

Autonomic Nerves in Rectum and Colon in Hirschsprung's Disease

A Cholinesterase and Catecholamine Histochemical Study*

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The autonomic nervous system innervates normal bowel in a complicated manner. Study of these nerves has been facilitated by histochemical techniques which enable separate identification of adrenergic- and acetylcholinesterase (AChE)-containing nerves. The latter are usually considered to be cholinergic, and while their presence in the muscle layers of the bowel wall is well known, little attention has been paid to their detailed distribution. It has usually been considered that the adrenergic nerves are also distributed directly to the muscle layers, but recent studies by Norberg (1964), Jacobowitz (1965), and Baumgarten (1967) have shown that these nerves are mainly associated with the myenteric ganglia. The ganglion cells themselves do not contain catecholamines, but possess variable amounts of AChE-activity (Koelle, 1955; Cauna *et al.*, 1961; Gunn, 1968).

In a classical case of Hirschsprung's disease, an undilated rectum is found to be devoid of ganglion cells on routine histology, though large nerve trunks are present. More proximal bowel appears normal. Recent studies of the morphology of nerves in aganglionic bowel from Hirschsprung's disease have been reported by Smith (1967) using silver staining, and by Ehrenpreis, Norberg, and Wirsén (1968) using catecholamine fluorescence. These workers suggested that the muscle layers themselves were virtually denervated, and that unopposed muscle activity was responsible for the contracted appearance of affected bowel. Meier-Ruge (1968), on the other hand, as a result of cholinesterase studies, suggested that the contraction was due to an increase in muscular

innervation, and implied that this always accompanied aganglionosis.

It is difficult to understand, from either of these conflicting ideas, why two patients with similar lengths of aganglionic bowel sometimes present in different ways. For example, one patient might present with a severe intestinal obstruction in the neonatal period, while another might be seen much later in life with a history of constipation. Furthermore, if muscular denervation is considered to be the mechanism responsible for the undilated distal bowel, a difficulty is raised by the condition known as Chagas' disease, where the ganglion cells of the myenteric plexus are destroyed by the organism *Trypanosoma cruzi*, yet the resulting aperistaltic bowel shows dilatation, not contraction.

Histochemical studies have shown that the larger nerve bundles in the intermuscular zone of aganglionic segments from cases of Hirschsprung's disease are AChE-positive (Kamijo, Hiatt, and Koelle, 1953; Adams, Marples, and Trounce, 1960; Niemi, Kouvalainen, and Hjelt, 1961), and that there is also an increase of AChE-positive nerves in the circular muscle (Niemi *et al.*, 1961; Meier-Ruge, 1968). A preliminary report by Bennett, Garrett, and Howard (1968) showed that the normal arrangement of adrenergic nerves around myenteric ganglia was absent in the affected segment of Hirschsprung's disease, but that there was a tendency for the numbers of adrenergic nerves in the muscle layers themselves to be increased.

The aim of the present investigation was to examine the distribution of the muscular nerves both in normal large bowel of children, and in different areas of gut resected from cases of Hirschsprung's disease. The results have been assessed in conjunction with the clinical presentation, in order to ascertain whether the pattern of

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distribution of the nerves helps to explain the clinical variability of the disease.

Materials and Methods

Gut resected because of Hirschsprung's disease from 19 children aged from 3 months to 2 years 8 months has been investigated. In addition, 4 segments of normal recto-sigmoid, removed at operation for other reasons from children aged from 5 months to 4 years, have served as controls.

Several small full-thickness blocks of gut were cut in a longitudinal direction from each of a number of different levels of resected bowel. The most distal blocks always came from the lower end of the rectum, but the levels of the other blocks depended on the length of bowel resected. Adjacent blocks from each level were placed cut-surface downwards on thin carding, to aid orientation and to minimize buckling. They were then immediately immersed in either a hexane/solid CO₂ mixture for rapid freezing, or in formol-phosphate-sucrose at 4 °C. for 4 hours. The formalin-fixed blocks were washed in phosphate-sucrose overnight and then rapidly frozen, as above.

Catecholamine fluorescence studies. Tissues from 18 cases of Hirschsprung's disease and the 4 controls were used. Adrenergic nerves were studied in 8 μ sections from freeze-dried blocks which had been heated with formaldehyde vapour (after Eränkö, 1967). The catecholamine fluorescence, thereby induced, was examined as described by Bennett *et al.* (1968) in a preliminary communication concerning 5 of the present cases of Hirschsprung's disease and 2 of the controls.

Cholinesterase studies. Tissues from 15 of the cases of Hirschsprung's disease and 3 of the controls were investigated.

AChE activity was studied by the Gomori (1952) modification of the Koelle and Friedenwald (1949) thiocholine technique. Acetylthiocholine iodide was used as substrate and iso-OMPA (tetraisopropylpyrophosphoramide) 3×10^{-6} M was used to inhibit non-specific cholinesterase activity (see Garrett 1966, 1967). Cryostat sections, cut at 10–12 μ from unfixed frozen blocks and also frozen-fixed blocks, were tested by the histochemical procedure over a range of incubation times, up to 24 hours. It was found that the best results were obtained by using unfixed blocks, mounting the sections on gelatine-faced slides, and fixing with formol-sucrose for 10 minutes before the histochemical procedure (as in Garrett, 1966). This contrasts with adult human salivary tissue, in which pre-fixed blocks gave the best results (Garrett, 1967).

Some sections, adjacent to those used in both the above methods, were stained by haematoxylin and eosin for conventional appearances.

Results

The results will be restricted to observations on

nerves in the circular muscle, the intermuscular zone, and the longitudinal muscle of the bowel. In light microscopical representations of nerves many variables influence assessment. Not all nerves are always made visible, separate nerves in the same field may sometimes represent recurrences of the same nerve, and orientation of the block influences appearances. Furthermore, each individual fibre seen is usually an aggregate representation of a Schwann-axon bundle rather than a single axon (see Garrett, 1966), and the presence of such gives no indication of the number of possible neuro-effector sites that would be recognizable by electron microscopy. No attempt has therefore been made to give an absolute assessment of the numbers of nerves in the tissues. Only large differences in the numbers of nerves are detectable, and therefore only such differences will be mentioned.

Acetylcholinesterase activity

Controls (see Fig. 1A). Ganglia and nerves were stained to advantage. All sections contained ganglia in the intermuscular zone, but the ganglionic masses were variable in size, structure, and AChE staining, and it was not possible to interpret the appearances in precise terms. This is not altogether surprising when one considers the immensely complex structural organization of the myenteric ganglia as revealed by electron microscopy (Richardson, 1958). In the present investigation the ganglion cells also varied both in size and AChE staining. Many cells showed moderate rather than strong staining, and some cells appeared to be negative. Much of the tissue between and around the cellular parts showed strong activity. This tissue was probably mainly axonal, but seldom showed fibre-like appearances. Nerve trunks, rich in AChE activity, were sometimes found passing through the longitudinal muscle layer to the ganglia; others were sometimes seen between ganglia.

In the circular muscle, moderate numbers of thin AChE-positive fibres were present among the smooth muscle cells. They radiated from the intermuscular zone to the mucosal layer, and were seen occasionally to connect with ganglia. In the longitudinal muscle there were somewhat fewer nerves which radiated down the long axis of this layer. The numbers of muscular nerves appeared to be relatively constant throughout the length of bowel from any one individual, but there were slight differences between one individual and another.

Adjacent to the mucosal surface of the circular

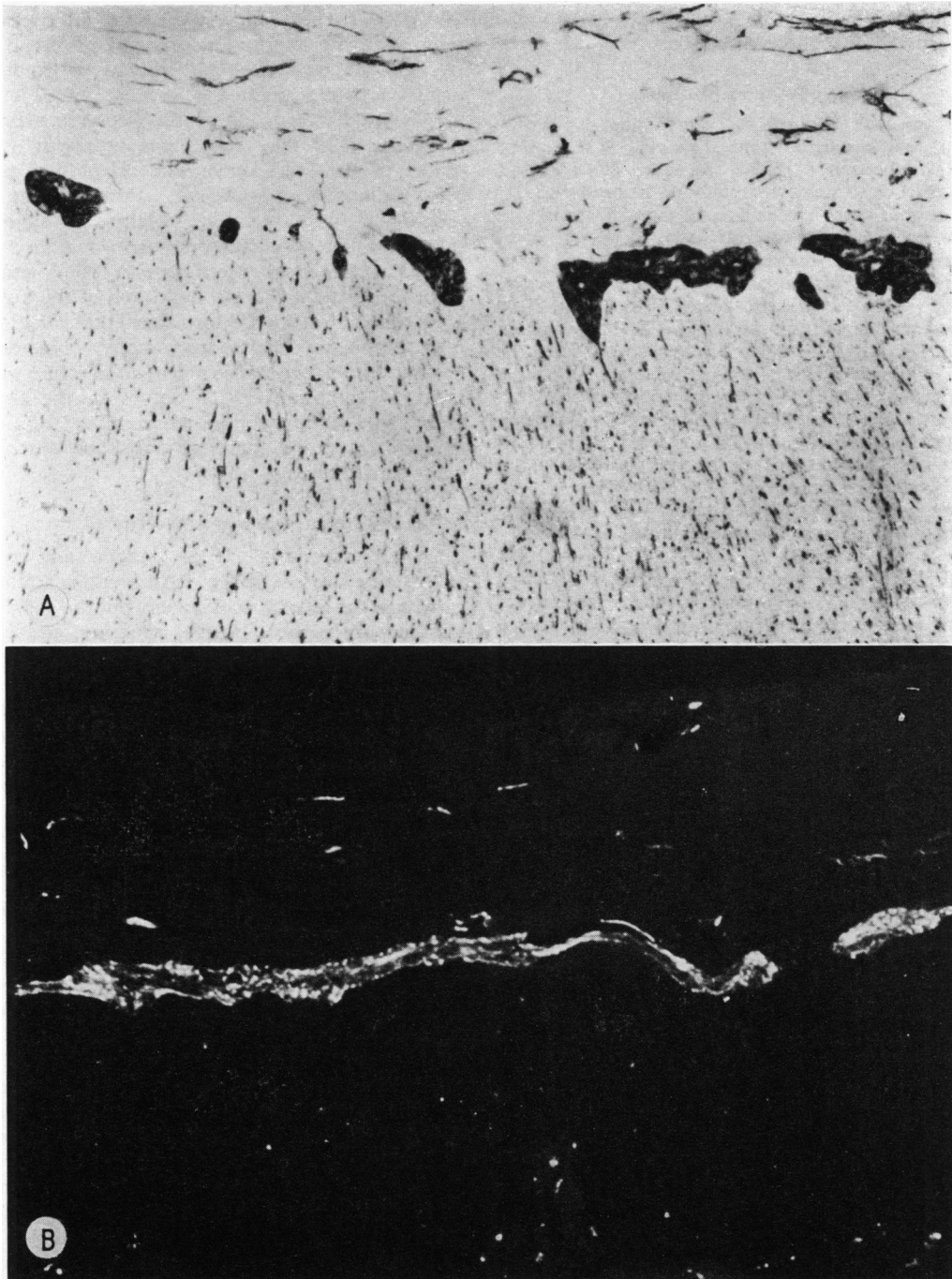


FIG. 1.—Normal rectum (circular muscle layer in the bottom half of each picture. (A) Stained for AChE activity (4-hour counterstained with haemalum), showing dark AChE-positive nerves in the longitudinal muscle and circular muscle. The ganglia between these two layers exhibit variable staining. ($\times 88$.) (B) Formalin-induced fluorescence showing fluorescent adrenergic nerves arranged in a complex manner within and between the myenteric ganglia. Few nerves are present in the muscle layers. ($\times 88$.)

muscle a number of small ganglia were present, and these ganglia frequently showed less AChE activity than in the intermuscular zone. Some of the cells were AChE-negative, and around such cells AChE-positive nerves were often evident.

The above findings have also been supported by studying AChE activity in the rectum and colon from 3 necropsy specimens of infants aged 18 months to 4½ years. Pictorially the results were less pleasing than in the surgical material; nevertheless, it was evident that the numbers of nerves and their pattern of distribution were relatively constant throughout the same length of bowel.

Hirschsprung's disease

(a) *Proximal tissue* (see Fig. 5A and B). It has not been possible in every case to examine satisfactorily tissues adjacent to the proximal cut edge of the resected specimens, because operative procedures have tended to damage them. Furthermore, whenever a colostomy has previously been established only tissue distal to the colostomy has been available for study. In 10 cases, ganglionic tissue was seen in the proximal parts of the resected specimens, but in all of these there were fewer AChE-positive muscular nerves than normal in the most distal parts of this ganglionic tissue. In fact, in only 3 cases were normal numbers of nerves seen in the most proximal parts of the ganglionic tissue. Sometimes the most distal ganglionic tissue showed only small infrequent ganglia in the intermuscular zone, and when this occurred there tended to be some overlap with large trunks, more characteristic of the aganglionic bowel. One case, in which the whole of the colon was affected by the Hirschsprung's process, showed a progressive loss of ganglia and muscular nerves in the lower end of the ileum.

Whenever ganglia were found, they showed a similar variable AChE staining to the normal ganglia.

(b) *Distal tissue* (see Fig. 2A, 3A, 4A, 5C and D and Table). No ganglia were found in the most distal tissue from the 15 cases examined, but AChE-positive nerve trunks were present in the intermuscular zone. The length of bowel that was aganglionic varied from case to case.

In the most distal aganglionic tissue there were large differences in the numbers of nerves in the circular muscle layer; in 7 cases there were many more nerves than normal, and in 5 the number of nerves was at the upper limit of normal. In all of these 12 cases the nerves tended to be more AChE-positive than normal, and also somewhat thicker, probably indicating that there were more axons in the

Schwann-axon bundles (see Garrett, 1966). These nerves often stained more strongly and more quickly than the large trunks in the intermuscular zone. In the 3 remaining cases fewer nerves than normal were found in the circular muscle. As one ascended the bowel there was always some decrease in the numbers of nerves in the circular muscle before reaching the ganglionic tissue, but the amount of this decrease and the length over which it occurred varied.

The numbers of AChE-positive nerves in the longitudinal muscle differed less from normal controls.

Summary of findings in Hirschsprung's disease.

From the foregoing study a distinct pattern of the distribution of the nerves emerges (see Fig. 5). Passing along the bowel in a caudad direction, gradual changes occur which may be divided into 4 regions.

(i) *Normal ganglionic bowel*, with normal numbers of nerves in the muscle layers (seen, however in, only 3 cases).

(ii) *Distal ganglionic bowel*, in which ganglia are present, sometimes apparently reduced in number and size. In the circular muscle nerves are invariably scanty.

(iii) *Proximal aganglionic bowel*, in which nerve trunks but no ganglia are seen in the intermuscular zone. In the circular muscle there are usually more nerves than in (ii) but less than in normal controls.

(iv) *Distal aganglionic bowel*, at which level more nerve trunks are seen in the intermuscular zone. In the circular muscle more nerves are present than in (iii), and are often more plentiful, more strongly AChE-positive, and thicker than in normal controls.

Sometimes regions (ii) and (iii) overlap and great differences may occur in the lengths of each region and the amount of change within them.

Catecholamine fluorescence

Controls (Fig. 1B). As described previously (Bennett *et al.*, 1968), fluorescent adrenergic nerves were found principally in association with the myenteric ganglia, and few fluorescent nerves were present in the muscle layers proper. The arrangement of the fluorescent nerves was complex and irregular within the ganglionic masses and not always precise. These nerves were usually most concentrated towards the periphery of the ganglia, some appearing to be associated with the ganglion cells which were non-fluorescent. Less conspicuous fluorescence was seen in association with the small ganglia on the mucosal surface of

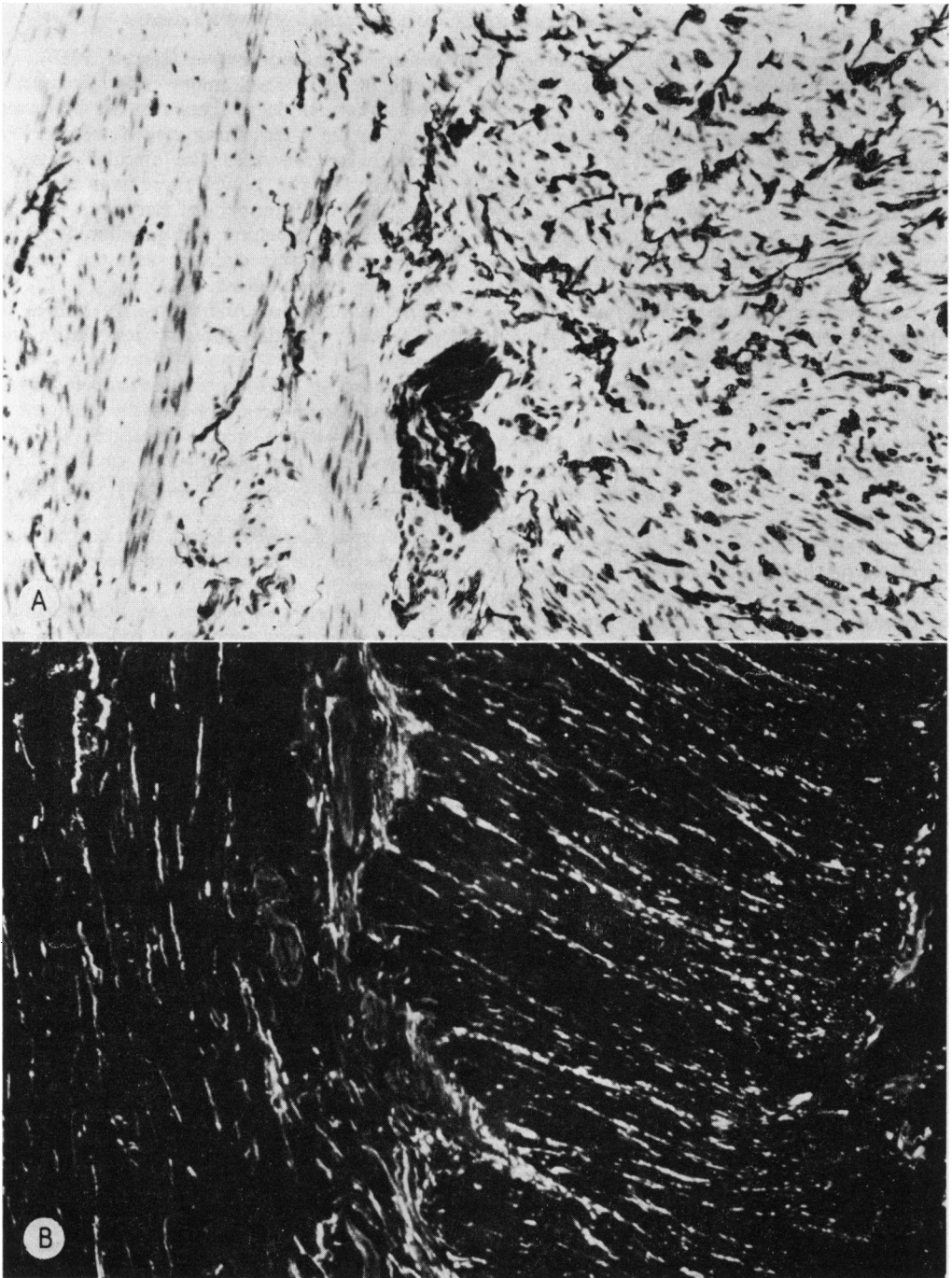


FIG. 2.—Distal aganglionic bowel from 2 cases of Hirschsprung's disease (circular muscle layer in the right half of each picture). (A) Stained for AChE activity (4 hours counterstained with haemalum), showing absence of ganglia, but a positive nerve trunk is present in the intermuscular zone. A dense arrangement of strongly positive nerves is present in the circular muscle, fewer nerves are present in the longitudinal muscle (graded +++ in the Table). ($\times 165$.) (B) Formalin-induced fluorescence, showing a dense arrangement of nerves in the circular muscle and to a lesser extent in the longitudinal muscle. The larger nerve trunks appear to be mainly non-fluorescent. ($\times 83$.) (This tissue contained the most adrenergic nerves; unfortunately, tissue was not available for AChE staining as well.)

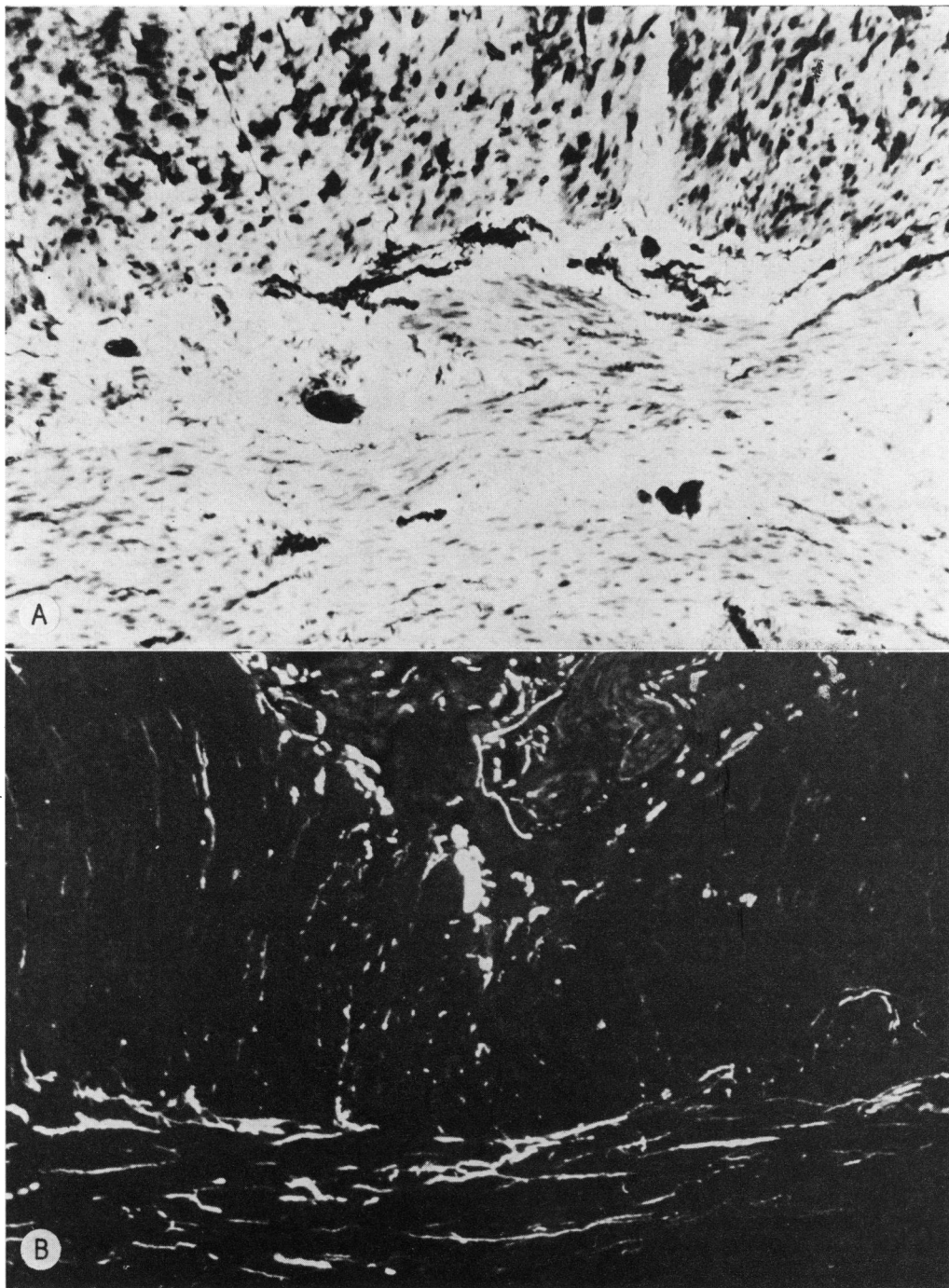


FIG. 3.—Distal aganglionic bowel from a case of Hirschsprung's disease (circular muscle layer in the top half of each picture). (A) Stained for AChE activity (4 hours counterstained with haemalum), showing a dense arrangement of thick, dark, somewhat overstained nerves in the circular muscle. Some thinner nerves are just becoming evident in the longitudinal muscle (graded+++ in the Table). ($\times 165$.) (B) Formalin-induced fluorescence, showing fluorescent adrenergic nerves in the same segment as (A). The nerves are more numerous in the longitudinal muscle than in the circular muscle but become more frequent again in the submucosa. ($\times 83$.)

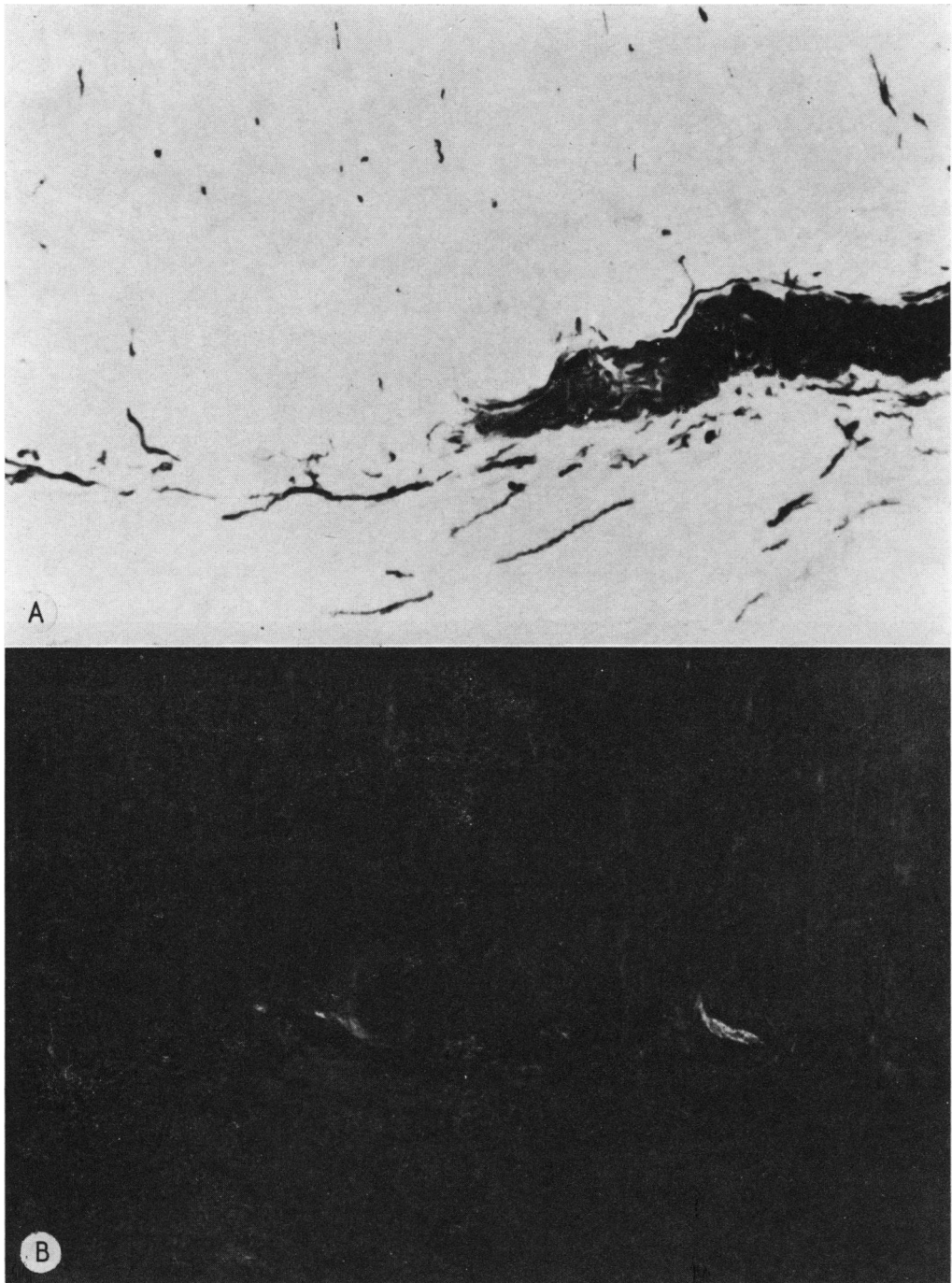


FIG. 4.—Distal aganglionic bowel from a case of Hirschsprung's disease (circular muscle layer in the top half of each picture). (A) Stained for AChE activity (4 hours faintly counterstained with haemalum), showing that despite the presence of a large positive nerve trunk in the intermuscular zone there are very few nerves in the muscle layers (graded \pm in the Table). ($\times 165$.) (B) Formalin-induced fluorescence, showing that very few adrenergic nerves are present. ($\times 165$.) This case showed no constricted segment on barium enema.

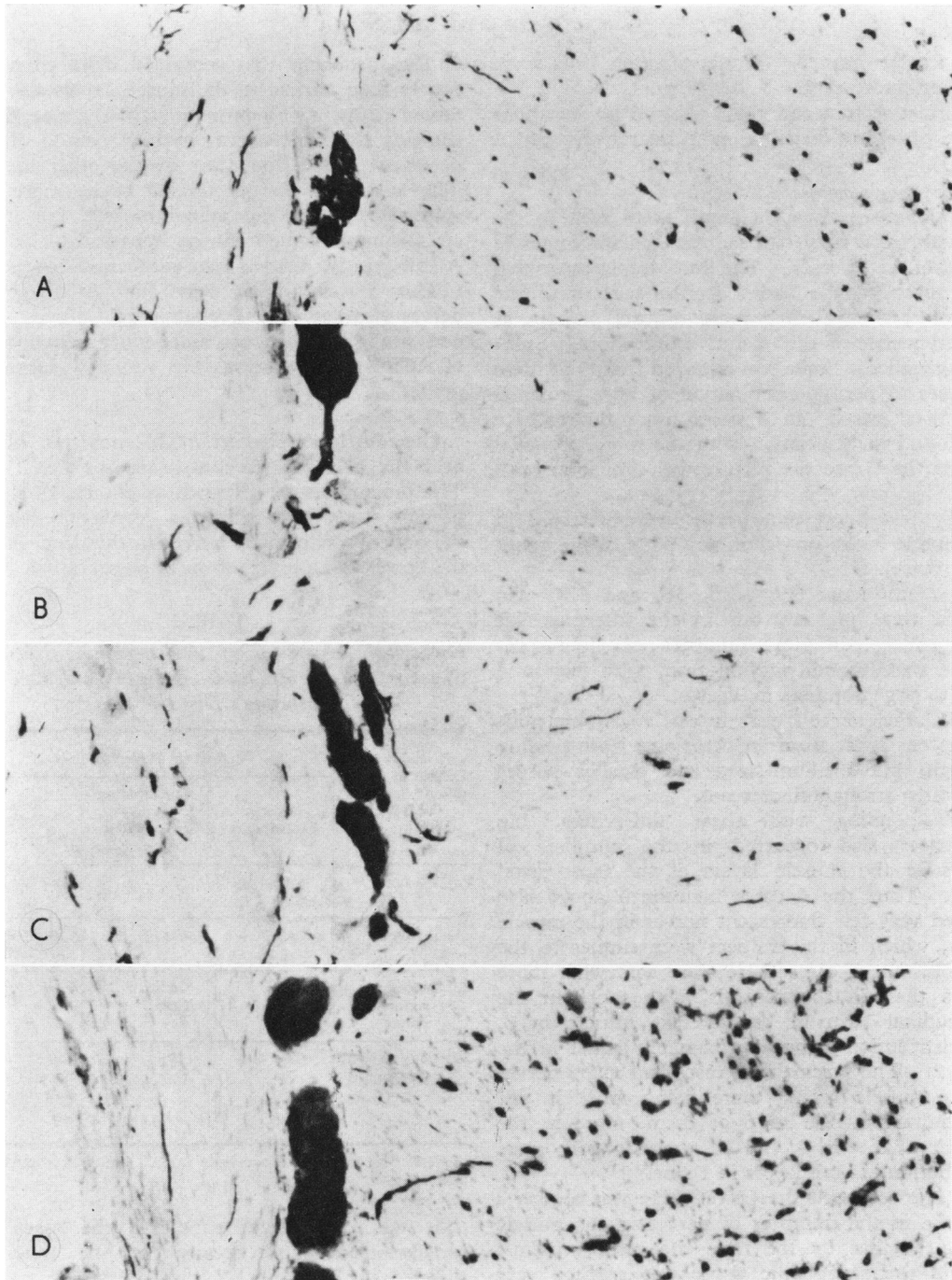


FIG. 5.—Segments of bowel from different levels in the same case of Hirschsprung's disease. All sections have been somewhat overstained for AChE activity in order to show up the smallest nerves and so help comparisons (counterstained with haemalum). (The circular muscle is on the right.) (A) Proximal ganglionic zone (24 hours incubation), showing a ganglion and normal numbers of nerves in the muscle layers. ($\times 109$.) (B) Distal ganglionic zone (24 hours incubation), showing a ganglion but very few nerves in the muscle layers. ($\times 109$.) (C) Proximal aganglionic zone (24 hours incubation), showing nerve trunks but few nerves in the muscle layers. ($\times 109$.) (D) Distal aganglionic zone (6 hours incubation), showing nerve trunks and many thickish, very positive, nerves in the circular muscle (graded ++ in the Table). ($\times 109$.)

the circular muscle. These ganglion cells were also non-fluorescent.

Normal rectum and colon showed no variations in the pattern of distribution at different levels.

Hirschsprung's disease

(a) *Proximal tissue.* Ganglia were seen in the most proximal tissues of the resected specimens in 14 out of the 18 cases. The fluorescence associated with these ganglia had a similar pattern to the controls but was often more diffuse, perhaps as a consequence of the operative procedure. Sufficient ganglionic tissue was removed from 9 of these 14 cases to permit examination of both proximal and distal parts. In 8 cases more fluorescence was found in association with the more proximal ganglia; in 1 case the picture was similar in both parts.

Very few fluorescent nerves were detected in the muscle layers proper in any parts of the ganglionic bowel.

(b) *Distal tissue* (Figs. 2B, 3B, and 4B). No ganglia were present, but in the intermuscular zone widely differing numbers of fluorescent nerves were found, varying from very few in 4 cases to large numbers in 9 cases.

The larger nerve trunks in this zone were non-fluorescent apart from an occasional fibre passing through, but medium-sized and smaller nerves were often strongly fluorescent.

Corresponding with these differences, big differences also occurred in the numbers of nerves in the muscle layers of the most distal tissue. Thus the 4 cases mentioned above also showed very few fluorescent nerves in the muscle layers, which in this respect were similar to the controls. In 5 cases there were somewhat more nerves than in the controls, with more in the longitudinal than in the circular muscle in 3, the distribution being approximately equal in the other 2. The 9 remaining cases had many nerves in the muscle layers, there being more in the longitudinal muscle in 6 of them, more in the circular muscle in 2, and an approximately equal distribution in both layers in 1 case.

As one ascended the bowel there was always a decrease in the numbers of nerves in the muscle layers, so that by the time the most proximal part of the aganglionic tissue was reached very few fluorescent nerves were usually found.

Correlation between adrenergic nerves and AChE-positive nerves, in distal aganglionic tissue. Corresponding tissues have been examined by both methods in 14 cases, and in this group all

of the specimens that contained more adrenergic nerves than normal in the muscle layers also contained many AChE-positive nerves. The tissues showing the most nerves by one method did not necessarily show the most by the other method. The adrenergic nerves tended to be more conspicuous in the longitudinal muscle, but in the circular muscle there always appeared to be more AChE-positive nerves than adrenergic nerves.

The 3 cases with very few AChE-positive nerves also had very few adrenergic nerves. One odd case is left which contained fairly large numbers of AChE-positive nerves but very few adrenergic nerves.

Correlation between AChE-positive nerves and the clinical presentation (see the Table). The previous paper (Bennett *et al.*, 1968) and the present study have shown a correlation between the patients with many nerves in the distal part of the bowel and a severe form of presentation.

TABLE

Numerical Assessment of AChE-positive Nerves in Circular Muscle of Distal Aganglionic Bowel from Hirschsprung's Disease (15 cases)

Length of Aganglionic Segment of Bowel	Clinical Presentation		
	Simple Constipation	Constipation and Intermittent Obstruction	Severe Neonatal Obstruction
(a) 1-2 cm. ..	Case 1 ± Case 2 ±		Case 8 + + +
(b) 5-7 cm. ..	Case 3 + +	Case 5 + +	Case 9 + + + Case 10 + + +
(c) Ending in sigmoid colon ..		Case 6 + + Case 7 + +	Case 11 + + +
(d) Long segment disease ..	Case 4 ±		Case 12 + + + Case 13 + + Case 14 + + + Case 15 + + +

Note: The number of nerves in normal bowel lies between + and + +.

It was difficult to subdivide the cases into satisfactory groups because of the numerous differences in this disease. Nevertheless, an attempt has been made by dividing the cases into 4 groups according to the length of aganglionic bowel present, and into 3 groups according to the severity of the symptoms. The gross numbers of AChE-positive nerves in the circular muscle of the most distal tissue have been assessed on a plus

and minus basis. From the Table it is evident that the cases with the most severe presentation tended to have the most nerves and the mildest cases tended to have the fewest nerves. In fact the 3 cases with the fewest nerves all failed to show a characteristic constricted segment on barium enema, including one in which the whole of the colon was aganglionic (see Fig. 4).

It is appreciated that other factors also influence the effects of this disease, and they will be mentioned in the discussion.

There was less correlation between the numbers of adrenergic nerves and the clinical presentation.

Discussion

The aim of this histochemical study was to demonstrate the distribution of AChE-positive and catecholamine-containing nerves in rectum and colon of children with and without Hirschsprung's disease. An attempt will be made in this discussion to correlate these morphological findings with the results of physiological experiments on muscular activity in both normal and aganglionic bowel. The significance of the variations in the autonomic nerve supply in different cases of Hirschsprung's disease will also be considered.

Cholinesterase histochemistry shows that there is a mixed population of myenteric ganglion cells which differ both in size and AChE activity in normal human rectum and colon. This is similar to the small intestine (Koelle, 1955; Cauna *et al.*, 1961) and large intestine (Gunn, 1968) in experimental animals. These cells contrast with the uniform population of large, strongly AChE-positive cells in conventional parasympathetic ganglia such as ciliary ganglia in animals (Koelle, 1955) and in the submandibular gland of the cat (Garrett, 1966) or human (Garrett, 1967). The myenteric ganglia therefore should not be considered as simple parasympathetic ganglia. The muscle layers in normal colon and rectum contained moderate numbers of strongly AChE-positive nerves in among the muscle cells. Most of these nerves were probably cholinergic efferents, for it is generally agreed that cholinergic autonomic nerves are the most strongly AChE-positive.

Formalin-induced fluorescence showed that adrenergic myenteric nerves were present in close relation with myenteric ganglion cells, but very few of these nerves were found in the muscle layers of normal bowel; thus confirming the findings of Norberg (1964), Jacobowitz (1965), and Baumgarten (1967). This seems to indicate that the sympathetic nerves exert an indirect effect on

bowel musculature through an influence on ganglion cell activity.

The bowel resected from cases of Hirschsprung's disease varied in the distribution of AChE-positive nerves at different levels from the same case, and large differences also existed between different cases. Distal zones of aganglionic bowel tended to contain more AChE-positive nerves in the circular muscle than normal. Ascending the bowel there was a reduction in the numbers of nerves and often the most proximal zone of aganglionic bowel contained fewer nerves than normal. Immediately above this, the most distal ganglionic bowel, sometimes called the 'transitional zone', usually had even fewer nerves in its muscle layers. These results are consistent with the manometric estimates of AChE activity by Kamijo *et al.* (1953), who found that there was much less activity in the more proximal ganglionic tissue in Hirschsprung's disease than in the distal aganglionic bowel, and also less than in a normal control. The observations suggest that the AChE-positive nerves in the distal aganglionic bowel originate in the sacral outflow, enter from below, and are distributed in a cephalad direction (see Smith, 1967).

Where the ganglion cells are absent in Hirschsprung's disease, there is a concomitant loss in the normal distribution pattern of adrenergic nerves, which then tend to be distributed to a variable extent through both muscle layers.

Before discussing the functional significance of these morphological findings, one must consider the physiology of the nervous control of muscular activity in normal colon and rectum; a complex and ill-understood subject. The sacral outflow of the parasympathetic system has a motor function which extends proximally from the anal sphincter to the mid-transverse colon; Garry (1933) showed that division of these nerves in the cat resulted in a dilated distal colon, with difficulty in bowel emptying, and these results were confirmed by Adamson and Aird (1932). The lumbar outflow of the sympathetic system, on the other hand, has an inhibitory activity, and stimulation of the lumbar sympathetic ganglia has long been known to cause inhibition of peristalsis in the colon (Langley and Anderson, 1895).

The finding that adrenergic nerves have a functional relation with myenteric ganglion cells rather than with the muscle cells directly (Norberg, 1964; Jacobowitz, 1965) makes it apparent that the control of bowel activity is far more complex than the usually held simple concept of a 'motor parasympathetic' and an 'inhibitory sympathetic' innervation of the muscular tissue. The myenteric

ganglion cells, as well as co-ordinating peristaltic waves, appear to be concerned with co-ordinating inhibitory sympathetic activity through their intimate connexion with the networks of adrenergic axons. Further, an inhibitory mechanism in the bowel wall, which may depend on a non-adrenergic system, has recently been postulated from pharmacological evidence in animals (Burnstock, Campbell, and Rand, 1966; Day and Warren, 1968) and also in the human (Bucknell, 1966), and this evidence suggests that a proportion of neurones in the bowel wall may in themselves be inhibitory in action.

The absence of ganglia in the distal segment of bowel, in Hirschsprung's disease, leads to an absence of normal peristalsis with a hold-up to the forward propulsion of bowel contents at the highest level of aganglionosis. Furthermore, the absence of the normal arrangement of ganglion cells and surrounding sympathetic nerves makes it likely that effective inhibitory mechanisms, inducing relaxation, are absent in the affected segment of bowel. This has been borne out by recent physiological experiments which have constantly shown an absence of recto-anal inhibitory reflexes in this condition (Lawson and Nixon, 1967; Howard, 1968). AChE-positive nerves, on the other hand, are often present in increased numbers in the circular muscular layer, especially in more distal segments. Their presence, in conjunction with the absence of a normal inhibitory mechanism, probably explains the contracted state of the bowel in a typical case of Hirschsprung's disease. Sometimes there is also an accompanying increase in the number of adrenergic nerves to the muscle layers, but even if these adrenergic nerves can produce an inhibition, it appears that the cholinergic nerves exert an overwhelming effect. Evidence that nerves do exert a predominantly contractile effect on the aganglionic bowel in Hirschsprung's disease was given by Bodian, Stephens, and Ward (1949), when they found that spinal anaesthetic produced relaxation of the distal segment, during barium enema examination in 3 cases. They considered that this finding indicated that there was a 'spastic obstructive factor'. Ehrenpreis (1946) had previously reported similar findings in 2 cases, relaxation being observed through a proctoscope.

The present morphological investigation of cases of Hirschsprung's disease suggests that, as well as the absence of ganglia and the increased numbers of AChE-positive nerves in the distal bowel, a further factor may be at least partly responsible for the severe bowel dysfunction.

Very few AChE-positive nerves were found in the muscle layers of the distal *ganglionic* bowel so that this zone, which may be of variable length, is probably deficient in propulsive power. This may accentuate the obstructive effect of the more tonic distal bowel.

The clinical presentation in this disease is variable. Bodian *et al.* (1949) commented that it was not possible to correlate the degree of intestinal obstruction in Hirschsprung's disease with the length of affected bowel. Similarly, Hope, Borns, and Berg (1965) stated that 'severity of symptoms are not just a matter of the length of aganglionic segment, but sometimes, given two babies with the same amount of bowel involved, one may be completely obstructed whilst the other is only slightly obstructed'. Does the present morphological investigation help to explain these clinical differences?

Though there is an absence of normal ganglia throughout a length of distal bowel in true Hirschsprung's disease, the present study shows that in the circular muscle of this segment, the numbers of AChE-positive nerves vary considerably. This wide range of AChE activity, extending from few nerves to many more than normal, is reflected in the results of manometric studies by Adams *et al.* (1960), for they also found a greater range of activity in aganglionic tissue from Hirschsprung's disease than in normal controls. This variation may account for some of the differences in obstructive symptoms. The greatest numbers of AChE-positive nerves were found in the circular muscle of distal aganglionic bowel from those children who had needed a colostomy for severe intestinal obstruction in the neonatal period. Patients presenting later in life had fewer AChE-positive muscular nerves extending a lesser distance along the aganglionic bowel. The 3 patients from this group with the fewest nerves in the muscular layers even showed no constricted segment on barium enema.

Finally, because of the wide variations that exist in the numbers of AChE-positive nerves in differing lengths of bowel from Hirschsprung's disease, it is advisable to have adequate morphological controls, using AChE histochemistry, whenever muscle from this disease is being investigated.

Summary

The distribution of adrenergic and acetylcholinesterase positive (cholinergic) nerves in the muscle layers of large bowel was studied in resected specimens from 23 young children: 4 normal

controls, and 19 cases of Hirschsprung's disease.

The number of cholinergic nerves in the circular muscle of the aganglionic bowel in Hirschsprung's disease varied from case to case, but a correlation was found between the number of such nerves and the severity of the disease.

The junctional zone between normal and aganglionic bowel, though containing ganglia, was deficient in muscular nerves.

The morphological findings are discussed in relation to functional activity in normal and aganglionic bowel, and it is suggested that more than one factor is responsible for the bowel mal-function in Hirschsprung's disease. Aganglionosis results in a failure of co-ordinated contraction and inhibition. Cholinergic muscular nerves are probably responsible for the typically contracted state of the distal bowel. In the junctional zone between normal and aganglionic bowel, the deficiency of muscular nerves probably means that this segment of bowel can exert only a poor propulsive force.

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