Constipation and congenital disorders of the myenteric plexus

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Summary: Full-thickness muscle biopsies have been taken from patients with severe disabling chronic constipation that has not responded to conservative measures. Assessment by neurohistochemical techniques has revealed that a range of neuronal dysplasias of the myenteric plexus are responsible in many cases; these include aganglionosis (Hirschsprung's disease), hypoganglionosis and hyperganglionosis. In cases considered unlikely to be Hirschsprung's disease on clinical grounds, the procedure used has often been anorectal myectomy; this has not only provided tissue for diagnosis but has also been of therapeutic value in most cases of hypoganglionosis and some cases reported as 'normal'.

Introduction

The first descriptions of a congenital disorder of the myenteric plexus in the hind-gut associated with a disturbance of bowel function were of congenital aganglionosis or Hirschsprung's disease (Whitehouse & Kernohan 1948, Zuelzer & Wilson 1948, Bodian *et al.* 1949). The early reports simply described an absence of ganglion cells and the presence of abnormally large nerve trunks between the muscle layers of the bowel wall, but the condition was shown eventually to be a complex and variable anomaly of the whole autonomic innervation of the aganglionic segment and the distal ganglionic segment (Garrett & Howard 1981).

Recent neurohistochemical studies of anorectal muscle from patients with severe, persistent constipation have revealed further examples of anomalies in the myenteric plexus which have been grouped together as congenital neuronal dysplasias. They include hypoganglionosis and hyperganglionosis and, like aganglionosis, they may be further classified into local or diffuse types depending on the length of affected bowel. There have also been recent reports of an association between aganglionosis and hyperganglionosis with other genetic disorders such as Down's syndrome and Sipple syndrome (Table 1).

This paper describes some of the clinical features associated with neuronal dysplasias of the hind-gut, and a plan of investigations which include neurohistochemical studies on anorectal biopsy specimens. The abnormalities of innervation are described and compared with the findings in normal bowel.

Table 1. Association of neuronal abnormalities of large bowel with other disorders

	Associated disorders
Aganglionsis	Congenital deafness Down's syndrome Failure of automatic ventilation (Ondine's curse) Sipple syndrome (Type 2a)
Hypoganglionosis	Aganglionosis
Hyperganglionosis	Aganglionosis Sipple syndrome (Type 2b)

Clinical material (Figure 1)

The investigation of paediatric or adult patients with chronic constipation begins with a detailed history and a general examination which should rule out metabolic disorders (e.g. hypothyroidism), drug therapy (e.g. cytotoxic agents) and gross neurological lesions (e.g. spina bifida) as a cause of bowel dysfunction. Detailed examination of the perineum and anorectal region in infants will occasionally disclose an anatomical anomaly such as an undiagnosed anal stenosis which needs urgent correction. A small number of cases may give histories with features suggestive of aganglionosis (Hirschsprung's disease). These features include failure to pass meconium during the first 24 hours of life,

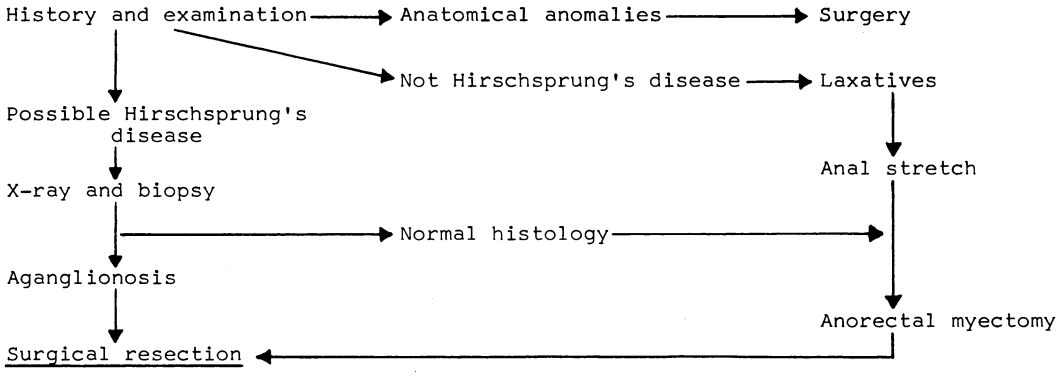

Figure 1. Plan of management of severe constipation

intermittent abdominal distension perhaps with vomiting, and constipation without overflow incontinence.

When the history suggests aganglionosis, barium enema examination and rectal biopsy are advised. A majority of cases referred with chronic constipation, however, have no history of intestinal obstruction although overflow incontinence, or faecal soiling, may be present. Laxatives and enemas are the first line of treatment and anal stretch under general anaesthesia is advised if they fail. Approximately 85% of our referred cases have been improved or cured by this stage of management. The remaining 15% with intractable constipation have undergone anorectal myectomy which was developed from a technique described by Lynn & Van Heerden (1975). With the patient in the lithotomy position the anal canal is dilated and stay sutures inserted above and below the mucocutaneous junction of the posterior third of the anal canal. The area is infiltrated with 1 : 200 000 adrenaline solution and a transverse incision made between the stay sutures. The internal sphincter is identified and the mucosa elevated from the muscle of the rectum for a distance of 6–7 cm. A full-thickness strip of muscle 0.5–1.0 cm wide is excised longitudinally from the rectum starting with the upper two-thirds of the internal anal sphincter through which a silk suture is placed to aid handling of the specimen, thereby avoiding the use of forceps. It also enables the proximal end of the specimen to be clearly identified by the pathologist who collects the tissue from the operating theatre. The mucosa is repaired with interrupted catgut sutures and no attempt is made to close the muscle defect. There has been no postoperative complication in approximately 70 operative procedures.

Neurohistochemical investigations

The techniques of examining the myenteric and submucosal nerve plexuses in anorectal biopsy specimens have been reviewed recently in detail (Howard & Garrett 1984). Briefly, we prefer the biopsy material to be frozen rapidly in hexane surrounded by dry ice immediately after surgical excision. Alternatively, the tissue is placed in a special formaldehyde fixative and stored at 0–4°C for four hours or overnight before processing in the laboratory. (Routine fixatives are damaging to enzymes and the preferred mixture is 4% paraformaldehyde, 0.08 mol/l cacodylate buffer pH 7.2, as in Karnovsky 1965.) Rapid identification of neurones and ganglia in the intermyenteric plexus is achieved by staining for acid phosphatase and nonspecific esterase (Howard *et al.* 1982). These techniques are particularly useful in cases of hypoganglionosis when ganglion cells are infrequent. The methods are those of Barka & Anderson (1962) and Davis (1959) respectively.

Acetyl cholinesterase staining (Gomori 1952, Garrett *et al.* 1969) is used to identify cholinesterase-positive nerves within the muscle layers, and is particularly important in diagnosis. Catecholamine fluorescence, on the other hand, gives valuable information on the innervation of the hind-gut by adrenergic nerves but is not as important in the diagnosis of neuronal disorders as are the previously described techniques. The catecholamine fluorescence material is as described by de la Torre & Surgeon (1976).

Muscle innervation of normal bowel (Figure 2)

The neurones and axons of the bowel wall are arranged chiefly in two plexuses named myenteric (Auerbach) and submucosal (Meissner). A rather less well defined plexus is found adjacent to the inner surface of the circular muscle (Henle).

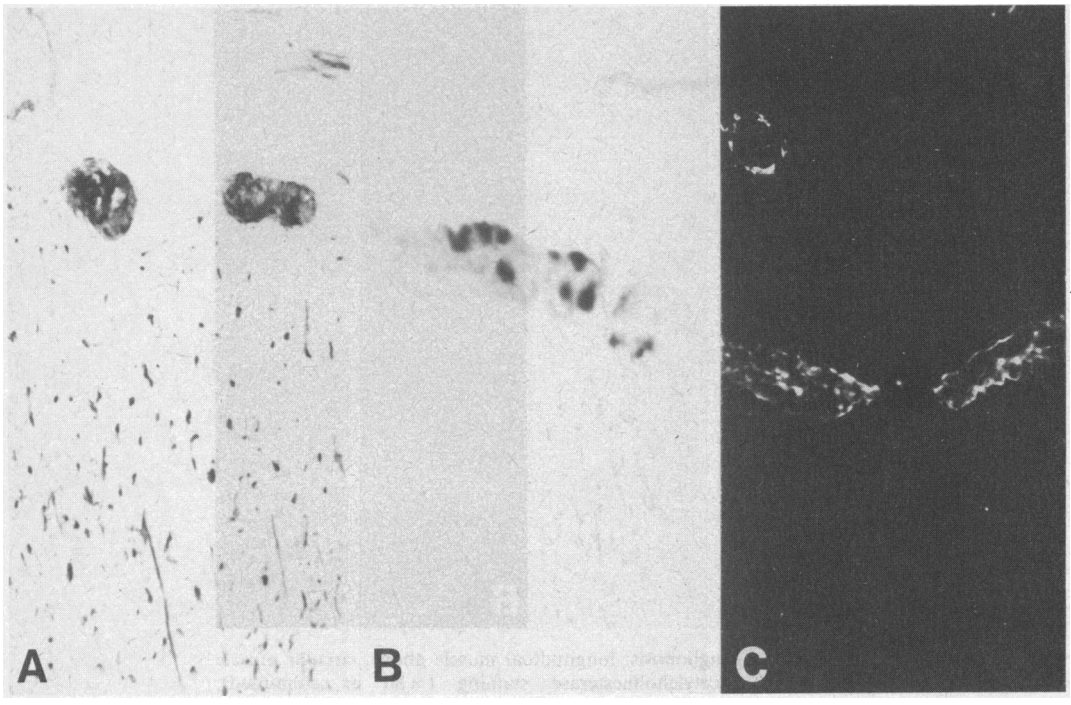

Figure 2. 'Normal' bowel: longitudinal muscle above, circular muscle below, showing ganglia in the intermyenteric zone, A, acetylcholinesterase staining (×210 as submitted); B, acid phosphatase staining (×200 as submitted); C, catecholamine fluorescence (×200 as submitted)

Electronmicroscopy of the groups of neurones known as ganglia shows the nerve cells to be arranged at the periphery and the remainder is composed of supporting cells plus large numbers of axons which contain a variety of vesicles. The ganglia vary in size and staining and their arrangement has been likened to primitive brain tissue (Howard & Garrett 1970). It has been suggested that the cholinesterase-positive cells, which are the majority of intramural neurones (Koelle *et al.* 1950), are the argyrophobe cells identified by silver staining techniques (Smith 1972) and that these are responsible for the motor innervation of the muscle layers. Argyrophil cells, on the other hand, may coordinate peristalsis through their intraneuronal axons. Catecholamine fluorescence has demonstrated that a majority of adrenergic nerves are arranged around the periphery of ganglia and few are found within the the muscle layers apart from those supplying blood vessels (Norberg 1964, Jacobowitz 1965, Garrett *et al* 1969). Moderate numbers of cholinesterase-positive nerves are present within the circular muscle with rather fewer in the longitudinal coat.

Peptide-containing neurones and nerves (e.g. vasoactive intestinal peptide (VIP), substance P, etc) have been identified throughout the gut. However the precise roles of these putative neurotransmitters are, as yet, largely a matter of speculation, although VIP – which is found especially in the neurones and postganglionic nerve endings in Meissner's plexus – may have a role in smooth muscle relaxation (Polak & Bloom 1979, Furness & Costa 1980).

Congenital aganglionosis (Hirschsprung's disease) (Figure 3)

Aganglionic segments of bowel in patients with Hirschsprung's disease usually start at the internal anal sphincter and extend cranially for a distance which varies from case to case. Occasional examples of 'skip' lesions have been reported (MacIver & Whitehead 1972, Martin *et al.* 1979, Chadarévian *et al.* 1982) but these are very rare. Aganglionosis is restricted to the rectum and sigmoid colon in approximately 75% of cases (Kleinhaus *et al.* 1979) but the severity of presenting symptoms does not necessarily depend on the length of affected bowel. The condition presents either as intestinal obstruction in infancy or as severe chronic constipation in older patients, and treatment consists of resection of

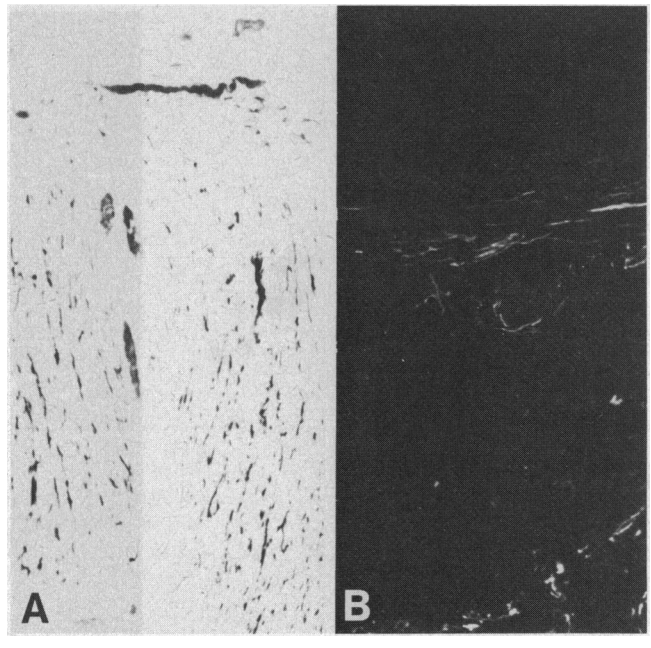

Figure 3. Aganglionosis: longitudinal muscle above, circular muscle below. A, acetylcholinesterase staining (×80 as submitted); B, catecholamine fluorescence (×90 as submitted)

the affected segment and restoration of bowel continuity with one of the several types of 'pull-through' operations. The definitive operation may or may not be preceded by colostomy.

Suspicion of aganglionosis from clinical signs, or from X-ray appearances on barium enema, is followed by histological confirmation from a rectal biopsy specimen.

Conventional histology with haematoxylin and eosin staining shows an absence of ganglia but large nerve trunks are found between the muscle layers of the rectum and these stain strongly for acetyl cholinesterase (Kamijo *et al.* 1953). Cholinesterase-positive nerves are easily identified within the muscle layers (Garrett *et al.* 1969). Increased acetyl cholinesterase activity is also found within the nerves of the lamina propria in a majority of cases that present early in life and this finding is used in the procedure of rectal biopsy by superficial suction techniques (Meier-Ruge 1974). However, occasional false-negative results are possible with mucosal biopsy (Van Der Staak 1981), and we have continued to confirm diagnosis on full-thickness muscle biopsies in a majority of our cases.

Neurohistochemical studies of resected aganglionic bowel have revealed complex anomalies in the autonomic innervation. This includes a variation in the distribution of cholinesterase-positive nerves at different levels in each specimen, the greater number of nerves being found in the most distal bowel. Proximal ganglionic bowel adjacent to the aganglionic segment tends to contain fewer nerves than normal within the muscle layers (Garrett & Howard 1981). The normal arrangement of adrenergic nerves is of course absent, but catecholamine fluorescence shows a very variable distribution of adrenergic nerves in both muscle layers (Bennett *et al.* 1968). Peptide-containing nerves are also reduced in aganglionic bowel, but the least reduction occurs in the most distal bowel (Bishop *et al.* 1981).

Electronmicroscopy of aganglionic bowel confirms the histochemical findings and also reveals neuro-effector junctions similar to those found in normal bowel, which suggests that the muscular nerves are functional within the aganglionic segments (Howard & Garrett 1970). These observations and the variability in the autonomic innervation help to explain the variable nature of clinical presentation.

'Hirschsprung-like' syndrome

A patchy distribution of ganglia in the hind-gut associated with signs of subacute intestinal obstruction has been reported in two infants by MacMahon *et al.* (1981). In both cases ganglion

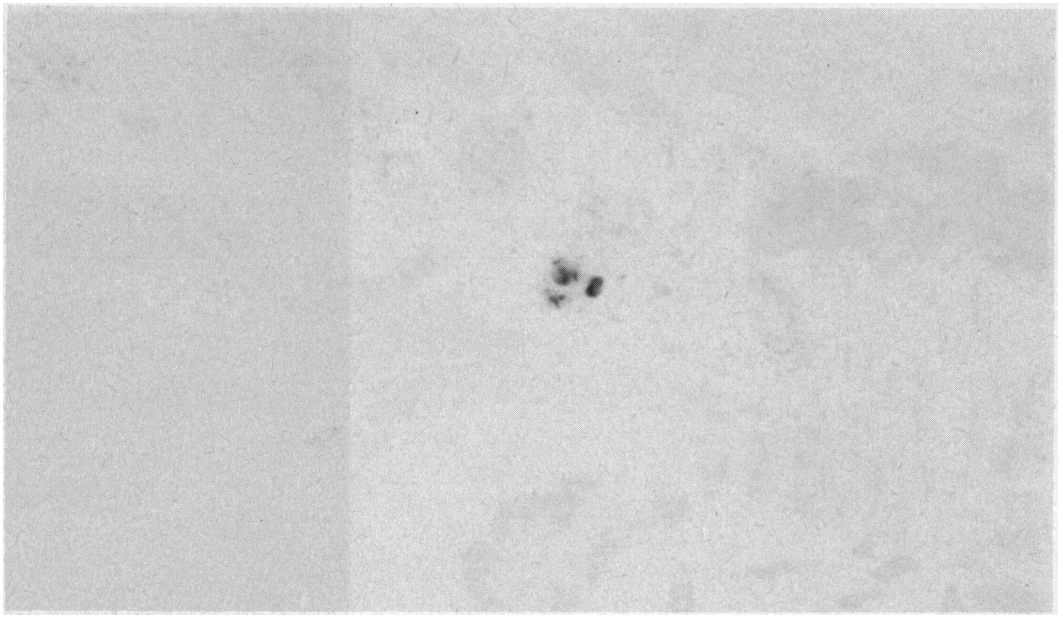

Figure 4. Hypoganglionosis: acid phosphatase staining – longitudinal muscle above, circular muscle below – showing a large extent of intermyenteric zone but only one very small ganglion is present (× 200 as submitted)

cells had been observed on suction biopsy specimens, but detailed examination after rectal resection showed zones of aganglionosis alternating with segments of bowel containing normal looking ganglion cells. The authors suggested that a persistence of the clinical signs of low intestinal obstruction after 'normal' suction biopsy should lead to a formal strip-biopsy of the rectum to exclude these rarer neuronal dysplasias.

Hypoganglionosis (Figure 4)

Twenty out of 60 patients with severe constipation were found to have reduced numbers of ganglion cells in the hind-gut after neurohistochemical analysis of anorectal myectomy specimens (Table 2). A majority of the cases had suffered with constipation from early childhood, with or without overflow incontinence, and 17 out of 20 have had relief from their symptoms after anorectal myectomy.

Diminished numbers of ganglion cells are well described in the 'transition' zone between normal and aganglionic bowel of patients with Hirschsprung's disease (Garrett *et al.* 1969, Meier-Ruge 1974), but there are several previous reports of hypoganglionosis without aganglionosis in patients presenting with 'idiopathic' constipation (Bentley 1964, Ehrenpreis 1970, Meier-Ruge 1974, Garrett & Howard 1981).

Neurohistochemistry of rectal biopsies in hypoganglionosis shows a few scattered ganglion cells in the intermyenteric zone and they are often associated with nerve trunks. The ganglion cells may be difficult to identify with conventional histological staining techniques which may therefore suggest a diagnosis of Hirschsprung's disease. Acid phosphatase staining is particularly useful for identifying the sparse neurones, although it may be necessary to search many sections for a correct diagnosis. There is generally a paucity of cholinesterase-positive nerves in the muscle layers, as has been described in the muscularis mucosae and lamina propria by Meier-Ruge (1974), which helps to differentiate the condition from Hirschsprung's disease in

Table 2. Results of neurohistochemical studies of anorectal biopsies from 60 patients with severe, chronic constipation

	No. of patients
'Normal innervation'	15
Minor abnormalities of nerves	7
Neuronal dysplasias:	
Aganglionosis	15●
Hypoganglionosis	20
Hyperganglionosis	3
	60

● including one case with hyperganglionosis more proximally

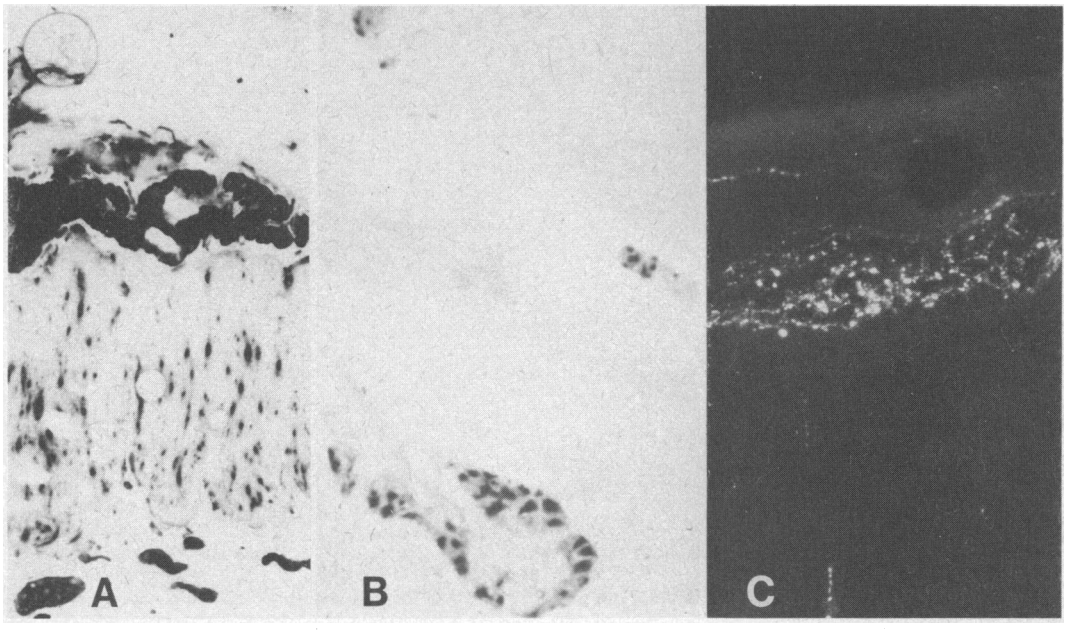

Figure 5. Hyperganglionosis: longitudinal muscle above, circular muscle below. A, acetylcholinesterase staining (× 52 as submitted); B, acid phosphatase staining. Note aberrant ganglia in longitudinal muscle in addition to the large ganglionic mass in the intermyenteric zone (× 132 as submitted); C, catecholamine fluorescence showing numerous adrenergic nerves in the intermyenteric ganglionic tissue (× 320 as submitted)

mucosal biopsies, but full-thickness biopsy of the anorectum is essential for accurate diagnosis. The length of affected bowel varies from case to case, and Munakata *et al.* (1978) described 4 cases confined to the rectum and 2 extending into the descending colon.

Hyperganglionosis (Figure 5)

Meier-Ruge (1974) first described the condition of hyperplasia of nerve plexuses in the bowel wall which is characterized by the formation of large ganglionic masses, the presence of isolated neurones within the lamina propria of the mucosa and within the muscularis mucosae, and an increased cholinesterase activity in nerve fibres within the lamina propria and the circular muscle. He called the condition neuronal colonic dysplasia, but we prefer the term hyperganglionosis. We have found that in common with other neuronal anomalies there is a tendency for cholinesterase activity to diminish in a cranial direction and that there are localized forms confined to the colon or more diffuse forms involving the small bowel (Garrett & Howard 1981).

Hyperganglionosis is usually associated with severe symptoms of bowel dysfunction, but may be overlooked because the presence of ganglia may be regarded erroneously as normal. Symptoms are present from early infancy (Schärli & Meier-Ruge 1981) and vary from severe constipation to intestinal obstruction, and fluid, electrolyte and protein loss from the colon may cause rapid deterioration in the patient's condition. Segmental contractions of the colon with poor peristalsis are typical findings on barium examination and anorectal inhibitory reflexes may be absent, as they are in Hirschsprung's disease (Schärli & Meier-Ruge 1981).

Association with other disorders

Congenital neuronal abnormalities of the gut may occur as combined lesions or may be found in association with other severe disorders (*see* Table 1). A segment of hyperganglionosis may occur in association with Hirschsprung's disease (Puri *et al.* 1977, Schärli & Meier-Ruge 1981; and one of the present cases, Table 2). Inadequate resection may therefore cause persistent bowel dysfunction after a 'pull-through' operation. Hypoganglionosis is usually present proximal to an aganglionic distal segment of bowel and if left after resection may lead to postoperative constipation.

Aganglionosis has been reported in association with Down's syndrome (Gravier & Sieber 1966),

congenital deafness and Waardenburg's syndrome (Cohen & Gadd 1982), Sipple syndrome (Verdy *et al.* 1982) and 'Ondine's curse' or failure of automatic ventilation (Stern *et al.* 1981).

Hyperganglionosis has also been reported in association with Sipple syndrome (Carney & Hayles 1977, Lemarec *et al.* 1980, Verdy *et al.* 1982), and bilateral phaeochromocytoma (Duffy *et al.* 1962).

Comment

The application of neurohistochemical techniques to the study of severe constipation has revealed a surprisingly wide variation in the patterns of intrinsic innervation in the hind-gut which range from aganglionosis to hyperganglionosis. These anomalies may occur either as single disorders or in a variety of combinations. We have found that conventional histology is unsatisfactory for the clear identification of these conditions and may even lead to misdiagnoses, particularly from biopsies of mucosa alone.

Our treatment of severe constipation has included submucosal anorectal myectomy, which was reported by Lynn & Van Heerden (1975) to be of value in the management of short-segment aganglionosis. Our experience suggests that the procedure is also useful for the treatment of hypoganglionic constipation and for the relief of symptoms in patients in whom no obvious anomaly has been detected in the neurohistochemical appearances of the myenteric plexus.

Although the methods described in this paper are satisfactory for diagnostic purposes, it seems likely that the future application of more sophisticated techniques of investigation will uncover an even wider range of innervation patterns in the gut and reduce further the number of cases currently classified as 'idiopathic' constipation.

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