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Internal Anal Sphincter

Observations on Development and Mechanism of Inhibitory Responses in Premature Infants and Children with Hirschsprung's Disease

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The relative importance of the internal and external sphincters to the maintenance of tone in the anal canal has been shown in previous studies of anal physiology (Gaston, 1948; Schuster *et al.*, 1965; Duthie and Watts, 1965).

The external sphincter is a striated muscle, but shows continuous activity on electromyography. Inhibition and stimulation is mediated by spinal cord reflexes, through the pudendal nerves and sacral segments of the spinal cord (Floyd and Walls, 1953; Porter, 1961). Voluntary control is possible over this part of the anal sphincter.

The internal sphincter is made up of smooth muscle fibres, continuous with the muscle layers of the rectal wall, and under resting conditions provides most of the tone of the anal canal (Duthie and Watts, 1965).

The internal sphincter is also under reflex control and a rise of tension in the rectal wall results in a decrease of tone—the relaxation reflex of the internal sphincter (Gowers, 1878; Denny-Brown and Robertson, 1935). Recently, Lawson and Nixon (1967) have emphasized that the internal sphincter is characterized by rhythmical activity. Inhibition of this activity accompanies the decrease in tone seen during the recto-sphincteric relaxation reflex.

Reflex response of the internal sphincter is absent in Hirschsprung's disease, while the reflexes of the external sphincter remain intact (Lawson and Nixon, 1967; Schnaufer *et al.*, 1967).

The possibility of using this abnormal response as a routine diagnostic procedure for Hirschsprung's disease was examined in the present study. The physiological responses of the internal sphincter were examined in 60 children who presented with evidence of bowel dysfunction. The symptoms varied in severity from those of acute intestinal obstruction to constipation alone. During this study physiological abnormalities were observed in the reflexes of premature infants, which showed similarities to those seen in patients with Hirschsprung's disease. On repeated examinations over several days, however, the physiological responses were found to change until normal reflexes were eventually established.

In order to help determine the nervous pathway through which the reflexes of the internal sphincter are mediated, we have examined normal bowel and aganglionic bowel from cases of Hirschsprung's disease by pharmacological and histochemical methods.

Physiological Study

Methods. Two methods were used to measure pressures within the anal canal—an air-filled system based on the principle of miniature balloons, and strain gauges mounted on thin strips of beryllium copper. The extreme sensitivity of the latter method was especially useful in detecting any rhythmical activity in the sphincters with very low pressures found in some infants during the neonatal period.

(*a*) *Air-filled system.* This method has recently been described in detail (Lawson and Nixon, 1967). A cylindrical brass probe, 5mm. in diameter, contains 2 or 4 air-filled chambers each 0·8 cm. in length. The chambers are covered with thin Paul's tubing and connected to pressure transducers (Solatron NT4-313 10 p.s.i.) by thin nylon tubing. An inflatable rectal balloon is incorporated, and pressure changes recorded on a modified Ofner 8 channel EEG recorder. A pressure change of 100 cm. H_2O results in a potential difference change of 3mV across each transducer. This is amplified and fed into the pen recorder.

(*b*) *Strain gauge system.* Two miniature silicon semiconductor strain gauges (Ether 2A-3A-120 P) were bonded to 0·008 cm. thick beryllium copper strips with a phenolic stoving cement (Bakelite cement J.11185).

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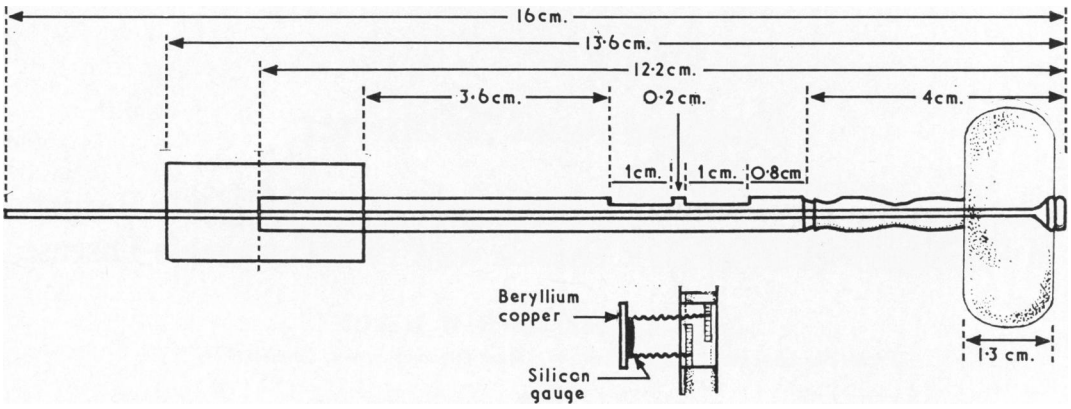

FIG. 1.—*Diagram of strain gauge probe, with exploded view to show gauge connexions.*

Each strip measured 12 mm. × 2 mm. These were mounted across 1 cm. gaps cut in an 8 cm. length of brass tubing of 5 mm. diameter. One end of each strip was bonded to the brass with epoxy-resin (Araldite), and a potential difference of 4½ volts was applied across each gauge (Fig. 1). The probe was covered with Paul's tubing and suspended in an air-tight chamber connected with a water manometer and sphygmomanometer hand pump. Calibration was performed at different pressures by measuring resistance changes in each gauge through a Wheatsone bridge circuit and voltmeter.

A pressure of 100 cm. H_2O resulted in a potential difference change of approximately 60 mV across each gauge.

As in the air-filled system, a rectal balloon was incorporated to lie approximately 5 cm. from the anal verge. The output from the Wheatstone bridge circuit was amplified and fed into an Ofner EEG machine.

Both types of probe were designed to measure rectal and anal canal pressures synchronously without moving the probe.

Electromyography. Surface electrodes and stainless steel clips were used to record action potentials of the external sphincter. These were amplified, shown on an oscilloscope screen, and written out by the Ofner pen recorder.

To obtain satisfactory tracings of internal sphincter activity in infants and small children, it is essential to reduce stimulation to a minimum. We therefore omitted the electromyograph electrodes during the first part of each recording. Restlessness obscures the rhythmical activity and responses of the internal sphincter. Sedation was not used.

Results. Recordings of 60 infants and children suspected of having Hirschsprung's disease were made using either the balloon or strain gauge systems (Fig. 2 and Table I). At the beginning of the study both probes were used on several patients for comparison purposes. Pressure measurements were found to be similar, but during the investigation of the very low pressures found at times in the smaller

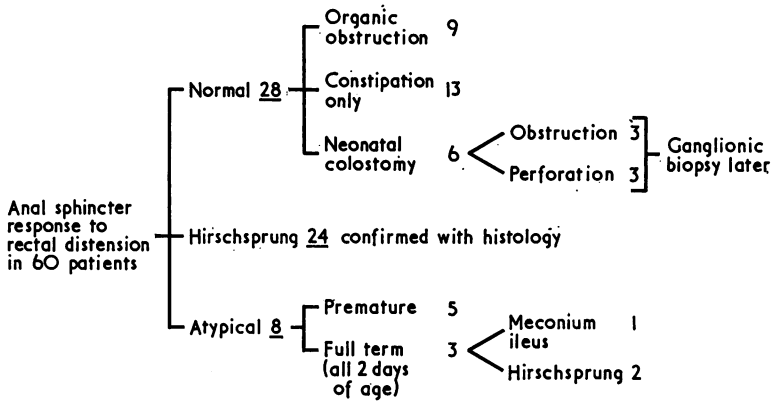

FIG. 2.—*Results of studies of anal sphincter responses in patients with bowel dysfunction.*

TABLE I

Ages of Patients in Anal Sphincter Studies

Normal responses (28)	6 days to 14 years
Hirschsprung responses (24)	4 days to 9 years
Atypical responses (8)	(*a*) Premature, 2 days to 10 days (5) (*b*) Full term, 2 days (3)

infants the greater amplification possible with the strain gauge method allowed easier recognition of any rhythmical activity that might be present.

Results fell into the following groups.

Normal sphincter physiology. 28 patients showed a normal internal sphincter response to rectal distension. Their ages ranged from 6 days to 14 years. All these recordings showed resting sphincter pressures greater than 30 cm. H_2O and rhythmical activity of 12-16 contractions per minute which could be inhibited by rectal distension. This inhibition of rhythmical activity was accompanied by a fall in pressure for 10-15 seconds in the older child (Fig. 3), but in smaller infants the mean pressure of the sphincter did not show a change on many occasions. Mechanical factors dependent on the relative sizes of the probes to the diameters of the anal canals at different ages may account for this difference in response.

Of the younger children in this group, 9 presented with vomiting and constipation within the first few weeks of life. Subsequent diagnoses of an organic cause for their symptoms were made at barium examination or operation and included meconium ileus, ileal atresia, ileo-caecal duplication, and hiatus hernia (Table II).

Examinations were made of 13 children because of constipation from early infancy. Internal sphincter responses were within normal limits, though changes of rectal inertia were often present (Callaghan and Nixon, 1964).

Six children had undergone laparotomy and colostomy during the neonatal period. All had presented with intestinal obstruction, and at laparotomy cones typical of Hirschsprung's disease had been found, but biopsies taken at sites selected for colostomy were ganglionic. 4 of these children were admitted to hospital 1 year later for definitive surgical treatment, but physiological examination revealed normal anal reflexes, and subsequent rectal biopsies were ganglionic. 2 patients were examined a few days after enterostomies had been fashioned. Normal physiology was shown, and biopsies indicated normal myenteric

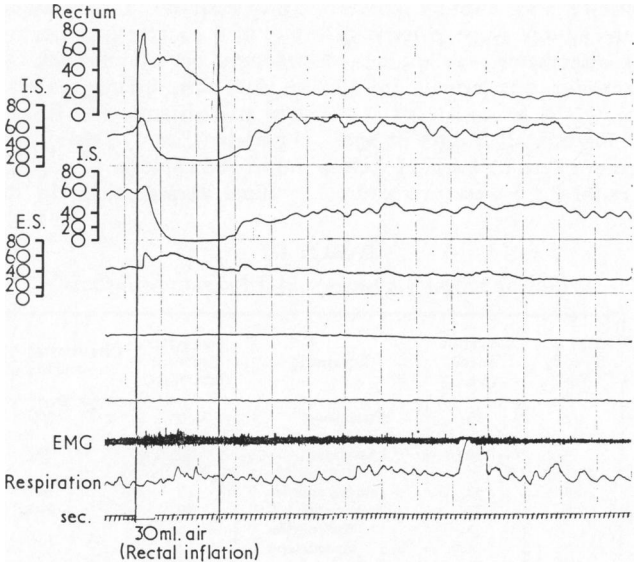

FIG. 3.—*Normal anal sphincter response to rectal distension by air in a boy of 10½ years. E.S. shows the external sphincter contraction corresponding to increased EMG activity. I.S. shows internal sphincter inhibition in the upper two-thirds of the anal canal. Units of pressure are cm. H_2O in Fig. 3-5.*

TABLE II

Normal Anal Sphincter Physiology in 8 Full-term Infants Under 8 Weeks of Age

Case No.	Sex	Age (dy.)	Diagnosis	Sphincter Pressure (cm. H_2O)	Rhythmical Activity (contractions/min.)	Recto-sphincteric Reflex
1	F	2	Meconium ileus	35	Nil	–
2	F	6	Hiatus hernia	60	12	+
3	M	8	Ileal atresia	55	16–20	+
4	F	9	Meconium ileus	55	12	+
5	F	12	Hiatus hernia	40	8–12	+
6	F	30	Ileo-caecal reduplication	30	12–16	+
7	M	39	Feeding problem	30	12–14	+
8	F	55	Hydrocephalus	55	12	+
Mean				45	13	

plexuses. The colostomies were therefore closed a few weeks later.

Hirschsprung's disease. Age range 4 days to 9 years. Tests in 24 patients showed internal sphincter activity which was unaffected by rectal distension, and diagnoses of Hirschsprung's disease were consequently made (Fig. 4). 8 of these children had colostomies established in the neonatal period. Histological proof of aganglionosis was not available at the time of the test, but was later obtained by biopsy.

Atypical results. 8 infants showed patterns of activity in the anal sphincter which were not typical of either normal sphincters or Hirschsprung's disease. Rhythmical activity was entirely absent except in one case where a slow wave pattern of low amplitude was just discernible. A sphincter response to rectal distension was absent in all cases.

Three were full-term infants, all 2 days of age. Operation showed meconium ileus in 1 and Hirschsprung's disease in the other 2. 5 were premature infants with intestinal obstruction. At the first examination they showed low sphincter pressures, no rhythmical activity, and absent recto-sphincteric reflexes (Table III). One child underwent operation for jejunal atresia and further examination was not possible. 3 showed signs of subacute intestinal obstruction for several days and repeat physiological tests were performed.

Repeated tests in 3 premature infants (Fig. 5a, b, c). Re-examination was carried out 5 and 6 days after their initial tests. Sphincter pressures were now 20-30 cm. H_2O higher than in the first examinations, and were recorded between 30 and 50 cm. H_2O. These values are close to the mean value of 45 cm. H_2O recorded in full-term infants (Table II). Rhythmical activity was now established with an average rate of 8 contractions per minute, slower by 5 contractions per minute than the average rate observed in full-term infants. Reflex sphincter relaxation was still absent apart from a transient response in 1 patient (Case 1, Table III) whose clinical condition improved rapidly.

Final examinations in the remaining 2 infants

TABLE III

Anal Sphincter Physiology in 5 Premature Infants

Case No.	Sex	Birth-weight (g.)	Age at Testing (dy.)	Gestation Period (wk.)	Diagnosis	Sphincter Pressure (cm. H_2O)	Rhythmical Activity (contractions/min.)	Recto-sphincteric Reflex
1	F	2502	2	35	Functional obstruction	0	Nil	–
2	F	2104	3	34	Functional obstruction	25	Nil	–
3	M	1820	5	33	Jejunal atresia	10	Nil	–
4	M	2047	6	37	Functional obstruction laparotomy	30	Nil	–
5	M	2360	10	38	Functional obstruction	25	6	–
Mean						18		

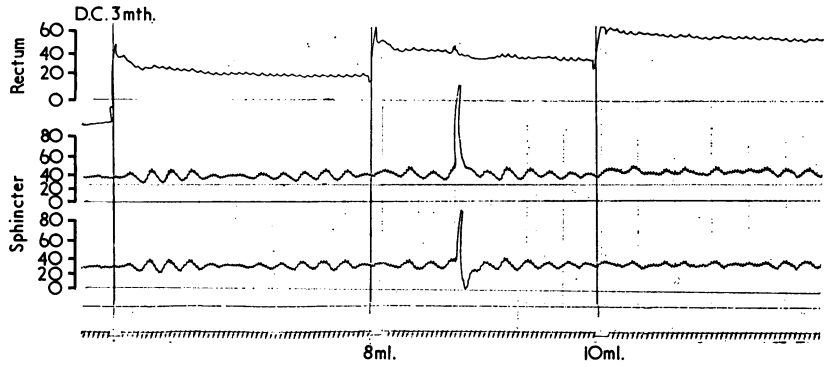

FIG. 4.—*Pronounced rhythmical activity of the anal sphincter in Hirschsprung's disease in an infant of 3 months of age. Rectal distension by 8 ml. and 10 ml. air has no effect.*

after further intervals of 6 and 11 days revealed normal rhythmical activity at 12 contractions per minute and a good reflex inhibitory response in the sphincter (Tables IV and V).

These changes in anal physiology observed over periods of approximately 2 weeks were accompanied by improvement in clinical signs. Abdominal distension disappeared, vomiting after feeds became infrequent, and bowel movements became regular.

Pharmacological Study

Method. Pharmacological responses of colon and rectum removed from 10 patients with Hirschsprung's disease were compared with those of normal ganglionic colon obtained at operations for unrelated conditions.

Full thickness longitudinal and circular muscle strips approximately 3 cm. long and 3-4 mm. wide were cut from the bowel under examination, and the mucous membrane removed. Each strip was suspended in an organ bath at 37°C. containing Krebs solution aerated with 95% O_2 and 5% CO_2. Movements were recorded on a smoked drum by a frontal writing level. Drugs used were acetylcholine perchlorate, adrenaline bitartrate, nicotine hydrogen tartrate, and dimethylphenylpiperazinium iodide (DMPP).

Results. All muscle contracted with acetylcholine but aganglionic tissue was less sensitive than normal bowel. The relaxation responses to adrenaline were similarly reduced in Hirschsprung's disease. Normal colon always responded to the ganglion-stimulating drugs, nicotine, and DMPP, with a relaxation. Tissue from the aganglionic and transitional zones of Hirschsprung's disease never showed this effect but showed either a contraction or no response at all (Fig. 6).

Histochemistry

Method. The catecholamine fluorescence technique of Falck and Hillarp was used to examine the adrenergic nerves within the myenteric plexuses of normal, transitional, and aganglionic bowel (Falck, 1962).

Small full thickness pieces of bowel were rapidly frozen as soon as the specimen was available at operation. After freeze drying, the tissue was treated with formaldehyde gas (Falck and Owman, 1965), embedded in paraffin, sectioned, and examined by fluorescence microscopy.

Using this technique, nerves containing noradrenaline are revealed by a pale green fluorescence. 5 cases of Hirschsprung's disease and 2 normal specimens have been examined in detail for this study.

TABLE IV

Changes in Anal Sphincter Activity in Premature Infant of 37 Weeks' Gestation (Case 4, Table III)

Age at Testing (dy.)	Sphincter Pressure (cm. H_2O)	Rhythmical Activity (contractions/min.)	Recto-sphincteric Reflex
6	30	Nil	−
11	50	8	−
22	50	12–16	+

TABLE V

Changes in Anal Sphincter Activity in Premature Infant of 34 Weeks' Gestation (Case 2, Table III)

Age at Testing (dy.)	Sphincter Pressure (cm. H_2O)	Rhythmical Activity (contractions/min.)	Recto-sphincteric Reflex
3	25	Nil	−
9	50	8–10	Transient
15	60	12	+

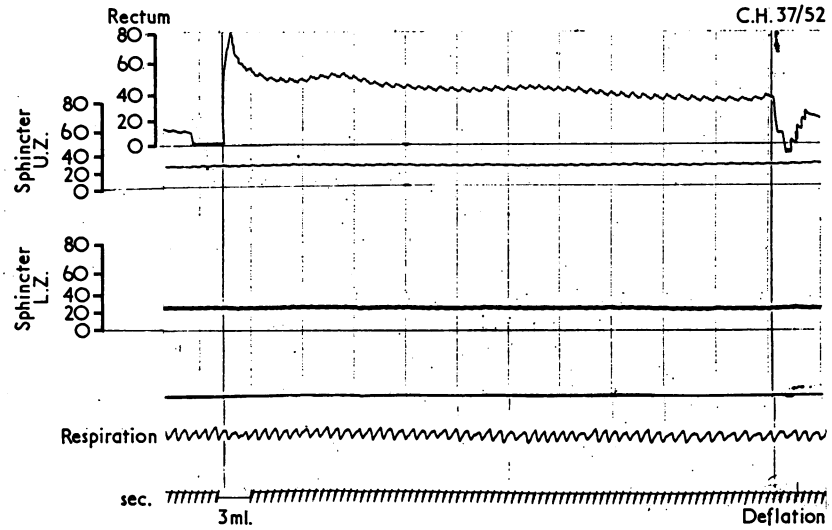

FIG. 5a.—*Pressure tracing from 6-day-old premature infant (Case 4, Table III) of 37 weeks' gestation. Rhythmical activity and response to rectal distension by 3 ml. air absent.*

Results. Normal bowel showed a pattern of nerve distribution seen previously in animals and human adults (Norberg, 1964; Jacobowitz, 1965; Baumgarten, 1967). Adrenergic nerves were sparse within the muscle layers but abundant in the myenteric plexus. Especially dense fluoresence was present in the region of each group of ganglion cells. No ganglion cell itself was fluorescent.

In aganglionic tissue, increased numbers of adrenergic nerves were seen, both in the intermuscular zone and in the muscle layers themselves (Bennett, Garrett, and Howard, 1968).

Dense areas of fluorescent tissue, as seen around ganglion cells in normal bowel, were not present in aganglionic zones, but in transitional areas the beginnings of such patterns were discernible in relation to small groups of ganglion cells.

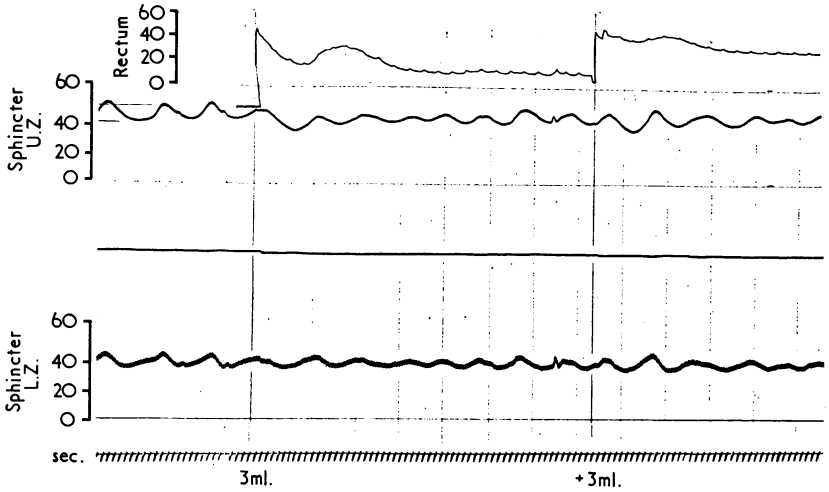

FIG. 5b.—*Same case as Fig. 5a at 11 days. Marked rhythmical activity at 8/min., unaffected by rectal distension by 3 ml. air.*

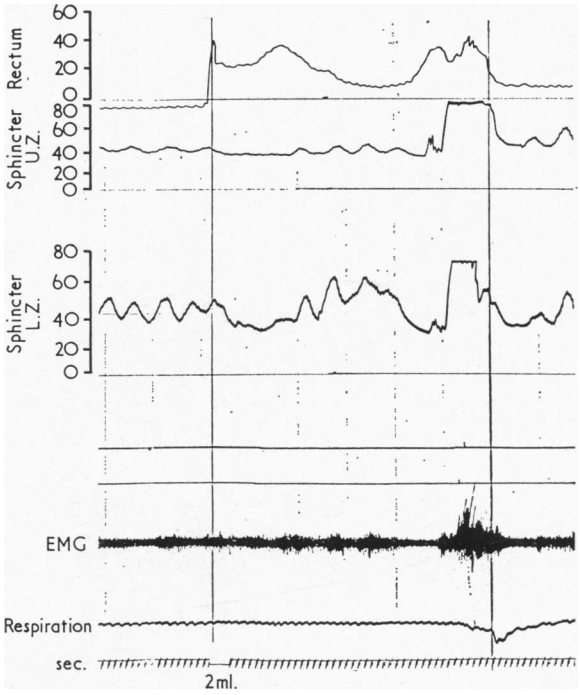

FIG. 5c.—*Same case as Fig 5a at 22 days. Rhythmical activity at 12/min. inhibited in upper (U.Z.) and lower zones (L.Z.) by rectal distension by 2 ml. air.*

Discussion

Normal physiology of the internal sphincter has been shown in this study to be established at the beginning of the neonatal period in infants of 40 weeks' gestation. Recto-sphincteric inhibitory reflexes were present in infants from 6 days after birth, together with normal rhythmical activity of 12 contractions per minute.

The anal sphincters of infants under 2 months of age are too small to allow any meaningful measurements of pressure to be made with our probes in different zones, as have been made previously in adults (Hill *et al.*, 1960; Bennett and Duthie, 1964). The pressures recorded in Tables II-VI represent therefore the maximum values in a gradient between the rectum and anal margin.

TABLE VI

Anal Sphincter Physiology in 9 Full-term Neonates with Hirschsprung's Disease (ages less than 7 weeks)

Case No.	Sex	Age (dy.)	Site of Transitional Segment	Sphincter Pressure (cm. H_2O)	Rhythmical Activity (contractions/min.)	Recto-sphincteric Reflex
1	F	2	Trans. colon	30	Nil	—
2	F	2	Splenic flexure	50	Nil	—
3	F	4	Sigmoid	55	10–12	—
4	M	8	Sigmoid	35	12	—
5	M	8	Ileum	50	8	—
6	F	10	Trans. colon	45	12	—
7	M	11	Sigmoid	80	10	—
8	F	27	Splenic flexure	30	9–12	—
9	M	57	Sigmoid	35	8–16	—
			Mean	45	11	

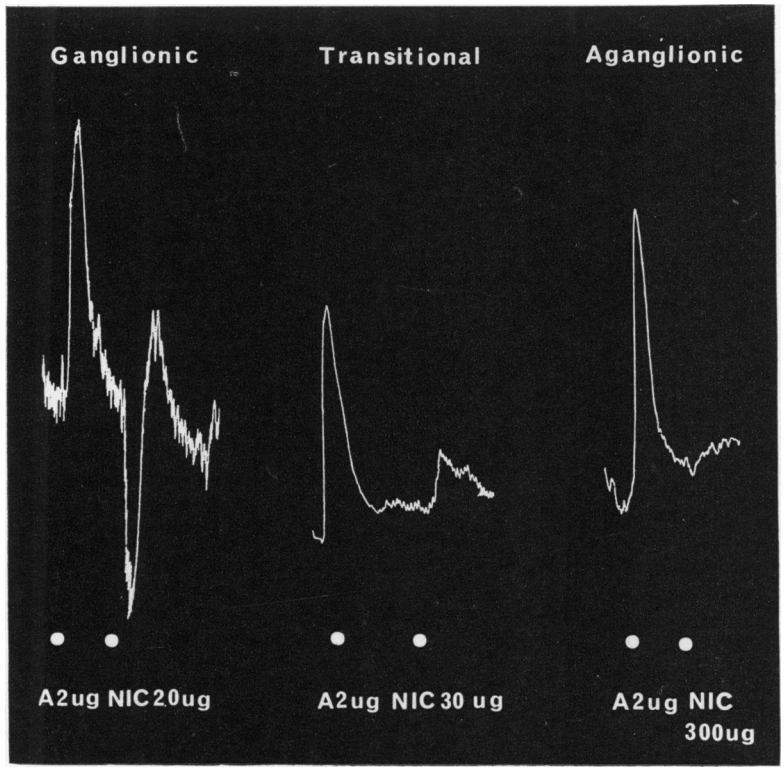

FIG. 6.—*Typical responses of colon muscle to acetylcholine (A) and nicotine (NIC). Ganglionic bowel shows a good relaxation to nicotine, while transitional tissue contracts. The aganglionic tissue has not responded.*

The mean value of 45 cm. H_2O seen in 8 infants under 2 months of age (Table II) is comparable with the values obtained in the maximal pressure zones of adults using thin open-ended tubes, e.g. Hill *et al.*, 1960: 45 cm. H_2O; Bennett and Duthie, 1964: 50 cm. H_2O.

These pressure values were measured after the probe had been in position within the anal canal for several minutes. During this time pressure gradually fell to a steady baseline. We believe that the pressure in this 'resting' state is due mainly to internal sphincter activity, while any rise above it is due to action of the external sphincter. Corroborative evidence for the view has been obtained in children paraplegic from meningomyeloceles. Though the pelvic floors showed absent reflexes and no tone, and the perineal areas were anaesthetic, the anal sphincters continued to show a pressure of around 45 cm. H_2O (E. R. Howard, unpublished observations).

Hirschsprung's disease was diagnosed in 24 of the children from an absent recto-sphincteric inhibitory reflex. This was possible in full-term infants from 4 days of life, when sphincter tone and normal rhythmical activity were shown to be present. These diagnoses were confirmed at a later date, when histology was available. There were no false positive or false negative results in patients over 4 days of age during this study.

The constant absence of the reflex in Hirschsprung's disease suggested that failure of inhibitory responses might also be present in the affected colon and rectum as well as in the sphincter, and that their absence might be as significant as any lack of co-ordinated contraction in the causation of the symptoms. Spasm is probably not a factor in Hirschsprung's disease, because in 9 infants examined before 2 months of age, the mean sphincter pressure was 45 cm. H_2O (Table VI), the same value as in the group without the condition.

Interpretation of the atypical results obtained in infants under 4 days of age is more complicated. 3 full-term infants showed normal sphincter tone but absent rhythmical activity and reflex responses. One of these had meconium ileus which could not have been differentiated from Hirschsprung's

disease on this test. All 5 premature infants showed absent reflexes and a mean sphincter pressure of only 18 cm. H_2O at the first examination (Fig. 7), though their ages ranged from 2 to 10 days. Normal physiology was not demonstrated in 3 of this group until about 2 weeks after admission, and followed a phase of normal sphincter tone and rhythmical activity which could not be inhibited by rectal distension. This temporary absence of the inhibitory mechanism suggested a cause for the bowel dysfunction sometimes seen in this age-group.

We examined the mechanism of smooth muscle inhibition in pharmacological studies of the large bowel. Muscle from normal colon and rectum responds to ganglion-stimulating drugs, such as nicotine and DMPP by a relaxation (Bucknell and Whitney, 1964; Wright and Shepherd, 1966). If myenteric ganglion cells are regarded as motor pathways of the para-sympathetic system, this response is difficult to understand. Wright and Shepherd considered that the effect might be produced through stimulation of post-ganglionic sympathetic nerves. In an investigation of aganglionic bowel from Hirschsprung's disease they noted a loss of inhibitory response, and suggested that this might be due to an ineffective or absent sympathetic system (Wright and Shepherd, 1965). Our pharmacological experiments have confirmed the failure of inhibition to ganglion-stimulating drugs, both in aganglionic and transitional zone tissue (Bennett and Howard, 1968). The histochemical studies, however, show that there is no lack of adrenergic nerves. Indeed there usually appears to be an increase, and histological examination has not shown any obvious abnormality of these nerve fibres. It therefore seems questionable whether the relaxation effect in normal colon produced by ganglion-stimulating drugs is caused by the release of noradrenaline from post-ganglionic sympathetic nerves.

Recent work using transmural stimulation on the caecum of the guinea-pig (Burnstock, Campbell, and Rand, 1966) and the ileum of rabbits and kittens (Day and Warren, 1968) has shown inhibitory responses which are not of sympathetic origin. This work has suggested the presence of non-adrenergic inhibitory neurones in the gut wall.

Absence of all neurones from the bowel wall, as in Hirschsprung's disease, may therefore lead to a loss of inhibitory reflexes; thus the demonstrable loss of the rectosphincteric inhibitory reflex in Hirschsprung's disease suggests that this is normally mediated through the myenteric plexus. This would explain its persistence even after destruction

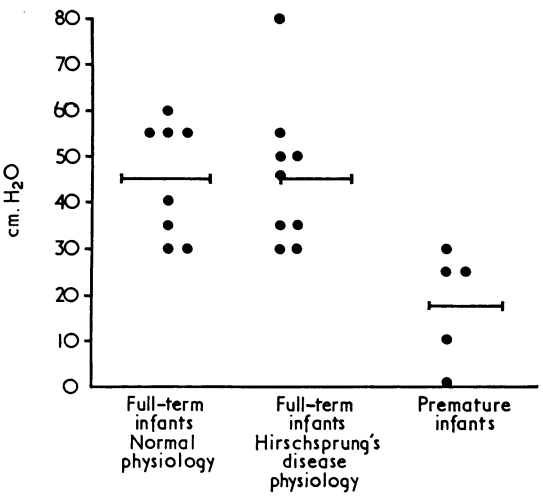

FIG. 7.—*Anal sphincter pressure in the neonatal period. The mean values are indicated in each group.*

of the cauda equina (Denny-Brown and Robertson, 1935).

Explanation for the absence of inhibition in premature infants may be provided by the recent histological evidence of immaturity of ganglion cells in the myenteric plexus (Smith, 1966). Maturation of these cells was found to depend on the length of pregnancy, and few immature cells were found in full-term infants. If a proportion of these ganglion cells is inhibitory in function, then these observations may account for the absent recto-sphincteric reflex in our premature infants.

Our study included 6 children who had presented with neonatal intestinal obstruction and who were found at operation to have coning of the bowel typical of Hirschsprung's disease. Normal physiology and ganglionic biopsies at a later date led to diagnoses of transient functional ileus. Immaturity of ganglion cells in the distal large bowel, with loss of inhibitory reflexes, may have been responsible for the Hirschsprung-like illness of these children. Muscle cell immaturity may be an additional factor to help explain the low sphincter pressures recorded at times in our premature infants at the initial examinations.

From these observations the mechanism for intestinal motility in the first few days of life of the premature infant appears to be precarious, and any adverse factor such as anoxia from respiratory distress (Dunn, 1963) or cerebral birth injury (Breton, Clay, and Gérard-Lefebvre 1959) may be enough to affect function to a variable degree and so

produce the clinical condition of functional intestinal obstruction.

Summary

Physiological examination of the internal sphincter has been performed in 60 children showing evidence of bowel dysfunction, varying in severity from simple constipation to acute intestinal obstruction.

Normal physiological responses were found in 28 children over 6 days of age, and this group included 6 children who had recovered from neonatal functional intestinal obstruction.

Hirschsprung's disease was diagnosed and confirmed in due course with histology, in 24 infants. 5 premature infants showed atypical physiological responses for up to 2 weeks.

Pharmacological and histochemical evidence is presented suggesting that normal inhibitory reflexes are mediated through neurones in the myenteric plexus. Immaturity of these cells may account for the bowel dysfunction seen in premature infants.

We wish to thank the staff of The Hospital for Sick Children for permission to study patients under their care, and Professor J. G. Murray, Dr. A. Bennett, and Dr. J. R. Garrett of King's College Hospital Medical School for the facilities that made the pharmacological and histochemical studies possible.

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