STUDIES IN HIRSCHSPRUNG'S DISEASE

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The morbid anatomy of Hirschsprung's disease is now firmly established. Swenson and Bill (1948) noted that the dilated colon which figures so largely in the earlier description of the disease terminated at a point proximal to the anus and was followed by a spastic segment. They concluded that the obstruction was due in some way to this segment and that the hypertrophy and dilatation of the bowel above was secondary to this obstruction.

Further work by Zuelzer and Wilson (1948), Bodian, Stephens and Ward (1949), Whitehouse and Kernohan (1948) and Swenson, Rheinlander and Diamond (1949) has shown that in the narrowed spastic segment there is a complete absence of the ganglion cells which form Meissner's and Auerbach's intramural plexuses. Instead there are large bundles of non-medullated nerve fibres occupying the position of the plexuses. This aganglionic segment extends from the anus up to the dilated part of the bowel. Examination of the dilated colon itself shows that there is hypertrophy of the muscle walls, but that the intramural ganglion cells are present in the normal quantities and arranged in the normal manner.

The abnormalities of function of this aganglionic segment are less clearly defined. Barium enema shows that this segment extends from the anus to the rectosigmoid junction or distal sigmoid colon; it appears narrow and can sometimes be seen to undergo segmentation. Proximal to this segment the bowel enlarges to form the typical megacolon. Using a technique which involves the recording of intracolonic pressures by means of multiple balloon at various levels in the lower bowel, Swenson *et al.* (1949) showed that there was increased tone with an absence of normal peristalsis in the narrowed segment. Davidson, Sleisenger, Steinberg and Almy (1955) described the effect of mecholyl on the activity of the colon in Hirschsprung's disease as recorded by catheters in the lumen of the gut. They noted that in records taken from the aganglionic segment of six patients with Hirschsprung's disease, mecholyl produced no change; whereas in 20 normal subjects there was no change in 11, and relaxation of the bowel in nine. In discussing their results they noted the possibility of using this method for mapping out the aganglionic segment of intestine, and they also commented on the absence of any increased sensitivity of the intestine to cholinergic drugs, such as is found in the aganglionic part of the oesophagus in achalasia of the cardia, and such as would be expected from Cannon's law of denervated structures. They conclude that this is due to the lesion being congenital rather than acquired.

It seemed that there was room for further investigation of the changes in the colon which occurred in Hirschsprung's disease. In the experiments which are described below, various properties of intestinal muscle obtained at operation from the aganglionic segment of gut from patients with Hirschsprung's disease and from a similar region in normal controls were examined to determine more accurately the nature of the derangement of function.

The following investigations were carried out:

(1) The response of the muscle strips to the action of various drugs *in vitro*;

(2) The response of muscle strips *in vitro* to electrical stimulation at various voltages and duration of stimulation;

(3) The presence or absence of ganglion cells in all specimens examined by the above methods was checked by ordinary histological means.

Response to Drug Stimulation

Method. Specimens of muscle both longitudinal and transverse were removed at operation from the aganglionic segment of bowel of patients with Hirschsprung's disease and from similar regions of the pelvi-rectal junction and upper rectum from patients with miscellaneous conditions. The mucous membrane was removed and strips of muscle were suspended in oxygenated tyrode at 37° C. The contractions of the muscle were recorded by a lever writing on a smoked drum.

Results. The response of the muscle strips to stimulation by drugs and the effect of various

blocking agents is shown in Tables 1, 2 and 3.

It can be seen that the normal muscle, and the muscle from both the aganglionic segments can all be stimulated by acetylcholine and that this response can be blocked by atropine but not by succinyl choline. Furthermore, muscles from all three sites will respond to nicotine with a contraction, although sometimes this response could only be obtained after the addition of eserine. It was also found that eserine would always increase the amplitude of the contraction with nicotine. The nicotine induced contraction could always be partially or completely blocked by hexamethonium and completely blocked by atropine. These findings hold true for both longitudinal and transverse muscle strips.

TABLE 1
THE RESPONSE OF LONGITUDINAL AND TRANSVERSE NORMAL MUSCLE FROM THE UPPER RECTUM AND COLON TO DRUGS

Stimulating Agent	Response	Blocking Agent		
		Hexamethonium (2·0 mg.)	Atropine (100 µg.)	Succinyl Choline (2·0 mg.)
Nicotine 400 µg.	+ (2) 0 (1)	B1	B1	0
Eserine 100 µg. + Nicotine 400 µg.	+ Increased response	B1	B1	0
Acetylcholine 20-50 µg.	+		B1	0
Noradrenaline 100 µg.	0 or fall in base line			
Adrenaline 100 µg.	0 or fall in base line			

50 ml. bath; + = contraction; B1 = block; 0 = no effect
Figures in brackets = number of experiments

TABLE 2
THE RESPONSE OF TRANSVERSE MUSCLE FROM THE AGANGLIONIC SEGMENT OF GUT IN HIRSCHSPRUNG'S DISEASE

Stimulating Agent	Response	Blocking Agent		
		Hexamethonium (2·0 mg.)	Atropine (100 µg.)	Succinyl Choline (2·0 mg.)
Nicotine 400 µg.	+ (4) 0 (1)	B1 (2)	B1 (2)	0 (2)
Eserine 100 µg. + Nicotine 400 µg.	+ (5) Increased in all specimens	B1 (3)	B1 (5)	0 (2)
Acetylcholine 20-50 µg.	+ (3)		B1 (3)	0 (2)
Noradrenaline 100 µg.	0 (1) Base line lowered (3)			
Adrenaline 100 µg.	0 (1) Base line lowered (3)			
5 Hydroxy tryptamine	Base line lowered (1)			

As for Table 1

TABLE 3
THE RESPONSE OF LONGITUDINAL MUSCLE FROM THE AGANGLIONIC SEGMENT OF GUT IN HIRSCHSPRUNG'S DISEASE

Stimulating Agent	Response	Blocking Agent		
		Hexamethonium (2·0 mg.)	Atropine (100 µg.)	Succinyl Choline (2·0 mg.)
Nicotine 400 µg.	+ (4) 0 (2)	B1 (1)	B1 (2)	0 (2)
Eserine 100 µg. + Nicotine 400 µg.	+ (4) Increased in all specimens	B1 (3)	B1 (2)	0 (1)
Acetylcholine 20-50 µg.	+ (3)		B1 (3)	0 (2)
Noradrenaline 100 µg.	0 (3) Base line lowered (1)			
Adrenaline 100 µg.	0 (2) Base line lowered (1)			
5 Hydroxy tryptamine 100 µg.	0			

As for Table 1

It is widely held that the stimulating action of nicotine is confined to the ganglion cell and that it has no action on either pre- or post-ganglionic nerve fibres or on smooth muscle. In the case of the normal gut, the response is as would be expected, but the contractions of the aganglionic segment from the subjects with Hirschsprung's disease, which could be consistently produced by nicotine, are more difficult to explain. The fact that the nicotine induced contractions could be blocked by atropine and augmented by eserine suggested that the nicotine was stimulating some structure within the colon wall which would release acetyl choline and which was associated with some cholinesterase system.

Response to Electrical Stimulation

In 1955 Paton described a method of stimulating the guinea-pig intestine by means of coaxial electrodes. He concluded that it was possible to distinguish by this method of excitation between pre-ganglionic, post-ganglionic and effector cell structures.

The nature of our specimen obtained at operation made this method of stimulation impossible, but it was considered worthwhile to investigate the nature of their response to electrical stimulation by another method.

Method. The strip of muscle was suspended in oxygenated tyrode at 37° C. Silver electrodes were applied to both ends of the strip and just before stimulation the level of the tyrode was lowered until the muscle and electrodes were completely uncovered. The muscle was then stimulated along its longitudinal axis and the contraction recorded on a revolving smoked drum.

The stimulator provided simple rectangular voltage pulses of variable duration (10 μsec. to 300 msec.) and amplitude (0 to 200 volts). Its output impedance was 300 ohms. In preliminary experiments the current wave form was observed simultaneously with the voltage wave form by means of a double beam cathode ray oscillograph. It was confirmed that the current wave form was also substantially rectangular except for the usual small spike of relatively short duration (associated with the capacitative component of the impedance between the electrode) at the rising edge of the pulse. The resistance of the electrodes and specimen ranged from 2,000 to 4,000 ohms. For example, for a pulse of amplitude 100 volts the current was of the order of 40 mA. With this longitudinal arrangement of the electrodes a considerable proportion of the total current presumably flows along the moist film over the surface of the specimen and does not contribute to the effective stimulus. The threshold voltage required for stimulation was determined by increasing the amplitude of single pulses in steps until a slight muscular contraction was observed. The tyrode level was then raised and the muscle allowed to rest for five minutes before another determination of threshold for a pulse of longer duration; thus intensity duration curves were constructed.

Results. Fig. 1 shows the intensity duration curve obtained from muscle from the upper rectum. It can be seen that the pattern produced with the longitudinal muscle differs a little from that obtained by stimulating transverse muscle, furthermore, after the muscle is atropinized there is a shift in the curve obtained from transverse muscle but not in that obtained from longitudinal muscle.

Fig. 2 shows the curves obtained in transverse muscle from the aganglionic upper rectum of patients with Hirschsprung's disease and it can be seen that all samples show a shift to the right and are similar to those found in atropinized normal muscle. However, the longitudinal muscle from patients with Hirschsprung's disease is similar to that from normals (Fig. 3).

Discussion

These experiments were undertaken to investigate some of the properties of aganglionic intestinal smooth muscle from subjects with Hirschsprung's disease.

The first group of experiments was concerned with the response of this muscle to various drugs. The response of the aganglionic muscle to nicotine was surprising; all the evidence obtained suggested that a structure was being stimulated which was capable of releasing acetylcholine, but extensive search failed to reveal any ganglion cells. It would appear, therefore, that the stimulating effect of nicotine as far as the intestine was concerned extended beyond the ganglion cells and that other structures could also be stimulated. Of the nature of these structures it is only possible to speculate, but the tissues which are most obvious or most likely to be concerned are the whorls of non-medullated nerve fibres which appear in the position usually occupied by Auerbach's plexus and it is not unreasonable to suggest that these structures are capable of being stimulated by nicotine to release acetylcholine.

Experiments by Kottegoda (1953) strongly suggest that nicotine is capable of releasing adrenaline-like substances from isolated rabbit ear and that this action can be blocked by hexamethonium. It seems that release of adrenaline from local chromaffin tissue is unimportant here as noradrenaline relaxes rather than contracts this muscle and the augmentation of the response by eserine and its blocking by atropine suggest a cholinergic rather than an adrenergic mechanism.

The hope of clarifying the situation by differentiating between stimulation of muscle and nerve by varying the durations of electrical pulse wave was disappointing. Small difference could be detected between the transverse muscle from the aganglionic segment on the one hand and normal gut on the other. As far as they went they suggested that it was not possible to stimulate the release of acetylcholine by electrical means as in normal intestine, but the differences were very small and the possibility of some form of artefact could not be excluded.

It can therefore be concluded that in the aganglionic segment of gut in Hirschsprung's disease, although there are no ganglion cells present, there are certainly structures which can release acetylcholine when treated with nicotine and cause the intestine to contract.

The most likely site of this acetylcholine release would be the whorls of tissue which appear to replace Auerbach's plexus in the wall of the gut. Whether these structures are capable of playing any part in intestinal activity is not clear, but the presence of some structure which resembles nervous tissue, and which is capable of releasing acetylcholine, may account for the absence of hypersensitivity of the intestine to cholinergic drugs. For to obey Cannon's law of the hypersensitivity of denervated structures the muscle should be truly denervated.

Summary

The response to drugs and electrical stimulation of strips of aganglionic intestine from patients with Hirschsprung's disease has been determined and compared with normal muscle from the same level.

The results show that there is present in the

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FIG. 1.—Intensity duration curves of normal transverse and longitudinal muscle from the upper rectum before and after atropine 1 : 500,000.
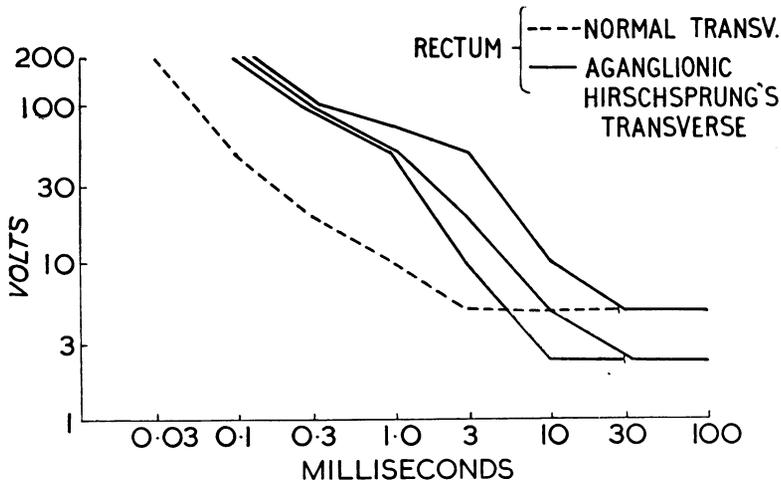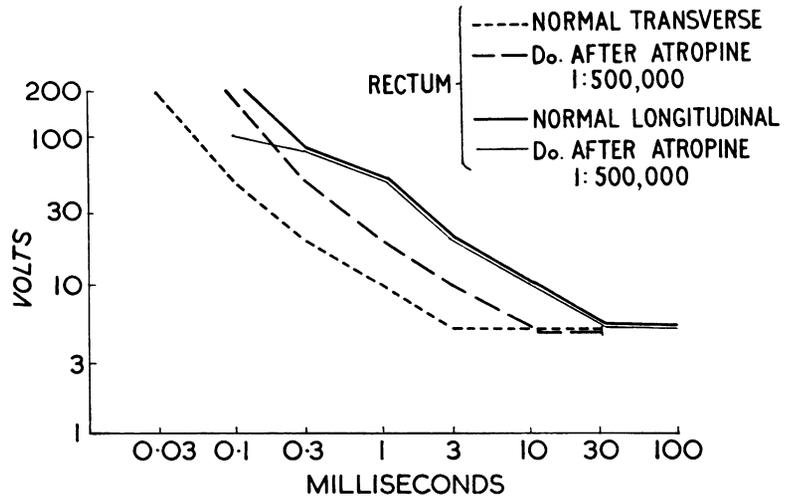
RECTUM
- - - - - NORMAL TRANSVERSE
- - - - Do. AFTER ATROPINE 1:500,000
——— NORMAL LONGITUDINAL
——— Do. AFTER ATROPINE 1:500,000

VOLTS

MILLISECONDS

RECTUM
- - - - - NORMAL TRANSV.
——— AGANGLIONIC HIRSCHSPRUNG'S TRANSVERSE

FIG. 2.—Intensity duration curves of transverse aganglionic muscle from the upper rectum of three subjects with Hirschsprung's disease compared with normal muscle.

VOLTS

MILLISECONDS

RECTUM
- - - - - NORMAL LONGIT.
——— AGANGLIONIC HIRSCHSPRUNG'S LONGITUDINAL

FIG. 3.—Intensity duration curves of longitudinal aganglionic muscle from the upper rectum of three subjects with Hirschsprung's disease compared with a normal.

VOLTS

MILLISECONDS
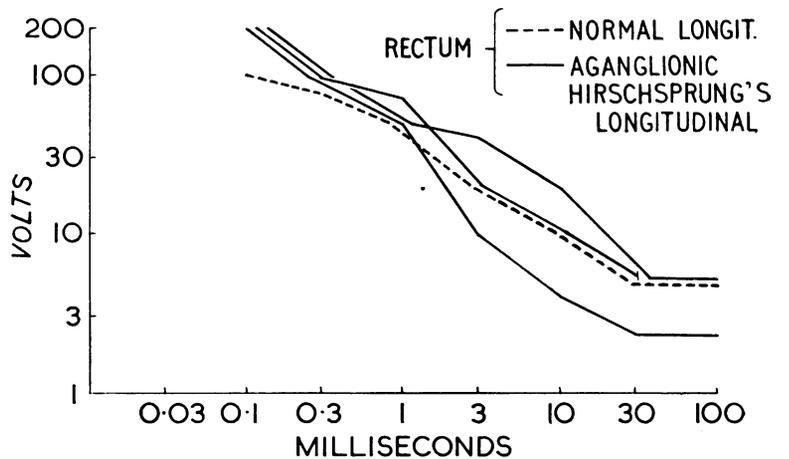
aganglionic segment some structure, which on stimulation with nicotine is capable of initiating a contraction mediated by acetylcholine release.

With electrical stimulation, no such acetylcholine release could be demonstrated.

The significance of these results is discussed.

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