

ALIMENTARY TRACT

Histological studies of Auerbach's plexuses of the oesophagus, stomach, jejunum, and colon in patients with achalasia of the oesophagus: correlation with gastric acid secretion, presence of parietal cells and gastric emptying of solids

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Abstract

Histological changes in the Auerbach's plexuses of the oesophagus, stomach, jejunum, and colon were analysed in a prospective study in 34 patients with achalasia of the oesophagus. At the distal end of the oesophagus ganglia cells were absent in 91% of cases as well as in the middle third of the stomach (20%). The Auerbach's plexuses were normal in the jejunum and colon. The results of gastric acid secretion showed that the peak acid output was significantly lower in achalasia patients compared with controls ($p < 0.001$). There was no correlation between the mean ganglion neuronal count in the gastric plexuses and the rate of gastric acid output ($r = 0.33$). Gastric emptying of solids was also evaluated, but there was no correlation between gastric emptying and the mean ganglion neuronal count in the gastric Auerbach's plexuses. The rate of gastric emptying of solids was similar in controls and patients with achalasia. These studies suggest that denervation of the oesophagus in patients with achalasia, which is a constant finding in several previous reports may extend beyond the oesophagus to the stomach in nearly half the cases.

Achalasia of the oesophagus is a primary motor disorder of the oesophagus characterised by a hypertensive gastrooesophageal sphincter with incomplete relaxation and aperistalsis of its body.¹⁻³ We and others have previously shown that these patients show an intrinsic denervation of the oesophagus, with absence of ganglion cells at the Auerbach's plexuses of the distal oesophagus.^{4,5} It has been usually assumed that this pathogenetic process is located in the oesophagus.⁶⁻⁹ There are few reports, however, suggesting that this denervation could affect organs other than the oesophagus.^{10,11}

The aims of the present prospective study were: (1) to determine the extent of denervation of the gastrointestinal tract by biopsies of the muscle layer from the oesophagus, stomach, jejunum, and colon obtained during surgery; (2) to analyse the gastric secretion in these cases and

correlate acid output with the presence of parietal cells and histological findings at the gastric Auerbach's plexuses, and (3) to measure the gastric emptying of solids and to correlate these results with the histological findings at the gastric Auerbach's plexuses.

Methods

PATIENTS STUDIED

In this study 34 consecutive patients (19 women, 15 men) with achalasia of the oesophagus were submitted to surgery. Mean age 42 years (range 20 to 65). In all cases, Chagas' serological examination was carried out and was negative in all patients.

DIAGNOSIS OF ACHALASIA

All patients had dysphagia with a mean duration of 3.8 years (range one to 19). Radiological examination revealed the typical findings of achalasia, and upper endoscopy excluded any other oesophageal or gastric lesion. In 33 patients it was possible to undertake oesophageal manometry.^{13,4} The mean lower oesophageal sphincter pressure in this group was 40.3 (3.5) mm Hg, while in our controls it was 20.1 (2.8) mm Hg.

PARIETAL CELL BIOPSIES

During endoscopy three biopsies of the upper third of the gastric mucosa (lesser curve, anterior face and posterior face) were taken, immediately fixed in 10% formalin, stained with haematoxylin and eosin (H&E) and blindly analysed by the same pathologist. The biopsies were classified as: (a) normal biopsies: normal appearance of the mucosa and numerous parietal cells seen at histology; (b) altered biopsies, with decrease of the number of parietal cells and chronic atrophy of the mucosa. The pathologist estimated the density of parietal cells by the count of these cells per section and calculated the percentage of decrease of parietal cells compared with normal findings.

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OPERATIVE TECHNIQUE AND BIOPSY PROCEDURE
 Surgery was by the abdominal route.³ An oesophagomyotomy 5 or 6 cm long was performed and a 3 mm wide and 6 to 8 mm long segment of oesophageal muscle taken at the anterior right border of this incision, always at the same level, which corresponded to the narrowed distal segment where the gastrooesophageal sphincter is located. Gastric biopsies were taken at the middle third of the anterior surface of the gastric wall. In 14 cases these were taken at the upper, middle, and lower third of the stomach. The biopsies at the upper third were taken 4 cm distal to the oesophagogastric junction; at the middle third in a midpoint from the lesser curvature to the greater curvature; at the lower third 4 cm proximal to the pylorus. The biopsies at the jejunum were taken at the midpoint between the Treitz ligament and ileocaecal valve. Those from the colon were obtained at the midpoint of the transverse colon, from one of the longitudinal tenias.

All biopsies were immediately fixed in formalin and sent for histological examination. The specimens were embedded in paraffin wax and sections 5 μ thick were cut at intervals of 50 μ and stained with haematoxylin and eosin, Van Gieson, blue methelene and argentic staining according to Bodian's technique. Eight to 10 sections were taken in each case. In controls the area of each section was measured in terms of the number of ganglion cells found per linear centimetre. It was not possible to calculate the number of ganglion cells in the achalasic patients because of the size of the biopsy for obvious reasons was much smaller than in the control group. The number of neurones in each ganglion was measured, determining the mean ganglion neurone cell count for each organ. This procedure was similar to that used by Misiewicz *et al*¹² The presence of other alterations such as inflammatory or eosinophilic infiltration at the plexuses were also determined.

As a control normal group, patients without gastrointestinal disease were examined at necropsy, and biopsies taken at the same sites as achalasic patients to determine the number of ganglion cells and the mean ganglion neurone cell count in each organ. In these cases the size of each biopsy was 20 \times 10 mm.

GASTRIC ACID SECRETION

Three months after surgery, gastric acid secretion test was carried out in 31 patients as previously described.¹³ Basal acid output and peak acid output was measured in each patient, and values were expressed in mmol/h. In five cases these measurements were repeated with an interval of one to two weeks. As controls, 42 adults (25 men and 17 women) mean age 44 years (range 18 to 64) were studied. All had no gastrointestinal symptoms and upper endoscopy was normal.

GASTRIC EMPTYING OF SOLIDS

Three months after surgery it was possible to measure gastric emptying of solids in 28 patients by the technique described previously.¹⁴ This

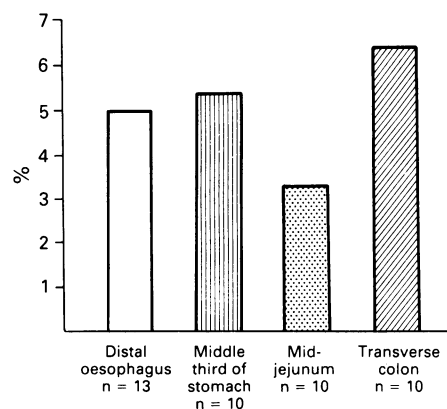


Figure 1: Mean ganglion neurone count in control subjects in four gastrointestinal organs.

study was carried out after labelling an egg with ⁹⁹Tc-sulphur colloid, introducing 1mCi (37 MBq) into the egg. A sandwich with mayonnaise was prepared which the patient ate in approximately five minutes together with 200 ml milk. This meal had weight of 300 g and contained 360 cal (46% carbohydrate, 30% fat and 24% protein). The stability was checked twice and showed 98% of activity remaining attached to the albumen at three and four hours post HCl incubation (pH 1.0 at 37°C). Imaging was performed with the patient lying supine under the gamma camera detector for two hours. Continuous imaging was done one frame/minute in all patients. The matrix was 64 \times 64 ward made. A region of interest was drawn manually around the stomach in the first and last images, avoiding intestinal loops.¹⁴⁻¹⁶ Twelve asymptomatic adult subjects served as controls.

Results

The histological findings at the Auerbach's plexuses of the gastrointestinal tract in control subjects are shown in Figure 1. At the distal oesophagus there were 2.8 ganglions per lineal centimetre, having five neurones per ganglion. At the middle third of the stomach there were 3.4 ganglions per lineal centimetre with 5.4 neurones per ganglion. At the middle jejunum there were 6.4 ganglions per lineal centimetre

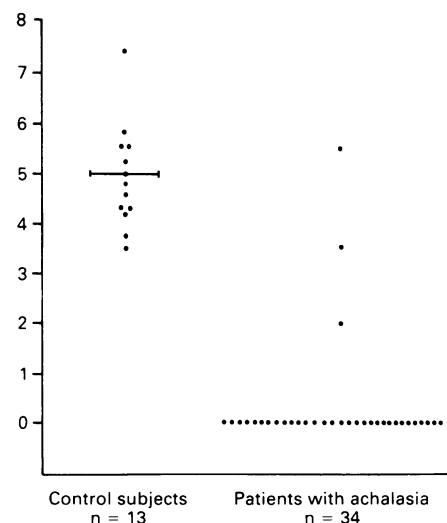
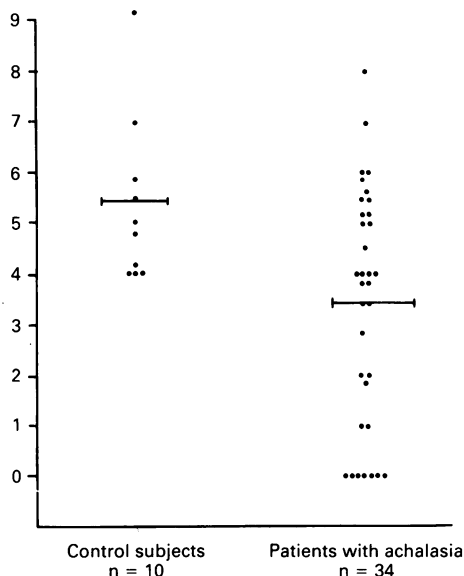


Figure 2: Mean ganglion neurone count at the distal oesophagus in controls and patients with achalasia.

Figure 3: Mean ganglion neurone count at the middle third of the stomach in controls and patients with achalasia.



with 3.3 neurones per ganglion. At the transverse colon there were 7.9 ganglions per lineal centimetre with 6.4 neurones per ganglion. Therefore, the ganglion concentration (quantity of ganglions per lineal centimetre) was increasingly progressive from the oesophagus (2.8) up to the transverse colon (7.9). The greatest neuronal density was observed at the midcolon and the lowest density at the middle jejunum. The distal oesophagus and stomach had similar neuronal density. In none of these controls inflammatory infiltration of the plexuses was observed. All 136 biopsies of achalasic patients included myenteric plexuses. In 31 of 34 patients (91.2%) oesophageal biopsy showed absence of neurones (Fig 2). In two cases the neuronal count was lower than normal and in one case (2.9%) the count was normal. Only one case with absence of neurones had minor eosinophilic infiltration of the Auerbach's plexuses. At the middle third of the stomach (Fig 3), in seven cases (20.5%) an absence of neurones was noted while in 10 (29.4%) the neuronal count was lower than in controls. In two cases lymphocyte infiltration of the plexuses was noted: in one the neural density was normal, while in the other, it was reduced. In the other 17 cases (50%) the neurone count was normal. In 14 patients (Table I) three gastric biopsies were taken: at the upper, middle and

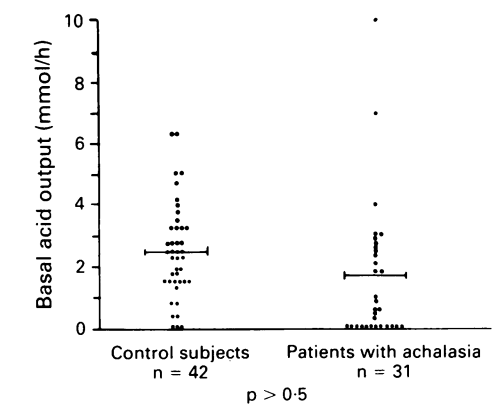


Figure 4: Basal gastric acid secretion in controls and patients with achalasia.

lower third. There was an increase in the mean ganglion neurone count from the upper third towards the lower third of the stomach.

All biopsies of the middle jejunum and transverse colon had similar neuronal density to control subjects and no inflammation was noted.

The results of gastric acid secretion in 31 patients and 42 controls are shown in Figures 4 and 5. Basal acid output (Fig 4) of control subjects was 2.49 (1.5) mmol/h, while in achalasic patients it was 1.55 (0.8) mmol/h ($p > 0.5$). Peak acid output in controls (Fig 5) was 22.5 (5.8) mmol/h, while in achalasic cases it was 13.0 (3.4) mmol/h, which was statistically lower ($p < 0.001$). There were five patients with basal acid output and peak acid output with no acid secretion. In all these patients the test was repeated at one or two week intervals and the results were exactly the same. The relationship between peak acid output and histological findings of the parietal cells at the oxyntic area is shown in Table II. In five patients no acid secretion was observed after histamine injection. Three had almost complete absence of parietal cells while two patients had normal counts. In the other two patients with peak acid output of 3.4 and 3.5 mmol/h, the parietal cell count was greatly diminished. In the remaining patients, parietal cell count was normal, independent of the peak acid output value. The relationship between peak acid output and histological findings at the Auerbach's plexuses of the middle third of the gastric wall is shown in Figure 6. There was no correlation ($r = 0.33$) between the

TABLE I

Patient	Mean ganglion neuronal count					
	Oesophagus	Stomach			PAO mmol/h	1/2 gastric emptying min
		Upper third	Middle third	Lower third		
1	0	0	0	2.5	0	112
10	0	2.3	1.7	2.7	22.6	118
11	0	1.3	2.0	1.0	3.5	301
14	0	1.0	3.4	4.0	23.2	133
19	0	4.0	4.5	4.0	11.2	108
20	5.5	4.5	5.0	5.0	-	-
21	3.5	4.7	5.1	6.0	9.9	145
22	0	4.5	5.3	6.5	18.2	-
23	0	4.5	5.3	5.5	6.3	95
24	0	3.0	5.4	7.3	4.8	220
25	0	6.0	5.5	6.0	12.7	-
26	0	7.2	5.8	4.7	0.8	111
31	0	6.0	6.0	5.8	-	-
32	0	2.5	3.7	5.0	7.9	207

\bar{x} : 3.7 \bar{x} : 4.2 \bar{x} : 4.8

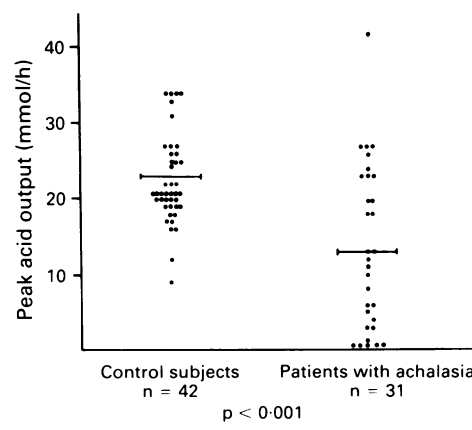


Figure 5: Peak acid output in controls in patients with achalasia.

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TABLE II Relationship between number of parietal cells and peak acid output (PAO)

Patient	PAO mmol/h	Parietal cells (%)
1	0	0
2	0	10
3	0	10
4	0	80
5	0	100
6	2.8	100
7	3.4	30
8	3.5	20
9	4.8	100
10 to 31	+5.1	100

mean ganglion neurone count at the gastric plexuses and the rate of the peak acid output.

The mean values of gastric emptying for solids in controls and in 28 patients with achalasia are shown in Table III. There were no significant differences comparing half time values or the percentage of retention at 60, 90, and 120 minutes in both groups ($p > 0.5$).

The relationship between gastric emptying of solids and histological findings of the Auerbach's plexuses at the middle third of the stomach is shown in Figure 7. There was no correlation ($r = 0.10$) between the mean ganglion neurone count and values of gastric emptying. The individual values of peak acid output and $t_{1/2}$ of gastric emptying in the 14 patients with biopsies at the distal oesophagus and at the upper, middle, and lower third of the stomach are shown in Table I. There was no correlation between the mean ganglion neurone count at the distal third of the stomach (antrum) and the half-time of gastric emptying of solids.

Discussion

The results of the present study suggest that classical histological findings showing denervation of ganglion cells in patients with achalasia is not limited to the oesophagus, but can extend beyond the gullet and involve the stomach in approximately half the patients. This denervation is not, however, seen at the distal gastrointestinal tract, having normal neuronal density at the jejunum and colon.

The aetiology of achalasia is unknown, although the pathological findings at the vagus nerve and oesophagus have been extensively studied.¹⁷⁻¹⁹ In the unique previous study in man determining the count of ganglion cells at the

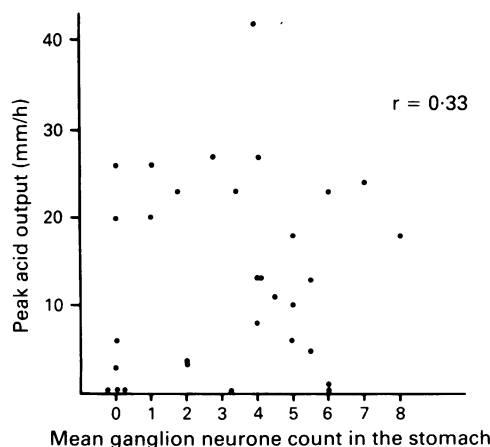


Figure 6: Correlation between peak acid output and mean ganglion neurone count at the middle third of the stomach in 31 patients with achalasia.

TABLE III Gastric emptying of solids in controls and in patients with achalasia of the oesophagus

Gastric emptying	Controls n=12	Achalasia n=28
t 1/2 (min)	158 (48)	133 (45)
Retention %		
60	84.7 (1)	84 (6.4)
90	72 (11.2)	65 (12.8)
120	61 (16.2)	52.7 (11.5)

distal oesophagus in controls and patients with achalasia,¹² Misiewicz *et al* found that in all but two of 14 patients, the mean count at the oesophagus was 0.3 or less and in nine it was zero, very similar to our results. In controls it was 1.9. Kumar and Phillips¹⁷ evaluated human myenteric plexuses in three neonates and six adults who had no achalasia. They studied mainly the interganglionic fascicles, but no mention was made about the count of neurones per ganglion. In two recent studies in the opossum,^{18,19} the authors measured the density of perikaria and ganglia, and found that it declined along the oesophagus. They also showed major differences in the anatomy of the plexuses in the different regions of the gut, which appear to be different from those in man.

There are various reports concerning the incidence of extraoesophageal vagal dysfunction in some patients with achalasia, based on an altered gastric secretory response to insulin stimulation.²⁰⁻²² A recent study, however, failed to show any change in gastric secretion in response to insulin in achalasic patients.²³ Dooley *et al*¹⁰ analysed 13 patients with achalasia determining the pancreatic polypeptide release after a modified sham feeding test, and found that six patients had normal response to modified sham feeding and seven cases exhibited no response, with no acid secretion and no release of pancreatic polypeptide, suggesting denervation of the stomach and pancreas without specifying whether this denervation is the result of altered vagal function or an alteration of the myenteric plexuses. Our findings support their hypothesis. In 13 of our patients with achalasia (42%), we found a very low acid output, below 10 mmol/h while in the remaining patients acid secretion

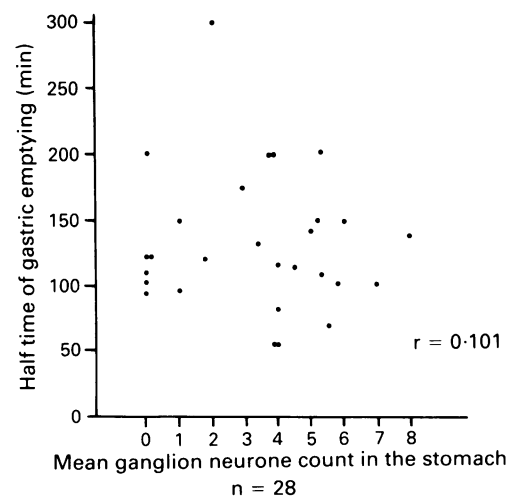


Figure 7: Correlation between half time of gastric emptying and mean ganglion neurone count at the middle third of the stomach in 28 patients with achalasia.

was normal. As gastric acid secretion is dependent on the mass of parietal cells, histological studies were carried out on the mucosa in these same patients, because it has been suggested that longstanding intrinsic denervation in patients with Chagas disease can lead to mucosal atrophy.²⁴ We could not find any significant alteration in the presence of parietal cells, however, and only five patients (14.7%) showed a chronic atrophy of the mucosa with a great decrease of the parietal cells count. In the remaining patients, parietal cells were present, even in two patients with complete absence of gastric acid secretion in response to histamine. Therefore, mucosal atrophy cannot be postulated as being responsible for gastric hyposecretion in these patients. We then examined the relationship between the presence or absence of neurones at the gastric Auerbach's plexuses, for evidence of intrinsic denervation and acid output, but we could not find any correlation between the decrease of acid secretion and changes of the neuronal density at the gastric plexuses.

A recent study of Eckard *et al*²⁵ evaluating the sham feeding response in controls and achalasia patients showed that all patients showed increase in acid secretion after sham feeding suggesting that vagal pathways controlling acid secretion appears to be intact in these patients. This finding confirms our results that the histological changes in achalasia patients are confined mainly to Auerbach's plexuses rather than vagal trunks.

Gastric emptying of solids was another parameter evaluated in patients with achalasia, which could reflect some damage of vagal innervation. We have found only one previous report of a single case with achalasia in which gastric emptying of solids was measured,²⁶ showing a delayed gastric emptying. In the study of Eckard *et al*,²⁵ measuring gastric emptying of liquids, they found a shorter emptying time in patients with achalasia and a tendency to a more rapid gastrointestinal transit time.

In our study gastric emptying of solids was evaluated showing that achalasia patients and controls have a similar rate of emptying. Considering that the gastric emptying of solids is mainly a function of the antrum, we took biopsies in 14 patients at the lower third of the stomach. We could not show any correlation between the mean ganglion neurone count of the antrum and gastric emptying of solids.

The histological analysis of the Auerbach's plexuses at the jejunum and colon were normal, with no evidence of denervation at these sites. In the study of Eckard *et al*,²⁵ they measured intestinal transit time, finding a more rapid transit in achalasia patients compared with controls, without statistical significance. Therefore, this report and our findings suggest that the mean sites of denervation in achalasia patients are the proximal segments of the digestive

system (oesophagus and stomach), as in Hirschprung's disease, where the denervation is located exclusively at the distal segment of the gastrointestinal tract. The careful evaluation of these two diseases remains a challenge for future studies.

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