin B. Roentgenographic diagnosis. in Holschneider AM, editor,Hirschsprung's disease. Stuttgart,New York: Hippokrates Verlag, Thieme-Stratton; 1982: pp 55-61.

Cywes S. Hirschsprung's disease. S Afr J Surg; 1967; 5(2): 65-68.

Dajani OM, Slim MS, Mansour A. Acquired hypoganglionosis after Soave endorectal pull-through procedure-a case report. Z Kinderchir; 1986; 41: 248-249.

Dalla Valla A. Richerche istologische su di un caso di megacolon congenito. Pediatria; 1920; 740-752.

Dalla Valla A. Contributo alla conoscenza della forme famigliare del megacolon congenito. Pediatria; 1924; 32:569-599.

Dale G, Bonham JR, Riley KWA, Wagget J. An improved method for the determination of acetylcholinesterase activity in rectal biopsy tissue from patients with Hirschsprung's disease. Clin Chim Acta; 1977; 77: 407-413.

Dale G, Bonham JR, Lowdon P, Wagget J, Rangecroft L, Scott DJ. Diagnostic value of rectal mucosal acetylcholinesterase levels in Hirschsprung's disease. Lancet; 1979; 1: 347-349.

Daudet M. Les Recidives pos-operatoires dan la maladie de Hirschsprung. Ann Chir Inf; 1970; 11: 137-140.

Davies MRQ , Cywes S , Rode H. The manometric evaluation of the Rectosphincteric reflex in total colonic aganglionosis. J Pediatr Surg; 1981; 16: 660-663.

Davies MRQ, Cywes S. Inadequate pouch emptying following Martin's pullthrough procedure for intestinal aganglionosis. J Pediatr Surg; 1983; 18: 14-20.
DeLorimier A. Comment on Hirschsprung's disease. Am J Surg; 1986; 152:55.

Denda T. Surgical treatment of Hirschsprung's disease: a modification of the Soave procedure. Geka Shinryo; 1966; 8:295-301.

Deodhar M, Sieber WK, Kiesewetter WB. A critical look at the Soave procedure for Hirschsprung's disease. J Pediatr Surg; 1973; 8(2): 249-253.

DeVries P. Results of treatment. in Stephens F, Durham-Smith E, Paul N, editors, in Anorectal malformations in children: 1988 update. Alan R Less; March of Dimes birth defect series; New York. 1988; 24(4): 481-500.

Dimler M. "Acquired" Hirschsprung's Disease. J Pediatr Surg; 1981; 16(6): 844-845.

Doctor BP, Toker L, Roth E, Silman I. Microtiter assay for actylcholinesterase. Anal Biochem; 1987; 166: 399-403.

Dorman G, Votteler T, Graivier L. A preliminary evaluation of the results of treatment of Hirschsprung's disease by the Duhamel-Grob modification of the Swenson procedure. Ann Surg;1967; 166: 783-791.

Dressler GR, Gruss P. Do multigene families regulate vertebrate development? Trends Genet; 1988; 4(8): 214-219.

Dubovy P, Sonkup T. Ultrastructural localization of non-specific cholinesterase activity in rat muscle spindles. Acta Histochem; 1987; 82(2): 159-170.

Duhamel B. A new operation for the treatment of Hirschsprung's disease. Arch Dis Child; 1960; 35: 38-39.

Duhamel B. Retrorectal and transanal pullthrough procedure for the treatment of Hirschsprung's disease. Dis Colon Rectum;1964; 7: 455-458.

Duthie HL, Watts JM. Contribution of the external anal sphincter to the pressure zone of the anal canal. Gut; 1965; 6: 64-68.

Earlam R. A vascular cause for aganglionic bowel - a new hypothesis. Dig Dis; 1972; 17: 559-571.

Earlam R. A vascular cause for Hirschsprung's disease? Gastroenterology; 1985; 88: 1274-1275.

Ebers L. Geschichte eines seltenen falles voon ileus. Hufelands J Prakt Heilk; 1836; 83/2:62.

Eckert CD, Hurley LS. Influence of various levels of hypervitaminosis A and zinc deficiency on teratogenesis and DNA synthesis in the rat. Teratology; 1979; 19: 279-284.

Edwards FA, Gibb AJ, Colquhoun D. ATP receptor-mediated synaptic currents in the central nervous system. Nature; 1992; 369(6391): 144-147.

Ehrenpreis T. Megacolon in the newborn. A clinical and roentgenological study with special regard to the pathogenesis. Acta Chir Scand (Suppl); 1946; 94: 112-187.

Ehrenpreis T. Acquired megacolon as a complication of rectosigmoidectomy for Hirschsprung's disease. Arch Dis Child; 1965; 40: 180-182.

Ehrenpreis T. Some newer aspects of Hirschsprung's disease. J Pediatr Surg; 1966; 1(4): 329-337.

Ehrenpreis T. Hirschsprung's disease. Hirschsprung's disease.Ehrenpreiss T ed. Chicago: Year Book Publishers; 1970:pp 58-60.

Eisenhammer S. Internal anal sphincterotomy plus free dilatation versus anal stretch with special criticism of the anal stretch procedure for haemorrhoids: the recommended modern approach for haemorrhoid treatment. Dis Colon Rectum; 1974;17: 493-521.

Elema JD, de Vries JA, Vos JM. Intensity and proximal extention of acetylcholinesterase activity in the rectosigmoid in Hirschsprung's disease. J Pediatr Surg;1973; 8(3): 361-368.

Ellman GC, Courtney KD, Andres V, Featherstone RM. A new and rapid calorimetric determination of acetylcholinesterase activity. Biochem Pharmacol; 1961; 7: 88-95.

Emery JL. Colonic retention syndrome (Megacolon) and immaturity of the intestinal intramural plexus. Proc Roy Soc Med; 1973;66: 222-223.

Emmanuel B,Padorr MP, Swenson O. Familial absence of the myenteric plexus (Congenital Megacolon). J Pediatrics; 1965; 67: 381-386.

Erickson HP, Inglesias JL. A six-armed oligomer isolated from cell surface fibronectin preparations. Nature (London); 1984;311: 267-269.

Erlich G, Viegas-Pequignot E, Ginsberg D, Sindel L, Soreq H, Zakut H. Mapping the acetylcholinesterase gene to chromosome 7q22 by fluorescent in vitro hybridization coupled with selective PCR amplification from a somatic hybrid cell panel and chromosome-sorted DNA libraries. Genomics; 1992; 13(4):1192-1197.

Fadda B, Pistor G, Meier-Ruge W, Hoffmann von Kapp - Herr, Muntefering H, Espinosa R. Symptoms, diagnosis and therapy of neuronal intestinal dysplasia masked by Hirschsprung's disease. Pediatr Surg Int; 1987; 2: 76-80.

Fadda B, Welskop J, Müntefering H, Meier-Ruge W, Enert J. Achalasia of the Internal sphincter. Pediatr Surg Int; 2: 81-85.

Fairgrieve J. Hirschsprung's disease in the adult. Br J Surg;1963; 50: 506.

Fenwick WS. Hypertrophy and dilatations of the colon in infancy. BMJ; 1900; ii: 564-567.

Fernandez HL, Stiles JR. Intra versus extracellular recovery of 16S acetylcholinesterase following organophosphate inactivation in the rat. Neuroscience Lett; 1984; 49: 117-122.

Ferrand C, Clarous D, Delteil C, Weber MJ. Cellular localization of the molecular forms of acetylcholinesterase in primary cultures of rat sympathetic neurones and analysis of the secreted enzyme. J Neurochem; 1986; 46(2): 349-358.

Filagamo G, Vigliani F. Ricerche sperimentali sulla correlazione tra estensione del territorio di innervazione e grandezza e numero della cella gangliare del plesso mienterico (di Auerbach) nel cane. Riv Pat Nerv ment; 1954; 75: 441-462.

Finney JMT. Congenital idiopathic dilatation of the colon. Surg Gynae Obstets; 1908; 6:624-643.

Fonkalsrud E. Acquired aganglionosis. Am J Surg; 1986; 152: 54.

Foster P, Cowan G, Wrenn EL. Twenty-five years experience with Hirschsprung's disease. J Pediatr Surg; 1990; 25(5): 531-534.

Foy C, Newton V, Weelesley D, Harris R, Read AP. Assignment of the locus for Waardenburgh syndrome Type 1 to human chromosome 2q37 and possible homology to the splotch mouse. Am J Hum Genet; 1990; 46: 1017-1023.

Fraser J. Surgical aspects of certain disturbances of involuntary nervous system met within alimentary tract. BMJ; 1926; i:359-364.

Frenckner B, Von Euler C. Influence of pudendal block on the function of the anal sphincter. Gut; 1975; 16: 482-489.

Frenckner B, Lindstrom O. Ano-rectal function assessed by manometry and electromyography after Endorectal pullthrough for Hirschsprung's disease. Z Kinderchir; 1983; 38: 101-104.

Friede RL. Enzymatic histochemical studies of senile plaques. J Neuropathol Exp Neurol; 1965; 24: 477-491.

Fujimoto T, Puri P. Persistance of Enterocolitis following diversion of the faecal stream in Hirschsprung's disease. Pediatr Surg Int; 1988; 3: 141-146.

Fujimoto T, Reen DJ, Puri P. Inflammatory response in Enterocolitis incidence in piebald mouse model of Hirschsprung's disease. Pediatr Res;1988: 24: 152-155.

Fujimoto T , Hata J, Yokoyama S, Mitomi T. A study of the extracellular matrix protein as the migration pathway of neural crest cells in the gut: Analysis in human embryos with special reference to the pathogenesis of Hirschsprung's disease. J Pediatr Surg; 1989; 24: 550-556.

Gabella G. Neuron size and number in the myenteric plexus of the newborn and adult rat. J Anat; 1971; 109(1): 81-95.

Gabella G. Hypertrophy of smooth muscle. Cell Tiss Res; 1975; 163: 199-214.

Gannon BJ, Noblett HR, Burnstock G. Adrenergic innervation of bowel in Hirschsprung's disease. BMJ; 1969; 111: 338-340.

Garrett JR, Howard ER, Nixon HH. Histochemical diagnosis of Hirschsprung's disease. Lancet; 1969; 2: 436.

Garry PJ, Routh JI. A micro method for serum cholinesterase. Clin Chem; 1965; 11: 91-96.

Garver K, Law JO, Garver B. Hirschsprung's Disease : A genetic study. Clin Genet; 1985; 28: 503-508.

Gee S. Idiopathic dilatation of the large intestine. St Barthol Hosp Rep; 1884; 20:19.

Gehring W. Homeoboxes in the study of development. Science (Wash DC); 1987; 236: 1245-1252.

Gershon MD ,Epstein MI , Hegstrand L. Colonization of the chick gut by progenitors of enteric serotonergic neurons: distribution, differentiation and maturation within the gut.
Dev Biol; 1980; 77: 41-51.

Getman DK, Eubanks JH, Camp S, Evans GA, Taylor P. The human gene encoding acetylcholinesterase is located on the long arm of chromosome 7. Am J Hum Genet; 1992; 51(1): 170-177.

Ghinelli C, Del Rossi C. Treatment of Hirschsprung's disease without colostomy. Pediatr Surg Int; 1993; 8: 27-30.

Goldberg E. An epidemiological study of Hirschsprung's disease. Int J Epidemiol; 1984; 13: 479-485.

Golligher JC, Duthie H. Surgical anatomy and physiology of colon, rectum and anus. Golligher J, editor. Surgery of anus, rectum and colon. 3rd edition. London: Balliere Tindall; 1975: pp 23.

Gordon H, Louw JH, Torrington H, Cywes S. A genetical study of Hirschsprung's disease. S Afr Med J; 1966; 40: 720-721.

Gorstein J. Assessment of nutritional status : effects of different methods to determine age on the classification of undernutrition. Bull WHO; 1989; 67: 143-150.

Goto S, Ikeda K. Histochemical acetylcholinesterase activity in the mucosa of resected bowel in Hirschsprung's disease. An analysis of 30 cases. Z Kinderchir; 1985; 40: 26-30.

Gowers WR. The automatic action of the sphincter ani. Proc Roy Soc Med; 1877; 26: 77-84.

Grassi J, Vigny M, Massoulie J. Molecular forms of acetylcholinesterase in bovine caudate nucleus and superior cervical ganglion: solubility properties and hydroophobic character. J Neurochem; 1982; 38: 457-469.

Gregory EJ, Hodges-Savola CA, Fernandez HL. Selective increase in tetramer (G4) AChE activity in rat hindlimb skeletal muscle following short term denervation. J Neurochem; 1989; 53(5):1411-1418.

Grimson KS, Vandergrift L, Dratz HM. Surgery for obstructed megacolon. 1 stage resection and ileostomy. Surg Gynae Obstets; 1945;b 80:164.

Grob M, Genton N, Vontobel V. Erfahrungen in der behandlung des megaclon congenitum und vorschlag einer neuen operationsteknik. Zentrabl Chir; 1959; 84: 1781-1789.

Grosfeld JL, Ballantyne TVN, Csicsko JF. A critical evaluation of the Duhamel operation for Hirschsprung's disease. Arch Surg;1978; 113: 454-461.

Gross ME, Tranquilli WJ. Use of alpha-2-adrenergic receptor antagonists. J Am Vet Med Assoc; 1989; 3: 378-381.

Haffner J, Schistaad G. Atresia of the colon associated with Hirschsprungs Disease. J Pediatr Surg; 1969; 4: 560-562.

Hall ZW. Multiple forms of acetylcholinesterase and their distribution in endplate and non-endplate regions of rat diaphragm muscle. J Neurobiol; 1973; 4(4): 343-361.

Hammond WS, Yntema CL. Depletions of pharyngeal arch cartilages following extirpation of cranial neural crest in chick embryos. Acta Anat; 1964; 56: 21-34.

Hamoudi AB, Reiner C, Boles T,McClung HJ, Kerzner B. Acetylthiocholinesterase staining activity of Rectal mucosa. Arch Path Lab Med; 1982; 106: 670-672.

Harmon RJ, Boston VE. Discordant Hirschsprung's disease in monozygomatic twins: a clue to pathogenesis. J Pediatr Surg; 1988; 23: 1034-1035.

Harrison M, Deitz D, Campbell J, Campbell T. Diagnosis and management of Hirschsprung's disease. Am J Surg; 1986; 152:49-54.

Hawkins HP. Remarks on idiopathic dilatation of the colon. BMJ; 1907; 1:477-483.

Hecker W, Holschneider A, Fendel H, Schauer A, Mester P, Beige H.Die chronische obstipation beim kind durch analsphincterachalasie. Dtsch Med Wschr; 1973; 98: 2334-2340.

Heider H, Brodbeck U. Monomerization of tetrameric bovine caudate nucleus acetylcholinesterase. Implications for hydrophobic assembly and membrane attachment site. Biochem J; 1992;281(1): 279-284.

Heiming G, Gluck M. Beobachtungen an Kindern mit angeborener dysganglionose des colons. Kinderarzt Prax; 1990; 21: 178-181.

Henry M, Snooks S, Barnes P, Swash M. Investigation of disorders of the anorectum and colon. Ann Roy Coll Surg Eng; 1985; 67: 355-360.

Hiatt R. The pathologic physiology of congenital megacolon. Ann Surg; 1951; 133: 313-320.

Hillemeier C, Biancani P. Mechanical properties of obstructed colon in a Hirschsprungs model. Gastroenterology; 1990; 99:995-1000.

Hinkel AS, Bender SW, Posselt HG, Meier-Ruge W, Stovewr B, Holschneider AM, Waag KL. Biochemische untersuchung der Acetylcholinesterase (AChE) an Rectumbiopsien und darmresektaten bei Morbus Hirschsprung. Monatschr Kinderheilkd; 1989; 137(12):824-827.

Hirschsprung H. Stuhltragheit zigheit neugeborener in folge von dilatation und hypertrophie des colons. Jahrb Kinderh; 1888; 27:1-3.

Hirschsprung H. 107 Falle von darminvagination bei kindern behandelt in Konigin Louise Kinderhospital in Kopenhagen warend der Jahre 1871-1904. Mitt Grensgeb Med Chir;1905; 14:525.

Hirobe S, Yokoyama J, Katsumata K. Development of the enteric nervous system in mammals revealed by neurofilament expression. Abstract 25;XXXV th Annual International conference of BAPS;Athens, Greece; 1988.

Hirobe S, Doody DP, Ryan DP, Kim SH, Donahoe PK. Ectopic class 11 major histcompatability antigens in Hirschsprung's disease and Neuronal Intestinal Dysplasia. J Pediatr Surg; 1992;27(3): 357-363.

Holland PWH, Hogan BLM. Spatially related patterns of expression of the homeobox containing gene Hox 2.1 during mouse embryogenesis. Development; 1988; 1021: 159-174.

Holschneider AM, Metzler EM. Elektromanometrische untersuchungen der kontinenzieistung nach rektoanalen fehlbildungen. Z Kinderchir; 1974; 14: 405-412.

Holschneider AM, Fendel H. Verleichende röntgenologische und elektromanometrische untersuchungen der chronischen obstipation. Z Kinderchir; 1974; 15: 76-89.

Holschneider AM. The problem of anorectal continence. Prog Pediatr Surg; 1976; 9: 85-97.

Holschneider A, Keller E, Streibl P, Sippell W. The development of anorectal continence and its significance in the diagnosis of Hirschsprung's disease. J Pediatr Surg; 1976; 11: 151-156.

Holschneider A, Borner W, Buurman O, Caffarena PF, v Issendorf H, Kaiser G, Khan O, Koepke W, Kolb F, Palua M, Pickard L, Potsch R, Raffensperger G, Schärli A, Schnaufer L, Waag L, Poschl U, Markwalder F. Clinical and electromanometrical investigations of postoperative continence in Hirschsprung's disease: An International Workshop. Z Kinderchir; 1980; 29:39-48.

Holschneider AM. Manometric Diagnosis. Holschneider AM, editor. Hirschsprung's disease. Stuttgart, New York: Hipprokrates Verlag, Thieme-Stratton; 1982: pp 72-86.

Holschneider AM. Postoperative results. in Holschneider AM, editor.Hirschsprung's Disease. Stuttgart, New York: Hippokrates Verlag, Thieme-Stratton; 1982: pp 237-240.

Holschneider AM. Elektromanometrische des enddarmes. Diagnostik und therapie der inkontinenz und chronischen obstipation.in Holschneider AM, editor. Munich: Urban and Swartzenberg; 1983: pp 141-207.

Holschneider AM. Treatment and functional results of anorectal continence in children with imperforate anus. Acta Chir Belg;1983; 83: 191-204.

Holschneider AM. Function and evaluation of sphincters. in Anorectal malformations in children: 1988 update. Stephens F, Durham Smith E, Paul N, editors. Alan R Less; March of Dimes Birth Defects series; New York: 1988; 24(4): 425-445.

Holst E, Helin I, Mardh PA. Recovery of Clostridium difficule from children. Scand J Inf Dis; 1981; 13: 41-45.

Howard ER, Nixon HH. Internal anal sphincter. Arch Dis Child;1968; 43: 569-578.

Howard ER, Garrett JR, Kidd A. Constipation and congenital disorders of the myenteric plexus. J Roy Soc Med; 1984; 77(Supp 3): 13-19.

Hultgren B. Ileocolic aganglionosis in white progeny of Overo spotted horses. J Am Vet Med; 1982; 180: 289-292.

Huntley CC, Schaffner L, Challa VR, Lyerly AD. Histochemical diagnosis of Hirschsprung's disease. Pediatrics; 1982; 69:755-761.

Hurst AF. The sphincters of the alimentary canal and the clinical significance. BMJ; 1925; 2: 145-151.

Hurst AF. Anal achalasia and megacolon. Guys Hosp Rep; 1934; 84: 317-350.

Hyde GA, DeLorimier AA. Colon atresia and Hirschsprung's disease. Surgery; 1968; 64: 976-978.

Ikawa H, Kim SH, Hardy Hendren W, Donahoe PK. Acetylcholinesterase and manometry in the diagnosis of the constipated child. Arch Surg; 1986; 121: 435-438.

Ikeda K. A new technique in the surgical treatment of Hirschsprung's disease. Surgery; 1967; 61: 503-508.

Ikeda K, Kume K, Nagasaki A, Suita S. Results of Z-shaped anastomosis for Hirschsprung's disease. Prog Pediatr Surg; 1975; 8: 97-108.

Ikeda K, Goto S. Diagnosis and treatment of Hirschsprung's disease in Japan: an analysis of 1628 cases. Ann Surg; 1984; 199:400-405.

Ikeda K, Goto S. Additional anomalies in Hirschsprung's disease. Z Kinderchir; 1986; 41: 279-281.

Inestrosa NC, Hall ZW. Characteristics of an 12 form of acetylcholinesterase in C2, a mouse muscle cell line. Soc Neurosci Abstr; 1981; 7: 302.

Irhe T. Studies on anal function in continent and incontinent patients. Scand J Gastroenterol; 1974; 25 (suppl): 7-59.

Irwin DA. The anatomy of Auerbach's plexus. Am J Anat; 1931; 49:141-166.

Ito Y, Donahoe P, Hardy Hendren W. Differentiation of intramural ganglia in the dissociated rectosigmoid of the rat: an organ culture study. J Pediatr Surg; 1977; 12(6): 969-975.

Ito Y, Sohma S, Hirano H. Light and electron-microscopic studies on acetylcholinesterase actvity in Auerbach's plexus of the developing rat colon. Histochemistry; 1984; 81: 209-212.

Ito Y, Tatekawa I, Nishiyama F, Hirano H. Ultrastructural localization of Acetylcholinesterase activity in Hirschsprung's disease. Arch Pathol Lab Med; 1987; 111: 161-165.

Iwai N, Yanagihara J, Tsuto T, Tokiwa K, Kalinski P, Takahashi T. Clinical and manometric assessment of anorectal function after Martins operation. Z Kinderchir; 1987; 42: 235-237.

Iwai N, Yanagihara J, Tokiwa K, Deguchi G, Perdzynski W, Takahashi T. Reliability of anorectal manometry in the diagnosis of Hirschsprung's disease. Z Kinderchir; 1988; 43: 405-407.

Iwai N, Yanagihara J, Tokiwa K, Takahisto T. Rectoanal pressure studies and postoperative continence in imperforate anus. Prog Pediatr Surg; 1989; 24: 115-120.

Jacobi A. On some important causes of constipation in infants. Am J Obst; 1869; 2:96.

Jacobs-Cohen RJ, Payette RF, Gershon MD, Rothman TP. Inability of neural crest cells to colonise the presunptive aganglionic bowel of ls/ls mutant mice: requirement for a permissive environment. J Comp Neurol; 1987; 255: 425-438.

Johnson DH, Davis H, Evans J. Hirschsprung's disease. Lancet; 1957; i: 1133-1134.

Jones DS. The origin of the vagi and the parasympathetic ganglion cells of the viscera of the chick. Anat Rec; 1942; 82: 185-197.

Joseph VT, Sim C. Problems and pitfalls in the management of Hirschsprung's disease. J Pediatr Surg; 1988; 23(5): 398-402.

Joppich I. Late complications of Hirschsprung's disease. In Holschneider AM, editor. Hirschsprung's disease. Stuttgart, New York: Hippokrates Verlag, Thieme-Stratton; 1982: 251-261.

Kadair GR, Sites JE, Crutchfield GF. Zonal colonic lymphoganglionosis. JAMA; 1977; 238: 1838- 1841.

Kamagata S, Donahoe PK. The effect of fibronectin on cholinergic differentiation of the fetal colon. J Pediatr Surg; 1985; 20:307-314.

Karnowsky MJ, Roots LA. Direct thiocholine method for cholinesterases. J Histochem Cytochem; 1964; 12: 219-221.

Kasa P, Rakonczay Z. Histochemical and biochemical demonstration of the molecular forms of acetylcholinesterase in the peripheral nerve of rat. Acta Histochem; 1982; 70: 244-257.

Kasai M, Susuki H, Watanabe K. Rectal myotomy with colectomy: a new radical operation with Hirschsprung's disease. J Pediatr Surg; 1971; 6: 31-41.

Kasai M, Suzuki H, O'Hi R, O'Htomo S. Rectoplasty with posterior triangular colonic flap - a radical new operation for Hirschsprung's disease. J Pediatr Surg; 1977; 12: 207-211.

Keighley M, Henry M, Bartolo C, Mortenson N. Anorectal physiology measurement : Report of a working party. Br J Surg; 1989; 76: 356-357.

Keiley E. Repeat pullthrough for Hirschsprung's disease: indications and results. Abstract A 39, XXXVI I th International congress of BAPS; Glasgow; 1990.

Keller H. The development of the intramural nerve plexus of the gastro-intestinal tract. Anat Embryol; 1976; 150: 1-6.

Kelly JH. Cineradiography in Hirschsprung's disease. J Pediatr Surg; 1969; 4: 538-546.

Kelly JH. The clinical and radiological assessment of anal continence in childhood. Aust N Z J Surg; 1972; 42: 62-63.

Kerremans R. Electrical activity and motility in the internal anal sphincter. Acta Gastroenterol Belg; 1968; 31: 465-482.

Khan A, Desjardins JG, Youssef S, Gregoire H, Seidman E. Gastrointestinal manifestations in Sipple syndrome in children. J Pediatr Surg; 1987; 22: 719-723.

Kimura K. Posterior saggital rectal myectomy for persistent rectal achalasia after the Soave procedure for Hirschsprung's disease. Abstract 58, 24th Annual meeting of the Canadian Association of Paediatric surgeons; September 10-13; 1992.

Kirby ML, Gale TF, Stewart DE. Neural crest cells contribute to aorticopulmonary septum. Science (Washington DC); 1983; 220:1059-1061.

Kleinhaus S, Boley SJ, Sherman M, Sieber WK. Hirschsprung's disease: a survey of the Surgical section of the American Academy for Pediatrics. J Pediatr Surg; 1979; 16(5): 588-597.

Klingman WO. The treatment of neurogenic megacolon with selective drugs. J Pediatr; 1938; 13: 805

Klotz DH, Velcek FT, Kottmeier PH. Reappraisal of the endorectal pullthrough operation for Hirschsprung's disease. J Pediatr Surg; 1973; 8(5): 595-600.

Kluck P, Ten Kate FJW, van der Kamp AWM, Tibboel D, Molenaar JC. Pathological explanation for postoperative obstipation in Hirschsprung's disease revealed by monoclonal antibody staining. Am J Clin Pathol; 1986; 86: 490-492.

Kluck P, Tibboel D, Leendertse-Verloop K, Van der Kemp AWM, Ten Kate FJW, Molenaar JC. Diagnosis of congenital neurogenic abnormalities of the bowel with monoclonal antineurofilament antibodies. J Pediatr Surg;1986; 21: 132-135.

Koenig J, Vigny M. Neural induction of 16S acetylcholinesterase in muscle cell cultures. Nature; 1978;271: 75-77.

Kostia J. Results of surgical treatment in Hirschsprung's disease. Arch Dis Child; 1962; 37: 167-168.

Kreiner I. Der histochemische Nachweis des morbus Hirschsprung nach Meier-Ruge. Zentralbl Allg Pathol; 1976; 120: 56-70.

Krishnamurthy S, Schuffler M. Severe idiopathic constipation associated with an obstruction: abnormality of the colonic myenteric plexus. Gastroenterology; 1985; 88: 26-34.

Kubota M, Ito Y, Taguchi T, Ikeda K, Ikadai H. Regional differences in the pattern of neurogenic responses in the aganglionic colon from congenitally aganglionic rats. J Pediatr Surg; 1989; 24: 911-919.

Kunde U, Bender SW, Posselt HG, Waag KL, Meier-Ruge W. Neuronale Intestinale dysplasie mit langstrekigem dundarmbefall: Probleme in diagnostik und therapie. Kinderarzt Prax; 1991; 22: 15-18.

Kuntz A. The development of the nervous system in birds. J Comp Neurol; 1910; 40: 389-408.

Kurer MH, Lawson JON, Pambakion H. Suction biopsy in Hirschsprung's disease. Arch Dis Child; 1986; 61: 83-84.

Lake BD, Puri P, Nixon HH, Claieaux AE. Hirschsprung's disease: An appraisal of histochemically demonstrated acetylcholinesterase activity on suction rectal biopsy specimens as an aid to diagnosis. Arch Path Lab Med; 1978; 102: 244-247.

Lake BD, Malone MT, Risdon RA. The use of acetylcholinesterase (AChE) in the diagnosis of Hirschsprung's disease and intestinal neuronal dysplasia. Pediatr Pathol; 1989; 9(3):351-354.

Lamont MA, Fitchett M, Dennis NR. Interstitial deletion of distal 13q associated with Hirschsprung's disease. J Med Genet;1989; 26: 100-104.

Lane P. Association of megacolon with 2 recessive spotting genes in the mouse. J Hered; 1966; 57: 29-31.

Larsson LT, Malmfors G, Wahlestedt C, Leander S, Hakanson R. Hirschsprung's Disease: a comparison of the nervous control of ganglionic and aganglionic smooth muscle in vivo. J Pediatr Surg; 1988: 431-435.

Law JL. Treatment of megacolon with acetylbetamethylcholine bromide. Am J Dis Child; 1940; 60:262-282.

Lawrence AG, van Wormer DE. Intussusception due to saegmental aganglionosis, JAMA; 1961; 153:143.

Lawson J, Nixon H. Anal canal pressures in the diagnosis of Hirschsprung's disease. J Pediatr Surg; 1967; 2: 544-551.

Lawson J. Observations on residual segment obstruction in treated Hirschsprung's disease. Prog Pediatr Surg; 1972; 4: 129-164.

Layer PG. Comparative localization of acetylcholinesterase and pseudocholinesterase during morphogenesis of the chick brain. Proc Natl Acad Sci USA; 1983; 80: 6413-6417.

Layer P, Kaulich S. Cranial Nerve Growth in birds is preceded by cholinesterase expression during neural cell migration and the formation of an HNK-1 scaffold. Cell Tiss Res; 1991; 265: 393-407.

Le Douarin N, Teillet M. Experimental evidence of migratory differences of neuroblasts. Dev Biol; 1974; 41:162-184.

Leenders E, Sieber WK. Congenital megacolon, observation by Frederick Ruysch 1691. J Pediatr Surg; 1970; 5:1-3.

Lemen R, Benson M, Jones JG. Absolute pressure measurements with hand dipped and manufactured oesophageal balloons. J Appl Physiol; 1974; 37(4): 600-603.

Lillie RD. Histopathologic techniques and practical histochemistry. 3rd Edition Lillie RD editor,. New York: McGraw Hill; 1965:545-546.

Lipson A, Harvey J. Three generation transmission of Hirschsprung's disease. Clin Genet; 1987; 32: 175-178.

Lister J. Abnormal arteries in Hirschsprung's disease. Arch Dis Child; 1966; 41: 149.

Loening-Baucke V. Sensitivity of the sigmoid colon and rectum in children treated for chronic constipation. J Pediatr Gastroenterol Nutr; 1984; 3: 454-459.

Louw JH, Barnard C. Congenital intestinal atresia, observations on its origin. Lancet; 1955; 2: 1065-1067.

Louw JH. The Duhamel operation for Hirschsprung's disease. S Afr Med J; 1961; 35: 1033-1036.

Louw JH. Jejunoileal atresia and stenosis. J Pediatr Surg; 1966; 1: 8-23.

Louw JH, Cywes S. Treatment of Hirschsprung's disease. S Afr J Surg; 1967; 5(2): 69-78.

Louw J.H. Total Colonic Aganglionosis. Can J Surg; 1978; 21:397-405.

Lynn HD, van Heerden JA. Rectal myectomy in Hirschsprung's disease: a decade of experience. Arch Surg; 1975; 110: 991-
994.

MacIver AG, Whitehouse R. Zonal colonic aganglionosis a variant of Hirschsprung's disease. Arch Dis Child; 1972; 47:233

Mahieu P, Pringot J, Bodart P. Defaecography: description of a new procedure and results in normal patients. Gastrointest Radiol; 1984; 9: 247-251.

Marfan A. De la constipation des nourissons et en pasrticular de la constipation d'origine congenitale. Rev Mens Mal de Lenf; 1895; 13: 153.

Martin LW, Altemeier WA. Clinical experience with a new operation (modified Duhamel procedure) for Hirschsprung's disease. Ann Surg; 1962; 156: 678-681.

Martin LW, Caudill DR. A method for elimination of the blind rectal pouch in the Duhamel operation for Hirschsprung's disease. Surgery; 1967; 62(5): 951-953.

Martin L, Torres A. Hirschsprung's disease. Surg Clin N Am; 1985; 65: 1171-1189.

Martucciello G, Bicocchi MP, Dodero P, Lerone M, Silengo-Cirillo M, Puliti A, Gimelli G. Total colonic aganglionosis associated with intestinal deletion of the long arm of chromosome 10. Pediatr Surg Int; 1992; 7(4): 308-310.

Massoulie J, Bon S. The molecular forms of cholinesterases in vertebrates. Annu Rev Neurosci; 1982; 5: 57-106.

McCabe L, Griffin LD, Kinzer A, Chandler M, Beckwith B, McCabe ERB. Overo lethal white foal syndrome: Equine model of aganglionic megacolon (Hirschsprung's disease). Am J Med Genet; 1990; 36: 336-340.

McHugh S, Diamant N. Anal canal pressure profile : A reappraisal as determined by rapid pullthrough. Gut; 1987;28: 1234-1241.

Meier Ruge W, Morger R, Rehbein F. Das hypoganglionäre Megacolon als beleitkrankheit bei morbus Hirschsprung. Z Kinderchir;1970; 8: 254.

Meier-Ruge W, Lutterbeck PM, Herzog B, Morger R, Moser R Scharli A. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. J Pediatr Surg; 1972; 7(1): 11-17.

Meier-Ruge W, Scharli A. The epidemiology and enzyme histotopochemical characterization of ultrashort Hirschsprung's disease. Pediatr Surg Int; 1986; 1: 37-42.

Meier-Ruge W. Das morphologische Erscheinungsbild der neuralen dysplasie des plexus submucosus. Kinderarzt Prax; 1990; 21:837-844.

Meier-Ruge W. Epidemiology of congenital innervation defects of the distal colon. Virchows Archiv A; 1992; 420: 171-177.

Meijers JHC, Tibboel D, van der Kamp AWM, van Haperen-Heuts CCM, Molenaar J. Cell division in migratory and aggregated neural crest cells in the developing gut: an experimental approach to innervation-related motility disorders of the gut. J Pediatr Surg; 1987; 22: 243-245.

Meijers J, Tibboel D, van der Kamp AWM, van Haperen-Heuts CCM, Molenaar JC. A model for aganglionosis in the chicken embryo. J Pediatr Surg; 1989; 24: 557-561.

Meunier P, Marechal J, Mollard P. Accuracy of manometric diagnosis of Hirschsprung's disease. J Pediatr Surg; 1978; 13:411-415.

Meunier P, Mollard P, Jaubert De Beaujeu M. Manometric studies of anorectal disorders in infancy and childhood and investigation of the pathophysiology of continence. Br J Surg; 1976; 63: 402-407.

Miller R, Bartolo D, Roe A, Mortensen N. Assessment of microtransducers in anorectal manometry. Brit J Surg; 1988; 75: 40-43.

Mishalany H, Woolley M. Postoperative functional and manometric evaluation of patients with Hirschsprung's disease. J Pediatr Surg; 1987; 22: 443-446.

Mishalany H , Suzuki H ,Yokoyama J. Report on the first International Symposium of Anorectal Manometry. J Pediatr Surg; 1989; 24: 356-359.

Mishalany H. Seven years experience with idiopathic unremitting chronic constipation. J Pediatr Surg; 1989; 24: 360-362.

Molander M-L, Bergdahl S, Husberg. Hirschsprung's disease: one year follow-up after modified Soave's operation. Z Kinderchir; 1989; 44: 348-351.

Molenaar J, Tibboel D, v.d.Kamp A, Meijers J. Diagnosis of innervation-related motility disorders of the gut. Prog Pediatr Surg; 1989; 24: 173-185.

Moore SW, Millar AJW, Rode H, Cywes S. Intestinal atresia and Hirschsprung's disease. Pediatr Surg Int; 1990; 5(3): 82-89.

Moore SW, Millar AJW, Rode H, Cywes S. Familial aspects of Hirschsprung's disease. Eur J Pediatr Surg; 1991; 1: 97-107.

Moore SW, Rode H, Millar AJW, Cywes S. Is tube caecostomy safe in the surgery of Hirschsprung's disease? S Afr J Surg; 1992; 30(3): 114-117.

Moore T, Landers DB, Lachman RS, Ament ME. Hirschsprung's disease discordant in monozygomatic twins: A study of possible enviromental factors in the production of congenital aganglionosis. J Pediatr Surg; 1979; 14: 151-161.

Morikawa Y, Matsufugi H, Hirobe S, Yokoyama J, Katsumata K.Motility of the anorectum after the Soave-Denda operation. Prog Pediatr Surg; 1989; 24: 67-76.

Munakata K, Okabe I, Morita K. Histologic studies of rectocolic aganglionosis and allied diseases. J Pediatr Surg; 1978; 13: 67-75.

Munakata K, Morita K, Okabe I, Sueoka H. Clinical and histologic studies of neuronal intestinal dysplasia. J Pediatr Surg;1985; 20(3): 231-235.

Murray RD, Ulysses B, Juhling McClung H, Heitlinger L, Rehm D.Cisapride for intractable constipation in children: observations from an open trial. J Pediatr Gastroenterol Nutr; 1990; 11(4): 503-508.

Nagasaki A, Sumitomo K, Shono T, Ikeda K. Anorectal myectomy after Ikeda's Z-Shaped anastomosis in Hirschsprung's disease. Prog Pediatr Surg; 1989; 24: 59-66.

Navarro J, Sonsino E, Boige N, Nabarra B, Ferkadji L, Mashako LMN, Cezard JP. Visceral neuropathies responsible for chronic intestinal pseudo-obstruction syndrome in Paediatric practice: analysis of 26 cases. J Pediatr Gastroenterol Nutr;1990; 11(2): 179-195.

Neilsen H, Madsen C. 13-25 years follow-up after Swenson's operation for Hirschsprung's disease. Prog Pediatr Surg; 1972; 10: 97-102.

Nirasawa Y, Yokoyama J, Ikawa H, Morikawa Y, Katsumata K. Hirschsprung's disease: catecholamine content, alpha-adrenoreceptors, and the effect of electrical stimulation in aganglionic colon. J Pediatr Surg; 1986; 21(2): 136-142.

Nivatvongs S, Stern H, Fryd D. Length of the anal canal. Dis Col Rect; 1982; 198: 600-601.

Nixon HH. Hirschsprung's disease. Arch Dis Child; 1964; 39: 109-115.

Nixon HH. Megacolon and other congenital abnormalities of the colon. in Golligher JC. Surgery of the anus, rectum and colon.3rd ed. London: Baliere Tindall; 1976: pp366-409.

Nixon HH. Hirschsprung's Disease - progress in management and diagnostics. World J Surg; 1985; 2: 189-202.

Nixon HH. Hirschsprung's disease. in Paediatric surgery. Spitz L, editor, London: Butterworths; 1990: 375-391.

Noden D. An analysis of the migratory behaviour of avian cephalic neural cells. Dev Biol; 1975; 24: 106-130.

Ogawa N, Sasaki Y, Misugi K, Shiraishi R, Nishi T. Histological study of the transitional zone in Hirschsprung's disease. J Jpn Soc Pediatr Surg; 1986; 22: 962-967.

Ohashi S, Okamoto E. An experimental study of the mechanism of rectosphincteric reflex with special reference to Hirschsprung's disease. J Pediatr Surg; 1984; 19: 278-280.

Ohmann U, Ehren H. Effects of luminal distention and obstruction on intestinal circulation. Pediatr Surg Int; 1986; 1: 4-9.

Okamoto E, Ueda T. Embryogenesis in intramural ganglia of the gut and its relationship to Hirschsprung's disease. J Pediatr Surg; 1967; 2: 437-443.

Okamoto E, Iwasaki T, Kakutani T, Ueda T. Selective destruction of the myenteric plexus: its relationship to Hirschsprung's disease, achalasia of the oesophagus and hypertrophic pyloric stenosis. J Pediatr Surg; 1967; 2: 444-453.

Okasora T, Okamoto E, Kuwata K, Toyosaka A, Ohashi S, Ueki S. Serum and erythrocyte AChE in Hirschsprung's disease. Z Kinderchir; 1983; 38: 298-300.

Omenn G, McKusick V. The association of Waardenburgh syndrome and Hirschsprung's megacolon. Am J Genet; 1979; 3: 217-223.

Parry CH. Singular and fatal accumulation of faeces . in Collection from unpublished medical writing of the late Parry CH. London: Underwood: Vol 2, pp 380 (Quoted from Ehrenpreiss 1970).

Paskins J, Lawson J, Clayden G. The effect of Ketamine anesthesia on anorectal manometry. J Pediatr Surg; 1984; 19:289-291.

Passarge E. The genetics of Hirschsprung's disease. N Eng J Med; 1967; 276: 138-143.

Patrick WJA, Besley GTN, Smith IL. Histochemical diagnosis of Hirschsprung's disease and a comparison of the histochemical and biochemical activity of acetylcholinesterase in rectal mucosal biopsies. J Clin Pathol; 1980; 33: 336-343.

Patterson PH. Environmental determinations of autonomic neurotransmitter function. Ann Rev Neurosci; 1978; 1: 1-17.

Payette RF, Tennyson VM, Pham TD. Origin and morphology of nerve fibres in aganglionic colon of lethal spotted (ls/ls) mutant mice. J Comp Neurol; 1987; 257: 237-252.

Peacock TB. Fatal constipation from dilatation of the colon. Trans Pathol Soc London; 1872; 23:104.

Pearse AGE, Polak JM, Rost FWD, Fontaine J, LeLievre C, Le Douarin N. Demonstration of the neural crest type 1 (APUD) cells in the avian carotid body, using a cytochemical marker system. Histochemistry; 1973; 34: 191-203.

Perelman A, Inestrosa NC. A simple assay to estimate the acetylcholinesterase molecular forms in crude extracts of rat skeletal muscle. Anal Biochem; 1989; 180: 227-230.

Perris R, van Boxberg Y, Lofberg J. Local embryonic matrices determine region specific phenotypes in neural crest cells.
Science; 1988; 241: 86-89.

Pickard LR, Santoro S, Wyllie R, Haller JA. Histochemical studies of experimental fetal intestinal obstruction. J Pediatr Surg;1981; 18(3): 256-260.

Pistor G, Hoffmann Von Kapp-Herr S, Grussner R, Munakata K, Müntefering H. Neuronal intestinal dysplasia. Pediatr Surg Int; 1987; 2:232-238.

Polley T, Coran A, Wesley J. A ten year experience with 92 cases of Hirschsprung's disease. Ann Surg; 1985; 202: 349-355.

Polley TZ, Coran AG, Wesley JR. The definitive management of Hirschsprung's disease with the endorectal pullthrough procedure. Pediatr Surg Int; 1986; 1: 90-94.

Polley T, Coran A. Hirschsprung's disease in the Newborn. Pediatr Surg Int; 1986; 1: 80-83.

Pryor GT, Schlesinger K, Calhoun WH. Differences in brain enzymes amoung five inbred strains of mice. Life Sciences; 1966; 5: 2105-2111.

Puri P, Nixon HH. Long term results of Swenson's operation for Hirschsprung's disease. Prog Pediatr Surg; 1972; 10: 87-95.

Puri P, Lake BD, Nixon HH, Mishalany H, Claireaux AE. Neuronal colonic dysplasia: an unusual association with Hirschsprung's disease. J Pediatr Surg; 1977; 12: 681-685.

Puri P, Blake R, Carrol R, Nixon HH. Relationship between functional and histologic appearances of developing ganglion cells in guinea pig rectum. J Pediatr Surg; 1980; 15(1): 42-47.

Rafferscheid P, Oehmichen M, Schweizer P, Flach A. Acquired segmental hypoganglionosis. Z Kinderchir; 1978; 23(1): 49-52.

Rafferscheid P, Flach A. Particular forms of Hirschsprung's disease:neuronal dysplasia of the intestine. Hirschsprung's disease.in Holschneider AM ed. New York: Thieme-Stratton; 1982: pp 133-142.

Rakonczay Z, Mallol J, Schenk H, Vicendon G, Zanetta JP. Purification and properties of the membrane-bound acetylcholinesterase from adult rat brain. Biochim Biophys Acta; 1981; 657: 243-256.

Rakonczay Z, Nemeth P. Change in the distribution of Acetylcholinesterase molecular forms in Hirschsprung's disease. J Neurochem; 1984; 43(4): 1194-1196.

Read MG, Read NW. The role of sensation in preserving continence. Gut; 1982; 23: 345-347.

Rehbein F, Von S, Zimmermann H. Results with abdominal resection in Hirschsprung's disease. Arch Dis Child; 1960; 35: 29-37.

Rehbein F, Morger R, Kundert JG, Meier-Ruge W. Surgical problems in congenital megacolon. J Pediatr Surg; 1966; 1: 526-533.

Rehbein F, Boos D. Surgical treatment of Hirschsprung's disease: Rehbein's procedure. in Holschneider AM, editor, Hirschsprung's disease. Stuttgart, New York: Hippokrates Verlag, Thieme-Stratton; 1982: 189-195.

Rescorla FJ, Morrison AM, Engles D, West KW, Grosfeld JL. Hirschsprung's disease: Evaluation of mortality and long term function in 260 cases. Arch Surg; 1992; 127: 934-942.

Rietra P, Slaterus KW, Zanen HC, Meuwissen SG. Clostridial toxin in faeces of healthy infants. Lancet; 1978; 1: 319.

Rintala R, Rapola J , Louhimo I. Neuronal Intestinal Dysplasia. Prog Pediatr Surg; 1989; 24: 186-192.

Robert B, Sassoon D, Jacq B, Gehring W, Buckingham M. Hox 7, a mouse homeobox gene with a novel pattern of expression during embryogenesis. EMBO J; 1989; 8: 91-100.

Robertson HE, Kernohan JW. The myenteric plexus in congenital megacolon. Proc Staff Meet Mayo Clin; 1938; 13: 123-125.

Robey S, Kuhajda FP, Yardley JH. Immunoperoxidase stains of ganglion cells and abnormal mucosal nerve proliferations in Hirschsprung's disease. Hum Pathol; 1988; 19(4): 432-437.

Rode H, Moore SW, Kaschulka ROC, Brown R, Cywes S. Degenerative leiomyopathy - a clinico-pathological study. Pediatr Surg Int; 1992; 7: 23-29.
Ross VP. The results of sympathectomy. Br J Surg; 1935; 23:433-443.

Rothman JP, Gershon MD. Phenotypic expression in the developing avian enteric nervous system. J Neuroscience; 1982; 2: 381-393.

Rothman TP, Gershon MD. Regionally defective colonization of terminal bowel by precursors of enteric neurones in lethal spotted mutant mice. Neuroscience; 1984; 12: 1293-1311.

Rotundo RL. Purification and properties of the membrane bound form of Acetylcholinesterase from chicken brain. J Biol Chem; 1984; 21: 13186-13194.

Ryan ET, Ecker JL, Christakis N, Folkman J. Hirschsprung's disease: associated abnormalities and demography. J Pediatr Surg; 1992; 27(1): 76-81.

Schärli A , Kiesewetter W. Imperforate anus : Anorectosigmoid pressure studies as a quantive evaluation of postoperative continence. J Pediatr Surg; 1969; 4: 694-703.

Schärli AF, Kiesewetter WB. Defaecation and continence:Some new concepts. Dis Colon Rectum; 1970; 13: 81-90.

Schärli A , Meier-Ruge W. Localized and disseminated forms of NID mimicking Hirschsprung's disease. J Pediatr Surg; 1981; 16:164-170.

Schärli A. The practical significance of manometry in pathology of the rectum and anorectum. Prog Pediatr Surg; 1989; 24: 142-154.

Schärli AF. Neuronal Intestinal Dysplasia. Pediatr Surg Int; 1992; 7: 2-7.

Schmidt JE. Uber Hirschsprungsche Krankheit, insbesondere irhe chirurgische behandlung. Klin Chir 61:682

Schnaufer L, Talbert J, Haller J, Reid N, Tobon F, Schuster MM. Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood. J Pediatr Surg; 1967; 2: 538-543.

Schneiderohn O. Die therapie der Hischsprungschen Krankheit. Z Kinderch 1915; 12:321.

Schocket E, Telok H. Aganglionic megacolon, Phaeochromocytoma, Megaloureter and Neurofibromatosis. Am J Dis Ch; 1957; 94:185-191.

Schofield D, Devine W, Yunis EJ. Acetylcholinesterase-stained suction rectal biopsies in the diagnosis of Hirschsprung's disease. J Pediatr Gastoenterol Nutr; 1990; 11(3): 221-228.

Schofield DE, Yunis EJ. Intestinal Neuronal Dysplasia. J Pediatr Gastroenterol Nutr; 1991; 12: 182-189.

Schultz L, Reiner C. Circumferential ulceration of the Ileum : An unusual cause of melaena in childhood. Am J Dis Ch; 1963;105: 375-380.

Schuster M , Hendrix T , Mendeloff A. The internal anal sphincter response. J Clin Invest; 1963; 42: 196-207.

Scobie WG, Mackinlay GA. Anorectal myectomy in treatment of ultrashort segment Hirschsprung's disease. Arch Dis Child; 1977; 52: 713-715.

Sereni F, Principi N, Perletti L, Sereni LP. Undernutrition and the developing rat brain. 1. Influence on AChE and succinic dehydrogenase activities on norepinephrine and 5-OH-tryptamine concentrations. Biology of the Neonate; 1966; 10: 254-265.

Shandling B, Gilmour RF. The anal sphincter force in health and disease. J Pediatr Surg; 1987; 22(8): 754-757.

She YX, Shi CR, Chen JZ, Wu Y. Observations on erythrocyte AChE activity in infants and children in Hirschsprung's disease. J Pediatr Surg; 1984; 19: 281-284.

Sherman J, Snyder M, Weitzman J, Jona J, Gillis DA, O'Donnell B, Carcassonne M. A 40 year retrospective study of 880 Swenson patients. J Pediatr Surg; 1989; 24(8): 833-838.

Shipton EA. Alpha-adrenergic agonists in anaesthesia and analgesia. S Afr Med J; 1991; 79: 578-579.

Shorvon PJ, Stevenson GW. Defaecography: setting up a service. Br J Hosp Med; 1989; 41: 460-466.

Sieber W. Hirschsprungs Disease. Paediatric Surgery. 4th edition, editor, Welch KJ, Randolph JG, Ravitch MM et al, eds. Chicago,IL: Year Book; 1986; 2: pp 995-1020.

Sieber-Blum M, Siber F, Yamada KM. Cellular fibronectin promotes adrenergic differentiation in quail neural crest cells in vitro. Exp Cell Res; 1981; 133: 285-295.

Siemers PT, Dobbins WO. The Meissner plexus in Crohns disease of the colon. Surg Gynae Obstets; 1972; 138: 39-42.

Sikorav JL, Kreji E, Massoulie J. cDNA sequences of Torpedo marmorata AChE: primary structure of the catalytiic subunit; co-existance of multiple 5'- untranslated regions. EMBO J; 1987; 6(7): 1865-1873.

Simpser E, Kahn E, Kenigsberg K, Duffy L, Markowitz J, Daum F. Neuronal Intestinal Dysplasia: Quantitative diagnostic criteria and clinical management. J Pediatr Gastroenterol Nutr; 1991; 12: 61-64.

Siplovich L, Carmi R, Bar-Ziv J, Karplus M, Mares AJ. Hirschsprung's disease in monozygotic twins. J Pediatr Surg; 1983; 18: 639-640.

Small DH. Non-cholinergic actions of AChE: proteases regulating cell growth and development. Trends Biochem Sci; 1990; 15:213-216.

Small D. Reply from Small. Trends Biochem Sci; 1990; 15: 338.

Smith B. Myenteric plexus in Hirschsprung's disease. Gut; 1967;8: 308-312.

Smith B. Pre and postnatal development of the ganglion cells of the rectum and its surgical implications. J Pediatr Surg; 1969; 3: 386-391.

Smith G, Cass D. Infantile Hirschsprung's disease- is barium enema useful? Pediatr Surg Int; 1991; 6: 318-321.

So HB, Schwartz D, Becker JM, Daum F, Schneider KM. Endorectal pullthrough without preliminary colostomy in neonates with Hirschsprung's disease. J Pediatr Surg; 1980; 15: 470-471.

Soave F. A New Surgical technique for the treatment of Hirschsprung's disease. Surgery; 1960; 56: 1007-1014.

Soave F. Hirschsprung's disease : A new surgical technique. Arch Dis Child; 1964; 39: 116-124.

Soave F. Long term results of operative treatment in Hirschsprung's disease. Z Kinderchir; 1977; 22: 267-279.

Soave F. Endorectal Pullthrough:20 Years Experience. J Pediatr Surg; 1985; 20: 568- 579.

Sonsino E, Mouy R, Focaud P, Cezard JP, Aigrain Y, Bocquet L, Navarro J. Intestinal pseudobstruction related to cytomegalovirus infection of myenteric plexus. N Eng J Med; 1984; 311(3): 196.

Soper TR, Miller FE. Modification of Duhamel procedure: elimination of rectal pouch and colorectal septum. J Pediatr Surg; 1968; 3: 376-385.

Soper RT, Miller FE. Congenital aganglionic megacolon. Arch Surg; 1968; 96: 554-562.

Sorenson K, Gentinetta R, Brodbeck U. An amphiphile-dependent form of human caudate brain nucleus acetylcholinesterase purification and properties. J Neurochem; 1982; 39: 1050-1060.

Soreq H, Zevin-Sonkin D, Razon N. Expression of cholinesterase gene(s) in human brain tissue: translational evidence for multiple mRNA species. EMBO J; 1984; 3(6): 1371-1375.

Soreq H, Lapidot-Lifson Y, Zakut H. A role for cholinesterases in tumorigenesis? Cancer Cells; 1991; 3(12): 511-516.

Soto JM, Soto RT, Aufses AH, Bleicher M, Krasna IH. Hirschsprung's disease : 25 year experience at the Mount Sinai Hospital (New York) and reviews of the literature. Mt Sinai J Med; 1977; 44: 241-256.

Sparkes RS, Sparkes MC, Kalina RE, Pagon RA, Salk DJ, Disteche CM. Separation of retinoblastoma and esterase D loci in a patient with sporadic retinoblastoma ans del (13)q 14.1q22.3. Hum Genet; 1984; 68: 258-259.

Spouge D, Baird P. Hirschsprung's disease in a large birth cohort. Teratology; 1985; 32: 171-177.

Springhall RJ, Keiley EM, Boyd SG. The nature of neurogenic damage to the external anal sphincter in children treated for Hirschsprung's disease. Pediatr Surg Int; 1990; 5: 131-133.

Sprinz H, Cohen H, Heatoin LD. Hirschsprung's disease with skip areas. Am Surg ; 1961; 153:143.

Staple T, McAlister WH, Anderson MS. Plexiform Neurofibromatosis of the colon simulating Hirschsprung's disease. Am J Roentgenol; 1964; 91: 840-845.

State D. Surgical treatment for idiopathic congenital megacolon. Surg Gynaecol Obstets; 1952; 95: 201-212.

Steichen FM, Talbert JL, Ravitch MM. Primary side to side colorectal anastomosis in the Duhamel operation in Hirschsprung's disease. Surgery; 1968; 64: 475-483.

Steichen M, Spigland A, Nunez D. The modified Duhamel operation for Hirschsprung's disease performed entirely with mechanical sutures. J Pediatr Surg; 1987; 22: 436-438.

Stephens FD, Smith ED. Classification,identification and assessment of surgical treatment of anorectal anomalies: Report of a workshop meeting. Wisconsin: Racine; 1984.

Stone WD, Hendrix TR, Schuster MM. Aganglionosis of the entire colon in adolescents. Gastroenterology; 1965; 48: 636-641.

Storsteen KA, Kerohan JW, Bargen JA. The myenteric plexus in chronic ulcerative colitis. Surg Gynae Obstets; 1953; 97: 335-343.

Strong R, Moore MA, Hale C, Wessels-Reiker M, Armbrecht HJ, Richardson A. Modulation of tyrosine hydroxylase gene expression in the rat adrenal gland by age and reserpine. Brain Research; 1990; 525: 126-132.

Stryker S, Telander J, Perrault J. Anoneorectal evaluation after colectomy and endorectal ileoanal anastomosis in children and young adults. J Pediatr Surg; 1985; 20: 656-660.

Sun WM, Read NW, Miner PB. Relation between rectal sensation and anal function in normal subjects and patients with faecal incontinence. Gut; 1990; 31: 1056-1061.

Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, Silman I. Atomic structure of acetylcholinesterase for Torpedo californica: a prototypic AChE-binding protein. Science; 1991; 253(5022): 872-879.

Suzuki H, Matanabe K, Kasai M. Manometric and cineradiologic studies on anorectal motility in Hirschsprung's disease before and after surgery operation. Tohuku J Exp Med; 1970; 102: 69-80.

Swenson O, Bill AH. Resection of rectum and rectosigmoid with preservation of the sphincter for benign spastic lesions producing megacolon: an experimental study. Surgery; 1948; 24: 212-220.

Swenson O. A new surgical procedure in the treatment of Hirschsprung's disease. Surgery; 1950; 28: 371-381.

Swenson O. Congenital Megacolon. Pediatrics; 1953; 12(1): 1-4.

Swenson O. Follow - up of 200 patients treated for Hirschsprung's disease during a 10 year period. Ann Surg; 1957; 146: 706-714.

Swenson O. Partial internal sphincterectomy in the treatment of Hirschsprung's disease. Am Surg; 1964; 160: 540-550.

Swenson O, Sherman JO, Fisher JH. Diagnosis of congenital megacolon: an analysis of 501 patients. J Pediatr Surg; 1973; 8: 587-594.

Taguchi T, Tanaka I, Ikeda K. Fibromuscular dysplasia of arteries in Hirschsprung's disease. Gastroenterology; 1985; 88: 1099-1103.

Tam P. An immunocytochemical study with Neuron Specific Enolase and Substance P of human ennervation. J Pediatr Surg; 1986;21: 227-232.

Tam PK, Boyd GP. New insights into physiologic abnormalities in Hirschsprung's disease by wholemount immunohistochemistry. J Pediatr Surg; 1991; 26(5): 595-597.

Tamate S, Shiokawa C, Yamada C, Takeuchi S, Nakahira M Kadowaki H. Manometric diagnosis of Hirschsprung's disease in the neonatal period. J Pediatr Surg; 1984; 19: 285-288.

Taylor B ,Beart R ,Phillips S. Longditudinal and radial variations of pressure in the human anal sphincter. Gastroenterology; 1984; 86: 693-697.

Teich S, Schisgall RM, Anderson KD. Ischaemic enterocolitis as a complication of Hirschsprung's disease. J Pediatr Surg; 1986; 21: 143-145.

Teitelbaum D, Qualman S, Caniano D. Hirschsprungs disease identification of risk factors for enterocolitis. Ann Surg; 1988; 207: 240-244.

Templeton RD, Lawson H. Studies in motor activity of large intestine: response to autonomic drugs. Am J Physiol; 1932; 101: 511-528.

Tepas JJ, Wyllie RG, Shermeta DW, Inon AE, Pickard LR, Haller JA. Comparison of histochemical studies of intestinal atresia in the human newborn and fetal lamb. J Pediatr Surg; 1979; 14: 376-380.

Ternberg J, Winters K. Plexiform neurofibromatosis of the colon as a cause of congenital megacolon. Am J Surg; 1965; 109: 663-665.

Testylier G, Gourmelon P, Clarencon D, Multon E, Fatome M, Viret J. Rapid postmortem decrease in ectocellular acetylcholinesterase activity in rat striatum as assessed by in vivo microspectrophotometry. Brain Res; 1991; 566(1-2): 159-165.

Thomas DFM, Fernie DS, Bayston R, Spitz L, Nixon HH. Enterocolitis in Hirschsprung's disease: a controlled study of the etiologic role of Clostridium Difficule. J Pediatr Surg; 1986; 21: 22-25.

Tibboel D, Meijers JHC, Kluck P, van der Kamp AWM, Ten Kate FWJ,van Haperen-Heuts JC, Molenaar J. Use of monoclonal antibodies for diagnosis and research in pathology of the enteric nervous system. A review. Dev Neurosci; 1987; 9: 133-143.

Tiffin ME, Chandler LR, Faber HK. Localized absence of the ganglion cells of the myenteric plexus in congenital megacolon. Am J Dis Child; 1940; 59: 1071-1082.

Tittel K. Uber eine angeborene Missbildung des dickdarmes. Wien Klin Wosenschr; 1901; 14: 903-907.

Tobon F, Reid NCRW, Talbert JL, Schuster M. Nonsurgical test for the diagnosis of Hirschsprung's disease. N Engl J Med; 1968; 278:188-193.

Toorman J, Bots G, Vio P. Acetylcholinesterase activity in rectal mucosa of children with obstipation. Virchows Arch A; 1977; 376: 159-164.

Touloukian RJ, Aghajanian G, Roth RH. Adrenergic hyperactivity of the aganglionic colon. J Pediatr Surg; 1973; 8: 191-195.

Touloukian RJ, Duncan R. Acquired aganglionic megacolon in a premature infant: report of a case. Pediatrics; 1975; 56(3): 459-462.

Towne BH, Stocker JT, Thomson HE, Chang JHT. Acquired aganglionosis. J Pediatr Surg; 1979; 14(6): 688-689.

Treves A. Idiopathic dilatation of the colon. Lancet; 1898; i: 276-279.

Trigg PH, Belin R, Haberkorn S, Long WJ, Plaskes J, Spitz L, Willital GH. Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung's disease. J Clin Pathol; 1974; 27: 207-213.

Tucker GC, Ciment G, Thiery JP. Pathways of avian neural crest migration in the developing gut. Dev Biol; 1986; 116: 439-450.

Tyohara T, Nada O, Ikeda K. Influence of ischaemia on noradrenergic nerves in the terminal colon of humans and rats. Eur Surg Res; 1986; 18(6): 349-355.

Ustach T, Tobon F, Schuster M. Simplified method for diagnosis of Hirschsprung's disease. Arch Dis Child; 1969; 44: 694-697.

Vane DW, Grosfeld JL. Hirschsprung's disease: experience with the Duhamel operation in 195 cases. Pediatr Surg Int; 1986; 1: 95-99.

Vaos G. Quantitative assessment of the stage of neuronal maturation in the developing human fetal gut. J Pediatr Surg; 1989; 24: 920-925.

Verder H, Staer Johansen K, Engbaek K. Anal tonometry: a diagnostic help in Hirschsprung's disease. Acta Pediatr Scand; 1973; 62: 59-65.

Verder H, Krasilnikoff PA, Scheibel E. Anal tonometry in the neonatal period in mature and premature children. Acta Pediatr Scand; 1974; 64: 592-596.

Verdiere M, Derer M, Rieger F. Mutiple molecular forms of rat superior cervical ganglion acetylcholinesterase: developmental aspects in primary cell culture and during postnatal maturation in vivo. Dev Biol; 1982; 89: 509-515.

Visi ES, Zseli J, Kontor E, Feher E, Verebelyi T. Characteristics of cholinergic neuroeffector transmission of ganglionic and aganglionic colon in Hirschsprung's disease. Gut; 1990; 31: 1046-1050.

Wade R, Royle ND. The operative treament of Hirschsprung's disease, new method with explanation of the technique and results of operation. Med J Aust; 1927; I:137-141.

Wakely PE, McAdams AJ. Acetylcholinesterase histochemistry and the diagnosis of Hirschsprung's disease. Pediatr Pathol; 1984; 2: 35-46.

Waterlow JC, Buzina R, Keller W, Land JM, Nichaman MZ, Tanner JM. The presentation and use of height and weight data for comparing the nutritional status of groups of children under the age of 10 years. Bull WHO; 1977; 55: 489-498.

Webb G, Keith CG, Campbell NT. Concurrent de novo Interstitial deletion of band 2 P 22 and reciprocal translocation 3-7 (P21q22). Clin Genet; 1988; 0: 125-127.

Webster W. Embryogenesis of the enteric ganglia in normal mice and in mice that develop congenital aganglionic megacolon. J Embryol Exp Morphol; 1973; 30(3): 573-585.

Weikert T, Rathjen FG, Layer PG. Developmental maps of actylcholinesterase and G4-antigen of the early chicken brain: Long distance tracts originate from AChE-producing cell bodies. J Neurobiol; 1990; 21(3): 482-498.

Weinberg A, Currarino G, Besserman M. Hirschsprung's disease and congenital deafness. Hum Genet; 1977; 38: 157-161.

Weinberg RJ, Kish WJ, Smalley JR, Brown MR, Putnam TC. Acquired aganglionosis of the colon. J Pediatr; 1982; 101: 406-409.

Weitzman JJ. Management of Hirschsprung's disease with the Swenson procedure with emphasis on long term follow up. Pediatr Surg Int; 1986; 1: 100-104.

Wenthold RJ, Mahler HR, Moore WJ. Properties of acetylcholinesterase from rat brain. J Neurochem; 1974; 22: 945-949.

West KW, Grosfeld JL, Rescorla FJ, Vane DW. Acquired aganglionosis: a rare occurrence following pull-through procedures for Hirschsprung's disease. J Pediatr Surg; 1990; 25: 104-109.

Weston JA. The migration and differentiation of neural crest cells. Adv Morphol US; 1970; 8: 41-114.

White HL, Wu JC. Choline and Carnitine transferases of Heart. Biochemistry; 1973; 12(5): 841-846.

Whitehouse FR, Bargen JA, Dixon CF. Congenital megacolon - favourable end results and treatment by resection. Gastroenterology; 1943; I: 922-937.

Whitehouse FR, Kernohan JW. Myenteric plexus in congenital megacolon. Arch Int Med; 1948; 82:75-111.

Wilson-Storey D, Scobie WG, Raeburn JA. Defective white blood cell function in Hirschsprung's disease : A possible predisposing factor to enterocolitis. J Roy Coll Surg Edin; 1988; 33: 185-188.

Wolgemuth D, Behringer RR, Mostoller MP, Brinster RL, Palmiter RD. Transgenic mice overexpressing the mouse homeobox containing gene Hox 1.4 exhibit abnormal gut development. Nature; 1989; 337: 464-467.

Wood JD. Electrical activity of the intestine of mice with hereditary megacolon and absence of ganglion cells. Am J Digest Dis; 1973; 18: 477-488.

Wood JD, Brann LR. Pharmacological analysis of rebound excitation of large intestine of piebald mouse model for Hirschsprung's disease. Dig Dis Sci; 1986; 31(7): 744-752.

Woolley DE. Sex differences in brain pseudocholinesterase activity in the rat. J Neurochem; 1963; 10: 447-452.

Wright C, Hogan B. Another hit for gene targeting. Nature; 1991; 350: 458-459.

Wu CS, Gan L, Jang JT. Conformation similarities of the globular and tailed forms of acetylcholinesterase for Torpedo californica. Biochem Biophys Acta; 1987; 911(1): 25-36.

Wyllie GG. Course and management of Hirschsprung's disease. Lancet; 1957; 1: 847-850.

Yamamoto M, Imamura K, Saji S, Sato T, Kashiki Y, Kunieda T, Sakate T. Manometric and histochemical studies in patients with Hirschsprung's disease and idiopathic constipation. Jap J Soc Pediatr Surg; 1977; 13: 529-536.

Yamataka A, Miyano T, Fujimoto H, Nishiye H. Sparse neuromuscular junctions in the normo-ganglionic bowel of Hirschsprung's disease- is it a causative factor for failed pullthrough operations. Abstract 59: 24th Annual meeting of the Canadian Association of Pediatric Surgeons; Ottawa; 1992.

Yancey A, Cromartie JE, Ford JR, Nichols RR, Saville AF. A modification of the Swenson technique for congenital megacolon. J Natl Med Ass; 1952; 44(5): 356-363.

Yntema CL, Hammond WS. The origin of the intrinsic ganglia of trunk viscera from vagal neural crest in the chicken embryo. J Comp Neurol; 1954; 101: 515-541.

Yntema CL, Hammond WS. Depletions and abnormalities in the cervical sympathetic system of the chick following extirpation of the neural crest. J Exp Zool; 1945; 100: 237-263.

Yoshioka K, Keighley M. Anorectal myectomy for outlet obstruction. Br J Surg; 1987; 74: 373-376.

Younkin SG, Rosenstein C, Collins PL, Rosenberry TL. Cellular localization of the molecular forms of acetylcholinesterase in rat diaphragm. J Biol Chem; 1982; 257: 13630-13637.

Yunis EJ, Schofield DE. Case 4. Intestinal Neuronal dysplasia in a case of sigmoid stenosis. Pediatric Pathology; 1992; 12: 275-280.

Zuelzer WW, Wilson JL . Functional intestinal obstruction on congenital neurogenic basis in infancy. Am J Dis Child; 1948; 75:40-64.

PUBLICATIONS FROM WORK PERFORMED FOR THIS THESIS

A. ARTICLES PUBLISHED

1. Intestinal atresia and Hirschsprungs disease. Moore SW, Millar A, Rode H, Cywes S. Pediatr Surg Int 1990 5(3)182-189
2. Familial Aspects of Hirschsprungs Disease. Moore SW, Rode H, Millar AJW, Albertyn R, Cywes S. Eur J Pediatr Surg 1991: 1:97-107
3. Is Tube caecostomy safe in the surgery of Hirschsprungs Disease? Moore SW, Rode H, Millar AJW, Cywes S. S Afr J Surg 1992: 30:3:114-117.
4. Familial and genetic aspects of Neuronal Intestinal Dysplasia and Hirschsprung's disease. Moore SW, Kaschula ROC, Cywes S. Pediatr Surg Int 1993
5. Acquired Aganglionosis following surgery for Hirschsprung's disease: a report of 5 cases during 33 years experience with pull-through procedures. Cohen MC, Moore SW, Neveling U, Kaschula ROC. Histopathology 1993: 22:163-168.
6. Long Term Clinical, Manometric and Histologic evaluation of obstructive symptoms in the postoperative Hirschsprungs patient. Moore SW, Millar AJW, Cywes S. J. Paediatr Surg 1994
7. A histological grading system for the evaluation of co-existing NID in Hirschsprung's disease. Moore SW, Laing D, Kaschula ROC, Cywes S. (Submitted 1993)
8. The effects of prolonged intestinal obstruction on the enteric nervous system (ENS) in Rats. Moore SW, Laing D, Melis J, Cywes S. J Pediatr Surg 1993.
9. Molecular forms of acetylcholinesterase in the diagnosis and management of Hirschsprung's disease. Moore SW, de Wet PM, Johnson G, Purves L. (Submitted 1993)

B. ABSTRACTS PUBLISHED FROM THIS THESIS

1. Clinical and Manometric evaluation following endorectal pullthrough for Hirschsprungs disease. Moore SW, Millar A, Cywes S. South African J Surg 1989; 27:154 (Abstract)
2. Acetylcholinesterase biochemical assay in the diagnosis and management of Hirschsprung's disease. de Wet P, Moore SW, Cywes S. S Afr J Surg 1991; 29:3: 134 (Abstract)
3. Complimentary tube caecostomy in the Soave Endorectal pullthrough procedure Moore SW, Millar AJW, Rode H, Brown RA, Cywes S. S Afr J Surg 1991; 29:3:129 (Abstract).
4. Secondary changes to the Enteric Nervous system (ENS) in an animal model of intestinal obstruction - the role of the Alpha-2 adrenergic receptor. Moore SW, Laing D, Melis J. S Afr J Surg 1992; 30:3:135 (Abstract)
5. The proximal ganglionated segment in Hirschsprung's disease - the value of a histological grading system in evaluating the enteric nervous system (ENS). Moore SW, Laing D, Kaschula ROC, Cywes S. S Afr J Surg 1993; 31(1)37 (Abstract).

C. FURTHER ARTICLES IN PREPARATION

1. The significance of Butyrylcholinesterase (BChE) activity in Hirschschsprung's disease and NID. Moore SW, Johnson G, de Wet P.
2. Increased AChE expression resulting from alpha-2-adrenergic receptor stimulation. Moore SW, Laing D, Melis J.

D. OTHER WORK ARISING FROM THIS THESIS

1. Acetylcholinesterase of human intestinal tissue affected by Hirschsprung's disease: effect of Magnesium chloride on isoforms. Johnson G, Moore SW, Purves L. (Article submitted 1992)
2. Magnesium effects on red blood cell Acetylcholinesterase - a modification for the Karnowsky-Roots technique. Moore SW, Johnson G, Laing D, Purves L, Kaschula ROC. (In preparation)

3. The role of impaired sensation in chronically constipated patients with overflow incontinence. Moore SW. S Afr J Surg 1992; 30:4: 192-193. (Abstract)

E. PRESENTATIONS AT SCIENTIFIC MEETINGS ON WORK DONE FOR THIS THESIS

1. Hirschsprung's Disease -long term follow up. Moore SW, Cywes S, Millar AJW, Ferreira M. 7th Biennial Congress of S.A.Association of Pediatric Surgeons, Cape Town 1986.
2. The significance of obstructed symptoms following Soave Endorectal pullthrough. Moore SW, Cywes S. 8th Biennial Congress of S.A.Association of Pediatric Surgeons. Pretoria 1988.
3. Clinical and manometric evaluation of the Soave Endorectal pullthrough. Moore SW, Millar AJW, Cywes S. Surgical Research Society May 1988.
4. Long Term Clinical, Manometric and Histologic evaluation of postoperative obstructive symptoms in Hirschsprung's surgery. Moore SW , Millar AJW , Cywes S. 37th International Congress of the British Association of Paediatric Surgeons , Glasgow , July 1990.
5. Tube Caecostomy in the surgery of Hirschsprungs disease. Moore SW, Lakhoo K, Rode H, Millar AJW, Brown RA, Cywes S. SAAPS Congress, Wilderness August 1990.
6. Tissue Acetylcholinesterase levels in Hirschsprungs Disease. De Wet P, Moore SW , Cywes S. SAAPS Congress, Wilderness, August 1990.
7. Secondary changes to the Enteric Nervous system (ENS) in an animal model of intestinal obstruction - the role of the alpha-2-adrenergic receptor. Moore SW, Laing D, Melis J. Poster presentation- Surgical Research Society , Cape Town 1992.
8. The proximal ganglionated segment in Hirschsprung's disease - the value of a histological grading system in evaluating the Enteric Nervous System (ENS). SAPA/ SAAPS Congress, Wild Coast, June 1992.

9. Secondary effects of prolonged intestinal obstruction on the enteric nervous system (ENS) in rats. Moore SW, Laing D, Melis J, Cywes S. Original paper presentation Combined meeting of Canadian and British Associations of Paediatric Surgeons, Ottawa, Canada, September 1992.

10. A histological grading system for the evaluation of co-existing NID in Hirschsprung's disease. Moore SW, Laing D, Kaschula ROC, Cywes S. Poster presentation 7th International Congress, World Federation of Associations of Pediatric Surgeons, Hamburg, Germany, September 1992.

11. A histological scoring system for assessing NID co-existing with Hirschsprung's disease. Invited address, International congress of gastrointestinal motility (GIMIC), Cologne, Germany, November 1993.

APPENDIX A

HIRSCHSPRUNG'S DISEASE STUDY

SURNAME CHRISTIAN

(Sticker if available)

1. White male 2. Female
3. Coloured male 4. Female
5. Asian male 6. Female
7. Black male 8. Female

DOB RACE/SEX CODE

*ANTENATAL COURSE: 1=Normal 2=Complicated 3=Unknown
*MODE OF DELIVERY: 1=NVD 2=Forceps 3=LUSCS 4=Other
*BIRTH WEIGHT: kg
*GESTATIONAL AGE: weeks

*ASSOCIATED ABNORMALITIES: 1=None 2=Down's 3=Microceph 4=Multiple 6=Other
*FAMILY HISTORY: 1=None 2=Sibling 3=Other relative
PATIENT SOURCE: 1=Maternity Hosp. 2=GP 3=Paediatrician 4=OPD 5=RXH 6=Other
*PLACE OF BIRTH: 1=City of Cape Town 2=<50km 3=50-100km 4=100-300km
5=300-600km 6=600-1200km 7=>1200km
***SYMPTOMS:** AGE AT ONSET: months. For less than 1 month, put '0'
DURATION: months. For less than 1 month, put '0'

*PRESENTATION: 1=Delayed passage of meconium 2=Constipation 3=Distension
4=Intestinal obstruction 5=Enterocolitis 6=Septicaemia
7=Neonatal appendicitis 8=Failure to thrive 9=Perforation
10=Pericolic abscess 11=Other

ABDOMINAL X-RAY: 1=Normal 2=Suggestive 3=Abnormal

BARIUM ENEMA: 1=Normal 2=Diagnostic 3=Abnormal
CONE LEVEL: 1=Recto-sigmoid 2=Desc colon 3=Splenic flexure
4=Transverse 5=Ascending 6=Total 7=Other
RECTAL BIOPSY: Histopathology Report Number:
1=Suction 2=Full-thickness 3=Other
1=Diagnostic 2=Suggestive 3=Normal 4=Other
*AGE AT DIAGNOSIS: months

COLOSTOMY/ILEOSTOMY: DATE:
SITE: 1=Sigmoid 2=Transverse 3=Ileostomy 4=Other
TYPE: 1=Loop 2=Divided

COMPLICATIONS OF COLOSTOMY: 1=None 2=Prolapse 3=Excoriation 4=Stricture
5=Intestinal obstruction 6=Faeculoma 7=Other
Procedure: 1=None 2=Prolapse reduction 3=Laparotomy 4=Dilatation 5=Other

*AGE AT OPERATION ONE: months. For less than one month, put '0'
*AGE AT OPERATION TWO: months.

TRANSITION LEVEL: Histopathology Report Number:
1=Recto-sigmoid 2=Sigmoid 3=Descending 4=Splenic flexure
5=Transverse 6=Hepatic 7=Total colon 8=Small bowel
TYPE OF SURGICAL PROCEDURE: .....
SURGICAL PROCEDURE: Date:
Duration of operation: Time operation started:
Time operation finished:

OPERATIVE DETAILS: 1=No difficulty 2=Technical difficulty 3=Haemorrhage
1=Cuff devided 2=Cuff not divided
Anastomosis: Anterior level in millimetres (mm)
Posterior level in millimetres (mm)
Suture material: 1=CCG 2=Vicryl 3=PDS 4=Other

*EARLY COMPLICATIONS:1=None 2=Haemorrhage 3=Cuff abscess 4=Caecostomy proble

5=Ileus 6=Stricture 7=Respiratory 8=Wound sepsis 9=Buttock excoriati
10=Leak 12=Micturition disturbance 13=Neorectum retraction 14=Other

*LATE COMPLICATIONS: 1=None 2=Diarrhoea 3=Colitis 4=Distension
5=Cuff stricture 6=Persistent excoriation 7=Encopresis
8=Constipation 9=Intestinal obstruction 10=Prolapse
11=Micturrition abnormality 12=Residual segment 13=Other

*SEXUAL FUNCTION: 1=Normal 2=Abnormal 3=not known

*PROCEDURES FOR COMPLICATION: 1=Laparotomy 2=Anal dilatation (single)
3=Repeated anal dilatation 4=Myectomy 5=Second procedure

*STOOL FREQUENCY: **EARLY** POST-OP: DAY: 1=Retention 2=1-5 3=5-10 4= >10
NIGHT: 1=Retention 2=1-5 3=5-10 4= >10

*MEDICATION USED POSTOP: 1=None 2=Methyl cellulose 3=Fybogel 4=Metamucil
5=Lactulose/Dulphalac 6=Senekot 7=Microlax enema 8=Dulcolax supposit
9=Fleet enema 10=Bowel washout 11=Other

FOLLOW-UP ASSESSMENT

*WEIGHT FOR AGE: 1= = >50% 2 = 10-50% 3 = 3-10% 4 = <3%ile
*HEIGHT FOR AGE: 1= = >50% 2 = 10-50% 3 = 3-10% 4 = <3%ile

*STOOL FREQUENCY: DAY: 1= <1/2d 2= <1/d 3= 1-5/d 4= 5-10/d 5= >10/
NIGHT: 1= <1/2n 2= <1/n 3= 1-5/n 4= 5-10/n 5= >10/

*STOOL CONSISTENCY: 1=Diarrhoea 2=Pasty 3=Normal 4=Hard 5=Alternating(1to4)

*STOOL TIMING: 1=Regular 2=Irregular

*STOOL CONTROL: 1=Normal 2=Skid marks 3=Slight soiling 4=Smea
5=Urgency 6=Urge incontinence 6=Incontinence 7=Oth

*STOOLING PATTERN MATURATION POST-OP: Time in months:

*SENSATION: 1=Normal 2=Uncertain 3=Absent

*DISCRIMINATION OF RECTAL GAS/LIQUID/SOLID:1=Normal 2=Reduced 3=Absent 4=Uns

*LONG-TERM MEDICATION: 1=None 2=Occasional therapy 3=Therapy dependent
1=Methyl cellulose 2=Fybogel 3=Metamucil 4=Lactulose/Dulphalac 5=Senekot
6=Dulcolax suppos. 7=Microlax enema 8=Fleet enema 9=Bowel washout 10=Other

*RECTAL EXAMINATION: 1=No prolapse 2=Prolapse
1=Tone normal 2=Tone reduced 3=Tone increased

1=Ampulla normal 2=Ampulla distended 3=Ampulla contrac

1=No faeces 2=Little faeces 3=Moderate faeces 4=Faecal loading

Reservoir 1=Good reservoir function
2=Small reservoir
3=No information

Length of Aganglionic segment 1=Recto-sigmoid 2=Sigmoid 3=Descending colon
4=Splenic flexure 5=Transverse colon 6=Hepatic fle
7=Total colon 8=Small bowel

Development 1=Normal milestones
2=Delayed milestones
3=Unsure

Schooling 1=Above average performance
2=Average performance 3=Performance affected

Extramural activities 1=No restriction 2=Mild/moderate
3=Restricted

Sibling relationships 1=Normal 2=Affected 3=Unsure

*MANOMETRY: Resting rectal pressure (mmHg)
Anal canal pressure (mmHg)

Internal sphincter relaxation: 1=present 2=absent 3=unsure

Sensation: 1=Increasing rectal fullness and desire to defecate
2=Increasing rectal fullness; no desire to defecate
3=Desire to defecate only

Propulsive waves: 1=Regular 2=Irregular 3=Diminished 4=Absent 5=Not te

Compliance: 1= 0-2 2= 2-4 3= 4-8 4= 8-10 5= >10 6=Not te

Anorectal fluctuations: 1=Regular 2=Irregular 3=Not tested

ASPB mm^2

Rectal contractions (frequency/mm)

Length of High Pressure Zone cm

Maximum Tol. volume ml Squeeze Pressure mmHg

Sensation volume ml

NID 1=Yes 2=No 3=No information

Outcome 1=Excellent 2=Fair 3=Poor 4=Death 5=Other

Obstructive symptoms 1=Yes 2=No

Follow up age ..... yrs

Interview 1=Yes 2=No

Interview -
Final Result -

APPENDIX B

TABLE B1

Reported Population and births (1957-1973)
Medical Officer of Health, Cape Town.*

YEAR	POPULATION OF GREATER CAPE TOWN	LIVE BIRTHS
1957	484 000	14 423
1958	491 570	14 108
1959	499 320	14 430
1960	531 730	16 536
1961	544 460	16 388
1962	558 790	16 428
1963	577 360	17 263
1964	590 960	18 201
1965	610 010	19 032
1966	634 780	18 726
1967	650 760	17 700
1968	657 000	20 892
1969	676 540	21 243
1970	679 513	20 995
1971	713 847	22 057
1972	730 592	22 063

TOTAL NUMBER OF LIVE BIRTHS	290 485

(* Data source: Dept of Statistics, Medical Officer of Health, Cape Town and Annual Reports MOH, Cape Town)

TABLE B2

Population and Births Cape Town 1973 - 1991 *

	Population			Live Births		
YEAR	MOH	RSC/DIV C	TOTAL POPULATION	MOH	RSC/DIV C	TOTAL
1973	747 755	437 000	1 184 755	22 058	11 599	33 657
1974	765 351	450 000	1 215 351	21 582	12 038	33 652
1975	783 390	462 940	1 246 330	19 898	12 426	32 325
1976	801 884	492 900	1 294 784	19 646	13 125	32 771
1977	820 842	523 510	1 344 352	18 961	14 106	33 067
1978	840 282	539 570	1 379 852	19 242	13 174	32 416
1979	860 211	555 770	1 415 981	19 698	13 182	32 880
1980	880 647	579 860	1 460 507	20 431	14 414	34 845
1981	901 599	602 910	1 504 509	22 908	15 445	37 353
1982	923 085	624 450	1 547 535	23 538	16 029	39 567
1983	945 117	648 970	1 594 087	24 478	16 827	41 305
1984	967 708	693 920	1 661 628	25 063	17 484	42 547
1985	990 875	897 510	1 888 385	24 573	18 634	43 207
1986	1 014 637	988 040	2 002 677	23 235	19 297	42 532
1987	1 140 677	958 520	2 099 197	22 585	19 428	42 013
1988	1 096 566	1 201 540	2 098 106	22 698	20 780	43 478
1989	1 118 119	1 230 156	2 348 275	22 721	22 556	45 277
1990	1 173 462	1 292 790	2 466 242	23 186	23 351	46 537

(* Data source: Dept of Statistics, Medical Officer of Health (MOH) and Regional Services Council (RSC/DIVC), Cape Town and Annual Reports MOH and RSC, Cape Town)

APPENDIX B

TABLE B3

PREMATURE BIRTHS GSH OBSTETRIC SERVICE #

Year	Births	< 37 weeks		< 2,5 kg	
1974	14 133	1 144	(8.1%)	2 023	(14.3%)
1975	15 610	1 459	(9.4%)	1 939	(12.4%)
1976	18 212	1 363	(7.5%)	2 421	(13/3%)
1977	18 489	1 498	(8.1%)	2 573	(13.9%)
1978	19 720	1 387	(7.0%)	2 649	(13.4%)
1979	19 779	1 435	(7.2%)	2 763	(13.9%)
1980	20 609	1 407	(6.8%)	2 731	(13.2%)
1981	22 059	1 721	(7.8%)	2 850	(12.9%)
1982	23 306	1 691	(7.2%)	3 041	(13.0%)
1983	24 572	1 986	(8.1%)	3 280	(13.3%)
1984	25 407	2 044	(8.0%)	3 598	(14.1%)
1985	26 020	2 106	(8.1%)	3 586	(13.8%)
1986	25 012	1 726	(6.9%)	3 348	(13.3%)
1987	25 030	1 604	(6.4%)	3 473 *	(13.8%)
1988	25 857	1 837	(7.1%)	3 569 *	(13.8%)

* Statistics for 1989 and 1991 omitted due to unreliability of data at the time of analysis

Data source: Official reports Groote Schuur Hospital

APPENDIX C

METHOD OF ACHE STAINING

References:

1. Karnowsky MJ, Roots LJ. Histochem Cytochem 1964; 12:219-221.
2. Meier Rüge W, Lutterbach BK, Herzog B, Morger R, Moser R, Scharli A. . Pediatr Surg 1972; 7(1):11-17.

Principle of Reaction

The solution contains acetylthiocholine which is hydrolised by acetylcholinesterase (AChE). The thiocholine liberated by hydrolysis reduces Ferricyanide to Ferrocyanide. This then combines with the copper ions to produce insoluble copper ferrocyanide. This is visible following counter staining of the slide.

Method:

1. Fresh surgical specimens are obtained and sections cut parallel to the colonic axis from selected sites.
2. Sections are then coiled, orientated on edge, mounted and snap frozen in liquid nitrogen.
3. 5μ sections are cut to check orientation.
4. 12μ sections are then cut and mounted on two cover slips ($\pm$ 8 sections per specimen).
5. Incubation medium is thawed and air removed.
6. 6 Sections are air dried for 20 minutes.
7. 7 Sections fixed in 4% cold neutral formalin at 0-4°C for a minimum of 5 minutes.
8. Rinse in distilled water.
9. Incubation with working solution at 39°C for a minimum of 90 minutes.
10. Rinse in distilled water.
11. Counterstain for 5 minutes (Haematoxyin and Eosin or Carazzi solution).
12. Stain blue in running tap water for 10-15 minutes.
13. Rinse in distilled water.
14. Dehydrate specimen and mount on slide with mounting medium (DPX or ENTECCAN, MERCK).

APPENDIX C

TABLE C1

REAGENTS FOR ACETYLCHOLINESTERASE STAINING:

SOLUTION A:

Acetylthiocholine iodide	0.02g
0.06M sodium acetate (anhydrous) 8.2g/litre	25.2ml
0.1M acetic acid (prepare fresh) 1ml/180ml DW (60mg/100ml)	0.8ml
0.1M sodium citrate 2.94g/100ml	2ml
30mM copper sulphate $5H_2O$ 0.75g/100ml	4ml
4mM Octamethylpyrophoramide tetraisopropylpyrophosphoramide (OMPA) 0.137g/100ml	0.8ml
Distilled water	3.1ml

Dispense 4.5ml per tube and freeze

SOLUTION B:

Potassium ferricyanide 5mM
0.16g/100ml DW

WORKING SOLUTION

Thaw two tubes Solution A - 9ml
Add 1ml Solution B.

APPENDIX D

SCORING SYSTEMS OF ANORECTAL FUNCTION

1. KELLY CLINICAL SCORE (Maximum score 6)

[Kelly (1969/1972)]

a)	2 Points	-	Normal control under all circumstances, with no accidents
	1 Point	-	Occasional escape of faeces or flatus with occasional major accidents
	0 Points	-	No control or frequent accidents (more than 50% of the time)
b)	2 Points	-	Always clean
	1 Point	-	occasionally stained
	0 Points	-	Always stained
c)	2 Points	-	Effective strong squeeze of puborectalis
	1 Point	-	Weak or partial squeeze of puborectalis
	0 Points	-	no contraction detectable

Point score interpretation:

Good = 5-6 points
Fair = 3-4 points
Poor = 0-2 points

2. Wingspread Scoring System

[Stephens, Smith (1984)]

Excellent / Very Good - Totally continent or very occasional stress related staining of underclothes, without constipation. Toilet trained with no medication.

Good - Rarely soils except during stressful exercise. Constipation managed with medication

Fair - Intermittent soiling. Urge incontinence. Frequent loose stools or constipation which requires enemas.

Poor - Constant faecal soiling and smearing. Constipation only responsive to enemas.

3. Holschneider Clinical & Electromanometric Score

[Holschneider A (1984)].

A. Frequency of stool

Frequent - 1-2 stools per day	2 points
Normal incidence - 3-5 per day	1 point
Frequent > 6 per day	0 points

B. Stool Consistency

Normal formed stool	2
Pasty stools	1
Liquid stools	0

C. Soiling

None	2
Stress incontinence	1
Constant	0

D. Appreciation of sensation

Normal	2
Feeling of fullness only	1
None	0

E. Anorectal resting rectal pressure

20-24 mmHg	2
14-19 mmHg	1
< 13 mmHg	0

F. Squeeze pressure

> 30 mmHg	2
20-29 mmHg	1
< 13 mmHg	0

G. Adaptation reaction

Normal	2
Low Amplitude	1
No reaction	0

Point score interpretation

12-14 points - good result
9-11 points - satisfactory results
5-8 points - adequate for social life with help
0-4 points - incontinent