Gastrointestinal Motility in Health and Disease

H. L. Duthie

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Gastrointestinal Motility in Health and Disease

Sixth International Symposium on Gastrointestinal Motility held in Edinburgh, Scotland, September 12th–16th, 1977

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Gastrointestinal Motility in Health and Disease

Proceedings of the 6th International Symposium on Gastrointestinal Motility, held at the Royal College of Surgeons of Edinburgh, 12–16th September, 1977

Edited by

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Contents

SECTION X GASTRIC EMPTYING AND ANTRODUODENAL CONTROL

SECTION XI COORDINATION IN THE OESOPHAGUS

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Section I
Interdigestive Migrating Complexes

1

Studies on the interdigestive (migrating) motor complex in man

G. VANTRAPPEN, J. JANSSENS, J. HELLEMANS, N. CHRISTOFIDES AND S. BLOOM

The interdigestive or migrating motor complex (MMC) occurs in most animal species that have been studied thus far. Little is known about the occurrence of an MMC-like phenomenon in man; and its characteristics, function, control and disorders in man have not been studied. It has been suggested that motilin plays an important part in the control of interdigestive motor activity in dogs[1]. The data on the mechanism of release and on the effect of meals on the serum motilin levels in man do not agree with observations in dogs[2,3]. The purposes of our investigations, therefore, were: (1) to study the MMC in normal subjects and patients with various gastrointestinal disorders; (2) to test the hypothesis that the absence of activity front leads to bacterial overgrowth in the small intestine; (3) to study the role of motilin in the control of human interdigestive motor activity.

The upper intestinal motility was studied manometrically by means of three perfused catheters with side openings spaced at 25 cm intervals. The proximal catheter was positioned under fluoroscopic control at about 15 cm below the pylorus; the middle one at about 10 cm below the angle of Treitz; and the distal one still 25 cm more caudally. Pressures were recorded in eighteen normal volunteers either after a continental breakfast of 450 kcal (mean duration of registration 10 h), or after an overnight fast of 12–14 h (mean duration of registration 7 h). The same type of motility study was performed in seventy-eight patients with various pathological conditions.

The analysis of the pressure records was carried out as follows: first the number of pressure peaks per minute was determined throughout the recording periods (850 h); the second step of the analysis consisted in identifying the activity front on the records; finally, several parameters of the activity front

(such as duration, rhythm, propagation velocity and duration of postprandial interruption) were determined. The identification of the activity front was based on the presence of the three following criteria: (1) appearance of a non-interrupted burst of pressure waves, produced at the basic electrical rhythm (the rhythm of the slow waves); (2) aboral progression of the activity front over all three or, at least, over the two distal recording orifices; (3) a period of complete quiescence following the activity front.

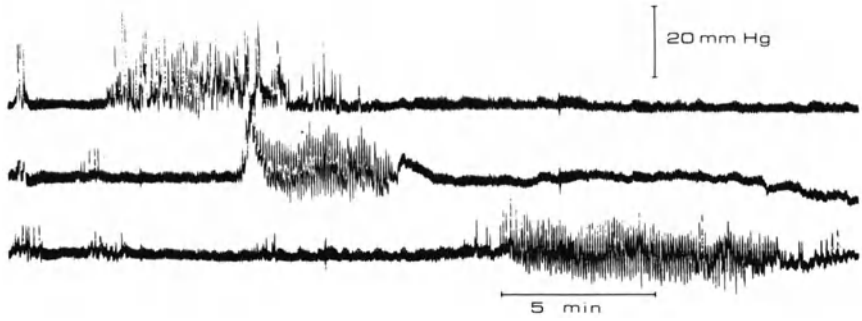

Figure 1.1

Figure 1.1 is an example of an activity front. The proximal catheter was located in the duodenum, catheters 2 and 3 were situated 25 and 50 cm more distally in the jejunum. The sequential nature of the rhythmic burst of pressure waves and the period of quiescence following that burst are obvious.

Our studies indicate that all normal subjects we examined presented regularly recurring MMC. The various phases of the complex could easily be identified in all tracings. Quantitative analysis of various parameters of the activity front (Table 1.1) indicate that it lasts for about 5 min. Its propagation velocity is slightly faster in the duodenum than in the upper jejunum. The calculated length of the front is 34 cm. The mean duration of a complete cycle is between 85 and 142 min, and the interval between a breakfast of 450 kcal and the appearance of the first complex is 214 min. These data all indicate that the interdigestive motility of man closely resembles that of dogs.

Table 1.1

	Duodenum	*Jejunum I*	*Jejunum II*
Duration (min)	5.11 ± 0.61	5.48 ± 0.40	5.90 ± 0.37
Contractions/min	11.61 ± 0.13	11.48 ± 0.14	11.30 ± 0.14
Progression velocity (cm/min)	7.65 ± 1.06	5.91 ± 0.79	
Calculated length (cm)	34.17 ± 4.35		
Duration of cycle (min)	112.50 ± 11.41 (mean ± SEM)		

In three normal subjects and five patients the effect of this motor complex on small intestinal propulsion was studied by simultaneous radiological and manometric observations. Contrast material was injected during the various phases of the complex through a supplementary catheter which ended 10 cm distal to the proximal recording orifice. These radiomanometric observations indicate that the burst of rhythmic pressure waves that constitutes phase 3 of the MMC corresponds to a series of propulsive contraction waves that follow each other in a rhythmic sequence. They clear the involved intestinal segment of the injected barium, suggesting that in man as well as in dogs they function as a 'housekeeper'[4].

If this is true it seems natural to speculate that absence of this clearing mechanism may result in accumulation of food remnants, desquamated cells and secretions, thus creating a medium favourable to bacterial overgrowth in the small intestine. Therefore, we studied a number of patients with bacterial overgrowth in the small intestine. A patient was considered to have bacterial overgrowth when the bile acid breath test was positive before, and became negative after, treatment with tetracycline for 5 days. The breath test was performed according to the method of Fromm *et al.*[5] Five μCi of [^{14}C]glycocholic acid were administered by mouth, and breath samples were taken before and 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after the administration of the radioactive label. A breath test was considered to be positive when two consecutive values were greater than the mean + 2 standard deviations.

Twelve patients with evidence of bacterial overgrowth in the small intestine and, as a control group, six patients with breath- and Schilling tests suggestive of ileal dysfunction were studied. Another control group consisted of nine patients with various gastrointestinal diseases but with a negative breath test. Of the twelve patients with bacterial overgrowth seven had a normal interdigestive motility pattern and five had no, or grossly disordered, MMC. The patients of the two control groups all had normal MMC. It thus seems that only five of the forty-five patients we originally studied had grossly abnormal interdigestive motility, and that all five had evidence of bacterial overgrowth. During the last few months the studies have been extended. We have studied thus far ninety-six human subjects, normal volunteers and patients with various pathological conditions. Only six patients were observed in whom the MMC was absent. All six had evidence of bacterial overgrowth in the small intestine.

The observation that absence or gross disorders of the MMC in chronically ill patients are always associated with evidence of bacterial overgrowth in the small intestine makes it very improbable that the association is fortuitous. The possibility cannot be excluded that the bacterial overgrowth by itself interferes with the small intestinal motor function and results in a disappearance of the activity front. However, seven of the twelve patients with evidence of bacterial overgrowth had a normal number of complexes, indicating that bacterial overgrowth by itself does not necessarily eliminate the activity front.

As this activity front is such a highly efficient mechanism of intestinal propulsion and is normally recurring so constantly throughout the interdigestive period, it seems logical to assume that the disorders of the MMC are the cause rather than the result of bacterial colonization. Further studies are needed to give direct proof of this conclusion.

Finally we studied, in collaboration with S. Bloom and N. Christofides, the role of motilin in the control of the MMC. Simultaneous pressure measurements and motilin assays at 15 min intervals were performed in fourteen normal subjects.

The fasting motilin concentrations varied considerably from one subject to another, as well as from one moment to another in the same subject. Eighteen activity fronts were studied before the subjects took breakfast. Sixteen of the eighteen 'fasting' activity fronts were associated with a motilin peak. The increment in serum motilin concentration amounted to about 25 pmol/l. The mean interval between the motilin peak and the onset of the activity front in the jejunum was 14 min 44 s.

An early postprandial motilin peak was observed in all nine subjects studied. It must be noted that the breakfast was always given immediately after an activity front had passed the distal recording orifice. Although this early postprandial motilin peak was as high as the fasting peak, it was never associated with an activity front. During the later postprandial period the serum motilin level returned to the postprandial level. The first postprandial activity front developed after a mean interval of 238 min. In five of the six subjects this first activity front was associated with a motilin peak; but this peak was rather small, amounting to 12 pmol/l only.

The fasting serum motilin levels in our patients with bacterial overgrowth and absence of MMC were within normal limits. As only one sample was taken we do not yet know whether or not motilin peaks occur in these patients.

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Discussion

D. A. W. Edwards (UK): Are you justified in saying that myoelectrical or mechanical activity is absent in some of the patients with bacterial overgrowth, because the method of recording by open tubes is not suitable for detecting mechanical activity of the wall or its electrical activity when the lumen is dilated and a column of fluid is present? The method is suitable only for recording from an empty lumen.

G. Vantrappen (Belgium): The lumen was dilated only in one patient who had systemic sclerosis. In the others radiological examination did not show an abnormal amount of fluid in the jejunum.

J. T. Farrar (USA): I would like to restate Dr Edwards' question as to whether the pressure recording is an adequate method of studying motor activity in dilated, distended loops. It may be that motor activity is present but is not recorded because of the capacious loops. You could prove this suggestion incorrect by telling us that other motor activity is normal.

Vantrappen: Yes, indeed. An apparently normal phase 2 pattern was recorded.

Z. Itoh (Japan): I would like to show you our result obtained from humans. The first slide shows comparison of period of contraction and quiescence between man and dog. The second slide shows the close relationship between the interdigestive gastric motor activity and plasma motilin increase.

Vantrappen: These results confirm our observations.

E. E. Daniel (Canada): Why should the peak in motilin level which you recorded occur 15 min prior to onset of the MMC in the upper intestine? After all, the MMC progresses throughout the small intestine and thus it will be present somewhere, whether motilin is elevated or not.

Vantrappen: The observation that the development of an activity front in the duodenum is preceded by a motilin peak suggests that the serum motilin peak may activate the mechanism that initiates the front but has nothing to do with the mechanism of propagation of the front. If the duodenum is more sensitive to motilin than lower parts of the intestine, the front will originate in the duodenum. Further studies are needed to prove this hypothesis. We are currently carrying out motilin infusion studies in human volunteers.

M. D. Schuffler (USA): If the absence of activity fronts causes bacterial overgrowth, then one would expect that they would still be absent following a course of antibiotics. If, on the other hand, bacteria were the cause, a course of antibiotics should be followed by reappearance of fronts. Did you re-test your five patients who had bacterial overgrowth and absent activity fronts after they were given a course of antibiotics?

Vantrappen: It is unlikely that the bacterial overgrowth causes the motility disorder, because seven of our twelve patients had bacterial overgrowth with a normal motility pattern. Only one patient was examined repeatedly after treatment with antibiotics, and the motility disorder was always present.

C. E. Code (USA): What was the nature of the motility recorded in the bowel of those patients with absent M complexes?

Vantrappen: A pattern resembling phase 2 activity.

J. H. Szurszewski (USA): Did you measure the levels of motilin in patients in whom there were no MMC?

Vantrappen: Yes, we did and they were in the normal range. What is being done right now is performing serial motilin assays to see whether or not motilin peaks are present in the fasting state in these patients.

M. Wienbeck (W. Germany): Why did just five of the twelve patients with bacterial overgrowth have disturbed motor activity?

Vantrappen: Seven of the twelve patients had other conditions which are known to induce bacterial overgrowth, such as fistulas. We suggest, but cannot yet prove, that the motility disorder may be the cause in the other five patients.

W. Silber (S. Africa): What were the clinical syndromes involved? I am interested particularly in the postgastrectomy afferent loop syndromes.

Vantrappen: Total gastrectomy, pseudo-obstruction, systemic sclerosis, Crohn's disease (without demonstrable stenosis) and 'functional, psychosomatic' intestinal disorder.

H. L. Duthie (UK): Are the changes in concentration of motilin associated with MMC in view of the large variation in motilin levels in different individuals?

Vantrappen: In each individual there was an association between an activity front and a motilin peak – at least for sixteen out of the eighteen activity fronts. Additional peaks, however, were observed, which were not associated with an activity front in the upper small intestine.

2
Migrating myoelectrical complexes in man

C. J. STODDARD, R. H. SMALLWOOD AND H. L. DUTHIE

Interdigestive migrating myoelectrical complexes, caudad moving bands of action potentials, were first described in the canine small intestine by Szurszewski in 1969[1]. The presence of similar complexes has since been demonstrated in the small intestine of a variety of animals[2,3,4]. These complexes are initiated in the stomach and then propagated along the small intestine. In fasting human duodenal motility studies each period of motor activity terminates in a burst of regular waves with a frequency identical to the duodenal slow wave frequency of 11–12 cpm[5,6], and these bursts of contractions have been shown to pass distally along the bowel[7]. However, studies of migrating myoelectrical complexes in the human small intestine have not been reported.

The aim of this project was to see if myoelectrical complexes could be recorded from the human duodenum.

METHODS

In ten consenting patients undergoing elective cholecystectomy, electrodes were attached to the serosal surface of the duodenum and the connecting wires exteriorized via a right hypochondrial stab incision, along with a corrugated rubber drain. The electrodes were constructed from 0.25 mm diameter stainless steel wire, 4 mm in length and implanted in the muscular wall of the duodenum in the transverse axis of the bowel, being held in position by a single plain 000 catgut suture. Initially only two electrodes were inserted in each patient, and from these monopolar recordings were made, the indifferent electrode being a metal disc coated with electrode jelly which was applied to the scarified skin over the right iliac fossa. In later experiments two pairs of electrodes were used, so that both bipolar and monopolar recordings could be obtained. The proximal pair of electrodes was inserted 1 cm beyond the pylorus and the distal pair at the junction of the second and third parts of the

duodenum (Figure 2.1). For each pair of electrodes the interelectrode distance was 1 cm, with the pairs of electrodes being 4–7 cm apart.

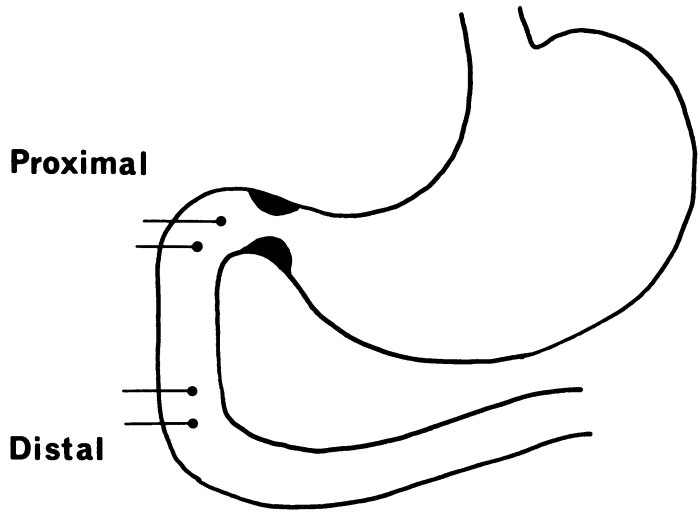

Figure 2.1 The sites of attachment of the proximal and distal pairs of electrodes. For each pair the interelectrode distance is 1 cm

Two myoelectrical recordings of 2–3 h duration were made from each patient between the second and fifth postoperative days. All recordings were made following a previous overnight fast of at least 12 h. Signals from the electrodes were fed into amplifiers with frequency responses within $\pm$3dB from 0.016 Hz to 300 Hz and displayed on a hot wire recorder (Hewlett–Packard). The amplifier outputs were also simultaneously recorded on magnetic tape for computer analysis. The drain and electrodes were removed uneventfully from all patients on the fifth postoperative day.

RESULTS

Recordings from two patients were unsatisfactory. Duodenal electrical signals could not be recorded, and the potentials detected indicated that these electrodes had become detached from the bowel.

Satisfactory recordings were obtained from the other eight patients. The mean duodenal slow wave frequency was 12.2 cpm (range 11.5–13.6). During each recording, the frequency of the slow waves varied by $\pm$0.4 cpm about the mean but at no time did the frequencies at the proximal and distal electrode sites differ by more than $\pm$0.2 cpm. The slow wave amplitude waxed and waned during each recording (Figure 2.2) with a range of 0.4–1.4 mV. The amplitudes of the waves were maximal when action potentials were superimposed on the basic electrical rhythm. In addition, the characteristic gastric

3 cpm electrical activity was recorded in the proximal duodenum in all patients, the amplitude of these signals usually being maximal during the period when random bursts of action potentials were seen on the duodenal slow waves.

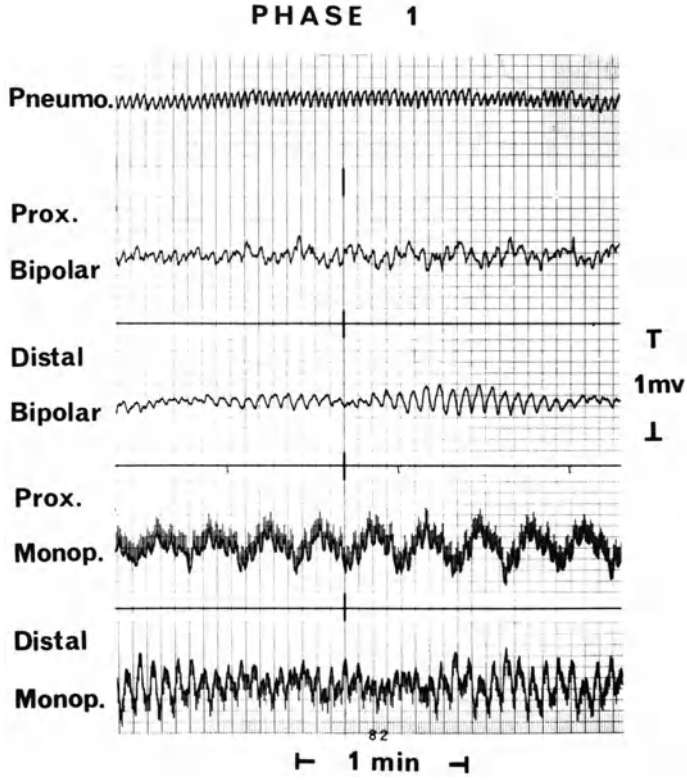

Figure 2.2 A record showing phase 1 activity. The top line of the tracing is the pneumogram and the other four are bipolar and monopolar electrical recordings from the proximal and distal pairs of electrodes. Electrocardiogram artefact is present on the monopolar recordings. Duodenal slow waves with a frequency of 13 cpm are present on all four electrical channels but are best seen on the bipolar recordings. No action potentials are present in this stretch of record. Gastric slow waves with a frequency of 3 cpm are superimposed on the duodenal waves recorded at the proximal electrode site

In seven of the eight patients bursts of action potentials superimposed on the duodenal slow waves were observed. As in the dog, three distinct phases of activity could be identified with respect to the action potentials. Phase 1 was characterized by slow waves without action potentials (Figure 2.2) and this was followed by phase 2 with randomly occurring bursts of action potentials (Figure 2.3), the bursts being of longer duration and the action potentials greater in number and larger in amplitude as time passed. Phase 2 was superseded by phase 3 when action potentials accompanied each slow wave (Figure

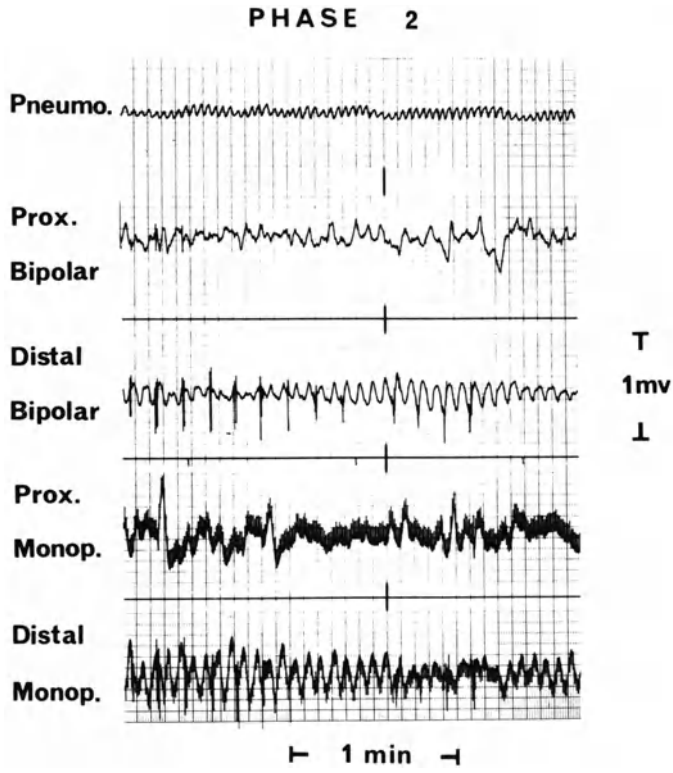

Figure 2.3 A record showing phase 2 activity. Action potentials are superimposed on some, but not all, of the duodenal slow waves

2.4). Phase 3 activity usually ended abruptly, being followed by a return of phase 1 activity. Szurszewski's original description of a migrating myoelectrical complex has been modified by other workers[8] to include a phase 4 period which is usually of short duration, and during which the action potential burst dies away. In this study of myoelectrical complexes in the human duodenum phase 4 activity was not seen, although on some occasions a few random action potentials were observed for up to 90 s after the termination of phase 3. The mean duration of each complete cycle (Table 2.1) was 41 min 56 s (±3 min 23 s). The mean duration of phase 2 was 5 min 40 s (±1 min 02 s) and the duration of phase 3, which showed the least variation in period,

Table 2.1 Duration of human duodenal myoelectrical complexes*

	Mean	*± 1 SEM*	*Range*
Total duration of one complex	41.56	3.23	22.00–64.35
Phase 2	5.40	1.02	2.30–20.10
Phase 3	4.44	0.20	2.20–8.40

* All times in minutes and seconds.

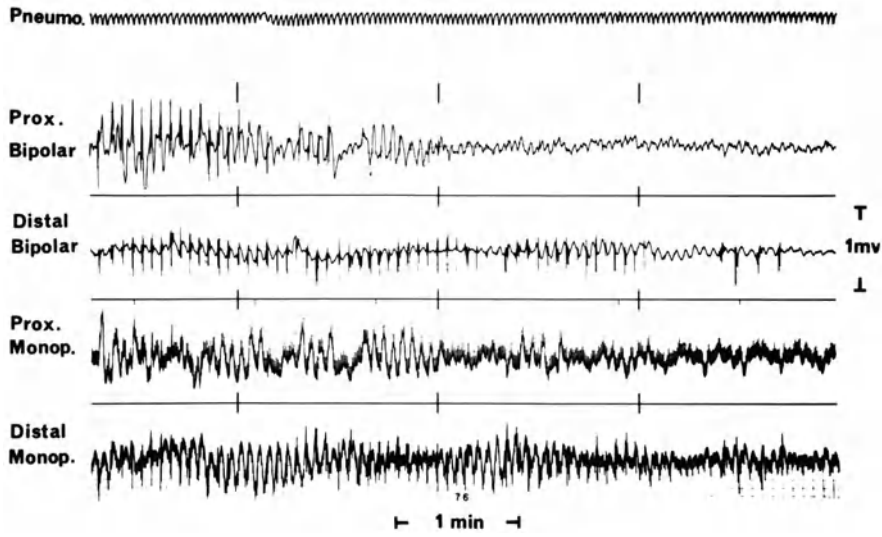

Figure 2.4 A record showing phase 3 activity. On the left-hand side of the record action potentials can be seen superimposed on every duodenal slow wave; they are seen most easily on the bipolar electrical recordings. The burst of action potentials terminates at the proximal recording site 170 s prior to the distal recording site

was 4 min 44 s ($\pm$20 s). For individual patients the duration of each complete cycle of activity was relatively constant, never varying by more than $\pm$21 %. There was no significant difference between the duration of the complexes recorded on the second and fifth postoperative days.

In five of the seven patients in whom myoelectrical complexes were observed, the action potential bursts migrated aborally. In these patients the spike activity terminated at the proximal electrodes prior to the distal electrodes by 70–190 s. The action potential bursts occurred simultaneously at the proximal and distal electrodes in the other two patients, but at no time was the spike activity observed first at the distal electrode sites.

Myoelectrical complexes were not observed in the remaining patient, but instead action potentials were regularly superimposed on the duodenal slow waves throughout each recording. The patient denied intake of either food or liquid prior to either study.

The passage of an electrical complex past an electrode caused measurable changes in duodenal slow wave frequency. On all but two occasions the frequency decreased, relative to the mean frequency, during phase 3 activity and then increased immediately after the complex had passed (Figure 2.5). The mean difference between the minimum and maximum frequencies during the passage of an electrical complex was 0.56 cpm (range 0.3–0.8 cpm). On two other occasions no change in slow wave frequency was observed.

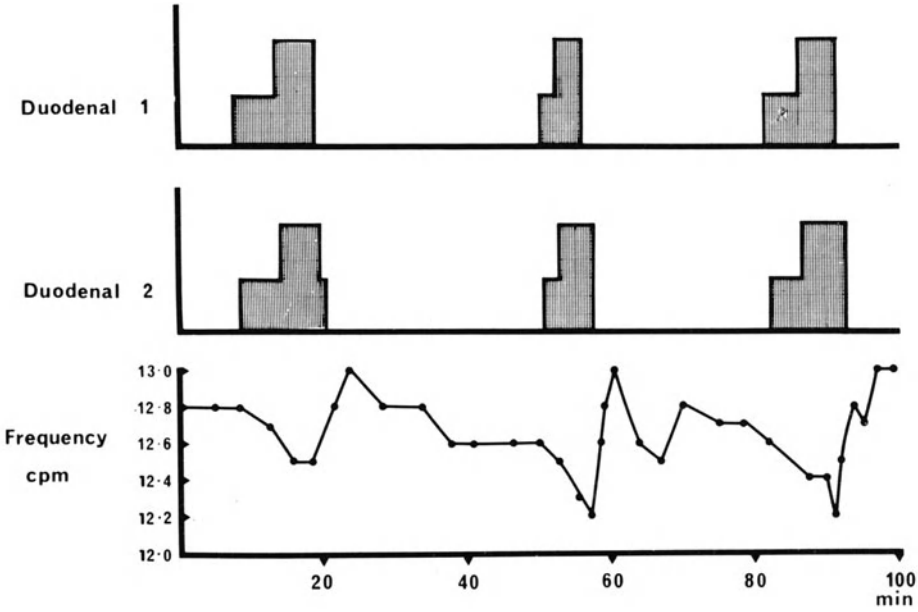

Figure 2.5 The change in duodenal slow wave frequency at the distal electrode site (Duodenal 2) associated with the passage of a myoelectrical complex. The top two lines are diagrammatic representations of a complex, phase 2 being represented by a single unit of shading and phase 3 by a double unit. The duodenal slow wave frequency falls during phase 3 and increases immediately after the complex has passed

After the end of the normal recording period, three patients were given 250 ml of milk to drink and a further 30 min recording made. In all cases duodenal action potentials were absent when the milk was administered. The ingestion of milk immediately interrupted the normal fasting pattern of duodenal electrical activity in all patients. Action potentials were observed 20–40 s after the commencement of drinking. They occurred simultaneously at both electrode sites and accompanied 30–80 % of the slow waves, there being a concomitant increase in slow wave amplitude. These changes persisted until the recording terminated.

DISCUSSION

These studies confirm that myoelectrical complexes exist in the human duodenum. They were recorded in seven of the eight patients from whom adequate electrical recordings were obtained. In five patients an aboral progression of the complexes was indicated by the commencement and termination of the action potential bursts at the proximal electrodes prior to the distal ones. The action potential bursts occurred simultaneously at both electrode sites on all occasions in the remaining two patients. This is probably because the interelectrode distance was too small for the caudad progression of the

spike bursts to be discerned. These bursts were identical to those recorded in the other patients, except for their temporal relationships. On no occasion were the spikes observed at the distal electrodes prior to the proximal pair.

The duration of the complete cycles of activity observed in this study is shorter than those recorded from the dog. These studies were conducted within five days of laparotomy, and although there was no significant increase in the duration of the cycles on the fifth postoperative day compared with the second day it may be that the duration of the cycles is affected by laparotomy. Recordings in the later postoperative period are unfortunately not possible using serosal electrodes, and further information is required from mucosal electrode studies. However, this technique has permitted the recording of duodenal myoelectrical activity, both in the fasting state and after milk, without patient discomfort and without any doubts as to the possible effects of intubation on resting electrical activity.

CONCLUSION

Migrating myoelectrical complexes exist in the human duodenum and this normal fasting pattern is interrupted by the ingestion of milk.

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Discussion

P. Bass: (USA) Was antral BER seen at the distal duodenal electrodes? Did the antral BER tend to disappear with time? Could the antral BER in the duodenum be related to the pathophysiology of the surgery?

C. J. Stoddard: (UK) Infrequently antral slow wave activity was detected at the distal electrode sites in some patients. It was seen at the proximal electrodes in all patients and here the amplitude of the signals was much greater.

The antral slow waves varied in amplitude during the recording period but did not tend to disappear as time passed. It is interesting that the maximal amplitude of the gastric slow waves was recorded during phase 2 activity of a myoelectrical complex in the duodenum. We think that this could be due to phase 3 activity in the antrum at this stage but do not have any electrical records to substantiate this theory.

We have previously recorded antral activity in the proximal duodenum using mucosal electrodes in patients who have not had surgery, and do not think that the antral waves here reported were related to the surgery. In addition, antral waves in the duodenum have been recorded in the absence of antral contractions and are not therefore due to mechanical artefact. We think that the pylorus does not act as an electrical insulator in man.

S. K. Sarna: (Canada) What do you think is the significance of the difference between phases 2 and 3? Would it really make a significant difference in net propulsion if reponse activity is absent over some cycles as in phase 2, as compared to phase 3, when it is present over all cycles?

Stoddard: During phase 2 the electrical excitability of the duodenal smooth muscle is not as great as phase 3, and the overall power of contraction will similarly be reduced. In terms of propulsive movements in the gut, phase 2 could be associated with incomplete contraction of the bowel wall and phase 3 with luminal occlusion and hence the major factor in forward movement of intestinal contents. However, I have no motility or cineradiographic evidence to support this hypothesis.

W. J. Dodds: (USA) In your records the spike activity was observed sometimes on the upstroke of the slow wave and sometimes on the downstroke, rather than at a constant site. Can you explain this observation?

Stoddard: I think the explanation for this depends on the recording technique. The macroelectrodes used detect the activity of many cells. The results obtained are therefore not analogous to intracellular recordings where the spike activity is observed at the time of maximum depolarization. The recording from many cells probably accounts for the variation in the position of the spikes seen in our records.

D. L. Wingate: (UK) There is a discrepancy between the cycle length in your studies (about 40 min) and those found by Dr Vantrappen (about 110 min). Is this a postoperative effect? If so, this is unlike the situation which we have

observed in dogs where cycle length is 'normal' postoperatively from the time when spike activity returns.

Stoddard: The durations of the complexes observed in the postoperative period in this study and also Professor Catchpole's were between 22 and 64 min. The variation in each patient between recordings made on the second and fifth days was only $\pm 15\%$ with no obvious trend for the duration to increase after 5 days. If the complex duration is shortened postoperatively then it certainly takes more than 5 days to return to the 'normal' reported in the previous paper. Unfortunately this recording technique does not allow recordings to be made after the fifth post-operative day.

K. Kelly: (USA) The pacesetter potentials (slow waves), as shown in your recordings, were more clearly detected by your distal electrodes than by your proximal electrodes. We have had a similar experience. Why is this so? Does this relate to the site of the duodenal pacemaker? Where is the duodenal pacemaker located in man?

Stoddard: The superimposition of gastric slow waves on top of the duodenal slow waves causes a marked variability in the 'base-line' and this makes the slow waves more difficult to observe at the proximal electrode recording site. Filtering out the 3 cpm activity at the proximal electrodes makes the duodenal activity just as easy to see here as at the distal site. I do not think that the differences are related to the site of the duodenal pacemaker. Our experimental model described does not allow us to locate the site of the duodenal pacemaker – this would require transverse section of the duodenum at different distances from the pylorus which is obviously inapplicable in this study.

J. Christensen: (USA) The slow wave frequencies reported, 12.2–12.8 cpm, are significantly greater than those reported previously in unoperated normal persons. Frequency varies with body temperature. Could this frequency that you report have been related to any degree of transient postoperative fever?

Stoddard: That is an interesting observation. It could easily explain the increased frequency of the waves but unfortunately I don't have the information regarding patients' temperatures at the time of each recording. The increased frequency observed in some patients would not affect the change in slow wave frequency associated with the passage of a myo-electrical complex.

E. E. Daniel: (Canada) Do you find phase-locking between control potentials from your two duodenal electrodes?

Stoddard: We have not as yet analysed the recordings for phase-locking.

E. Atanassova: (Bulgaria) Have you any impression for the healing of the duodenal muscle wall after removing the implanted electrodes?

Stoddard: The electrodes were removed with ease and without any complications in all patients. As the electrodes are only loosely attached to the serosal surface of the duodenum with plain catgut we expect that the damage to the wall is negligible.

R. E. Condon: (USA) Did you by chance make recordings during operation or in the recovery phase from anaesthesia? And, if so, did these records differ from those taken during 2–5 days postoperative?

Stoddard: Our recording apparatus is in a fixed room away from the operating theatre so we did not make recordings earlier and I cannot answer your question.

3

The interdigestive myoelectrical complex and other migrating electrical phenomena in the human small intestine

P. FLECKENSTEIN, F. KROGH AND A. ØIGAARD

A regularly occurring interdigestive myoelectrical complex has so far been demonstrated in the small intestine of a number of experimental animals[1–3]. The myoelectrical activity may be divided into three or four phases, the 'active phase', that is, the myoelectrical complex, consisting of regular spike activity with the frequency of slow waves. The complex propagates slowly in the distal direction and when it reaches the colon another complex appears in the pyloric region.

Other migrating phenomena such as the 'peristaltic rush' and 'minute-rhythm' have been described from non-physiological studies from various animals[3,4].

The purpose of the present work was to investigate these electrical phenomena in man. Such investigations require simultaneous recording of spike potentials from several electrodes along a considerable section, or preferably along the whole small intestine.

MATERIALS AND METHODS

Eight young healthy volunteers were examined. The probe used for the recordings consisted of a 220 cm long pvc tube, with an outer diameter of 5.6 mm (Figure 3.1). Bipolar electrodes were formed by winding the distal end of long copper wires around the circumference of the tube. The remaining parts of the wires were within the lumen of the tube. Inside the tube was also a thin polyethylene catheter, through which a latex balloon could be inflated at the tip of the tube in order to facilitate the transport through the small intestine. The probe was introduced transnasally. (The probe is commercially available from Ing. I. Tullin, A/S Sondeteknik, Hedeparken 1[XI], 2750 Ballerup, Denmark.) Each electrode pair was connected with an amplifier and

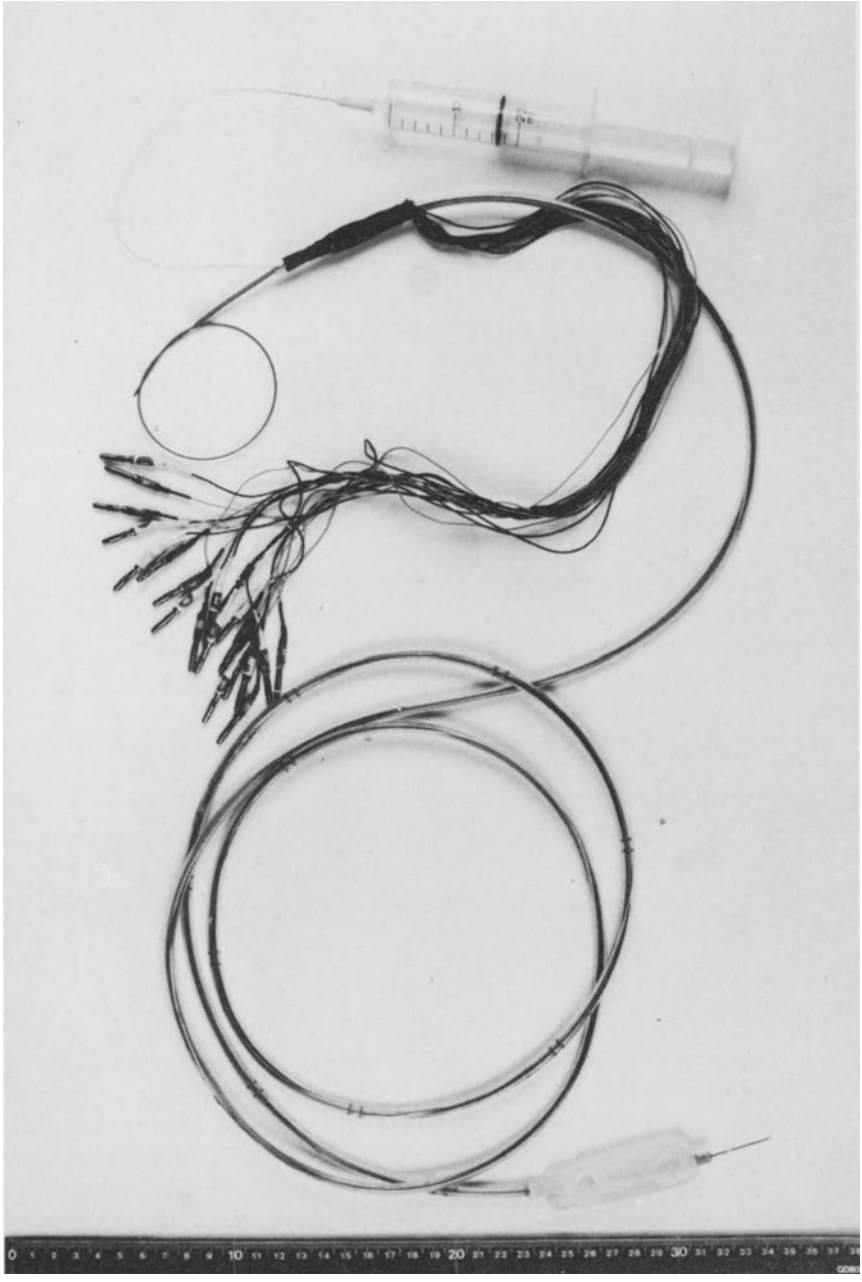

Figure 3.1 Probe for intraluminal registration of electrical potentials. Interelectrode distance 5 mm (bipolar electrodes) and distance between each pair of electrodes 10 cm. Inflatable balloon at the tip and inserted metallic guide wire

curve-writer. Upper frequency limit 700 Hz, time constant 0.015 s, paper speed 3 mm/s.

Recordings were carried out after a fasting period of 16 h for solids and 12 h for liquids. The total observation time was 96 h; range 2–28 h.

RESULTS

The following three different migrating phenomena have been observed:

1. Myoelectrical complex
2. Minute-rhythm
3. Peristaltic rush

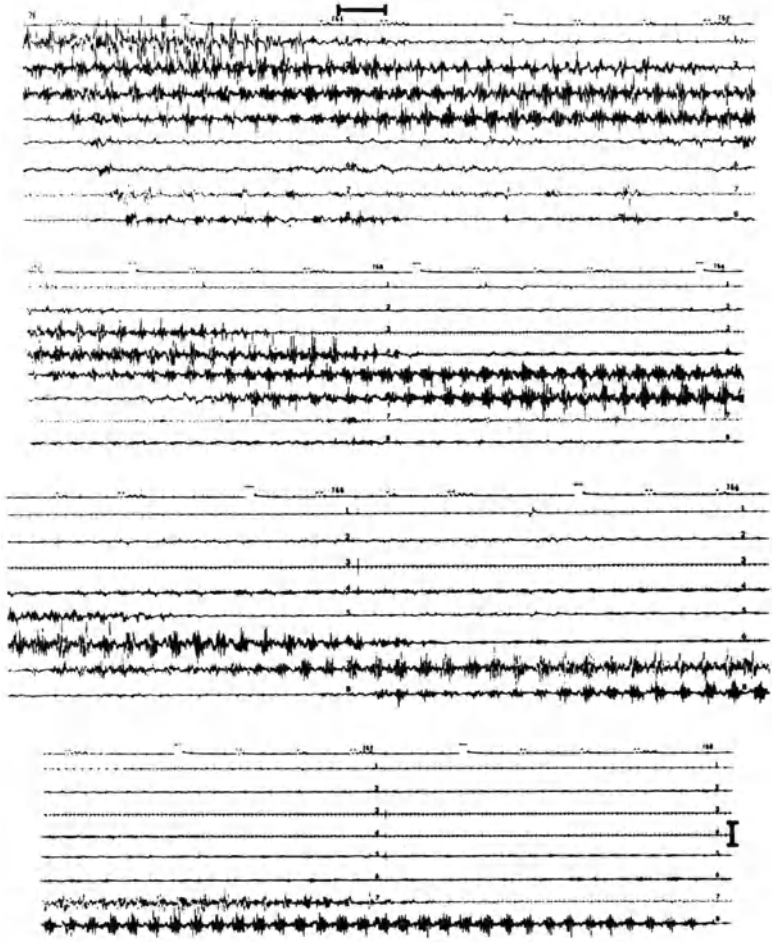

Figure 3.2 Consecutive recordings of a migrating myoelectrical complex from eight pairs of bipolar electrodes placed at intervals of 10 cm in the upper human small intestine. Note the similar appearance of spike potentials from the various leads. Time marking 10 s; vertical bar 0.3 mV; paper speed 3 mm/s; TC 0.015 s

1. Myoelectrical complex

In the fasting state all subjects examined showed a myoelectrical complex which did not significantly differ from that observed in animals (Figure 3.2).

Figure 3.3 gives a schematic representation of a myoelectrical complex from the most proximal 100 cm of the small intestine. Various characteristics may be deduced from the figure: duration of the complex at the individual electrode levels; velocity of propagation of the front of the complex (v_1 and v_2), the length of the activated segment (l_1 and l_2) etc. Based on thirty-two

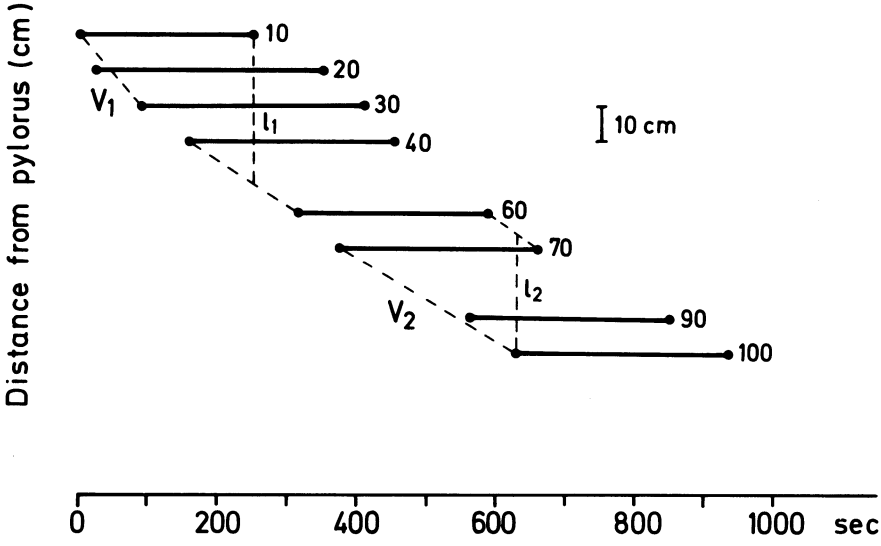

Figure 3.3 Schematic representation of a migrating myoelectrical complex from the human small intestine. Zero time represents time of onset of phase III in the most proximal tracing (10 cm from the pylorus). v_1 and v_2 indicate propagation velocity of the front of the complex. l_1 and l_2 represent sections of the intestine activated at one time. Note the difference in inclination between v_1 and v_2 and the difference between l_1 and l_2. Numbers at the end of horizontal lines indicate approximate distance from the pylorus

complexes the duration appears to increase in the distal direction, values ranging from 5–8 min. The velocity of the front of the activity appears to decrease from the duodenum (12 cm/min) to a point 80 cm distal to the pylorus (6 cm/min). The extent of the active bowel segment was 41 cm just below the pylorus and 29 cm just distal to the ligament of Treitz.

As shown in Figure 3.2, there is a change in amplitude and duration of the individual spike potentials during the active phase, with a relatively smaller amplitude and a shorter duration at the beginning and at the end of the phase.

2. Minute-rhythm

In the proximal jejunum a typical motility pattern was observed during approximately one-third of the recording period. It consisted of small bursts

of several spike potentials propagating distally over a section of the jejunum (approximately 100 cm; Figure 3.4). These potentials reappeared in a regular rhythm with a period duration of 80 s (SD 21 s).

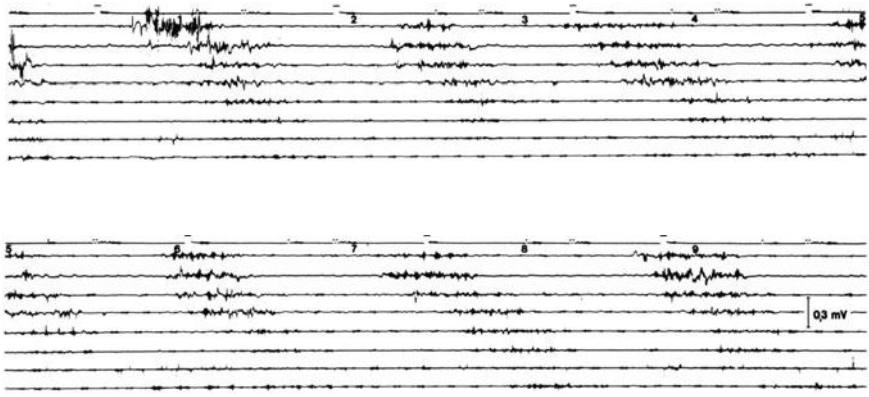

Figure 3.4 Recording showing bursts of migrating spike potentials reappearing at regular intervals ('minute-rhythm'). Recording during 10 min from the upper human small intestine. Same technique as in Figure 3.2

3. Peristaltic rush

In almost all subjects examined, single spike potentials with a great amplitude and a short duration were observed migrating rapidly through a short section of the upper small intestine (Figure 3.5). These potentials were observed during the irregular phase (see below). Duration of an individual rush was 5.0 s (SD $\pm$ 1.6 s) and their propagation velocity was calculated to be greatest immediately distal to the pylorus (5 cm/s).

Diurnal variations

Figure 3.6 shows the activity in a normal subject from ten electrodes along the entire small intestine. A total of thirty-two complexes were observed by this technique during 24 h of recording in two subjects. The mean duration from one complex to the onset of the next was 110 min. As in animals, the complex is part of an activity cycle consisting of an inactive phase with practically no spike potentials (phase I), followed by an irregular phase with increasing spike activity (phase II). During this phase minute-rhythm was usually present in the jejunum. This phase was followed by the active phase (phase III) with the migrating band of regular spike activity; i.e. the myoelectrical complex.

Reaction to food

In two cases the active phase was observed to terminate abruptly after intake

of 500 cc of milk, followed by a period with irregular spike activity throughout the small intestine.

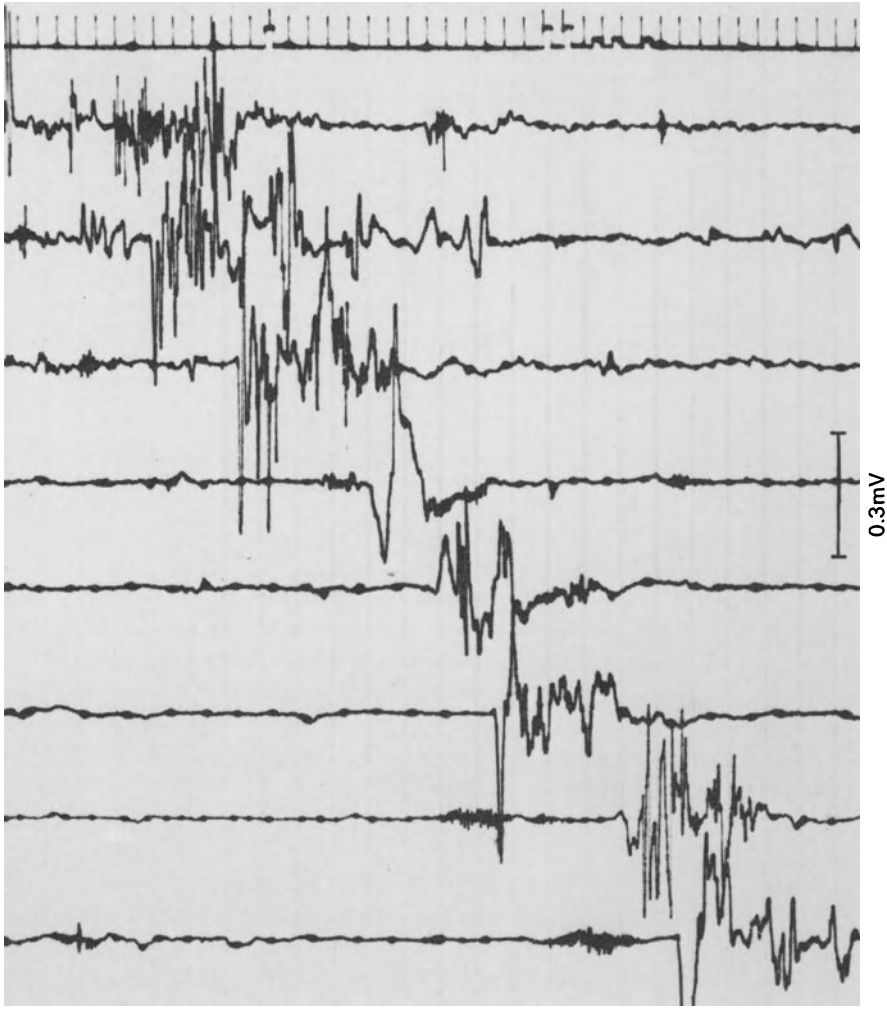

Figure 3.5 'Rush' of spike potentials recorded from the upper human jejunum. Same technique as in Figure 3.2. Time marking in seconds

DISCUSSION

Myoelectrical complex

It appears that in humans this activity is very similar to the previous observations in other animals. The time interval from onset of one complex to onset of the next complex has been reported to be 80–110 min in dogs[1–3]. According

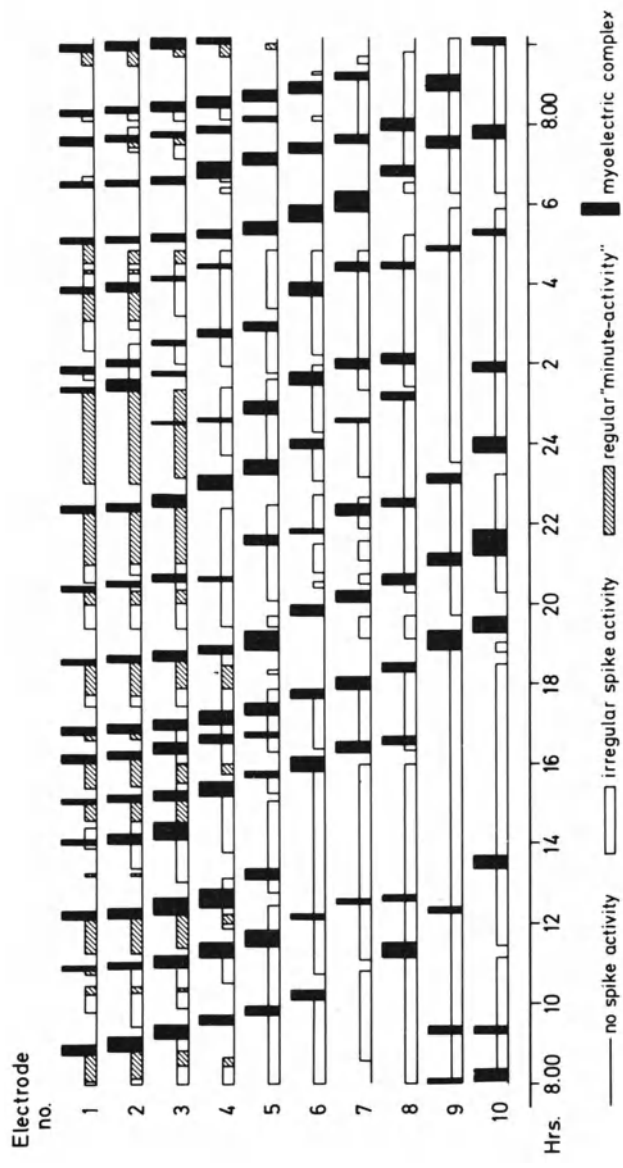

Figure 3.6 Schematic representation of fasting myoelectrical activity in the entire human small intestine recorded continuously during 26 h

to our observation, this time interval is around 110 min in humans. In both species most complexes propagate through the entire small intestine, thus only minor quantitative differences were observed between the myoelectrical complex in dog and man. Our preliminary investigations indicate that after food intake the complex is interrupted, both in man and dog[3–5].

Minute-rhythm

This phenomenon does not appear to have been described previously in the intact bowel. It appears with great regularity with a burst of several spike potentials every 1–2 min and this activity covers about one-third of the total fasting observation time. In this respect man seems to be more related to the cat, since *in vitro* experiments with colon preparations from the cat by Christensen *et al.*[6] have shown an activity corresponding rather closely to the human minute-rhythm.

This activity also seems to fit into the classification proposed by Golenhofen[7] with second-, minute- and hour-rhythm in all types of smooth muscle. The myoelectrical complex in this way corresponds to the hour-rhythm.

Rush

Has been described only from rather unphysiological animal experiments. Its propagation velocity seems to correspond well with the velocity of slow waves and our preliminary cineradiographic observations indicate that isolated rapid waves of contraction in the proximal part of the small intestine are identical to the electrical rush. We assume that the rush may have some transport function.

CONCLUSION

Three characteristic electrical migrating phenomena have been observed in the intact human small intestine: (1) the myoelectrical complex propagating through the entire small intestine; (2) minute-rhythm; and (3) rush present only in the proximal small intestine.

Acknowledgement

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Discussion

T. Y. El-Sharkawy (Canada): In one of your slides some migrating complexes started in the upper duodenum but stopped somewhere before the terminal ileum. Also some complexes did not start in the upper duodenum but somewhere lower down. We have observed such complexes in the dog. How frequently do you see such 'incomplete' complexes?

P. Fleckenstein (Denmark): This material does not allow for calculations on the frequency of such 'incomplete' complexes. As appears from the figure, however, most complexes traverse all the way through the small intestine.

D. L. Wingate (UK): I believe that the minute-rhythm can be seen in dogs if you space the electrode at 10 cm intervals as you have done in man.

Fleckenstein: I agree. I am sure the minute-rhythm is also present in dogs.

G. Stacher (Austria): You showed a diagram with 24 h duodenal activity. Can you tell us if your subjects were asleep during the night hours and did you control for sleep?

Fleckenstein: The slide showed activity in the entire small intestine. This subject slept during the hours 10 pm to 6 am. It appears that sleep does not affect the myoelectric complex.

N. E. Diamant (Canada): Does the 'minute-rhythm' occur during the irregular spiking activity which occurs after feeding?

Fleckenstein: No, we have not observed that.

S. K. Sarna (Canada): Could the presence of a 2 m long tube in the small bowel simulate the presence of food and could minute-rhythm be related to the presence of the tube?

Fleckenstein: After feeding we have not observed the minute-rhythm. Since the myoelectrical complex is normally disrupted by feeding, it is not likely that the tube would simulate the presence of food.

J. T. Farrar (USA): I believe that the 'minute-rhythm' has been recorded before in intact man but not described as well as you have done so. Bursts of activity at one point have been seen in early balloon recordings. Franz Ingelfinger and I observed bursts of sound at intervals of $1\frac{1}{2}$–2 min. Using a telemetering capsule, we observed bursts of activity at the same frequency. We thought that this activity was more prominent in patients with the functional bowel syndrome.

D. Frommer (Australia): Did you have the opportunity of examining the effect of nausea on the electrical rhythm?

Fleckenstein: No, we did not.

4

Effect of feeding and of gastrin on the interdigestive myoelectrical complex in man *(Abstract)*

J. HELLEMANS, G. VANTRAPPEN, J. JANSSENS AND T. PEETERS

The interdigestive myoelectrical complex (IDMEC) has been identified in the small intestine of several animal species and its motor equivalent in man has been described by our group. Feeding stops the complex in dogs and rats but not in sheep. Some consider gastrin to be involved in this inhibition while others propose insulin as the major inhibitory factor. The purpose of this study was to examine whether feeding is able to stop the complex in man and whether gastrin is involved in this inhibition. Intraluminal pressures were measured by means of three perfused catheters with recording orifices 25 cm apart, the proximal orifice being located at about 10 cm above the angle of Treitz. Nine normal subjects were fed a continental breakfast of about 450 kcal just prior to the onset of the pressure recording. The interval between the meal and the appearance of the first activity front of the IDMEC was 213 $\pm$ 12 min (mean $\pm$ SEM) whereas in the fasted state the activity front recycled every 112.50 $\pm$ 11.41 min (mean $\pm$ SEM) ($p < 0.001$). Three patients with Zollinger–Ellison syndrome and three patients with pernicious anaemia (serum gastrin levels between 380 and 1500 pg/ml) were also examined. Interdigestive complexes were recorded in all six patients. The characteristics of the activity front in these patients were not different from those of normal subjects (see Table 4.1).

The effect of intravenous infusion of different doses of pentagastrin on the IDMEC was examined in each of six normal subjects. Infusion rates from 0.05 to 1 μg/kg/h were studied and their effect on small intestinal motility and gastric acid secretion was measured for periods of 16–18 h. In each subject doses of pentagastrin which induced more than half the maximal acid out-

put were unable to block the IDMEC. High doses of pentagastrin (1 μg/kg/h), however, invariably stopped the complex. It is concluded that feeding stops the IDMEC in man. The hypothesis that gastrin is the major inhibitory factor is difficult to reconcile with the occurrence of interdigestive complexes in patients with hypergastrinaemia and with the results of the pentagastrin infusion studied.

Table 4.1

	Duodenum	*Jejunum I*	*Jejunum II*
Duration (min)	4.46 ± 0.66	4.34 ± 0.58	4.64 ± 0.90
Contractions/min	11.24 ± 0.17	11.02 ± 0.15	10.92 ± 0.43
Progression velocity (cm/min)	8.30 ± 0.17	5.90 ± 2.64	
Calculated length (cm)		24.63 ± 5.80 (mean ± SEM)	

Discussion

A. Bennett: (UK) Does acid entering the duodenum affect the myoelectrical complex, and if so could this influence a direct affect of pentagastrin on the myoelectrical complex?

J. Hellemans: (Belgium) The hypothesis that acid entering the duodenum can elicit the complex certainly merits further investigation. Indeed, in man, acid seems to release motilin and this in turn is in some way related to the complex. However, in our pernicious anaemia patients, the complex was able to start off without any acid stimulus. During the infusions of pentagastrin, the gastric acid secretion increased, but most if not all of the acid was aspirated via a gastric tube, and not allowed to enter the duodenum. It is unlikely that this acid influenced the results to a large extent.

N. W. Weisbrodt: (USA) Could the MMC be interrupted in those patients with hypergastrinaemia by feeding or by infusion with pentagastrin?

Hellemans: We could only try this in one patient with hypergastrinaemia. Feeding inhibited the myoelectrical complex in this patient in the usual way.

W. J. Snape, Jr.: (USA) We have shown that colonic spike and motor activity are stimulated more by a 1000 cal meal than by a 450 cal meal. Did you evaluate whether a larger meal delayed the interdigestive motor complex more than the 450 cal meal?

Hellemans: We did not do these experiments in man. In dogs, rather extensive studies have been done in our laboratory on the influence of the quantity and quality of the food administered. Dr Eeckhout will report on the results later (Chapter 6).

J. S. Davison: (UK) Have you considered the possibility that there may be a nervous component? In this context it would be of interest to know the time course for the onset of inhibition – a rapid blocking of an already established IDMEC would support the notion of a nervous mechanism. Have you attempted to measure this?

Hellemans: Recording was started immediately after the meal. At the moment the complex had always already been inhibited in the upper intestine. The studies on dogs in our laboratory show within a few minutes an effect of feeding on the IDMEC in the upper small intestine. However, our experiments have not been designed to find direct evidence in favour of a nervous component bringing about the inhibition, or against it.

C. F. Code: (USA) A comment on the mechanism of interruption of the activity front. Distension of a balloon in the stomach will immediately interrupt the activity front in the stomach and upper small bowel. Also, the local injection of a volume of 5–10 ml into a portion of the bowel displaying the activity front immediately interrupts the activity front at the site of the injection. Vagotomy interferes with interruption of activity fronts. All these observations implicate neural mechanisms in the interruption of activity fronts.

5
Postoperative gastrointestinal complexes

B. N. CATCHPOLE AND H. L. DUTHIE

Numerous studies have been made of upper gastrointestinal tract motility in man after laparotomy: radiographic[1], pressure[2], and myoelectrical[3] recording techniques amongst them. Some by their nature do not lend themselves to prolonged gathering of data but simply sample events from time to time; other studies, although continuous, have been for fairly brief periods. We took the opportunity, therefore, to record intraluminal gastrointestinal pressures continuously for periods of up to 4 days after abdominal surgery. Particular study was made of the timing of the return of gastric and duodenal activity after closure of the abdomen, the evolution of patterns of motility, and the effects of drugs and of the surgery itself.

METHODS

A double-lumen plastic tube with appropriate single side-holes (20 cm apart) in each lumen was passed via the nose at the completion of abdominal surgery, and positioned to record pressure in the gastric antrum and in the duodenum in its distal second part. Both lumens of the tube were slowly perfused with water and connected to strain gauges recording on an ink-writing polygraph within 2 h (with one exception) of closure of the abdominal incision. Patients received nothing by mouth during the periods of study except for sips of water occasionally towards the end of the recording period. Approximately 650 h of recordings, from eleven male patients, are available for study. Of these, five patients had truncal vagotomy and pyloroplasty (TV + P), three partial (Billroth I) gastrectomy (PG), and three other manoeuvres (hemicolectomy, resection of aortic aneurysm, and bypass procedure for carcinoma pancreas). However, only three of the gastric records were adequate for analysis for various surgical (e.g. excision of the distal part of the stomach) or technical reasons.

Morphine, its associated alkaloids and pethidine (meperidine) were given as postoperative analgesics according to need. One patient being mechanically respired received diazepam, pancuronium and phenoperidine from time to time. Water and electrolyte balance was maintained in all patients by intravenous therapy. 50 ml of 50% glucose was given to two patients intraduodenally, and in a further diabetic patient, recording took place when his blood sugar was elevated.

RESULTS

Duodenal activity

This had returned in all patients within 2 h of abdominal closure with the exception of one patient whose connection to the recorder was delayed; his activity was first recorded 3 h after completion of surgery. The minimum period of recorded activity was 1 h following a truncal vagotomy. Since the type of duodenal activity did not appear different after differing types of surgery, only clear exceptions to this rule will be discussed.

From the time it was first recorded, duodenal activity usually took the form of bursts or complexes of rhythmical contractions (phase III[4]), producing pressures of up to 50 mmHg between which no activity was recorded (Figure 5.1). In ten of the eleven patients recovering from their anaesthetic, the appearance of the complex was spontaneous, while the other patient receiving 8 mg morphine thereupon initiated a complex – although all patients had had

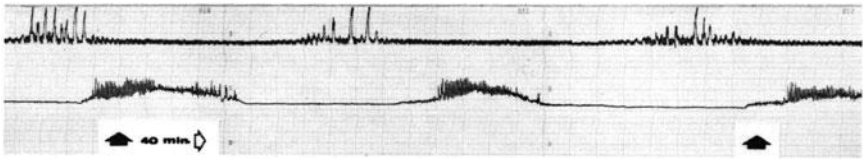

Figure 5.1 Established gastric and duodenal complexes. The period between black arrows is 40 min. The trace was taken 20 h after laparotomy from a patient who had had a vagotomy and pyloroplasty 9 months previously. Upper trace—stomach. Lower trace—duodenum

Table 5.1 Complex appearance in a 'drug-free' period

Type of surgery	*Mean duration of complex* (min ± SEM)	*Mean frequency of complex* (min ± SEM)	*No. of complexes measured*
TV + P	8.6 ± 0.46	59 ± 4.3	12
TV + P	5.3 ± 0.44	40 ± 3.1	10
TV + P	6.0 ± 0.20	36 ± 1.4	18
TV + P	5.0 ± 0.12	28 ± 0.85	22
Laparotomy 9/12 post TV + P	8.9 ± 0.22	24 ± 1.2	26
Billroth I gastrectomy	4.2 ± 0.30	35.2 ± 3.8	23
Billroth I gastrectomy	6.4 ± 0.18	50.1 ± 2.0	28
Billroth I gastrectomy	5.9 ± 0.16	42.2 ± 1.8	25

morphine or pethidine premedication and sometimes further doses during anaesthesia.

Recordings of complex duration (phase III) and cycle length (phases I–IV inclusive) after truncal vagotomy or partial gastrectomy – measured at least 6 h after any analgesic or other drug had been given – are shown in Table 5.1. The other patients' records were not able to meet this criterion of a 'drug-free' state.

Contraction frequency during complexes was easily counted and varied between 10 and 12/min. In four patients this rose from 10 or 11 to 11 or 12/min respectively over 12 h postoperatively, while in the other patients it remained constant throughout the duration of the recordings. Certain complexes of the same individual showed an initial rise of tone (pressure) before rhythmical contractions began, while others consisted only of contractions without rise of tone (Figure 5.2). During the rhythmical contractions of the duodenum during a complex, mean pressure occasionally fluctuated, suggesting a further rhythm with a periodicity of between 0.4 and 1.8 min (Figures 5.3a, 5.3b).

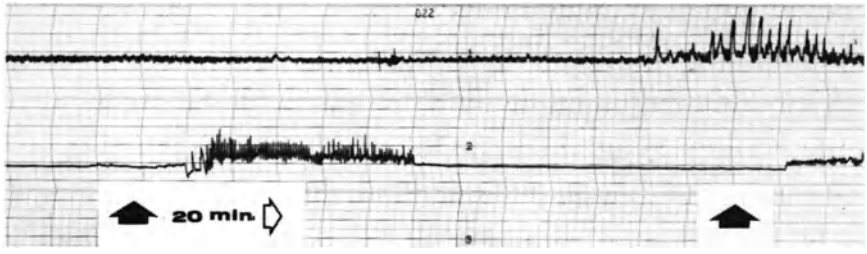

Figure 5.2 The upper tracing shows one gastric complex; the lower shows a complete duodenal complex and the beginnings of another. No gastric complex is related to the first duodenal complex. The second duodenal complex shows a rise of tone before significant contractions begin. From the same patient as Figure 5.1, taken 24 h after laparotomy

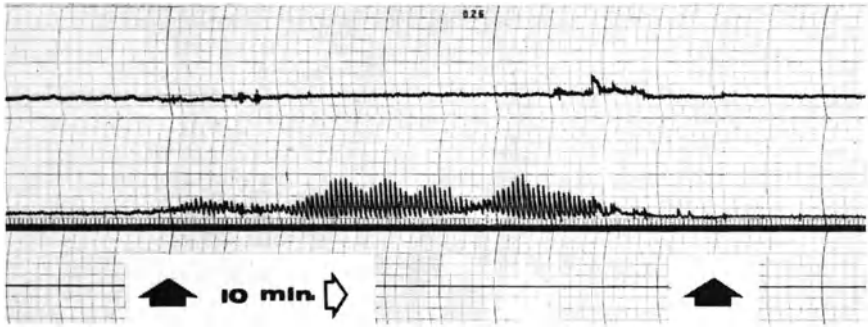

Figure 5.3a The upper tracing shows the end of a gastric complex followed by a duodenal complex recorded in the lower tracing. Note the phasic nature of the mean duodenal pressure during this complex. From a patient 7 h after the completion of a cholecystjejunal and jejunojejunal anastomosis for carcinoma pancreas

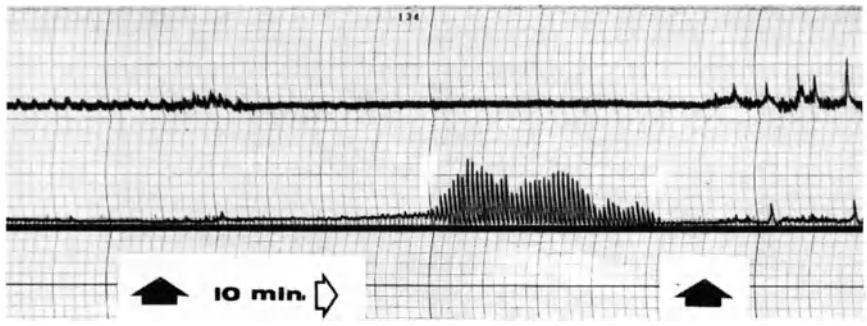

Figure 5.3b From the same patient as Figure 5.3a, 29 h after laparotomy. The phasic change in mean pressure is again shown

Morphine (and related alkaloids) significantly reduced cycle length but less often complex-duration (Figure 5.4). The change was much less dramatic after pethidine (Figure 5.5).

Additional contractions between complexes after a drug were either not seen or seen only very occasionally.

However, morphine in some patients so increased the frequency of the complexes that two or three might follow so closely for 15 min after the

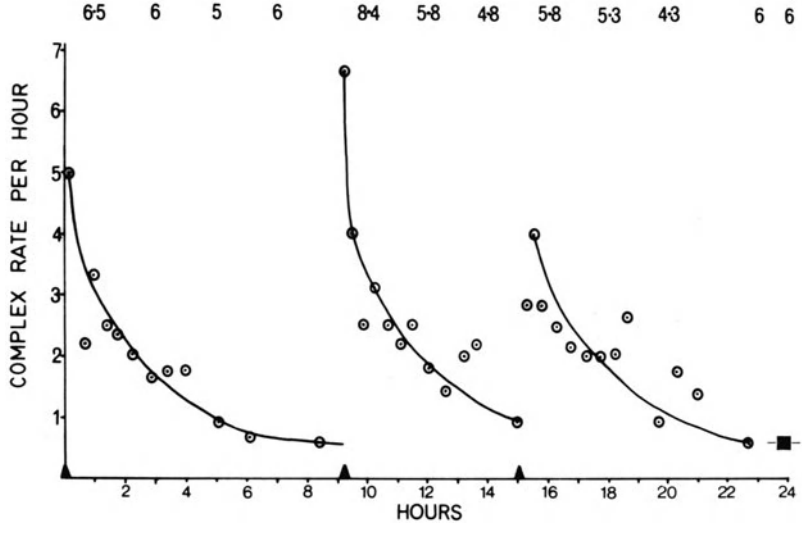

Figure 5.4 The effects on the complex cycle rate in one patient after three injections of 8 mg morphine (at the arrowheads) over a period of 24 h – 32 h following Billroth I gastrectomy – are shown. Complex cycle rate was calculated by dividing the time between the onset of complexes (in minutes) into 60 min. All complexes are plotted. The mean duration of complexes for each 2 h period is given (in minutes) in the upper line of figures (6.5, 6, 5, etc.). The –■– symbol at the extreme right of the graph indicates the mean complex rate during the following 8 h while the patient was not receiving morphine

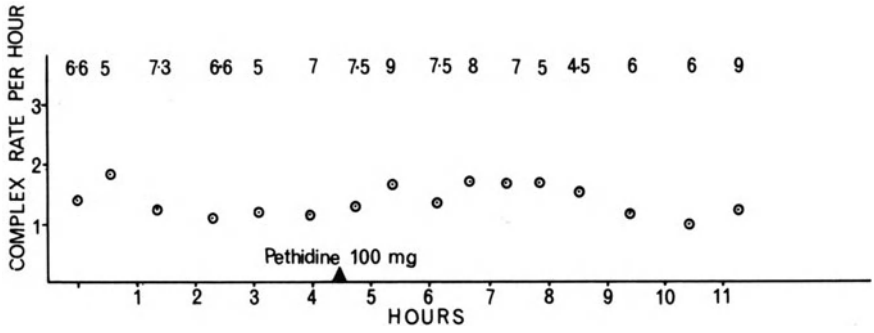

Figure 5.5 Complex rate (calculated as for Figure 5.4) before and after an injection of 100 mg pethidine (meperidine). All complexes are plotted and their duration (in minutes) is given by the figures above each. From a patient 26 h after completion of Billroth I gastrectomy

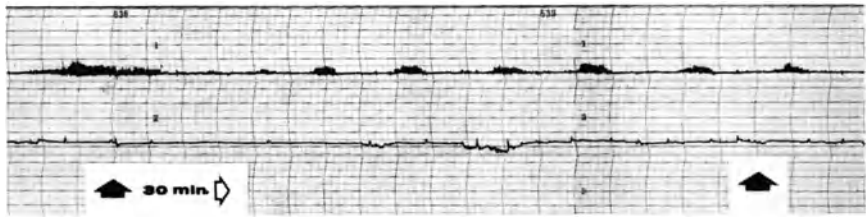

Figure 5.6 The upper trace from the duodenum shows a period of short frequent complexes following 8 mg morphine. The lower trace shows no antral activity. From a patient 24 h after truncal vagotomy and pyloroplasty for perforation of a chronic duodenal ulcer

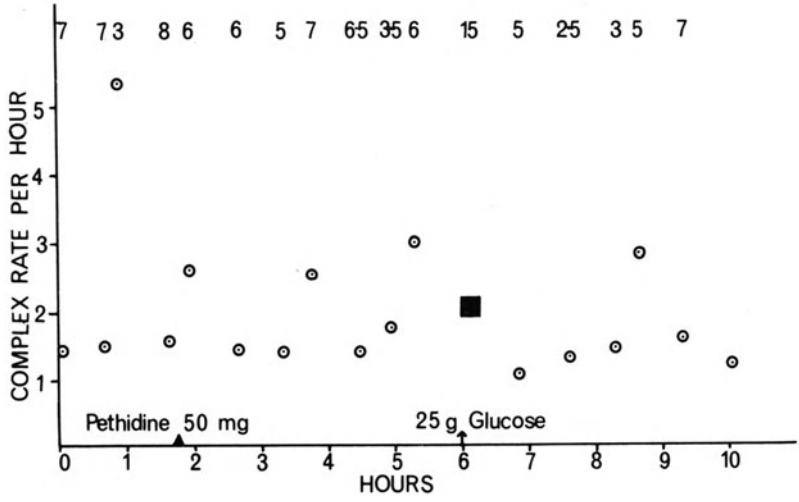

Figure 5.7 The effect of intraduodenal glucose (25 g in 50 ml water injected over 5 min) 4 h after an injection of 50 mg pethidine. The ■ symbol represents a 15 min period of continuous duodenal activity. The duration of each complex (in minutes) is given by the figures above each complex. From a patient 39 h after a Billroth I gastrectomy

injection, that the separate components of this 15-min complex were barely discernible. In one patient rapid short complexes followed one another for periods of 60 min (Figure 5.6). Rises of gut tone in complexes did not occur more often after morphine than those developing spontaneously.

Although sips of water failed to disrupt duodenal complexes, an intraduodenal injection of 50 ml of 50% glucose in two patients caused continuous duodenal activity for 15 min, followed by a resumption of the patients' usual complex pattern (Figure 5.7). In one diabetic patient, blood sugar fluctuations from 4.9 to 10.5 mmol/l did not alter the complex pattern. Another patient receiving pancuronium, phenoperidine and diazepam during a period of mechanical ventilation had fifty-seven complexes in 16 h lasting a mean of 4.33 (SEM $\pm$ 0.24) min.

Gastric activity

This appeared after duodenal activity was well established. In the three patients who had had (a) truncal vagotomy and pyloroplasty, (b) laparotomy after a truncal vagotomy 9 months previously, or (c) cholecystenterostomy gastric contractions were first apparent after 9 h, 2 h, or 6 h respectively. Activity began as isolated contractions or continuous low pressure contractions (Figure 5.8) which aggregated into discernible complexes 15, 8 and 14 h subsequently.

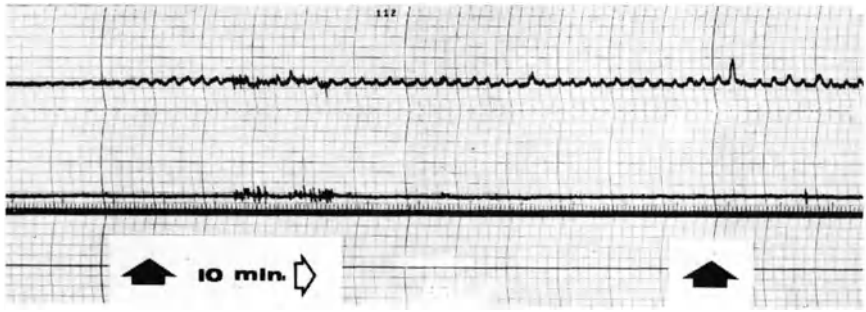

Figure 5.8 The onset of gastric contractions is seen in the upper trace. The second trace, apart from some interference, shows no duodenal activity. From the same patient as Figures 5.3a and 5.3b, 24 h after laparotomy

Complexes were variably related to those of the duodenum (Figure 5.1), the two sometimes being synchronous, and the gastric complex sometimes preceding the duodenal. When both gastric and duodenal complexes were established, the gastric complex might fail to occur despite the regular appearance of that of the duodenum (Figure 5.2). Usually the gastric complex was one-half to three-quarters of the duration of that of the duodenal when they were both well established. In the patient (a) who had been vagotomized at

laparotomy, desultory gastric contractions occurred throughout the record in addition to complexes.

Morphine or Omnopon® given before the development of gastric contractions did not result in their appearance even though there was a duodenal response. However, once established, the drug's effect on the gastric complexes was similar to that on the duodenal complexes, rate increasing *pro rata* with duodenal rate, while complex-duration remained proportionately constant. Pethidine had a similar effect on the gastric as it did on the duodenal complexes.

DISCUSSION

Szurszewski[5] described a migrating electric complex in the intestine of the fasting dog. Many observers have since confirmed and extended these original observations[4] and recorded them in man[6]. After abdominal surgery when the patient is still fasting, gut activity returns taking up the form of discrete complexes. Duodenal motor activity usually returns within an hour or two of abdominal closure, well before gastric contractions appear. This delay in the return of gastric motility after abdominal surgery was recognized many years ago[1] and has been shown to be a consequence of disordered electrical control activity (basic electrical rhythm) of the stomach[3].

The aggregation of contractions in stomach and duodenum into complexes was seen in patients with or without intact vagi. It is apparent that the occurrence of a duodenal complex is not dependent on the previous occurrence of a gastric complex, as duodenal complexes are well established before the gastric ones appear. Further, the duodenal complex is inconstantly related to the gastric complex (Figure 5.1), still appears when the gastric does not (Figure 5.2) and is unaffected by partial gastrectomy.

Although truncal vagotomy was found by Marik and Code[7] to disturb the appearance and shorten the length of canine duodenal complexes, the findings in man recorded here are in accord with Weisbrodt and colleagues'[8] observations in the dog that vagotomy has little or no effect on the fasting complex pattern. However, after both truncal vagotomy and partial gastrectomy, the duration of the duodenal complexes and of the cycle length were variable (see Table 5.1) from patient to patient, the former tending to be longer and the latter shorter than in Vantrappen's[6] normal subjects. Although measurements of these variables were made at least 6 h after any type of drug, it is possible that an operative and postoperative accumulation of morphine or other alkaloid was responsible. However, the regularity of the complex cycles at around 40 min for over 12 h in the patient shown in Figure 5.7, without any form of analgesic, casts some doubt on this explanation.

The increasing frequency of duodenal contraction, and therefore of the electrical control activity noted in some patients after laparotomy, may be a normal variable, as is seen in the dog. The types of complexes varied from

patient to patient and in the same patient – tone first rising in some before significant contractions occurred, while in others virtually maximal contractions began precipitously. The significance of these differences is not clear. It seems impossible to detect segmentation from propulsive contractions by the recording technique used here, and whether the apparent waxing and waning of mean pressure has significance is also unclear.

The effects of morphine and its related alkaloids were dramatic and in sharp contrast with those of pethidine. Neither drug, in doses used for analgesia, gave rise to spasm, nor was the complex pattern different in the vagotomized and the non-vagotomized patients. Daniel and his colleagues[9] found ileal spasm in man after morphine, and transient slight inhibition of ileal activity in all patients receiving intravenous pethidine, but this was not comparable to dosage techniques used here. The way in which morphine shortens the gastric and duodenal complex cycles suggests that it is acting at least in part on the centre responsible for the rhythmicity, since the cycle time was shortened without significantly affecting the duration of the complex.

The mechanism of control of these fasting complexes remains obscure. Their characteristics seem in general to be similar in man to those of other animals although much further work in man is indicated. The conclusion that they are centrally regulated seems to be inescapable, but the level of control is undetermined as yet. Clearly gastric and intestinal complexes are closely related but not interdependent. Some drugs with a central action, e.g. morphine, phenoperidine and diazepam, increase the complex rate. An elevated blood glucose, however, does not appear to alter the fasting pattern in man, and neither does intravenous feeding in the dog[10]. It is tempting to believe that gut activity might be continuous if it were not 'turned off' by some mechanism acting perhaps via the sympathetic autonomic nervous system, but splanchnicectomy in animals[11] does not suggest that the terminal control mechanism is quite so simple. However, the fundamental similarity of periodicity of the interdigestive or fasting complex in unoperated man[6], of REM sleep[12], and of oral activity[13] indicates the presence of a body rhythm involving structures outside the abdomen.

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Discussion

G. Scott: (Canada) Had a Hollander test been performed on the vagotomized patients to prove that their vagus nerves had been divided?

B. N. Catchpole: (Australia) No. However, one patient had had his gastro-oesophageal area widely resected for carcinoma 9 months before further laparotomy. In this case total vagotomy seems almost certain to have been performed.

N. W. Weisbrodt: (USA) Can the same criteria be used to identify a complex in all instances? How do you define a complex? Do the morphine-induced 'complexes' migrate?

Catchpole: I define a complex, recorded by a pressure-measuring technique, as an aggregation of gut contractions, without activity before or after the aggregation. We have not studied the possibility that morphine-induced complexes migrate.

S. K. Sarna: (Canada) We observed in our cholecystectomy postoperative patients a significant disruption of gastric ECA for about 24 h. Did you observe any disorganized frequency of contraction in your complexes? If not probably vagotomy abolished this disorganization.

Catchpole: The return of gastric ECA activity appears to be delayed many hours after surgery in these studies whether vagotomy had been performed or not.

H. L. Duthie: (UK) In all these presentations the length of the period of activity is 5–10 min, whether the records are pressure or electrical. The difference between the groups is the duration of phase I which is 5 min after morphine, 30 min after operation and 90 min in intact subjects. Thus the control of the rhythm of complexes is separate from the control of the active phase (activity front).

Catchpole: Agreed.

E. Atanassova: (Bulgaria) Section at the level of sphincter pylori leads to a dissociation of the spike activity between the stomach and duodenum. The spike activity of the duodenum exceeds by about 70% that of the normal duodenum. The spike activity of the duodenum following antrectomy increases also. I expected this in man too.

It is another question if the antrectomy is made 1–2 cm above the sphincter pylori. We performed transection of the stomach at the level of the incisura angularis followed by anastomosis. This results in a slowing of the slow potential rhythm of the distal segment. The spike potentials burst in this slowed rhythm, and in the same rhythm they appeared with the duodenal slow waves. At the end of the first week smooth muscle cell regeneration ensures a unification of the slow potential rhythm of the two segments. Spikes burst in this normal rhythm in the duodenum too. This investigation suggests a regulatory role of the stomach and the duodenal spike activity.

This can explain the good results by the patients with antrectomy 1.5–2 cm above the sphincter pylori. Moreover, in recent studies we showed the regeneration of the intrinsic nervous system in the transected and re-anastomosed stomach.

6

The effect of different test meals on the interdigestive myoelectrical complex (MMC) in dogs *(Abstract)*

C. EECKHOUT, I. DE WEVER, J. HELLEMANS
AND G. VANTRAPPEN

Feeding disrupts the interdigestive myoelectrical complex (MMC) in several animal species. The purpose of our study was to investigate: (1) the relation between the quantity of food and the duration of the disruption of the MMC; (2) the duration of disruption of the MMC after equicaloric amounts of milk-proteins, carbohydrates, long chain triglycerides (LCT) and medium chain triglycerides (MCT); (3) the duration of disruption of the MMC after mixtures of these pure components.

The experiments were performed in five mongrel dogs in which eleven bipolar electrodes were sewn on to the serosa of the small intestine at equal distances between the gastroduodenal junction and the terminal ileum. When, after an overnight fast, an activity front had reached the distal part of the intestine, the dogs were fed. The period of disruption was determined as the interval between the time of feeding and the reappearance of the first phase III that progressed normally.

In a first series of experiments the dogs were fed commercial dog food in a dose of 30, 60, 90 kcal/kg body-weight. A linear relation was found between the quantity of food and the duration of the disruption (for all dogs $r = 0.93$). The slope of the regression lines was approximately the same in all dogs.

In a second series the three major food components were fed in equicaloric amounts of 30 kcal/kg body-weight. Carbohydrates were given as sucrose, fat as arachis oil and proteins as Alburone (consisting of 95% of its caloric value as milk-proteins). The disruption of the MMC after feeding the small volume of oil lasted longer than after sucrose and much longer than after the greater volume of proteins (Table 6.1). Feeding 10 kcal/kg of MCT produced

Table 6.1 Duration of disruption of the MMC by the different test meals used

	Commercial dog food			*Pure food components*				*Mixtures*		
								I	*II*	*III*
Quantity (kcal/kg body-weight)	30	60	90	Arachis oil 30	Sucrose 30	Milk-protein 30	MCT 10	Arachis oil 30 Sucrose 30 Milk-protein 30	Arachis oil 30 Milk-protein 30	MCT 10 Milk-protein 30
Duration of disruption (min)	324 ± 23*	561 ± 31*	799 ± 33*	354 ± 65*	195 ± 7*	101 ± 6*	621 ± 57†	134 ± 16†	171 ± 15†	325 ± 31†

* The values represent the mean ± SEM for five experiments in five dogs
† The values represent the mean ± SEM for three experiments in three dogs

a longer effect than 30 kcal/kg of LCT (the dose of MCT was reduced because 30 kcal/kg of MCT caused vomiting in some dogs). These data suggest that the chemical nature of the food is more important than its amount.

In a third series of experiments we studied the duration of the disruption of the MMC after three different mixtures of these pure components. One could expect the effect of a mixture to be the sum of the effects of its components, or to be equal to the effect of the most potent inhibitor, but both proved to be wrong since much shorter disruptions of the MMC were found.

For mixture I the values were in the range of these for pure milk-proteins; for mixtures II and III the duration of the disruption was about half that caused by pure arachis oil or pure MCT.

The first and second MMC after the test meals frequently started at a lower level on the intestine than those recorded after an overnight fast.

CONCLUSIONS

1. There is a linear relationship between the amount of food and the period of disruption of the MMC, at least in the range of normal food quantities.
2. The physicochemical composition of the food is much more important than its amount in determining the duration of the disruption.
3. The effect of a mixture cannot be calculated from the effect of its components.
4. MCT has a potent disruptive effect on the MMC.
5. There is no receptor mechanism for calories regulating the duration of the disruption of the MMC after feeding.
6. The first and second MMC after feeding start at a lower level of the intestine: this suggests that the mechanism regulating the MMC continues to recycle during digestive activity, but that its manifestation at the effector organ is suppressed.

Discussion

C. Pope: (USA) Did you try the effect of sham feeding (with a cervical oesophagostomy) on the MMC?

C. Eeckhout: (Belgium) We have not done such studies.

M. A. Cook: (Canada) I wonder whether the long duration of inhibition seen after oil or triglycerides is a consequence of the hormonal control exerted on the MMC. Oil would, of course, cause the release of GIP which may produce the inhibition. Alternatively the insulin which would be released by GIP may play a role. Could you comment on the possible hormonal influences on the disruption of the complexes?

Eeckhout: We do not have direct measurements of GIP. The effects of feeding on motilin are still controversial. Although other groups and ourselves have been looking for hormones regulating the MMC or its disruption after feeding, direct evidence is still lacking. It should also be emphasized that feeding may act on the MMC through different mechanisms. We looked for gastrin and insulin during most of the experiments. After the oil test meal and MCT, the serum insulin levels remained unchanged, and thus insulin cannot explain the long inhibition of the MMC. In comparison to fat, sugar produces a large rise in serum insulin with a rather short duration of disruption of the MMC. The insulin level could not be correlated to the duration of the disruption intravenous infusion of 20% glucose for several hours at 125 ml/h.

D. Frommer: (Australia) How were the animals fed or forced to eat the test mixtures? The animal's psychological attitude to the food might well be a very important factor in the disruption of the MEC.

Eeckhout: The test meals were put in a metal cup and eaten by the dogs spontaneously. Force-feeding was never necessary. No disgust was shown for any of the test meals except in the case of repeated administration of MCT. The fact that the dogs had been fasted facilitated their feeding. It is difficult to evaluate the influence of nausea in dogs; vomiting did not occur.

S. K. Sarna: (Canada) It is generally known that fatty meals empty more slowly from the stomach. Do you think the inhibition of MMC is due to the chemical nature of fat, or due to the longer physical presence of food.

Eeckhout: Fat indeed slows down gastric evacuation and can remain in the stomach for a long time. We therefore infused the same quantity of arachis oil 30 kcal/h directly into the duodenum over a period of 1 h. The results were very similar to those in the other experiments; the duration of the disruption being 381 $\pm$ 20 min, mean $\pm$ SEM.

C. F. Code: (USA) Bowel needs volume in its contents to have all servomechanisms operational. Did you try the effects of inert volume increases by fibre or metamucilin on the complex?

Eeckhout: Yes; we fed one dog in a few experiments 200 cc of an agar-agar gel cut in pieces. No disruption of the MMC occurred.

7

The effects of exogenous cholecystokinin and pentagastrin on myoelectrical activity in the small intestine of the conscious fasted dog

D. L. WINGATE, H. H. THOMPSON, E. A. PEARCE AND A. DAND

The most consistent feature of the migrating myoelectrical complex (MMC) of the small intestine of carnivores is its abolition by oral feeding[1,2]. Two studies[3,4] have been published reporting the effects of exogenous pentagastrin on the fasting myoelectrical pattern; the resemblance of the effects of gastrin to the effects of food led both groups to suggest that endogenous gastrin is the agent responsible for the switch of pattern from fasting to feeding. The effects of cholecystokinin (CCK) on myoelectrical activity in the small intestine are less well-documented, but one recent study[5] showed an effect of exogenous CCK similar to that of pentagastrin. Since CCK is also released by food, and is a structural analogue of gastrin, a similarity of action on digestive smooth muscle seems plausible.

During the last 3 years, we have developed a system for the automated analysis of recorded myoelectrical activity from the small intestine of conscious animals[6]. This system will not only provide a quantitative estimate of spike activity, but also a quantitative estimate of patterns of spike activity, both in terms of spike content and duration[7]. We report here the results of a quantitative study of the effects of pentagastrin and CCK on myoelectrical activity in the conscious fasted dog with implanted electrodes. The aim of the study was to submit the hypothesis that one or other of these peptides is responsible for the feeding change to quantitative as opposed to qualitative analysis.

METHODS

Animals

Four trained Labrador retriever dogs were the subjects of this study. Each had been fitted with an array of monopolar electrodes implanted along the serosa of the stomach and small intestine up to 6 months prior to study. The electrode leads were taken to a cannula sutured to the abdominal wall and this cannula also served as the remote reference electrode. All dogs were in good health at the time of study and during the studies, while supported in a frame, were conscious, alert, and apparently contented.

Drugs

The peptides used were pentagastrin (Peptavlon, ICI) and highly purified CCK (GIH Labs., Karolinska, Stockholm). For use these agents were diluted with normal saline and delivered using a Harrard constant-infusion syringe pump.

Experimental design

All animals were fasted 18 h prior to the commencement of the study. Where there was evidence of persistent residual feeding activity at the onset of the study, these studies were rejected; this happened on two occasions during this series.

The total duration of each study was 6 h. During the first 2 h, a saline infusion was administered to the animal at a slow rate (24 ml/h). At the end of this time the infusion of the peptide was started and continued for 2 h; following this there was a further 2 h saline infusion. Thus, for each animal there was an initial 2 h period which served as a control for the subsequent 2 h peptide infusion. The 2 h saline infusion following the peptide allowed an estimate of rapidity of recovery from the effects, if any, of the peptide.

Each of the four animals received one infusion of pentagastrin at each of six different doses (0.125, 0.25, 0.5, 1.0, 2.0, 4.0 μg/kg/h), and one infusion of CCK at each of five different dosages (0.125, 0.25, 0.5, 1.0, 2.0 U/kg/h). Thus each animal underwent eleven studies in all, totalling 66 h for each animal, and 264 h of study overall.

Recording and analysis of data

In each animal, signals were recorded from electrodes situated at the distal duodenum (channel 1), mid-jejunum (channel 2), and distal ileum (channel 3). Recording was carried out with a Medilog 4-24 recorder, on Phillips C 120 cassettes. Playback and analysis of the data was carried out as described previously by us[6]. Gastric and duodenal electrodes were also monitored

directly through a pre-amplifier and amplifier, on a Medelec fibre-optic recording oscilloscope.

Data were analysed in a number of different ways. Total spike activity was automatically computed on reply[6] for each of the three 2 h periods at each electrode site in each study. At the same time, quantitative analysis of patterns of activity was also obtained on line during tape replay[7]. The analysis system is shown in Figure 7.1. The incidence of migrating complexes was judged visually from the histogram of spike activity during the study. The 'activity front' of a migrating complex identified by its sharp peak of spike activity (phase III), exceeding 100 spikes/min, followed by a period of absent spike activity (phase I). The temporal incidence of migrating complexes was then plotted for each study. Slow wave frequency (basic electrical rhythm) was calculated directly from the chart recording using 5 min samples of activity once an hour during the study.

Statistical analysis of the data was undertaken using standard procedures for calculating linear regression, mean and standard deviation, and *t*-tests for paired data where appropriate.

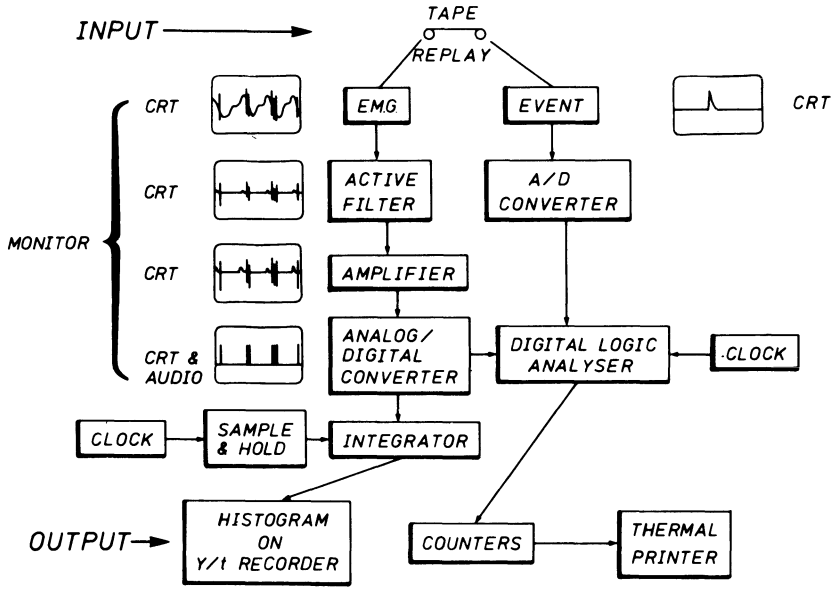

Figure 7.1 Block diagram of system for analysing tape-recorded myoelectrical activity on high-speed replay

RESULTS

Total spike activity

There was no effect of pentagastrin on total spike activity in relation to dose

in either duodenum, jejunum, or ileum. By contrast, there was a significant relation between increasing spike activity in the jejunum and CCK dosage ($p < 0.001$) (Figure 7.2). There was no dose-dependent relationship between CCK and spike activity in the duodenum or ileum.

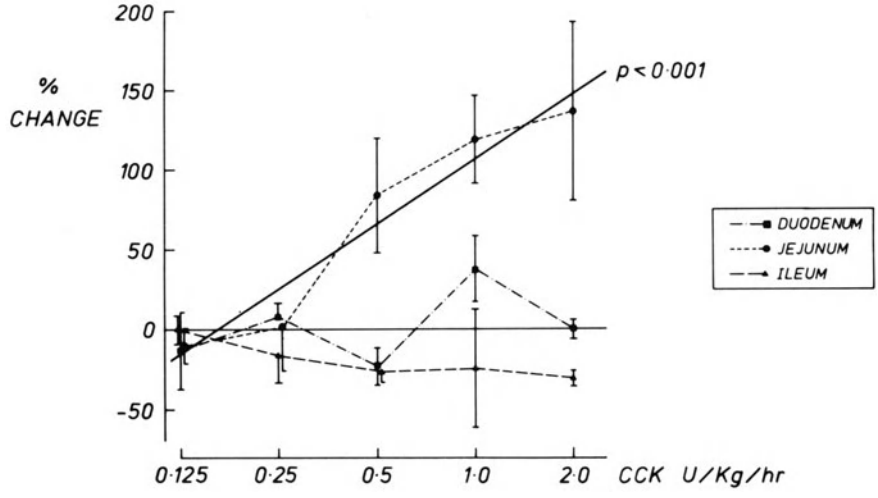

Figure 7.2 Effect of CCK on spike activity ($n = 4$). Spike activity is expressed as the percentage change in total recorded spikes between the 2 h peptide infusion period, and the preceding 2 h saline infusion. Each point is the mean ± SEM of four separate studies. The solid line is the calculated linear regression of change in spike activity against log dose of CCK

Quantitative patterns of spike activity

The only significant dose-dependent trend in the pattern of spike activity with pentagastrin was a significant ($p < 0.05$) increase in the duration of type 2 activity in the jejunum, but not in the duodenum or the ileum (Type 2 activity being similar to Phase 2 activity as defined by Code and Marlett[2]). CCK had the same effect on the jejunum but, in addition, there was a significant ($p < 0.05$) dose-related increase in the duration of Type 2 activity in the duodenum with CCK. Neither peptide appeared to alter significantly the duration of activity types in the ileum.

Migrating complexes

The effects of both peptides on migrating complexes were somewhat surprising. Above the threshold dose (pentagastrin 0.5 μg/kg/h, CCK 0.5 U/kg/h) both peptides appeared to diminish or abolish migrating complexes in the duodenum and jejunum (Figure 7.3). However, neither peptide consistently abolished migrating complexes in the ileum (Figure 7.4). This phenomenon is

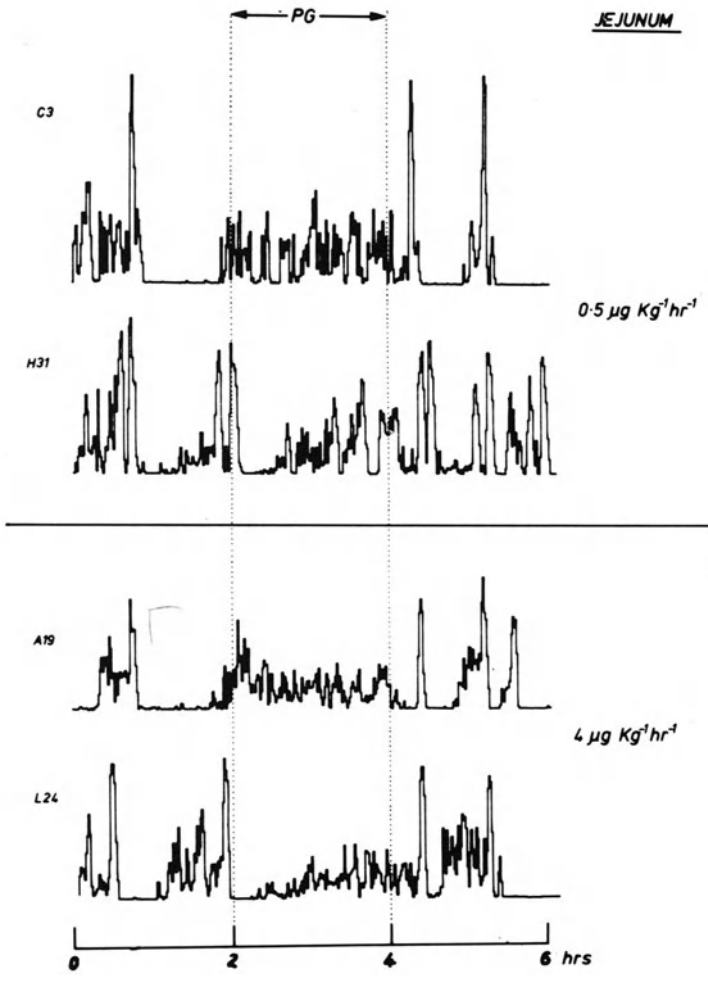

Figure 7.3 (a)

Figure 7.3 Histograms of jejunal spike activity: (a) during four studies at two different dose levels of pentagastrin (PG); and (b) during four similar studies at two dose levels of CCK

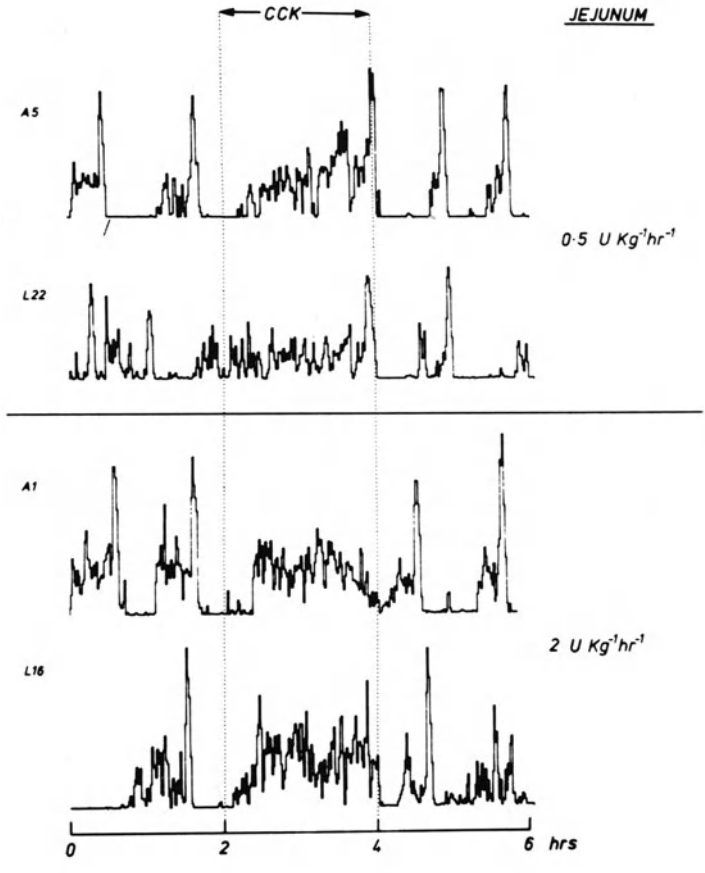

Figure 7.3 (b)

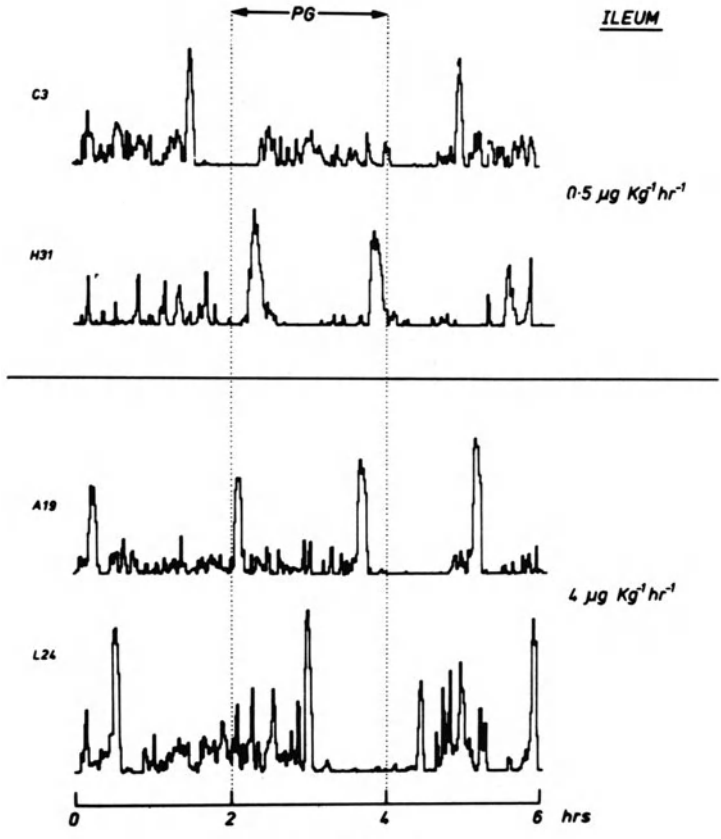

Figure 7.4 (a)

Figure 7.4 Histograms of ileal spike activity during the same studies as shown in Figure 7.3 with (a) pentagastrin, and (b) CCK

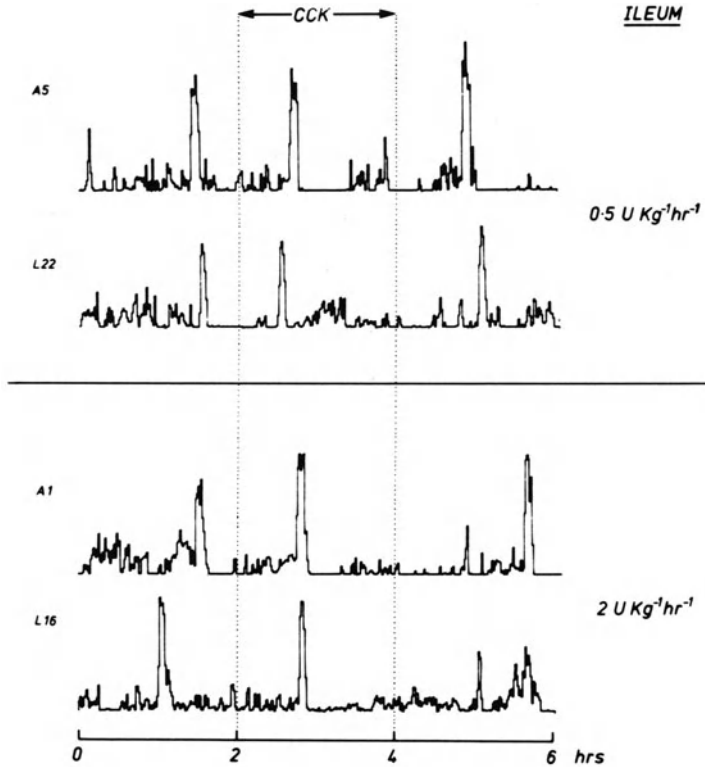

Figure 7.4 (b)

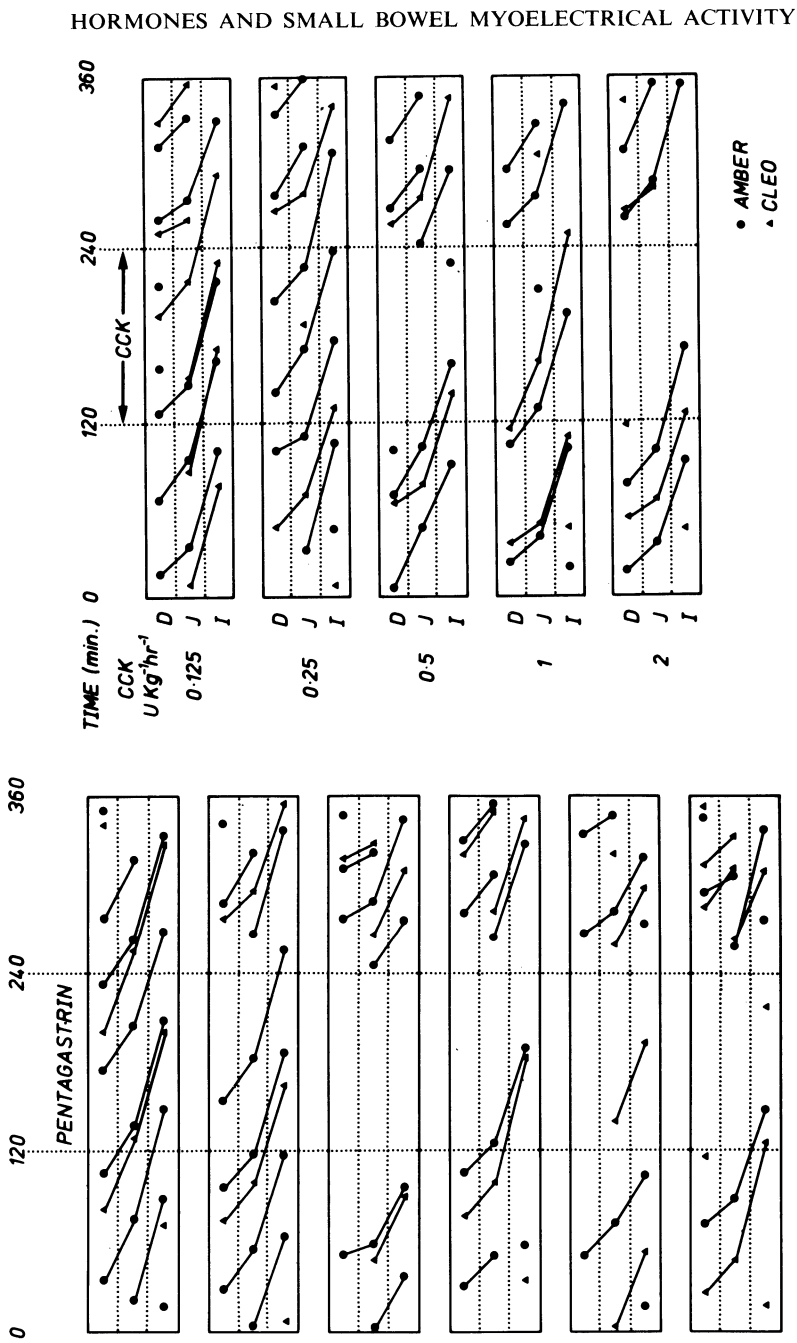

Figure 7.5 Incidence of migrating complexes in two animals during the complete sequence of studies with (a) pentagastrin, and (b) CCK. Each point represents a single migrating complex, and where the migratory sequence has been identified, points are joined by solid lines. D, J, I = duodenal, jejunal and ileal sites respectively. Both sets of studies show abolition of duodenal complexes during peptide infusion above a threshold (0.25) dose, with persistence of ileal complexes

illustrated here by plots of the incidence of migrating complexes in two animals during control periods and infusions of CCK and pentagastrin (Figure 7.5) which show that migrating complexes persist in the distal intestine during peptide infusion at maximal doses.

Slow wave frequency

Pentagastrin significantly accelerated both the gastric ($p < 0.001$) and duodenal ($p < 0.01$) pacemaker; this was dose-related. By contrast CCK had no consistent effect either on gastric or duodenal pacemakers; there was some suggestion of an inhibitory effect but this did not reach statistical significance.

DISCUSSION

The effects of those two peptides differ in a number of respects from those of oral feeding. A meal significantly increases total jejunal *and* ileal spike activity (compared with a fasting control period), abolishes migrating complexes at *all* levels of the small intestine, including the distal ileum, and significantly *depresses* the gastric, but not the duodenal pacemaker[8]. Thus the hypothesis that one or other of these peptides, as the endogenous analogue, is responsible for the feeding pattern has not survived quantitative testing.

But if the results of the study have answered one question they raise others which deserve consideration. Systematic testing of the dose-responsiveness of intestinal myoelectrical activity has not been attempted in this way before, and the results present problems in data interpretation. Some motor effects of peptides are undoubtedly stoichiometrically dose-related, but some appear to be 'on–off' or 'all-or-nothing'. CCK has been shown to have dose-dependent secretory and threshold motor effects[8]. The migrating complex appears to be an 'all-or-nothing' effect; the complex seems to be either present or absent with little or no intermediate gradation. Our data suggest a threshold dose for both peptides for this effect.

In addition, the use of statistical calculation may be misleading. The data shown in Figure 7.6 when calculated against the logarithm of the dose, produce a statistically significant ($p < 0.001$) linear regression (Figure 7.2). Inspection of Figure 7.6 suggests a sharp change in response over a narrow dose range (0.25–0.5 U CCK/kg/h) with a flat response above and below this point. This coincides with the dose level at which migrating complexes are affected by CCK. Thus it seems possible that the effect on total spike activity may be an 'all-or-nothing' effect. By contrast, at other levels in the intestine, changes in patterns of spike activity occur without significant increase or decrease in total spike incidence; a phenomenon of reorganization rather than 'stimulation' or 'inhibition'.

We interpret our data as showing that the prime effect of these two peptides is the *partial* disconnection of the intestinal smooth muscle from the extra-

enteric centre[10] which controls fasting activity, rather than a gross stimulation of spike activity. This disconnection deranges the pattern of spike activity in the proximal and mid-intestine to mimic the effect of food. Our studies also show that other factors – neural or humoral – must mediate the response to food.

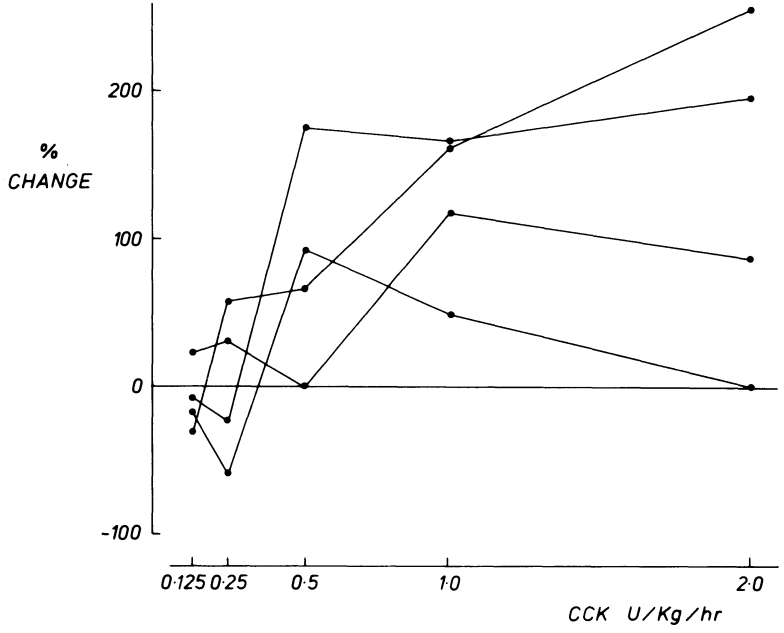

Figure 7.6 Data on spike activity as in Figure 7.2, except that each point is the result of a single study, and the CCK infusion dose is shown on a linear, not logarithmic, scale. Solid lines join the data points derived from the same animal

Acknowledgements

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Discussion

M. Weinbeck: (W. Germany) Did you look at the effect of the peptides in fed animals, because the action of peptides on intestinal transit may then be different? CCK is known to hasten gastrointestinal transit after feeding, e.g. after a barium meal, whereas its effect on the MMC suggests a slowing of propagated activity in fasted animals.

D. L. Wingate: (UK) We have not tested the peptides in fed animals – it is difficult to design an experiment without knowing the endogenous levels of CCK and although we think our infusions bracket physiological levels, we do not have an assay. We do not know the exact propulsive correlates of the pattern induced by CCK infusion, and how this differs from the feeding pattern.

Z. Itoh: (Japan) What kind of CCK did you use?

Wingate: GIH CCK.

Itoh: Then GIH CCK is pure?

Wingate: No, but relatively pure compared to Boots CCK. It was the purest CCK we could afford in the quantities needed for a systemic study.

E. E. Daniel: (Canada) After a meal, both gastrin and CCK, as well as other hormones, are released. Can you rule out the possibility that a combination of CCK plus gastrin could have reproduced the effects of feeding?

Wingate: To test all the permutations of combining the peptides at different doses would be a formidable undertaking. We do not think that the combination will reproduce the effects of feeding, because there are some aspects of the response to food which are not produced by either of these peptides or by motilin or secretin, which we have tested. If there is a digestive hormone – which is probably a naive concept – the likeliest candidate, on the basis of our present studies is glucagon.

S. K. Sarna: (Canada) What do you think is the site of action of these peptides? Would you agree that it is local, since neither pentagastrin nor CCK were able to disrupt MMC in ileums?

Wingate: In broad terms, using the model of an extra-enteric timing centre to control MMC, we speculate that these peptides disconnect the gut from the clock, as some kind of switching-off effect. If so, our evidence suggests that this only operates reliably in the proximal and mid-intestine, but not in the distal intestine.

J. T. Farrar: (USA) Previous studies, although not conclusive, have suggested that CCK acts to inhibit duodenal muscle contractions. Do you interpret your experiments to indicate that CCK inhibits either duodenal spiking or aboral movement through the duodenum?

Wingate: In our studies, CCK does not increase duodenal spike activity, but it changes the pattern from cyclical activity to intermittent spiking. It is very probable that this has the effect of slowing duodenal transit in comparison with the propulsive effect of a migrating complex, so our

results are consistent with the idea of inhibition of transit under some circumstances.

C. F. Code: (USA) On your records it appeared to me that CCK did cause the disappearance of the activity front you would have expected after cessation of the injection.

Wingate: It is quite true that in the 2 hours of saline infusion following the infusion some derangements of MMC are induced, but the changes are difficult to interpret and not statistically significant. CCK infusion probably stops an MMC starting during the infusion, although not invariably; but, unlike food, it does not stop an MMC which has already started from reaching the distal ileum.

8

Longitudinal contractions in the jejunum of fasting dogs

C. MENDEL, A. POUSSE, J. C. SCHANG, J. DAUCHEL AND J. F. GRENIER

The function of the longitudinal movements in the coordination of the motility of the small bowel remains unclear despite numerous studies. Most of these have been performed *in vitro*, and they gave conflicting results. The longitudinal contractions of the intestine have been considered as purely artefactual consequences of the circular contractions[1,2] or, on the contrary, as active and efficient movements[3–5]. Because of the technical difficulties encountered, only a few studies have been performed *in vivo*[6].

A method has been developed for the simultaneous recording of the longitudinal and transverse components of mechanical activity, together with electrical activity, in the non-transected intestine. Using this method, the purpose of the present study was to analyse the patterns of longitudinal contractions in the jejunum of fasted dogs.

MATERIAL AND METHODS

Eight mongrel dogs, weighing 12–15 kg and fasted for at least 48 h, were used in these experiments. The same procedure was carried out on each animal. The peritoneal cavity was opened by a xipho-umbilical laparotomy. A jejunal loop, situated at about 20 cm from the ligament of Treitz was carefully exteriorized without being transected (Figure 8.1) and was put on a metallic grid.

The intraluminal pressure was measured by using a spherical, water-filled vinyl balloon 1.5 cm in diameter. The balloon was introduced into the lumen of the exteriorized loop through a small enterostomy made about 60 cm from

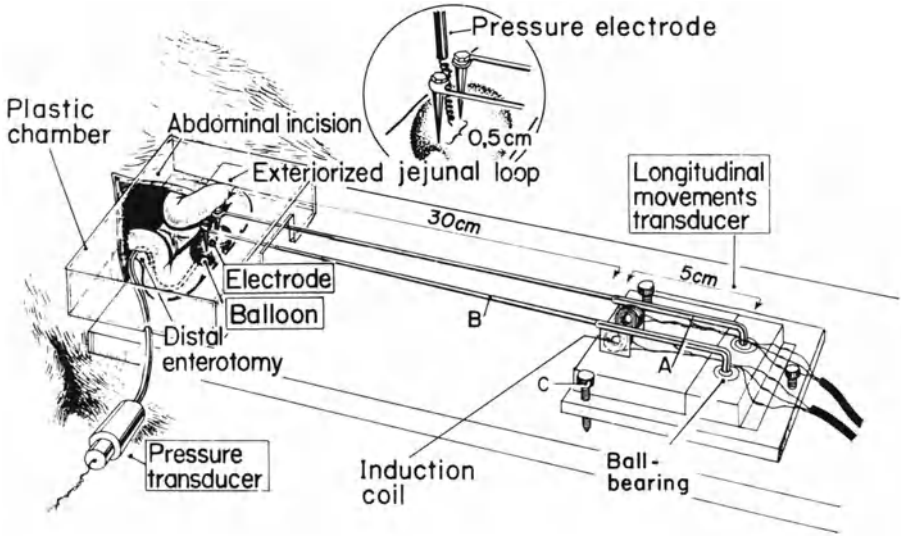

Figure 8.1 Simultaneous recording of longitudinal (transducer) and transverse (intraluminal balloon) components of mechanical activity, together with electrical activity (pressure electrode), in a non-transected exteriorized jejunal loop in anaesthetized dog (see text for details)

the ligament of Treitz. The distance between the balloon and the distal enterostomy, at least 30 cm, prevented the latter influencing intestinal motility at the level studied. The balloon was inflated with about 1.2 ml of water in order to obtain a mean differential pressure just above zero, to ensure close contact with the intestinal wall without distension of the lumen. This contact made sure that the balloon was acted on only by transverse contraction, and not by intraluminal pressure changes. The balloon was connected to a pressure differential transducer (Schlumberger H-50) by means of a flexible, water-filled catheter.

The transducer for detecting longitudinal movements was set up on the horizontal surface supporting the exteriorized loop. This apparatus essentially consisted of two movable segments. A rigid one (A) could move only in the horizontal plane, and an induction coil was fixed on it. A flexible one (B) acted as a stylet, following the entire movement of the serosa; however, only its longitudinal component acted on the rigid segment and was therefore detected by the transducer. The two stylets' points were put on the intestinal serosa on the antimesenteric border, at a distance of 0.5 cm from each other and equidistant from the site of the balloon's contact with the intestinal wall. The apparent weight of the stylet on the intestine was adjusted to about 1 g with three screws (C). One of the coils was fed an alternating high-frequency current. The intensity of the induced current in the second coil was proportional to the distance between the coils. During longitudinal movements, the

displacements of the stylets never exceeded 1 mm and the length of the stems (B) was 30 cm. Therefore the relationship between the movement and changes in the induced current could be considered as linear within a 1% error.

The rubbing constraining the segments' displacements was minimized by the use of ball bearings, which rendered the transducer essentially isotonic. This device, the care taken in filling the balloon and catheter with water, and the slow frequency of intestinal movements all made the lag in the measurements of these movements negligible.

Electrical activity was measured with a monopolar pressure electrode made from silver–silver chloride wire 0.2 mm in diameter. The electrode was in contact with the serosa half way between the two stylet points. The metallic grid on which the exteriorized loop was set served as a reference electrode.

The loop was protected against drying and cooling by a plastic chamber, and a heating lamp placed above the set-up ensured an almost constant temperature of about 38 °C. The mechanical and electrical activities were recorded simultaneously on a rectilinear Beckman Dynograph. A DC input was used for the mechanical activities, and a RC input for the electrical activity with time constants ranging from 0.01 to 1 s.

Recording began 15 min after the end of the handling of the intestine and continued for at least 90 min. Longitudinal movements were analysed in relation to the temporal patterns of transverse contractions, to electrical control activity (ECA), and to the occurrence of spike bursts. In two dogs we studied the effects on longitudinal motility of Verapamil, which inhibits electrical spiking activity[7]: 2 ml of a solution of Verapamil (5×10^{-6} mg/ml) was injected over 2 min into a mesenteric artery of the loop during intense spiking activity.

RESULTS

During the 90 min of recording, the patterns of the electrical response activity (ERA) very closely resembled those of the migrating myoelectric complex (MMC) identified in fasted dogs by Szurszewski in 1969[8]. However, the recording time was not long enough to permit observation of the complete cycle of the MMC. Nevertheless, the three characteristic phases of the MMC could be identified. Phase I, absence of spiking, was observed in all eight experiments. However, its duration could not be established, because of the relatively short recording time. Complete sequences of phase II, increasing occurrence of spike bursts, and of phase III, intense spiking activity, were seen in six experiments. The total duration of phases II and III was 16 ± 5 min, and that of phase II alone was 5 ± 2 min. In both experiments with Verapamil, its administration interrupted phase III.

The same three phases were seen for transverse contractions as for the spike bursts. Thus transverse contractions followed exactly the patterns of the MMC.

Longitudinal movements showed a very different picture, as exemplified in Figures 8.2–8.7. They were almost always present whether or not the transverse contractions were present.

During phase I – transverse quiescence without spike bursts – the longitudinal contractions were usually present (Figure 8.2). They showed no apparent correlation with the ECA, usually spanning several control wave

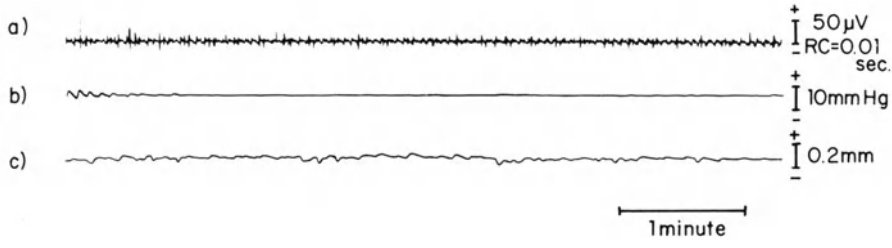

Figure 8.2 Electrical and mechanical activities in the jejunum. Phase I, characterized by the absence of spike bursts (a) correspond to transverse quiescence (b) and to irregular longitudinal movements (c)

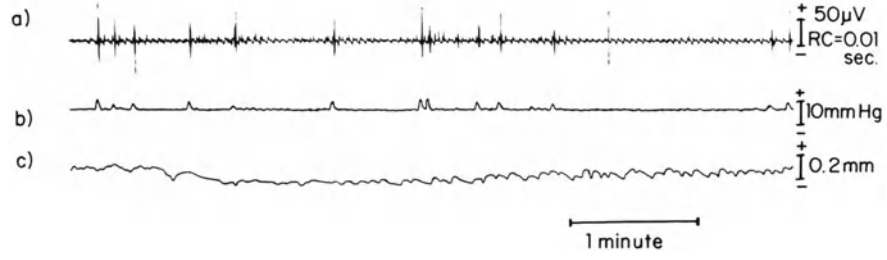

Figure 8.3 Electrical and mechanical activities in the jejunum. Phase II, characterized by intermittent electrical spiking activity (a). Transverse contractions correlate exactly with the occurrence of spike bursts (b), longitudinal contractions show irregular patterns (c)

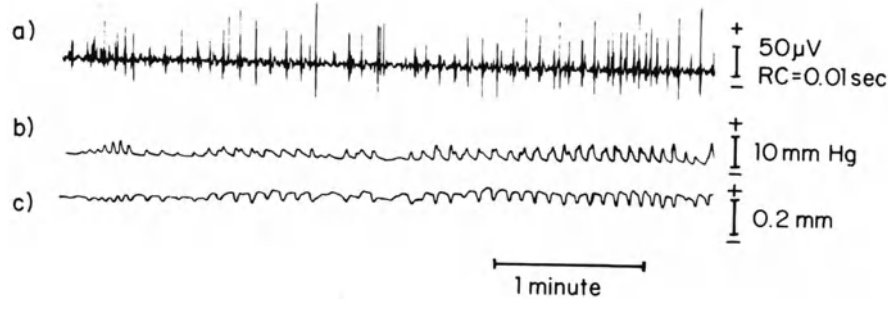

Figure 8.4 Electrical and mechanical activities in the jejunum. Phase III, characterized by uninterrupted spike bursts (a). Transverse (b) and longitudinal (c) contractions are rhythmic and strongly correlated

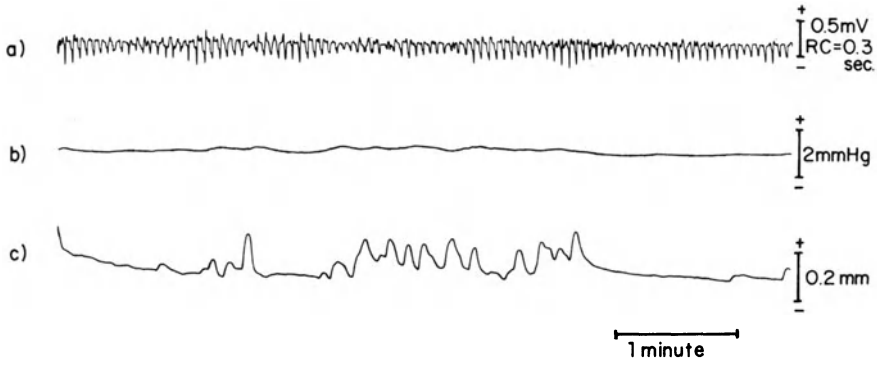

Figure 8.5 ECA and mechanical activities in dog jejunum (phase I). During transverse quiescence (b), sequences of longitudinal contractions (c) spanning several control wave cycles (a) are separated by short periods of quiescence

cycles. Short sequences of longitudinal quiescence were not accompanied by any modification of the electrical activity (Figure 8.5).

During phase II, no significant change in longitudinal movements could be detected (Figure 8.3). When transverse contractions occurred, the longitudinal contractions were usually out of phase both with these and with spike bursts, or were even absent (Figure 8.6).

During phase III, a very different picture of longitudinal movements

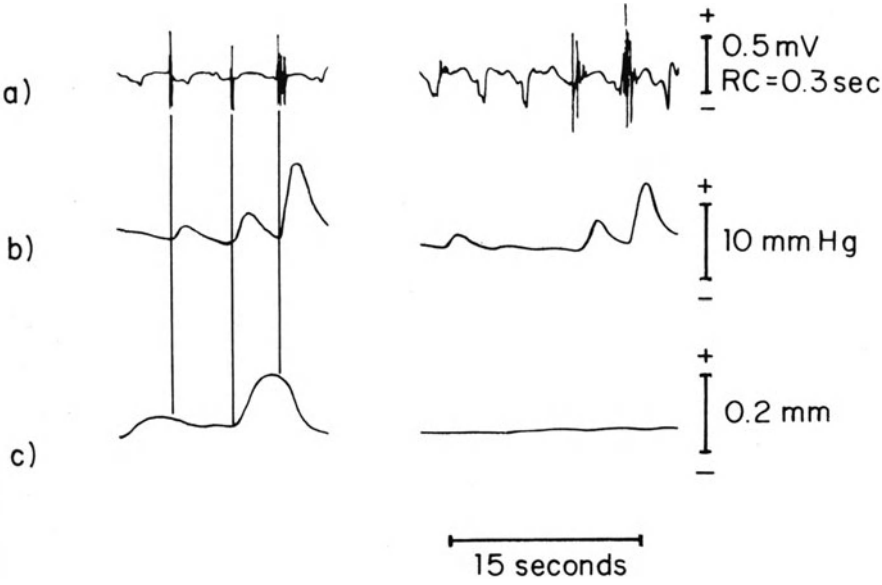

Figure 8.6 Electrical and mechanical activities in dog jejunum (phase II). Spike bursts (a) trigger transverse contraction (b). Longitudinal contractions (c) are out of phase (left) or absent (right)

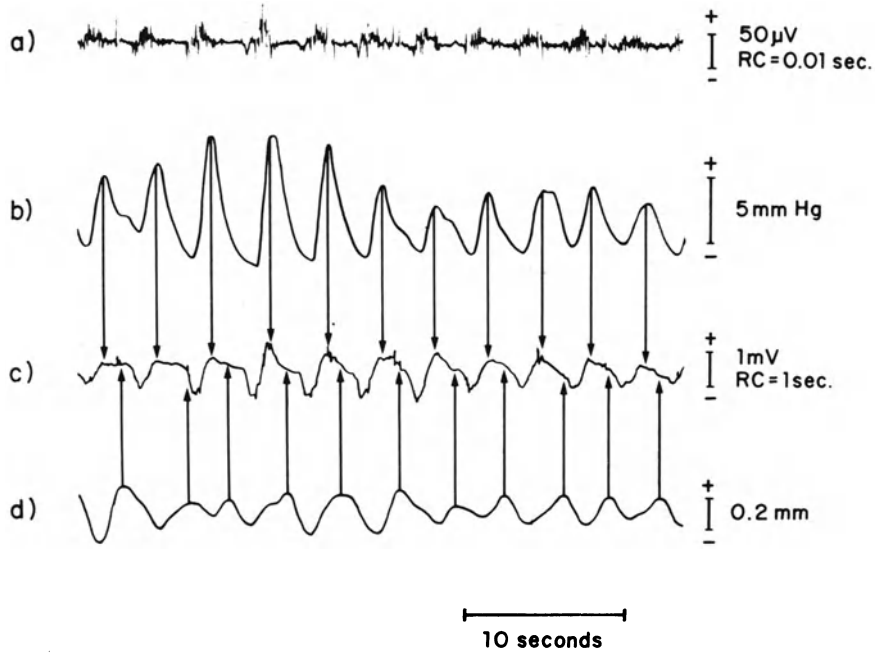

Figure 8.7 Electrical and mechanical activities in dog jejunum (phase III). During uninterrupted spiking activity (a): transverse contractions (b), electrical control wave (c) and longitudinal contractions (d) are strongly correlated. Transverse contractions maximize during control wave depolarization and longitudinal contractions during control wave repolarization

appeared. They showed a highly regular pattern of recurring contractions closely correlated with the transverse pattern (Figure 8.3). Transverse and longitudinal contractions had the same frequency, i.e., that of the ECA. However, they were sequential, transverse contraction reaching its maximum during the depolarization phase of the control wave cycle and longitudinal contraction during its repolarization phase (Figure 8.6).

Verapamil, administered at the beginning of phase III, gradually reduced the intensity and occurrence both of spike bursts and transverse contractions. Longitudinal contractions were not suppressed, but returned to the disorganized patterns of phases I and II (Figure 8.8).

The pressure changes in the intraluminal balloon due to transverse contractions never exceeded 10 mmHg. We attempted to test whether a strong pressure increase in the intraluminal balloon had some artifactual effect on the longitudinal movements. Up to 0.4 ml of water was added into the balloon by a 2 s infusion, a period which corresponded to the mean duration of pressure increase due to transverse contraction. The resulting pressure increase was more than 15 mmHg without any observable effect on the recorded longitudinal displacements.

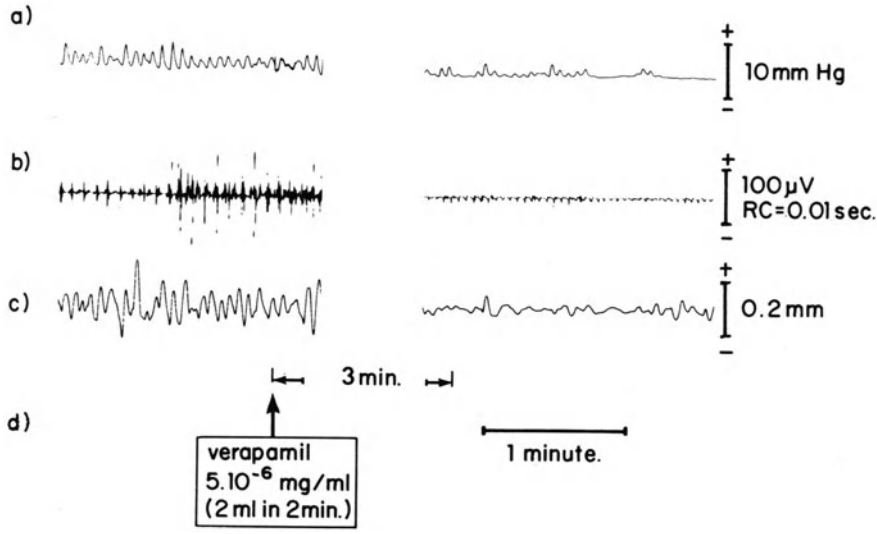

Figure 8.8 Effects of Verapamil administered during phase III, on electrical and mechanical activities in dog jejunum. Transverse contractions (a) and spike bursts (b) are reduced, longitudinal movements (c) return to disorganized patterns

DISCUSSION

We believe that the described method separated the longitudinal and transverse components of jejunal motility for two reasons: (a) long periods of longitudinal contractions were often recorded during transverse quiescence, which was attested by the lack of electrical spiking activity; and (b) the pressure changes in the intraluminal balloon due to transverse contractions never exceeded 10 mmHg, while a superimposed pressure of 15 mmHg did not alter the longitudinal tracings.

Despite the relatively short recording time (90 min), the three characteristic phases of the MMC[8] were identified in the exteriorized jejunal loops of the anaesthetized dogs. The transverse contractions correlated exactly with the MMC patterns. This result agrees with the description by Itoh *et al.*[9] of hunger contractions in dogs, recorded *in vivo* with extraluminal force transducers sewn onto the serosal surface of the intestine.

Longitudinal contractions exhibited very different patterns. They were usually dissociated from the transverse ones and were even present during transverse quiescence. This kind of activity occurred during phases I and II of the MMC. Longitudinal contractions seemed thus to be independent of the occurrence of spike potentials and were not related to the electrical control wave cycles. Dissociated longitudinal and transverse mechanical activities have been reported by several authors[10–14] who have shown that the longitudinal and circular muscle layers of the small bowel can contract independently of each other. Sustained contractions of the longitudinal muscle

without circular muscle contractions were observed by Tsuchiya[15] and by Melville *et al.*[13]. The method used by Melville *et al.* presented some similarities with ours in that the longitudinal displacements were assessed by measuring changes in the distance between points marked in India ink on the serosal surface.

Correlated longitudinal and transverse contractions have often been reported. However, reports have been conflicting. As early as 1899, Bayliss and Starling[16] observed that, in anaesthetized dogs, the contractions of the two muscle layers were simultaneous. The same conclusion was reached by Cannon[17], Alvarez[18], Hukuhara and Fukuda[19], and Bortoff and Ghalib[20]. On the other hand, Trendelenburg[21] observed a 90° phase lag between contractions of the two muscle layers, and Feldberg and Solandt[22] reported slight phase lag. Using radiopaque markers attached to the serosa, Tasaka and Farrar[6] observed, *in vivo* in the dog, movements in the longitudinal and transverse planes which were out of phase. These apparent contradictions may be explained by the different experimental conditions in which the reported works were performed. The dissociated patterns reported by some authors may correspond to the results we obtained during phases I and II of the MMC, while the correlated contractions observed by others could be related to our results during phase III. In the latter case our results agree with those of authors reporting sequential contractions.

Anuras *et al.*[10] have shown that in the duodenum of opossums and cats, longitudinal muscle is dominated by an excitatory cholinergic nerve and circular muscle by a non-adrenergic, non-cholinergic inhibitory nerve. However, the mechanisms that correlate or dissociate the longitudinal and transverse contractions remain unclear. Some explanation may be obtained from the morphology of the spike bursts in the different sequences of the MMC. What characterizes phase III of the MMC is the occurrence of spike bursts that are not only uninterrupted but also much more intense and thus perhaps of a different nature. These strong spike bursts may constitute the mechanism of coordination of intestinal motility or, at least, be related to such a mechanism. An argument favouring such an assumption is the effect of Verapamil, which reduced the spiking activity and simultaneously disorganized the longitudinal contractions.

Acknowledgement

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Discussion

A. Bortoff: (USA) Several laboratories have shown that circular muscle contraction produces an elongation of the intestine which resembles longitudinal relaxation but is really a mechanical artefact. Likewise inflation of an intraluminal balloon results in longitudinal shortening which is not due to active longitudinal contraction. Have you eliminated the possibility of such artefacts in your recordings?

C. F. Mendel: (France) The possibility of such artefacts cannot be completely eliminated. However, they cannot account for the following observations:

(1) longitudinal contractions occurring during transverse quiescence.

(2) dissociated longitudinal contractions or longitudinal quiescence during transverse intermittent activity.

Moreover, balloon inflation, increasing intraballoon pressure by more than 15 mm Hg produced no apparent alteration in longitudinal movements.

Thus it may be assumed that the longitudinal displacements produced by transverse contraction must have been much smaller than the spontaneous longitudinal movements recorded and were therefore masked.

J. T. Farrar: (USA) Dr Bortoff and I have disagreed for years as to the timing of longitudinal contractions. I believe that the longitudinal and circular contractions are out of phase and this is, we think, supported by the observation of Paul Bass and the classic experiments of Kosterlitz. It seems to me that your records support this position though I am admittedly biased.

Can you comment on the function of these longitudinal contractions?

Mendel: We have no explanation on the function of dissociated longitudinal movements. Coordinated longitudinal contractions perhaps may contribute to peristalsis.

J. D. Wood: (USA) Dr Bortoff is certainly correct in stating that passive mechanical interactions between circular and longitudinal axes of the intestine occur and should be taken into account when interpreting motility records. Secondly, there is evidence for spike-free cholinergic activation of the longitudinal muscle layer. Could this be the case in your experiments?

Mendel: This could indeed be the case in our experiments.

E. E. Daniel: (Canada) Balloon distension as used in your method may cause distal inhibition by non-adrenergic inhibitory mechanisms during part of your experiment. This may have been overcome at times during intensive activity. This may have affected the occurrence of longitudinal contractions and prevented coordination of electrical events with longitudinal movements.

(1) Have you any comments?

(2) Also in the jejunum you may have been beyond the region of phase-locking. This too would lead to poor correlation between electrical

activity and longitudinal contraction and between contractions in the two axes.

Mendel: (1) This work has shown that transverse contractions as measured with the balloon were always correlated with occurrence of spike bursts. Thus electromyography will account for transverse contractions detection. Further studies will therefore be performed without the use of an intraluminal balloon.
(2) This is an important point. The question arises whether phase-locking is necessary for such correlations.

J. Christensen (USA): Could you see that the longitudinal contractions moved along the jejunum across the field in which you were recording?

Mendel: The method used, i.e. measurement of distance change between two points, in the longitudinal arc of the intestine, contraction propagation cannot be recorded. During intense activity fronts, moving contractions have been noted visually. However, such visual observation cannot separate longitudinal and transverse components of the movement.

C. F. Code (USA): It does seem to me that with our conventional recordings we are not recording the electrical counterparts of contraction in the longitudinal muscle of the small bowel. I think we have recorded longitudinal muscle electrical activity in the fundus of the stomach but not in the small bowel. Your records seem to bear this out. What is your view?

Mendel: Spike bursts have been recorded together with pendular contractions in the longitudinal muscle of rabbits' duodenum (Gonella, J., 1970, J. Physiol. Paris, **62**, 447). Our records showed no electrical counterpart of longitudinal contractions during dissociated motor activity. During the intense activity front, much more rapid spikes were present in each burst. Part of these spikes may perhaps be related to longitudinal contractions.

9

Laxative effects on small intestinal electrical activity of the conscious dog

W. D. ATCHISON, G. J. KLASEK AND P. BASS

In an earlier study[1], we described a unique pattern of intestinal spiking activity recorded from the conscious dog in response to castor oil and its constituent fatty acid, ricinoleic acid. This pattern consisted of repetitive clusters of spike potentials which migrated over the jejunum, and was unlike that produced in a digestive or interdigestive state, or following an equal dose of a non-cathartic oil, triolein. Other investigators have described a similar electrical phenomenon in the rabbit ileum with ricinoleic acid[2], cholera toxin[3] and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)[4]. These agents also alter intestinal fluid absorptive systems, and are all known to be diarrhoeagenic[5–8].

The present study was undertaken to compare the effects of phenolphthalein and magnesium sulphate – two commonly used laxatives – on intestinal electrical activity. This study could further demonstrate that the unique diarrhoeagenic pattern in the small bowel reported for castor oil[1] was common to other laxatives.

METHODS AND MATERIALS

Extracellular, silver unipolar recording electrodes were implanted in the four mixed-breed dogs of either sex weighing between 10 and 15 kg as previously described[1,9]. Briefly, eight electrodes were implanted on the serosal surface of the jejunum in two groups of four at two intestinal sites. The first group was placed approximately 6 cm caudad to the ligament of Treitz, and the second group was placed on the mid-jejunum, 60 cm caudad to the ligament of Treitz. The four electrodes in each group were spaced at 2.5 cm intervals.

The following drugs were used in the course of this study: castor oil (Ruger Chemical Co.), phenolphthalein diphosphate-tetrasodium salt (Sigma Chemical Co.), and magnesium sulphate-heptahydrate (Mallinckrodt A.R.).

Experimental protocol

Following an 18 h fast, unipolar electrical potentials were monitored with a Beckman Dynograph set at a time constant of 0.3 s. Immediately after passage of the migrating myoelectrical complex (MMC) over the most caudal electrode[10], 40 ml of castor oil, or 30 ml of phenolphthalein (150 mg free base), magnesium sulphate (30% w/v), or sodium chloride (0.9% w/v) was administered by gastric tube, and electrical recordings monitored for the next 2 h. To avoid emesis, magnesium sulphate was administered in two 15 ml doses separated by a 20 min interval. All three laxatives produced diarrhoea within 8 h.

Recordings during the fasted state were obtained for a 2 h period beginning immediately after the MMC swept over the electrodes on the mid-jejunum, while fed studies were obtained for a 2 h period immediately following feeding of 200 g of canned dog food (Vets, Perk Food Company, Inc., Chicago, Ill.). All experiments were randomized and duplicated in each animal.

Data analysis

All data were visually inspected, and one electromyogram from each group of four was selected for quantitative analysis. These two channels were selected based on clarity of recording. The same channels were used for data quantification throughout the experiment. The total number of basic electrical rhythm (BER) with superimposed spike potentials for the duration of the 2 h recording sessions were counted for both areas monitored. Comparison of the total number of spike potentials at the two locations by Student's paired *t*-test[11] indicated no significant difference, so the data were pooled. The total number of BER accompanied by spike potentials produced by each condition were compared by analysis of variance, and the means compared using Duncan's multiple range test[11].

As previously described[1], the various patterns of spike potentials observed were quantified by measuring intervals between adjacent spike-containing BER and scoring them in one of four time-intervals: 0–5, 5–10, 10–20, and > 20 s. The results were plotted as the mean total number of BER with associated spike potentials which occurred in each time-interval for each treatment. These data were compared by means of Chi-square contingency analysis[12]. This test compared the number of spike occurrences as well as their time-distribution. Chi-square analyses were necessary since spike potential activity was skewed to the left and statistics of normal distribution were inappropriate.

RESULTS

Occurrence of spike potentials

The total number of BER with associated spike potential produced during the interdigestive, digestive and drug states are shown in Table 9.1. The spike potentials produced in response to castor oil were equivalent to that produced by feeding, but were significantly increased when compared to the interdigestive state ($p < 0.05$). The administration of isotonic saline, phenolphthalein, or magnesium sulphate produced spiking activity which was statistically equivalent ($p > 0.05$) to the interdigestive state.

Table 9.1 The number of spikes produced during a 2 h interval: a comparison of fasted, fed, and drug states

Drug condition	*Mean number of BER with spikes generated in 2 h post-dosage** (± SEM)	*Comparison of means of Duncan's range test*†
Interdigestive (fasted)	413.6 ± 67.3	
Phenolphthalein	478.3 ± 85.2	
NaCl‡	565.4 ± 63.5	
Magnesium sulphate	623.0 ± 78.4	
Castor oil	707.8 ± 115.3	
Digestive (fed)	846.5 ± 72.4	

* Each mean represents the average of two replicates at two sites for four animals
† Any two means not paralleled by the same line are significantly different ($p < 0.05$)
‡ 0.9% w/v

Total spiking activity at the upper jejunal site was compared over four consecutive ½ h intervals for castor oil and the interdigestive state (Table 9.2). In response to castor oil, a relatively uniform level of spiking activity was observed during all four time-intervals. In contrast, during the interdigestive state, only the last hour had an increased level of spike activity. This increase may be attributed to the passage of the MMC.

Table 9.2 Comparison of electrical spiking activity during four ½ h intervals during the interdigestive state or following castor oil administration

	Time (min)			
Condition	*T*0–30	*T*31–60	*T*61–90	*T*91–120
Interdigestive	16.1 ± 9.0*†	43.7 ± 17.4	194.6 ± 24.6	191.9 ± 31.3
Castor oil	126.8 ± 27.4	183.2 ± 39.3	201.1 ± 22.5	229.4 ± 18.5

* Each value is expressed as mean number of spike potentials (±SEM) observed during the ½ h recording period
† Each value represents an average of two replicates for each animal, and $n = 4$ for each condition

Patterns of spike potentials

Distinctly different patterns of electrical spiking activity were observed under the conditions studied (Figure 9.1). The cyclical interdigestive pattern of basal, pre-burst (not shown) and burst activity (Figure 9.1, top two panels), and the random electrical spiking activity characteristic of the digestive state (third panel) were confirmed in all four dogs. Both of these patterns have been described previously.

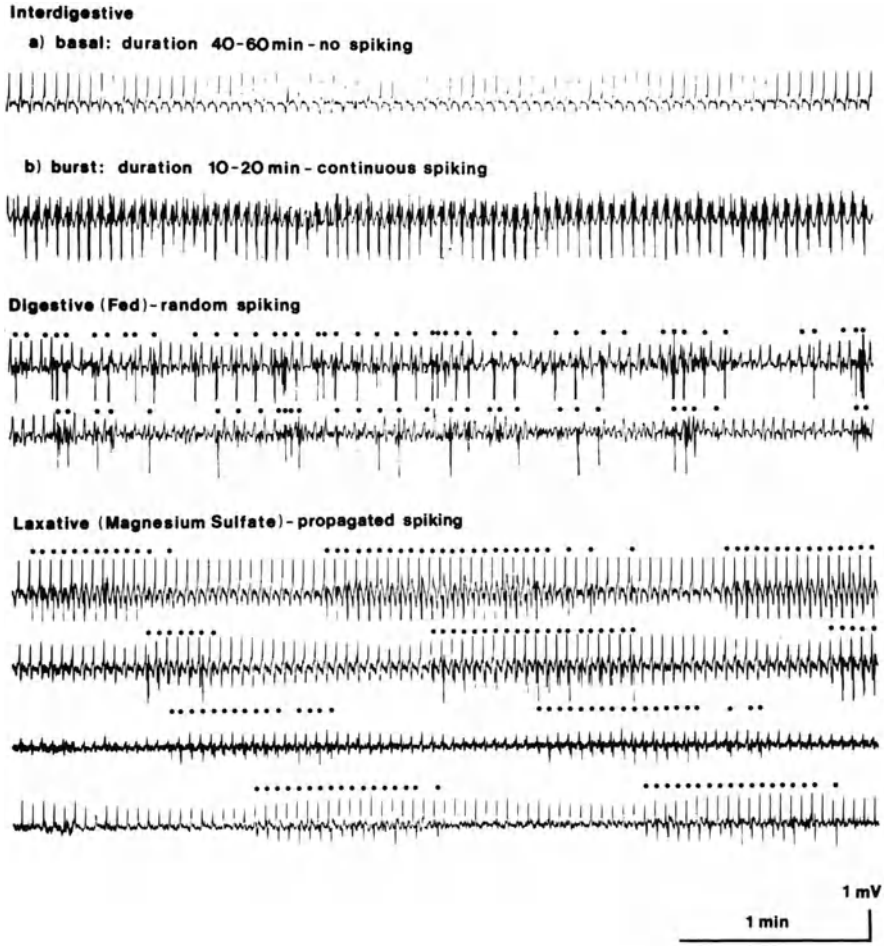

Figure 9.1 Representative samples of electromyograms recorded during interdigestive (a) basal and (b) burst, digestive, and laxative-induced states. Electrode placement is on the upper jejunum. Dots (●) have been placed over each spike potential in the lower two panels. The time and voltage scale are shown; time constant = 0.3 s. Four distinct patterns of spiking activity are shown. Spike potential activity in response to the laxative occurs in distinct bursts on continuous BER. The propagation of these waves of activity is illustrated

Administration of isotonic saline produced an alteration of the interdigestive state similar to that seen following feeding, but the normal interdigestive pattern returned within a few hours.

A third pattern of electrical activity was observed following oral administration of castor oil, magnesium sulphate, or phenolphthalein (Figure 9.1, bottom panel). This pattern consisted of recurrent bursts of 3–15 spike potentials associated with consecutive BER. These waves of spike potentials appeared at both intestinal sites and migrated caudad over all four electrodes at each site. The time of onset and duration of this laxative-induced pattern (LIP) varied for each cathartic. For castor oil a digestive pattern was initiated immediately upon dosing. This pattern was converted into a LIP within approximately 40–60 min and was observed for as long as 72 h. The LIP onset following magnesium sulphate generally occurred within 15–20 min of dosing; however it seldom lasted the duration of the recording session, and normal interdigestive patterns were not altered on succeeding days. Very little delay in LIP onset was observed following phenolphthalein. The LIP with this drug occasionally lasted 24–48 h.

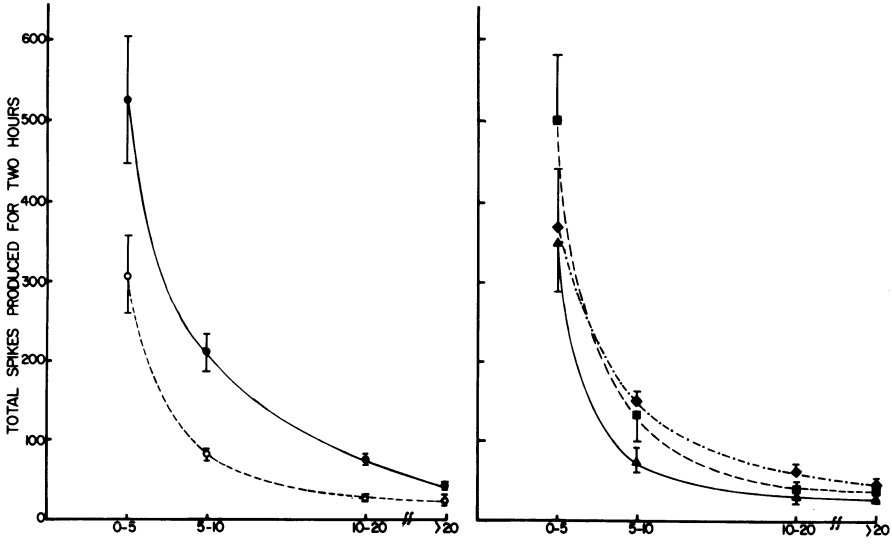

Figure 9.2 A comparison of the distribution of spike potentials which were recorded from the jejunum as a function of the time interval between adjacent spikes for the interdigestive (– – ○ – –) and digestive states (——●——) (left panel), and for magnesium sulphate (·–·–◇–·–·), castor oil (– –■– –), and phenolphthalein (——▲——) (right panel). Inter-spike intervals were measured for the 2 h recording session and grouped into one of four time categories: 0–5, 5–10, 10–20, and > 20 s. Each distribution consists of pooled data at two jejunal sites with two replicates per treatment, per animal; $n = 4$ animals. The points represent the mean total number of spike potentials (±SEM) that occurred in each time-interval. Note that the laxative distributions all fall between those of the interdigestive and digestive states.

The distribution of spike potential activity was compared using a Chi-square contingency test, which compared both the number and distribution of spiking occurrences in the 2 h period (Figure 9.2). The pattern of spike potential activity produced following castor oil, magnesium sulphate and phenolphthalein differed from those produced in the fasted state or following feeding or NaCl administration ($p < 0.05$). The patterns produced during the digestive and interdigestive states differed between themselves ($p < 0.01$), and the distributions of spike potential activity produced by the various laxatives also differed among themselves ($p < 0.05$).

DISCUSSION

Though phenolphthalein, castor oil, and magnesium sulphate have been used clinically as laxatives for years, the mechanism by which they produce catharsis is still poorly understood. Phenolphthalein and castor oil, classified as 'stimulant cathartics', are purported to produce their effects by 'irritation', of the gastrointestinal tract. Through 'irritation', the drugs are then assumed to increase intestinal contractile activity, thereby moving the luminal contents at a more rapid rate, and decreasing the absorption of fluids from the small bowel. However, no studies to date have conclusively demonstrated a stimulant action of these drugs on intestinal motility, and recent evaluation of the literature[13] does not substantiate this claim.

In addition to motility, an absorptive component to the action of these laxatives may exist. Phenolphthalein has been shown to inhibit Na-K ATPase[14] and may act in part by decreasing intestinal absorption[15,16]. Similarly, castor oil has been shown to affect absorption and membrane functional integrity nonspecifically[17–19]. Thus, both the absorptive membrane and the smooth muscle of the small intestine are apparently affected by these agents.

The mechanism of action of magnesium sulphate is also poorly understood. Historically, magnesium sulphate laxation has been attributed to poor absorbability resulting in intestinal hyperosmality; this mechanism is also unproven[13]. Magnesium sulphate, like ricinoleic acid, produces decreases in total gastrointestinal motility and hypertonic mucosal solutions of magnesium sulphate reduce net intestinal water absorption[20]. More recently, Harvey and Read[21] have speculated that magnesium sulphate acts through the release of cholecystokinin (CCK), but this has yet to be proven by measuring CCK blood levels. It has been shown that intraduodenal administration of bolus amounts of the C-terminal octapeptide of CCK produces a biphasic inhibitory effect on duodenal contractility similar to that produced by ricinoleic acid[22]. So conceivably, both laxatives may act by releasing an endogenous substance.

Previously, we indicated that castor oil, and its active constituent ricinoleic acid, produced a novel pattern of intestinal spiking activity in the dog[1]. This

LIP, consisting of repetitive bursts of 3–15 spike potentials which migrated the length of the jejunum, also occurs with phenolphthalein and magnesium sulphate. However, the three laxatives did have a different latency to onset of the LIP. While phenolphthalein and magnesium sulphate had a relatively rapid onset, a 40–60 min interval preceded the LIP onset for castor oil. This delay may reflect inhibition of stomach emptying of the oil. An analogous pattern, referred to as the migrating action potential complex (MAPC) has been described by Mathias *et al.* The presence of the MAPC was detected in ligated ileal loops following administration of ricinoleic acid[2], cholera toxin[3] and $PGF_{2\alpha}$[4]. Thus, a number of diarrhoeagenic agents produce altered patterns of intestinal spike potentials, which may be indicative of the diarrhoeal state.

Whether the LIP is a result of other drug-induced actions, or represents a primary action of the cathartic, is not yet clear. The diarrhoeal actions of many laxatives have been attributed to accumulation of intestinal fluids, either through active secretion or inhibition of fluid and electrolyte absorption. Fluid accumulation in the bowel could produce distension, resulting in the observed laxative pattern. Mechanical distension of the small intestine has been shown to initiate propulsive wave patterns using *in vitro* systems[23–25], so it is conceivable that the patterns observed here could result from the inhibition of fluid absorption systems.

Alternatively, the electrical patterns themselves may in part be responsible for the diarrhoeal effects of these agents. In the absence of other intestinal electrical activity, these migrating waves could be responsible for rapidly moving the intraluminal contents caudad. The narcotic analgesic-induced incidence of spike potentials are an example of the contributions which electrical patterns can provide to an altered gastrointestinal state – i.e., prolonged transit time[26].

It was previously demonstrated that castor oil and ricinoleic acid produced no stimulation in electrical activity when compared to a fed state[1]. In this study this lack of stimulation is confirmed, and extended to include magnesium sulphate and phenolphthalein. In fact, total spike potential activity in response to phenolphthalein and magnesium sulphate was statistically indistinguishable from a fasted state or a sodium chloride test meal. Thus, 'irritation' or 'stimulation' of the gastrointestinal tract does not follow administration of laxatives, and plays no significant role in their cathartic actions.

In conclusion, laxatives possess multiple effects. They can inhibit fluid absorption, produce non-specific membrane effects which may alter absorption, or affect smooth muscle function. Any of these factors may contribute to their diarrhoeagenic action.

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Discussion

K. Kelly: Did you measure and correlate the occurrence of spikes on slow waves as detected by *adjacent* electrodes in the various experimental conditions? Laxative-induced spikes might show a 'peristaltic' pattern, whereas food and triolein might not.

G. Gullikson: (USA) This was not done, though it is something to keep in mind for future studies. We instead selected just one electrode in each group of four to quantify in terms of inter-spike intervals. We merely made the observation of migration down the bowel by visual inspection.

H. L. Stockley: (UK) It is generally assumed that castor oil is hydrolysed to ricinoleic acid before acting. Was there a delay in onset of action in your experiments with castor oil compared to ricinoleic acid?

Gullikson: There was a latency of onset for the migrating pattern of approximately 40–60 min for *both* castor oil and ricinoleic acid. We ascribed this delay primarily to the delay of emptying of the oil from the stomach.

A. Bennett: (UK) Castor oil is one of the few substances that acts on the small intestine. What effects are there of other laxatives, such as bisacodyl, on the small intestine? Have you measured colonically active substances on electrical changes in the colon?

Gullikson: Our laboratory has seen the same type of migrating spike potentials following the administration of magnesium sulphate and phenolphthalein. Although we have not tried bisacodyl, I would not be surprised to see the same type of activity. These migrating spike potentials are most clearly seen in the jejunum as compared to other parts of the small bowel. We have not implanted electrodes in the colon in order to observe the pattern, as from our experience it would then be quite difficult to obtain a distinctive record.

J. Christensen: (USA) A moment ago you mentioned phenolphthalein. Is it a laxative in the dog? I ask because it is not in the cat.

Gullikson: Diarrhoea was produced in all five dogs in a study in our laboratory in which 150 mg of phenolphthalein was administered orally.

10

Migrating myoelectrical complexes: disruption, enhancement and disorganization

L. BUENO AND Y. RUCKEBUSCH

Migrating myoelectrical complexes (MMC) first identified in the small intestine of the fasted dog as 'a caudad-moving band of large-amplitude potentials'[1] have been recorded in several other species including rats, guinea-pigs, rabbits, pigs, cattle and horses[2]. They have been also observed to operate in the human duodenum on the basis of mechanical[3,4] and electrical recordings[5].

Common characteristics of the MMC pattern are:

1. an alternation of periods of quiescence and electrical spiking activity occurring at regular intervals;
2. the existence of two consecutive phases, one of irregular spiking activity (ISA) followed by regular spiking activity (RSA) when all slow waves have associated action potentials[6,7];
3. the distal propagation of the phase of RSA at a velocity positively related to the length of the small intestine[8];
4. the intermittent flow of the intestinal contents, two thirds of which takes place in the 4–6 min preceding the occurrence of phases of RSA[9,10]; and
5. the persistence of these complexes after vagotomy[11] and removal of the splanchnic nerve supply, although major components of the MMC (number of complexes, velocity of propagation and duration of ISA) will be modified[12].

We have tested the hypothesis that the MMC pattern which corresponds essentially to spike activity in circular muscle layer[13] is an intrinsic activity of the small intestine which can be influenced by the amount of digestive bulk and modified specifically in different pathological situations.

METHODS

Ten dogs, four pigs, twelve sheep and four ponies on normal *ad libitum* diets, or being fed one or two meals a day, were prepared with chronic electrodes and/or cannulae. Some animals were equipped with an electromagnetic flow transducer inserted 10 cm below one jejunal electrode site, and this transducer was connected to an electromagnetic flowmeter (Nycotron Drammen, Model 375, Norway). The electrical activity was either recorded directly or was integrated at 20 s intervals by linear integration of the amplitude and duration of the spiking activity[14], while flow of digesta was simultaneously registered on a polygraph. Continuous recordings (24 h/day) were started 10–14 days after surgery and were continued for several weeks.

In sheep, a piece of silastic tubing was looped around the jejunal segment and both ends exteriorized via a polyvinyl chloride tube. This enabled partial or complete experimental occlusion of the bowel to be accomplished by pulling on the ends of the silastic tubing. A flow bypass of a segment of the intestine was achieved by connecting extracorporally two 'T' cannulae placed 4 m apart in the jejunum. An increase in the digestive bulk was obtained by the infusion via the orad cannula of digestive contents taken from another animal[15] at a rate of 150 or 300 ml/h.

Diarrhoea could be induced either by sudden changes of diet, such as giving an excess of grain as opposed to hay, or by perfusion into the duodenum of hyperosmotic D-mannitol. It was also observed in animals with strongyloides infestation[16].

The time necessary to re-establish normal patterns of motility was studied after several different surgical procedures using general anaesthesia.

The disappearance of the MMC pattern for a few hours with the obliteration of both the phases of quiescence and RSA is termed *disruption*. A continuous random high level of ISA is recorded until the occurrence of a phase of RSA followed by quiescence is re-established.

Enhancement refers to the presence of supernumerary complexes on the proximal part of the small intestine as well as the propagation along its distal part of complexes which usually fade out before reaching the ileum[17].

Disorganization of the MMC pattern consists of repetitive groups of three to twenty spike bursts more or less fused and propagated at high velocity. The pattern of recovery is similar to that following disruption.

RESULTS

Disruption of the MMC pattern

In dogs receiving a daily meal of 600 g of canned food containing 20% dry matter, the duration of the phase of ISA varied between 30 and 70 min, and this was negatively correlated with the time which elapsed after feeding, i.e. 6–24 h. Immediately after feeding the mean flow rate was tripled (from

129 ± 17 ml/h to 436 ± 125 ml/h) and was accompanied by continuous spike activity 30–50% greater than that in the ISA phase. In pigs with free access to food the mean daily intake was 30–40 g dry matter per kg live-weight ingested in ten to fourteen feeding periods. The mean digesta flow rate was increased by 50% after each snack without any disruption of the complexes. When the ration was supplied in two meals per day, the flow of digesta suddenly increased by 300% and the MMC pattern was disrupted for 2–3 h (Figure 10.1).

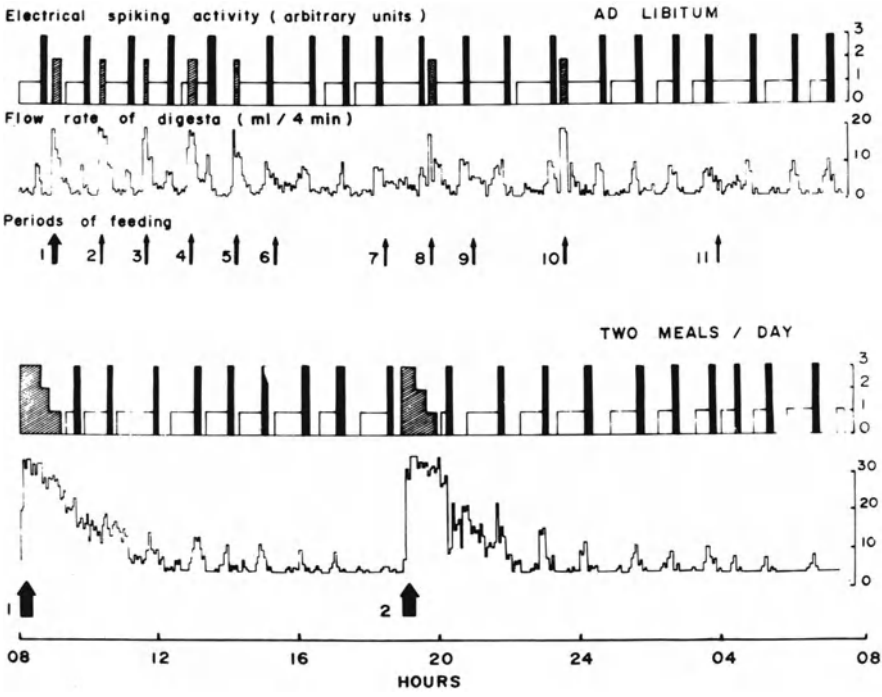

Figure 10.1 Patterns of spike activity and digesta flow rates of digesta in proximal jejunum of the pig when fed *ad libitum* or given two meals a day. High levels of irregular spike activity (hatched columns) are related both to the periods of feeding (arrows) and the increased flow rates. Disruption of the complexes shown by white columns (irregular spike activity) and black columns (regular spike activity), occurs when the flow rates rise above 300 ml/h

In sheep, an increased duration of the phase of ISA also corresponded to an increased flow rate and beyond 500 ml/h the phases of quiescence and RSA disappeared. This pattern of activity was similar to that recorded after feeding in dogs (Figure 10.2).

Enhancement of the complexes

Both in dogs and sheep, the mean number of complexes identified in the proximal jejunum and propagated on the distal part of the small intestine

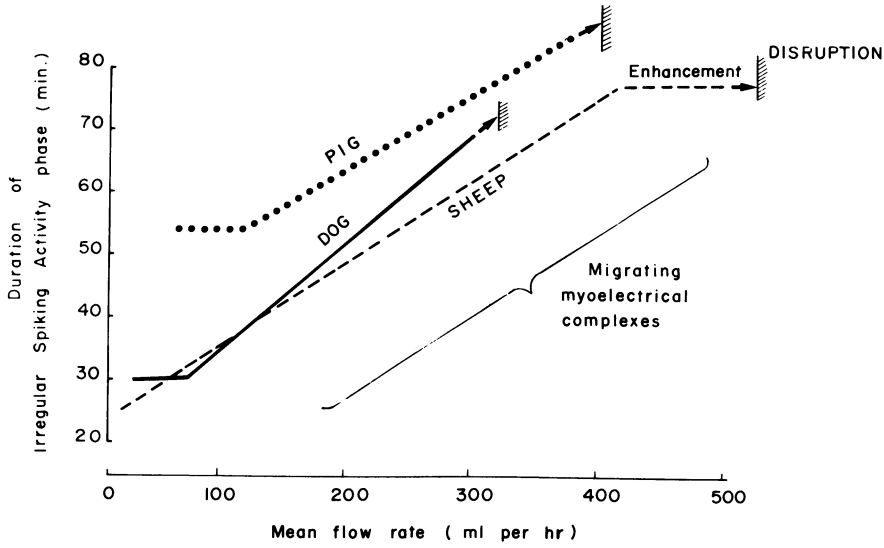

Figure 10.2 Relationship between flow rate of digesta and the duration of irregular spike activity in the pig, dog and sheep. Disruption of the cyclic patterns in the proximal jejunum occurs in pigs and dogs when the flow rates increase. An infusion of additional digesta at the rate of 300 ml/h is needed before such disruption will occur in sheep

increased from 60 to 90% when digesta flow rate was increased. On a normal diet of hay with a mean digesta flow rate of 392 ± 87 ml/h in sheep, only twelve of the seventeen to nineteen complexes starting at the duodenum reached the ileum. A 10–20% increase in food intake allowed all the duodenal complexes to reach the ileum. With a further increase of food intake providing a mean digesta flow of 510 ± 121 ml/h, eight to twelve supernumerary complexes started at the jejunal level each day (Figure 10.3). In the pony allowed oats for 10 h/day, the number of complexes recorded on the proximal jejunum averaged four to six. When receiving hay for the same period, the bulk of the digesta was nearly tripled and twelve to fourteen complexes were identified.

Disorganization of the MMC pattern

The periods of quiescence were replaced by a high level of ISA in the orad segment when the intestinal lumen was partially occluded in sheep. In this case the spike activity consisted of repetitive groups of spike bursts. In contrast, well-patterned phases of RSA developed on the aborad segment during partial occlusion.

After excess ingestion of grain in the herbivores, the duration of the ISA increased and tended to become continuous for 12 h. The phases of RSA then disappeared and spike activity in repetitive groups of spike bursts invaded the whole small intestine for the next period of 12 h (Figure 10.4). Short phases of RSA and subsequent inactivity reappeared when recovery was underway.

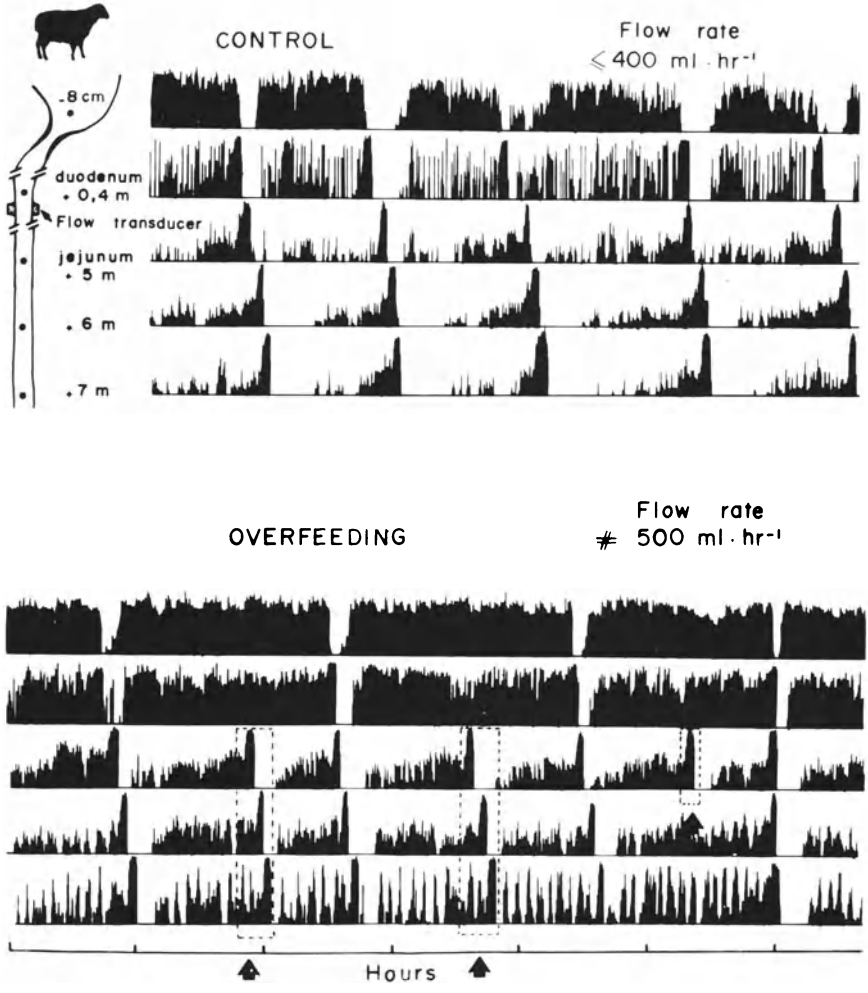

Figure 10.3 Enhancement of the MMC pattern in sheep. Electrical spike activity recorded at five sites from the antrum to the proximal jejunum under normal or overfed conditions. When feeding with an intake of 1200 g/day of hay (control), complexes started in the duodenum at intervals of 60–90 min and lasted 50% of the recording time in the jejunum. With increased food intake (1500 g/day) a higher level of irregular spike activity both in duration and intensity occurred in the duodenum (arrows)

Inhibition of electrical spike activity by laparotomy and surgical procedures, such as colonic resection, was less marked in the duodenum than in other parts of small intestine both in dogs and sheep, and affected more the phases of ISA than that of RSA. The period of inhibition ended when the irregular contractions slowly amassed to form complexes and progressively moved down the small intestine.

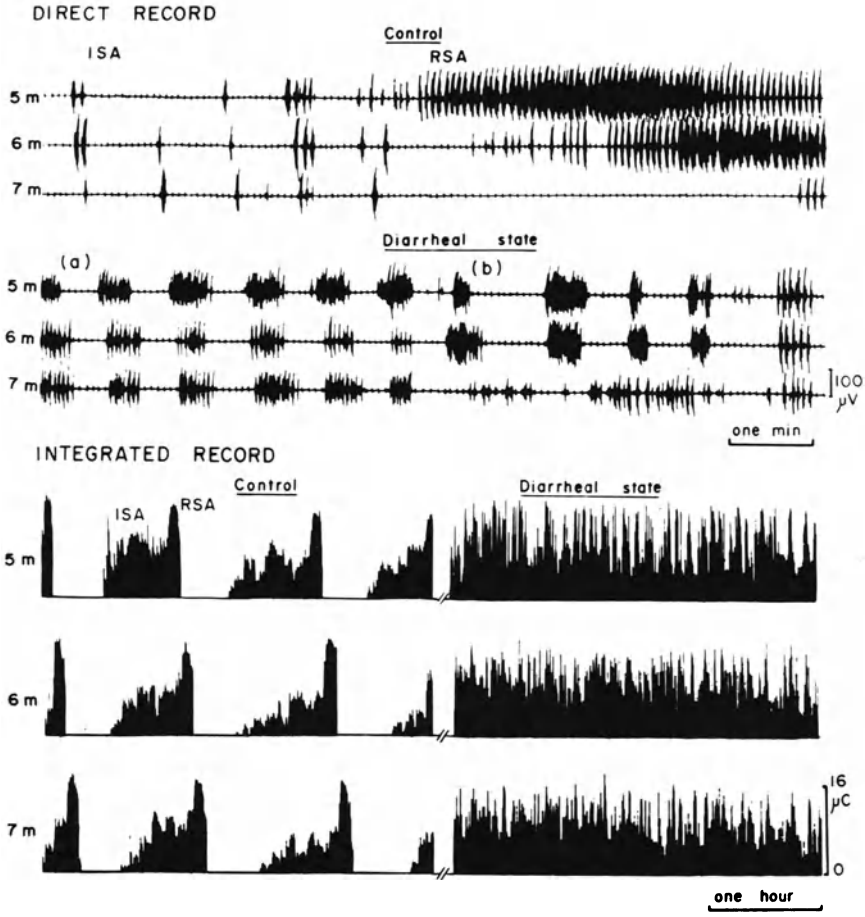

Figure 10.4 Disorganization of the MMC pattern shown in the jejunum 5 m from the pylorus during the diarrhoeal state in sheep. Electrode sites at 1 m intervals were used for direct and integrated records. In the healthy animal, spike bursts moved at a high velocity (30 cm/s) during the phase of ISA. During the RSA, the spike bursts occurred regularly and the phase moved at low velocity (40 cm/min). Two patterns of continuous spike activity are shown at the onset of the diarrhoeal state: spike bursts in repetitive series (a), or more condensed (b). The changes of temporal organization of the electrical activity are illustrated by integrated record

DISCUSSION

The results of this study of four different species showed a natural tendency for the electrical spike activity of the small intestine to occur as spike bursts which are irregularly and then regularly superimposed on the slow waves. These phases are then followed by a period of inactivity. The passage of digesta occurred intermittently during the phase of ISA, its propulsion being more efficient near the peak of high pressure gradient level induced by the

phase of RSA. The phases of ISA, which occupied about 50% of the recording time and are followed by the slow migration of the phases of RSA, are able to propel about 200 ml/h of digesta in pigs, dogs and sheep. Although removal of the extrinsic nerve supply modifies the intensity of spike activity, the ISA phases and the frequency of the complexes by altering the duration of the ISA phases[1,2], the MMC pattern persisted.

Three different possibilities are available to deal with the increased flow of digesta delivered into the duodenum:

1. the duration of the phase of ISA can be increased at the expense of the phase of quiescence;
2. all the complexes beyond the proximal area may continue on to the distal part of the bowel; or
3. the number of complexes at the jejunal level can be increased.

The sudden increased flow of digesta resulting from gastric emptying when the ration is divided in only one or two meals a day cannot be achieved in this manner. The complexes (ISA + RSA phases) will be disrupted and the flow is carried out by a continuous but irregular and high level of spike activity in which sometimes two or three spike bursts may be fused.

The disorganization of the MMC pattern which occurs with the onset of the diarrhoea, with the occlusion of the intestinal lumen or during recovery from surgical procedures, consisted of repetitive groups of spike bursts. The decrease in the level of spike activity and the occurrence of periods of inactivity promote the development of the two successive phases of ISA and RSA, i.e. the normal MMC pattern.

These studies indicate that propulsive activity of the small intestine is directly mediated by the MMC, both the duration and level of the ISA phases as well as the intervals between the RSA phases being related to the volume of food residues and chyme. Sudden increases in the volume of contents, as well as different pathological situations, lead to either disruption or disorganization of the MMC pattern.

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Discussion

D. L. Wingate: (UK) Although you have demonstrated effects of large volume and flow in sheep or pig, do you think this is important in carnivores where small volume meals such as arachis oil cause great disruption?

Y. Ruckebusch: (France) My hypothesis is that the basic MMC pattern, i.e. ISA phase followed by RSA phase, may be considered as an ultraradian rhythm of the small bowel in all species. About the factors involved in the disruption: the sudden increase in volume of digestive contents is a major one, the nature of the diet being the second one via release of insulin, glucagon, etc. It is true that the dog is more sensitive than other species to disruption but the volume of a meal is nearly doubled at the jejunal level by gastric bilio-pancreatic secretions and thus we have to be careful about our deductions. In the case of oil, whatever the volume, there is not a disruption of the MMC pattern but an inhibition of the electrical spike activity[7]. After feeding, the spike activity is continuously irregularly superimposed on the slow waves and the intensity is about twice that seen during the phase of ISA of a myoelectric complex.

E. Atanassova: (Bulgaria) We succeeded in influencing the duodenal spike activity by electrical stimulation. At the end of a normal migrating complex we began a stimulation with impulses with a frequency of 15 Hz. We evoked spike activity all through the quiescent period. The next migrating complex began; in other cases after the migrating complex began (phase 2) we stimulated with a frequency of 50 Hz. We succeeded in suppressing this period of spike activity. Further investigations of ours showed the role of the intrinsic nervous system in regulating the gut spike activity.

Ruckebusch: The persistence of the MMC pattern after vagotomy and section of the splanchnic nerve does not mean that they are not involved in regulating the propulsive function of the small intestine. Enhancement of the ISA phase by direct stimulation of the gut at 15 Hz, or its inhibition at 50 Hz, cannot be compared to changes in the volume of digestion.

J. S. Davison: (UK) I would like to make a point about volume receptors. It should be noted that the only receptors we have seen which could subserve this function are in fact tension receptors, which respond not only to stretch but also to active contraction of the muscle wall. Hence the influence of a given volume depends on the underlying tension in the muscle and may therefore vary from test to test. If such receptors are responsible for the suppression of MMC after a meal they might also account for the phasic nature of the interdigestive complex; that is each burst of activity will activate the inhibitory mechanism and lead to suppression of the complex.

Ruckebusch: I agree, and possibly the phase of quiescence that followed the phase of RSA is a period of inactivity linked to the inhibition subsequent to the active contraction of the circular muscle layer during the 5–7 min period of RSA.

Section II
Intestinal Polypeptides

11

Treatment of diverticular disease with hydrophilic colloids

M. A. EASTWOOD AND A. N. SMITH

There have been radical changes recently in the treatment of diverticular disease, mainly resulting from the introduction of dietary fibre into the regimen. The rationale of treatment is based on observations that diverticular disease is characterized by a low stool weight, prolonged intestinal transit time and raised intracolonic pressure, all of which are thought to be secondary to a reduced dietary fibre intake[1]. The object of treatment therefore is geared to the reduction of the enhanced intraluminal pressure, and all the various agents used in treatment would be expected to share in this.

However, the problem is what fibre should be taken. The usual fibre that is suggested is cereal fibre in the form of bran; but it is not clear what form of bran is advisable, nor is it known if other fruit and vegetables are of equal value. There are several approaches to the selection of fibre: on the one hand one can select the fibre's chemical constituents, whether its content are cellulose, pectin, hemicellulose or lignin. However, this information is not readily available. Alternatively a physico-chemical approach can be applied, using the water-holding capacity of the fibre, and to a lesser extent the cation-exchange capacity. Such measurements, however, are provisional in their value in predicting their effect on bowel function, in that the fibre may be metabolized by faecal flora. This leads to the production of hydrolytic end-products which may of themselves be absorbed from the caecum or may have biological effects of their own[2].

To this end, we have compared three hydrophilic materials which can be used in the treatment of diverticular disease: bran, which should pass along the gastrointestinal tract without alterations by bacteria; lactulose which is a synthesized disaccharide unabsorbed in the small intestine and hydrolysed by bacteria in the caecum; and Fybogel, an ispagula hydrophilic colloid.

The patients were all recruited from the X-ray department; i.e. they were not reporting specifically with symptoms applicable to diverticular disease. Each patient was interviewed by a dietitian and in addition kept a diary during the week of study. Stool was collected for one week, barium-impregnated markers were ingested and the transit time measured by fluoroscopy of the stool, in order to count the pellets in the stool. This method, incidentally, allows an assessment of the reliability of the stool collection. The patients also had a colonic motility test with an open-ended tube passed to 25 cm and recording at three points, 25, 20 and 15 cm from the anal verge. All these investigations were conducted before treatment and after 3 weeks of treatment. For their treatment, 20 g of bran, 20–30 ml lactulose or 2 g of Fybogel were taken, the dosages being those recommended by the manufacturers.

The effects of bran, Fybogel and lactulose were invariably to increase the stool weight, bran by an average of 30%; for Fybogel and lactulose the increase was of the order of 30–100%. Bran always reduced the intestinal transit time to approximately 50% of the initial recorded time. Fybogel and lactulose reduced the transit time to a variable extent.

A comparison was made of two types of cereal bran, one of a coarse nature with a water-holding capacity of 6 g of water per gram of fibre, and a second fine bran had water-holding capacity of 2.4 g of water per gram of fibre. The effect of both was to increase stool weight. The coarse bran, however, resulted in a decrease both of basal and post-food motility index; whereas the effect of fine bran was to increase the intracolonic pressure[3]. Fybogel increased the basal motility index and the post-food motility index by 25–40%. The effect of lactulose on motility index was varied and no consistent pattern was obtained. Incidentally all the patients claimed striking clinical improvement.

Here we have a situation in which therapeutic agents, which might have been expected in general to decrease intracolonic pressure, in fact failed to change (or even increased) intracolonic pressure. Furthermore there was no uniform relationship between symptoms and pressure. This raises some doubt about the overall importance of the 'features' of diverticular disease, i.e. low stool weight, the prolonged transit time and the raised intraluminal pressure. The stool weight and transit time for our forty-six patients with diverticular disease are in fact in the same range as are normal population studies in the same part of Edinburgh. These figures are derived from a study to define the characteristics of the population from which our diverticular patients come. The mean daily excretion of stool for the diverticular disease patients before treatment was 100 g (range 20–190 g/24 h), which contrasts with the population (age-range 16–80 years) with an output of 80 g/24 h (range 20–280 g/24 h). The mean transit time is of the order of 2–3 days with a range of 1–7 days. The mean transit time in the diverticular disease patients before treatment was 50 h (range 24–160 h). This contrasts with the normal population with a mean transit time of 72 h (range 25–168 h). The motility index for the

overall diverticular disease group showed a wide range. The basal motility was 1000 (range 0–4000) and the motility index after food 2100 (range 600–7000.)

This represents a considerable range of results both for stool weight, transit time and colonic motility index. The variable measured responses to bran, lactulose and Fybogel contrast to the overall clinical satisfaction of the patients.

The identification of patients in the past as having the hallmark signs of diverticular disease, i.e. low stool weight, prolonged transit time and a raised motility index, may more correctly be that of patients with special features which facilitated their identification.

Acknowledgements

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Discussion

N. Painter: (UK) I agree that some patients with diverticula have no high pressures; possibly their bowel is fibrosed and no longer active. Fine bran is less effective than coarse bran clinically but as there is no 'dose' the patient only has to take more bran if he is eating fine bran. We have had patients complain of pain on Fybogel.

J. De Carle: (Australia) To what symptoms were you referring when you said the patients 'improved'? This is a very poorly defined clinical entity.

M. A. Eastwood: (UK) These patients were recruited from the X-ray department rather than from the gastrointestinal unit clinic. The symptoms that led to having a barium enema vary – diarrhoea, abdominal pain, constipation. The only patients excluded were those with bleeding. A diary of symptoms and a clinical description of symptoms was obtained before and after treatment. Clinical improvement is vague but we are suggesting that these measurements such as motility index are also selected.

A. G. Johnson: (UK) Is it possible that your patients in Edinburgh are already eating a high residue diet?

Eastwood: No. Our diet surveys both for normal subjects, and patients with diverticular disease, show a very low intake of vegetable fibre.

J. Weinreich: (Denmark) I agree that coarse bran decreases the motility index, but it does not necessarily mean that the cause of colonic diverticula is bran deficiency in diet. Do you agree? I mean that the diverticula are age-dependent. Do you agree? Example: the meat-eating Eskimos do not have diverticula and they did have a very short transit time.

Eastwood: I agree that success with treatment has no aetiological implications. The development of diverticula is very age-related.

A. N. Smith: (UK) If diverticular disease patients report to a surgeon, this group (probably with secondary obstruction) have higher pressures, prolonged transit times and respond by having a lowering of their intraluminal pressure to bran. What I think my colleague is saying is that when he or a radiologist recruits cases, more generally found to have diverticula on medical check up – then there are many cases without raised pressure or transit times which are prolonged or faecal residues which are reduced. Therefore diverticula may not depend on pressure-pulsing effects; they arise from a change in the wall of the bowel. The pressure rise comes later when thick muscle acts as a barrier; this may be a secondary or sub-group. Even then the pressure rise is not central to the story because some agents which increase it (at this stage) nevertheless make patients feel better. We feel the pressure theory is not so exclusively part of the diverticular disease story as it has been developed up to now.

T. G. Parks: (N. Ireland) I agree that patients with diverticular disease often have a normal transit time, and this is true whether estimated by radio-opaque pellets or sodium chromate.

DISCUSSION

C. F. Code: (USA) We have now had the 'motility index' referred to this morning in three different ways. I think if this expression is to be used meaningfully then the authors should state to what 'motility index' refers.

W. J. Snape, Jr.: (USA) I would like to comment on Dr Code's point on the motility index. The motility index should not be used as the only expression of colonic contractility. We have found in patients with the irritable bowel syndrome, a disease that is probably related to diverticular disease, that the motility index was increased equally in both normal subjects by cholecystokinin or pentagastrin. However, the frequency of the contractile response following the hormonal stimulation was different in patients with the irritable bowel syndrome.

Eastwood: The question still remains if the colonic motility changes relate to symptoms or the genesis of diverticular disease. I suspect these are separate entities.

12

Control of motor activity in the lower oesophageal sphincter by motilin

I. AIZAWA, K. HIWATASHI, I. TAKAHASHI AND Z. ITOH

Recently it has been reported that motilin increases lower oesophageal sphincter (LES) pressure[1,2], but the physiological significance of its effect on the LES has not been elucidated. A perfused catheter method has generally been used to measure LES pressure; however, this method is not suitable for long-term measurement on conscious animals. To accomplish this, we have studied LES motor activity using force transducers chronically implanted on the serosal surface of the canine LES[3]. The present study was designed to determine the effect of motilin on the LES in the light of physiological changes in motor activity, both of the LES and stomach.

MATERIALS AND METHODS

As previously reported[3], the dog LES is simply a thickening of the muscle at the gastro-oesophageal junction, and a transducer sutured to the thickened muscle exhibits changes in motor activity of the LES which are similar to those measured by a traditional perfused catheter. In the present paper, therefore, we will only briefly describe the method of implantation of a transducer on the LES.

Strain gauge force transducers (transducers) were constructed in our laboratory according to a method described by Bass and Wiley[4], but with some modifications as reported previously[5]. The transducers were calibrated by hanging weights, which varied between 10 and 400 g. They were sterilized by boiling in water for 30 min before implantation.

Five mongrel dogs weighing 9–12 kg were used in this study. After they were anaesthetized with intravenous injections of Nembutal (25 mg/kg bodyweight), the abdominal cavity was opened. A transducer was sutured onto the

serosal surface of the LES. The index finger, inserted into the gastro-oesophageal junction through a gastrotomy made on the anterior wall of the stomach, was used as a guide. Two other transducers were similarly sutured; one on the serosal surface of the gastric body and the other on the antrum, to measure the contractile force of the circular muscle in each case. Lead wires of the transducers were bundled together, pulled out through a stab wound in the abdominal wall at the left subcostal margin, taken through a subcutaneous tunnel, and then through a skin incision between the scapulae. A stainless steel cannula was implanted in the stomach between the corpus and antrum[6]. This cannula was taken through a stab wound of the abdominal wall and fitted with a removable cap. Gastric pressure could be relieved through this cannula. A silastic tube (medical grade, Fr. size 6.5, Dow Corning, Midland, Mich., USA), for infusion of drugs and withdrawal of blood samples, was inserted into the superior vena cava from the external jugular vein, and its outer end was sutured to the skin[7]. All surgical procedures were performed under aseptic conditions. After the operation, the lead wires were assembled to a connector, and harness made of canvas cloth was put on the dog to protect the lead wires and the silastic cannula.

Contractile activity detected by the three transducers was simultaneously recorded on two polygraphs (RM-45, Nihon Kohden Kohgyo Co., Ltd, Tokyo, Japan). One was modified to have a paper speed of 1 mm/min. The second recorder, with paper speeds of 10–30 mm/min, was used to resolve details whenever desired.

Blood samples (1.0 ml) for motilin determination were drawn into heparinized disposable syringes from the silastic cannula at 15, 30, 60 and 120 min after the beginning of each meal and at intervals during the interdigestive period. Enzymatic degeneration of the motilin was prevented by adding 0.1 ml of Trasylol (10 000 KU/ml) to the sample. Plasma was separated in a centrifuge at 3000 rpm at 4 °C, and then stored at −30 °C until assay. Radioimmunoassay for motilin was carried out as reported in Reference 8.

Experiments were done in the conscious state 2 weeks after operation. Dogs were fed once a day at 10.00 p.m. with dog food (Gaines Meal, dry type, 20 g/kg body-weight) soaked with 200 ml hot water.

After all experiments were completed, autopsies were performed, and correct placement of the transducers was confirmed histologically.

Data obtained in the present study were analysed statistically with Student's t-test, and paired data differences having $p < 0.05$ were accepted as significant.

RESULTS

LES contractile pattern in the interdigestive state

It was found that the interdigestive contractions in the LES and the stomach

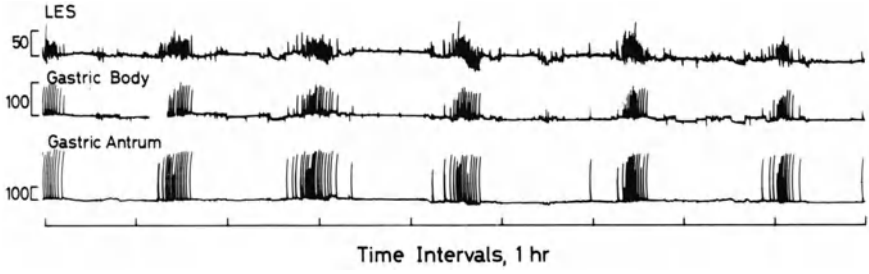

Figure 12.1 A 9 h record of contractile activity of LES and stomach during interdigestive state

Table 12.1 Duration of natural interdigestive contractions in the LES

Dog No.	*Contractions*	*Duration time* (min) *Quiescence*
A	26.5 ± 2.7*	73.8 ± 6.6
B	24.8 ± 3.1	67.4 ± 5.9
C	28.8 ± 3.6	80.5 ± 8.8
D	26.4 ± 1.9	78.3 ± 3.3
E	28.5 ± 2.7	82.4 ± 9.6

* Mean of ten observations in each of the five dogs ± SE

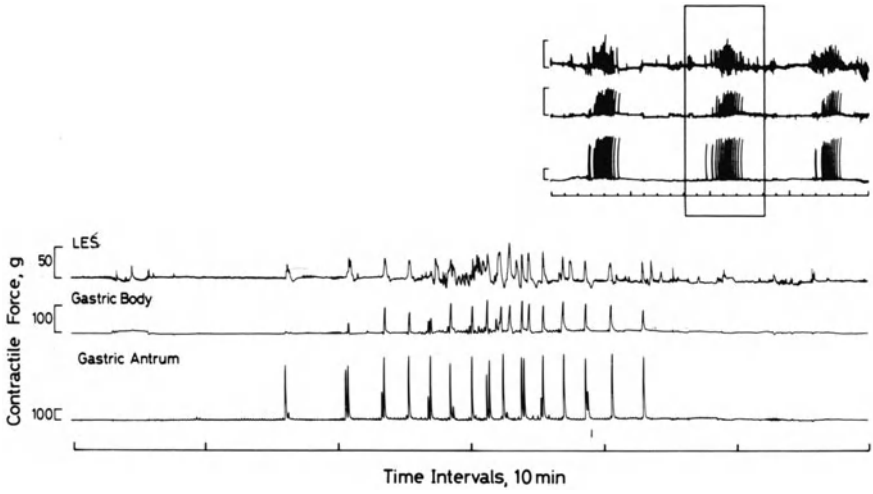

Figure 12.2 Single episode of interdigestive contractile activity of LES and stomach. The insets in this and Figure 12.5 show same record and adjacent episodes recorded at slower speeds

began in all dogs at an average of $14\frac{1}{2}$ h after feeding, and occurred at regular intervals until the next meal. Interdigestive episodes in the LES lasted for approximately 25 min, recurred at approximately 80 min intervals, and coincided with those of the stomach, as shown in Figure 12.1. Details of contraction duration and interval are shown in Table 12.1 for five individual dogs.

As seen in Figure 12.2, an LES episode consisted of 15–30 individual contractile waves of high amplitude. Frequency of the waves was 0.86 ± 0.09 min in five dogs.

Recordings at speeds up to 30 mm/min clearly show the synchronism between the LES and the gastric body contractions; this is shown in Figure 12.3. The coefficient of correlation between LES and the gastric body contractions was 0.98.

Interdigestive contractions did not change in amplitude, frequency, or duration at any of the positions even after the pressure decrease which followed opening of the gastric cannula, as shown in Figure 12.4.

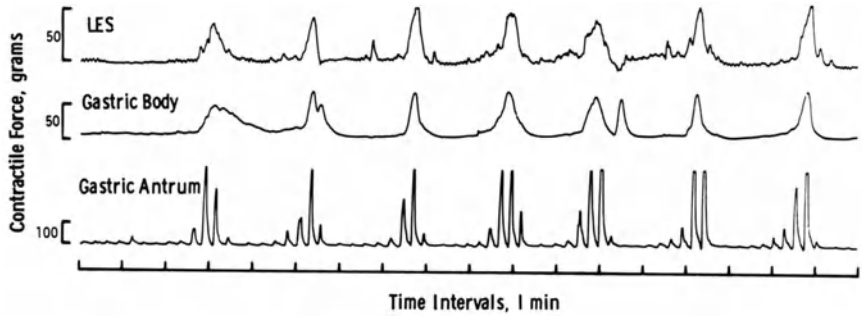

Figure 12.3 Relations between contractions of LES and gastric body during the interdigestive period

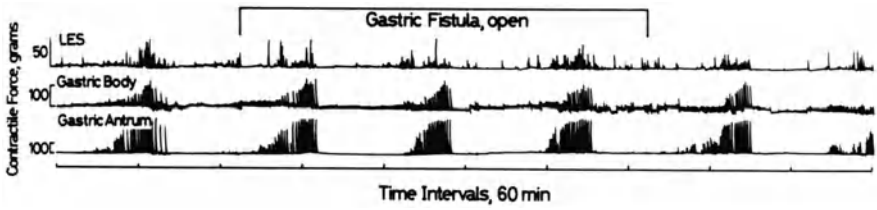

Figure 12.4 Interdigestive contractions of LES and stomach showing absence of effect of open gastric cannula

Effect of exogenous motilin on LES motor activity

Since the interdigestive contractions occurred at regular intervals, intravenous infusion of motilin at 0.9 μg/kg/h was started 10 min after termina-

tion of a natural contraction and continued for 40 min, in order to distinguish motilin's effect on the LES from the natural contractions. The contractile pattern induced by motilin was the same, both in frequency and amplitude, as that observed naturally in all three positions during the interdigestive state. Figure 12.5 shows the end of a natural episode and a motilin-induced episode. Detailed comparisons are summarized in Table 12.2.

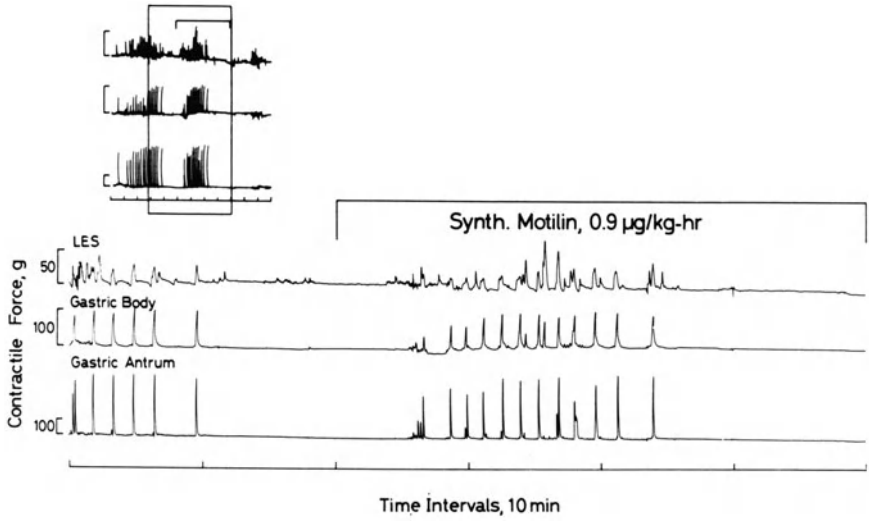

Figure 12.5 Effect on LES and stomach of intravenous infusion of synthetic motilin at 0.9 μg/kg/h for 40 min. First part of tracing shows natural contractions

Table 12.2 Comparison of the motor activity of natural and motilin-induced contractions in the LES

	Natural	*Motilin-induced*
Duration (min)	27.0 ± 3.0*	26.4 ± 4.8†
Contractile force (g)	47.3 ± 5.2	46.1 ± 3.7
Frequency (contractions/min)	0.86 ± 0.09	0.84 ± 0.08

* Mean of eight observations in each of the five dogs ± SE
† Mean of five observations in each of the five dogs ± SE

When the dose of motilin was increased the amplitude of contractions did not change, but the latency between the initiation of infusion and the beginning of contractions decreased. The inverse relation between motilin dose and this latency is indicated in Table 12.3. A similar response to motilin was also observed in the stomach, as reported previously[9,10]. Motilin had no significant effect on motor activity in the LES or the stomach during the digestive state.

Table 12.3 Dose response relationship between motilin and contractions in the LES

Dose of motilin (μg/kg/h)	*Onset time after infusion* (min)
0.3	10.5 ± 2.0*
0.9	4.1 ± 0.6
2.7	1.8 ± 0.07

* Mean of five observations in each of the five dogs ± SE

Relationship between plasma motilin concentration and LES motor activity

Increase of plasma motilin concentration correlated closely with the increased motor activity associated with the LES and stomach episodes during the interdigestive state. During the digestive state, motilin concentration in plasma never exceeded 35 pg/ml regardless of motor activity. The elevated level of plasma motilin concentration observed during the interdigestive state (291.3 ± 96.4 pg/ml) remained for approximately 20 min and then abruptly decreased to a low level (81.4 ± 40.6 pg/ml). This close correlation between increase in plasma motilin concentration and LES motor activity was observed during the interdigestive state in all of the five dogs (Figure 12.6). When animals were fed, there was a plasma motilin concentration decrease to a low

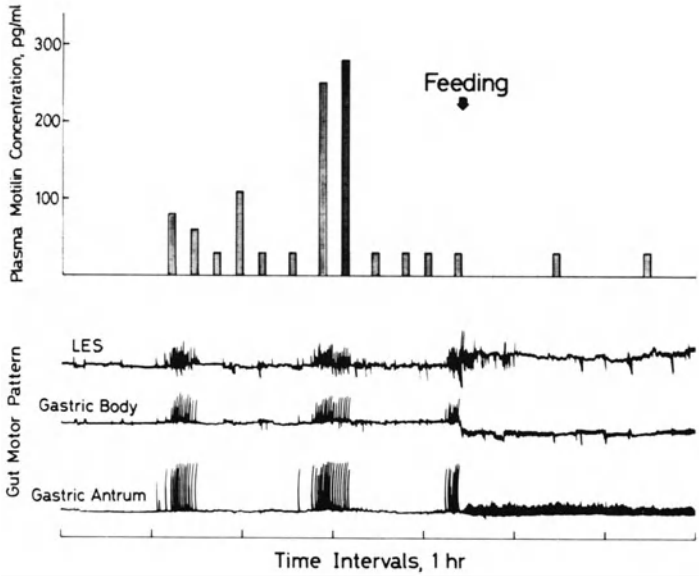

Figure 12.6 Plasma motilin level (columns) variation with LES and gastric motor activity during interdigestive state and after feeding

level, a transient increase immediately after the meal, and a continuous low level as long as the digestive motor pattern continued.

DISCUSSION

Fyke *et al.*[11] first demonstrated that there is a sphincter between the oesophagus and the stomach by measuring their respective intraluminal pressures. At present, LES pressure is generally measured in clinical studies by a pull-through technique with perfused assembled catheters. The practical technique reported by Dodds *et al.*[12] is now accepted and used widely to measure LES pressure in man. However it is difficult to apply this technique to conscious animals, especially when long-term continuous changes in LES pressure are to be obtained. In order to overcome this difficulty, we developed a new technique to measure changes in LES contractile activity in a chronic preparation[3]. As reported previously[3], a transducer attached to the thick muscle of the LES produced results similar to those obtained by perfused catheters. Rinaldo *et al.*[13] studied LES motor activity by using strain gauge force transducers sutured over the sphincter in the dog. They reported that the muscle thickening was actually a physiological sphincter between the oesophagus and the stomach. Our findings are compatible with theirs; however, they did not describe long-term changes in LES contractions.

There are no reports in the literature describing continuous long-term changes in LES motor activity in man or experimental animals. This is due partly to the technical limitations mentioned above. The results obtained in the present study, and in our previous studies[3], indicate the importance of observing simultaneous long-term changes in LES and gastric motor activity in conscious animals. The traditional pull-through technique will measure LES pressure for only a moment and is not suitable for continuous changes. In this respect, our studies are the first records of simultaneous long-term changes in the LES and stomach.

The present study clearly demonstrated that the LES and the stomach contract simultaneously during the interdigestive state. Contractile episodes in the stomach, together with LES contractions, are a part of the interdigestive caudad-migrating contractions which we reported previously[10]. We consider, therefore, that the LES contractions during the interdigestive state may furnish the most proximal seal for interdigestive gastric contractions. On the other hand, Diamant *et al.*[14] studied relationships between LES pressure and gastric contractions and demonstrated the direct correlation between the organ and its associated sphincter in pressure changes. They suggested that elevating gastric pressure causes an increase in sphincter pressure. But as shown in this study contractile pattern in the LES did not change even after gastric pressure was released by opening the cannula. These motor changes in the LES during the interdigestive phase with minimal physiological disturbance have not heretofore been reported.

Jennewein *et al.*[2] studied the effect of motilin on the LES in anaesthetized dogs, and showed that motilin induced phasic contractions in the LES and the stomach. Meissner *et al.*[1] investigated the dose response of the LES to motilin in dogs. They indicated that an unusual feature of the LES response to motilin was repetitive contractions; however, the naturally occurring pattern of the LES during the interdigestive phase is essentially a repetitive one. Further, these authors[1,2] did not clearly demonstrate similarity between motilin-induced contractions in the LES and the naturally occurring interdigestive contractions. We have previously reported[10] that motilin induces motor activity in the canine gastrointestinal tract similar to the naturally occurring interdigestive contractions migrating from the stomach to the terminal ileum. In this report we demonstrate that motilin induces the same motor response in the LES as that observed in naturally occurring contractions of the LES.

Eckardt and Grace[15] measured LES pressure and plasma motilin level in man, and found no correlation between LES pressure and plasma motilin concentration. Their failure to demonstrate close correlation may have been a result of making only a single measurement of motilin concentration in the blood, because plasma motilin level is high only during interdigestive contractions in the LES and stomach. We have recently reported high plasma motilin level during contraction episodes in interdigestive period and fluctuation in coordination with interdigestive gastric contractions[16,17]. In the present study, we have shown that the plasma motilin level is elevated when the LES and stomach contract and low when they are quiescent. These results indicate that the canine LES and the stomach are under the control of plasma motilin during the interdigestive period.

Acknowledgements

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Discussion

K. Kelly: (USA) I have always considered the contractions of the LES sphincter to be tonic. Are you saying that they are intermittently phasic?

I. Aizawa: (Japan) Yes they are. They contract repetitively during interdigestive contractions.

N. E. Diamant: (Canada) Although there is an early demonstrable relationship between gastric and LES contractions in the dog, it is also present in the human. This is not easily demonstrated in the adult but is very obvious in the infant and small child.

T. Y. El-Sharkawy: (Canada) Do you have any ideas on the possible mechanisms that control the cyclical variations of motilin levels associated with your interdigestive contractions?

Aizawa: No, we have no idea about the cyclical change of motilin.

D. O. Castell: (USA) How did you arrive at the motilin dose used in this study? Did you measure serum motilin levels during the infusion, and were they similar to those occurring spontaneously in the interdigestive phase; i.e. is this a 'physiologic' or a pharmacologic dose? In our numerous studies in humans we often see spontaneous increases in LES pressure in association with apparent gastric contractions. This would seem to confirm your data that LES pressure-increases are part of the interdigestive motor complex in man.

Aizawa: Yes, we measured plasma motilin levels during the intravenous infusion of synthetic motilin at a rate of 0.3 μg/kg/h. The value obtained was similar to that during the interdigestive contractions. We considered that the motilin dose used in our study is within the physiological range.

J. Christensen: (USA) I am concerned about the localization of the LES transducer. Did you see relaxations with swallowing in every animal?

Aizawa: Yes, we saw relaxations with swallowing in every animal.

S. K. Sarna: (Canada) Contractions in the antrum are controlled in time and space by electrical control activity. Your studies show almost simultaneous contractions in the antrum, body and LES. What do you think controls the timing of contractions in the gastric body and LES?

Aizawa: I have no idea about your question. However, even a gastric cannula keeps open; the similar co-ordinated contraction between them was observed. I personally consider that the LES and the gastric body have a similar receptor for motilin.

E. E. Daniel: (Canada) Your data do not include the possibility that your strain gauges may have been on fundal sling fibres; these relax on each swallow and contract with the fundus. By distending a balloon proximally in the duodenum, one can find places at which only the LES responds, not the fundus. Have you tried this?

Aizawa: No, we have not.

J. T. Farrar: (USA) Is it not possible that your oesophageal transducer is recording activity of gastric muscle fibres which, in the dog, may extend superiorly to the

lower oesophagus? I am suggesting that the 'oesophageal' recording may, in fact, be of gastric activity rather than reflecting intrinsic oesophageal muscle activity at that level.

Aizawa: No, it is not. We confirmed that LES muscle activity obtained by the transducer method correlated with LES pressure change measured by the unfused catheter method.

13

The role of the cholinergic nervous system in the gastrointestinal response to motilin *in vivo*

H. S. ORMSBEE III AND S. S. MIR

The controls over the pattern of motor activity in the interdigestive state of dog and man have not been fully elucidated. Carlson and co-workers[1] postulated that extrinsic nerves were responsible for the initiation and the propagation of the interdigestive migrating myoelectric complex. Their experiments in dogs with Thiry-Vella loops demonstrated that continuity of the jejunal bowel wall was not an important factor in the control of the migrating complex. Carlson's hypothesis received support from the work of Weisbrodt *et al.*[2], which showed that the myoelectric complex did not occur on a denervated Thiry-Vella loop. However, Marik and Code[3] and Weisbrodt *et al.*[4] demonstrated the presence of interdigestive migrating complexes following truncal vagotomy. Therefore, the vagus nerve may not have a primary role in initiating and maintaining these complexes although it may be involved in the timing of each complex. Other factors, such as circulating levels of gastrin[3], also appear to be instrumental in the control of interdigestive motor activity.

The role of humoral factors in the control of interdigestive complexes was strengthened when Itoh *et al.*[5] demonstrated that the polypeptide motilin could precipitate a contractile complex in the stomach and duodenum. This complex was propagated along the intestine like the natural complex. The motilin-induced pattern was initiated at a time when the natural complex was not expected to be present on the stomach. This unique action of motilin has been confirmed by Wingate *et al.*[6] who proposed that motilin interacts with a control centre for interdigestive state motor activity by stimulating receptors in the duodenum which in turn send afferent impulses to the control centre. Efferent pathways from the centre are then excited, and a new interdigestive complex is initiated in the stomach and duodenum. This hypothesis suggests

that motilin may initiate the interdigestive migrating complexes by an interaction with nerves. The pathways for such a complex neurohormonal interaction are as yet undefined.

The present study attempted to define the site of action of motilin by examining the interaction between the polypeptide and the cholinergic nervous system. Motilin responses were observed in conscious, fasted dogs before and after atropine, hexamethonium and bilateral thoracic truncal vagotomy.

METHODS

Seven healthy, mixed-breed dogs (10–15 kg) were used. Under pentobarbital sodium anaesthesia (30 mg/kg intravenously) five dogs were each implanted with four extraluminal strain gauge force transducers. The transducers were sewn on the gastric body and antrum (15 and 3 cm, respectively, from the pylorus), the mid-duodenum (9 cm from the pylorus) and the proximal jejunum (24 cm beyond the ligament of Treitz). In two additional dogs, seven extraluminal strain gauge force transducers were sewn on the stomach and small intestine at the same locations as mentioned above, as well as farther down the small intestine (50, 100 and 190 cm beyond the ligament of Treitz) to record distal jejunal and ileal motor activity. Each transducer had its long axis parallel to the transverse axis of the intestine to record contractions of the circular smooth muscle layer. The dogs were allowed to recover for 10–14 days before beginning the experiments. They were maintained on solid laboratory chow and water was provided *ad libitum*.

Following an 18 h fast, circular smooth muscle contractile activity was recorded on an 8-channel Hewlett-Packard 7758 A recorder for 4–8 h per experiment. Interdigestive motor activity was observed for one or more cycles of the natural interdigestive contractile complex. This pattern of contractile activity is characterized at any one site by a quiescent period lasting about 60 min, when few contractions occur, followed by a period with increased contractions which culminates in a burst, a period of maximal contractile activity lasting about 5–15 minutes. This pattern of activity migrates throughout the length of the small intestine and is repetitive or cyclic at each site along the stomach and small intestine. Synthetic motilin (100 ng/kg intravenously), which was kindly supplied by M. Fujino, Takeda Chemical Industries, Ltd., Osaka, Japan, was administered 30 min after the natural interdigestive burst period had traversed the duodenum. Atropine sulphate (0.1 mg/kg subcutaneously) or hexamethonium bromide (13 mg/kg/10 min intravenously) was administered immediately after the subsequent natural interdigestive burst had again traversed the duodenum. Motilin injection was repeated 30 min later. These studies were performed at least twice in each dog.

After the initial studies with atropine and hexamethonium, four of the dogs underwent bilateral transthoracic truncal vagotomy. The dogs were allowed to recover for 1 week and were maintained during this period and for all

subsequent studies on a liquid diet. The liquid diet consisted of one can (384 ml) Pet® evaporated milk (Pet, Inc., Grocery Products Division, Saint Louis, Missouri 63166) mixed with water and one-half tube (60 g) Nutri-cal® (Evsco Pharmaceutical Corp., Buena, New Jersey 08310), a high-calorie dietary supplement, per day. Following an 18 h fast contractile activity was recorded during one or more cycles of the interdigestive migrating contractile complex. Motilin was again administered 30 min after a natural interdigestive burst had passed along the duodenum. These studies were repeated at least three times at weekly intervals following vagotomy.

Contractile activity from the force transducers in each animal was analysed manually for the number and force of contractions during a 30 min period following each injection of motilin. This information was used to compute a motility index (MI) according to the formula:

$$MI = (N_1 \times 1) + (N_2 \times 2) + (N_3 \times 4) + (N_4 \times 8) + (N_5 \times 16)$$

where N equals the number of contractions within any one of five particular force ranges. The force ranges used were 5–10 g, 10–20 g, 20–40 g, 40–80 g, and over 80 g; these were then multiplied by a factor which gave more weight to the larger amplitude contractions. This method has been described previously[7]. Comparisons of motility indices and all other data were made using Student's t-test.

RESULTS

Typical patterns of interdigestive state contractile activity were apparent in all dogs (Figure 13.1). Each burst was followed by a basal or quiescent period which preceded the return of the next burst. The period of the interdigestive complex in the jejunum was 86 ± 4 min (Table 13.1). In the animals with seven force transducers, the natural interdigestive complex appeared to migrate down the entire length of the small intestine.

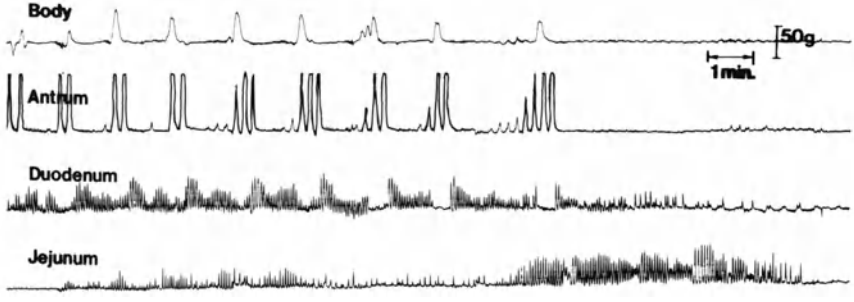

Figure 13.1 Circular muscle contractile activity observed during a natural interdigestive burst. Locations of the force transducers shown at the left are described in the text. Time and force bars are shown at the upper right

Table 13.1 Period of the natural and motilin-induced interdigestive complex

	Natural cycle (min)*		*Motilin-induced cycle* (min)†		*Natural cycle with motilin given during the cycle* (min)‡	
	A§	*B*§	*A*	*B*	*A*	*B*
Period	86 ± 4	88 ± 4	118 ± 11	113 ± 12	172 ± 13	163 ± 12
No. of experiments	21	16	14	16	14	16
No. of dogs	4	4	4	4	4	4

* This cycle is the time between the end of two natural bursts on the jejunum
† This cycle was measured from the end of the motilin-induced burst on the jejunum to the end of the next natural burst on the jejunum
‡ This cycle is the time between the end of two natural bursts on the jejunum, with a motilin-induced complex present in between
§ *A* = Pre-vagotomy; *B* = post-vagotomy

Rapid intravenous injections of synthetic motilin were followed usually within 30 s by a pattern of contractile activity which closely resembled the natural interdigestive burst period (Figure 13.2). The motilin-induced burst appeared to migrate down the length of the small intestine. The period of the motilin-induced complex (that is, the time from the end of a motilin-induced

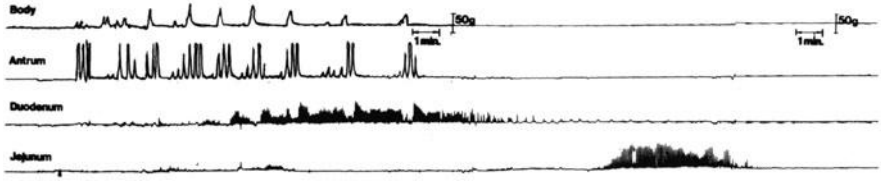

Figure 13.2 Circular muscle contractile response to motilin injection (100 ng/kg, intravenously, at arrow). Locations of the force transducers, time and force bars are shown as described in Figure 13.1

burst on the jejunum to the end of the subsequent natural burst on the jejunum) was 118 ± 11 min (Table 13.1). Thus, when motilin was administered after a natural complex, the next natural complex appeared 172 ± 13 min after the previous natural complex. This is exactly two cycles later. Motility index (MI) values for the stomach, duodenum and jejunum calculated for the first 30 min following motilin administration are presented in Table 13.2.

Table 13.2 Motility index values for each condition of the experiment*

	Gastric body	*Gastric antrum*	*Duodenum*	*Jejunum*
Motilin	152 ± 16	463 ± 35	1392 ± 129	657 ± 82
control	23(6)†	24(6)	25(6)	22(6)
Atropine	14 ± 4§	18 ± 4§	133 ± 37§	8 ± 6§
and motilin	11(6)	11(6)	10(5)	9(5)
Hexamethonium	58 ± 15§	195 ± 21§	120 ± 35§	22 ± 15§
and motilin	11(4)	12(5)	11(5)	9(4)
Post-vagotomy	198 ± 71	224 ± 53‡	1233 ± 107	647 ± 43
motilin	4(3)	6(3)	14(4)	15(4)

* Values given are mean ± SEM for 30 min analyses

† The numbers below each motility index represent the number of experiments performed, with the number of animals used given in parentheses

‡ $p < 0.01$ compared to motilin control

§ $p < 0.001$ compared to motilin control

Administration of atropine or hexamethonium significantly reduced the MI values for motilin-induced motor activity (Table 13.2). When motilin was given in the presence of atropine or hexamethonium few contractions were observed in any organ. When such a weak motor response was observed, the

onset of the response was not different from the onset of the control motilin response. In the presence of atropine or hexamethonium motilin-induced complexes never migrated down the bowel (Figures 13.3 and 13.4).

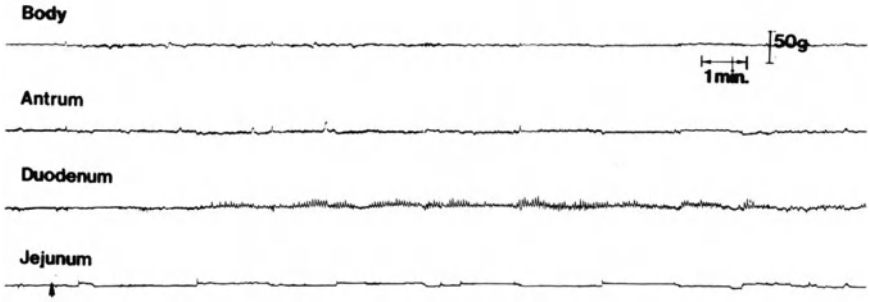

Figure 13.3 Circular muscle contractile response to motilin injection (at arrow) after treatment of the dog with atropine (0.1 mg/kg, subcutaneously). Locations of the force transducers, time and force bars are as described in Figure 13.1

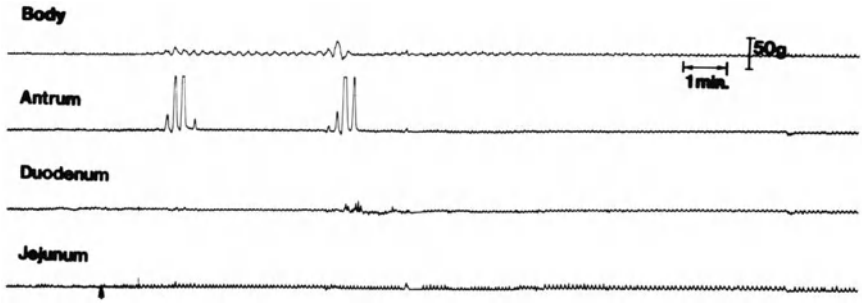

Figure 13.4 Circular muscle contractile response to motilin injection (at arrow) after treatment of the dog with hexamethonium (13 mg/kg/10 min, intravenously). Locations of the force transducers, time and force bars are as described in Figure 13.1

Following truncal vagotomy, interdigestive contractile complexes which were observed in these dogs were qualitatively similar to those observed in the intact dogs (Figure 13.5). The burst phase of contractile activity again appeared to migrate along the length of the small intestine. The period of the interdigestive complex was 88 $\pm$ 4 min (Table 13.1). This was not significantly different from the period of the natural complex before vagotomy.

When motilin was given to the vagotomized dogs a contractile complex was produced which resembled the natural interdigestive complex (Figure 13.6). Increased motor activity was observed in the gastric body, antrum and in the duodenum, and this activity progressed along to the proximal jejunum. Motility index values were unchanged when compared with pre-vagotomy values for the gastric body, duodenum and jejunum. The antral MI was significantly decreased from its corresponding pre-vagotomy value (Table 13.2).

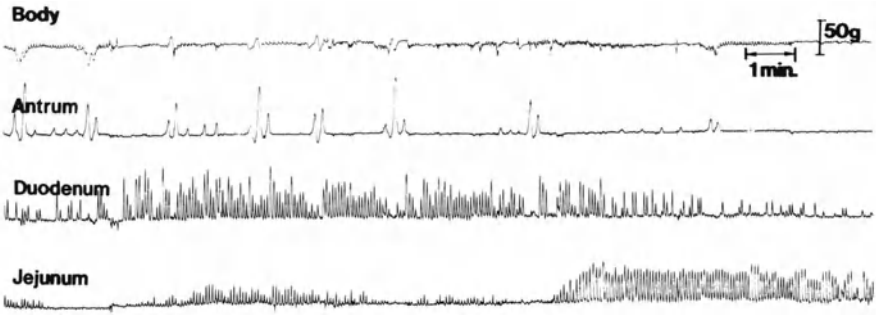

Figure 13.5 Circular muscle contractile activity observed after truncal vagotomy during a natural interdigestive burst. Locations of the force transducers, time and force bars are as described in Figure 13.1

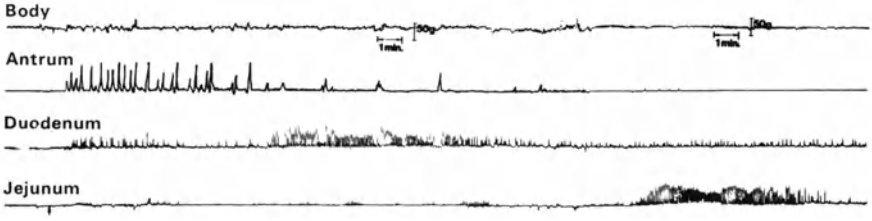

Figure 13.6 Circular muscle contractile response to motilin injection (at arrow) 1 week after truncal vagotomy. Locations of the force transducers, time and force bars are as described in Figure 13.1

When the motilin-induced interdigestive complex was examined in one dog on the distal jejunum and ileum 71% of the complexes failed to migrate to the ileum. Prior to vagotomy, 40% of the motilin-induced complexes failed to migrate to the ileum in this dog. After vagotomy, the period from the motilin-induced complex to the subsequent natural interdigestive complex was 113 ± 12 min (Table 13.1), a value which was not different from that determined before vagotomy.

DISCUSSION

Our results confirm the work of Itoh *et al.*[5,8] and Wingate and associates[6] demonstrating the ability of motilin to induce a premature interdigestive migrating myoelectric or contractile complex in the canine gastrointestinal tract. Similar results are obtained whether an intravenous infusion of the polypeptide, as in their experiments, or a bolus injection, as in our experiments, is given. It appears that once sufficient blood level of exogenous motilin is reached, the characteristic response proceeds. Even though the period from the end of the motilin-induced complex to the end of the next natural burst is somewhat longer and more variable than the period of the natural complex, the motilin-induced cycle appears to substitute for one

natural complex. Thus, motilin can induce a cycle of the interdigestive complex without affecting the overall timing of the occurrence of the next natural cycle. The gastrointestinal tract responded with one natural complex followed by a motilin-induced complex followed by another natural complex, as though three natural complexes occurred in succession.

Following atropine or hexamethonium, motilin-induced contractile activity was significantly reduced and no migrating complex was ever observed. Occasionally an initial contractile response of the antrum was observed in some of the dogs indicating that motilin may have stimulated the smooth muscle directly, or that motilin may have released acetylcholine from a pre-ganglionic or post-ganglionic site which acted upon unblocked nicotinic or muscarinic receptors. Since the complex never really developed in the antrum or duodenum when the dog had been treated with atropine or hexamethonium, its onset (which is observed as a burst pattern) would appear to require the presence of acetylcholine at pre-ganglionic or post-ganglionic sites or both. Although there is evidence *in vitro* for a direct action of motilin on rabbit and human antral and jejunal smooth muscle[9], such an action could not be shown in isolated tissue from the guinea pig, rat, pig or dog[9,10]. In contrast, when motilin responses were examined in the isolated vascular perfused canine stomach[11] or isolated stomach and duodenum[6], the responses were blocked by atropine, hexamethonium and by tetrodotoxin. From this evidence Cook *et al.*[11] suggested that the contractile response to motilin was mediated through the pre-ganglionic release of acetylcholine. The present experiments support a pre-ganglionic cholinergic site of action for motilin in the chronic unanaesthetized dog. Furthermore, the release of acetylcholine from pre-ganglionic cholinergic nerve terminals is necessary for the appearance of a full-blown burst in the stomach and duodenum and for the migration of the whole complex down the small intestine. Thus, the present data do not support the suggestion of Mukhopadhyay *et al.*[12] that different neural or hormonal mechanisms may control the onset and migration of the interdigestive myoelectric complex.

It is clear from the present experiments that neither the natural interdigestive migrating complex nor the motilin-induced migrating complex require an intact vagus to propagate along the bowel. This agrees with the work of Marik and Code[3] and Weisbrodt *et al.*[4] on the effect of vagotomy on the myoelectric complex. Marik and Code's analysis showed altered cycle periodicity following vagotomy. This was not observed in Weisbrodt's study or in our dogs. We did, however, observe an apparent significant decrease in antral motility during the post-vagotomy motilin complexes. Disruption of fasting antral motor activity following vagotomy has been reported by Khan and Bedi[13], and by Walker *et al.*[14]. Such altered antral motility may be one reason for the gastric stasis observed in vagotomized dogs fed solid food[15].

Although the exact nature of the control over interdigestive state contractile activity will require further studies, our working hypothesis is as follows.

Decreased gastric distension following a meal[16] or a decreased serum gastrin level[3] stimulates the release of motilin from the duodenum. Motilin release may also be stimulated by an increased intraduodenal pH in the dog[17], since this is how its effects were originally discovered. Once released, motilin acts via an acetylcholine-dependent mechanism, probably by stimulating pre-ganglionic cholinergic neurons in the myenteric plexus, to initiate an inter-digestive complex in the stomach and upper duodenum. While the burst in the duodenum is occurring, the adjacent small bowel begins to increase its activity. This activity may be mediated through nervous reflexes located in the myenteric plexus or through non-vagal extrinsic nerves.[2] Thus, a control centre[6] or biological clock mechanism appears to be turned on by motilin, after which a series of unknown neurohumoral mechanisms produces the migration of the complex. This may be mediated through a series of non-vagal feedback loops which alternatively excite and inhibit adjacent areas of the small intestine. Whatever pathway is responsible for the migration of the complex, one necessary component appears to be the pre-ganglionic or post-ganglionic release of acetylcholine. We suggest that the myenteric plexus plays an important role in the co-ordination of the interdigestive migrating complex.

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Discussion

H. L. Duthie: (UK) We have now seen three demonstrations of minute rhythms in the activity front: Dr Fleckenstein, Dr Aizawa and Dr Ormsbee – certainly in the upper part of the stomach. Is there no change in the length of the active part (phase III) of the complex after vagotomy?

H. S. Ormsbee: (USA) The only alteration in activity we observed after vagotomy was in the antral motility index. There may have been a change in the length of the burst on the body, antrum, and duodenum, but we did not specifically look for this. Also, the precise pattern of duodenal activity during the burst may have been altered by vagotomy even though the motility index analysis did not show it.

J. D. Wood: (USA) We heard earlier a conclusion that the clock or endogenous oscillator seemed to continue to run when the MMC was converted to the digestive state; i.e. the clock continues to run although there is no alarm. Does this seem to be also during blockade of the response by atropine?

Ormsbee: Our data would tend to support this conclusion, but it is still too early to tell.

Z. Itoh: (Japan) How long did the atropine-induced inhibition last?

Ormsbee: Our preliminary data suggest that, at a dose of 0.1 mg/kg subcutaneously, the natural burst may be inhibited for at least 200 min.

N. E. Diamant: (Canada) Atropine in our dogs not only abolishes the myoelectric complex but all other motor activity including the feeding pattern. Therefore, can the effect of atropine be anything other than a blocker of the final common excitatory muscarinic receptor? That is, does the atropine effect differentiate the complex induced by motilin from a naturally occurring one?

Ormsbee: No, it does not differentiate between the two. Both the natural and the motilin-induced complexes are blocked by atropine.

G. Charbon: (Netherlands) If I remember the text on one of your slides correctly, the effect of atropine is much more pronounced than that of vagotomy. Would you care to comment on that?

Ormsbee: Blockade of cholinergic muscarinic receptors with atropine inhibited the migrating complexes induced naturally or by motilin. These complexes were both present following truncal vagotomy. We presume that the atropine effect is strictly a peripheral one.

M. A. Cook: (Canada) (1) Was there a threshold dose of motilin below which no MMC was elicited and, conversely, did increasing the dose above the threshold achieve any different effect? (2) If there was no difference in the complex once initiated by any dose of motilin, do you feel that this polypeptide is acting as the trigger of an all or none event?

Ormsbee (1) Frequently, doses of 50 ng/kg intravenously failed to elicit a

migrating complex. Doses of 100 ng/kg intravenously and above, all appeared to initiate complexes similarly. (2) Yes.

V. Rayner (UK): Does vagotomy alter minute rhythm of stomach contraction that you show? I would like to comment that in the pig truncal vagotomy caused total abolition of the minute rhythm recorded by extraluminal strain gauge transducers implanted on the antrum of the pig.

Ormsbee: We have found a reduced antral motility index, but not totally abolished antral contractile activity following truncal vagotomy. We have not looked at how the rhythmicity or periodicity of these antral contractions may be altered by vagotomy.

J. S. Davison (UK): Returning to the role of the vagus nerve, have you attempted to stimulate the nerve, for example with implanted electrodes?

Ormsbee: Not in the unanaesthetized dog.

14

VIP antagonizes motilin-induced antral contractions *in vitro*

U. STRUNZ, P. MITZNEGG, S. DOMSCHKE, W. DOMSCHKE, E. WUNSCH AND L. DEMLING

Both vasoactive intestinal peptide (VIP) and motilin are candidate hormones of the gut[1]. Both are released by acid in the duodenum in man[2,3] and dog[4,5]. Only for motilin were stimulated endogenous serum levels comparable to those seen after motilin infusion resulting in motor effects of the gut[6,7]. Thus motilin may well become an established gut hormone.

The abundance of VIP effects reported in the last 7 years were almost all elicited by exceedingly high exogenous peptide doses. Since endogenous serum levels can now be determined by radioimmunoassay, all those effects await critical reconsideration.

The significance of VIP serum levels is becoming more and more questionable since, in 1976, VIP was demonstrated by immunohistochemistry not only in endocrine cells of the gut, but also in nervous tissue of the gut wall[8]. Distribution of immunoreactive VIP along nervous structures invited speculation about a possible role for VIP as a peptidergic neurotransmitter substance of the non-cholinergic, non-adrenergic inhibitory nerves[9] of the gut. These nerves are widely distributed all over the body[9], but have been especially demonstrated and studied at the site of the lower oesophageal sphincter[10] and the pylorus[11].

We report on the inhibitory VIP action on basal and motilin-stimulated motor activity of the pyloric antrum (but not duodenum), *in vitro*; the lack of effect of secretin, compared to VIP; and on the resistance of inhibitory VIP action to common antagonists, which would be in keeping with a possible role for VIP as a peptidergic inhibitory neurotransmitter.

MATERIALS AND METHODS

Male rabbits (2–2.5 kg body-weight) were killed by cervical dislocation;

circular muscle strips (3 × 15 mm) prepared immediately proximal to the intermediate sphincter[12] of the pyloric antrum and were freed from mucosa. Strips were suspended in a 10 ml organ bath containing Tyrode solution at 37.5 °C and continuously aerated with 5% CO_2 in O_2. Composition of the solution (g/l): NaCl 8.01, KCl 0.26, $MgCl_2 . 6H_2O$ 0.211, $NaHCO_3$ 1.0, NaH_2PO_4 0.055, $CaCl_2 . 2H_2O$ 0.255, glucose 1.0. Contractions were magnified ten times by a writing lever, and were recorded isotonically on a kymograph. Drugs were added and withdrawn with the bath fluid.

The VIP was the kind gift of Professor Mutt, and was free of cholecystokinin and secretin. Natural (GIH) and synthetic secretin, as well as natural (kindly supplied by Professor J. C. Brown) and synthetic position-13 norleucine-substituted motilin were used. Since both natural and synthetic peptides were found to act identically, no further differentiation was made. Other drugs included atropine sulphate, pheniramine, phenoxybenzamine hydrochloride, propranolol hydrochloride and tetrodotoxin. All peptides and drugs were freshly prepared prior to use and administered in volumes not exceeding 100 μl.

Doses necessary for half maximal responses (D_{50}) were calculated from the means of the concentration-response data subjected to the linear transformation of the Michaelis–Menten equation recommended by Dowd and Riggs[13].

RESULTS

Pyloric antrum: basal activity

Circular muscle of the pyloric antrum exhibited rhythmic basal phasic contractions. VIP, but not secretin, abolished basal activity (Figure 14.1). Lowest

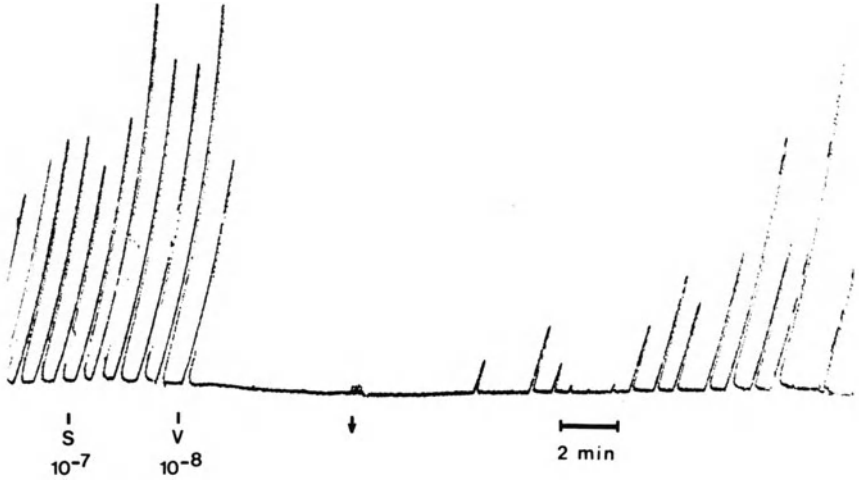

Figure 14.1 Basal contractions of rabbit circular pyloric antral muscle *in vitro*. Inhibition by VIP (V; 10^{-8} M), but not by secretin (S; 10^{-7} M). Vertical bar: addition of peptide to the bath fluid. Inverted arrow: washout of peptides

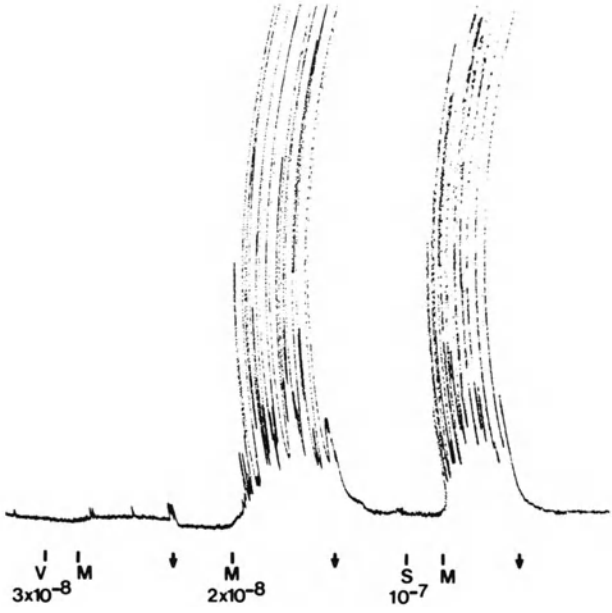

Figure 14.2 Inhibition of motilin (M; 2×10^{-8} M) induced contractions of rabbit circular pyloric antral muscle by VIP (V; 3×10^{-8} M), but not by secretin (S: 10^{-7} M). Vertical bar: addition of peptide to the bath fluid. Inverted arrow: washout of peptides

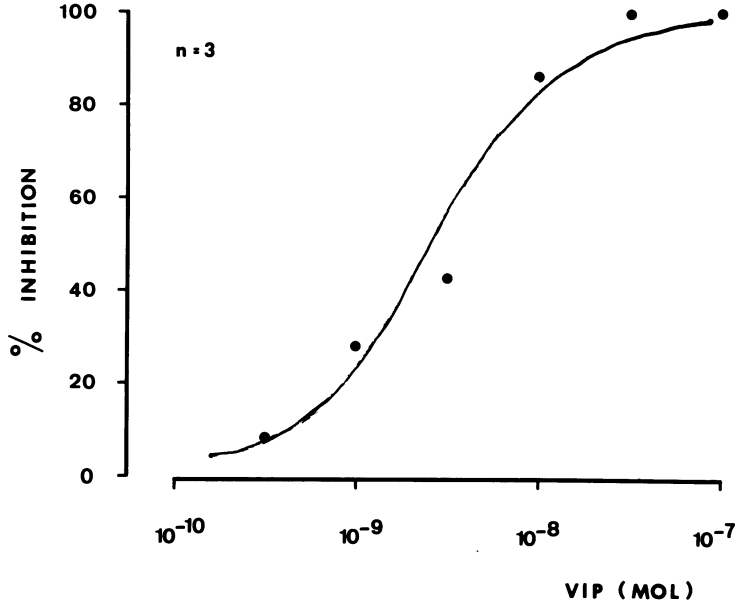

Figure 14.3 Log concentration response curve for inhibition of motilin-induced contractions (constant dose 2×10^{-8} M) of rabbit circular pyloric antral muscle by VIP. Each point represents the mean of three experiments

concentration for complete inhibition was about 5×10^{-9} M of VIP, whereas secretin up to 10^{-7} M remained ineffective. After washing out of VIP, basal activity returned.

Pyloric antrum: stimulated by motilin

Motilin induced phasic and tonic contractions of pyloric antral muscle. VIP, but not secretin, abolished motor action stimulated by motilin (Figure 14.2). When VIP was added after a constant dose (2×10^{-8} M) motilin, motilin contractions were dose dependently diminished (Figure 14.3). The calculated D_{50} for this inhibitory VIP action was 2.9×10^{-9} M.

Figure 14.4 Rabbit circular pyloric antral muscle. Tetrodotoxin (T; 10^{-6} M) fails to affect inhibitory VIP (V; 5×10^{-8} M) action on motilin-induced contractions (M; 2×10^{-8} M). Additional calcium ions (Ca^{2+}; 5.4×10^{-3} M) restore phasic activity. Vertical bar: addition of drug to the bath fluid. Inverted arrow: washout of drugs

Pyloric antrum: pharmacological analysis

The inhibitory action of VIP on motilin-induced contractions was not affected by either: the anticholinergic agent atropine (10^{-6} M), the alpha-blocking agent phenoxybenzamine (10^{-6} M), the beta-blocking agent propranolol (10^{-6} M), or the histamine H_1-antagonist pheniramine (10^{-6} M). In addition, VIP remained effective after blocking axonal conduction with tetrodotoxin (10^{-6} M) (Figure 14.4). Additional Ca^{2+} ions (5.4×10^{-3} M) restored phasic activity suppressed by VIP (Figure 14.4).

Duodenum: basal and stimulated activity

Neither VIP nor secretin in concentrations higher than 10^{-7} M affected basal phasic activity or tone of duodenal muscle. Moreover, both peptides failed to influence duodenal contractions due to motilin (Figure 14.5).

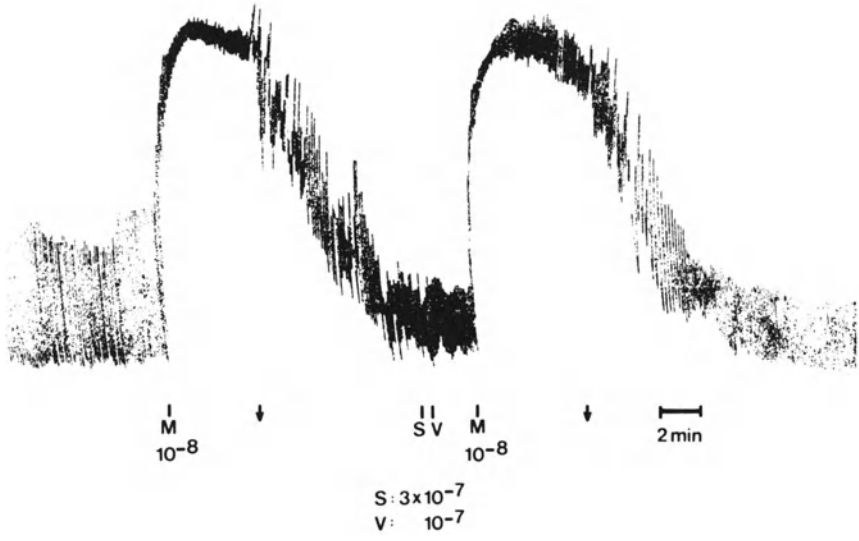

Figure 14.5 Rabbit duodenal muscle. Lack of effect of VIP (V; 10^{-7} M) and secretin (S; 3×10^{-7} M) on contractions due to motilin (M; 10^{-8} M). Vertical bar: addition of peptide to the bath fluid. Inverted arrow: washout of peptides

DISCUSSION

Motor effects suggesting a possible role for motilin in normal digestive physiology are contraction of lower oesophageal sphincter[6] and initiation of cyclic interdigestive motor activity of the gut[7]. As to the mode of action of this peptide, potentiating interaction with acetylcholine seems to be an important principle[14]. This interaction might explain why motor effects due to

motilin can be inhibited by atropine *in vivo*[15] but not in muscle strips *in vitro*[16]. *In vitro*, the only drug shown to counteract motilin is verapamil[16] which is believed to interfere with membranal calcium transport.

VIP is the first peptide reported to inhibit motilin-induced motor activity. The inhibition was not affected by anticholinergic, antiadrenergic, or antihistaminergic (H_1) agents. This is in accordance with earlier observations on unstimulated smooth muscle preparations[17]. A possible action of VIP on ganglia of non-cholinergic, non-adrenergic nerves might also be excluded since axonal conduction blockade by tetrodotoxin did not exert any influence on the inhibitory effect of VIP. Thus, a direct mode of action of VIP on antral smooth muscle cells can be assumed.

Additional calcium ions restored motilin-induced contractile activity previously absolished by VIP. This observation needs further differentiation, since calcium ions are likely to act by themselves unspecifically on contractile elements. A verapamil-like calcium antagonistic property of VIP, however, cannot be excluded.

The concentration of VIP needed to elicit an inhibitory effect on basal and on stimulated motor activity was found to be around 10^{-9} M. It should be noted that effective doses of acetylcholine or motilin are of the same order of magnitude in our *in vitro* system.

It is well known that muscle preparations of different gut segments exhibit varying susceptibility to VIP[17]. So, the lack of effect of VIP on rabbit duodenal muscle, in spite of high sensitivity of neighbouring tissue to the peptide, is in keeping with previous results. Of all factors said to be responsible for these local differences in excitability of gut muscle, regional differences in innervation seem to be most likely. With this in mind, we speculate that the observed local predominance of action of VIP may reflect local predominance of inhibitory nerve fibres.

1. VIP, but not secretin, inhibits basal and motilin-stimulated contractions of rabbit pyloric antral, but not duodenal, muscle *in vitro*.
2. This inhibitory action of VIP was resistant to anticholinergic, antiadrenergic, antihistaminergic (H_1) as well as neuronal blockade.
3. Low effective concentration, localization as well as mode of action are supporting the hypothesis that VIP might be a peptidergic inhibitory neurotransmitter.

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Discussion

Z. Itoh: (Japan) According to studies we made in our conscious-dogs system, VIP did not inhibit the interdigestive (natural) contractions and motilin-induced contractions. Do you have any comment in this respect? By the way, the similar dose of VIP did inhibit the digestive contractions.

U. Strunz: (Germany) The point I was trying to make at the beginning was that, as far as we can say, normal circulating VIP levels are much lower than those seen after infusions resulting in VIP effects. For example, only recently we have shown that for inhibition of pentagastrin-stimulated lower oesophageal sphincter contraction in man, levels of VIP have to be around 200 pmol/l, whereas peptide serum levels after stimulation by acid in the duodenum rose from a basal value of around 1 pmol/l to 10 pmol/l. Therefore, a local inhibitory action of VIP on gut muscle seems to be more likely.

A. Bennett: (UK) The difference between the longitudinal and circular muscle was also my question. So have you looked at VIP on the circular muscle of the duodenum?

Strunz: No, we have not done this.

S. Anuras: (USA) In my experiment with opossum duodenum, it showed that VIP reduced tension in circular muscle, but increased tension in longitudinal muscle; this is quite different from your finding. So, I think it will be difficult to draw a conclusion that VIP may be a non-adrenergic transmitter, because VIP has a stimulatory effect in the longitudinal muscle in opossum duodenum.

Strunz: In 1970, Piper showed that VIP did not affect longitudinal muscle of rat duodenum and guinea pig ileum. On the other hand Maklouf, in 1974, reported about a stimulatory effect of VIP on longitudinal muscle of guinea pig duodenum. This effect, however, could be blocked by tetrodotoxin. So there certainly are special differences; moreover, this contractile effect seemed to be nerve-mediated and therefore not to be comparable with the action of VIP I was talking about. By the way, VIP only recently is said to be free from CCK. I wonder if this contractile effect might be due to CCK.

M. A. Cook: (Canada) This is just a comment. Unpublished studies from our laboratory have shown that in all tissues which respond to motilin from dog, rabbit and guinea pig, the antagonists tetrodotoxin and atropine always abolish the action of motilin. We have shown this in stomach, duodenum and ileum in all three species.

Strunz: There are two groups independently reporting, in 1975 and 1976, that muscle strips prepared from the canine gastrointestinal tract do not respond to motilin – neither to the natural nor to the synthetic one. The same was found in the guinea pig. So I have to assume that different motilins have been used.

E. E. Daniel: (Canada) (1) Since verapamil is a non-selective antagonist to most agents which contract the gut by inducing Ca^{2+} spikes, the question arises whether

VIP is similarly non-selective. Have you tested this? (2) In other systems *in vivo* (stomach, LES, intestine) motilin has been shown to act by release of pre-ganglionic cholinergic sites (and possibly post-ganglionic cholinergic sites). Are you satisfied that you have ruled out such a site of action *in vitro*?

Strunz: VIP indeed seems to act as unspecifically as the non-selective C^{2+} antagonist verapamil, since acetylcholine-induced muscle contractions also were inhibited by VIP. As to the second question, atropine inhibits motor effects due to motilin *in vivo*, but not on isolated muscle strips *in vitro*. This observation *in vivo* might be explained, as it was done for gastrin by Dr Bennett in Banff, with an inhibition of background tone of acetylcholine, having a permissive role. This seems quite reasonable since last year it was shown that both acetylcholine and motilin potentiate each other's contractive activity. Because of this potentiating interaction, the anticholingergic action of atropine might, *in vivo*, greatly diminish the effects of motilin.

W. Sircus (UK): If I may interpolate a pertinent clinical observation: a characteristic of the clinical manifestation of vipomata is the occurrence of periods of prolonged ileus with atony of the whole gut from duodenum to rectum. In a recent case of ours studied in detail (Lennon, Sircus and Bloom, to be published) the atonic ileus persisted for a period of months and was associated with a high degree of hyper-VIPaemia. The patient had multiple hepatic metastatic vipomata.

Section III
Neurotransmitters

15

Release of [^{3}H]noradrenaline by electrical stimulation of human isolated taenia coli

J. HOUGHTON AND A. BENNETT

The gastrointestinal tract receives adrenergic innervation which can be demonstrated anatomically and histologically[1–4]. Ganglion-stimulating drugs or electrical pulses relax human gastrointestinal muscle, particularly if cholinergic responses are prevented by drugs. However, relaxations to nicotine are blocked by conventional adrenolytic drugs but those to electrical stimulation are usually unaffected[5–11]. The present study was therefore undertaken to determine whether electrical stimulation releases noradrenaline from human gastrointestinal muscle.

METHODS

Specimens of macroscopically normal human colon (eight patients) were collected in Krebs solution equilibriated with 5% CO_2 in O_2, as soon as possible (5–45 min) after surgical removal for cancer or diverticulitis of the large bowel. Tissues were either used immediately or stored overnight (+5 °C). After removing the mucosa and submucosa, the muscle was cut along the taenia into strips 1–2 mm wide and about 30 mm long. The strips were subjected to the following procedures carried out in sequence at 37 °C in 15 ml Krebs solution, with ascorbic acid 10 μg/ml and EDTA 20 μg/ml added to reduce oxidation of the amines.

1. Pre-incubation in solution for 20 min.
2. Incubation for 40 min with ^{3}H-NA (10 μCi/ml) and NA standard (10

μg/ml). Before incubation 0.15 ml was taken for measurement of the initial activity.

3. Three 15 ml washes, 5 min each.
4. Five 15 ml washes, 5 min each with phenoxybenzamine (10 μg/ml).

The Krebs solution contained (g/l): NaCl, 7.1; $CaCl_2 . 6H_2O$, 0.55; KH_2PO_4, 0.16; KCl, 0.35; $MgSO_4 . 7H_2O$, 0.29; $NaHCO_3$, 2.1; dextrose, 1.0.

Each strip was tied at the lower end to a tissue holder incorporating the electrodes for stimulation; the upper end was attached to an isotonic transducer. Tissues were then set up in 10 ml isolated organ baths and allowed to equilibrate with Krebs solution (37 °C, bubbled with 5% CO_2 in O_2) for 1 h before starting stimulation. Usually four strips were used: the control was stimulated every 20 min at 11.5 V/cm, 0.2 ms and 8 Hz for 30 s. In the other three tissues, one parameter only was altered (i.e. voltage, pulse width or frequency). Square-wave pulses of alternating or single polarity were delivered across the tissue between pairs of 5 mm^2 silver electrodes 1 cm apart. The voltage drop was measured in Krebs solution on an oscilloscope.

The bath fluid was removed and replaced with fresh solution 1 min before, and 2 min after, beginning stimulation. Aliquots (0.3 ml) were mixed with 3 ml Unisolve scintillation cocktail (Koch–Light) and counted in plastic minitubes for 20 min in a Packard Tri-Carb scintillation counter. Dpm values were calculated by the automatic externalization ratio method. In some experiments the NA was absorbed on alumina and counted after elution.

In the control tissue both the resting and stimulated ^{3}H-label release decreased over the period of the experiment. Ideally, only one or two stimulations per tissue should be used because the release of NA in the presence of phenoxybenzamine probably reduces the 'readily releasable' storage pool. Because of the limited supply of human tissue each strip was used for a maximum of eight stimulations. We therefore measured the stimulated release (i.e. actual stimulated release less the mean of resting release in the periods immediately before and after that stimulation) as a percentage of release from a control tissue stimulated at constant parameters throughout the experiment. Since the amount released can vary (due to differences in tissue size, NA storage pools, and the incorporation of the label), release from test tissues stimulated at the control parameters (11.5 V/cm, 0.2 ms, 8 Hz) does not exactly match the release from the control tissue.

RESULTS

Nicotine (1–8 μg/ml) relaxed the strips of taenia coli. Release of ^{3}H-label increased with the dose (three experiments, Figure 15.1) although the maximally effective concentration varied from 4 to 8 μg/ml. Electrical stimulation generally caused a relaxation of taenia coli despite pre-treatment with phenoxybenzamine which usually prevented the responses to nicotine. When phenoxybenzamine was not added (two experiments) the response to electrical

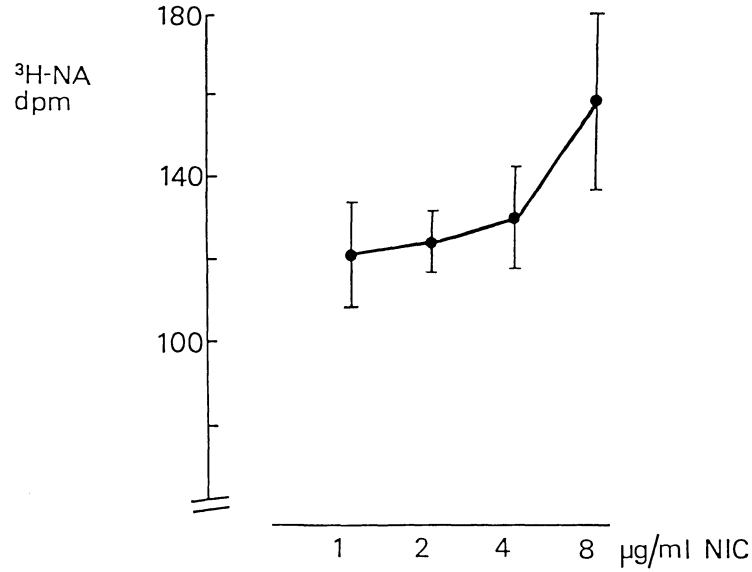

Figure 15.1 Release of ^{3}H-label from human taenia coli increased with the concentration of nicotine (1–8 μg/ml) added to the bath for 2 min. The vertical axis represents the release in response to nicotine compared to the release from a control tissue stimulated at 0.2 ms, 11.5 V/cm, 8 Hz for 30 s ($n = 3$)

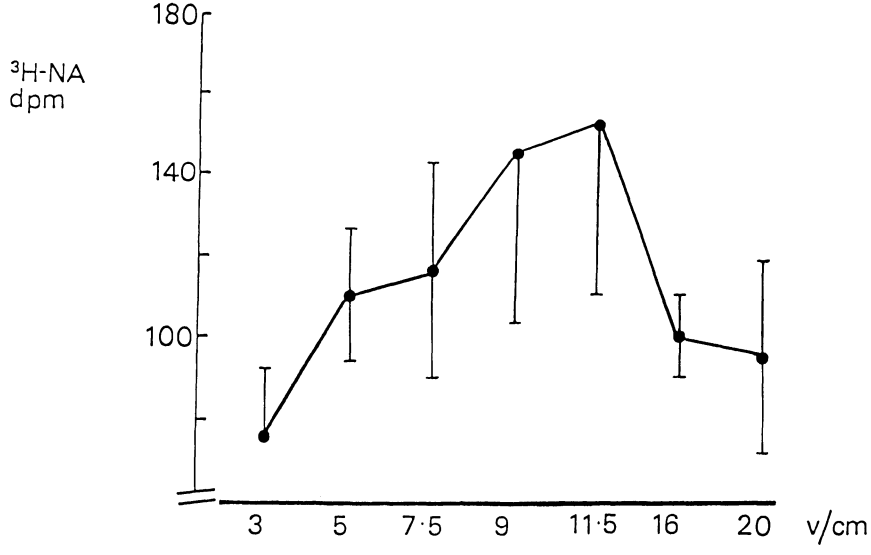

Figure 15.2 Release of ^{3}H-label increased up to 11.5 V ($r = 0.95$; $p < 0.01$) and then decreased. Each point represents the mean $\pm$ SE; $n = 6$–8) of the percentage change in release compared to the control tissues stimulated as in Figure 15.1

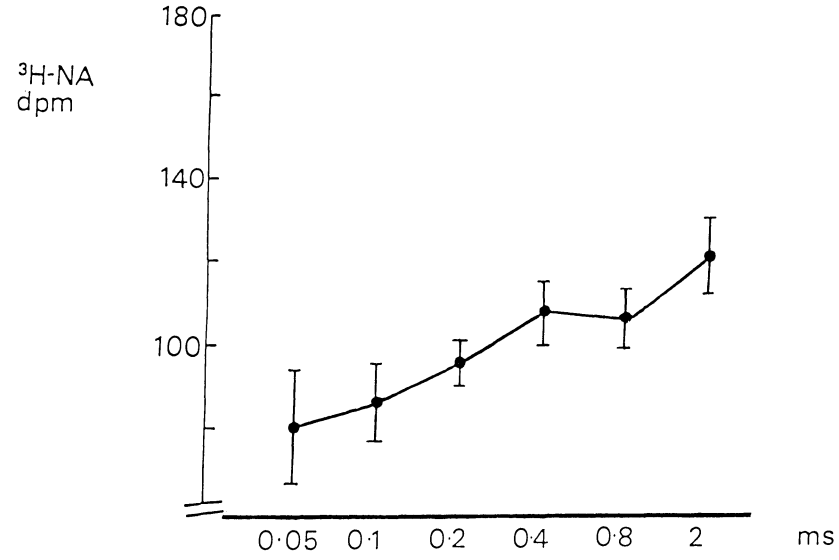

Figure 15.3 ^{3}H-label release increased with the pulse width ($r = 0.93$; $p < 0.01$; $n = 6$–8) compared with controls stimulated as in Figure 15.1

stimulation was usually triphasic: relaxation, an after-contraction on switching off the stimulator, followed by a prolonged relaxation.

Increasing the voltage from 3 to 11.5 V/cm, changed the overflow of ^{3}H-label from 87% of control at 3 V/cm to 145% at 11.5 V/cm ($r = 0.95$; $p < 0.01$) (Figure 15.2). Above 11.5 V/cm the release of ^{3}H-label decreased to 107% control, and also decreased the release on the next stimulation if the voltage was above 9 V/cm. Release of ^{3}H-label varied with pulse width (80% control at 50 μs to 122% at 2 ms, $r = 0.93$; $p < 0.01$), although the increase was relatively small at each increment (Figure 15.3). Total overflow of ^{3}H-label was greater at higher frequencies (165% at 16 Hz compared with 82% at 0.25 Hz; Figure 15.4), but the release per pulse was less (250% and 87% of the control at 0.25 and 16 Hz respectively).

DISCUSSION

Most previous studies have found that low-frequency stimulation of intramural nerves causes non-adrenergic inhibition of human gastrointestinal muscle, whereas ganglion-stimulating drugs cause adrenergic inhibition[5,10]. The authors in the latter reference demonstrated that under certain conditions an adrenergic pathway contributes to the response; at 0.3 ms and 16 Hz oxprenolol reduced the relaxations, but at higher pulse widths and lower frequencies no significant effect could be shown. They thought that the effect of electrically stimulating the non-adrenergic nerves usually overshadows the

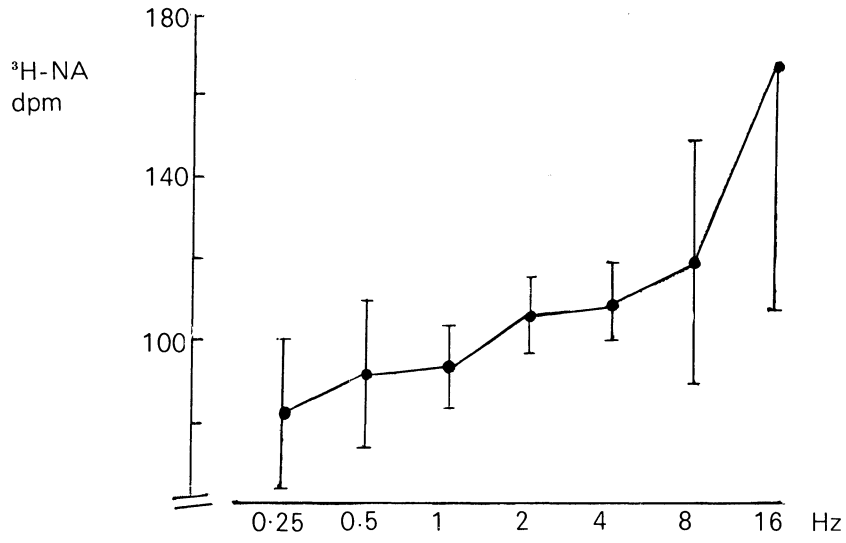

Figure 15.4 ^{3}H-NA increased with the frequency of stimulation ($r = 0.98$; $p < 0.001$; $n = 6$–8), compared with controls stimulated as in Figure 15.1

adrenergic component, thus explaining the failure in most studies to inhibit electrically induced relaxations with adrenolytic drugs. Our work confirms this view since ^{3}H-label is released both by nicotine and electrical stimulation. ^{3}H-label release increases with increasing voltage up to 11.5 V/cm, probably because more nerve fibres are stimulated; the decrease above this voltage may be due to depletion of the NA pool available for release. The substantial release of label with pulses up to 2 ms does not seem to contribute to the relaxation, which is apparently due to maximally effective activation of non-adrenergic nerves.

^{3}H-label output per pulse decreased markedly as the frequency was increased, particularly at 8 and 16 Hz. This is contrary to the work in other animal tissues where facilitation has been observed at higher frequencies (guinea pig and mouse vas deferens[12–14]; rabbit vas deferens and portal vein[15–17].

The mechanisms controlling colonic motility are incompletely understood. The present study indicates that NA is released by stimulation of intramural nerves, but its role as an initiator or modulator of muscle activity still has to be determined.

Acknowledgement

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Discussion

E. E. Daniel: (Canada) (1) Since many mono-aminergic nerves (5-HT, dopamine etc.) can take up noradrenaline, uptake and release from a nerve does not establish it as noradrenergic. Can you comment? (2) What is the evidence that non-adrenergic inhibitory nerves are stimulated submaximally by 0.3 ms and 16 Hz? In other systems this is not the case.

J. Houghton: (UK) We agree with your first comment and have just been looking at total release of ^{3}H-label from the pre-incubated tissue. Stockley and Bennett (1977) have shown that at 0.3 ms and 16 Hz there is a substantial reduction of the response by adrenolytic drugs. We think, therefore, that at these parameters the non-adrenergic does not overwhelm the adrenergic effect, since these responses are reduced, but at other parameters they are not usually affected.

J. Christensen: (USA) Have you done any studies on catecholamine fluorescence to support your conclusion?

Houghton: I have tried using catecholamine fluorescence but have not found it to be sufficiently reproducible in human gut to undertake studies to determine depletion of transmitter by electrical stimulation.

M. A. Cook: (Canada) Have you measured NA output in the presence of compounds presumed to block the non-adrenergic–non-cholinergic inhibitory nerves such as the methylxanthines or pyridyl isatogen (PIT)?

Houghton: No, we have not measured the release in the presence of any antagonists. The next paper in this meeting may have some bearing on the use of PIT.

J. D. Wood: (USA) I was concerned that β-blockers abolished the inhibitory response to nicotine when it has been demonstrated that the non-adrenergic–non-cholinergic neurons also have nicotinic cholinergic receptors. Could you comment on this?

Houghton: This blockade of responses to nicotine has been previously reported by various authors. For example, Stockley and Bennett found that oxprenolol alone was sufficient to prevent nicotine-induced relaxation in human taenia coli.

S. Anuras: (USA) We have done catecholamine fluorescent staining in the cat and opossum colons. We could only demonstrate adrenergic nerve fibres around the arteries. I am wondering if you have done any such experiments to localize adrenergic nerve fibres in the taenia coli?

Houghton: Several groups (e.g. Jacobowitz, Baumgarten, Bennett *et al.*) have looked at catecholamine fluorescence in human gut, and found the results in the colon to be similar to many other mammalian species with adrenergic nerves around myenteric ganglion cells, but not in smooth muscle.

16

2-2′-Pyridylisatogen antagonizes adenosine 5′-triphosphate but not nerve-mediated relaxations in human isolated taenia coli

HELEN L. STOCKLEY

Relaxations to electrical field stimulation in human isolated taenia coli are mainly non-adrenergic[1–3]. Such relaxations in isolated intestine from laboratory animals have been attributed to 'purinergic' nerves[4], but in human taenia coli adenosine 5′-triphosphate (ATP) sometimes causes contraction or biphasic responses instead of simple relaxation; quinidine, which was used by Burnstock *et al.*[5] to antagonize ATP, inhibited (non-selectively) relaxations to catecholamines, ATP and electrical stimulation[2].

2-2′-Pyridylisatogen tosylate selectively antagonized relaxations of guinea pig isolated taenia caeci induced by ATP but did not inhibit relaxations mediated by non-adrenergic nerves casting doubt on the 'purinergic nerve' hypothesis[6]. The following experiments suggest that ATP is unlikely to act as a non-adrenergic inhibitory transmitter in human isolated taenia coli.

METHODS

Strips of taeniae were dissected from surgically removed specimens of ascending or sigmoid colon, and set up under a load of 1 g in isolated organ baths in Krebs solution at 37 °C bubbled with 5% CO_2 in O_2 as described previously[3]. Square-wave pulses of alternating polarity (1 ms, 17 V measured in Krebs solution) were delivered at 1, 2, 4, 8 or 16 Hz in 10 s trains from 0.5 cm^2 silver plate electrodes 1 cm apart. Isotonic responses magnified 10- to 20-fold were registered on pen recorders.

Since 2-2′-pyridylisatogen base is poorly soluble in water, it was dissolved

in a minimum amount (about 0.05 ml/mg) of polyethylene glycol 400 (PEG) at 40 °C; saline (0.15 M NaCl) was then added to give a final concentration of 2 or 5 mg/ml. Matching control solutions of PEG in saline were also prepared. Later, the tosylate salt was obtained and used as a solution in saline (1 mg/ml). The other drugs, also dissolved in saline, were ATP (disodium salt), carbachol chloride, (−) hyoscine hydrobromide, (−) noradrenaline bitartrate (with 100 μg/ml of (−) ascorbic acid), and prostaglandins E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$ tromethamine salt); potassium chloride was dissolved in water. Concentrations are expressed in terms of the free base or acid, except for KCl.

RESULTS

Twenty-seven taenia strips from eleven specimens were predominantly relaxed by ATP (90–7200 μg/ml); one contracted at once, whereas most, after relaxing for 20–30 s, tended to contract while ATP remained in the bath. Because tachyphylaxis to ATP developed readily, submaximally effective doses were given at intervals longer than 15 min. Saline or 5% PEG in saline (both 0.5 ml) caused no significant changes in relaxations to ATP (180–720 μg/ml) which were 110 ± 7% of control (mean ± SEM, $n = 4$), noradrenaline (200 or 400 ng/ml) which were 131 ± 14% of control ($n = 4$) or electrical stimulation which were 89 ± 10%, 88 ± 8% and 94 ± 7% of control at 1, 2 and 4 Hz respectively.

Preliminary experiments indicated that there was no qualitative difference between the actions of 2-2′-pyridylisatogen (10–50 μg/ml) and 2-2′-pyridylisatogen tosylate (1–255 μg/ml): both relaxed the muscle at lower concentrations (10 and 18 μg/ml respectively) than were required to antagonize ATP. When hyoscine (1 μg/ml) was used to prevent cholinergic contractions and enhance nerve-mediated relaxations, no suitable stimulant was found to restore the muscle 'tone'. PGE_2 (500 ng/ml) or $PGF_{2\alpha}$ (1 μg/ml) failed to cause contraction in the presence of 2-2′-pyridylisatogen (10 μg/ml) although control strips were stimulated (one experiment each). In the presence of 2-2′-pyridylisatogen (10 or 50 μg/ml) KCl (2 mg/ml) contracted the muscle to near its initial level but in two control strips relaxations to noradrenaline or ATP were reduced in the continuous presence of KCl. When hyoscine was not used and tone was regenerated with carbachol, variable and relatively high concentrations of 2-2′-pyridylisatogen were needed to inhibit ATP: 2-2′-pyridylisatogen tosylate 45 μg/ml (*c.* 200 μM), equilibrated with the tissue for approximately 1 h, was the lowest consistently effective concentration. Its effects on submaximal relaxations to ATP and electrical stimulation are compared in Figure 16.1, carbachol (1–6 μg/ml) being used to counteract the reduction of tone caused by 2-2′-pyridylisatogen. Two other experiments, in which maximally effective concentrations of carbachol (8 or 16 μg/ml) only partly contracted the muscle, yielded similar results. 2-2′-pyridylisatogen

reduced or prevented relaxations to ATP ($p < 0.05$, Student's t-test for paired data), but its effect varied considerably between strips. Nerve-mediated relaxations were not significantly changed and, although relaxation with noradrenaline was reduced more than with ATP in two experiments, it was unchanged in one and increased in another preparation, thus the overall effect was not significant ($0.1 < p < 0.2$).

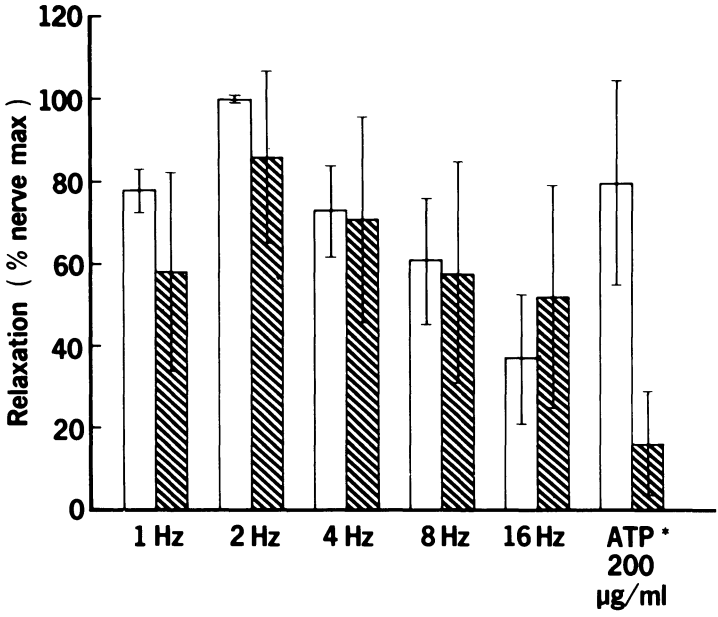

Figure 16.1 Electrically induced relaxations (1 ms, 17 V/cm, 10 s trains) and submaximal relaxations to ATP (200 µg/ml) are expressed as percentages of maximum relaxation to electrical stimulation (±SEM) before (open columns) and after 2-2′-pyridylisatogen tosylate (45 µg/ml) when the tone had been restored to within 20% of its initial level with carbachol (hatched columns). 2-2′-Pyridylisatogen tosylate antagonized relaxations to ATP ($p < 0.05$) but not to electrical stimulation

DISCUSSION

In human taenia coli strips both 2-2′-pyridylisatogen base and tosylate exerted two distinct effects: muscle relaxation and antagonism of ATP as reported in guinea pig taeniae[6]. Higher concentrations were needed than in guinea pig tissue, possibly because penetration was reduced in the thicker human taeniae. In both tissues, carbachol sometimes failed to cause substantial contractions in the presence of 2-2′-pyridylisatogen: the reason is unclear but it is further evidence of non-selectivity.

Despite relatively high concentrations of 2-2′-pyridylisatogen relaxations due mainly to non-adrenergic nerve stimulation were not significantly altered.

These results are consistent with the findings in guinea pig taenia caeci[6] and do not support a neurotransmitter role for ATP.

Acknowledgements

I thank the Wellcome Trust for support (grant to Dr A. Bennett, Department of Surgery, Kings College Hospital Medical School, London, where the experiments were performed), Drs K. J. Elcome (Wellcome Foundation Ltd.) and M. Spedding (Sunderland Polytechnic) respectively for 2-2′-pyridylisatogen and its tosylate.

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Discussion

J. Christensen: (USA) Is the effect of pyridylisatogen on ATP-relaxation confined to ATP? That is, does it antagonize relaxations by isoprenaline or dopamine?

H. L. Stockley: (UK) One of my slides showed that noradrenaline was not antagonized by pyridylisatogen but there was antagonism in some strips and also with higher concentrations of pyridylisatogen. Spedding *et al.*[6] reported that neither noradrenaline nor isoprenaline was antagonized in guinea pig taeniae. I have no information about dopamine.

J. Van Nueten: (Belgium) Have you any information on the effect of dipyridamole on the relaxation induced by electrical stimulation of the human taenia coli? A potentiation will be an indication that adenosine may be involved as an inhibitory neurotransmitter.

Stockley: No.

Christensen: From what parts of the colon did the strips come? I ask because there could be different responses in different regions.

Stockley: The strips came from the ascending and sigmoid regions and no differences between strips from the two regions were noted.

S. Anuras: (USA) Have you seen any contractions of taenia coli by electrical field stimulation?

Stockley: Yes. These results were reported to the 4th International Symposium on Gastrointestinal Motility in Banff. Cholinergic contractions occur during electrical stimulation, and also non-cholinergic contractions occur following relaxations when stimulation ceases.

L. Marzio: (Italy) In previous work (Lanfranchi *et al.*, *Proceedings of the Fifth Symposium on Gastrointestinal Motility*) we showed a stimulatory motor action on sigmoid colonic muscle *in vivo* with a pure β-adrenergic agent (Isoprel). Have you ever seen contraction with noradrenaline in your preparations?

Stockley: No. I, and others, have reported small contractions to adrenaline and noradrenaline in human intestinal strips, but this seems to be an α-adrenoceptor mediated response.

M. A. Cook: (Canada) (1) Do you have any dose–response data for the antagonism of ATP by pyridylisatogen which would allow you to speculate on the competitive nature (or otherwise) of the interaction? (2) In your tissue does pyridylisatogen have any effect on the contractile response to acetylcholine?

Stockley: (1) No, but poor penetration of the tissue by pyridylisatogen resulting in slow equilibration might be the factor masking such relationships in human tissue, as they have been reported in guinea-pig taenia[6]. (2) Acetylcholine has not been studied, but carbachol, a close analogue of acetylcholine, was used to recontract the muscle after pyridylisatogen. This was used in preference to acetylcholine because it is not hydrolysed by tissue cholinesterase and can therefore cause a sustained contraction. Sometimes with 45 μg/ml pyridylisatogen (and usually

with higher concentrations) carbachol, in maximally effective concentrations, was unable to re-contract the muscle to its initial level, suggesting that antagonism of some type was occurring.

17

Stimulatory actions of adenosine triphosphate in dog intestine

T. F. BURKS AND M. N. GRUBB

There is considerable evidence[1] that the intestine contains neural inhibitory pathways which are neither adrenergic nor cholinergic. The non-adrenergic inhibitory fibres may form the efferent links of descending inhibitory reflexes[2] which are important in the control of intestinal transit and which may participate in responses to drugs.

Burnstock[3] has reviewed data which suggest that the principal active substance released by the non-adrenergic inhibitory neurons is a purine nucleotide, probably ATP. Because the postulated purinergic inhibitory neurons have been studied in several vertebrate species, and due to the convincing elegance of many of the experiments, there has been a temptation to assume the existence of intestinal purinergic inhibitory neurons in all vertebrate species. The presumption of purinergic inhibitory neurons in a particular species requires at a minimum that the purine nucleotides cause inhibition of the intestine.

The present experiments were conducted to determine the effects of purine nucleotides on motor activity of the small intestine of the dog. The technique of vascular perfusion of isolated intestinal segments was employed because it preserves functional integrity of the intramural nerves[4] and, by administration of substances by bolus intra-arterial injections, allows brief exposure of the tissues to the putative neurotransmitter in a manner which mimics endogenous transmitter action. Preliminary experiments revealed that in this preparation the purine nucleotides cause contractions of the intestinal smooth muscle. The

mechanisms by which the excitatory effects occur were explored pharmacologically.

METHODS

Adult dogs of either sex, weighing 10–25 kg, were anaesthetized with intravenous thiopental sodium (15 mg/kg) and barbital sodium (250 mg/kg). The small intestine was exposed through a short mid-line incision. One arcade of mesenteric artery supplying a small (5–8 cm) section of intestine was cannulated with polyethylene tubing. The vasculature of each segment was perfused at a constant rate by means of a Sigmamotor T8 peristaltic perfusion pump with warm Krebs bicarbonate solution bubbled with 95% O_2–5% CO_2. After perfusion with the physiological salt solution was established, the ends of the segment were ligated and it was removed from the dog. A small latex balloon was tied into the lumen of the segment. Intraluminal pressure was measured from the intestinal preparation by a Statham P23Db pressure transducer connected to a Beckman R511 Dynograph recorder. Arterial perfusion pressure was recorded from a T-junction between the pump and the artery by a Statham P23 Db pressure transducer. Mean perfusion pressure was maintained at 60–70 mmHg at a flow rate of 9–13 ml/min.

Aqueous solutions of stimulatory drugs were administered as boluses by injection in volumes of 0.01–0.1 ml into the arterial cannula. Responses were measured as increases in intraluminal pressure. Inhibitory drugs were dissolved in small volumes of distilled water and were added in appropriate concentrations to the reservoir of Krebs solution. In most experiments, each stimulatory agent was tested in two preparations from each dog: one perfused with control Krebs solution and the other perfused with Krebs solution containing an inhibitory agent.

In experiments with hemicholinium, transmural electrical stimulation was employed to exhaust neuronal stores of acetylcholine.[5] Each agonist was tested during perfusion of the intestinal segment with control Krebs solution. After the control responses were obtained, electrical stimulation (7 Hz for 10 s at intervals of 60 s) was initiated and perfusion with hemicholinium (2 μg/ml) was begun. The agonist drugs were re-tested after the segment became refractory to electrical stimulation.

The purine compounds employed were adenosine triphosphate sodium (ATP), adenosine diphosphate disodium (ADP), adenosine monophosphate (AMP), adenosine, adenine, inosine, and guanosine triphosphate sodium (GTP). Other stimulatory agonists employed were bethanechol chloride, dimethylphenylpiperazinium iodide (DMPP), 5-hydroxytryptamine creatinine sulphate (5-HT), and amino acids 4–11 of substance P. Dosages of these agents were calculated as the salt forms. The inhibitory drugs used were tetraethylammonium chloride, tetrodotoxin, atropine sulphate, and hemicholinium-3-bromide.

RESULTS

Responses to purine compounds

Adenosine and the adenine nucleotides (ATP, ADP and AMP) caused contractions of the intestinal segments. The contractions generally consisted of tonic increases in baseline pressure with superimposed phasic contractions, as illustrated for ATP in Figure 17.1. Inhibitory effects or relaxation of the intestinal segments were not observed. The threshold stimulatory dose of ATP,

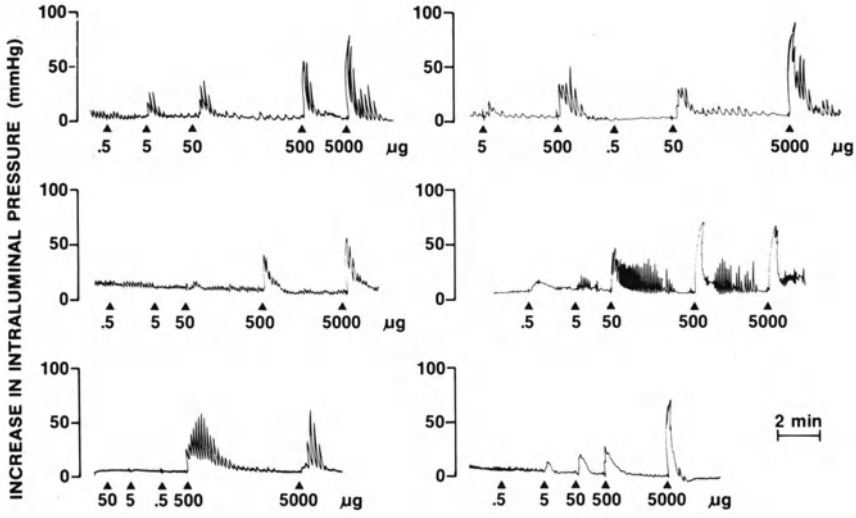

Figure 17.1 Representative intestinal responses to ATP. The responses illustrated were obtained in preparations from six different dogs. ATP was injected in each case as an intra-arterialbolus

the most potent of the purine compounds, was 0.5–5 μg and the maximum response occurred with doses of 500–5000 μg. The order of potency as intestinal stimulants was ATP > ADP > adenosine > AMP. Adenine, inosine and GTP were completely inactive.

Tachyphylaxis to ATP

To determine whether ATP acts upon a specific receptor, intestinal segments were perfused with 25 μM ATP to induce receptor desensitization[6] or tachyphylaxis. During continuous exposure to ATP, responses of the intestine to bolus doses of ATP were reduced (Table 17.1). Responses to other intestinal stimulants were not altered by perfusion with ATP.

Table 17.1 Effects of desensitization with 25 μM ATP on intestinal responses

Agonist	*Intestinal response* (mmHg)*		*No. of experiments*	*p*†
	Control	*ATP perfusion*		
ATP, 500 μg	35 ± 6	10 ± 3	6	<0.05
Bethanechol, 5 μg	49 ± 11	53 ± 12	6	NS‡
DMPP, 5 μg	68 ± 10	60 ± 12	6	NS
Substance F, 1 μg	82 ± 9	83 ± 14	6	NS

* Each value = mean ± SEM
† p = level of probability, Student's t test, paired comparison
‡ NS = not statistically significant

Neuronal blockade

Segments were perfused with tetrodotoxin (100 ng/ml) to determine whether the stimulatory effects of ATP result from direct actions on smooth muscle or are neurally mediated. To ascertain that the concentration of tetrodotoxin employed was appropriate for blockade of neuronal function without non-specific depression of smooth muscle, parallel experiments were performed with control substances. The control stimulatory drugs employed were 5-HT and DMPP, which act largely through neural mechanisms[7], and bethanechol and substance P, which act directly on smooth muscle. During perfusion with tetrodotoxin, intestinal responses to ATP, 5-HT and DMPP were nearly abolished, while responses to bethanechol and substance P were essentially unaffected (Table 17.2).

Table 17.2 Effects of neuronal blockade with 100 ng/ml tetrodotoxin on intestinal responses

Agonist	*Intestinal response* (mmHg)*		*No. of experiments*	*p*†
	Control	*Tetrodotoxin*		
ATP, 500 μg	37 ± 5	4 ± 2	6	<0.01
5-HT, 1 μg	46 ± 8	8 ± 2	6	<0.01
DMPP, 5 μg	56 ± 12	2 ± 2	6	<0.01
Bethanechol, 25 μg	100 ± 10	103 ± 10	6	NS‡
Substance P, 10 μg	109 ± 22	94 ± 15	6	NS

* Each value = mean ± SEM
† p = level of probability, Student's t test, paired comparison
‡ NS = not statistically significant

Muscarinic cholinergic blockade

Evidence that the intrinsic neural elements activated by ATP release acetylcholine was provided by the use of atropine, a muscarinic cholinergic receptor antagonist. As can be seen in Table 17.3, perfusion with atropine (25 ng/ml) inhibited responses of the intestine to ATP and to control substances tested.

As this experiment did not rule out the possibility of atropine-induced non-specific depression of the smooth muscle, another technique was employed to establish that the neurons acted upon by ATP are cholinergic.

Table 17.3 Effects of muscarinic receptor blockade with 25 ng/ml atropine on intestinal responses

Agonist	*Intestinal response* (mmHg)*		*No. of experiments*	*p*†
	Control	*Atropine*		
ATP 5000 μg	58 ± 9	13 ± 6	6	<0.01
Bethanechol 25 μg	109 ± 9	18 ± 5	6	<0.01
DMPP 25 μg	120 ± 11	53 ± 20	6	<0.01
5-HT 5 μg	56 ± 10	12 ± 3	6	<0.01

* Each value = mean ± SEM
† p = level of probability, Student's t test, paired comparison

Depletion of neuronal acetylcholine

Hemicholinium blocks synthesis of acetylcholine by preventing neuronal uptake of choline[5]. During perfusion of intestinal segments with hemicholinium (2 μg/ml) and after pre-formed stores of acetylcholine had been exhausted by electrical stimulation, responses to ATP were reduced (Table 17.4). Responses to 5-HT and DMPP, which also act upon cholinergic neurons[7], were similarly inhibited by hemicholinium. Responses to bethanechol, which acts directly upon smooth muscle cholinergic receptors, were not altered.

Table 17.4 Effects of 2 μg/ml of hemicholinium on intestinal responses

Agonist	*Intestinal response* (mmHg)*		*No. of experiments*	*p*†
	Control	*Hemicholinium*		
ATP, 5000 μg	53 ± 7	19 ± 8	7	<0.05
5-HT, 25 μg	69 ± 3	42 ± 7	6	<0.05
DMPP, 25 μg	63 ± 6	19 ± 7	6	<0.05
Bethanechol, 25 μg	77 ± 13	61 ± 11	5	NS‡

* Each value = mean ± SEM
† p = level of probability, Student's t test, paired comparison
‡ NS = not statistically significant

Nicotinic cholinergic blockade

To determine whether the site of neuronal action of ATP is pre- or post-ganglionic, responses to ATP and control intestinal stimulants were measured in the absence and in the presence of tetraethylammonium, a ganglion-blocking drug. Perfusion of intestinal segments with tetraethylammonium

(1 mg/ml) did not significantly alter responses to ATP or 5-HT, but completely blocked responses to the ganglionic stimulant, DMPP (Table 17.5).

Table 17.5 Effects of ganglion blockade with 1 mg/ml tetraethylammonium on intestinal responses

Agonist	*Intestinal response* (mmHg)*		*No. of experiments*	*p*†
	Control	*Tetraethylammonium*		
ATP, 5000 μg	88 ± 30	75 ± 16	5	NS‡
DMPP, 5 μg	84 ± 20	0 ± 0	5	<0.01
5-HT, 25 μg	93 ± 26	86 ± 28	5	NS

* Each value = mean ± SEM
† p = level of probability, Student's t test, paired comparisons
‡ NS = not statistically significant

DISCUSSION

The adenine nucleotides, ATP, ADP and AMP, and adenosine, produced purely excitatory responses in dog isolated intestine. The prototype and most potent nucleotide, ATP, appears to interact with a specific neuronal receptor to produce contractions of the intestine. The specificity of the receptor is suggested by the order of potency of the purine compounds, by the total lack of activity of GTP, and by the selective inhibition of responses to ATP in the presence of receptor desensitization. The ATP receptor may be associated with post-ganglionic cholinergic neural elements. Responses to ATP were inhibited by tetrodotoxin, atropine and hemicholinium, indicating that post-ganglionic cholinergic neural function is necessary for the intestinal action of ATP. Ganglionic blockade did not affect responses to ATP, which indicates that ATP does not act directly on a nicotinic cholinergic receptor or by activation of pre-ganglionic parasympathetic fibres. Other drugs, including 5-HT[7] and cholecystokinin[8], appear to activate post-ganglionic cholinergic neural elements by actions upon specific non-nicotinic receptors in the wall of the intestine.

There have been previous reports of excitatory actions of purine nucleotides in gastrointestinal tissues. Rebound contractions after initial inhibitory responses have been observed in rat stomach strips, guinea pig taenia coli, rat duodenum and rat ileum[9,10]. Purely excitatory responses have been noted in intestinal preparations from lower vertebrate species, such as toads, lizards and goldfish[10]. Stimulatory effects of adenine analogues have recently been described in longitudinal muscle of pig stomach[11]. Unlike the dog intestine, however, ATP-induced contractions in pig stomach were not affected by tetrodotoxin and appeared to result from direct effects on the smooth muscle.

While the purine nucleotides may serve as neural inhibitory modulators of intestinal motility in some species[9], that possibility in dog small intestine

seems unlikely. However, it should be pointed out, as Burnstock[3] has, that purine nucleotides may be involved as excitatory neurotransmitters in certain species. Because of the low order of potency of the adenine nucleotides as intestinal stimulants, a physiological role as an excitatory neurotransmitter in the dog is at present difficult to postulate. But it is possible that estimates of potency obtained with exogenously administered adenine nucleotides are unreliable because of their rapid degradation into adenine or inosine[12,13]. Endogenously released ATP may be relatively more effective and, if so, could possibly serve a modulatory role in intestinal motility. The presence of a specific neuronal receptor for ATP argues in favour of a role for ATP in physiological events.

Acknowledgement

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Discussion

M. A. Cook: (Canada) Since you are delivering your agonist by way of the arterial system, which contains an efficient ATP and adenosine uptake system, I wonder if you have studied the methylene-substituted esters of adenosine which are resistant to breakdown?

T. Burks: (USA) We have tested the effects of the alpha, beta-methylene and beta, gamma-methylene analogues of ATP. The alpha, beta-methylene derivative, which is not dephosphorylated, mimicked the effects of ATP. The beta, gamma-methylene derivative, which is subject to hydrolysis to ADP, was less potent.

J. D. Wood: (USA) Which muscle layer is activated by ATP?

Burks: We have used extraluminal strain gauges oriented in the transverse and longitudinal axes of the gut while recording intraluminal pressure with our balloon technique. Responses recorded from the balloon corresponded exactly to those recorded from transversely arranged strain gauges, so we believe the contractions are of the circular muscle layer.

H. L. Stockley: (UK) Is there tachyphylaxis to this stimulant action of ATP as there is with the inhibitory effects?

Burks: Yes, tachyphylaxis occurs readily, either after the injection of a large bolus dose or during perfusion with ATP. Tachyphylaxis is not a problem and advantage was taken of the phenomenon in the receptor desensitization experiments.

E. E. Daniel: (Canada) Some years ago we showed that intra-arterial ATP and ADP very briefly inhibited spiking in dog intestine; I wonder if your mechanical recording techniques may not have missed a very transient inhibition. Have you recorded electrically to make sure a very transient inhibition did not occur?

Burks: We can see inhibitory responses to bolus intra-arterial doses of norepinephrine and isoproterenol. It is possible, however, that a very transient initial inhibition would not be recorded. Your suggestion that we measure electrical activity is an excellent one as it would provide an index of motility not subject to mechanical artefacts.

J. S. Davison: (UK) I would endorse what Dr Daniel has just said. If the contractions are rebound excitations, following a brief period of inhibition which was not being recorded, then they might be dependent upon prostaglandin release as in other species. Have you examined, therefore, the action of prostaglandin synthesis inhibitors such as indomethacin?

Burks: We believe that the contractions induced by ATP are not caused by prostaglandin release. We have tested the effects of indomethacin under conditions which prevent prostaglandin formation in our preparation. Indomethacin did not affect responses to ATP.

N. E. Diamant: (Canada) (1) Can your recording system in fact pick up relaxations, for example, induced by adrenergic agents, and have you checked this? (2) There

appeared to be a significant delay between ATP injection and the appearance of the contraction that seemed greater than the delay following cholinergic stimulation. Is it possible you are missing, for technical reasons, a transient relaxation and are seeing a rebound after contraction associated with this?

Burks: As you have observed, in most of our preparations there was little pre-existing tone at the time ATP was administered. Intraluminal pressure at rest ranged from 0 to 5 mmHg. Under those conditions, it would be possible to overlook relaxation. In some preparations, however, tone was greater, yet relaxation responses were never observed. We have tested the effects of adrenergic amines and we can see their inhibitory effects. Relaxation responses can also be observed with E series prostaglandins.

18

Effect of dopamine on gastric motility in man: evidence for specific receptors

G. A. LANFRANCHI, L. MARZIO, C. CORTINI, L. TRENTO AND G. LABÓ

In previous work[1], we showed that dopamine induces an increase of the motor activity of human sigmoid colon; the response was antagonized neither by α- or β-adrenergic blocking agents, nor by atropine.

The aim of the present investigation was to study the effect of dopamine on the motility of the human stomach. Dopamine, a precursor of noradrenaline, is an important neurotransmitter in the central nervous system. There is increasing evidence that dopamine may have peripheral actions of its own on the cardiovascular system[2,3] and on the gastrointestinal tract. An inhibitory response was constantly observed on the LES of the opossum *in vivo* and *in vitro*[4,5] and on the intraluminal pressure of the proximal stomach in the dog and in man[6]. By contrast, dopamine induced a stimulatory effect on the lowermost part of the oesophageal body in the opossum[5], on the sigmoid colonic motility in man[7], on exocrine pancreatic secretion[8] and on plasma glucagon levels in man[9].

The aim of this work is to study the effect of dopamine on the electrical and mechanical activity of the human gastric antrum and to determine whether this effect is opposed by sulpiride, a specific dopaminergic antagonist[10].

METHODS

Electrical and mechanical activity of the gastric antrum was recorded in twelve subjects; they all gave their informed consent to the procedure.

Table 18.1 Modifications of the pressure wave parameters in the different periods of the experiment (Mean ± SE)

	Frequency/min		*Duration/min*		*Amplitude*		*Percentage duration*		*MI*	
	Proximal	*Distal*	*Proximal*	*Distal*	*Proximal*	*Distal*	*Proximal*	*Distal*	*Proximal*	*Distal*
Basal	1.5 ± 0.3	1.2 ± 0.2	17.6 ± 1.1	7.9 ± 1.0	14.9 ± 2.1	43.8 ± 10.0	41.8 ± 9.3	19.1 ± 4.1	665 ± 236	1051 ± 347
Dopamine	0.5 ± 0.3*	0.8 ± 0.3 ns	8.2 ± 3.8*	4.5 ± 1.4*	5.4 ± 2.4†	12.3 ± 4.7†	14.2 ± 8.1†	12.9 ± 6.0 ns	206 ± 151†	351 ± 203†
After dopamine	1.2 ± 0.3 ns	1.2 ± 0.2 ns	17.5 ± 2.6 ns	7.6 ± 1.3 ns	14.4 ± 2.2 ns	40.8 ± 14.7 ns	35.0 ± 10.3 ns	18.2 ± 9.2 ns	515 ± 233 ns	1125 ± 370 ns
Dopamine + sulpiride	0.9 ± 0.3 ns	1.1 ± 0.2 ns	13.6 ± 3.4 ns	7.6 ± 0.9 ns	11.4 ± 2.6 ns	51.7 ± 15.5 ns	29.7 ± 9.6 ns	21.5 ± 5.7 ns	546 ± 205 ns	794 ± 227 ns

Recordings were obtained in twelve cases for the distal balloon and in nine cases for the proximal balloon due to technical reasons.
ns = not significant; * $p < 0.05$; † $p < 0.01$

Recordings were obtained by means of a nasogastric probe with two small (2.5 × 1 mm) rubber, air-filled balloons mounted at the terminal side, 10 cm apart. Electrical activity was recorded by a monopolar suction-type silver electrode, attached to the tube, on the opposite side of the distal micro-balloon. The time-constant used to record the potential was 0.1 s; the signals obtained were amplified and fed into a recorder. The tube assembly was introduced through the mouth and placed in such a way that the recording device lay in the gastric antrum; the position was always checked by X-ray control.

The records were analysed by measuring the number, amplitude and duration of each pressure wave. The results were conventionally evaluated by the motility index (MI), obtained by multiplying the mean amplitude of the waves by the percentage duration of activity[11].

The electrical activity was recorded in eight subjects. The only parameter evaluated was the frequency per minute of the electrical control activity (ECA).

All the experiments were carried out in the afternoon; patients were allowed continental breakfast early in the morning. Recordings were begun 30–65 min after the introduction of the probe, then basal motility was recorded for 30 min; during this period intravenous saline was infused in the cubital vein of the arm. Subsequently, dopamine was administered at the dose of 5 μg/kg/min for 10 min. This dose was chosen on the results of a previous investigation[7] on colonic motor activity, showing that 5 μg was the lowest dose capable of obtaining a significant motor modification without side-effects. The motor response of dopamine was evaluated during the 10 min administration only because of the immediate cessation of the effect at the suspension of the drug infusion. After an interval of 30 min, dopamine infusion at the same dose was repeated, with the pre-treatment as bolus intravenously of sulpiride at the dose of 100 mg.

Heart rate and blood pressure were monitored during the whole experiment. All the parameters evaluated during the basal period were compared with those recorded during dopamine, during the period after dopamine and during dopamine + sulpiride.

Changes were tested for significance by the Student's t test for paired data. Results were expressed as mean ± SE.

RESULTS

The parameters evaluated in the different periods of the experiment are reported in Tables 18.1 and 18.2. The modifications of the slow wave frequency are shown in graph form in Figure 18.1.

Basal period

In the 30 min basal tracings, ECA and pressure waves were regularly recorded.

Motor activity was lower in the proximal balloon in comparison to the distal one regarding the mean amplitude, while frequency and duration of the pressure waves were higher at the proximal level of recording. The mean frequency of the ECA was 2.9 ± 0.1 cpm and slow waves occurred at regular time-intervals.

Table 18.2 Modifications of the cardiovascular parameters in the different periods of the experiment (Mean ± SE) in 12 subjects

	Heart rate	*Systolic pressure*	*Diastolic pressure*
Basal	79 ± 3	126 ± 6	83 ± 3
Dopamine	86 ± 4†	131 ± 4 (ns)	83 ± 2 (ns)
Dopamine + sulpiride	87 ± 5†	135 ± 7*	85 ± 3 (ns)

ns = not significant; $*p < 0.05$; $\dagger p < 0.01$

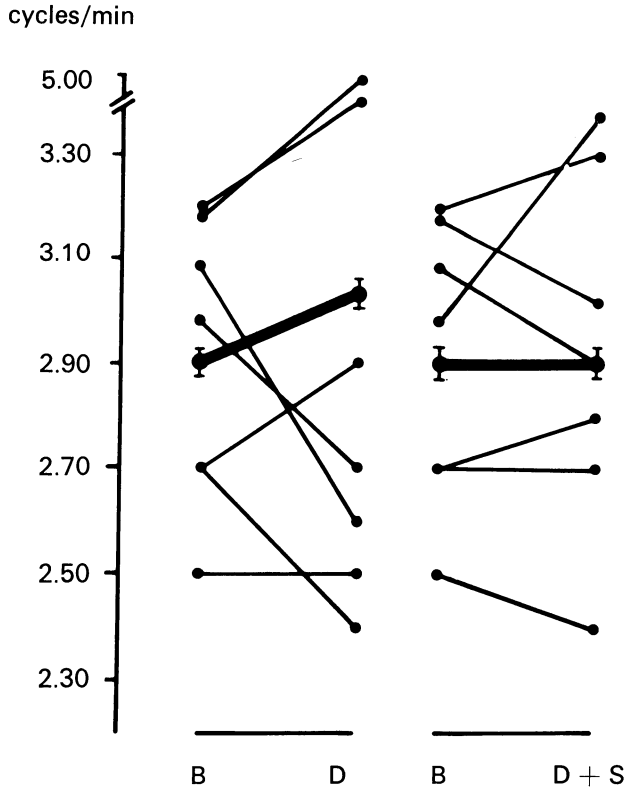

Figure 18.1 Variations of the ECA frequency of individual cases (light bar) and of the mean ± SE (heavy bar) of seven subjects (B = basal; D = dopamine; S = sulpiride)

Dopamine period

The infusion of the drug produced a prompt decrease in the number, amplitude and duration of the pressure waves; in some cases their complete disappearance was observed (Figures 18.2–18.4). The inhibitory effect persisted for all the infusion period; the decrease of the MI was significant at both the levels of recording (Figure 18.5).

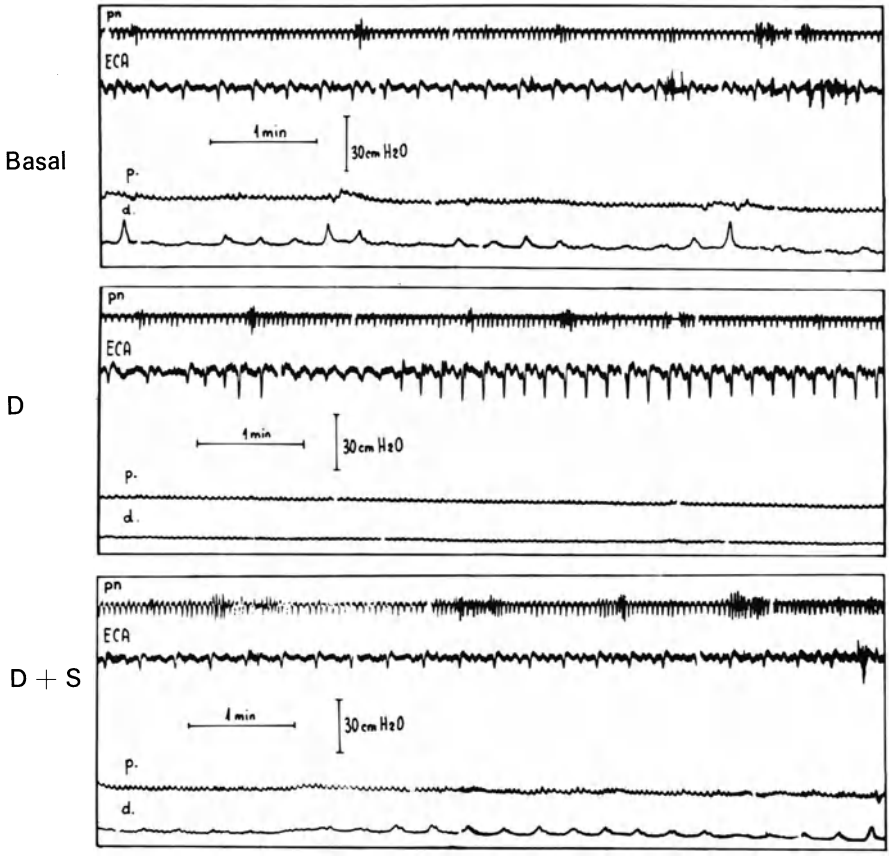

Figure 18.2 Myoelectrical activity and pressure recorded from the antrum. The ECA frequency is present at 3.0 cpm in the basal recording; there is an increase to 5.0 cpm during dopamine; no change during dopamine + sulpiride. No mechanical activity is present at the proximal (p) and distal (d) balloons during dopamine. Pneumogram (pn) at the top of each tracing. (Abbreviations: as Figure 18.1)

Different changes of the ECA were recorded. The mean frequency of the ECA increased but not significantly (Figure 18.1). The analysis of every case showed that in three subjects the ECA frequency increased (Figure 18.2); in

three cases a decrease was observed (Figure 18.3); in one the frequency did not change; in the last case, a complete disappearance of the ECA for 4 min associated with sporadic delayed potentials was recorded (Figure 18.4). In the whole group the intervals of the slow waves occurred irregularly so that the distribution of the ECA frequency was scattered (Figure 18.6).

Heart-rate and systolic blood-pressure increased, but only heart-rate increase reached a statistically significant level (Table 18.2).

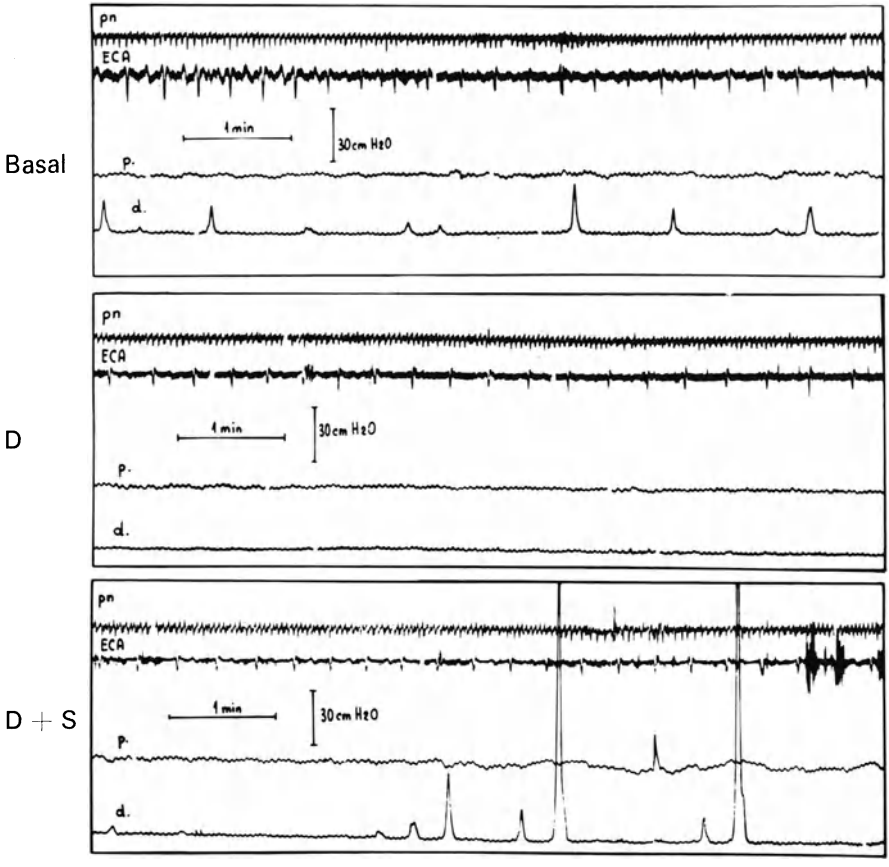

Figure 18.3 Myoelectrical activity and pressure recorded from the antrum. The ECA frequency is present at 3.2 cpm in the basal recording; there is a decrease to 2.7 cpm during dopamine; no change during dopamine + sulpiride. No mechanical activity present at the proximal (p) and distal (d) balloons during dopamine. Pneumogram (pn) at the top of each tracing. (Abbreviations: as Figure 18.1)

Post-dopamine period

In this 30 min period a progressive increase of the mechanical activity was observed, with a return to the basal levels (Table 18.1).

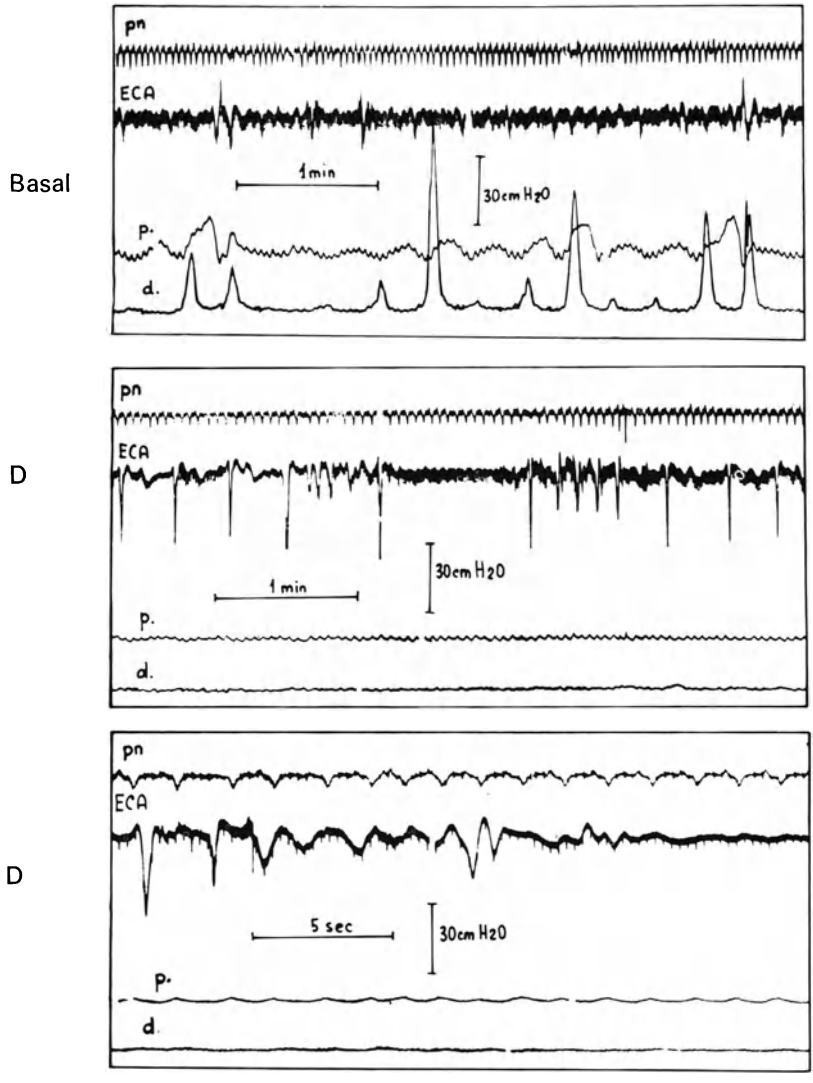

Figure 18.4 Myoelectrical activity and pressure recorded from the antrum. The ECA frequency is present at 3.0 cpm in the basal recording; during dopamine premature and delayed potentials are recorded. Complete inhibition of the mechanical activity at the proximal (p) and distal (d) balloons during dopamine. Pneumogram (pn) at the top of each tracing. The last tracing is recorded at higher speed. (Abbreviations: as Figure 18.1)

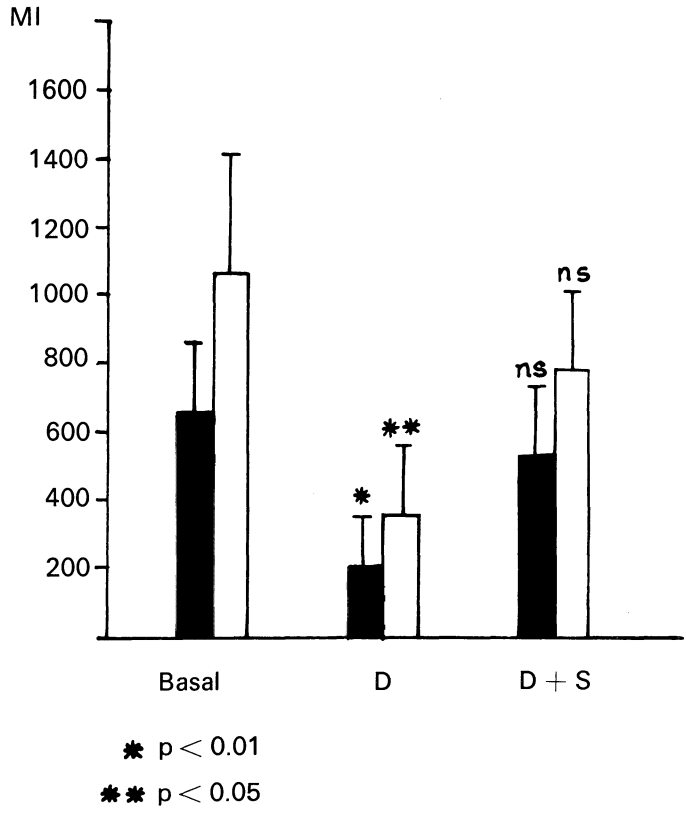

Figure 18.5 Variations of cumulative MI during dopamine and during dopamine + sulpiride. Values are mean ± SE of nine subjects for the proximal balloon (white bar) and of twelve patients for the distal balloon (black bar). (Abbreviations: as Figure 18.1)

These tracings showed a progressive normalization of the slow waves, associated with the return of the ECA frequency to the basal values.

Dopamine plus sulpiride period

The administration of sulpiride before dopamine infusion prevented the electrical and mechanical modifications observed during the dopamine period (Figures 18.1, 18.5). On the contrary, sulpiride did not prevent the increase of the heart-rate and of the systolic blood-pressure (Table 18.2).

DISCUSSION

The effect of dopamine on the stomach has already been assayed by Valenzuela[6]; this author measured intragastric pressure in dogs with gastric

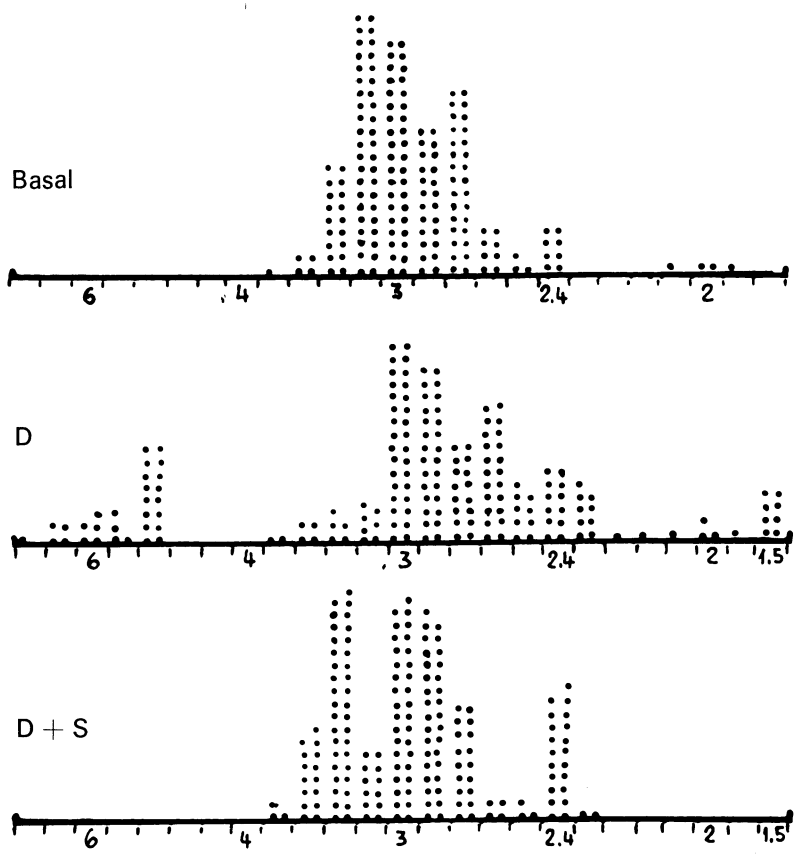

Figure 18.6 Variations of the distribution of ECA frequency of seven subjects during dopamine and during dopamine + sulpiride. Black points represent the frequency of each control wave. (Abbreviations: as Figure 18.1)

fistulas by means of a big flaccid balloon. Dopamine caused a decrease in intragastric pressure, not blocked by α- and β-receptors antagonists, but by pimozide and metoclopramide, two known dopaminergic antagonists. The conclusion of this investigator was that dopamine may be the physiological neurotransmitter for receptive relaxation of the stomach.

Our results demonstrate that dopamine induces an inhibitory effect on the mechanical activity of the human gastric antrum: therefore these findings are consistent with the possibility that dopamine may also play a role in the regulation of the antral motility.

The inhibitory effect could be due to conversion or release of noradrenaline, with activation of gastric adrenergic receptors. This possibility seems unlikely because sulpiride abolished the inhibitory action of exogenous dopamine. The specificity of dopaminergic inhibition is supported also by the

experiment of Valenzuela[6], because pre-treatment with α- and β-adrenergic blockers did not significantly reduce the inhibitory effect of dopamine. On the other hand in a previous investigation[7], we demonstrated that the stimulating effect of dopamine on colonic sigmoid motility in man was prevented neither by adrenergic antagonists (phentolamine and propranolol) nor by anticholinergic drugs (atropine).

An intriguing aspect of dopaminergic effect is the inhibitory action on LES and gastric muscle and the stimulatory action on the lowermost part of the oesophagus and on the sigmoid colon. These findings may suggest the possibility of two different dopaminergic receptors, with contrasting effects on motor activity, similar to the adrenergic system.

In the present study the inhibitory effect could be due to the stimulation of dopaminergic receptors in the chemotrigger zone of the medulla oblongata, or to activation of dopamine receptors in the stomach.

Because the effect of dopamine is also observed in isolated oesophageal muscles[4], it seems likely that the action of dopamine is peripheral. Furthermore, in the opinion of De Carle and Christensen[4] the receptor is in the muscle itself, rather than in the intramural nerves, for the effects of dopamine are not tetrodotoxin-sensitive.

The modifications of the ECA observed during dopamine infusion were variable. In two cases the pattern of the control potentials looked like the type observed after administration of anticholinergic or sympathomimetic drugs[12], i.e. premature control potentials followed by disappearance of control waves, increase in frequency and alteration of the amplitude and morphology of the slow waves. In the other subjects, no reproducible variations in frequency were recorded, even if the mean ECA frequency was increased. However, the effect seemed to be due neither to excitation of adrenergic receptors nor to inhibition of cholinergic agents, because the electrical response to dopamine was in every case antagonized by sulpiride.

The cardiovascular effects observed in the present study agree with our previous one[7] but differ from other reported results. The study of Goldberg[3] in normal subjects showed that intravenous infusions of dopamine ranging from 1 to 10 μg/kg/min did not induce any modification of heart-rate and of mean arterial blood-pressure; with higher infusion rates, arterial pressure increased (probably for an α-adrenergic effect) and heart-rate decreased. On the contrary, our findings show an increase in heart-rate and systolic blood-pressure, not prevented by sulpiride; therefore it seems that these effects are not mediated by specific dopaminergic receptors.

It is concluded that, as defined in terms of the effects of agonists and antagonists, a gastric dopamine receptor has been demonstrated by this study with an inhibitory effect on motor activity of the antrum and a variable influence on the ECA.

However, the physiological role of dopaminergic receptors remains to be determined, because the doses infused in our study are pharmacological.

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Discussion

P. Bass: (USA) Why was sulpiride used instead of metoclopramide or haloperidol?

G. A. Lanfranchi: (Italy) The study was carried out in man, so haloperidol was not used for ethical reasons, due to the possible onset of side-effects. Metoclopramide is able to induce a strong motor response in the stomach which, at least partly, is not dependent on antagonism to dopamine, but is probably cholinergic.

J. Van Neuten: (Belgium) One important difference between sulpiride and metoclopramide in studies on gastrointestinal tissues is the potentiation of the release of acetylcholine, observed with the latter compound in the electrically stimulated guinea pig ileum.

J. De Carle: (Australia) I support not using metoclopramide. In our *in vitro* studies of dopamine on oesophageal smooth muscle we were unable to block its effects with metoclopramide.

R. W. McCallum: (USA) We recently had the opportunity to study oral L-dopa's effect on gastric emptying in man using a gamma camera technique and a semi-solid meal. 1000 mg L-dopa orally, significantly prolonged gastric emptying time while metoclopramide inhibited this prolongation.

Lanfranchi: Thank you for this comment, which supports our results.

E. E. Daniel: (Canada) Your results showed an increased heart-rate, not blocked by sulpiride. This suggests an adrenergic stimulation by dopamine and may account for some of the variability of effects of dopamine on ECA, since there may have been an adrenergic, as well as a dopaminergic, effect. Phentolamine or propranolol might have regularized dopamine effects on ECA. Have you tried them?

Lanfranchi: No, we did not try. However, the constant regularization of ECA by sulpiride suggests a specific dopamine effect.

J. A. J. Schuurkes: (Holland) A great variety of motility indices are used. Could you define yours? Do you use the same definition as used by Dr Ormsbee or Dr Eastwood?

Lanfranchi: Our motility index is calculated by multiplying the percentage duration for the mean amplitude of the pressure waves, as is reported in our paper. I do not know the motility indices used by these authors.

A. J. Van Merwyk: (UK) All patients showed a decrease of motor activity. How do you explain an increase in ECA in some patients when the motor activity was decreased?

Lanfranchi: I think that it is generally accepted that there is no constant relationship between ECA frequency and motor activity.

19

Is dopamine an endogenous inhibitor of gastric emptying?

J. M. VAN NUETEN AND P. A. JANSSEN

Gastric motility depends mainly upon the myogenic activity of the gastric wall; it is modulated by intrinsic and extrinsic nerves, but also by locally produced hormones[1,2]. Among the candidates for mediating gastric relaxation, a possible role has been proposed for dopamine in the dog's stomach[3]; such a role would be in line with other effects of dopamine at gastrointestinal sites[4,5]. The present study was designed to assess whether or not dopamine acts as a local modulator of gastropyloric function in the guinea pig.

MATERIAL AND METHODS

The experiments were performed on isolated stomachs taken from fasted guinea pigs killed by cervical trans-section; the oesophagus, the first 10 cm of the duodenum, the vagal trunks and the coeliac axis with its gastric branches were dissected out together with the stomach[6]. The gastrointestinal content was removed by repeated washing. After ligating the oesophagus, the stomach was filled with 20 ml of saline and suspended in 200 ml of oxygenated (95% O_2; 5% CO_2) Krebs–Henseleit solution maintained at 37 °C. A glass cannula was placed into the duodenum (2 cm below the pyloric sphincter) and connected to an ultrasonic transit time device, based on the Doppler pulse principle. The recording cannula was further connected to a bottle of saline, ensuring a constant hydrostatic pressure of 6 cm saline in the stomach. With this system, previously used for measuring volume displacements associated

with peristaltic reflex activity in the guinea pig ileum[7], changes in gastric content can be continuously recorded; emptying and filling of the stomach correspond to contraction and relaxation of the gastric wall, respectively. The vagal trunks and the coeliac axis were passed through platinum-ring electrodes, connected to a stimulator (Janssen Scientific Instruments SU_1); electrical stimulation consisted of trains (10 s) of square wave impulses (1 ms, 50 mA,

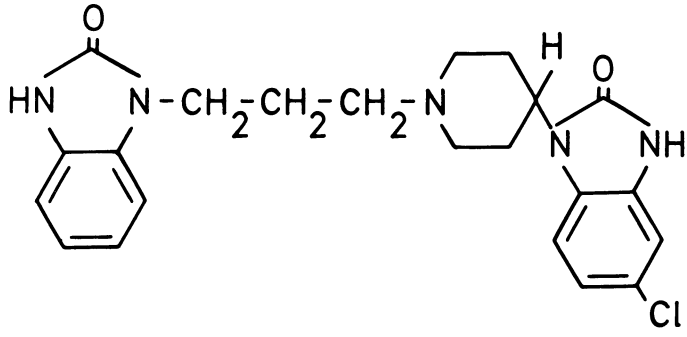

Figure 19.1 Chemical structure of domperidone (5-chloro-1-{1-[3-(2,3-dihydro-2-oxo-1*H*-benzimidazol-1-yl)propyl]-4-piperidinyl}-1,3-dihydro-2*H*-benzimidazol-2-one

10 Hz). The following pharmacological agents were used: atropine, domperidone (Figure 19.1), dopamine, haloperidol, noradrenaline, phenoxybenzamine, phentolamine and tetrodotoxin. The drugs were added to the bath solution in 0.5–1 ml of saline; all concentrations are expressed as final bath concentrations. For statistical analysis Student's *t*-test for paired observations was used; *p*-values smaller than 0.05 were considered to be significant.

RESULTS

Control responses (Figure 19.2)

In the experimental conditions used, the stomachs display various degrees of irregular spontaneous activity, emptying alternating with filling (Figure 19.2; upper). Further relaxation of the gastric wall can be obtained with electrical stimulation of the sympathetic nerves contained in the coeliac trunk (Figure 19.2; middle, left) in untreated preparations; in atropinized stomachs, stimulation of the vagi also caused an increase in gastric content (Figure 19.2; middle, right). Addition of both dopamine and noradrenaline (Figure 19.2; lower) to the bath solution caused pronounced filling of the stomach. For the further experiments equiactive concentrations of dopamine (2.5 μg/ml) and of noradrenaline (0.08 μg/ml) were used throughout; these concentrations first were shown to cause 60% of the maximal inhibition which could be obtained with both amines.

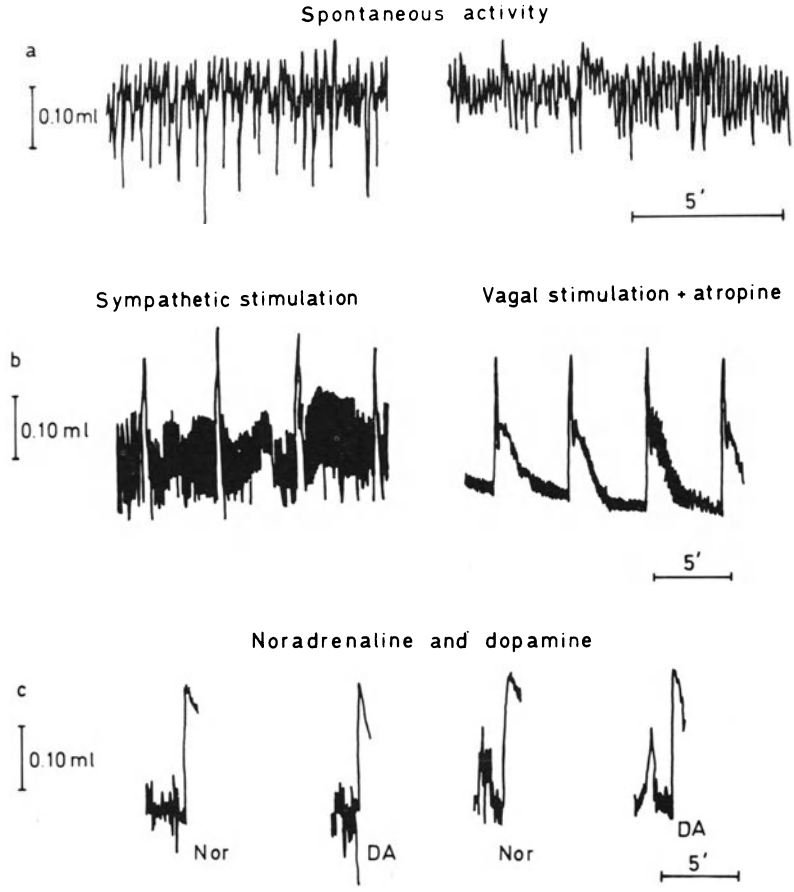

Figure 19.2 Typical experiments on the isolated guinea pig stomach. Relaxation and contraction are measured as an increase (upward deflection) and decrease, respectively, in gastric volume. Spontaneous activity (a); decrease in gastric volume induced by sympathetic nerve stimulation and vagal inhibitory stimulation (b), and by exogenous noradrenaline and dopamine (c)

Effect of domperidone

Domperidone caused a significant dose-dependent inhibition of the relaxations evoked by dopamine. Higher concentrations were required to inhibit significantly the response to sympathetic nerve stimulation. Only with the highest concentration of domperidone used could a significant depression of the response to exogenous noradrenaline and to vagal stimulation (in atropinized stomachs) be obtained (Figure 19.3; Table 19.1).

Concentrations of domperidone lower than those required to significantly reduce the inhibitory effect of dopamme, augmented and regularized the spontaneous changes in gastric content (Figure 19.4).

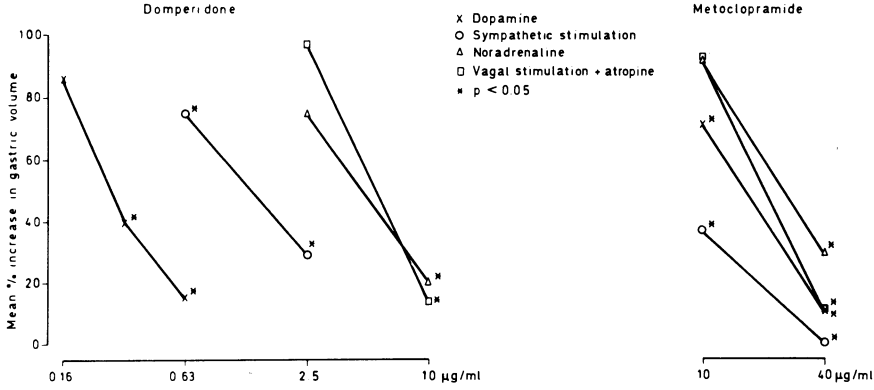

Figure 19.3 Effect of domperidone and metoclopramide on increase in gastric volume evoked by exogenous dopamine and noradrenaline, by sympathetic nerve stimulation and by vagal inhibitory nerve stimulation. Experiments performed on isolated guinea pig stomachs. Data shown as means of the peak effects ($n = 5$)

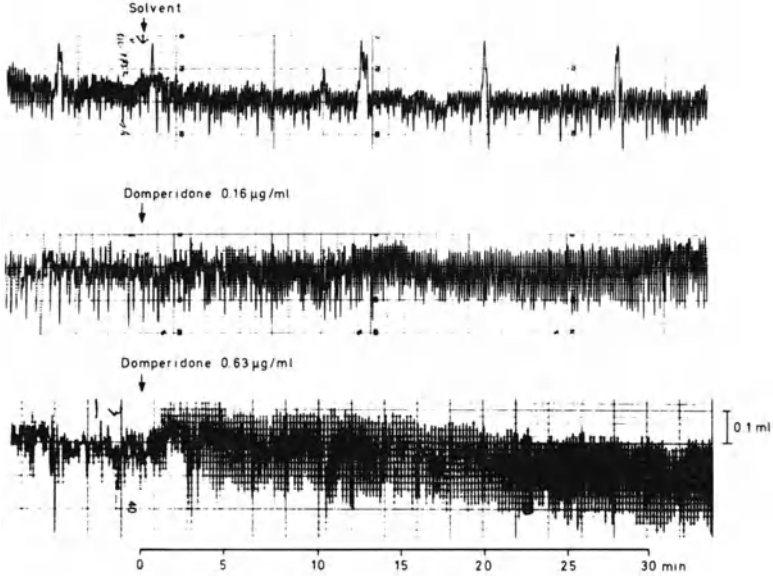

Figure 19.4 Effect of increasing concentrations of domperidone on spontaneous activity of the isolated guinea pig stomach. Spontaneous activity recorded as changes in gastric volume

Table 19.1 Effect of blocking agents on inhibitory responses and spontaneous activity in the guinea pig stomach*

Compound	*Regularization of spontaneous activity*	*Inhibition of increase in gastric volume due to*		
		Dopamine	*Noradrenaline*	*Sympathetic stimulation*
Domperidone	++++	+++	+	++
Haloperidol	+++	+++	no effect	not examined
Metoclopramide	++	+++	+++	++++
Phentolamine	no effect	+++	++	+++
Phenoxybenzamine	no effect	+++	++	not examined

* Shown as relative potencies when compared to the depression of the relaxation induced by dopamine

Effect of α-adrenolytic drugs

Phentolamine and phenoxybenzamine abolished the inhibitory effect of dopamine and sympathetic nerve stimulation at lower concentrations than those required to antagonize the response to exogenous noradrenaline (Table 19.1); they had no effect on the spontaneous activity of the preparations.

Effect of haloperidol

Haloperidol inhibited the increase in gastric content caused by dopamine, but not that evoked by noradrenaline (Table 19.1). At concentrations which antagonize the effect of dopamine, the drug caused an increase in the spontaneous volume displacements of the isolated stomachs.

Effect of metoclopramide

Metoclopramide inhibited all relaxatory stimuli applied. The response to sympathetic stimulation was significantly depressed at lower concentrations of metoclopramide than the response to dopamine and to exogenous noradrenaline; there was no significant difference in the antagonism by metoclopramide of the response to dopamine, noradrenaline and vagal stimulation (in atropinized preparations) (Figure 19.3; Table 19.1). At the highest concentration used (40 μg/ml), metoclopramide usually regularized the spontaneous activity of the isolated stomachs.

Effect of tetrodotoxin

At 0.16 μg/ml, tetrodotoxin depressed the inhibitory effect of dopamine by 78%, but did not significantly affect the inhibitory response to noradrenaline.

DISCUSSION AND CONCLUSIONS

The present study demonstrates that, as in the dog[3], there are dopaminergic receptors in the guinea pig stomach, which mediate inhibition of the spontaneous gastric activity. This conclusion is drawn from the observation that the inhibitory effect of dopamine can be specifically antagonized by the well-known inhibitor of dopaminergic receptors, haloperidol. Domperidone has potent anti-emetic properties, and also prevents symptoms of delayed gastric emptying and of gastro-oesophageal reflux without producing psychotropic or neurological side-effects[8–10]; although *in vitro* it is a potent antagonist of striatal dopamine receptors when tested in the [^{3}H]-haloperidol binding assay, domperidone cannot reach brain dopamine receptors in *in vivo* conditions (Laduron and Leysen, unpublished observations). The present experiments indicate that domperidone, at low concentrations, causes specific inhibition of the dopaminergic receptors, the activation of which causes relaxation of the gastric wall. In the guinea pig stomach, domperidone appears to be both more potent and more specific than metoclopramide in that regard. In addition, domperidone inhibits the response to sympathetic nerve stimulation, and at higher concentrations, than to noradrenaline; this dissociation in the degree of inhibition is seen also with metoclopramide and with α-adrenolytic drugs.

The inhibition by domperidone of the response to sympathetic nerve stimulation, exogenous noradrenaline and vagal nerve stimulation (in atropinized preparations) occurs at higher concentrations than those required to antagonize the dopamine-induced relaxation. It can be related to non-specific properties of higher concentrations of the compound among which α-adrenergic blockade and interruption of post-ganglionic conduction (Van Nueten, unpublished observations). Thus the different peripheral effects of domperidone concur to disconnect the gastric smooth muscle from inhibitory neurohumoral control mechanisms and help explain the potent effect of the drug in stimulating gastric function *in vivo*.

The inhibitory effect of dopamine on gastric motility is antagonized also by α-adrenolytic drugs, in concentrations lower than those required to influence the response to exogenous noradrenaline, but similar to those needed to inhibit the relaxation evoked by sympathetic nerve stimulation. It is markedly reduced by tetrodotoxin in a concentration which does not affect the response to exogenous noradrenaline. The logical explanation for these observations is that exogenous dopamine causes release of endogenous noradrenaline, and that the latter is responsible for most of the inhibition noted. The present experiments do not, however, rule out the possibility that in the guinea pig stomach, as in certain other tissues[11,12], α-adrenergic blocking agents such as phentolamine have a high affinity for dopaminergic receptors.

A major finding in the present study is that domperidone, in concentrations lower than those required to antagonize the effect of exogenous dopamine,

augments and regularizes the spontaneous activity of the isolated stomach; similar results were obtained with haloperidol. In view of the dose-dependency of the phenomenon, it is likely that the augmentation of the rhythmic activity is due to the dopaminergic blocking properties of domperidone and haloperidol. These observations thus mean that a dopaminergic link is involved in the feedback loop controlling the mechanical activity of the gastric wall. The dopaminergic receptor involved could modulate the activity of either smooth muscle cells or intrinsic nerves, or determine the release of other endogenous inhibitors (adenosine?). It must be different from the receptor sites reached by exogenous dopamine, since α-adrenolytic agents have no effect, and metoclopramide relatively little effect on the spontaneous activity. An alternative explanation is that augmentation by domperidone of the spontaneous activity is due to interference either with another endogenous inhibitor (inhibitory peptides?) or with a step in the relaxatory process beyond the interaction between inhibitors and the smooth muscle cell membrane. In the former case, the present experiments exclude the adrenergic and the vagal inhibitory components.

Acknowledgements

The skilful assistance of Mr L. Helsen is greatly appreciated. The authors wish to thank Dr P. M. Vanhoutte for valuable discussion.

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Discussion

P. Blower: (UK) Have you determined whether guanethidine or any other adrenergic neurone blocking drug is capable of blocking the response to dopamine?

J. M. Van Nueten: (Belgium) No, we have not yet. This is one of the experiments we planned to perform as soon as possible.

M. Wienbeck: (W. Germany) Dr Weihranch from Mainz has demonstrated that domperidone increases lower oesophageal sphincter (LES) pressure. This provides indirect evidence that dopamine plays a role in man (as has been demonstrated before in the opossum). This could be thought of as support for the ideas presented in Dr Van Neuten's paper.

Van Nueten: Yes, this is in complete agreement with our results on the guinea pig.

R. A. Hinder: (S. Africa) In canine *in vivo* experiments we have shown that domperidone causes sustained strong action potentials and enhances the gastric emptying of a digestible solid (radioactive liver), but does not appear to have as pronounced an action on the gastric emptying of solutions of 5% dextrose.

Van Nueten: The kind of food may change the motility state of the stomach before treatment; this may influence your results.

A. J. Van Merwyk: (UK) Domperidone on 1 cm strips of isolated perfused human taenia coli showed decreased electrical activity and motor activity measured by strain gauge. The dosage used was 2×10^{-5} and 1×10^{-4} g/ml.

Van Nueten: As shown in the summarizing slide (Figure 19.3), these high concentrations will produce non-specific effects. The regularization of the spontaneous activity of the guinea pig has been observed with a 100 times lower concentration.

Section IV
Effect of Diet on Motility

20

Low residue diet affects motility of the duodenum as well as the colon

J. A. McLEISH AND A. G. JOHNSON

An insidious change of diet has occurred during this century in the more developed countries. The refining, processing and packaging of foods has resulted in increased consumption of concentrated carbohydrate and fat, especially saturated fat and cholesterol, and a reduction in the intake of vegetable fibre. This dietary modification has been blamed for the emergence of many gastrointestinal disorders[1]. Early investigators concentrated on such disorders of the colon because of the accessibility of the sigmoid to probes and cannulas. Results suggested that some of the effects of changed diet may be due to disordered motor function of the colon: abnormal patterns of motility have been demonstrated in many symptomatic or 'functional' disorders, as well as organic disease, and, in some, can be corrected by increasing fibre content of the diet. Furthermore, experimental animals fed on high-fat, low-residue diets have developed similar abnormal motility patterns[2].

Disorders of the upper gastrointestinal tract occur in the same people and the same populations as colonic motor dysfunction and in some cases abnormal motility has been demonstrated. Organic disease of colon and duodenum, for example, may co-exist, and patients suffering from 'irritable colon syndrome' often have symptoms referred to the epigastrium but no demonstrable upper gastrointestinal pathology. Abnormal motility patterns have been demonstrated both in symptomatic and organic upper gastrointestinal disorders, but little is known about the relationship between motor function of this end of the gut, and either diet or colonic motility. It is possible that the motor disturbance is widespread throughout the gut, and may result from a specific alteration in diet.

This preliminary study was undertaken to answer two questions. (1) What are the relative effects of dietary fibre depletion and increased dietary fat on

colonic motility? (2) Does dietary modification also affect upper gastrointestinal motility and, if so, is the change comparable to that seen in the colon?

METHODS

Protocol

Fifteen New Zealand White rabbits were studied. Each animal underwent an initial assessment of both resting and stimulated motility. It was then fed on one of three formulated diets for 30 weeks. At the end of this period both resting and stimulated motility were reassessed. At the conclusion of the second assessment, the effects of maximal stimulation were observed before removing the colon for further examination.

Motility assessment

All experiments were performed in the same manner, by the same investigators, in the same place, and commenced at the same time in the morning.

Food was withdrawn 12 h before the assessment. Anaesthesia was induced with nitrous oxide and fluothane, and maintained with intravenous α-glucochoralose, 40 mg/kg. The peritoneal cavity was entered through a midline incision, and two fine, water-filled, open-ended, pvc cannulas were introduced into the lumen of the bowel: the upper through a gastrostomy into the second part of the duodenum; the lower through a caecostomy into the proximal colon. The bowel was returned to the peritoneal cavity and the abdominal walls approximated. The cannulas were connected to Statham Pressure Transducers, the signal amplified, and recorded by an ultraviolet light recorder.

Two stimuli were used. Prostigmine 0.02 mg/kg was injected intravenously as a bolus, and Boots cholecystokinin (CCK), 5 U/kg was infused intravenously during a 10 min period.

Intraluminal pressures were recorded during consecutive 10 min periods, before and after (or during) stimulation. One hour was allowed to elapse before the first stimulation, and the interval between stimuli was 30 min.

Activity was quantitated by determining the motility index (MI) for each 10 min period. This figure was obtained by adding the products of height and duration of all waves during the period, and dividing by 10^3.

Diets

The three diets comprised a standard rabbit diet (SD) which acted as a control, a high-fat diet (HFD), and a low-residue diet (LRD). The two special diets were formulated so that, as far as possible, they differed from the control

only in the content of fat and fibre respectively (Table 20.1). The carbohydrate content of the high-fat diet was necessarily reduced to maintain a constant caloric value.

Table 20.1 Analysis of diets (percentages)

	Lipid	*Fibre*	*Carbohydrate*	*Nitrogen*
Standard diet	5.3	11.7	43.2	2.9
High-fat diet	26.2	12.7	18.2	2.8
Low-residue diet	3.9	3.7	53.9	2.6

RESULTS

Proximal colon

The results of the initial assessment of colonic motility are represented in Figure 20.1. The mean motility index for each dietary group is shown, plus or minus one standard deviation. The resting activity in the 10 min period prior to either stimulus was similar in the three groups, ranging between 4.6 ± 4.6

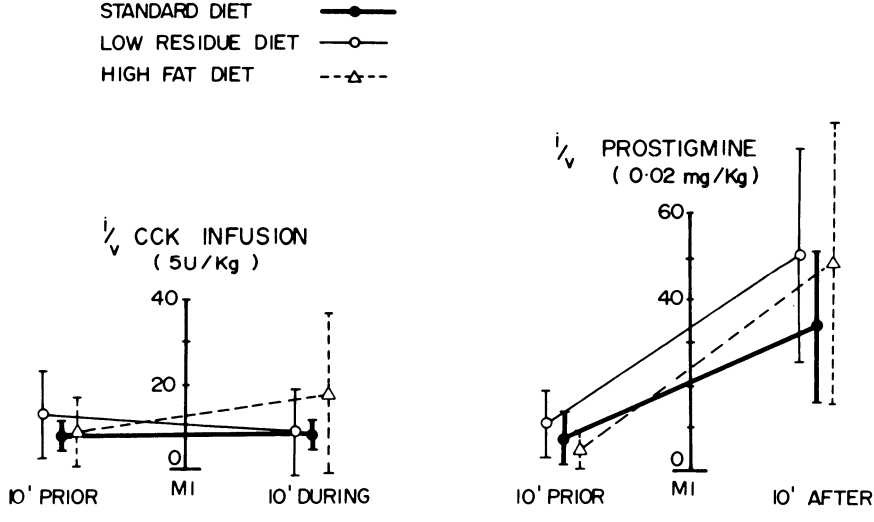

Figure 20.1 Colonic motility index with response to stimulation before starting diets

and 12.9 ± 10.3. CCK infusion did not alter the activity of any of the three groups, but injection of prostigmine resulted in a significant increase in activity of similar magnitude in all groups.

At reassessment (Figure 20.2) the resting colonic activity was again similar in all groups and of the same magnitude as that seen at the initial assessment.

The MI ranged between 8.8 $\pm$ 7.8 and 18 $\pm$ 13. Again there was no change in activity in any of the groups during CCK infusion, and activity was increased in all three following prostigmine injection. However on this latter occasion, the mean MI of the LRD group was 143 $\pm$ 64, almost three times greater than that of the SD group (54 $\pm$ 58) and the HFD group (53 $\pm$ 40) respectively.

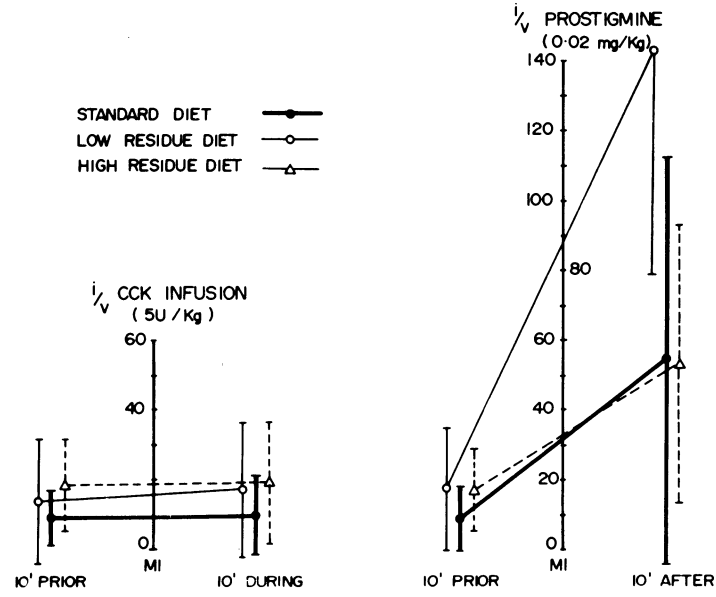

Figure 20.2 Colonic motility index with response to stimulation after 30 weeks on diets

The response to stimulation by each animal was obtained by determining the difference in the MI for the 10 min period before and during (or after) stimulation. The mean responses of the SD group (45 $\pm$ 51) and HFD group (37 $\pm$ 31), at reassessment, were similar to the mean response of the animals at initial assessment (36 $\pm$ 21). However the mean response of the LRD group to prostigmine was 125 $\pm$ 65 after diet, and this was significantly greater than that shown at initial assessment ($p < 0.01$).

Duodenum

The initial assessment of duodenal activity is represented in Figure 20.3. Resting activity was infrequent, means ranging from 0 to 2.1 $\pm$ 3. There was no activity in the duodenum of seven animals prior to CCK infusion, and in nine prior to prostigmine. The mean motility indices of all three groups were increased during CCK infusion but the variation within the groups was high, and the differences between the means were not significant. Increased activity

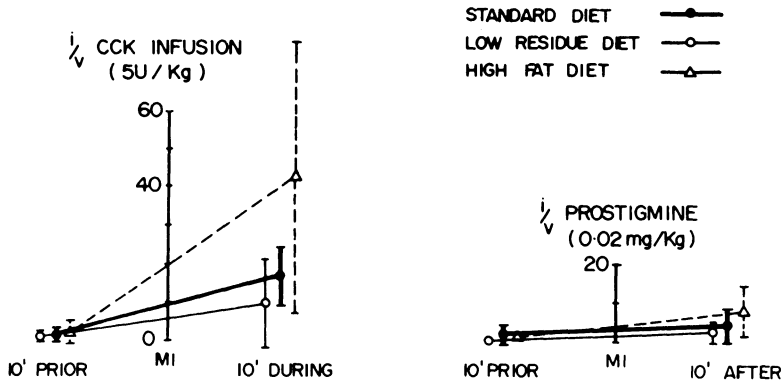

Figure 20.3 Duodenal motility index, with response to stimulation before starting diets

also occurred in all groups during the period following the injection of prostigmine.

At reassessment (Figure 20.4) activity in the resting duodenum was again infrequent: none was recordable in nine animals prior to CCK infusion and in twelve prior to the injection of prostigmine. The mean MI of the LRD group (51 $\pm$ 24) during CCK was higher than that of the SD group (33 $\pm$ 18) and the HFD group (29 $\pm$ 26), but variation was wide and the differences were not statistically significant. The mean MI of the LRD group (30 $\pm$ 21) was also greater following prostigmine injection than that of the SD group (7 $\pm$ 8) or HFD group (12 $\pm$ 12) but again the differences were not significant because of the wide range within groups.

The mean responses of the LRD group to both stimuli were significantly greater at reassessment than those shown by the animals prior to the diet

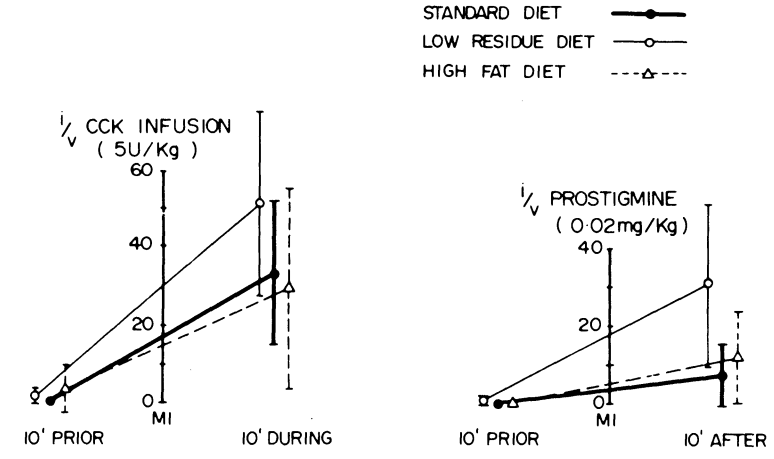

Figure 20.4 Duodenal motility index, with response to stimulation after 30 weeks on diets

(50 ± 25 compared to 19 ± 24) during CCK infusion ($p < 0.05$), and 30 ± 20 compared to 3 ± 4 following prostigmine ($p < 0.01$). The responses to CCK of the SD group (33 ± 18) and the HFD group (26 ± 27) were not significantly different from the responses at initial assessment. Neither were the responses to prostigmine of either group (7 ± 8 and 12 ± 12 respectively).

Animal weight

There was no relationship between the weight of each animal and either its resting motility or its response to stimulation. The mean weight of the LRD group was 3.3 kg at initial assessment, slightly higher than that of the control (SD) group (3.1 kg). However at reassessment the LRD group averaged 4.0 kg, a mean increase of 23%, compared to the final weight of the control animals which averaged 4.8 kg, a mean increase of 57%.

Stool weight

Four random 24 h stool collections were made. The mean stool weight of the LRD group was 28.2 g/24 h. This was less than half the mean weight of the stool passed by the control group (64.3 g/24 h). The mean weight of the stool passed by the HFD animals was 31.8 g/24 h.

The effects of maximal stimulation

Intravenous injection of boluses of prostigmine resulted in the production of marked haustral pattern in the colon. On occasions, spasm occurred across two to three haustral segments, but despite marked bulging no diverticula were seen. There were no differences between the appearances of the colons of the three groups.

Colon morphology

The mean length of the colons from caecum to the part where the taeniae blend from the LRD group was 21.9 cm. This was slightly greater than that of the controls (20.5 cm) and that of HFD group (18.9 cm) but proximal, mid and distal colonic diameters were similar. All measurements were taken with the colon distended under constant pressure. Histological examination of transverse and longitudinal strips from the colons revealed no differences between the groups either in thickness or structure. There was no evidence of diverticulum formation.

DISCUSSION

Depletion of dietary fibre has resulted in significantly increased responses by

the duodenum both to CCK and prostigmine, and by the colon to prostigmine. In neither the duodenum nor colon was there apparent disturbance of wave frequency or form. The increases in MI were brought about predominantly by increases in the heights of the waves. This accounted entirely for the increase in MI in the duodenum during CCK infusion, and in the colon in response to prostigmine injection. In both situations there was no change in the duration of activity during that period. The increased duodenal response to prostigmine resulted from increases in both peak height and duration of activity. It should be noted that intraluminal pressures reflect segmenting activity; and that disturbances in other activity complexes may co-exist.

The mechanism whereby dietary fibre depletion brings about increased gut sensitivity to stimulation remains unclear. In man, the response is non-specific, indicating that the disorder is primarily muscular. The increased activity in the rabbit has occurred in response both to the cholinergic drug, prostigmine, and CCK. The preparation of the hormone was impure and the response may have been due to a mixture of gastrointestinal hormones. The response therefore appears to be non-specific also in the rabbit, although the possibility remains that the effective gastrointestinal hormones are acting through cholinergic pathways.

Explanations of motility changes based on local colonic factors brought about by small, hard, slow faeces are hardly adequate for the functional disturbance elsewhere. The changes in stool may be secondary – it is interesting that the response of the LRD group was greater than that of the HFD group despite similar stool weight and appearance. Fibre influences intraluminal cation exchange[3], bile acid content and bacterial flora, but the relationship between smooth muscle sensitivity and each of these factors is not known.

Clinical extrapolations from animal experiments should be made with caution. However it is interesting that the pattern of increased response, in the presence of unaltered resting activity, produced by these fibre-depleted animals, is the same as that found clinically in patients with diverticular disease and 'irritable bowel' syndrome.

Both patterns apparently result from fibre depletion, as the clinical abnormalities occur in patients from societies where a low fibre diet is eaten, and can be corrected by increasing dietary fibre content.

If the magnitudes of the abnormalities are also comparable in patients, then it would be surprising not to find similar motility disturbances accounting for the upper gastrointestinal symptoms that accompany functional colonic disease.

Acknowledgements

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Discussion

H. J. Ehrlein: (Germany) The motility of the colon of rabbits is a very complex event. Four types of motility must be differentiated: (1) local segmentation; (2) slowly moving peristalsis; (3) movements of the haustra; and (4) mass movements. We have found that by recording internal pressure in the colon of rabbits, only peristalsis and mass movement can be detected. I have some doubt if records of internal pressure in an anaesthetized rabbit present a real picture of the complex events of colon motility. Therefore I think it would be better if the experiments were performed in unanaesthetized animals and if segmentation and movements of the haustra would be taken into account.

J. McLeish: (UK) I agree that the motility is complex and not well represented by intraluminal pressure changes. However, we were looking at a change in smooth muscle response, not an alteration in the type of motility.

A. Bennett: (UK) Are physostigmine and CCK necessarily similar things? The answer to this depends on whether CCK cuts through a cholinergic mechanism; is this so? What happens if you use a direct muscle stimulant *in vivo* or if you look at strips of muscle obtained from the treated animals?

McLeish: We did not use a direct muscle stimulant *in vivo*. We have measured the isometric responses of isolated colonic taenia strips to acetylcholine and pure CCK, and are currently interpreting these.

D. L. Wingate: (UK) It has recently been shown that there is a marked circadian variation in colonic function in the rabbit mediated by aldosterone, resulting in alternation of hard and soft stools. In view of this, do you think that the rabbit is a suitable model? And also, since diurnal variation might mark real differences, did you make the comparative studies at the same time of day?

McLeish: All assessments have commenced at 9 a.m. I think the basal colonic function is more susceptible to circadian variation than is the response to stimulation.

A. Dubois: (USA) Did you try to determine the mechanism by which the duodenal and colonic motility is altered by the low residue diet? Did you find macroscopic or microscopic changes in the duodenum and/or colon?

McLeish: There were no differences in the weights, lengths, diameters, mural thickness, nor microscopic features of the colons. There was no obvious macroscopic difference of the duodenums.

Z. Itoh: (Japan) Have you looked at the purity of the CCK preparation you used? I ask this because Boots' CCK is contaminated with fairly large amounts of secretin and motilin.

McLeish: We were aware that the Boots' CCK is impure, and that it was a relatively non-specific stimulus.

J. Weinreich: (Denmark) Your low-residue diet was also low in fat, so you could very well correlate your results to the fat intake as well.

McLeish: The low-residue diet had a similar fat content to the standard diet. The

reason for studying two special diets was to separate the effect of increased fat, and fibre depletion.

D. Kirk (UK): Are you studying the caecum or the colon proper? The rodent colon is more distinct from the caecum than that of the human. Human diverticular disease is concentrated in the sigmoid.

McLeish: The colon – this segment of the rabbit's gut is not structurally dissimilar to the human colon. The rabbit caecum is quite distinct, and has no reasonable analogue in man.

H. L. Duthie (UK): Have you any explanation for the fact that the responses in both duodenum and colon have increased in all three groups although low residue has most effect? Is this because of the increase in weight of the animal, or because of the second laparotomy needed to make the recordings?

McLeish: The small changes in response by the other two groups are not significant because of wide range within groups. The effects of prior surgery are unlikely to have influenced the response at reassessment, as the cannula tips were well distal to the site of insertion. It is possible that increasing age in rabbit results in increasing responses.

Y. Ruckebusch (France): Is recording the intraluminal pressure in the anaesthetized rabbit during 10 min sufficient to give reference? In both duodenum and proximal colon, there are ultradian patterns which may persist under anaesthesia.

McLeish: Measurements were begun 1 h after induction, and after this interval there was only small variation in basal activity. The responses to stimulation showed wide variation but were short-lived, occurring mainly in the 10 min period following prostigmine injection, and only during the CCK infusion.

21

Effects of 16,16 dimethyl prostaglandine E_2 on the integrated response to a meal

C. JOHANSSON AND K. EKELUND

Natural prostaglandins (PG) have well-documented effects on gastrointestinal secretion and motility[1]. From a clinical viewpoint two of their properties are of special interest due to their possible implication in peptic ulcer treatment: namely, the inhibition of the gastric acid secretion by PGs of the A and E series[2,3], and the prevention of experimental ulcers by numerous PGs[4]. In recent years stable, synthetic analogues of PGE_2 have been available, which after oral intake give a dose-dependent and sustained inhibition of the basal and stimulated gastric acid secretion[5–7]. Less is known about their actions on the secretory and motilic functions in the intact human gastrointestinal tract. The purpose of this study was to examine the effects of 16,16 dimethyl prostaglandin E_2 (16,16 diMe PGE_2) on the integrated gastrointestinal response to a mixed meal.

METHODS

A multiple indicator dilution technique developed by our group[8–11] was used for simultaneous measurements of secretory, motor and absorptive functions. By this method (Figure 21.1) the gastric content is marked by one indicator, and the duodenal content by three different indicators. The concentrations of the mixed indicators in timed collections of jejunal contents are used to calculate the flow volumes at the jejunal sampling level 70 cm distal to the pyloric sphincter. The advantage of the technique lies in the possibility of determining the post-prandial variations in flow volumes and intestinal transit.

Eight healthy male subjects were examined twice. 140 μg 16,16 diMe PGE_2 (1.4 ml alcoholic stock solution diluted with 8.5 ml saline) or alcoholic saline (control experiments), was instilled through the gastric tube 30 min prior to

meal intake. 300 ml of a mixed liquid formula, containing (per 100 ml) 3.6 g protein, 6 g fat, 5 g lactose and 10 g glucose, was used as a test meal.

Means are given ± SEM. Significance tests were made on paired differences according to Snedecor.

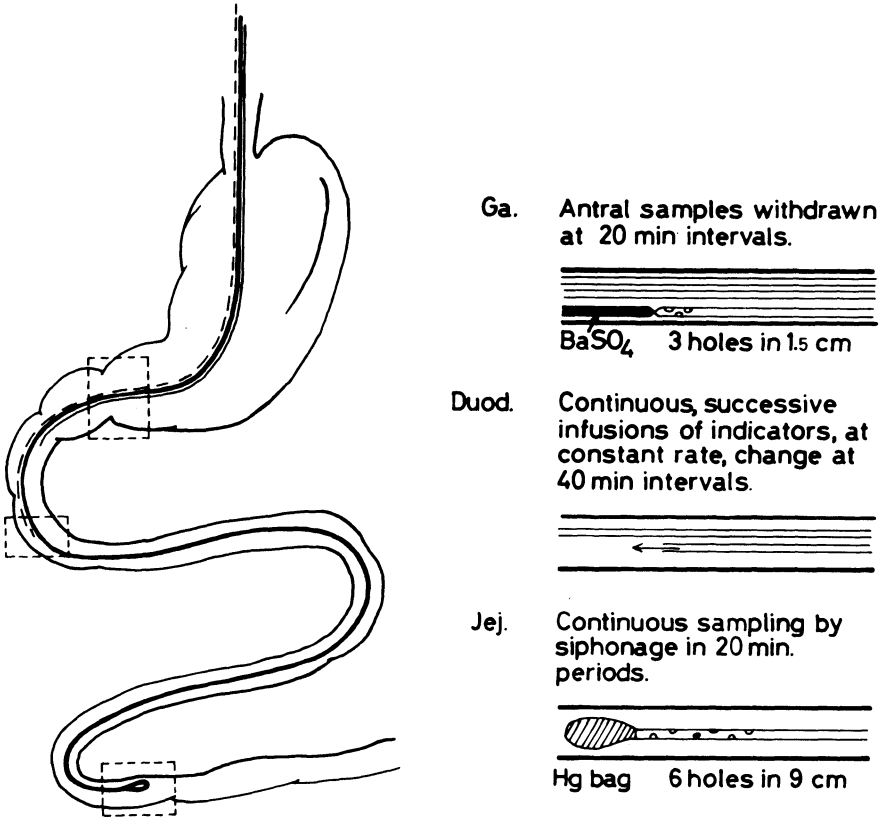

Figure 21.1 Experimental procedure. The three tubes end respectively in the gastric antrum, in the duodenum descendens and in the jejunum 70 cm distal to the pyloric sphincter. Polyethylene glycol (PEG) is used as a test meal marker. The duodenal contents are indicated by three markers: unlabelled vitamin B_{12} and vitamin B_{12} labelled ^{57}Co and ^{58}Co

RESULTS, COMMENTS

Gastric elimination of test marker (Figure 21.2)

The time to empty 50% of the test meal marker, $T\frac{1}{2}$, was shorter in the experiments with 16,16 diMe PGE_2 than in controls (22 ± 3 min *vs* 39 ± 2 min; $p < 0.001$). The fractional elimination rate during the continued experiments was however lower, and therefore the time for complete elimination of the marker (183 ± 10 min) did not differ from the controls (183 ± 9 min).

We are inclined to interpret the early enhanced gastric emptying rate in terms of a delayed inhibition rather than in terms of an increased propulsion.

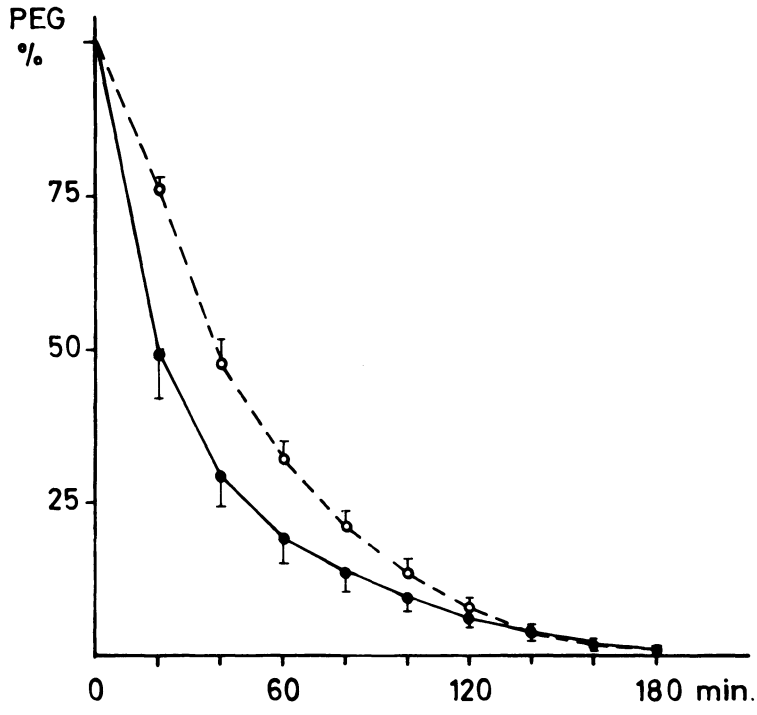

Figure 21.2 Gastric elimination of test meal marker (PEG), Mean ± SEM; $n = 8$

Such a delay could result from a relaxation of the pyloric sphincter, a known effect of PGE_2, or be related to the impaired digestion and absorption of the meal components.

Gastric acid response (Figure 21.3)

The amount of acid emptied together with the meal was reduced from 35.6 ± 6.6 mE in the controls to 0.6 ± 0.3 mE ($p < 0.001$).

The marked and sustained inhibition of the gastric acid response to a test meal confirms a previous report by Ippoliti *et al.*[6]. Despite the absence of duodenal markers in the gastric samples we cannot exclude the possibility that part of the reduction was caused by neutralization from duodenal reflux which, due to the arrest of the bile flow (see below) was uncoloured for considerable periods of time in the test experiments.

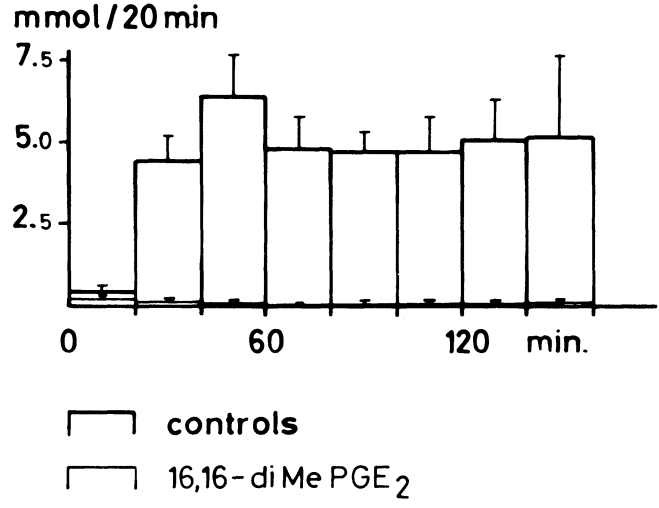

Figure 21.3 Gastric emptying of acid. Mean ± SEM; $n = 8$

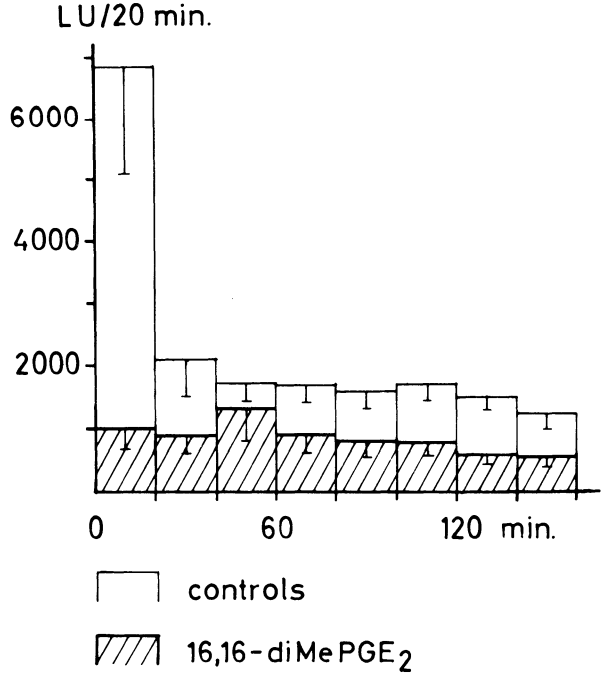

Figure 21.4 Output of pancreatic lipase. Mean ± SEM; $n = 8$

Pancreatic enzyme output (Figure 21.4)

The pancreatic response to the meal was decreased; the output of lipase amounting to 43 $\pm$ 11 % of that in control experiments ($p < 0.02$).

The early inhibition, which is most pronounced, could be a direct effect of the analogue. It cannot be explained by differences in acid loads which by then are negligible. The later moderate reduction in enzyme output may reflect the smaller acid load entering the duodenum and more extensive enzyme inactivation during prolonged transit time (see below).

Bile flow (Figure 21.5)

A complete arrest of the biliary flow was recorded in all experiments with the PGE_2 analogue. The arrest lasted to the end of the experiments in three subjects and for a variable time in the other five.

This effect has not been described hitherto. The mechanism behind the arrest needs further investigation; from the present data it can be concluded that the action is exerted at a level higher than sphincter of Oddi, since pancreatic enzymes were continuously present in the jejunal contents.

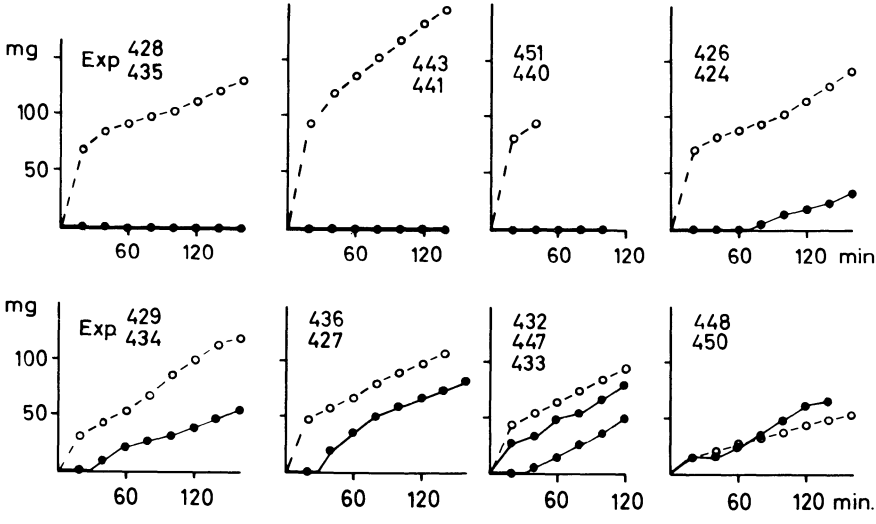

Figure 21.5 Cumulative output of bilirubin. Abolition of gall-bladder emptying after intragastric administration of 140 μg 16,16 diMe PGE_2 prior to meal. ○—○ = control experiment; ●—● = 16,16 diMe PGE_2

Intestinal transit (Figure 21.6)

The transit time is the time it takes for the indicator to transit the 70 cm long intestinal segment. The transit time was significantly prolonged by the PGE_2

analogue during the time period 60–160 min after meal intake ($p < 0.005$–0.05). In several subjects the continuous slowing of the propagation rate was followed by an acceleration of the rate towards the end of the experiments.

The marked slowing of the intestinal propagation induced by 16,16 diMe PGE_2 is compatible with the decrease of the intestinal muscular tone[12] and the relaxation of the circular muscle[13], which are known effects of PGs. A slowing of the intestinal transit by PGE_2 and PGF_2 is suggested in the results from perfusion studies[14,15] although these data were not conclusive. The sudden acceleration of the propagation rate towards the end could be the experimental correlate of the passage of a loose stool after the end of the study, which was experienced by some subjects. No side-effects were observed during the experiments, in contrast to the immediate diarrhoeas reported after the higher doses of PG analogues used to induce abortions.

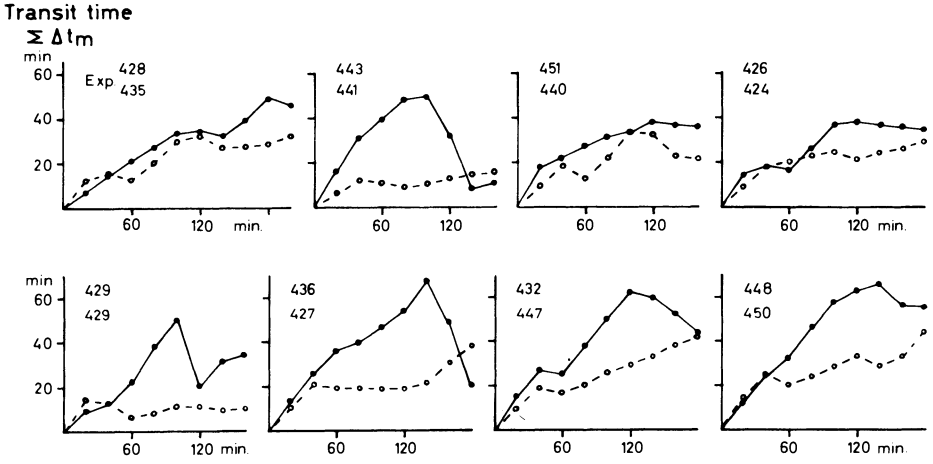

Figure 21.6 Intestinal transit time. Intragastric 16,16 diMe PGE_2 slows the propagation of the intestinal contents in the proximal intestine. ○—○ = control experiment; ●—● = 16,16 diMe PGE_2

Intestinal absorption (Table 21.1)

Only 27 ± 5% of the test meal energy was absorbed during transit of the meal in the proximal 70 cm intestine, compared to 62 ± 6% in the controls ($p < 0.001$). As demonstrated in Table 21.1, the absorption of glucose + lactose was as equally depressed as that of fat and protein, indicating that an impaired digestion is insufficient explanation of the absorptive defect. The absorption of glucose + lactose is relatively even more decreased than suggested by the figures, as the longer transit times should promote the absorption in the segment[16]. A moderate and similar net dilution during transit of the test meal was observed in the test and control experiments.

Table 21.1 Net absorption in the proximal 70 cm intestine. Percentage of emptied test meal. Mean ± SEM; $n = 8$

	Control	16,16 *diMe* PGE_2	p-*level*
Carbohydrates	71.4 ±4.7	28.1 ±5.1	<0.001
Fat	61.0 ±6.6	21.0 ±4.1	<0.001
Protein	53.3 ±2.7	22.1 ±3.2	<0.001

CONCLUSIONS

The complexity of the system under study makes it difficult to separate direct actions of the analogue from secondary effects – and to evaluate correctly the durations of its actions. The present results, like those from perfusion studies, indicate that the different secretory, motor and absorptive functions have different dose–response relationships, which should be defined. The various actions do not occur to the same extent in each subject. This needs to be examined further by comparing the effects of intragastric and intraduodenal administration.

The effects of 140 μg 16,16 diMe PGE_2 instilled into the stomach 30 min prior to the intake of a meal can be summarized as follows:

on secretory functions: an almost complete and long-lasting inhibition of the gastric acid secretion; a 60% reduction of the pancreatic enzyme output most marked early after meal intake; no effect on the net intestinal secretion;

on motor functions: a complete arrest of the bile flow to the intestine lasting from 20 min to 3 h; a marked and long-lasting slowing of the intestinal propagation rate; an early enhanced gastric emptying rate followed by slowing;

on the absorption: a decreased absorption of the meal in the proximal intestine, not related to impaired digestion.

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Discussion

A. Dubois: (USA) I have a comment and questions regarding this very elegant paper. First a comment. This study stresses again the importance of simultaneously evaluating gastric emptying and gastric secretion when evaluating antisecretory agents. My first question is: Could the increased initial emptying that you observed after PG be related to a decreased intragastric and intraduodenal acidity? Acid is known to slow emptying. My second question is: What is the evidence that prostaglandin was equally active throughout the emptying of the meal?

C. Johansson: (Sweden) The answer to your first question is no. During the first 20 min the differences in emptied acid between control and test experiments were too small to account for the different emptying rate. We do not know if the PG analogue was equally active throughout the emptying of the meal, and cannot conclude whether the slower fractional emptying rate after the first 20 min in the test experiments is an effect of the analogue or for example a result of lower gastric volumes. The long-lasting inhibition of the acid secretion after topical administration of the PGE_2 analogue points to a local effect, possibly through a second reaction, in which case a constant stimulation is hard to obtain.

N. W. Weisbrodt: (USA) Since PGE_2 inhibits digestion and absorption of carbohydrates, lipids and protein perhaps the duodenal receptors responsible for controlling gastric emptying and small bowel motility are not activated. Do you feel that this could explain the rapid gastric emptying? Also, do you have any data on whether the pattern of small bowel contractile activity is that of the fasted or fed type during the influence of PGE_2 and food? Perhaps the abrupt return of rapid small bowel transit is due to the presence of phase 3 of a MMC and the slow transit due to the presence of phase 1 of the MMC.

Johansson: As to your first question I think a deficient digestion might delay the inhibition of the gastric emptying. We should like to use combined techniques to be able to translate the transit time to pressure waves and electrical activity.

A. Bennett: (UK) Prostaglandin E compounds stimulate fluid secretion in the intestine and this is consistent with your finding that some subjects passed loose stools. How do you explain this in relation to the slowing of upper intestinal transit in your subjects?

Johansson: The passage of loose stools occurred after the end of the studies and coincided in time more with the acceleration of the propagation rate towards the end than with the slowing during the second and third hour. Until it has been shown that prostaglandin-induced diarrhoeas result from one single mechanism, we can speculate that a maximal distension of the intestine, a sudden acceleration of the propagation rate, a decreased absorption and an increased intestinal secretion may provide explanations for the rapid transit.

A. R. Cooke: (USA) I wonder whether the increased gastric emptying and decreased transit time could be due to the high dose of PG seen by the stomach, since you administer this drug intragastrically. What is known about the absorption and elimination of the PG analogue and what would happen if you administer, for example, intracolonically?

Johansson: Data from animal experiments indicate that the gastric antisecretory effect may be mediated by local actions of the analogue on the gastric mucosa. On the other hand analogue is active after intraduodenal administration and has systemic effects after high oral doses. The pharmacokinetics of these analogues are not fully known. Although care is taken to avoid passage of the analogue into the duodenum during and after its instillation – an unknown and varying proportion is emptied to the duodenum (the intestinal effects have a wider individual distribution than the gastric in this series). I do not know what would happen after intracolonic administration but I think it is necessary to compare different routes of administration.

Dubois: Don't you think that acid, whether secreted by the stomach or introduced with a meal, will slow gastric emptying? The differences in gastric emptying produced by any anti-secretory agent should be assessed with the same intragastric acidity.

Johansson: I am not convinced that gastric acid secreted and emptied after a meal slows the gastric emptying rate, since this is not supported by our data from studies of meals with varying protein content. Neither am I convinced that the acid secreted by the stomach in response to a meal, and acid introduced with a meal, have comparable effects on gastric emptying. The second part of your question is I believe correct from a theoretical point of view, provided that manipulation of the gastric pH also includes care to ensure that the gastric volume is kept the same.

22

Influence of nutritive density of a meal on gastric emptying in duodenal ulcer patients

R. BITTNER, H. G. BEGER, M. MEVES, E. KRAAS AND H. GOGLER

Reviewing the literature, Hunt and Stubbs[1] concluded that in normal persons the greater the nutritive density of a meal, the less is the volume transferred to the duodenum.

The purpose of this investigation was to study, in duodenal ulcer patients, the effect on gastric emptying of two meals with different energy content using an isotopic technique[2].

MATERIALS AND METHODS

Patients and test meals

Eighteen consecutive patients (five female, thirteen male, mean age 42 years, mean body-weight 72 kg) with chronic duodenal ulcer were included in the trial. The studies were carried out preoperatively during hospitalization for surgical treatment. Every patient was tested on two different days:

1. After eating a low-calorie (semi-solid) meal: one scrambled egg, 50 g of lightly buttered roll, 250 ml caffein-free instant coffee with 100 ml milk (proteins: 12.7 g; fat: 17.5 g; carbohydrates: 31.1 g). Volume: 450 ml. Energy: 333 kcal $\simeq$ 0.72 kcal/ml. Osmolarity: 194 mosm/l.
2. After eating a high-calorie (liquid) meal: chemical defined diet (Fresubin®) (proteins: 12.3 g; fat: 3.8 g; carbohydrates: 80 g). Volume: 300 ml. Energy: 403 kcal $\simeq$ 1.34 kcal/ml. Osmolarity: 522 mosm/l.

Measurement of gastric emptying

The two standard meals were labelled with 50 microcuries of ^{51}Cr as sodium chromate. (In the semi-solid meal the ^{51}Cr was given to the patient in the milk–coffee mixture. In order to guarantee an equal distribution of the ^{51}Cr, the patient was required to consume the various components of the meal in a definite manner.) Radioactivity from the gastric area was recorded using two highly sensitive stationary scintillation detectors, which were mounted above and below the fundic region of the patient in supine position. The counting rates measured by the two detectors were summed up and transferred into semi-logarithmic scale. The rate of gastric emptying was expressed graphically by plotting percentage decrease in radioactivity from the maximum count against time.

The regression equation was calculated and from it the half emptying time ($T\frac{1}{2}$) obtained, i.e., the time taken for the volume of meal to diminish by half.

All time-measurements were related to the start of recording, not to the start of the meal. The time between starting the meal and starting recording ranged from 6 min (liquid meal) to 12 min (semi-solid meal).

RESULTS

Figure 22.1 shows the mean emptying patterns for the eighteen tested patients after having eaten the high-calorie meal and after having eaten the low-calorie meal. In every patient the low-calorie meal leaves the stomach more rapidly than the high-calorie meal. The mean $T\frac{1}{2}$ for the low-calorie meal is 38 ± 17 min and the mean $T\frac{1}{2}$ for the high-calorie meal is 70 ± 33 min. The difference is statistically significant ($p < 0.001$, Student's t test).

Figure 22.2 shows that there is a linear relation between $T\frac{1}{2}$ of the low-calorie meal and $T\frac{1}{2}$ of the high-calorie meal. This means that the longer the half-emptying time of a low-calorie meal is in one patient, the longer the $T\frac{1}{2}$ of a high-caloric meal is in the same patient. The coefficient of correlation is statistically of high significance $r = 0.866$ ($p < 0.001$). In addition to this fact you can see that the slower the high-calorie meal leaves the stomach the greater is the difference between $T\frac{1}{2}$ of the high-calorie meal and the $T\frac{1}{2}$ of the low-calorie meal (Figure 22.3). The coefficient of correlation is statistically significant $r = 0.91$ ($p < 0.001$).

The analysis of percentage decrease of impulse-rate per 5 min-intervals shows quite different emptying patterns for the two meals (Figure 22.4). After the low-calorie meal the percentage decrease of emptying is highly rectilinear ($r = 0.91$) during the whole test period. However, the high-calorie meal shows an increase of the emptying rate during the first three 5 min-intervals and only after the 15th minute is there a rectilinear decrease ($r = 0.887$). As a result of these measurements it is possible to calculate the energy delivery to the duodenum per 5 min-interval according to the formula:

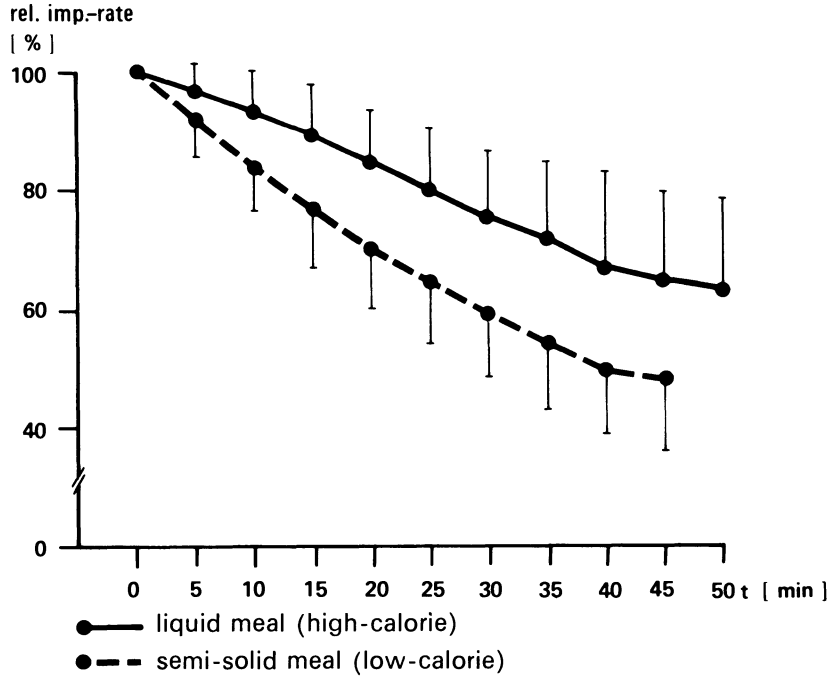

Figure 22.1 Percentage emptying of the two meals. Each point is the mean ± SEM of the measurements of eighteen patients

$$\frac{\text{total volume (ml)}}{100\ (\%)} \times \frac{\text{percentage decrease}}{5\text{ min-interval}} \times \frac{\text{energy (kcal)}}{1\text{ (ml)}}$$

As Figure 22.5 shows, during the first 15 min the energy delivery to the duodenum in the high-calorie meal is smaller than in the low-calorie meal. The course of further measuring shows that the number of calories that leaves the stomach after the high-calorie meal is greater than after the low-calorie meal only at the end of the test period.

In fourteen patients gastric acid analysis could be performed. Figure 22.6 shows that chronic duodenal ulcer patients with a slow half-time of gastric emptying also have a lower peak acid output. The coefficient of correlation is $r = 0.73$ ($p < 0.01$).

DISCUSSION

In accordance with Barker, Cochrane, Corbett, Hunt and Roberts[3] who tested normal persons and Chaddock, Carlson and Hamilton[4] who tested Rhesus monkeys our study demonstrates that also in duodenal ulcer patients the high-calorie meal with a high osmotic pressure leaves the stomach more

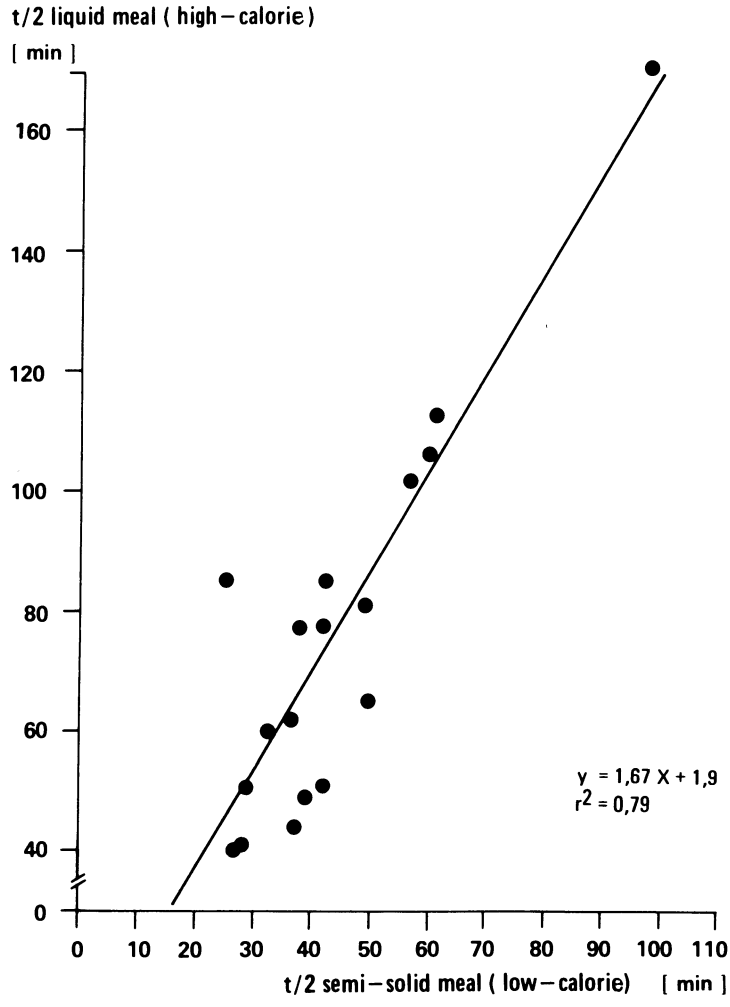

Figure 22.2 Relation between $T\frac{1}{2}$ of the low-calorie meal and $T\frac{1}{2}$ of the high-calorie meal

slowly than the low-calorie meal with a low osmotic pressure. The content of protein and fat in the two meals is relatively small. Therefore the high content of carbohydrates in the liquid meal is responsible for the higher osmolarity of this meal. In 1905 Carnot and Chassavant[5] found that the higher the osmotic pressure of a solution is, the slower its gastric emptying. Based on this and his own experiences Hunt[6] postulated osmoreceptors in the duodenum. The excitation of these receptors may partially inhibit the pumping action of the gastric antrum and will probably augment the motor activity of the proximal part of the duodenum, so increasing the resistance to the transfer of the gastric contents to the duodenum.

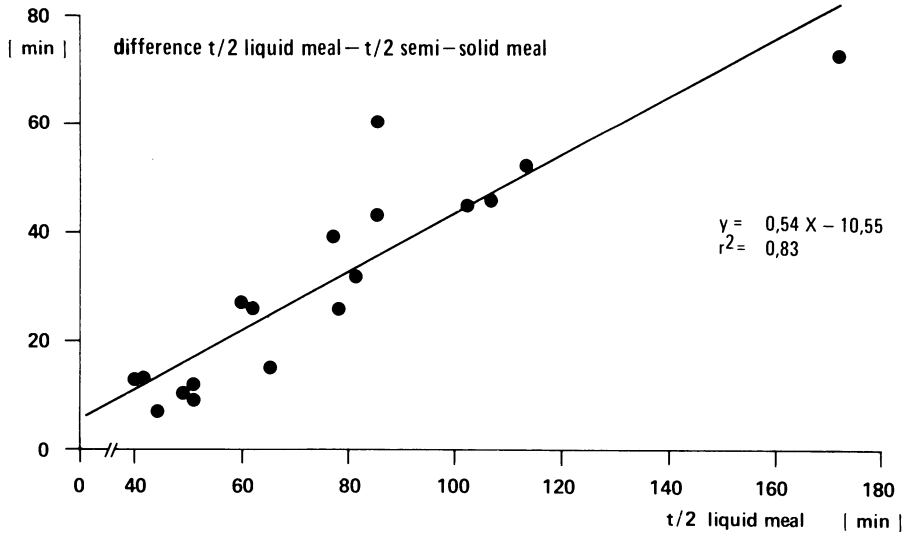

Figure 22.3 Relation between $T\frac{1}{2}$ of the high-calorie meal and the difference $T\frac{1}{2}$ high-calorie meal – $T\frac{1}{2}$ low-calorie meal.

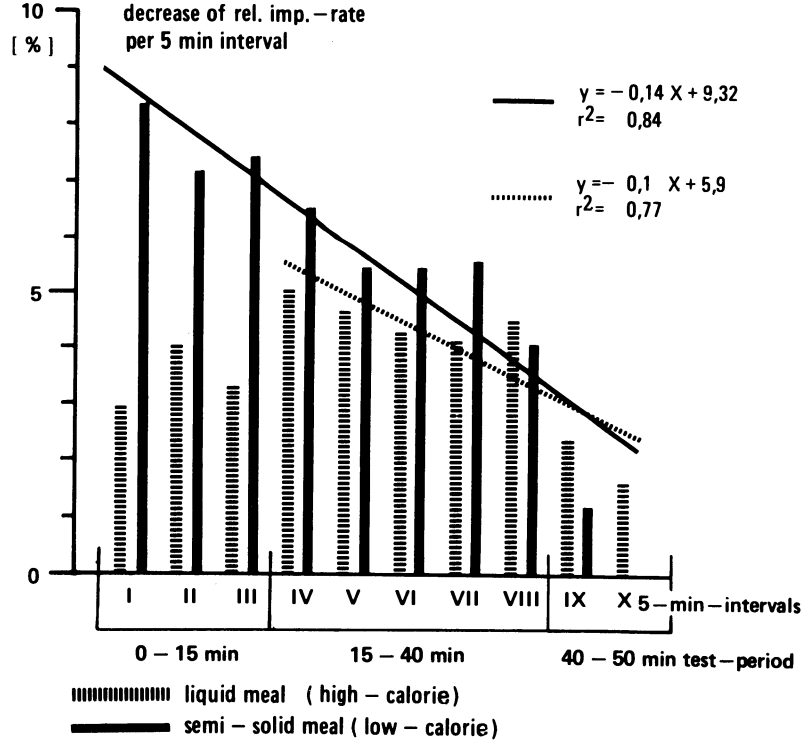

Figure 22.4 Percentage decrease of emptying per 5 min interval

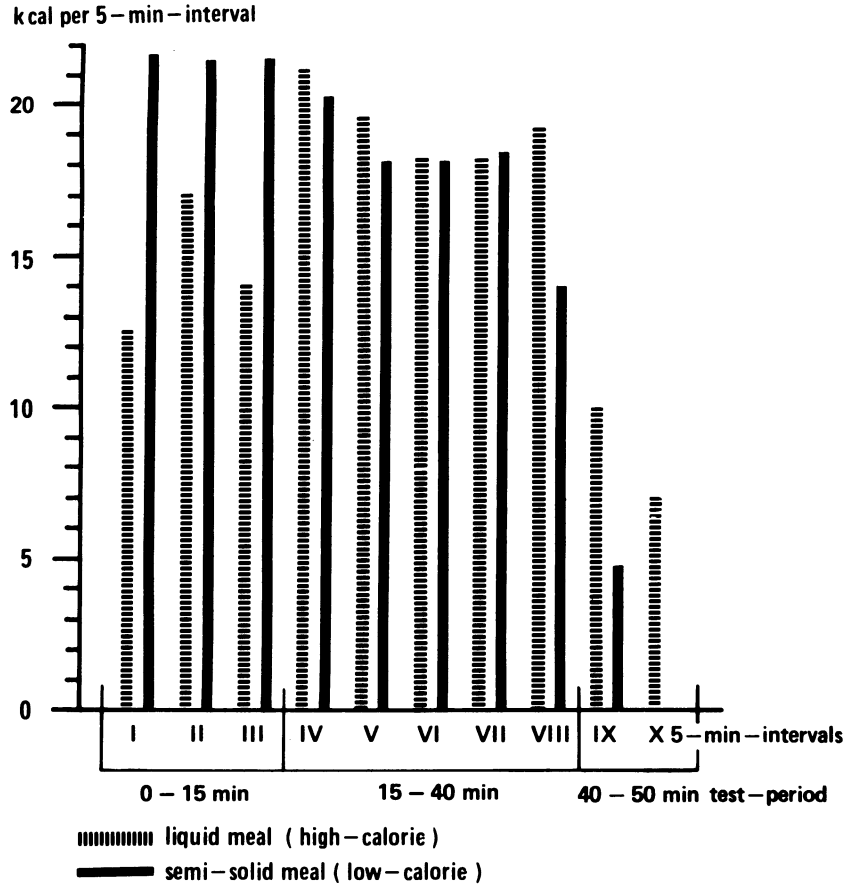

Figure 22.5 Calculated number of kcal leaving the stomach per 5 min-interval

The analysis of the percentage emptying of the two meals proves that only after eating the more physiological low-calorie meal do the emptying patterns follow an exponential function. The emptying patterns of the high-calorie meal, however, show two phases: in the first 15 min the emptying rate is small and even increases slowly, but after the 15th minute there is a rectilinear decrease as in the low-calorie meal. In addition to that fact the percentage emptying of the high-calorie meal up to the 45th minute is smaller than the percentage emptying of the low-calorie meal. This may lead to the conclusion that already the first portion of the high-calorie meal reaching the duodenal receptors triggers by feedback-mechanism neural and/or hormonal controls to slow gastric emptying. It must be supposed that the aim of this regulation of gastric emptying is to control the energy delivery to the duodenum, providing an optimal absorption of the food. This hypothesis is supported by the results demonstrated in Figure 22.5. Despite the nearly twofold energy con-

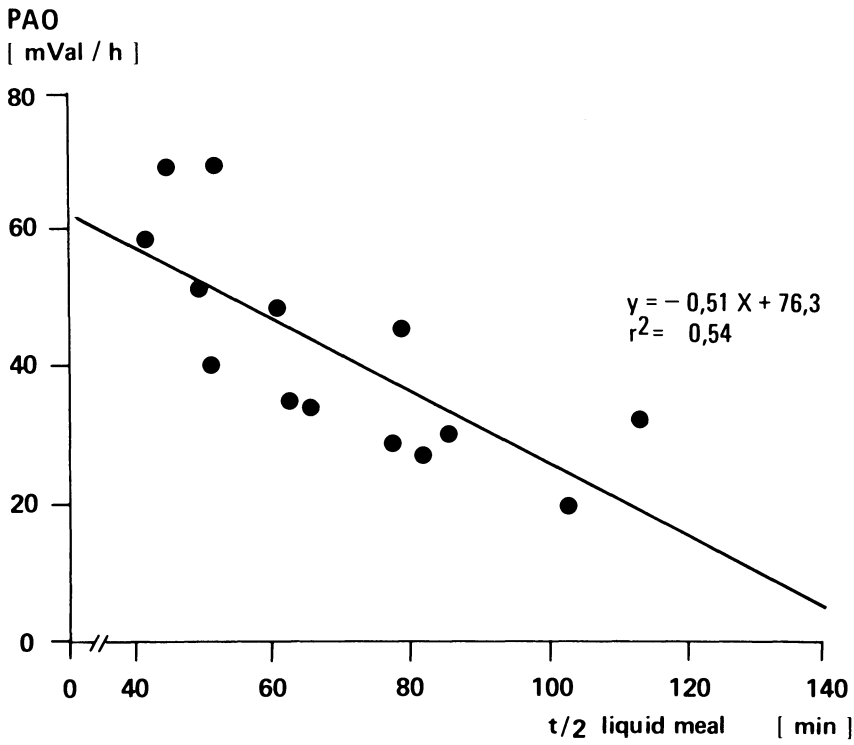

Figure 22.6 Relation between $T\frac{1}{2}$ of the high-calorie meal and peak acid output

tent of the liquid meal compared with the semi-solid meal the calculated number of calories delivered to the duodenum in the first 15 min is even smaller, and in the following minutes is held relatively constant.

Although all the patients investigated had the same disease, the measurements of gastric emptying rate showed great individual differences. Except in patients with severe pyloric obstruction there was a close correlation between velocity of gastric emptying and peak acid output. This indicates that chronic duodenal ulcer disease can also be associated with normal $T\frac{1}{2}$ of gastric emptying as well as with normal gastric acid secretion.

Acknowledgements

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Discussion

R. C. Heading: (UK) Some years ago we found that ^{51}Cr partially adsorbed to the solid component of a mixed meal – and could be eluted off the solid by further addition of fluid. Do you think this aspect of the behaviour of chromium contributed to the differences you have shown between your two meals and, in particular, could greater elution into secretions explain your PAO emptying rate correlation?

R. Bittner: (Germany) We performed preliminary studies to clarify this problem. The semi-solid meal was homogenized and centrifuged. Measurements of radioactivity showed an equal distribution of the ^{51}Cr in both fractions.

C. F. Code: (USA) There were many variables between the contents of the two meals – volume, fat, carbohydrate. Does this not make the results difficult to interpret?

Bittner: We agree with you that there are several variables in the meals. However, this study is based on the experiences of Hunt. Following him we may accept that the nutritive density is an important factor determining gastric emptying. The different amounts of the various components of a meal, e.g. fat, carbohydrates, and likewise small differences in the volume, are of secondary importance.

A. Dubois: (USA) Isotopic methods allow measurement of the emptying of the isotope, not of any component of the meal. We should not talk about emptying of 'energy' or fat or protein but, instead, of emptying.

Bittner: Of course with an isotopic technique we can only measure radioactivity. But the patients had to eat the meal in a certain manner providing an equal distribution of the ^{51}Cr, so we can infer from emptying of the isotope to emptying of the meal by analogy.

H. L. Duthie: (UK) May I suggest that your study has shown the effect of osmolarity on gastric emptying and not the calorie content. The initial delay in emptying of high osmolar meal (also high calorie) may be a dilution phase, and when the second phase occurs both meals will have been diluted in the stomach by the gastric secretion. This would explain the correlation between the peak acid output and emptying rate – the greater the acid output the more quickly will the meal be diluted and so can empty faster.

Bittner: We concluded that gastric emptying is regulated by osmoreceptors and in so far we have studied the effect of osmolarity. We do not think that a dilution phase is responsible for the initial delay. On the contrary, the correlation between high PAO and rapid emptying proves that dilution cannot be the cause of slow emptying. Dilution causes a decrease of the high osmotic pressure of the liquid meal, thus resulting in diminished stimulation of osmoreceptors. Therefore parallel with the increasing dilution, gastric emptying is going faster in the course of measuring.

23

The effect of vagotomy on gastric emptying of solid and liquid components of a meal

J. W. MILLAR, G. P. McLOUGHLIN, I. B. MACLEOD AND R. C. HEADING

The effects of various surgical procedures on gastric emptying have been studied by several groups of workers in recent years[1–9]. Some measure of agreement is now emerging, following recognition that apparently conflicting results may be explained by the fact that emptying patterns of liquids and solids may differ, and that emptying rates during the first few minutes after a meal may bear no relationship to the rates occurring thereafter. There have been few studies in which emptying of the liquid and solid components of a mixed meal have been measured simultaneously, and in this paper we report our use of a scintigraphic technique to study this in patients who have undergone truncal vagotomy and pyloroplasty or highly selective vagotomy on account of chronic duodenal ulceration.

METHODS

Four groups of male subjects were studied. Ten healthy volunteers formed a control group for comparison with twelve patients with uncomplicated duodenal ulcers (DU), twelve patients who had undergone highly selective (proximal gastric) vagotomy (HSV) and twelve patients who had undergone truncal vagotomy with Heineke–Mikulicz pyloroplasty (TVP). All the operations were performed by one of the authors (I.B.M.) and the gastric emptying measurements were made 6 months postoperatively. Pre- and postoperative and secretion data were available for sixteen of these patients and were consistent with satisfactory vagotomy according to the criteria of Carter *et al.*[10] All postoperative patients were also evaluated clinically by two observers and

symptoms assessed according to the Visick criteria[11]. The observers had no knowledge of the nature of the patients' surgery nor of the results of gastric emptying studies.

Gastric emptying measurements were performed using a double isotope scintiscanning method[12] which permits simultaneous study of liquid and solid components of a standard meal. After fasting overnight, the subject was given a meal of 20 g cornflakes, 15 g sugar and 150 ml milk which also contained indium-113m DTPA as a marker of the liquid phase and approximately thirty small paper particles impregnated with technetium-99m sulphur colloid and coated with plastic, which acted as a solid marker. Ten minutes after taking the meal, the subject was positioned under a double-headed rectilinear scanner with the opposed detectors coupled to appropriate display, area selection and computer equipment which allowed quantitation of the proportions of total intra-abdominal activity of each isotope that remained within the stomach. Subsequent scans were performed at 30, 60, 90 and 120 min after ingestion of the meal and were restricted to the stomach area only. Each scan took about 7 min, during which time the patient was supine; between scans, he sat in a chair. Smoking was prohibited on the morning of study and none of the subjects were receiving drugs which were thought to have any influence on gastrointestinal motility.

Successive scans on each patient were used to determine emptying rates for the two markers. For the liquid marker, the rate was calculated as an emptying half-time or as a simple exponential rate constant since the emptying pattern was approximately monoexponential. However, the solid marker emptied in a linear rather than an exponential manner with time and the emptying rate was determined by linear repression of the observed points and expressed as percentage ingested marker emptied per minute. These calculated emptying rates were used in statistical comparisons of the patient groups.

RESULTS

Figure 23.1 depicts the findings in control subjects and illustrates the differing emptying patterns of the liquid and solid markers. In the DU group (Figure 23.2), similar patterns were observed, with liquid emptying on average being slightly faster and solid emptying slightly slower than in controls. Comparing the HSV group with controls (Figure 23.3), slightly greater mean emptying of both markers was seen at 10 min, but subsequent emptying rates were almost identical to the control rates. The TVP group (Figure 23.4) exhibited greater early (0–10 min) emptying of both markers than in control subjects, although the subsequent emptying rates appeared slower. However, statistical comparisons by Student's t tests of the controls with each of the three patient groups in respect of the amounts of marker emptied at 10 min and the 10–120 min emptying rates demonstrated significance ($p < 0.05$) only for early emptying of the liquid marker in TVP patients.

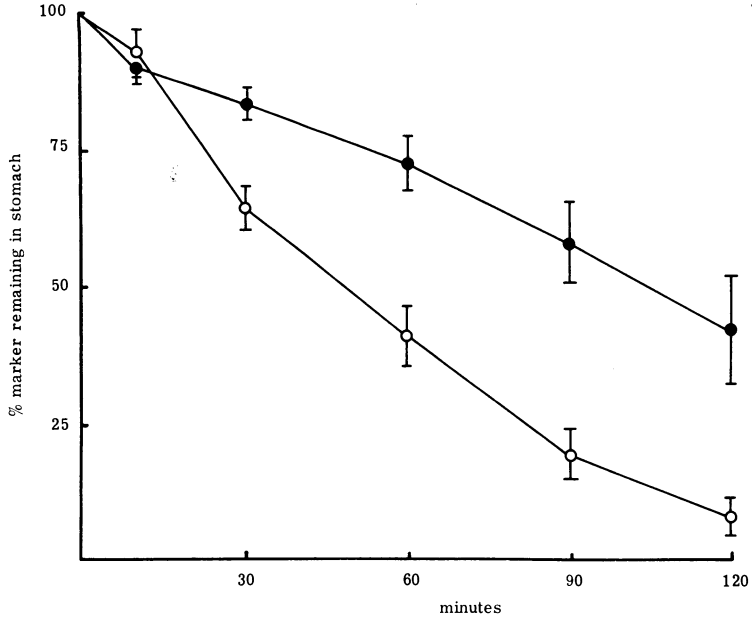

Figure 23.1 Gastric emptying of liquid (○) and solid (●) markers in ten control subjects. Error bars are ±SEM

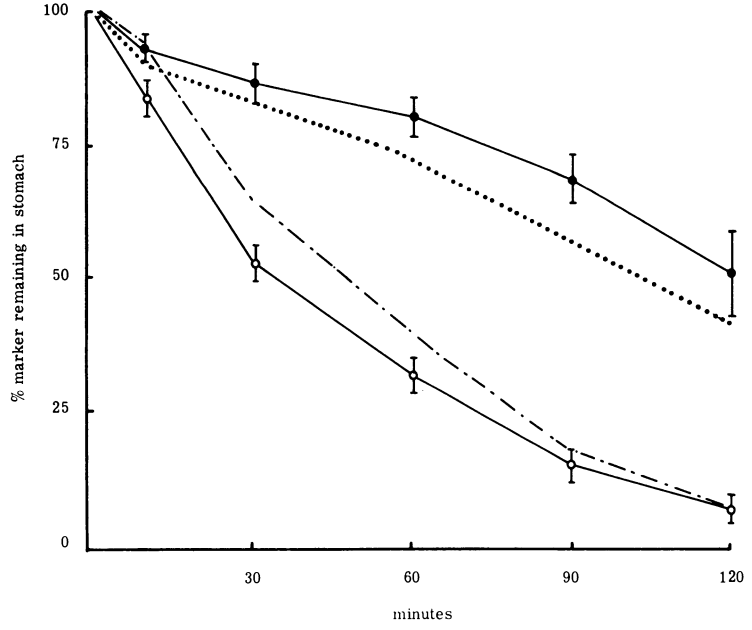

Figure 23.2 Gastric emptying of liquid (○) and solid (●) markers in twelve DU patients. Error bars are ±SEM. Broken lines represent emptying in control subjects shown in Figure 23.1

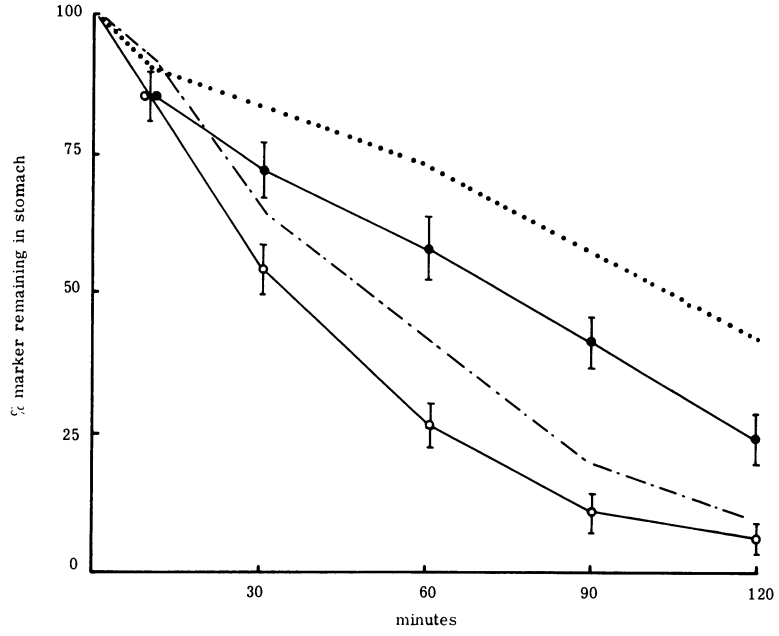

Figure 23.3 Gastric emptying in twelve HSV patients. Symbols as for Figure 23.2

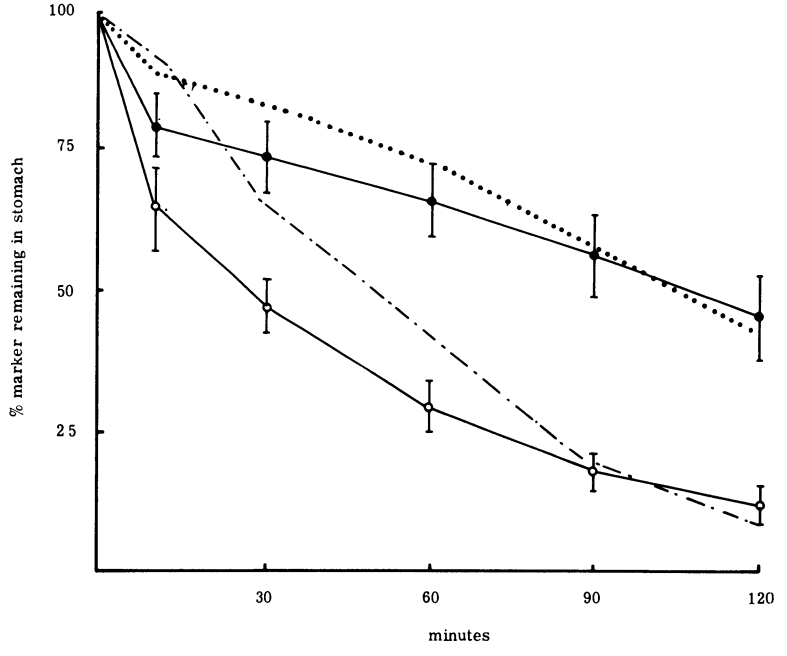

Figure 23.4 Gastric emptying in twelve TVP patients. Symbols as for Figure 23.2

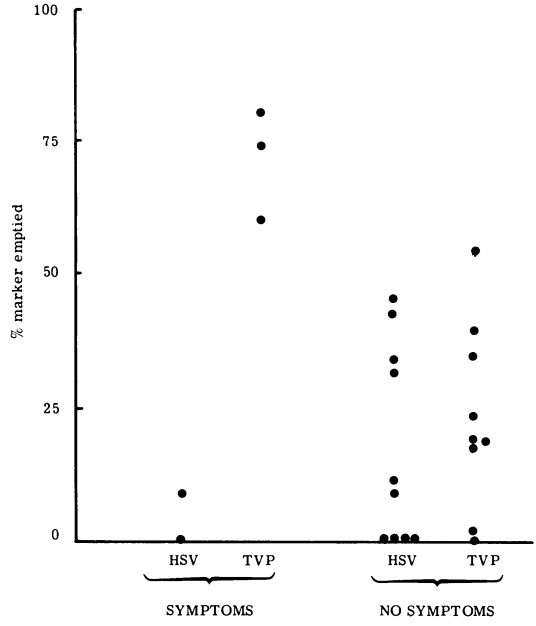

Figure 23.5 Early gastric emptying of liquid marker in HSV and TVP patients with or without postoperative symptoms

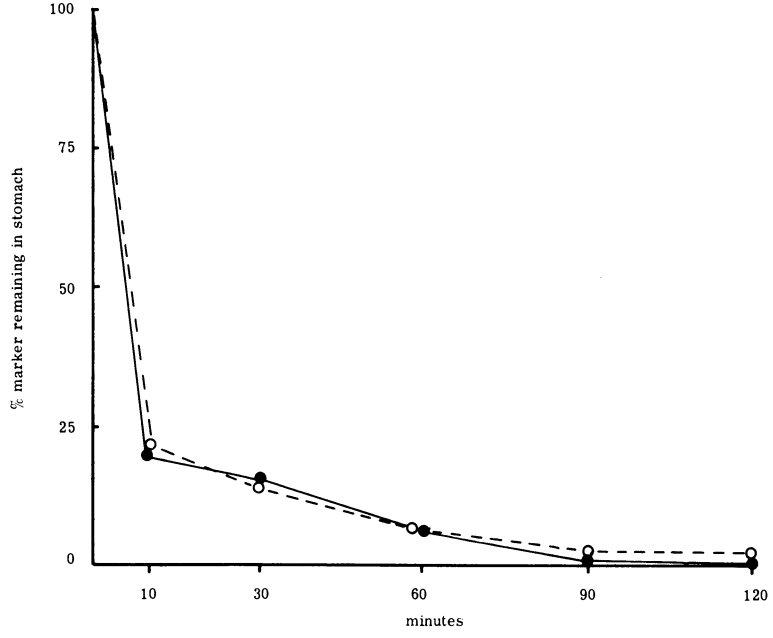

Figure 23.6 Gastric emptying of liquid (○) and solid (●) markers in one TVP patient with severe diarrhoea and post-prandial abdominal discomfort

The relationship of early emptying to symptoms of diarrhoea and/or post-prandial nausea and fullness in the postoperative patients is shown in Figure 23.5. The patients with symptoms were sufficiently affected to warrant classification as Visick grade III or IV in the opinions of the two assessors working independently. A clear association between the occurrence of rapid early emptying and symptoms was identified in the TVP patients ($p < 0.01$; two-sample rank test) but two HSV patients with normal early emptying were also symptomatic.

Figure 23.6 shows the emptying results recorded from the patient with the greatest incapacity from postoperative symptoms, who was classified Visick Grade IV due to post-prandial epigastric discomfort and diarrhoea. In addition to very rapid early emptying of both markers, this TVP patient was the only one in the study to lack all differentiation between solid and liquid marker emptying. This was an atypical pattern after TVP and contrasts with the group results shown in Figure 23.4, which demonstrate that this operation did not usually destroy the solid–liquid emptying differential.

DISCUSSION

In several respects, the results of this study are in accord with the findings of larger studies published by others. One may reasonably expect that emptying after TVP will be more abnormal than after HSV and this seems generally agreed[2,3,7,9]. However, the present results emphasize the importance of the early period of emptying as the time when the greatest departure from the normal pattern may occur. Rapid early emptying after TVP has been consistently observed with fluid meals[1,2,13,14] but conflicting results have been reported with solid meals[5,7,15,16]. Our results seem to reconcile these discrepancies in that an abnormality of early emptying was seen after TVP in respect of the liquid component of our mixed meal, together with a lesser abnormality for solid which was not statistically significant.

In comparison with TVP, HSV produced less disturbance of early emptying in spite of the gastric body denervation and consequent loss of receptive relaxation that occurs with both. Pyloroplasty thus makes a significant contribution to the early emptying abnormality after vagotomy although experimental studies in dogs indicate that pyloroplasty without vagotomy has no effect[14].

The normality of emptying in our HSV patients during the 10–120 min period conforms with the findings of others[7,9]. In the TVP group, slower emptying rates were recorded both for the solid and liquid markers and we suspect the differences from controls were real, although in this study they did not attain statistical significance. We also failed to detect statistically significant differences between the control and DU patients. Faster emptying in DU than in normal subjects has been described in some reports[17,18] although others have noted little or no difference[7,19]. It is possible that differences

between DU and normal subjects would be more readily demonstrable with meals of higher energy density than that used in the present study[20].

The preservation of the differential between solid and liquid marker emptying in all but one of our TVP patients indicates that this aspect of gastric emptying, presumably a function of the antrum, is not dependent on an intact pylorus. However, the one exception demonstrates that TVP may sometimes disrupt antropyloric function more radically, and it is therefore of interest that major symptoms occurred in this patient.

An association of symptoms with rapid early emptying of a solid meal has been observed previously[15] although no association has been reported by others[7,21]. Certainly it is clear that not all symptomatic patients exhibit abnormal early emptying. Nevertheless, symptomatic patients with rapid early emptying might be helped if a more normal emptying pattern could be restored and identification of such patients and of acceptable pharmacological or surgical means of reducing abnormally rapid early emptying would seem to be worth pursuing.

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Discussion

A. Dubois: (USA) This very elegant radioisotopic method does not allow, by definition, any measurement of the volume of intragastric contents. Do you not think that differences in water and acid secretory rates in duodenal ulcer and vagotomized patients could account for some of the changes or absence of changes that you observed?

R. C. Heading: (UK) I think the only change possibly attributable to differing gastric secretions would be the apparent slowing of solid emptying in duodenal ulcer patients in comparison with controls. However, we did not obtain statistical significance for this difference in any case, so I think speculation about the reasons for it would not be justifiable.

V. Rayner: (UK) Is the early rapid emptying after truncal vagotomy and pyloroplasty a function of the truncal vagotomy, that is the lack of vagal control, or the pyloroplasty? In pigs we find an early rapid emptying of liquid test meals of acid (0.1 M HCl) or glucose (500 m M) but not of water after truncal vagotomy without pyloroplasty.

Heading: I cannot answer your question on the basis of our studies. However, my understanding of the literature is that rapid early emptying results from vagotomy and is enhanced when pyloroplasty is added.

M. A. Eastwood: (UK) The method used differentiates solid and liquid phases. To what extent is the differentiation due to the shape of the stomach? Would the same differentiation be obtained if the homogenized meal were placed in a teapot and slowly poured out? Would a marker on a colloid which disperses through the gastric contents and yet reflects the solid phase to some extent, be of value?

Heading: This raises the question of 'what do we mean by solid?' I believe the important point is to distinguish between fluid material and particles. A colloidal suspension would behave as a fluid – large particles or lumps of food behave differently. We have not tried the teapot test.

R. W. McCallum: (USA) A technical question. You mentioned making gamma camera counts every 30 min with the patient sitting between times. In our experience trying to make sure that the stomach is exactly relocated on each occasion is very difficult – how can you be sure that you are not also counting some emissions from the duodenum or elsewhere?

Heading: This is a problem for everyone doing this type of measurement. We use reference points on the subject's costal margin and reposition with reference to these. Thus we try to count the same area each time. I am sure that some duodenal emissions are inevitably included for some subjects, but this may be less important than we used to believe. If we determine emptying rates on a basis of areas deliberately selected to be too large, we obtain very similar results to those obtained by counting from the proper area. So area selection, though important, is not so critical as we formerly believed.

H. J. Ehrlein: (Germany) A comment about solid markers. Solid markers as normally used are particles which the stomach cannot grind and dilute. If you introduce

plastic spheres with a diameter of 6–8 mm together with fluids or pulpy materials into the stomach of dogs, the spheres remain in the stomach until it is totally emptied. Only the emptied stomach produces contractions strong enough to press the spheres into the duodenum. Your pieces will be small enough to pass the relaxed pylorus; they will be emptied at random. Solids in the food, however, are materials which can be crushed and diluted by the stomach. They leave the stomach only when they have become fluid. Therefore, the normal events in emptying of solids can only be measured with materials which can be crushed and diluted.

Heading: I entirely agree that physiological reality is as you describe – ingested solid is converted to fluid. In consequence, measurements based on labelled liver or similar food initially reflect the way the stomach deals with particles, and later on reflect the way it empties fluids. I think it is important to recognize this as the reality, but we believe that our approach offers a reproducible means of obtaining some indication of the way the stomach deals with solid particles.

24

The effect of bran on colonic myoelectrical function in diverticular disease

I. TAYLOR AND H. L. DUTHIE

The incidence of diverticular disease of the colon presents a growing problem in Western society, with a gradually increasing incidence. It is now seen in approximately 70% of barium enema examinations in patients over the age of 70 and is present in 20% of people over the age of 40 both in Britain and North America[1]. It has been estimated that 10% of these patients have symptoms related to diverticula. Recently a good deal of both clinical and experimental evidence has accumulated to implicate a chronic low-fibre diet in the aetiology[2–3].

In a previous study we were able to demonstrate that bran given to patients with established diverticular disease not only improves symptoms but also reverses the abnormal myoelectrical disturbances which are present[4]. These patients have now been studied after a prolonged period of bran therapy, and the findings are presented and compared both to the pre-treatment and intermediate treatment values.

METHODS

Twenty patients with established diverticular disease were initially included in the study and sixteen were available for subsequent prolonged follow-up assessment. For the first 4 weeks of the study they received 18 g of bran per day (three bran tablets three times a day) and the assessments repeated. They were continued on a maintenance dose of 12 g bran per day for 30–39 weeks (mean 34.8 weeks) and the assessments repeated in sixteen patients. The remaining four patients did not re-attend for objective testing but claimed to be free of symptoms.

The following assessments were carried out:

1. Symptom score

A standard questionnaire was used to evaluate each patient's symptoms and a symptom score derived. Degree of pain, bowel habit and amount of distension were all considered.

2. Stool weight

The stool weight was calculated as the mean for a 5-day collection and expressed as g/day.

3. Transit time

Twenty small radio-opaque pellets were taken by the patient and the time for 80% of the pellets to pass was regarded as the intestinal transit time (Hinton's method)[5].

4. Percentage motility

Thin, open-ended tubes were used to measure changes in intraluminal pressure as previously described[6]. The tubes were introduced via a sigmoidoscope, and care was taken to ensure that they recorded from a similar area of the colon on each occasion as measured in centimetres from the anus.

5. Electrical activity

An intraluminal suction probe was used to record the myoelectrical activity in the rectosigmoid region. This probe has been described previously[6] and had incorporated within it the thin, open-ended tubes; thus pressure and electrical measurements could be obtained from the same region of bowel.

Monopolar recordings were made with the indifferent electrode placed on scarified skin over the thigh. The pressure and electrical recordings were suitably amplified and recordings obtained for 1 h. In the analysis of the electrical recordings particular attention was paid to the frequency of the rapid electrical rhythm and to the percentage of recording time during which it was present. The percentage motor activity was calculated by adding together the duration of all waves and expressing the sum as a percentage of recording time.

RESULTS

Symptom score

All patients had an improved symptom score after treatment for 1 month; 60% were entirely symptom-free. Following prolonged bran therapy only

three patients complained of symptoms referable to diverticular disease. Two had a lower symptom score than noted after 1 month of treatment, and one had a higher score. This patient thought he was more constipated than previously, although this was not confirmed either by stool-weight or transit-time estimations. Hence 85% of patients were entirely symptom-free (zero symptom score) after prolonged bran therapy.

Stool weight (Table 24.1)

The mean stool weight prior to treatment was 79 ± 7.3 g. Following bran tablets for 1 month there was a statistically significant increase (mean 121 ± 7.1 g) but the mean daily stool weight after prolonged treatment was not statistically greater than the value after 1 month (mean 124 ± 8.0 g).

Transit time (Table 24.1)

The mean transit time before treatment was 96.6 ± 7.1 h. This was statistically significantly decreased after 1 month (56.1 ± 4.1 h). However there was no further statistically significant reduction in transit time following prolonged bran therapy (55.5 ± 4.5 h).

Table 24.1 Mean stool weight, intestinal transit times and motility (± SEM) before therapy, after 1 month and after prolonged bran therapy

	Before treatment	*One month's treatment*	*Prolonged treatment*
Stool weight (g/day)	79 ± 7.3	121 ± 7.1 ($p < 0.001$)	124.1 ± 8.0 ($p < 0.001$)
Transit time (h)	96.6 ± 7.1	56.1 ± 4.1 ($p < 0.001$)	55.5 ± 4.5 ($p < 0.001$)
Motility (%)	14.2 ± 3.1	6.5 ± 0.8 ($p < 0.02$)	8.4 ± 1.0 ($p < 0.02$)

Percentage motility

The normal mean percentage motility obtained by this method is 7.5%[6]. Prior to treatment there was a high mean percentage motility (14.2 ± 3.1%). This fell to within normal limits after treatment for 1 month (6.5 ± 0.8%). The mean value after prolonged treatment was 8.4 ± 1.0%, which did not represent a statistically significant difference in the individual patient from that obtained after 1 month's treatment. There was no apparent difference in either the shape or type of pressure waves obtained.

Electrical activity

The rapid electrical rhythm characteristically seen in patients with diverticular

disease was noted in sixteen of the patients prior to treatment. After treatment for 1 month it was still present for a proportion of the recording time in eight patients, although in the remainder the electrical rhythm returned to normal (Figure 24.1).

Following prolonged treatment it was recognized in only two patients, mean frequency 0.215 Hz (12.9 cpm) and 0.225 Hz (13.5 cpm), with an incidence of 32.3% and 6.9% respectively. In all other patients the electrical activity was similar to that previously recorded in normal subjects.

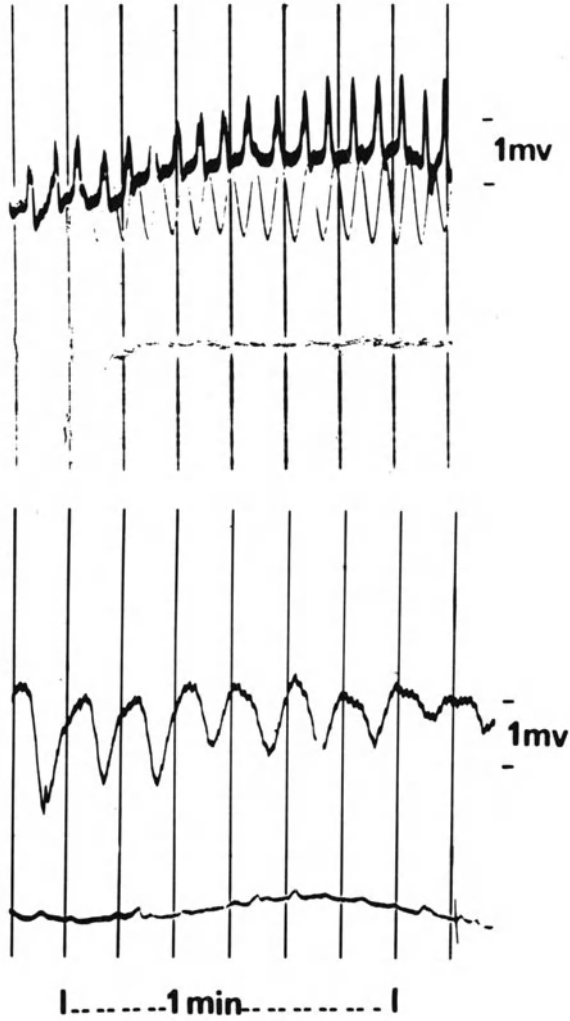

Figure 24.1 The top trace shows the myoelectrical activity in a patient with diverticular disease before treatment. The mean frequency is 14 cpm. The bottom trace shows the slow wave activity restored to normal frequency after 1 month's treatment with bran

In these two patients the rapid rhythm had also been recognized at 1 month with an incidence of 21.5% and 29.2%. Thus, of seven patients who had a persistent rapid rhythm present after 1 month's treatment, in five this could no longer be recognized after prolonged treatment.

DISCUSSION

Diverticular disease of the colon is one of several disorders characteristic of modern Western civilization, and a diet deficient in cereal fibre is generally regarded as one of the main predisposing factors. There is no doubt that the addition of fibre to a refined diet results in shortened transit times and increased stool weight, but what physiological response this achieves in diverticular disease is not yet established. The purpose of this study was to determine the effect of bran on colonic smooth muscle pathophysiology, both in the short and long term.

After treatment for 1 month we noted improvements in all assessments of colonic smooth muscle activity. Hence the improvement in symptomatology was associated with a significant increase in stool weight and decrease in transit time; the raised intracolonic pressure fell and the rapid electrical activity was seen less frequently. These improvements were maintained with continuing treatment, and when the patients were re-assessed after 9 months only two had any evidence of abnormal myoelectrical smooth muscle activity.

It is interesting that after prolonged treatment there did not appear to be a more rapid transit time or a greater stool weight than that achieved after 1 month's treatment. Apparently once the optimum conditions have been obtained these are maintained provided the diet is continued. It is also intriguing that a disease which presumably takes decades to develop in the presence of a hostile low bulk environment can be reversed in less than 1 year of maintenance therapy with bran. The muscular abnormality is readily improved provided an appropriate regime is followed, adding more weight to the hypothesis that diverticular disease results from abnormal environmental stress rather than a primary colonic disorder.

In conclusion it is clearly important to ensure that patients with diverticular disease continue with a high roughage diet, even though they may be asymptomatic, to ensure continuing improvement and prevent relapse.

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Discussion

J. McLeish: (UK) Did your patients have symptoms referable to the upper gastrointestinal tract? If so, were they improved after the course of bran tablets?

H. L. Duthie: (UK) Our patients all had pain in the left iliac fossa as a criterion of activity of the diverticular disease, and this was the main indicator of symptomatic relief. Incidentally, some had upper abdominal symptoms which did show improvement with treatment with bran.

W. J. Snape, Jr.: (USA) What is the frequency of the calonic contractions in patients with the fast slow-wave activity and is there a relationship between the slow waves and simultaneous occurring contractions?

Duthie: Colonic contractions, when present simultaneously with slow waves, occur at the same frequency as the slow waves whether rapid or in the normal frequency band. Most measurements were made with only one electrode and one pressure recording tip so that I cannot comment on simultaneous contractions.

J. Christensen: (USA) In a patient with the high-frequency slow waves, does the high frequency turn on and off or is it constantly present? If on and off, is the change abrupt or graded?

Duthie: The higher frequency of slow waves is intermittent and may begin either abruptly or with a gradual increase in amplitude.

A. M. Connell: (USA) What was the nature and quantity of the fibre in your control and high residue diets?

Duthie: The control diet had about 4 g of fibre and the high residue diet about 10 g including the added bran.

B. N. Catchpole: (Australia) People who are given bran pass a lot of flatus. (1) What is the source of gas? (2) Is the gas the cause of the reduced transit time when the patient is on bran?

Duthie: We find that patients taking bran tablets have less flatus than with unprocessed bran. I would speculate that the extra gas is swallowed air.

A. J. M. Brodribb: (USA) We have also studied intrasigmoid pressure in patients with diverticular disease before and after 6 months treatment with bran. I would suggest that one of the most marked effects of bran in diverticular disease is to reduce the number of abnormally high pressure peaks. While bran halved the number of pressure waves of 15–20 mmHg, it reduced the number of waves greater than 60 mm to approximately one-tenth of that before treatment. When results are expressed as percentage motility or a motility index, this effect is not demonstrated.

A. N. Smith: (UK) A comment: the bran tablets seem to win. Perhaps this is because they are more regularly taken (it is difficult to check how much fibre is in a diet or whether this is consistently taken). Bran was better than Normacol and antispasmodic. Bran could, by bulk, etc. act on the muscle, but by filling up the colon could dilute something.

Section V
Methods of Analysis

25

Methods of analysing rhythmic electrical potentials in the gastrointestinal tract

D. A. LINKENS

The clear visual evidence of regular oscillations in electrical potentials recorded from many parts of the gut has prompted attempts to measure the frequency of these 'slow waves' accurately. It is desirable to replace manual measurement of such frequencies by automated methods in order to remove the tedium and time involved. A more important reason for automated methods, however, is to enable precise objective values to be given to measurements which will facilitate discussion between research groups involved in this work. Manual or automatic methods of counting a number of cycles per unit time do not give good accuracy, and are not included in this paper. Similarly the use of narrow pass-band analogue or digital filters as an indication of the presence of rhythms is also not discussed. In the context of medical electrical recording the presence of large amounts of artifacts, particularly at the lower frequency spectrum near to the 'slow wave' frequency band, gives problems in the use of narrow pass-band filters.

The subject of frequency analysis is of considerable interest in many technological disciplines and there is considerable scope for cross-fertilization between medical and scientific disciplines at this point. In particular one could mention the areas of mechanical vibrations[1], telecommunications[2] and control system identification[3]. It will be seen that the methods which are now briefly described have largely been developed and applied in such disciplines. The five techniques are summarized, with their relative merits and disadvantages, and some typical results using gut signals are presented.

FIVE METHODS OF FREQUENCY ANALYSIS

In assessing the various methods a number of criteria should be applied both to the data which are available and to the information which the technique

provides. For the data the number of cycles of oscillation is a very important parameter. Similarly both long- and short-term variations in the instantaneous period of the slow wave are crucial, as is the amount of noise or artifacts added to the signal. The wave-shape of the data is important and is related to the number of harmonic components present, while the simultaneous existence of more than one rhythm sets a further constraint.

Fast Fourier transforms

In recent years the widespread use of digital computers has made this method very popular whenever it is required to measure accurately the frequency components of a signal. In the Fourier transform approach a signal which is characterized by a number of data values versus time is represented by a frequency spectrum comprising a graph of frequency values plotted horizontally and the amplitude of each frequency component plotted vertically. The use of analogue methods to mechanize the Fourier transform has now been almost completely superseded by digital methods which use the so-called FFT algorithm developed by Cooley and Tukey[4].

The FFT method is theoretically based on noise-free data of infinite duration, but in spite of these limitations it has been used successfully to quantify noisy, finite-length medical data in many applications. In terms of accuracy of frequency measurement the crucial factor is the number of cycles of data present. Thus, for n cycles of data recorded and transformed, the discrimination in frequency will be $100/n\%$. It is also necessary that the frequency should not vary during this number of cycles if a sharp spectral peak is to be obtained in the transform. This problem is illustrated in Figure 25.1, which shows an FFT on a human colonic recording which had both frequency variability and a small number of cycles available. There is a spread of peaks around 5 cpm. Frequency components in an FFT having a wide 'spread' rather than a single sharp peak can thus be caused by factors such as small number of cycles, variations in instantaneous frequency and/or amplitude, and the presence of large artifacts near the particular frequency.

The presence of large amounts of noise can cause difficulty in determining the significance of peaks in the FFT spectrum. To reduce the spurious peaks caused by artifacts one requires either long time transforms or the averaging of successive transforms, but this aggravates the problems already referred to. Averaging of adjacent peaks in a single spectrum can be used to determine significance of a peak, but this then degrades the frequency accuracy proportionately[5]. The visual detection of significant peaks in the FFT spectrum is easy but subjective. An objective assessment of significance is not straightforward, but two ideas may be useful. First, for pure sinusoidal components in the presence of Gaussian noise one can quantify peak significance simply; particularly if the spectrum is plotted with a log amplitude vertical axis[6]. Medical noise is not, however, Gaussian and in gut recordings there is a

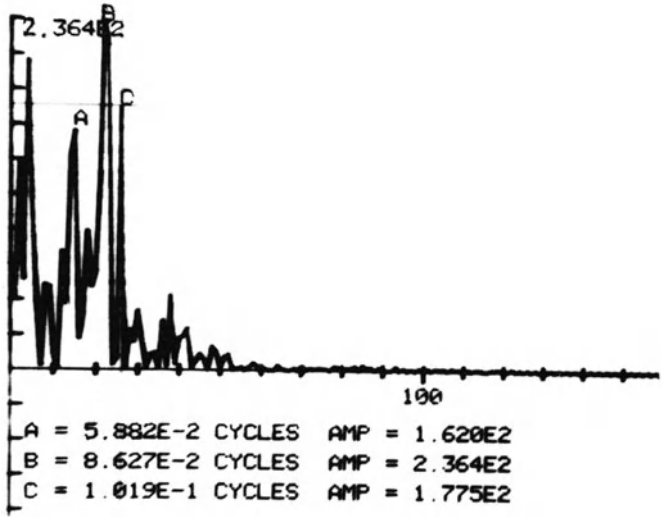


Figure 25.1 FFT on a 512 point (2 Hz or 120 cpm sampling rate) stretch of human colonic data

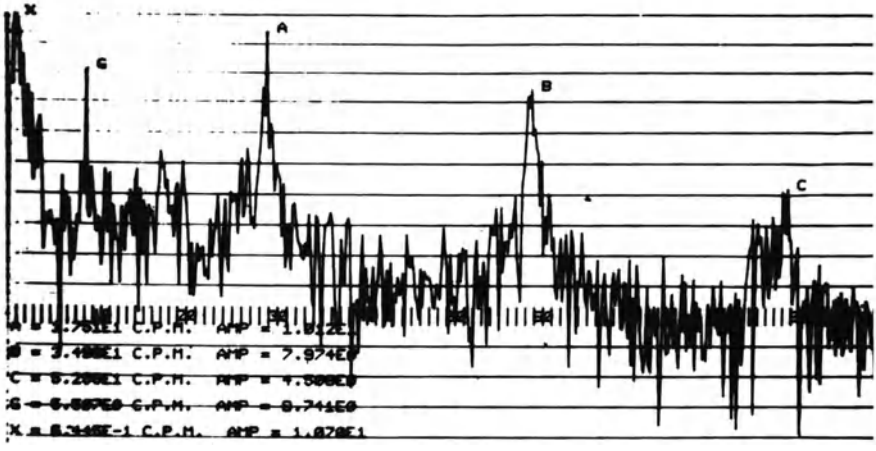


Figure 25.2 Log amplitude-squared FFT spectrum of a canine duodenal signal showing peaks at gastric and duodenal frequencies

preponderance of noise at low frequency values. Another idea therefore is to compare the signal spectrum with the mean and standard deviation of the noise spectrum, and on the basis of this to print out the significant peaks in the signal[7].

It is clear from the basis of the method that multiple rhythms can be detected. An example of this is shown in Figure 25.2, which demonstrates the clear detection of a gastric frequency marked G on a recording taken from a human duodenum. This figure is a long amplitude spectrum, and shows its ability to detect harmonic components marked here as peaks B and C. It also illustrates the large amount of low-frequency artifact near the peak labelled X.

Fast Walsh transforms

Fourier transforms assume that a signal can be represented by a summation of sinusoidal components each with a different frequency. In the mathematics of this transformation a large number of multiplications involving sine and cosine functions are required for each FFT. These are time-consuming and would be expensive to implement on a dedicated micro- or mini-computer system.

In contrast, a Walsh transform assumes that a signal can be represented by a summation of discrete functions similar to square waves[8]. When used with a Cooley–Tukey algorithm a fast Walsh transform (FWT) is a very fast process involving simple additions and subtractions instead of multiplications involving sine and cosine expressions. If the main requirement in the data analysis is the measurement of frequency components then a very simple FWT system can be used. If the data are 'squared' using a zero-crossing detector so that logic '1' and '0' are allocated to positive and negative sections of the signal respectively, then no analogue to digital conversion equipment is required. Figure 24.3 shows an FWT using this technique on a canine gastric recording[9]. It has given clear peaks at the fundamental and second harmonic frequencies, but two general observations arising from Walsh transforms can also be made. These are that the overall background 'noise' components are increased and that 'spurious' peaks often occur, particularly at the high-

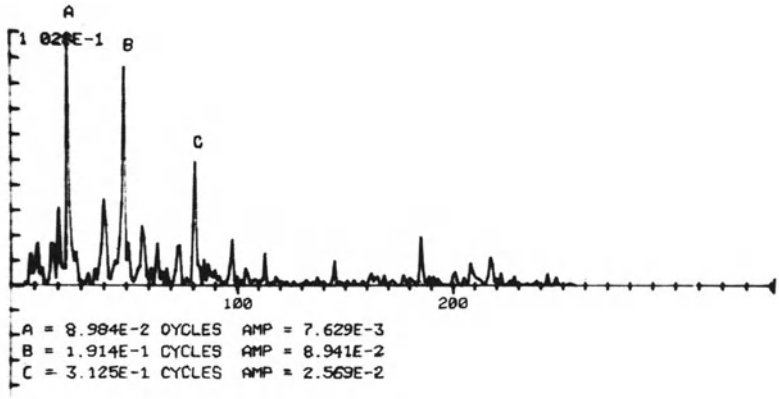
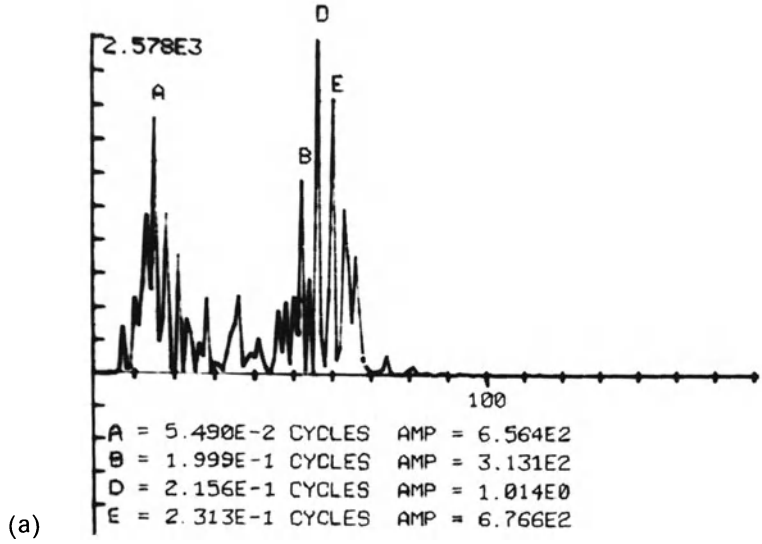
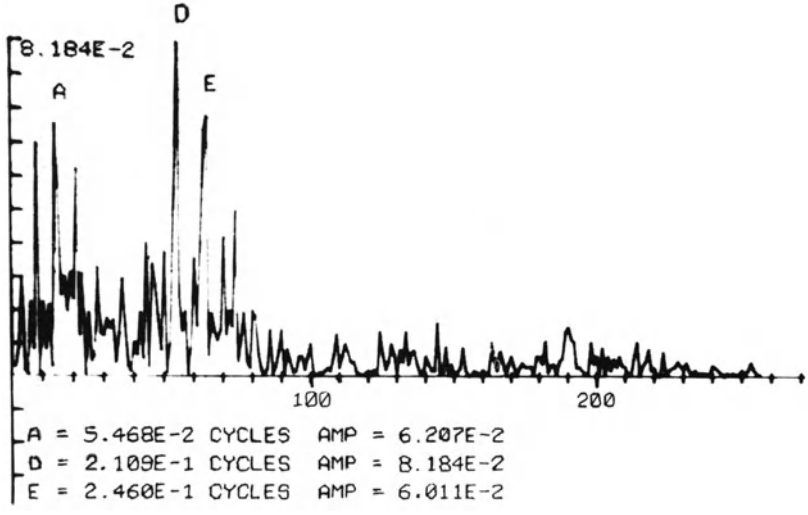


Figure 25.3 FWT of a canine gastric signal showing fundamental and second harmonic peaks



(a)



(b)

Figure 25.4 Human colonic transforms: (a) FFT; (b) FWT

frequency end of the spectrum. These differences from the FFT method are clearly visible when using regular data from gastric and small intestinal recordings but are not so apparent when using colonic data. Figure 25.4 shows FFT and FWT plots from the same stretch of human colonic data. Two rhythms were present at about 3 and 12 cpm but both FFT and FWT show considerable spread of components around these frequencies.

This method has been implemented on a simple microprocessor system (DEC 7341) using no analogue to digital conversion equipment, but reading out the FWT spectra onto a laboratory oscilloscope via two digital to analogue channels[10]. A further point of interest is that the same microprocessor system has also been used to perform digital filtering using forward and inverse FWT.

Auto-regressive (AR) modelling

This technique has been in use for some years for the identification of noisy signals in industrial processes[11]. More recently it has been used by medical researchers in the analysis of EEG, although the emphasis has usually been on the detection of abnormalities in the EEG pattern rather than on the determination of rhythm frequencies[12]. It has been applied in Sheffield to typical gut recordings and shows promise of being a method that will give accurate estimations of frequencies using only a few cycles of data, unlike the FFT and FWT methods[13].

The method assumes that the data being analysed can be represented as a Gaussian white noise source operated on by a filter expressed in a digital format referred to as a z-transform notation. Given a specified complexity of filter, referred to as the 'order of the model', the algorithm produces a best least-squares fit to the original data by adjustment of the model coefficients. The amount of error is determined by the difference between the model output and the data, and is referred to as the 'residuals'. The technique is successful if the residuals are substantially white noise, and this is checked by observing the autocorrelation function.

The second part of the method is to determine the frequency components from the model coefficients. This is done by factorizing the z-transform polynomial of the model and plotting the roots on a z-plane diagram. The roots have real and imaginary parts, and a rhythm frequency is indicated by roots which lie near the unit radius circle on the z-plane. These concepts are illustrated in Figure 25.5 which shows artificially generated data comprising a sine wave plus its second harmonic, together with noise corruption. The z-plane representation of the autoregressive model analysis is shown in Figure 25.5b, in which the points marked 'A' represent the fundamental frequency. The actual value of frequency is simply related to the angle that the particular point makes with the origin of the diagram. The points marked 'B' indicate the second harmonic frequency, while points 'C' and 'D' represent the noise

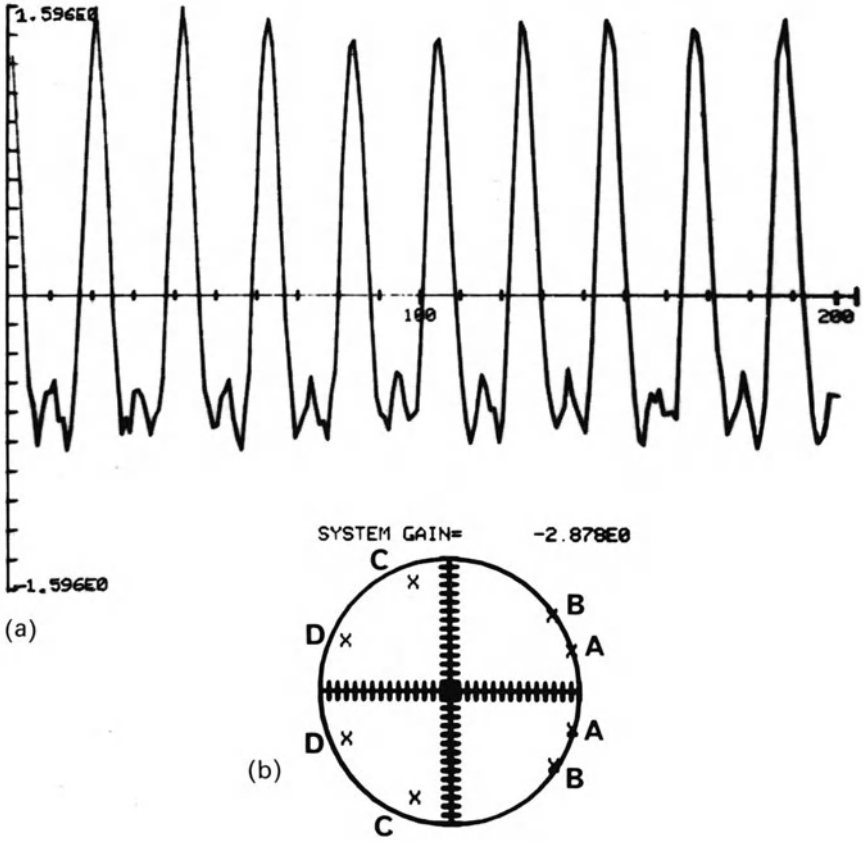


Figure 25.5 AR modelling on simulated data: (a) data comprising fundamental, second harmonic and noise; (b) z-plane plot

components. A number of comments can now be made about the z-plane diagram. Relevant rhythms will give points which are close to, but not exactly on, the unit circle. The distance inside the unit circle is inversely proportional to the amplitude of the rhythm, so that points very close to the circle indicate components with a large amplitude. This provides a simple test of significance for component frequencies, and in fact values of distance from the origin of between 0.95 and 1.0 appear in gut work to be a good indication of significance. It can also be seen that relevant components appear in the right-hand side of the diagram, while high-frequency noise terms are located on the left-hand side. These remarks enable a simple criterion to be applied which gives numerical print-out of relevant frequencies together with an indication of their amplitudes. Points having a magnitude of 0.95–1.0 and located in the right-hand side are read out. This eliminates both high- and low-frequency noise artifact.

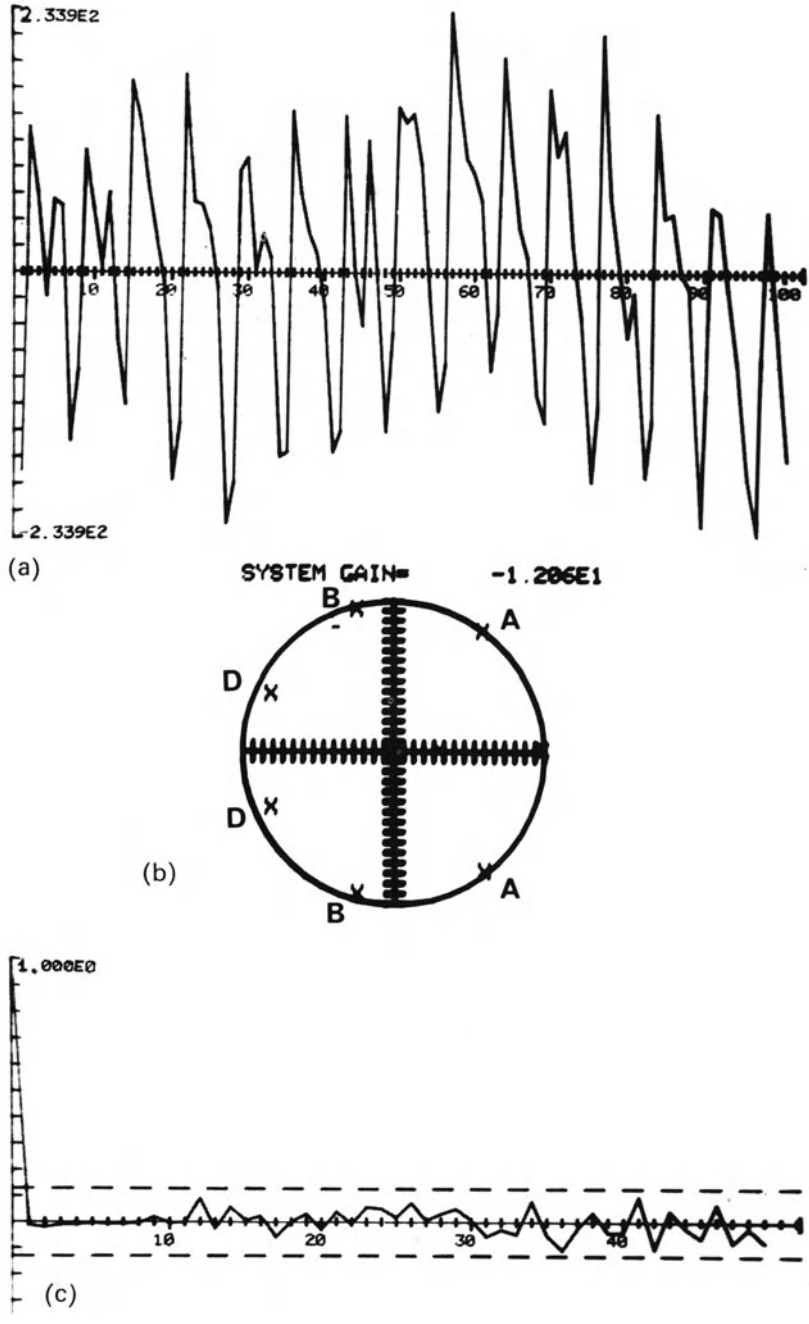


Figure 25.6 AR modelling on canine duodenal data: (a) 100 points of data; (b) z-plane plot; (c) residuals

The order of model must be selected, and this depends on the type of data being processed. Basically the order must be high enough to allow for the probable number of relevant rhythms plus harmonics together with sufficient points for noise representation. This can be seen in Figure 25.6 which shows the data, z-plane diagram and residuals for a canine duodenal recording. An FFT of 512 points from the same recording gave fundamental and second harmonic components of 17.7 and 35.6 cpm respectively with a frequency discrimination of 0.24 cpm from about 75 cycles of data. The z-plane points 'A' and 'B' represent the equivalent frequencies which gave values of 17.4 and 34.8 cpm using an eighth-order model and 100 data points or 15 cycles only. In this example a low-order model was sufficient to accurately identify the data and give residuals which were substantially white noise as indicated by the autocorrelation function plot of Figure 25.6c.

It was found that the order of the model and the number of data points had to be increased if there were many harmonics or large noise artifacts. Model

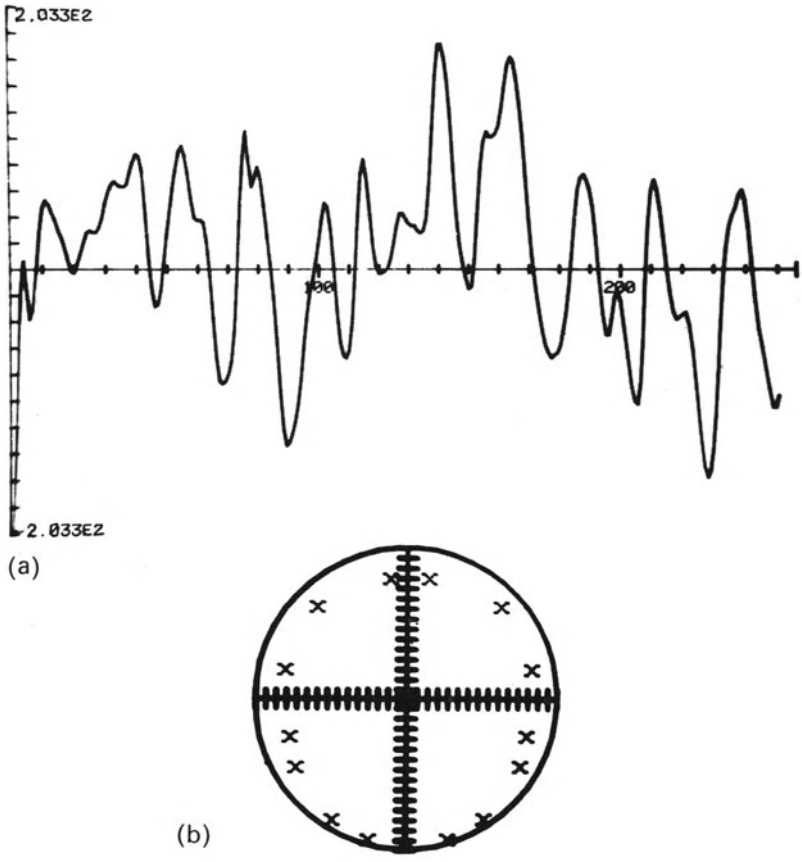


Figure 25.7 AR modelling on human colonic data: (a) 250 points of data; (b) z-plane plot

orders of about 15 and 250 data points were capable of dealing with the majority of signals from canine and human gastrointestinal recordings. A further example is shown in Figure 25.7 for a human colonic record which gave an FFT with considerable spread around 5 cpm (see Figure 25.1). The autoregressive method using 250 data points gave a frequency component at 5.28 cpm together with a smaller probable harmonic component at 10.8 cpm.

The autoregressive method appears to give good results on gastrointestinal data, offering numerical print-out of relevant frequencies on short stretches of data covering 5–15 cycles of information. Although the algorithm includes a matrix inversion at present there is no reason why it should not be modified to make use of an iterative updating method that works successively on incoming data points.

Phase-lock loop

This technique has been widely used in radio communications for the recovery of signals imbedded in noise, and considerable theoretical analysis has been attempted on phase-lock loops[2]. Although the method has been applied to gastrointestinal recordings[14], it has only recently been instrumented using cheap integrated circuit electronics[15].

A phase-lock loop comprises a feedback control system in which the frequency and phase of a high-accuracy oscillator are adjusted to match the in-

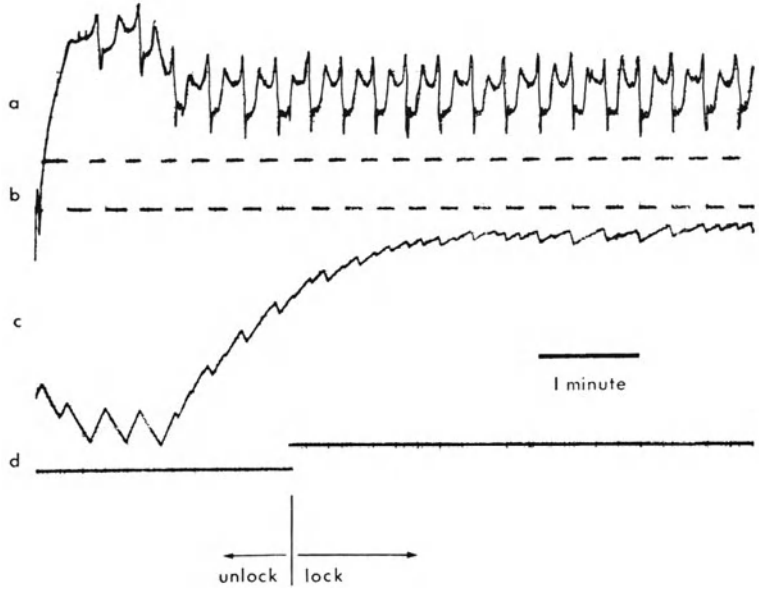


Figure 25.8 Recording from phase-lock loop system showing: (a) human gastric signal; (b) output from voltage-controlled oscillator; (c) filtered output from lock detector; (d) lock/unlock indicator

coming noise-contaminated data. When the loop has locked onto the signal the output from the internal oscillator gives a noise-free signal which can be used to give a measurement of signal frequency plus its variance. A further feature is that a signal can be derived which gives an indication if the system is locked onto a signal. In this way the technique provides objective information on the statistics of the frequency content in the data. Figure 25.8 shows the dynamic response of a phase-lock loop system used on a human gastric mucosal recording. After a transient disturbance in the incoming data the lock/unlock indicator shows that a satisfactory lock condition can be obtained within a few cycles of the disappearance of the transient disturbance.

The phase-lock loop method shows promise of being a simple analogue method which gives fast tracking of frequency changes in gastrointestinal rhythms. It should be noted that the internal oscillator frequency range must be limited to one octave (i.e. ratio of maximum frequency to minimum frequency must be less than 2) and similarly the incoming data must be band-pass filtered with the same limits. If this is not done locking can occur onto an integer multiple or sub-multiple of the signal frequency. From this it is apparent that the technique cannot deal with the case of multiple rhythm frequencies or harmonics of a single frequency.

Raster scanning

This technique is similar to a method used in determining the period of circadian rhythms[16]. Electronic ramp generators are used to sweep the beam across the Y-axis of a fibre-optics recording oscilloscope. The signal being analysed is connected to the z-modulation of the oscilloscope so that when a peak occurs a bright spot appears on the screen. If the ramp generator frequency is equal to the data frequency and the X-axis scan is very slow then a series of horizontal dots is recorded. If the data frequency varies then the dots will give an inclined line which eventually disappears off the edge of the screen. The use of nine ramp generators in parallel gives the appearance of continuous lines in this case and makes the visual read-out easier to assimilate.

The method requires the ramp generators to be set to the average value of the data frequency, and can clearly only deal with a single frequency. It does however, give a clear visual indication of changes in instantaneous frequency as can be seen in Figures 25.9 and 25.10. Figure 25.9 is a raster scan readout from a canine duodenal recording. Relatively small changes in frequencies at the beginning of this record are followed by a decrease in frequency at point 'A' immediately prior to the onset of a migrating electrical complex which causes disorganization of the slow-wave frequency at point 'B' onwards. Figure 25.10 shows a raster scan read-out from a 64 relaxation oscillator model of the small intestine[17]. The read-out is from an oscillator well away from the duodenal plateau and has considerable frequency fluctuations[18]. It has been found that when non-linear oscillators with different frequencies are

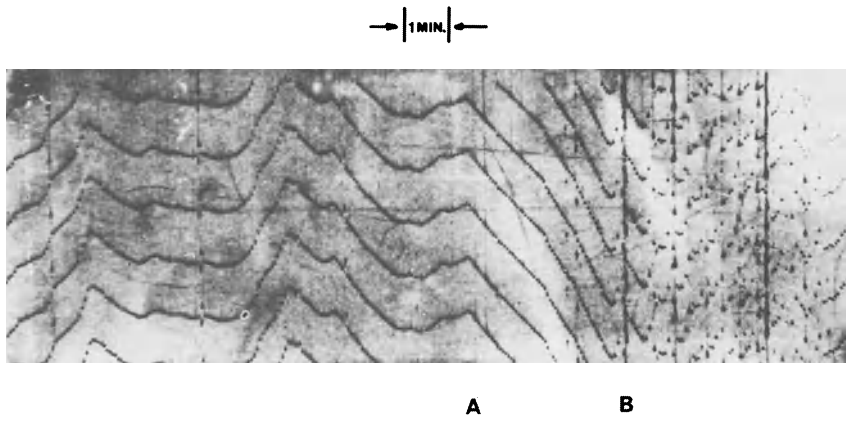


Figure 25.9 Raster scan display from a canine duodenal recording. Point 'B' marks the onset of a migrating electrical complex

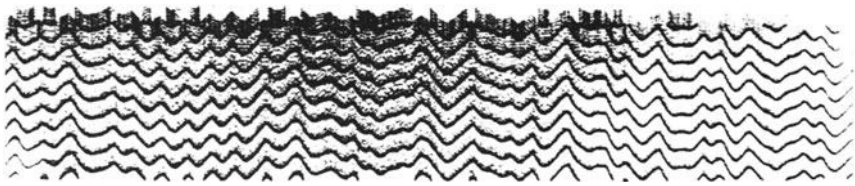


Figure 25.10 Raster scan display of the output of a single oscillator in a 64 relaxation oscillator electronic model of the small intestine. The oscillator represents a section of the small intestine where frequency entrainment does not occur, and hence frequency fluctuations exist

coupled together weakly, modulations can occur both in amplitude and frequency[19]. Such variations in frequency can be seen clearly in Figure 25.10 and almost sinusoidal modulation conditions exist for considerable lengths of time.

CONCLUSIONS

It is clear, from the description of the five methods which have been applied to gastrointestinal data in Sheffield, that each technique has relative advantages and disadvantages. In choosing the best method for a particular application account must be taken of factors such as the length of data, amount of artifact, presence of harmonics components and multiple rhythms. In Table 25.1 an attempt is made to summarize the characteristics of the techniques, in order to enable a researcher to choose the best method for his current requirement from the ever-increasing tool-kit of methods for analysing the frequency content of noise-contaminated gut data.

Table 25.1 Summary of five methods of frequency analysis

Method	*Advantages*	*Disadvantages*
FFT	*Established	Difficult to quantify
	Clear visual information	Many cycles for accuracy†
	Many spectral components	Time-varying data difficult to follow
	Fast	
FWT	*Simple algorithm	Many cycles
	Suitable for microprocessors	Spurious high-frequency components†
	Filtering easy	Better on discrete signals
	Good for frequency	No good for amplitude
AR modelling	*Few cycles only	Complex algorithm
	Smaller number of data points	Pre-filtering may be needed
	*Easy quantification	Convergence not guaranteed†
	Simple significance indication	
	Multiple components	
Phase-lock loop	Simple analogue hardware	One component only†
	Variance estimation	Harmonic locking
	*Fast estimation	
Raster scan	Simple analogue hardware	No direct read-out†
	*Time variations easily seen	Noise difficulty
		Single component only

* Indicates main advantage
† Indicates main disadvantage

Acknowledgements

All the methods described in this paper have been applied to data obtained in conjunction with Professor H. L. Duthie of the University Department of Surgery, Sheffield. The Walsh transform approach was developed in conjunction with Dr Z. B. Temel and the autoregressive method with Dr S. P. Datardina, both of the Department of Control Engineering. The phase-lock loop system is due to Dr R. H. Smallwood and the raster scan approach to Dr B. H. Brown, both of the Department of Medical Physics.

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Discussion

W. J. Snape: (USA) (1) If there are two fundamental frequencies of slow waves in the colon which have similar frequencies to a fundamental and second harmonic, how can one differentiate the two possibilities using the FFT? Can one do this by evaluating the power at each frequency? (2) Are smoothing windows (Hanning or Ham window) of use in FFT programmes? (3) When you determined the frequency of your slow waves you used the highest peak as the absolute frequency. Since the peak is a point that has been calculated by the FFT programme, would it not be better to use a statistical method encompassing all the points in the frequency distribution as the frequency determination?

D. Linkens: (UK) (1) Visual examination of the data can easily distinguish these cases. Harmonic content is indicated by a fixed wave shape, whereas two rhythms with approximately 2:1 ratio will show continual changes in wave-shape. Using FFT analysis the cases can be distinguished if many cycles of data are available. (2) Smoothing windows reduce the 'spreading' of spectral components caused by having short stretches of data. They also degrade frequency discrimination which in gut analysis is a major disadvantage. (3) Various methods of spectral smoothing can be used to reduce random fluctuations in the height of the components. These methods also degrade frequency discrimination and are not recommended for signals which give a stable frequency and/or amplitude for only a few cycles of data.

26

Frequency analysis of electrical activity in dog colon

K. L. BOWES, N. L. SHEARIN, Y. J. KINGMA AND Z. J. KOLES

In vivo electrical activity of the colon has been studied in a variety of species by several authors[1–4]. In all such studies electrical control activity (slow waves, pacesetter potential) has been noted to be present during only a portion of the recording time, to consist of two frequencies and to be marked by varying degrees of irregularity. These findings are in striking contrast to studies of electrical control activity in the stomach and the small bowel where electrical control activity is omnipresent, exists essentially as only one frequency and is usually regular[5,6]. Our studies suggest that, as in the upper gastrointestinal tract, electrical control activity (ECA) in the dog colon is ubiquitous, relatively regular and consists essentially of only one frequency. The higher frequencies observed visually appear to be due to the recording electrodes picking up multiple, loosely coupled oscillators with each oscillating at the basic frequency.

METHODS

In vivo studies

Three to eight sets of platinum-irridium bipolar electrodes were inserted subserosally at operation into the colon of eight healthy dogs (10–20 kg) using sodium pentobarbital anaesthesia. Each bipolar electrode consisted of two electrodes (5 × 0.4 mm) 5 mm apart. The electrode leads were brought to a type 1910 Amphenol socket mounted in a stainless steel cannula that was implanted into the anterior abdominal wall[7]. In five dogs, one to three extraluminal strain gauge force transducers were sutured onto the serosa of the colon[8]. Colonic electrical and mechanical activity were recorded for up to 2

months following operation. Recordings were made under fasting (54 h) and post-prandial (37 h) conditions. In an additional twenty-one experiments a 1 h recording was made after the intramuscular injection of prostigmine (0.05 mg/kg).

Fifty-eight strips of colon (2 × 5–8 cm) were obtained at operation from healthy dogs. Strips were taken from all portions of the colon, both longitudinal and circular directions. The mucosa was removed and each strip placed in a tissue bath (38 °C) and irrigated with oxygenated (95% O_2; 5% CO_2) Krebs Ringer solution at 5–7 ml/min. Five to eight monopolar silver–silver chloride glass pore electrodes spaced 1 cm apart were mounted in the top of the tissue bath[9,10]. A large silver electrode in the tissue bath served as the neutral electrode.

The electrodes were gently pressed onto the exposed circular muscle side of the colon strip until a satisfactory electrical record was obtained. In a separate group of experiments, recordings of isolated strips of colon muscle were made using bipolar and large monopolar electrodes. The bipolar electrodes were identical to those used *in vivo* and were inserted subserosally or into the circular muscle layer. The monopolar electrodes (6.4 × 5 mm) were introduced directly into the circular muscle.

Recordings

All recordings were made on a Dynagraph R411 polygraph and stored on magnetic tape. The filters were set to give a time constant of 1 s and a high-frequency cutoff at 32 Hz.

In vivo–in vitro studies

At operation a fine (0.075 mm diameter) Teflon-coated silver wire, of which 1 mm was exposed, was inserted into the longitudinal muscle of dog colon. In these experiments only the high-frequency cutoff was set at 0.3 Hz. The tissue was transplanted into the *in vitro* chamber and recordings made.

Power spectral analysis

The analogue records, usually about 30 min long, were digitized using a Hewlett Packard 2109 computer. Samples were collected from appropriately prefiltered recordings by the computer at the rate of 2.5/s and Fourier transformed in blocks of 256 consecutive samples. Approximately fifteen transformed segments resulted from this procedure for each recording. Subsequent to their calculation, transformed segments were averaged to produce a raw spectrum for the recording. The smoothed spectrum was derived from the raw spectrum by four passes of a 0.25, 0.5, 0.25 moving average filter applied to the coefficients of the raw spectrum. Smoothed spectra were plotted on an LA 36 DEC writer II terminal and the dominant frequencies determined.

Simulation of colon electrical activity

Noise-free signals exhibiting the basic wave-form obtained from *in vitro* experiments were used in simulation experiments. The sum of pairs of signals of this kind, with varying frequencies and varying degrees of phase-locking, were used to obtain the type of wave-form observed when one electrode would record both.

On a small digital computer, four relaxation oscillators (of slightly different frequencies), the bi-directional coupling between which could be adjusted arbitrarily, were simulated. Recordings were made of the individual and summed outputs of the simulated oscillators.

RESULTS

In vivo

In approximately half of the records obtained, no recognizable regular ECA frequency could be visually discerned. Such records were, however, never

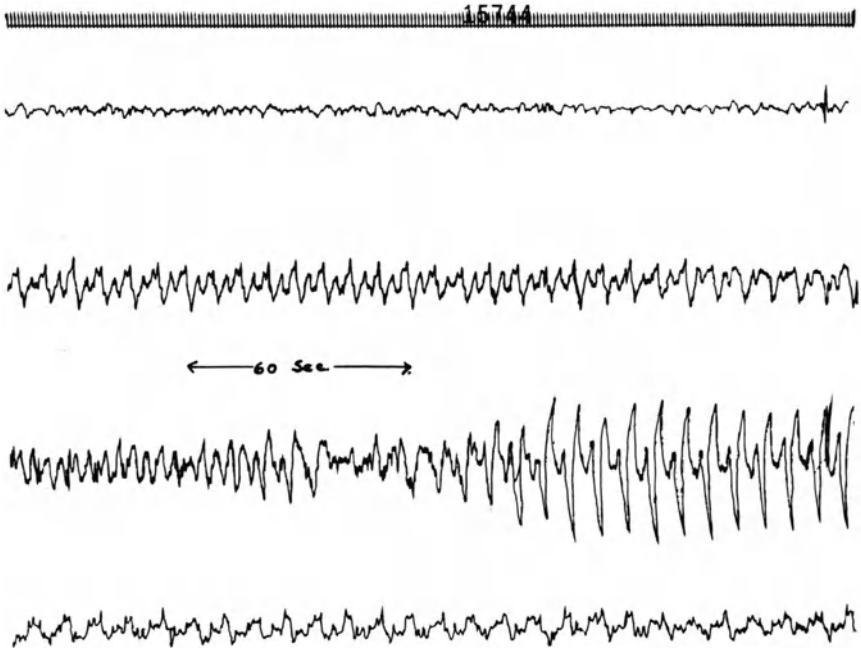

Figure 26.1 Recording *in vivo* from four bipolar electrodes 3 cm apart in the canine left colon. The top (first) channel displays a noisy record with no recognizable regular frequency. In the second channel is displayed a regular signal that could be interpreted as either 8 or 16/min. In the third channel a high frequency on the left gradually converts into the lower frequency of 8/min. A regular frequency of 8/min is present in the bottom (4th) channel

'silent'. A great deal of irregular 'noise' was present. When an apparently regular frequency could be recognized, it appeared to exist at a relatively high frequency of 10–16/min, and at a relatively low frequency of 4–8/min (Figures 26.1, 26.2). The higher frequencies were more common, being present in approximately 25–35% of all records; the lower frequencies were present in 15–20%. Very low frequencies (1/min) or very high frequencies (greater than 30/min) were rarely recognized. It was not uncommon to note one frequency at one site and the other frequency at a different site at the same time in the same animal. The same site often exhibited both frequencies at different times. On some occasions a change from one frequency to the other could be noted. A high frequency would change to a lower one by the fusing of two adjacent waves to produce a larger wave that appeared at half the earlier frequency (Figure 26.2). The converse could also occur. In many records both frequencies could be recognized simultaneously at the same location.

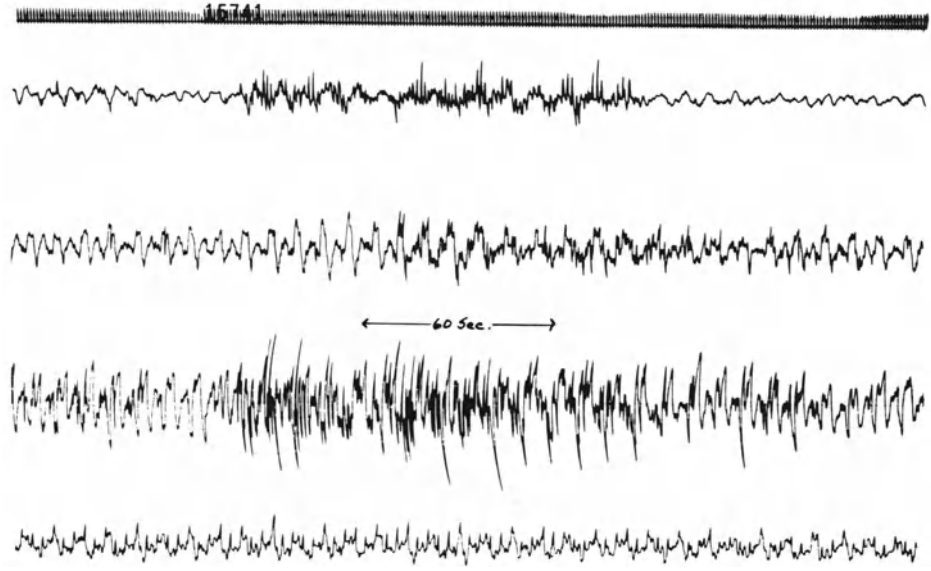

Figure 26.2 Recording *in vivo* from four bipolar electrodes 3 cm apart in the canine left colon. Response activity appearing at any time in the ECA wave-cycle can be seen in channels 1 and 3. Channel 2 demonstrates the conversion from a high frequency to a lower one

Electrical response activity (ERA) was observed both spontaneously and after stimulation by a meal or prostigmine. Frequently, ERA appeared at any time during the control wave cycle. At times virtually continuous spiking could be noted. Intermittent bursts of spike activity could also be recognized. The frequency of such bursts was highly variable but at times appeared at up to 50/min (Figure 26.2).

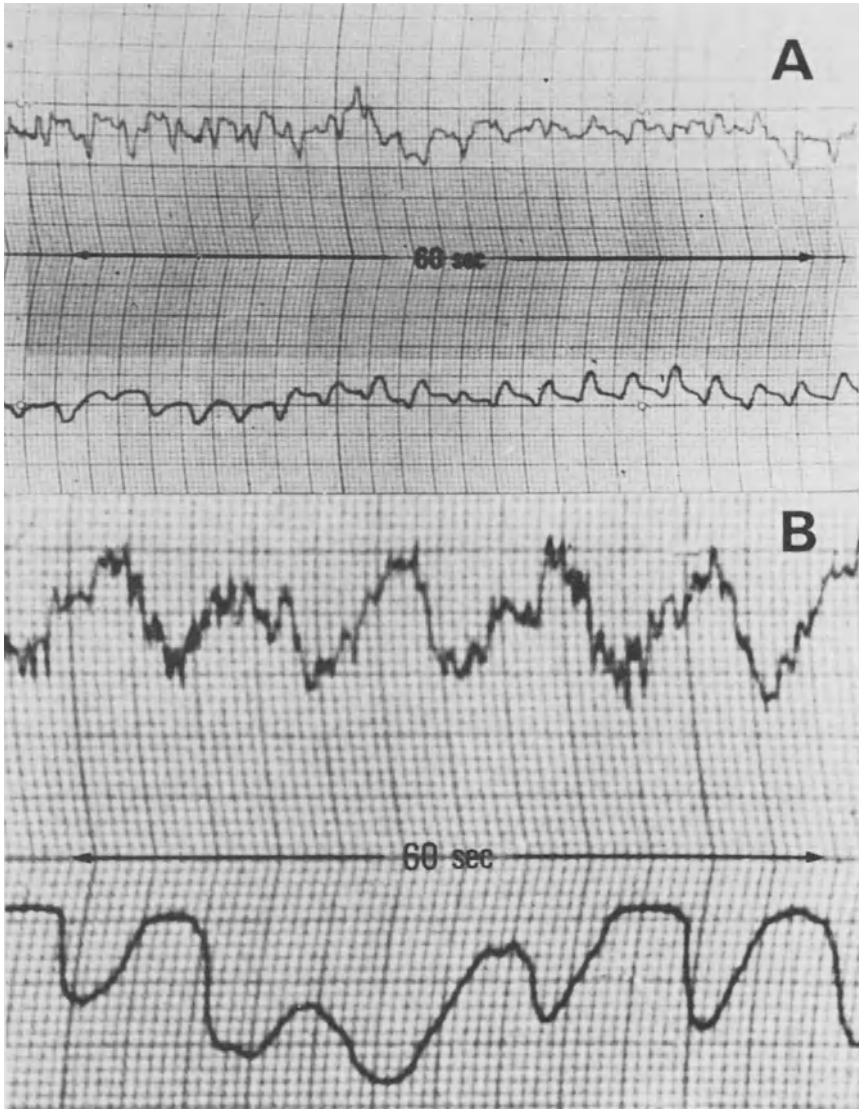

Figure 26.3 *In vivo* electrical and mechanical activity in the colon: (a) No recognizable regular electrical frequency is present in the top channel; mechanical activity in the bottom channel at the same site demonstrates contractions at appproximately 16/min; (b) Electrical (top) and mechanical activity (bottom) at 5/min

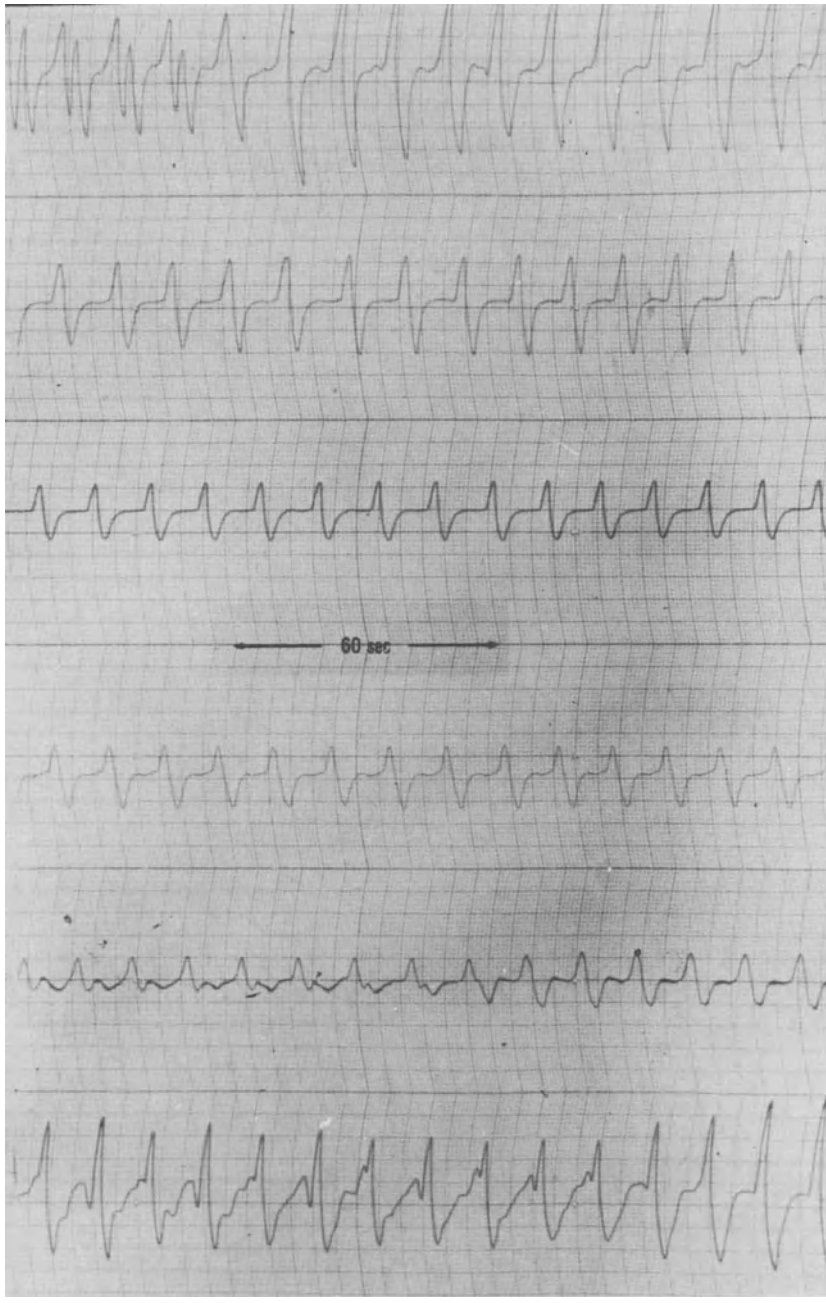

Figure 26.4 Electrical activity *in vitro* (glass-pore electrodes): a regular, easily recognizable frequency of 5/min is present

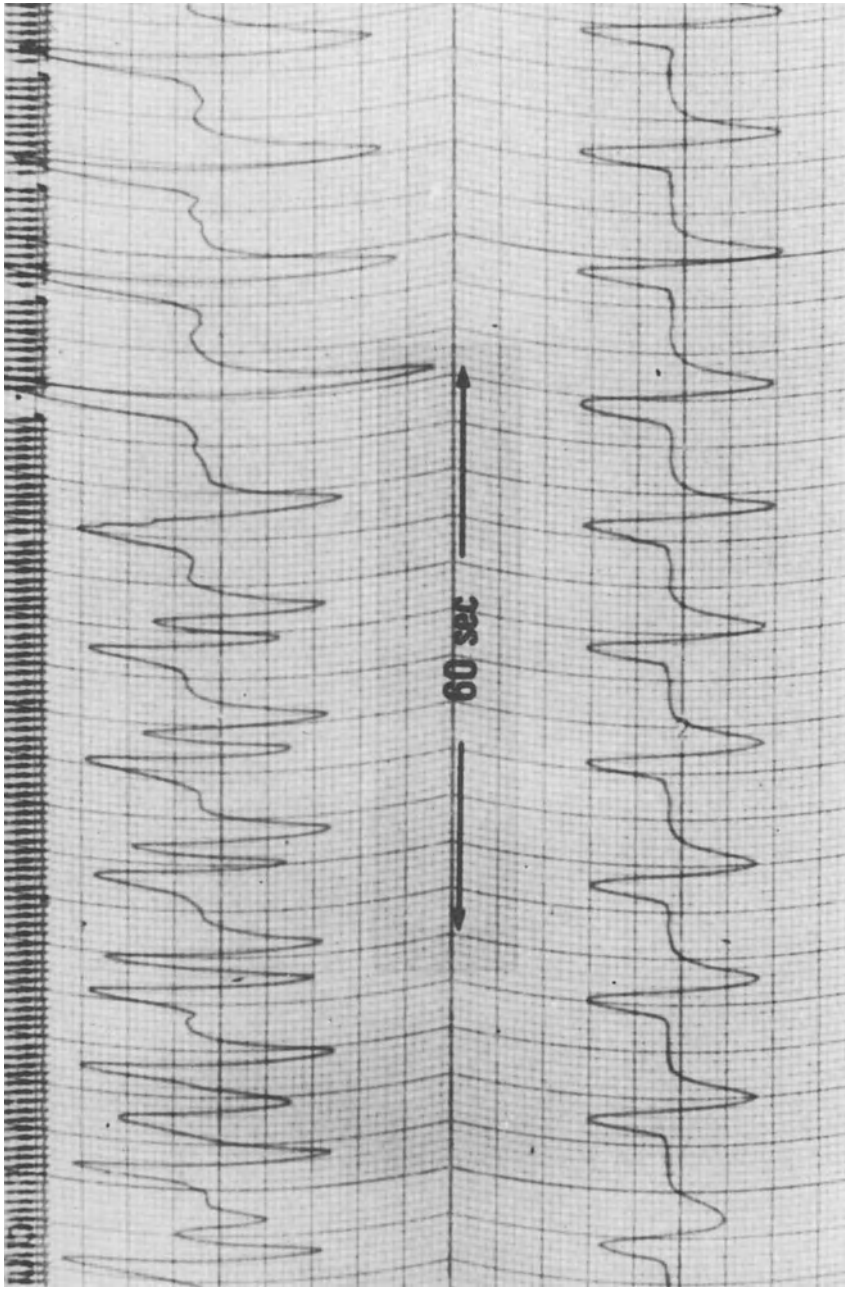

Figure 26.5 Conversion of a high frequency to a lower frequency, more 'ideal', signal by apparent fusion of two high-frequency signals

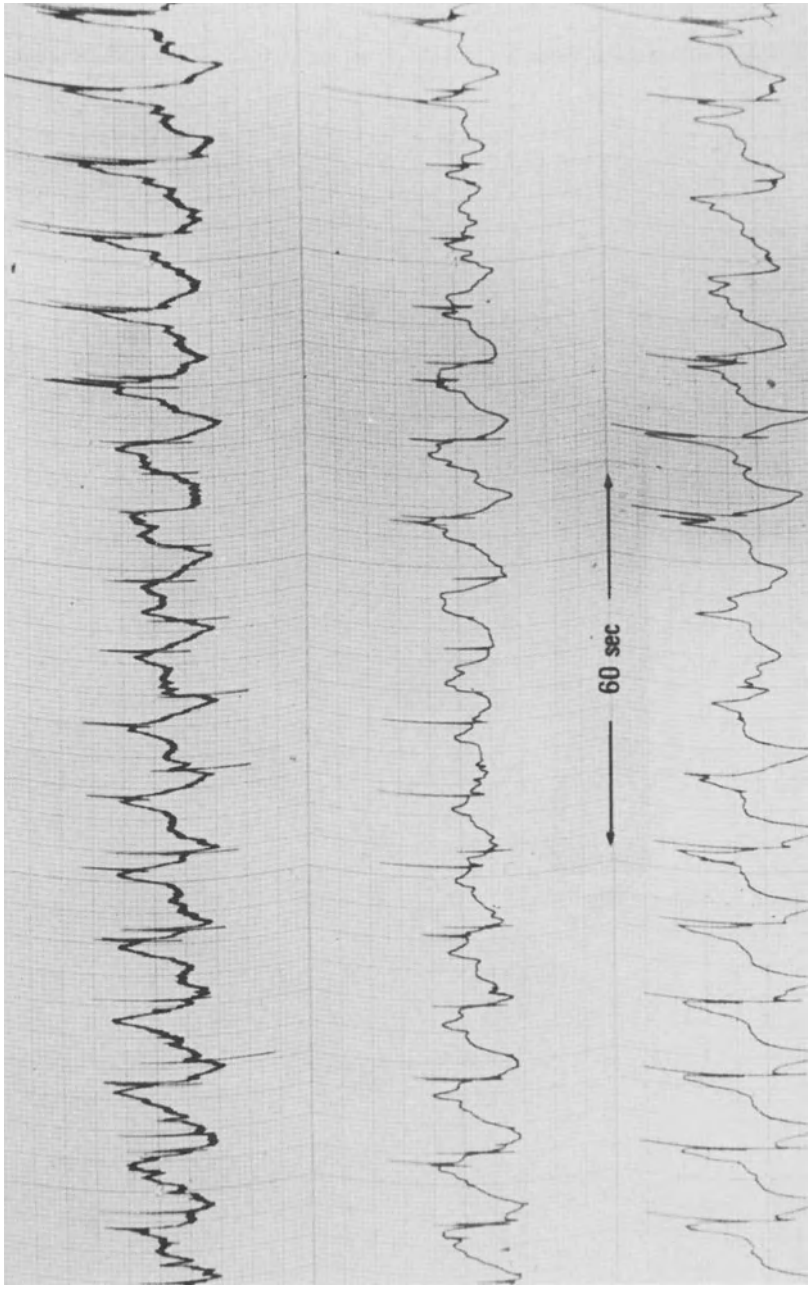

Figure 26.6 Electrical activity *in vitro* (glass-pore electrodes): ERA appears anywhere on the small, poorly defined ECA cycle but appears near the point of maximal amplitude of larger, better-defined ECA cycles

Contractions of the colon, as monitored by the strain gauge transducers, were noted at both a high (10–16/min) and low (4–8/min) frequency. Both frequencies of contractions were noted occasionally during the basal state (Figure 26.3). When the colon was stimulated with prostigmine and contracting strongly the lower frequency (4–8/min) was noted in virtually all records. The higher frequency was observed in less than 1% of records taken during stimulation of the colon. High frequencies of contractions only appeared as low-amplitude contractions when the colon was relatively quiescent.

In vitro

Virtually all records obtained *in vitro* demonstrated an ECA frequency of 4–7/min (Figure 26.4); only rarely were higher frequencies noted. Transition of the higher frequency into the lower more common frequency could be discerned. When this occurred, it appeared to be due to the melding of two of the faster waves together to form a larger wave at the lower frequency (Figure 26.5).

ERA appeared both spontaneously and after stimulation with prostigmine. When the wave-form approached its maximal amplitude and more ideal form, ERA appeared at the peak of the wave. If the wave deteriorated and became more complex, spikes could be noted distributed along the control wave, and in some such records spikes could be noted at any point in the cycle (Figure 26.6).

The excellent record obtained with monopolar electrodes is demonstrated in Figure 26.7a. A marked deterioration in the signal was noted when bipolar or large monopolar electrodes were used (Figure 26.7b). Apparent higher frequencies could be noted. A return to the original recording method using pore electrodes once again gave the lower frequency an excellent wave-form (Figure 26.7c).

In vivo–In vitro studies

A low frequency of approximately 5/min was observed in the *in vivo* colon. When the colon was transposed to the *in vitro* situation and records made, using the same electrode, an almost identical frequency was obtained (Figure 26.8).

Spectral analysis

In both *in vivo* and *in vitro* records, the computer analysis revealed a single fundamental frequency at approximately 4–7/min (Figures 26.9, 26.10). Harmonics of this frequency were seen in both types of records. Higher frequencies were noted in the power spectra only as multiples of the lower frequency.

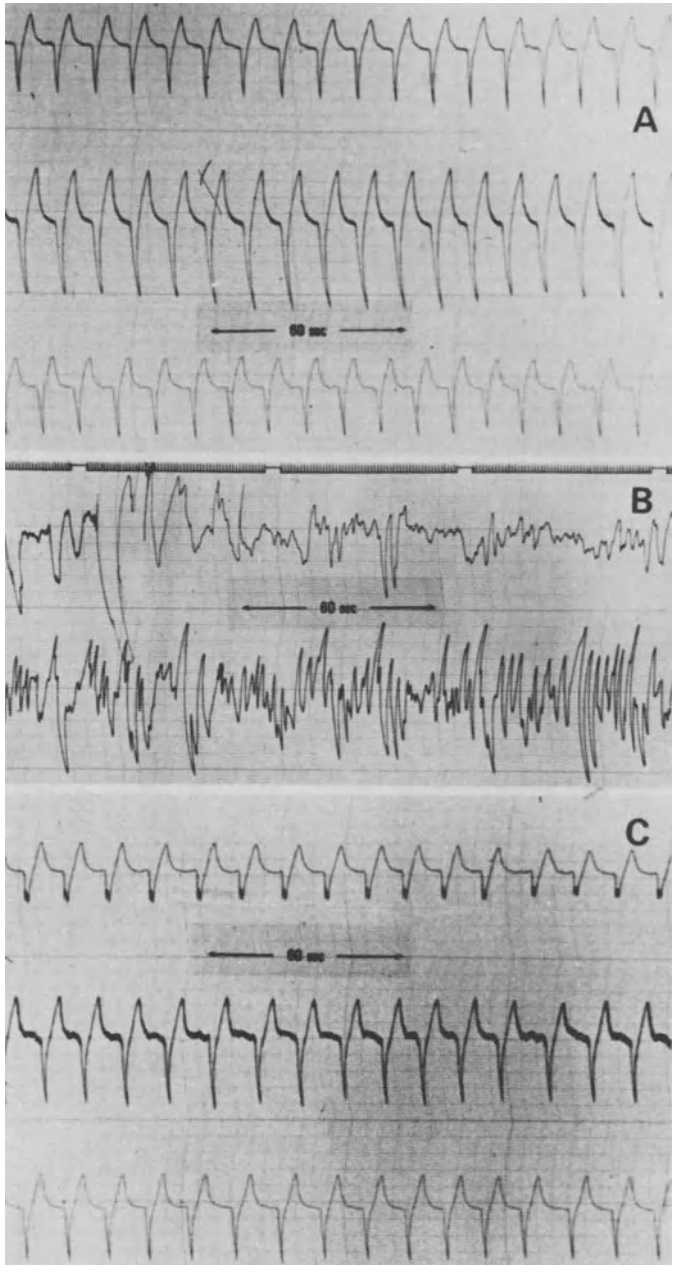

Figure 26.7 *In vitro* electrical activity recordings from colon strip using: (a) glass-pore electrodes; (b) bipolar electrodes (top) and monopolar electrodes (bottom); (c) glass-pore electrodes

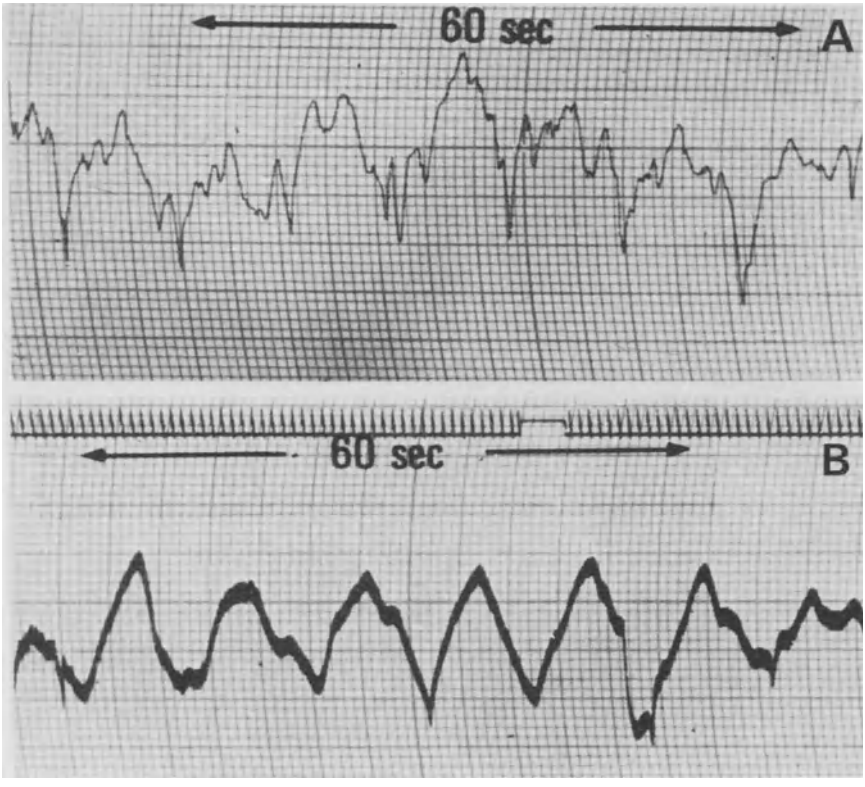

Figure 26.8 *In vivo* and *in vitro* electrical activity record obtained from the same specimen of colon using a silver electrode with 1 mm tissue contact: (a) *in vivo* at operation and (b) *in vitro*

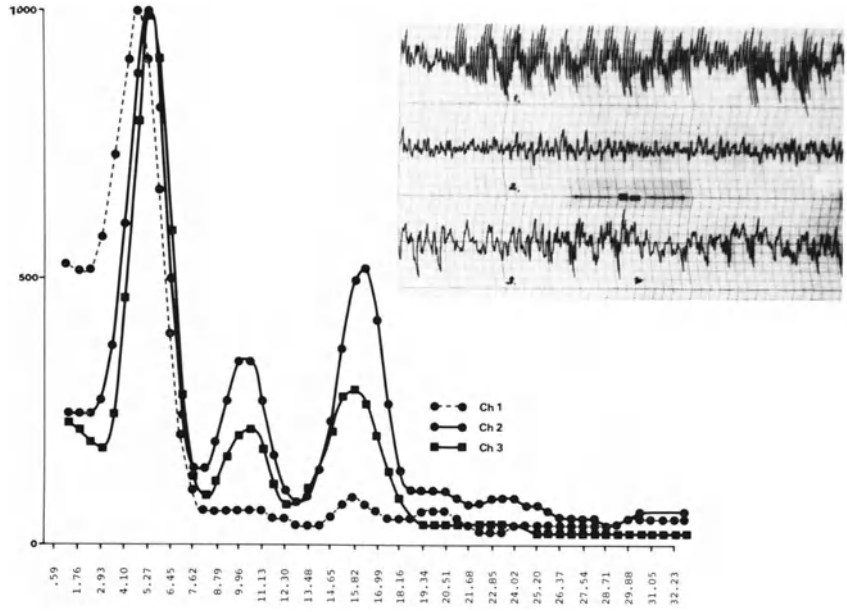

Figure 26.9 Power spectrum of colonic electrical activity *in vivo*. A fundamental peak of approximately 5/min with subsequent peaks at multiples of this frequency are seen

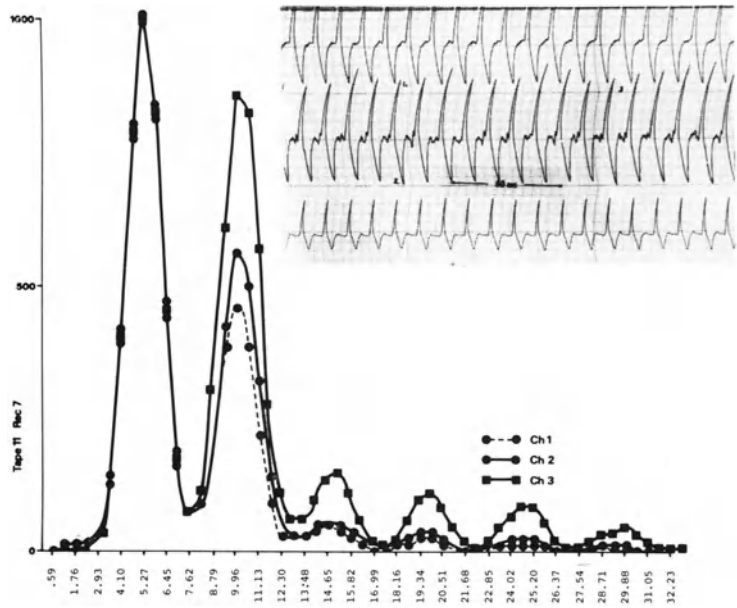

Figure 26.10 Power spectrum of colonic electrical activity *in vitro*. A fundamental peak of approximately 5/min with subsequent peaks at multiples of this frequency are seen

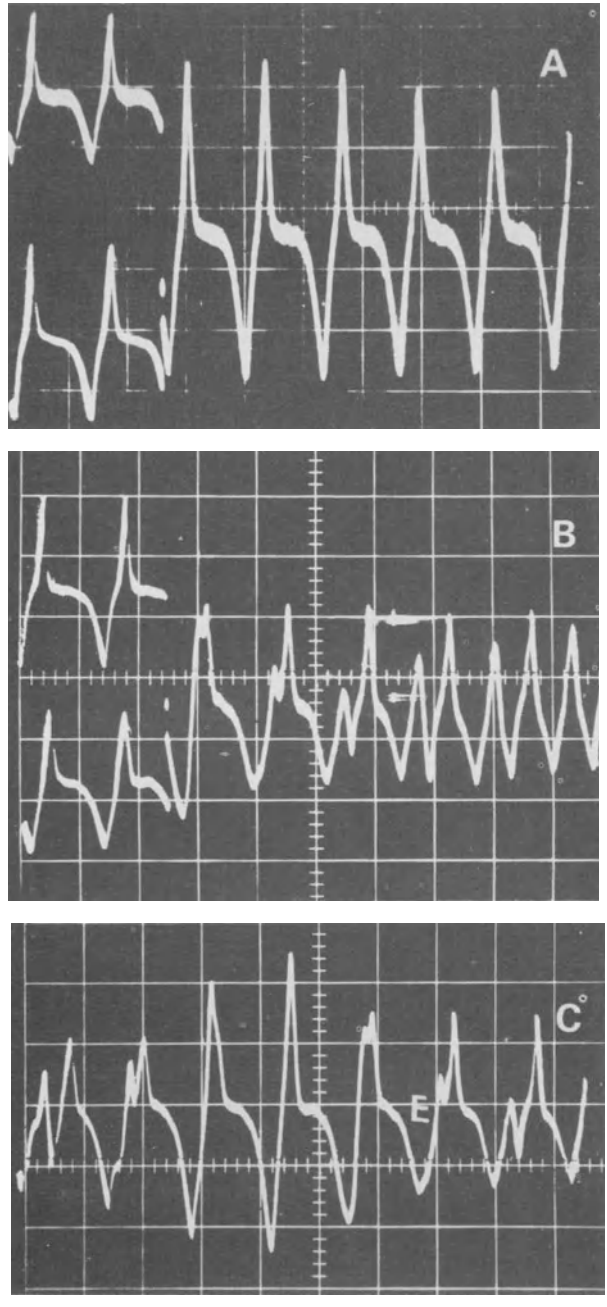

Figure 26.11 Analogue summation of pairs of *in vitro* wave-forms: (A) same frequency (zero phase shift); (B) different frequency (20%); (C) slightly different frequency (5%)

Simulation of colon electrical activity

Wave-forms similar to the higher frequencies recorded *in vitro* were synthesized by taking the sum of pairs of signals of the basic wave-form obtained in the *in vitro* experiments (Figure 26.11).

When four oscillator signals were summed on the computer without coupling, the sum of the signals appeared to contain frequency components much higher than those of the contributing signals (Figure 26.12). When the

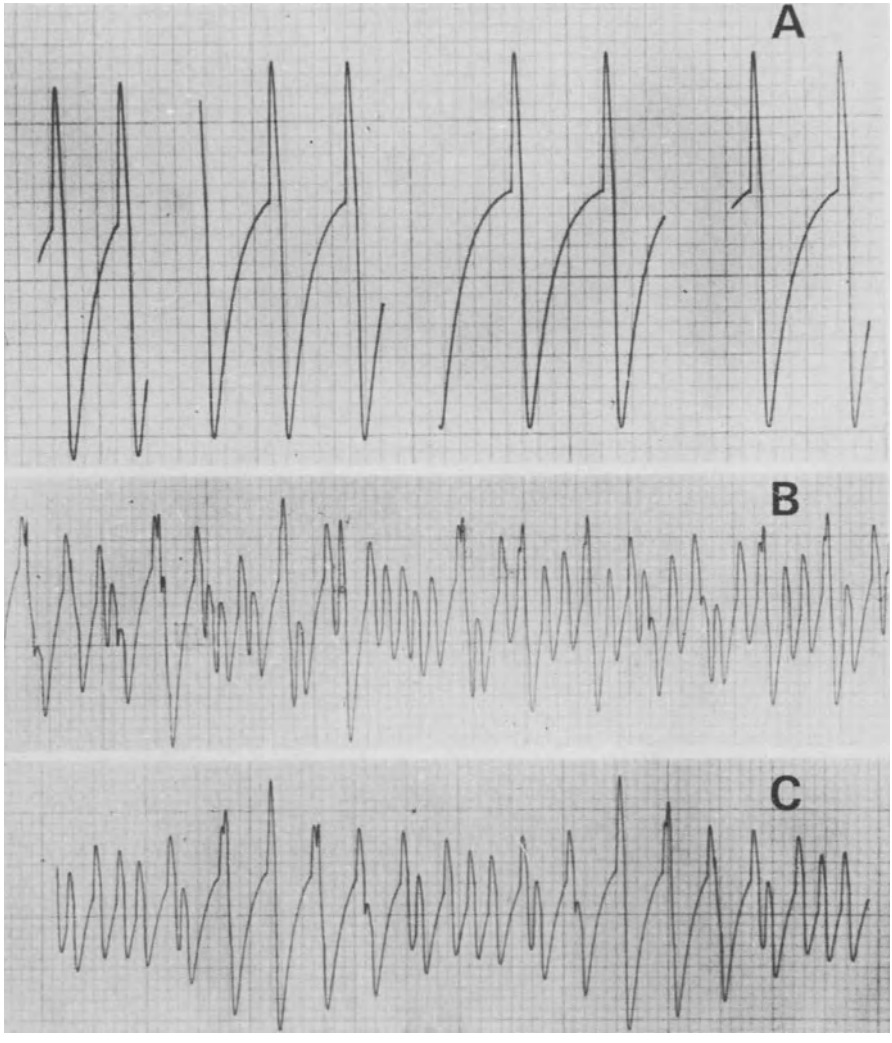

Figure 26.12 Simulation of record obtained by four oscillators: (A) of four different frequencies; (B) slight coupling between oscillators; (C) no coupling between oscillators

coupling was gradually increased, the sum signal would show increasing intervals during which phase-locking would occur. An apparent waxing and waning effect could be observed when the coupling factor was small (Figure 26.13).

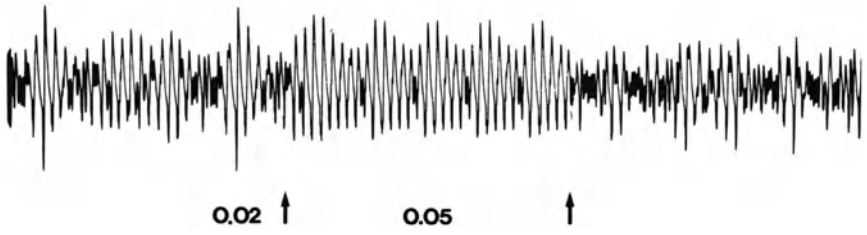

Figure 26.13 Simulation of record obtained by four oscillators using varied coupling between oscillators

DISCUSSION

Our visual analysis of canine colon electrical activity presented results similar to those reported by several other investigators in a variety of species[1–4]. Recognizable colon electrical control activity was present only part of the recording time, and different frequencies could be observed in different locations at the same time or at the same site at different times. It is possible that either two relaxation oscillators of markedly different frequencies could be responsible for such findings. Linkens *et al.* have demonstrated, by mathematical modelling of human colorectal myoelectrical activity, that two different frequency oscillators are not necessary to produce two frequencies[11].

Three observations that we made from our records support the concept of an essentially single-frequency oscillator. First, a completely silent record was never observed. When a recognizable frequency was not present, a great deal of apparent 'noise' was observed. The second observation was the apparent presence of two frequencies simultaneously. Finally, it was evident on many occasions that a higher frequency could gradually convert to a slower one by melding of two adjacent waves into a larger wave at a lower frequency. It seemed to us that the simplest explanation for such findings would be the simultaneous recording (by each recording electrode) of multiple oscillators having approximately the same frequency, with a variable degree of coupling between them. Our further studies support this concept.

If ECA in the colon is important in determining mechanical events, the latter would be expected to appear at the same frequency as the ECA when maximal stimulation is present. Certainly in the stomach and small bowel the maximum contraction frequency approaches that of the ECA. We therefore looked at ERA and mechanical activity *in vivo*. ERA appeared to bear little or no relationship either to the 'fast' or 'slow' electrical control activity wave. When intermittent bursts of response activity were seen they were noted at

frequencies up to 40–50/min, a frequency of ECA that we had never noted. We concluded that these findings indicated either that ERA was not related to ECA or that the ERA we were recording came from multiple, poorly coupled oscillators.

In an attempt to clarify this issue we looked at mechanical activity as recorded by serosal strain gauges. Both high- and low-frequency contractions were noted. All high-frequency contractions, however, were of very low amplitude. When the colon was maximally stimulated, the low-frequency component was observed in virtually all records. The strain gauges could easily cover several poorly coupled oscillators, each of which could control independent contractions. During strong contractions, coupling should be maximal and the frequency seen should be that of the fundamental ECA. This suggests that the low-frequency ECA is the only important one in the genesis of contractions.

Contrasting greatly to the *in vivo* studies were our *in vitro* recordings. ECA was omnipresent and almost always at the lower frequency. Only rarely were higher frequencies present and, when noted, the characteristic melding of wave-forms to produce a larger wave seen *in vivo* was noted even more clearly *in vitro*. Electrical response activity usually appeared once each cycle and at the peak of each control wave. There appeared to be a characteristic pattern of positioning of electrical response activity on electrical control waves. When the wave-form was distorted or present at a higher frequency, ERA appeared randomly on the slow wave. When the ECA had its most characteristic form and was of maximal amplitude, ERA was located at the time of maximal slow-wave amplitude.

One major difference in the techniques of recording *in vivo* and *in vitro* was in the area of contact of the electrode with the smooth muscle. Our *in vitro* recordings had a very small area of contact, whereas our *in vivo* recordings had a relatively large area. With small contact areas fewer oscillators would be 'seen' and thus a purer record obtained. We tested this concept by comparing the record obtained *in vitro* with our standard technique to that obtained *in vitro* by using bipolar electrodes similar to those used *in vivo* and large monopolar electrodes. In both instances marked deterioration of the record was observed, and records similar to those noted *in vivo* were obtained.

Is it possible that the higher electrical frequencies are actually present *in vivo* and transformation to the *in vitro* situation with separation from the remainder of the colonic musculature and nerves results in their absence? On the digital computer, the power spectra of our *in vivo* and *in vitro* signals were calculated. In both cases, peaks appeared at high and low frequencies. The high-frequency peaks were always present as a multiple of the lower-frequency peak, and we believe they represent harmonics due to the non-sinusoidal wave forms. Our *in vivo–in vitro* studies also indicated that essentially the same frequency is present in both situations.

In order to confirm our hypothesis that the electrodes commonly used for

obtaining electrical signals from the colon often register the signals from multiple, poorly coupled oscillators we have used two approaches. First we have taken noise-free signals exhibiting the basic wave-form obtained from *in vitro* experiments. Taking the sum of pairs of signals of this kind (whether phase-locked or poorly coupled) we could synthesize wave-forms similar to those recorded by electrodes that apparently register multiple frequencies.

Secondly, we have simulated on a small digital computer four relaxation oscillators (of different frequencies) the bi-directional coupling between which could be adjusted arbitrarily. Taking the sum of the four simulated oscillators signals we observed that without coupling the sum of the signals appeared to contain frequency components much higher than those of the contributing signals. When the coupling was gradually increased the sum signal would show increasing intervals during which phase-locking would occur. When the coupling was slight an apparent waxing and waning effect could be observed.

The experiments reported in this paper indicated that canine colonic ECA is always present at only one frequency for any given site. Unlike the upper gastrointestinal tract where coupling is tight, coupling is frequently poor in the colon. Recording electrodes that are in contact with a large area of colonic

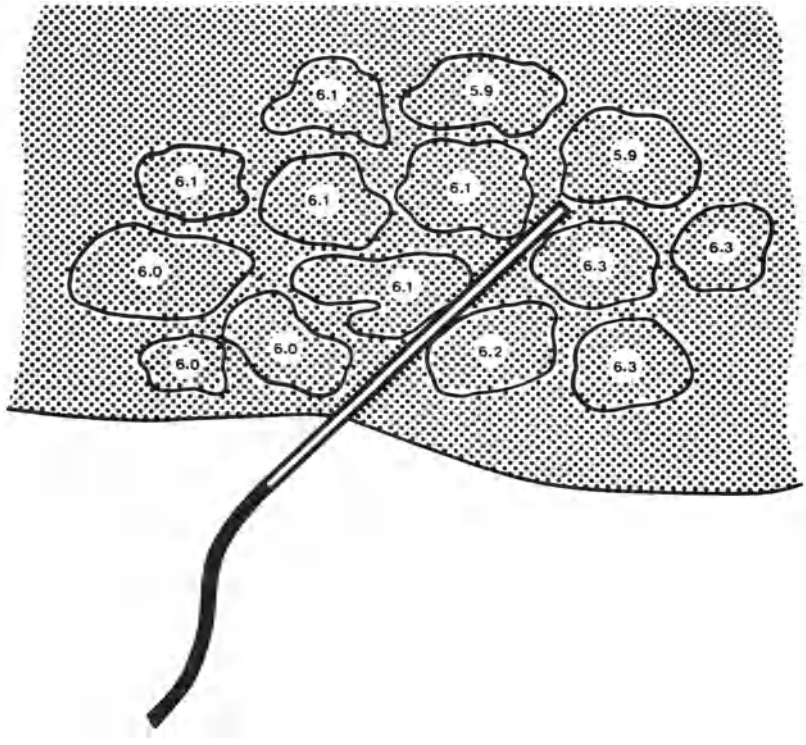

Figure 26.14 Concept of poorly coupled oscillators. One large electrode would be in contact with multiple, poorly coupled oscillators each of approximately the same frequency

musculature record events from several oscillators (Figure 26.14). In the absence of tight coupling, a record characterized either by apparent high frequencies or 'noise' and the absence of any recognizable regular frequency will be obtained. Tight coupling would be necessary for any significant contractile activity, and under these circumstances recordings would be more likely to reflect the true frequency. The increased incidence of the lower frequencies in patients with spastic colon may be explained on this basis[3].

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Discussion

D. L. Wingate: (UK) The techniques for deducing ECA frequency from the wave-forms are complex, as are the wave-forms. In the small bowel we have found that analysis of inter-spike intervals demonstrates the ECA frequency very clearly. Could such a technique be used to determine the frequency of ECA activity in the colon, which still seems to be a matter of dispute?

K. L. Bowes: (Canada) Unfortunately we have not found such measurements to help. Spiking in the *in vivo* colon can appear to be continuous. At other times, when intermittent bursts are seen, the intervals are extremely variable, and frequencies determined by such intervals range from 1 to 50 min. We feel that the recording electrodes *in vivo* pick up activity from multiple, poorly coupled oscillators and thus the frequency of the spike bursts does not accurately reflect the true ECA frequency.

D. Linkens: (UK) If the concept of weakly coupled oscillators with drifting frequencies is correct then integer multiples of the basic rhythm of about 5 cpm should be preferred values. Have you seen, not only integer ratios of 2, but higher integers? If so, for what percentage of time are they present?

Bowes: They are present in almost all power spectral analyses done on *in vivo* and *in vitro* colon.

Y. Ruckebusch: (France) Have you found different patterns of electrical and/or mechanical activity of the canine colon related to its proximal and distal parts? Our recordings showed, on the proximal part, that each spike burst is associated with a transient increase in pressure. More distally, i.e. on the transverse colon, spike bursts occurred in series and the pressure changes are more sustained. On distal colon, still more marked coalescence of both the spike bursts and pressure exist. Do you find different frequencies related to the proximal or distal parts?

Bowes: We found only slightly different frequencies present in different parts of the colon. To date we have not observed the associations you describe.

27

Gastroduodenal coordination: a computer analysis *(Abstract)*

S. K. SARNA, R. KITAI, K. MUNIAPPAN, L. MARZIO, E. E. DANIEL AND W. E. WATERFALL

Four extraluminal strain gauges and four bipolar electrodes were surgically implanted, one set on the pylorus, one in the antrum (2–4 cm) and two on the duodenum (<2 cm and 8–10 cm) in six dogs, to record mechanical and electrical activities before feeding, after feeding and after drugs. All the records were analysed on a Nova 830 minicomputer for the frequency content of signals. The pylorus behaved primarily as an extension of the antrum in the sense that it had electrical and mechanical frequencies mostly in the range of 4–5 c/min; the same as antrum but with some phase-lag. It did not show any relaxation in response to feeding. Both the duodenal electrodes showed a frequency component at antral frequency both in fasting and fed states. Spontaneous contractions of the duodenum in the fasting state were at its inherent frequency of 18–20 c/min; after feeding, however, the contractions at this rate were either inhibited or reduced, and the duodenal mechanical activity showed a dominant frequency component at antral frequency. After feeding, the component of antral frequency in the duodenal electrical activity increased. Pentagastrin (4 μg/kg/h) induced a pattern similar to that after feeding, but it did not inhibit duodenal contractions at its inherent rate to the same extent as feeding. Antral frequencies were still present in the duodenal electrical records when atropine abolished all mechanical activity. Secretin (4 units/kg/h) only temporarily (<10 min) abolished or reduced the amplitude of contractions after a meal. When they reappeared, secretin enhanced the gastroduodenal coordination in the sense that a stronger component of antral frequency was present in duodenal mechanical activity. This study shows objectively that, during gastric emptying, duodenal contractions are coordinated with antral contractions. This coordination may involve both

the nerves crossing over from the antrum into the duodenum, and the interaction of relaxation oscillators across the pylorus – the nerves causing inhibition and antral oscillators driving the duodenal oscillators to cause excitation. Alternatively, the periodic turning off of inhibition timed by the antral control potentials or antral contractions (through ganglia) could result in duodenal contractions at antral rate. In the absence of this coordination, the high-frequency duodenal contractions may appear as an obstruction to antral emptying, and cause reflux. Inadequate coordination of antral–duodenal coordination during pentagastrin infusion and temporary inhibition of contractions by secretin may account for delays in gastric emptying of liquids reported by Cooke.

This study was supported by the Medical Research Council of Canada.

Discussion

M. Wienbeck: (W. Germany) (1) Your Fourier analysis looked to be much clearer than that of Dr Linkens; did you use a trick to get your clean frequency spectrum? (2) In some of your spectra there were many other frequencies besides those on which you commented; is it justifiable to neglect these other frequencies?

S. K. Sarna: (Canada) (1) It is just the method of representation. The Fourier analysis gives the strength of various frequencies at discrete intervals and we have represented these by vertical bars. (2) These other frequencies are present because of the limitations of the FFT method and because most biological signals show an appreciable variation with time. With some knowledge of the signal being analysed the relevant frequencies can easily be separated from spurious frequencies; e.g. in duodenal records one would be looking for frequency peaks close to the duodenal rate of 17–19/min and frequency peak at the critical rate which has been determined separately.

A. R. Cooke: (USA) Did your strain gauges cover only the pylorus or did they include the distal antrum, since they do cover a relatively large area?

Sarna: Our strain gauges were about 5–6 mm wide. The pylorus in the dog is about 1 cm in length. We believe our strain gauges were within this area. Sometimes an additional electrode was positioned just 2–3 mm distal to this strain gauge, and it recorded the duodenal pattern of electrical control activity.

C. F. Code: (USA) I was pleased to see that your computer analysis has displayed the same coordination between antral and duodenal bulb contractions after ingestion of meals as was previously reported by Allan Pool and Code, and Bedi and Code and agree that the coordination is accomplished by neural rather than smooth muscle connections between the two areas. It does seem to me that your data, despite your own interpretation, do indicate that the messages between antrum and duodenum are neural rather than pacesetter potential (PP) or smooth muscle in origin. We never did observe the coupling between the two areas – the duodenal PP showed a continually changing relationship to the antral PP, indicating that the antral PP was not driving the frequency of the duodenal PP.

Sarna: Our data show that both neural and myogenic systems are involved in bringing about the coordination. The neural systems inhibit duodenal contractions at its inherent rate, while its contractions that are coordinated with pyloric and antral contractions are brought about by the increase in excitability of duodenal muscle due to antral relaxation oscillators driving the duodenal relaxation oscillators. With your visual analysis you could not have picked up the effect of this driving in records of your duodenal electrical control activity. We found an antral component in duodenal records even when the dog was fasted and showed no gastric contractions, and after atropine.

M. A. Cook: (Canada) You showed changes in the degree to which the antral oscillators drive the duodenal oscillators, presumably reflecting changes in coupling between them. Are these changes under neural control and, if so, how does a transmitter exert this control?

Sarna: Most of the evidence in this study points out that the driving effect across the pylorus is myogenic in nature. It is definitely non-cholinergic, since it was present even after atropine.

G. Charbon: (Netherlands) You presented data from one dose of pentagastrin. Would the quality of the information be improved by using pentagastrin at different dose levels, thus establishing the dose-dependence of the effects you observed?

Sarna: We used the higher of the two doses that had been used by Allan Cooke since we wanted to elucidate the mechanism of slowing down of gastric emptying by pentagastrin which increases both the rate and the strength of gastric contractions. We agree with you that it will be useful to know the dose–response curve of pentagastrin.

A. G. Johnson: (UK) (1) In your recordings it appeared that when the antrum was quiescent there was sometimes pyloric activity which seemed to be related to duodenal activity. (2) Did you know if, and when, the pylorus closed completely?

Sarna: (1) Generally the pylorus contracted only at the antral rate. When the antrum was quiescent, the pylorus was also quiescent. In some cases, in one or two dogs, we did find that the duodenum could drive the pylorus. The frequency of pyloric contractions increased under these circumstances, but it was not the same as that of the duodenum. There was thus frequency pulling but no entrainment. (2) No, with our extraluminal strain gauges we cannot tell about the complete closure of the pylorus.

28

Computer analysis of electrical and mechanical activity of stomach, duodenum and caecum over long periods

E. HIESINGER, H. HOERNICKE AND H. J. EHRLEIN

The electrical and mechanical activity of the gastrointestinal tract can be measured by electrodes or transducers and can be transferred by filters and amplifiers as an analogue signal. In the cases of electrical control activity (ECA), of electrical response activity (ERA) and contractions only a few parameters are of interest. These are for example: time of occurrence, amplitude and duration as well as their mutual time-relationships. For experiments over long periods, the detection and evaluation of even these reduced data exceed the manual capacity without the aid of a computer. ECA, ERA and contractions must first be recognized. For this recognition, we use electronic circuits, which cause an interrupt in the computer. By this interrupt, a special circuit will be activated which detects and stores the necessary data. Subsequent analysis can then be done by conventional means of display, classification and statistical treatment.

METHODS

Detection of the electrical control activity (ECA)

When measuring with monopolar, extracellular electrodes, the ECA from dogs begins with a fast rise and stays at this high level (plateau-phase) for a certain time (Figure 28.1a). Using bipolar extracellular electrodes, the first derivative will be measured (Figure 28.1b). The initial rise can be easily recognized with a Schmitt trigger circuit. To avoid interpretation of artifacts as an ECA, we developed a circuit which rectifies the signal (Figure 28.1c) and inhibits further triggering over a certain time-interval after an ECA has

been detected (Figure 28.1d). In this way the detection of the ECA is independent of polarity, and most of the artifacts are eliminated. An interrupt-impulse (Figure 28.1e) indicates to the computer that a circuit is to be activated.

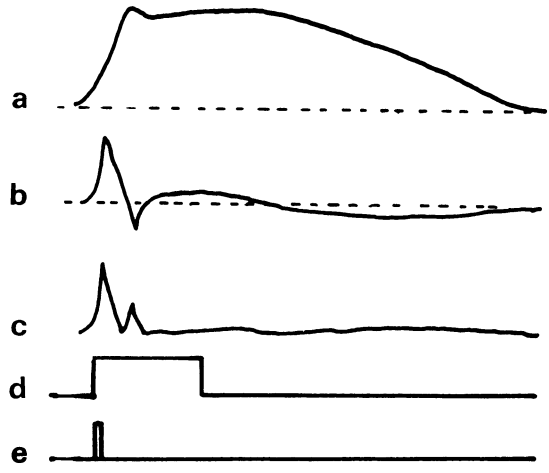

Figure 28.1 Detection of the ECA. (a) ECA monopolar; (b) ECA bipolar; (c) rectified bipolar ECA; (d) triggering inhibition; (e) interrupt impulse

Detection of the electrical response activity (ERA)

The ERA (Figure 28.2a) can occur during the plateau-phase of the ECA. If

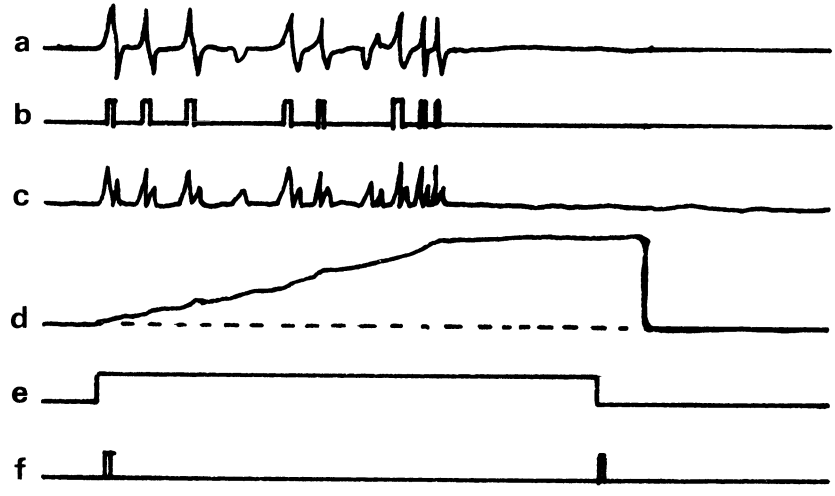

Figure 28.2 Detection of the ERA. (a) Filtered ERA; (b) Schmitt trigger impulses; (c) rectified ERA; (d) integration of the rectified ERA; (e) triggering inhibition; (f) interrupt impulses

every single spike is to be detected, a Schmitt trigger circuit can be used (Figure 28.2b). If, however, the ERA bursts should be treated as an entity, we use a similar circuit as for the detection of the ECA. The ERA (Figure 28.2a) will be rectified (Figure 28.2c) and integrated (Figure 28.2d). At the occurrence of the first spike, an interrupt impulse (Figure 28.2f) will be given to the computer. For a certain time-interval, the triggering of interrupts is inhibited. This time-interval will be prolonged with each spike (Figure 28.2e). The end of the inhibition time will be announced to the computer by a second interrupt impulse (Figure 28.2f). The integral of the spikes will be held for a certain time and will be reset to zero after the computer has accepted its analogue value. The time between the first and the second interrupt impulse represents the duration of the ERA plus the adjustable inhibition time.

Detection of contractions

Analogue signals from extraluminal strain gauges or intraluminal pressure transducers (Figure 28.3), are fed into so-called peak-picker circuits. The smallest voltage (U_{min}) between two contractions and the maximum voltage (U_{max})d uring the contraction are stored in two separate peak-detectors (peak-picker). The start of the contraction is recognized when an adjustable level is exceeded. The occurrence of the maximum is detected when the difference between the contraction signal and the stored peak signal is below a second level. In both cases an interrupt signal is produced which causes the computer to store the minimum and maximum voltage, and the time of occurrence. The smallest detectable amplitude is given by these two adjustable levels. This eliminates small fluctuations of the signal which have not been caused by contractions.

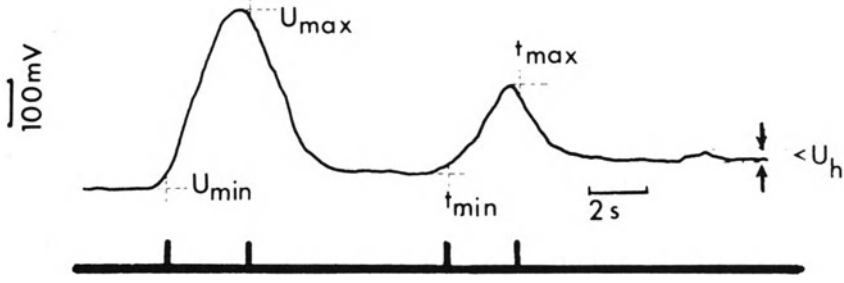

Figure 28.3 Contraction of the smooth muscle with interrupt impulses and stored maximum and minimum voltage

Evaluation by computer

For detection and evaluation we use a laboratory computer (Dietz Mincal 621) with real-time clock, interrupt input, analogue–digital converter, disc,

graphical display and printer. The electronic interfaces produce interrupts, which activate circuits. By these circuits the instant of the occurrence of the interrupts and the analogue data are stored. Obviously error signals are rejected by plausibility control programmes. The data are stored on a disc. Values to control the experiment are issued on printer or display. To be sure that no events are lost, the circuits for the detection of the events have the highest priority. For the evaluation of the stored data, modular software packages allow graphic display, classification and statistical analysis. This can be done either on a beat-to-beat basis or for given intervals (for example 15 min) within the analysis period (for example 24 h).

RESULTS OF ANIMAL EXPERIMENTS

Measurements were made with extraluminal chronically implanted strain gauges and bipolar Ag–AgCl electrodes. Electrodes and strain gauges were sewn to the caecum of three rabbits, to the antrum and gastroduodenal junction (GDJ) of another three rabbits, to the antrum and GDJ of two dogs and to the proximal duodenum of one of these dogs.

ECA in dogs

By examining the ECA variation after more than 12 h of starvation, it was found that:

(a) There are periods of up to 10 min with a slow variation of the ECA interval. The intervals were 10.3 ± 0.7 s at the stomach. During this time, no contractions occurred.
(b) In other periods a few contractions occurred, followed by a pause of about 1–3 min. The ECA intervals following the contractions were delayed to 16–20 s (Figure 28.4).
(c) The same delay was also recognized at the proximal duodenum, where the ECA interval was prolonged from 3.1 s to 3.3–3.5 s.
(d) Sometimes a tachygastria[5] occurred at the stomach with an ECA interval of 4.44 ± 0.02 s (Figure 28.5). These intervals were different from the duodenal ECA intervals of 3.1 s. During this ECA acceleration no contractions occurred.
(e) After feeding, when each ECA was followed by a contraction, the ECA was rather constant again, but at a higher level (12.1 ± 0.6 s at the stomach).
(f) Time course: the method allows the display of the time course of the ECA. This display shows the different phases as well as faulty measurements (Figure 28.6). Short tachygastria phases, which are normally not detected, can be seen by a reduction of the ECA interval. Errors can be determined by counting the ECA intervals, which are not in the region of

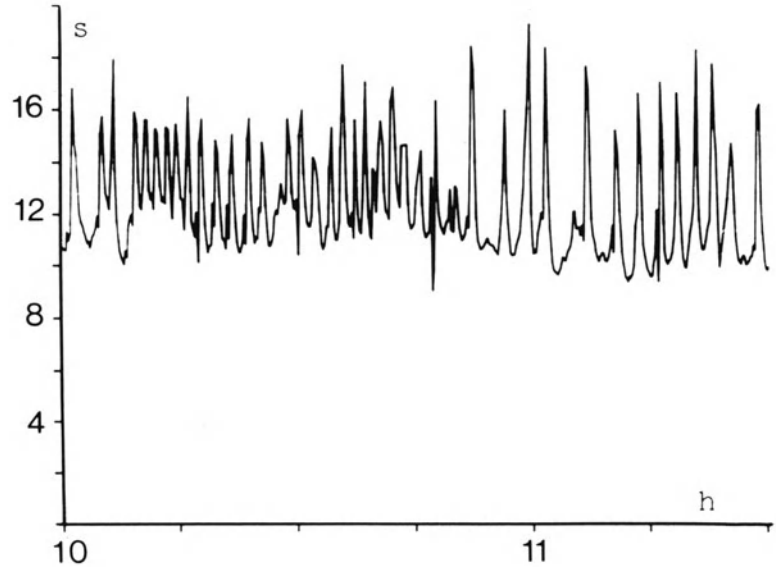

Figure 28.4 Prolongation of the ECA intervals when single contractions occur, measured at the antrum of a dog

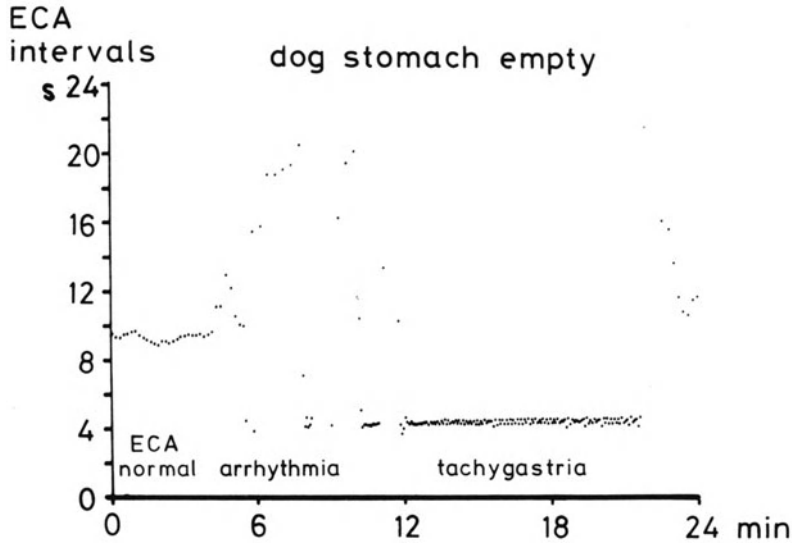

Figure 28.5 Tachygastria rhythm with an ECA interval of 4.44 s, measured at the antrum of a dog

9–20 s and which are not caused by tachygastria. Figure 28.6 shows the ECA over 8 h from 9.00 a.m. to 5.00 p.m. During this period, over 2500 ECA intervals were measured. The great variation of the ECA before feeding and the smaller variation after feeding (f) can be seen. A short tachygastria period (t) is present at 1.05 p.m., followed by a pause of 39 s. Twenty-four ECA intervals are either below 9 s (e) or over 20 s and were determined as errors. Thus, the error rate was about 1 per cent.

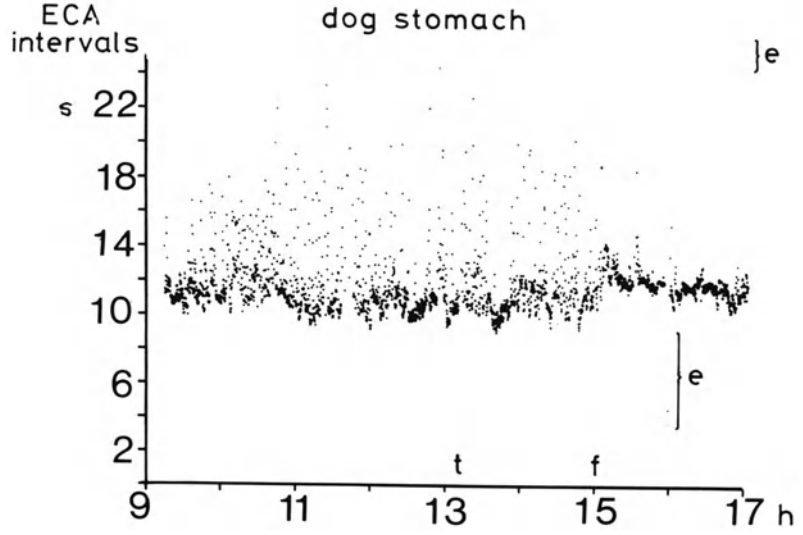

Figure 28.6 ECA intervals, measured at a dog's antrum from 9.00 a.m. to 5.00 p.m. (t) short tachygastria period at 1.05 p.m. (f) feeding of the dog at 3.00 p.m. (e) faulty measurement

Time-differences between events

Measuring the time-interval between the occurrence of the ECA at the antrum (2.5 cm before the GDJ) and at the GDJ, we found 0.7 ± 0.1 s. On the other hand the corresponding values for the contractions at the same points showed time-differences of several seconds with a very strong variation. Sometimes the contraction at the GDJ occurred before that at the antrum. This means that between the beginning of the ECA and the contraction, there is a highly variable latency period. This latency period measured at the antrum of a dog showed a mean value of 1.8 s with a variation of ± 0.9 s.

Relations between integral values of ERA bursts, amplitudes of the contractions, duration of the ERA bursts and duration of the contractions

A strong relation was recognized between the occurrence of the ERA and the occurrence of contractions. Only weak relationships were present between the

duration of the contractions and the duration of the ERA bursts, as well as between the integral value of the ERAs and the amplitude of the contractions. The duration of the ERA bursts and the durations of the contractions generally were shorter at the GDJ than at the antrum. For example the duration of the ERA bursts of rabbits, measured over 24 h, was 5.5 $\pm$ 1.5 s at the GDJ and 9 $\pm$ 1.5 s in the antrum.

Periodicity of contractions

Not every ECA is followed by a contraction; therefore the time-difference between two contractions is in accordance with the time difference between two, or several, ECAs. This can be well seen when plotting the distribution of

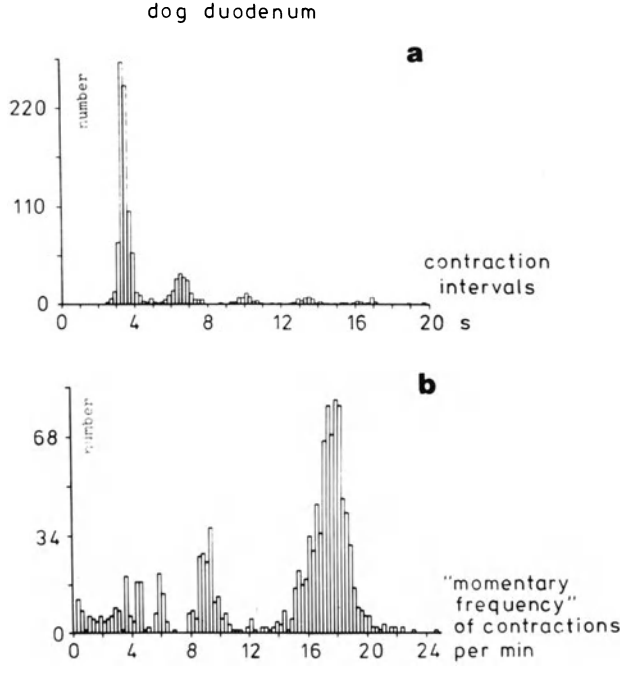

Figure 28.7 (a) Distribution of the intervals between two contractions, measured at a dog's proximal duodenum over 8 h. A basal rhythm of 3–3.5 s can be seen, which is in accordance with the ECA intervals. (b) Distribution of the 'momentary frequencies' per minute of the same data. The multiples of the basal rhythm are obscured

time differences between two contractions (Figure 28.7a). In plotting the distribution of the 'momentary frequencies' (Figure 28.7b), a maximum of 18/min appears, which corresponds to the maximum of 3.3 s in Figure 28.7a. But the equivalents of the smaller maxima shown in this Figure are hidden. The distribution of intervals between contractions provides an indirect

method for determining the ECA interval. Figure 28.8 shows such a distribution measured in the stomach of a rabbit. We can conclude that the ECA interval must be 13–16 s.

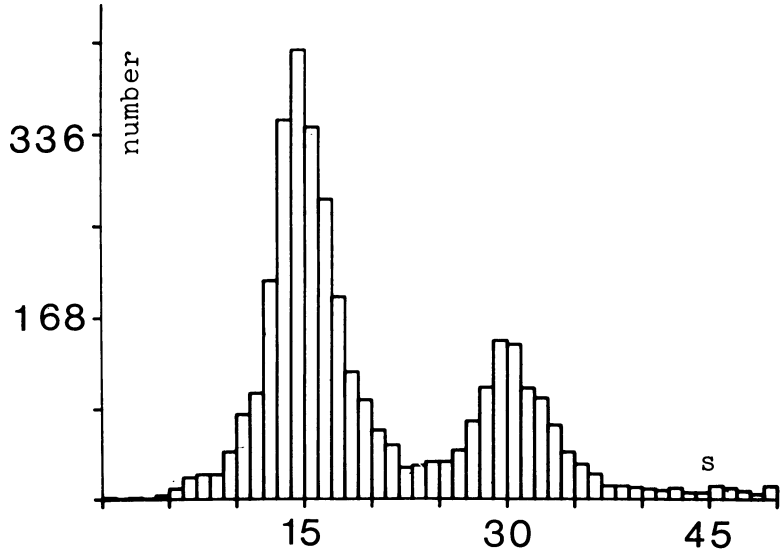

Figure 28.8 Distribution of the intervals between two contractions, measured at a rabbit's stomach over 48 h. A basal rhythm of 13–16 s can be seen

DISCUSSION

For analysing gastrointestinal motility records usually the number of electrical and mechanical events are counted and their relationships are visually evaluated. The following problems, among others, call for an automatic analysis:

1. The analysis should be reproducible, and independent of subjective criteria.
2. For long periods, the vast amount of data can only be analysed with the aid of a computer.
3. An exact analysis of time-differences by hand is only possible from traces taken at high paper speed, which makes analysis expensive. Nevertheless a reproducible determination of the beginning and the maximum of a contraction remains difficult.

The main problem in automatic analysis is the detection of the events. If the events must still be detected by a human operator and the essential parameters are given to the computer (for example by punched cards), no essential shortening of the analysis time or improvement of the reproducibility can be expected. If the computer has to detect the events directly from analogue signals, rapid processors and large data fields are needed. Therefore only short

intervals can be examined, or only a few parameters of only one analogue signal can be analysed. The system for detection and evaluation, which is presented here, is a combination of hardware and software, in which hardware interfaces make the routine work for detection. By this means the computer is relieved of unessential data. It has no velocity problems and can therefore be programmed in a higher programming language. Large data fields are not needed, and data from several transducers can be analysed over long periods. The use of this system is limited to the condition in which the interesting events can be separated by filters from other events or from disturbances. In rabbits, this method of measurement was successfully tested by analysing ERA and contractions of the stomach and the caecum. The ECA of the rabbit's stomach could not be analysed by our methods, because it is very weak, as in most herbivores[7]. Nevertheless the basal rhythm could be derived from the time-intervals between contractions. In dogs our method was useful for analysing contractions of the stomach and the duodenum, and for analysing the ECA of the stomach. In the duodenum of dogs, the frequency content of the ECA rise and of the ERA spikes are nearly the same. Therefore it is only possible to separate ECA and ERA by very steep filters. This has been effected by using analogue tape recorders[8] with high-speed replay.

The application of a threshold of the peak-picker gives reproducible criteria for contraction parameters. The measured and stored amplitude will be exactly the difference between the maximum and the minimum value. Baseline drifts are eliminated.

The great advantage of our method is its ability to treat each event (ECA, ERA or contraction) as a separate entity. This gives the opportunity to study properties of single contractions, as well as their time-relationships to other events. In this way it is possible to find hidden arrhythmias as, for example, short ECA tachygastrias or prolongation of ECA intervals after contractions. The greater accuracy of measurement, the greater reproducibility and the greater quantity of data lead to results that demonstrate the advantage of automatic event detection and computer evaluation.

Acknowledgements

This work was supported by grants (Ho 105/18 and 21) of the Deutsche Forschungsgemeinschaft. We are grateful to J. Becker, W. W. v. Maltzahn, H. W. Seitner and G. Tolkmitt for development and construction of the peak-picker.

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Discussion

S. K. Sarna: (Canada) This appears to be a useful method for long-term studies of ECA and ERA. In your present study you have applied this method to gastric and duodenal signals which are relatively noise-free. Have you used this technique on colonic signals which are noisy?

E. Hiesinger: (Germany) We have not used this method for measuring electrical activity of the colon, therefore I can only give a general statement. On the electrical signals, there are not only ECA and ERA, but there are also small fluctuations of the baseline, artifacts and noise. When analysing the frequency content of a signal through a Fourier transform, it can be shown that ECA and ERA have only a small spectrum. Using band-pass filters with a small bandwidth, most of these disturbances are eliminated. Because noise has a very wide spectrum, but ECA and ERA need only a small spectrum, in this small spectrum noise should not play such a dominant role.

D. L. Wingate: (UK) Your paper demonstrates the ease and simplicity of interval measurement but it depends, using the slow wave, on knowing at what point each slow wave begins. If the wave-form changes during the study, this may produce changes in apparent interval, which do not reflect real changes in frequency.

Hiesinger: Indeed, it is necessary to know with which accuracy the ECA intervals are measured, and to control this by visual inspection of the record. Because we use bipolar electrodes, the bipolar signal is maximal, when the first derivative of the ECA rise has its maximum. The trigger level is between the beginning of the ECA rise and the maximum of the first derivative. Then the maximal interval error is about this time, which is in the order of magnitude of a half-second at the stomach of a dog. Usually, the trigger level is very stable and these changes do not play a significant role for the interpretation of these experiments.

D. Linkens: (UK) In one of your computer analyses you indicate apparent cyclic variations in instantaneous frequency of large values. Is it possible that such changes are caused by wave-shape alterations and are therefore spurious?

Hiesinger: Your question probably concerns Figure 28.4, where we demonstrated that the ECA intervals are prolonged when single contractions occur. We recognized this prolongation in all our experiments from dogs, and we tested these plots also by visual inspection of the records with the same result. Changes in the ECA intervals, caused by spurious wave-shape alterations, cannot explain a prolongation of the ECA intervals from 10 s to more than 16 s at the stomach of a dog. They can only explain variations of about a half-second. That this prolongation occurs rhythmically corresponds to the rhythmical occurrence of a few contractions.

29

Real time analysis of colonic myoelectrical rhythms in disease

C. F. DARBY, P. HAMMOND AND I. TAYLOR

The irritable colon syndrome is a very common diagnosis in gastroenterological departments[1]. It is, however, a diagnosis which is made on the basis of exclusion of all other possible pathology. Unfortunately, to date, colonic motility studies do not appear to be sufficiently specific to be of value in the diagnosis of the irritable colon[2,3].

Recently, in an attempt to provide extra diagnostic information, a good deal of interest has centred on the myoelectrical changes in colonic smooth muscle, both in the normal[4] and pathological colon[5–7]. These normal and abnormal electrical patterns can, with some difficulty, be recognized and interpreted by eye. However, if they are to be utilized for routine clinical diagnostic and prognostic purposes some form of automatic data analysis is required.

One possible solution to this problem is the use of Fast Fourier analysis and the subsequent presentation of this information to the clinician in the form of a frequency histogram[8]. However, the use of a remote centralized computer, in general, requires that the slow-wave activity be recorded and then analysed retrospectively, so that results cannot be correlated directly with clinical observation at the time of recording.

The problem of analysing slow-wave activity in our gastroenterological department has largely been overcome by producing our own frequency spectrum analyser.

METHOD

The instrument is based on two Motorola M6800 microcomputers. The spectrum analyser produces an analysis in 1 min epochs of the electrical activity

recorded from intraluminal electrodes placed within the rectosigmoid region of the patient. The results of the analysis are available for immediate clinical assessment in the form of a frequency histogram displayed on a cathode ray oscilloscope.

Simultaneously, a recording of the slow-wave electrical activity and the resulting frequency analysis is also made on a chart recorder (Figure 29.1). This facility allows retrospective analysis of both the recorded slow-wave activity, and its frequency components, as well as providing long-term patient records.

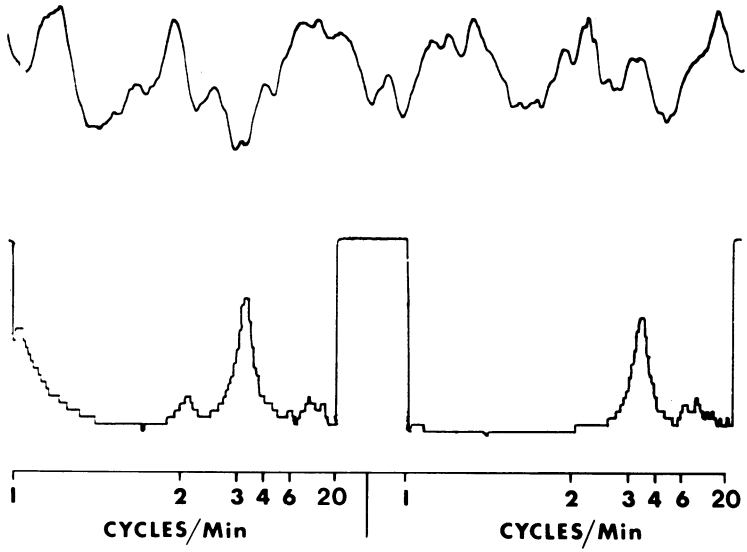

Figure 29.1 Electrical recording from the rectosigmoid in a patient with the irritable colon syndrome. The top trace is the electrical slow-wave rhythm with a predominant frequency of 3 c/min. The bottom trace is the automatic frequency analysis of each 1 min epoch of the electrical slow-wave activity

One major advantage of this system is the ability to correlate the results of slow-wave analysis with clinical observation at the time of recording.

Ten subjects with no known colonic pathology, and twenty patients classified clinically as having the irritable colon syndrome were studied by means of a mucosal suction electrode at 12–20 cm from the anus[4]. This electrode was introduced into the rectosigmoid through a sigmoidscope with the subject or patient lying on his or her left side. The patient then assumed the supine position.

After 15 min, to allow the effects of the insertion of the mucosal electrode to pass, monopolar recordings were made from the internal electrode and an indifferent electrode placed on the scarified skin of the right thigh. This electrical activity was then analysed by means of a real-time frequency spectrum analyser.

Motility was recorded by means of a 1.0 mm internal diameter open-tipped catheter terminated directly opposite the measurement electrode. A 0.15 molar saline solution was perfused through the catheter at a rate of 5 ml/h by means of a constant perfusion pump. The proximal end of the catheter was terminated with a Statham strain gauge pressure transducer.

After suitable amplification and signal processing, the slow-wave activity, slow-wave frequency analysis, pneumograph, and motility were recorded on paper by means of a four-channel chart recorder.

In addition to the chart recorder, a magnetic tape recorder was occasionally used to record the slow-wave activity. This facility allowed a 're-run' of a particularly interesting patient record.

All of the results recorded on the paper chart were analysed visually, and particular note was made of the percentage incidence of each of the cyclic frequencies present during the recording session.

RESULTS

A frequency spectral analysis of the electrical slow wave activity in the recto-sigmoid region of thirty patients reveals cyclic components of less than 1 c/min to more than 20 c/min. Although some of these cyclic components are close to multiples of others, they do not appear to be harmonically related, as the higher frequencies have been observed when the fundamental is absent. Also some components are more frequently observable than others, particularly in those patients having the irritable colon syndrome.

Normal subjects

Although we have observed a considerable variation in the incidence of slow-wave activity in subjects without pathology of the colon, we have found that the percentage of time that each of these individual cyclic components is present produces a characteristic curve. This curve (Figure 29.2) has as its most obvious characteristic two peaks, seemingly caused by a marked reduction in the slow-wave activity at approximately 5 c/min. This observation explains why the slow-wave activity, when analysed by eye, has been described in the past as having two separate frequency components, one at approximately 3 c/min and the other at 6–7 c/min. It now seems that a large number of individual cyclic components in varying amplitude and phase relationship are responsible for these two maxima.

Irritable colon syndrome

In those patients diagnosed clinically as having the irritable colon syndrome, the incidence of each of the individual cyclic components appears to be different (Figure 29.3).

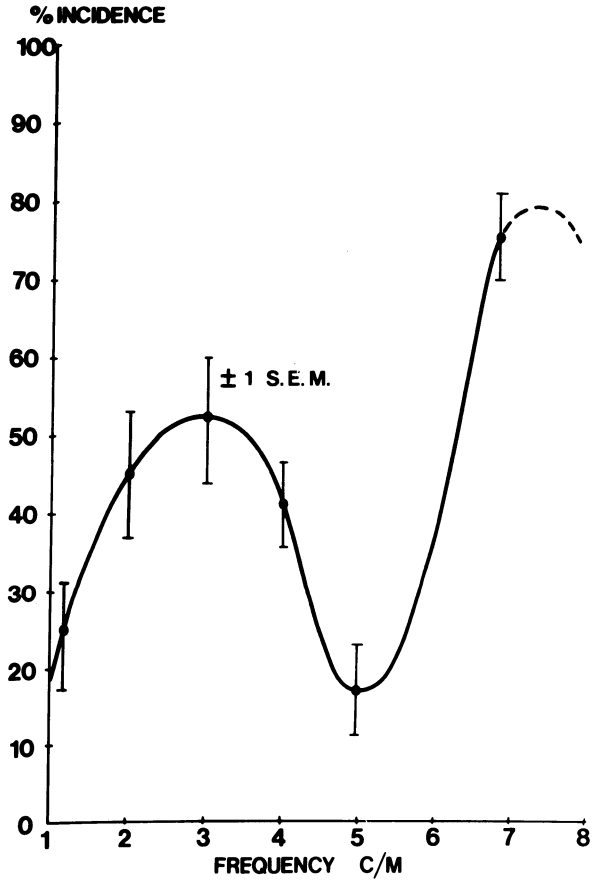

Figure 29.2 Graph showing the mean percentage incidence of each electrical slow-wave frequency component obtained from the rectosigmoid from a group of eight normal subjects. Note the two peaks of incidence at approximately 3 c/min and 6–7 c/min.

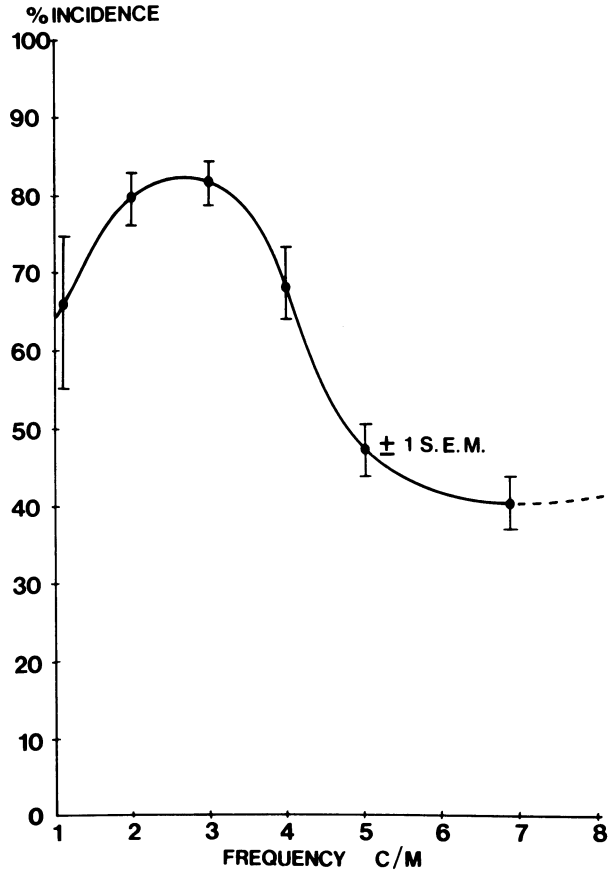

Figure 29.3 Graph showing the mean percentage incidence of each slow-wave frequency component obtained from the rectosigmoid region in a group of thirteen patients with the irritable colon syndrome. Note the increased incidence of 3 c/min slow-wave electrical activity and the smaller incidence at 6–7 c/min.

Patients with irritable colon syndrome show an increase both in the incidence and amplitude of those low-frequency components between 1 and 4 c/min and a marked decrease at 6–7 c/min. This observation has allowed us to suggest that the ratio of the incidence of slow-wave activity at 3 c/min to that at 6–7 c/min may be a useful criterion when used in the diagnosis of patients having the irritable colon syndrome.

An initial study to test our slow-wave classification technique has been made on the slow-wave activity of two sets of patients. The first, a control set of eight patients free from known pathology of the colon; the second, a set of thirteen patients classified clinically as having the irritable colon syndrome. The ratio of the incidence of the 3 c/min activity to 6–7 c/min activity is shown in Figure 29.4. As can be seen, there appears to be a separation between the sets of data points for the two groups of patients. Patients with irritable colon syndrome have a higher ratio of 3 c/min to 6 c/min activity (mean value 2.1) than normal subjects (mean value 0.78).

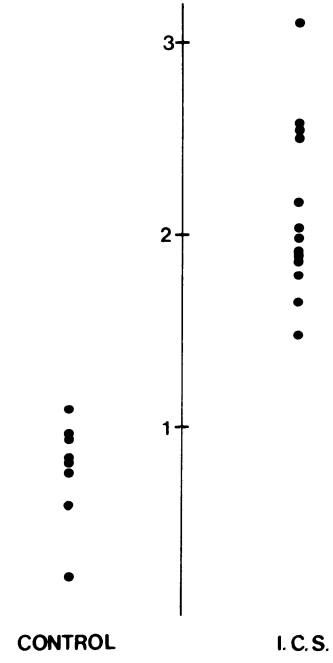

Figure 29.4 The ratio of the incidence of 3 c/min to 6–7 c/min electrical slow-wave activity in a group of eight normal subjects and a group of thirteen patients with the irritable colon syndrome. Note that there is a separation of the data points, and patients with the irritable colon syndrome have a significantly higher ratio than normal subjects

DISCUSSION

The irritable colon syndrome is a disorder characterized by a combination of diarrhoea, constipation and abdominal pain. In patients with the irritable

colon syndrome there appears to be a specific abnormality in the colonic slow-wave activity.

By its very nature, visual analysis of colonic slow-wave activity can only produce, at best, poor estimates of the true frequency and percentage activity of each of the individual cyclic components recordable from within the colon. For this very reason we have chosen to analyse the slow-wave activity, both visually and by means of a specially constructed frequency spectrum analyser, in the hope of obtaining further insight into colonic function.

Our initial findings suggest that there are many more individual cyclic components recordable from within the colon than has been previously reported. However, these cyclic components are in general of lower amplitude and occur less frequently than the two most often visually observed components[4,9] at 3 and 7 c/min.

Our estimates of the mean percentage incidence of both the 3 c/min and the 6–7 c/min activity for normal subjects are significantly higher than those mean values recorded in previous studies[4,6].

In patients with the irritable colon syndrome we confirm previous reports of an increase in slow-wave activity at 3 c/min. However, our investigations reveal that this is only one of several cyclic components that increase, both in amplitude and incidence, in patients with this disorder. We have also observed in the same patients a marked decrease in the incidence of slow-wave activity at 6–7 c/min. This major difference in slow-wave activity appears to be specific to this condition[10]. It appears hopeful that a ratiometric measurement made at the two principal frequencies of the electrical slow-wave activity may allow a positive diagnosis to be made for those patients suffering from the irritable colon syndrome, rather than the present method of diagnosis by exclusion.

Eventually, it is hoped that this diagnostic method, or a logical extension of it, may assist both in the prognosis and follow-up assessment of patients.

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Discussion

C. F. Code: (USA) Is any special preparation of the patient necessary before you take your records? After the patients with irritable colon syndrome have been treated for weeks or months is there a change in the 3–6 c/min ratio?

C. F. Darby: (UK) No special preparation is used. Our initial results suggest that with symptomatic improvement the ratio of 3–6 c/min does not return towards the normal pattern.

W. J. Snape, Jr.: (USA) I am very happy that Dr Darby and his associates were able to confirm our findings. I would caution him about using myoelectrical recordings as a routine clinical test at this point. We have found some overlap between normal subjects and patients with the irritable bowel syndrome. However, the technique may be an important way of classifying patients for clinical therapeutic trials.

Darby: We are pleased to take note of Dr Snape's comments. However, we are hoping that our ratio-metric classification technique will provide greater discrimination between normal subjects and those patients having the irritable colon syndrome. Indeed our initial results suggest that even our simplest classification technique produces more consistent results than those obtained by visual analysis of the slow-wave activity.

Section VI
Myogenic and Neural Factors

30

Specific impedance of longitudinal and circular muscle from cat proximal jejunum

A. BORTOFF

Although electron microscopic studies have demonstrated the presence of gap junctions or nexuses between smooth muscle cells of the circular muscle layer of the small intestine, they have failed to reveal any significant number of nexuses between muscle cells in the longitudinal layer[1,2]. If the gap junction is involved in electrically coupling smooth muscle cells, one would expect to find an inverse relationship between the number of such junctions and the specific junctional impedance of the tissue. In the case of the intestine, circular muscle would be expected to have a considerably lower specific junctional impedance than longitudinal muscle. We investigated this relationship using a modification of a method for measuring tissue impedance originally devised by Tomita[3].

METHODS AND MATERIALS

The method consists essentially of measuring the impedance of strips of tissue in a narrow chamber perfused first with Tyrode solution and then with Tyrode solution which has had half of its NaCl replaced by sucrose. Knowing the specific resistances of both solutions, and their resistances in the chamber, the specific impedance of the tissue can be calculated. The main advantage of this method is that it avoids the use of either isotonic sucrose or oil, which are commonly used to replace the extracellular fluid between the measuring electrodes. Sucrose, probably because it leaches ions from the tissue, results in impedance measurements which are high and which decrease with time; while oil, which leaves a shunting layer of electrolyte around the tissue, results in impedance measurements which are low.

The actual measuring procedure consists first of threading a strip of tissue into a Lucite chamber slightly larger than 1 mm in diameter and 3 cm in length. Platinum black electrodes, 10 mm apart, pass perpendicularly through the chamber in such a way that each makes contact with the tissue in the chamber. One end of the tissue is fixed in the chamber below the lower electrode and the other end, above the upper electrode, is attached by means of a silk thread to a strain gauge transducer which is used to monitor tension during the impedance measurements. After the tissue is in place the transducer is raised until the tissue just begins to develop passive tension. If any active tension is recorded during an experiment the tissue is immediately discarded. The chamber is first perfused with Tyrode solution and then Tyrode–sucrose solution at a flow-rate of about 5 ml/min and a temperature of either 24 or 37 °C. Because of the relatively large temperature effect on impedance the temperature is maintained at ± 0.1 °C by adjusting the flow-rate. The perfusing fluid is removed by suction as it leaves the top of the chamber.

The schematic of the circuit for measuring impedance is shown in Figure 30.1. Alternating current is passed between the electrodes from an oscillator operating in the frequency range of 50–20 kHz. The magnitude of the current is limited to 5×10^{-7} A by a current-limiting resistor. The current is converted to a proportional voltage by the current to voltage amplifier, and is measured by one channel of a two-phase/vector lock-in amplifier. Since the input of the current-sensitive amplifier is at virtual ground, the voltage drop across the tissue is essentially identical to the voltage measured at the current-injecting electrode with respect to ground. This voltage is measured by the second channel of the lock-in amplifier. The absolute value of the impedance

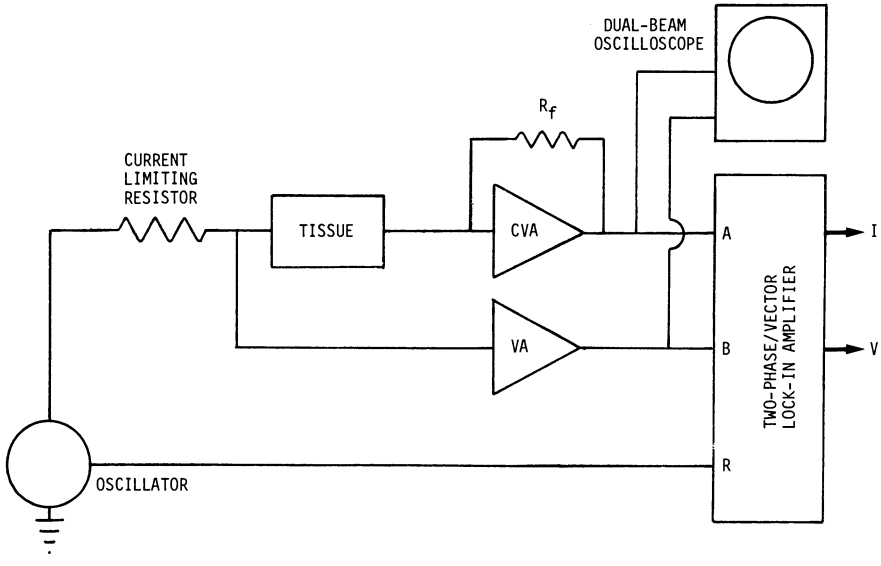

Figure 30.1 Schematic of the circuit for measuring tissue impedance. See text for details

at any frequency is obtained by dividing the measured voltage by the current. Although corresponding phase angles can also be measured with the lock-in amplifier, the resultant phase angle of the impedance is too small ($<5°$) to be of any value in these particular experiments.

After the measurements are made the specific impedance of the tissue is calculated for each frequency. Since the tissue and the solution perfusing the chamber are electrically in parallel, the measured impedance, expressed as impedance per unit of volume, X, is related to Y, the actual tissue impedance in the chamber, and R, the specific resistance of the solution perfusing the chamber, by the following equation, using Tomita's[3] notation:

$$X = \frac{a\,R \cdot Y}{a\,R + Y}$$

where a is the reciprocal of the fraction of space occupied by the solution in the chamber. The equation can be rearranged in terms of tissue impedance in the chamber as:

$$Y = \frac{a\,R_1X_1}{a\,R_1 - X_1} = \frac{a\,R_2X_2}{a\,R_2 - X_2}$$

where R_1 and R_2 are the specific resistances of Tyrode and sucrose – Tyrode respectively, and X_1 and X_2 are the corresponding specific impedances of tissue plus solution in the chamber. a can be determined from this relationship:

$$a = \frac{X_1X_2\,(R_2 - R_1)}{R_1R_2\,(X_2 - X_1)}$$

The specific tissue impedance, Z, is related to Y by:

$$Z = (1 - 1/a)\,Y$$

Impedances were determined for strips of longitudinal and circular muscle removed from the first 3–5 cm of cat jejunum. The data were plotted as specific impedance in Ω cm against log current frequency. The first group of experiments was done at 24 °C in an attempt to reduce the incidence of spontaneous contractions in the longitudinal muscle preparations. The second group of experiments was done at 37 °C where slightly more than 50% of the measurements on longitudinal muscle preparations were successfully completed. Spontaneous contractions did not occur in freshly dissected circular muscle preparations at either temperature.

RESULTS

The data obtained at 24 °C are shown in Figure 30.2. Each point represents the mean of six muscle strips taken from three cats. The bars represent +SEM. In both muscle preparations the impedance tends to plateau at both the low and the high current frequencies. This is consistent with Tomita's

measurements on the guinea pig taenia coli and it is also consistent with Tomita's electrical analogue of smooth muscle represented by the equivalent circuit in the inset. Here, R_m represents the myoplasmic resistance, R_j the junctional resistance and C_j the junctional capacitance. In such a circuit the capacitive reactance would be infinite at very low frequencies and zero at very high frequencies. Thus, at low frequencies all the current passes through R_j and the measured impedance is equal to $R_m + R_j$, while at high frequencies R_j is effectively shorted out by C_j and the measured impedance is equal to R_m. If the measured impedance at 50 Hz approximates the low-frequency impedance plateau and that at 20 kHz represents the high-frequency plateau, then $R_m + R_j$ is 457 and 311 Ω cm, while R_m is 214 and 185 Ω cm for longitudinal and circular muscle, respectively. R_j, which is the difference between the low- and high-frequency values, is 243 and 126 Ω cm for longitudinal and circular muscle, respectively. The differences between the impedances at 20 kHz are found to be insignificant ($p > 0.1$) by the Mann–Whitney U test, while the difference at 50 Hz is found to be significant ($p < 0.01$) by the same

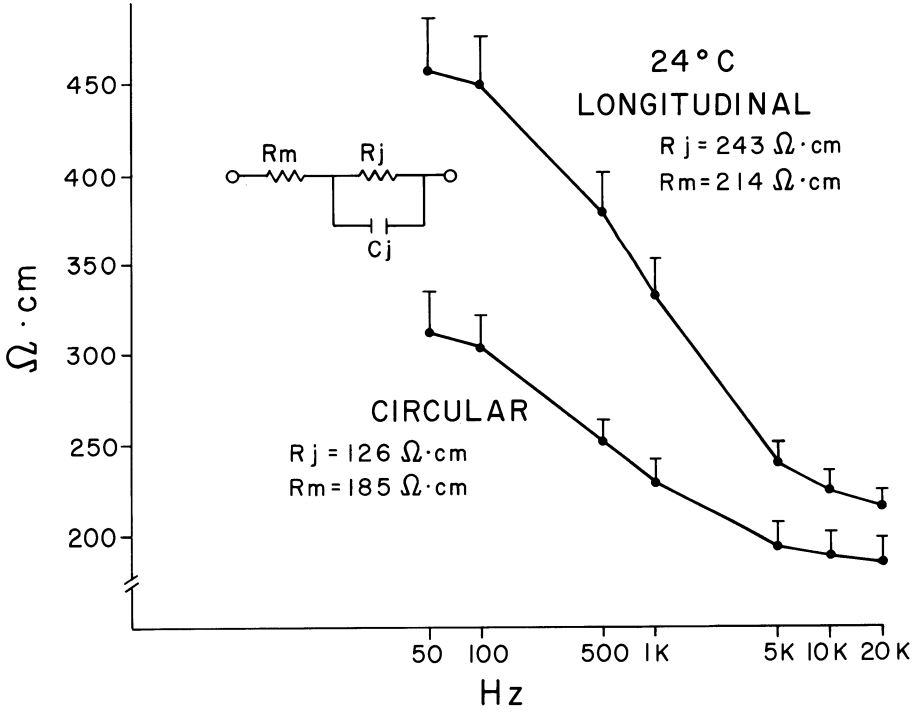

Figure 30.2 Specific tissue impedance measured at different frequencies from 50 Hz to 20 kHz. Each point represents the mean of ten strips of tissue from three cats. Bars represent +SEM. Differences between curves at 10 and 20 kHz are not significant at the 0.05 level. Inset shows assumed equivalent circuit of tissue. R_m = myoplasmic resistance; R_j = junctional resistance; C_j = junctional capacitance. Impedance measurements made at 24 ± 0.1 °C

test. Thus it is apparent that at 24 °C the myoplasmic resistances of longitudinal and circular muscle are equal, but the junctional impedance of longitudinal muscle is significantly higher than that of circular muscle.

Not surprisingly, the data obtained at 37 °C show the same relationship. They are plotted in Figure 30.3, in which each point represents the mean of ten strips of tissue obtained from a total of three cats. Again, the differences between the high-frequency impedances are not statistically significant ($p > 0.2$) while that between the low-frequency impedances is highly significant ($p < 0.001$) both by Student's t-test and the Mann–Whitney U test. The lower resistances in this second set of experiments can be entirely attributed to the higher temperature at which the impedances were determined.

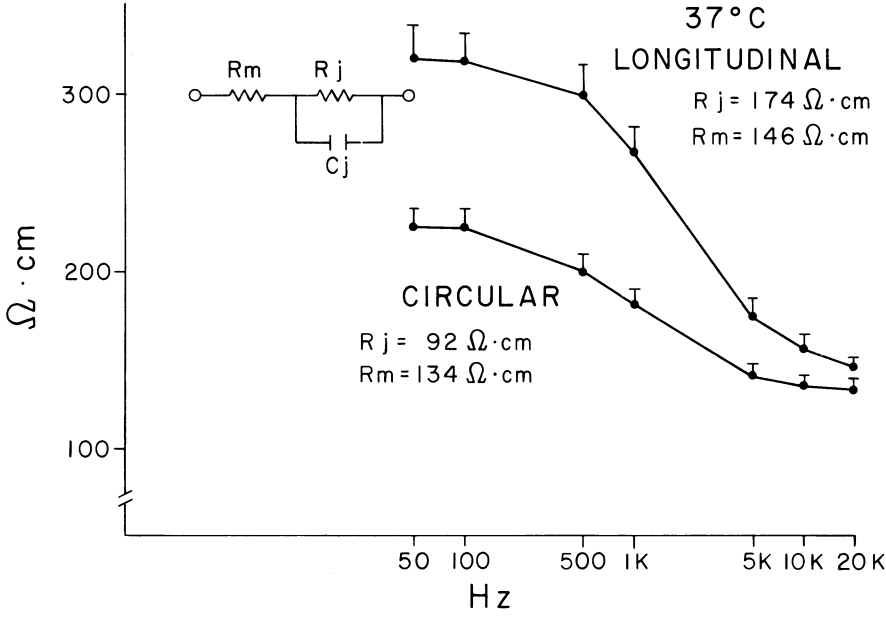

Figure 30.3 Specific tissue impedance measured at different frequencies from 50 Hz to 20 kHz at 37 ± 0.1 °C. Symbols have same meaning as in Figure 20.3. Differences between curves at 10 and 20 kHz are not significant at the 0.05 level

DISCUSSION

The results of these experiments indicate that the myoplasmic resistances of the two muscle layers from cat jejunum are essentially equivalent, but the junctional resistance of longitudinal muscle is almost twice that of circular muscle.

The values reported here may be compared to those obtained by Tomita[3], who used a similar method to estimate the specific myoplasmic and junctional resistances of guinea pig taenia coli. At temperatures between 25 and 28 °C Tomita obtained values of 180 Ω cm for R_m and 190 Ω cm for R_j. According

to our measurements the ratio of the resistivity of Tyrode solution at 37 °C to that at 25 °C is approximately 0.8. Using this conversion factor to extrapolate Tomita's values to 37 °C, we obtain 144 Ω cm and 152 Ω cm for R_j. The value for R_m is essentially the same as we obtained at 37 °C and that for R_j is intermediate between the values reported here for longitudinal and circular muscle.

How do these values compare to those determined for other types of mammalian muscle? Boyd and Martin[4] calculated a specific resistance of 125 Ω cm for mammalian myoplasm at 37 °C, a value which is reasonably close to the average of 140 Ω cm reported here. Total specific longitudinal resistance, $R_m + R_j$, for mammalian Purkinje fibres has been reported to range from 105 to 181 Ω cm[5–7], while that for mammalian ventricular working muscle has been found to be approximately 470 Ω cm at body temperature[7,8].

Thus our values for both circular and longitudinal muscle are well within the range of values reported for those tissues in which the cells are electrically coupled by relatively low resistance pathways. In circular muscle the low resistance pathway probably involves the nexus, although other structures may also be involved. The anatomical basis for electrical coupling in longitudinal muscle remains to be resolved.

Acknowledgement

This work was supported in part by USPHS Grant AM-06958 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

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Discussion

M. Pescatori: (Italy) A simulation of mechanical events occurring during propulsion seems to be a good complementary method for investigating some controversial aspects concerning the temporal relationship and the role of the two layers when peristalsis occurs. In the rabbit distal colon *in vitro* (isometric isovolumic preparation) the following parameters were recorded: intraluminal pressure, force exerted by the segment and changes in morphology of the intestinal loop (films were taken by a cine-camera) during spontaneous and electrostimulated propulsion. Circular and longitudinal muscle strips of the same tract were also examined and then tension–velocity and tension–length relationships computed. In theoretical studies a mathematical model was developed, describing the same events observed experimentally. The parameters of the resulting equation, which describes the intestinal muscle contraction, were obtained from the measurements from the strips. The results were as follows: As far as the pressure and force wave-forms were concerned, the outputs of the model were extremely similar to the tracings obtained by the experiments: the force take-off preceded the pressure take-off and the time-lags between the two peaks were quite the same. Furthermore, looking at morphology, velocity of the peristaltic wave and maximum segment radius decrease were the same in experimental and model results. We supposed in the model the two muscle layers being simultaneously activated (according to the experimental hypothesis of Bortoff and Ghaliba), the activation being greater for the circular than for the longitudinal coat (according to the experimental data of Frigo, Torsoli *et al.*). As the simulation of propulsion gave good results, we can conclude that this model is an adequate complementary method for analysing and testing experimental findings, especially if controversial. Furthermore, it relates two different experimental areas (strips and segment) and could be a part of a more complex description of both electrical and mechanical activity.

C. F. Darby: (UK) Is the impedance constant for a range of current injection levels?

A. Bortoff: (USA) Impedances are constant over a current amplitude range of 10^{-7} to 10^{-6} A. We have not used current amplitudes greater than 10^{-6} A in order to avoid the possibility of electrically stimulating the tissue.

D. O. Castell: (USA) Do we know that the number of smooth muscle cells in a given sized muscle strip is the same for both circular and longitudinal muscle; that is, is there any difference in muscle density between the two muscle layers?

Bortoff: Light microscopy indicates virtually no difference in cell diameter or extracellular space, although we have not yet carried out a comparative electron microscopic study of the two muscle layers.

31

Colonic mechanoreceptor input to neurons in the coeliac plexus and superior mesenteric ganglion *(Abstract)*

D. L. KREULEN AND J. H. SZURSZEWSKI

Studies were performed to elucidate the relationship between colonic motility and electrical activity in neurons of the coeliac plexus (CP) and superior mesenteric ganglion (SMG). *In vitro* preparations consisted of the abdominal pre-vertebral ganglia of guinea pigs, attached to the colon by its vascular supply and nerve trunks. Electrical activity of neurons was monitored with intracellular microelectrodes, and nerve fibres were stimulated with external electrodes. Patterns of connections to neurons were characterized both by stimulation of nerve trunks and by air distension of proportions of the colon. Neurons in both the CP and SMG received mechanosensitive excitatory synaptic input from the colon. This input consisted of excitatory post-synaptic potentials (EPSPs) and action potential. The level of synaptic input returned to control levels after the air had been expelled from the colonic lumen. Repetitive stimulation (15 Hz) of nerve fibres between the ganglia and the colon was followed by a brief period of reduced synaptic input. The SMG received excitatory synaptic input from all portions of the colon, however, the neurons appear to be spatially distributed according to the source of the input. There were groups of cells which received synaptic input via only the lumbar colonic nerves, and there were other groups of cells which received synaptic input via only the more orad coeliac nerves. In addition, there were cells which received synaptic input via both orad and aborad sources. Only a very few if any of these neurons received input from the CNS via the greater splanchnic nerves. Some neurons in the coeliac ganglia also received excitatory synaptic input from the colon. In the neurons tested, this input came via the coeliac nerves and not via the lumbar colonic nerves. These data demonstrate that

neurons in both the CP and SMG receive mechanosensitive synaptic input from the colon.

Acknowledgement

This study was supported by NIH Grants AM-NS 17632 and T 32 RL 7111-02.

Discussion

J. S. Davison: (UK) (1) Did afferent input from the colon to the SMG and the coeliac ganglion increase during spontaneous contractions and distension; that is could the input be from 'tension' receptors? (2) Have you examined the input from mechanoreceptors and chemoreceptors in the stomach and in particular in the duodenum?

J. H. Szurszewski: (USA) (1) Yes, afferent input is correlated with spontaneous contractions. This information has recently appeared in *Gastroenterology*. (2) No; at least not with the stomach and duodenum attached to the solar plexus, but we have found that stimulation of nerve bundles running between the gastrointestinal organs and the solar plexus produced synaptic responses in the noradrenergic neurons in the solar plexus.

N. W. Weisbrodt: (USA) Do you see any responses which would indicate a spatial representation or distribution within a ganglion?

Szurszewski: Yes. Our data are preliminary – there appears to be a spatial representation of different regions of the colon and other regions of the gastrointestinal tract in the solar plexus.

J. S. Davison: (UK) The following *in vivo* observation on the inferior mesenteric ganglion of the guinea pig emphasizes the importance of long spinal pathways in reinforcing the peripheral reflexes Figure 1 shows an average record of a response recorded in a colonic nerve while stimulating another colonic nerve with single shocks. The ascending mesenteric and hypogastric nerves have been cut, but the lunbar splanchnic nerves are intact. As the upper trace shows, the response consists of an early

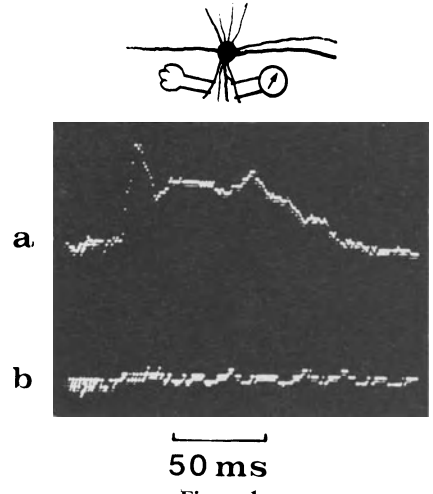

Figure 1

peak followed by a long late wave. Both components are blocked by 1% nicotine applied to the ganglion (lower trace). This concentration did not affect the compound action potential in nerves connecting with the ganglion. The late wave is progressively reduced by sectioning the lumbar splanchnic nerves in turn. When all are severed, only the early peak remains. These observations show that the peripheral pathway through the inferior mesenteric ganglion initiates the reflex, but is reinforced and prolonged by a longer pathway through the spinal cord which also relays in the ganglion.

32
Electrical activity of myenteric neurons: comparison of results obtained with intracellular and extracellular methods of recording

J. D. WOOD AND C. J. MAYER

INTRODUCTION

The enteric nervous system is an independent integrative system that programmes and coordinates the various patterns of motility of the gastrointestinal musculature. The neural circuitry of this system receives sensory information from chemo- and mechanoreceptors, processes the information and generates output that is appropriate for control of the effector system. These are functional properties that are usually associated only with the central nervous system, and in fact, both the structural and functional characteristics of the enteric ganglia resemble the central nervous system much more than the autonomic ganglia outside of the alimentary tract[1].

Intracellular and extracellular methods of electrical recording from single neurons have proved to be valuable tools for investigation of the neurophysiology of the central nervous system, and now both of these methods have been successfully applied in studies of the ganglia of the enteric nervous system. In the present paper, we report new results that we have obtained from Auerbach's plexus with the intracellular recording method, and relate these findings to earlier observations that we made with extracellular recording methods.

METHODS

The methods for intracellular and extracellular recording of electrical activity in Auerbach's plexus *in vitro* have been described in detail in earlier publications[2,3].

RESULTS

We have reported previously that both spontaneous and stimulus-evoked patterns of action potential discharge can be recorded extracellularly from single neuronal units within myenteric ganglia in intestinal segments *in vitro*[2,4-6]. We report here intracellular studies on guinea pig myenteric neurons that also revealed spontaneous and stimulus-evoked spike discharge, some of which appeared to be counterparts of the extracellularly recorded electrical activity.

The extracellularly recorded neuronal units can be classified into the following categories on the basis of the pattern of spike discharge:

A Mechanosensitive neurons
   1. Mechanoreceptors
      (a) Slowly adapting
      (b) Fast adapting
   2. Tonic-type enteric neurons

B Single-spike neurons

C Burst-type neurons
   1. Steady bursters
   2. Erratic bursters

Mechanoreceptors

Three kinds of myenteric neurons respond with an increase in rate of discharge to mechanical distortion of the ganglion[4-6]. One mechanosensitive unit behaves like a typical slowly adapting mechanoreceptor, and another discharges like a fast-adapting mechanoreceptor (Figure 32.1A,B). No counterparts of slowly adapting and fast adapting mechanoreceptors have been identified with the intracellular method of recording. Intracellular studies have revealed a type of neuron in which the action potentials are followed by prolonged hyperpolarizing after-potentials of several seconds' duration (Figure 32.1C). The properties of these cells were first described by Hirst and co-workers[7] who referred to them as AH cells and by Nishi and North[8] who called them Type II cells. The soma of this type cell does not appear to receive synaptic input and, on this basis, these authors suggested that the AH or Type II neurons might be sensory neurons.

The characteristics of the somal membranes of AH or Type II myenteric neurons are reminiscent of neurons in invertebrate nervous systems[9] in which the soma supplies processes to the synaptic neuropil, but does not participate in the transmission of synaptic and spike information. The AH or Type II cells may be analogous to invertebrate neurons in that action potential discharge in the soma is incidental and that information processing occurs in the neuropil.

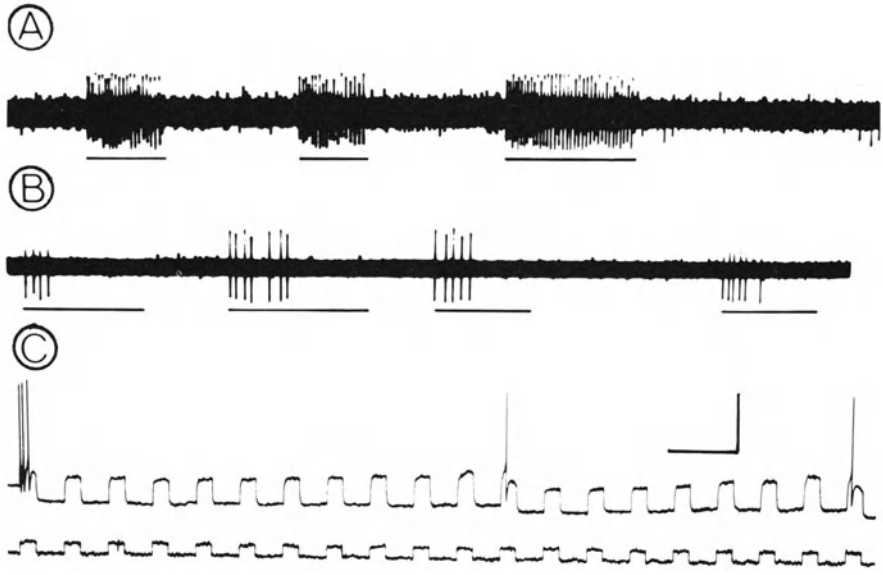

Figure 32.1 Comparison of extracellular records of myenteric mechanoreceptors with an intracellular record from a ganglion cell postulated to be a sensory neuron. (A) Extracellular record of a slowly adapting mechanoreceptor. (B) Extracellular recording of a fast adapting mechanoreceptor. Both receptors were in myenteric plexus of cat small bowel. Mechanical stimulus was a transient forward–reverse movement of a glass-Pt electrode indicated by horizontal bars on records. (C) Intracellular record of postulated sensory neuron in myenteric plexus of guinea pig jejunum. Upper trace is transmembrane voltage; lower trace indicates current of depolarizing pulses injected through the recording electrode. Prolonged hyperpolarization followed spikes elicited by current pulses. Increase in amplitude of the trans-membrane voltage to constant current pulses applied after the spikes reflected progressive increase in input resistance as after-hyperpolarization decayed. Calibration: time scale for *A–B*, 2 s and for *C*, 0.75 s; vertical scale for *A–B*, 100 μV and for *C*, 33.5 mV and 8.25×10^{-10} A

Tonic-type neurons

Tonic-type myenteric neurons (Figure 32.2A) are activated by mechanical stimulation to discharge long trains of spikes 30–40 s in duration[4,5]. The discharge frequency of this type of cell is independent of the intensity of mechanical stimulation, and the cell continues to discharge in a set pattern after the stimulus is removed. The discharge behaves like an all-or-none event. Once the neuron is activated during mechanical stimulation, it discharges a stereotyped train of spikes independent of the original stimulus. Close coupling between slowly adapting mechanoreceptors and tonic-type neurons has been observed on multi-unit–extracellular recordings[2,4,6]. These properties suggest that tonic-type neurons are associative neurons that are activated by input derived from intramural mechanoreceptors.

Figure 32.2B illustrates the intracellular equivalent of the tonic-type neuron. After impalement of the neuron with the recording electrode, electri-

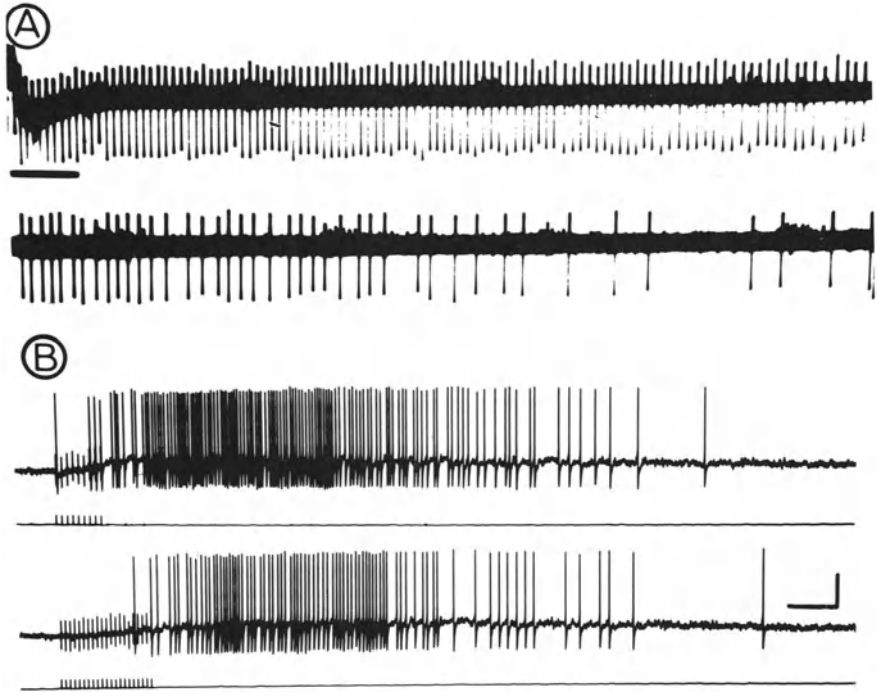

Figure 32.2 Comparison of extracellular and intracellular records from tonic-type enteric neurons. (A) Discharge pattern of a tonic-type neuron recorded extracellularly from Auerbach's plexus of cat small intestine. The duration of the spike discharge was much longer than the duration of stimulation (horizontal bar). Mechanical stimulus was transient forward–reverse movement of glass-Pt electrode. (B) Intracellular records of depolarization and tonic discharge of spikes in a myenteric neuron of guinea pig jejunum evoked by electrical stimulation of inter-ganglionic fibre tract. The duration of spike discharge of each of the two responses was much longer than duration of train of stimulus pulses. Upper trace, trans-membrane voltage; lower trace, stimulus marker. Calibration: time-scale for *A*, 0.75 s and for *B* 0.45 s; vertical scale for *A*, 67 μV and for *B*, 20 mV

cal stimulus pulses applied to one of the inter-ganglionic fibre tracts with an extracellular stimulating electrode elicited a sustained depolarization and a prolonged discharge of action potentials that continued for several seconds after termination of the stimulus. The graded depolarization which occurred at the onset of stimulation in this type of cell appeared to result from activation of axons that synapse with the cell. The mechanisms that account for the relatively slow onset and prolonged nature of these synaptic potentials are unclear. They could involve slow release of transmitter, long diffusion distances, slow kinetics of transmitter–receptor interaction, or a combination of these.

Recently proposed neural models of peristalsis require sustained discharge by a particular type of enteric neuron to account for the observed delays of several seconds between stimulus and coordinated responses, and to account

for sustained neural influence at the effector[1,10]. The behaviour of the tonic-type neurons fits the requirement for a neuronal unit whose functional significance would be production of either prolonged excitation or inhibition at neuronal and neuroeffector junctions within the gut wall[1].

Single-spike neurons

Single-spike units show continuous discharge of action potentials at relatively low frequencies with no consistent pattern when the activity is recorded extracellularly (Figure 32.3A). These ganglion cells do not require synaptic input for spontaneous discharge, because they continue to generate spikes in the presence of elevated concentrations of magnesium ions[11]. However, the discharge pattern is influenced by synaptic input because spike-interval statistics are altered in presence of elevated magnesium[11]. The single-spike neurons have cholinergic receptors that are activated by nicotine in the presence of elevated magnesium[11].

Figure 32.3 Comparison of extracellular and intracellular records from single-spike neurons. (A) Discharge pattern of a single-spike unit recorded extracellularly from Auerbach's plexus of cat small intestine. (B) Intracellular recording of spontaneous spikes and EPSPs in a myenteric neuron of guinea pig jejunum. Calibration: time-scale for *A*, 1.1 s and for *B*, 1 s; vertical scale for *A*, 100 μV and for *B*, 40 mV

The intracellular counterpart of the single-spike neuron is illustrated in Figure 32.3B. Intracellular recordings from these cells show continuous discharge of spikes. There is large variation in duration of inter-spike interval and this produces broad, platykurtic inter-spike-interval histograms that closely resemble inter-spike-interval histograms for the extracellularly recorded activity[6,12]. The spontaneous single spikes that are detected by intracellular electrodes are superimposed upon a fluctuating baseline that appears to reflect synaptic bombardment of the cell membrane, because all of the activity is abolished by the nerve-blocking drug, tetrodotoxin.

Burst-type neurons

In extracellular studies, burst-type neurons periodically discharge bursts of spikes with silent interburst intervals (Figure 32.4). The burst-type cells are classified as either steady bursters or erratic bursters on the basis of regularity of the time-intervals between bursts[1].

The steady bursters (Figure 32.4A) discharge with relatively low variance of inter-burst time interval, and when long inter-burst intervals occur they are distinct multiples of the shortest inter-burst intervals[13]. The steady bursters do not require synaptic input for the discharge pattern and behave as if the timing of the bursts is determined by an endogenous oscillatory pacemaker mechanism[11].

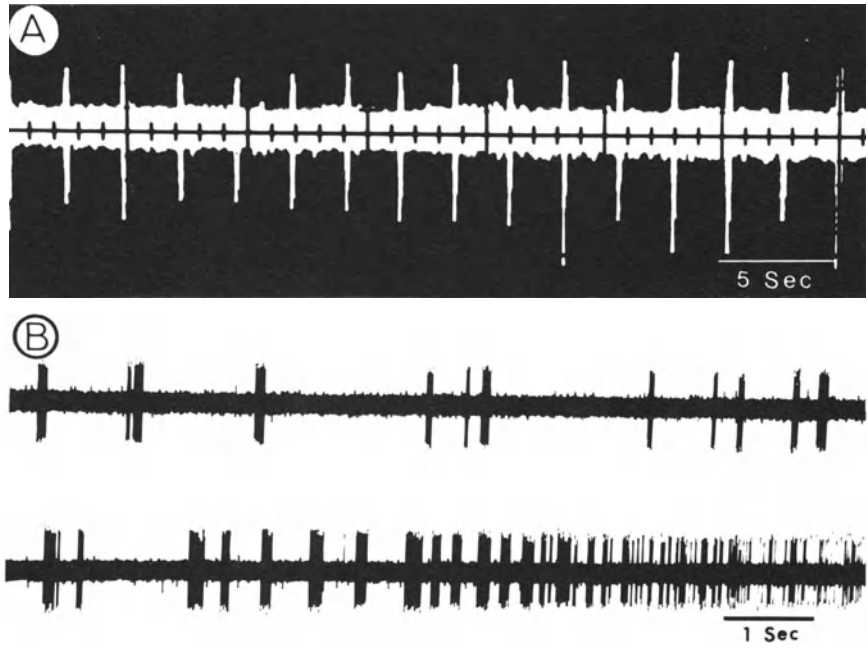

Figure 32.4 Examples of discharge of burst-type neurons recorded extracellularly in Auerbach's plexus. (A) Example of a steady burster in cat jejunum. (B) Example of a continuous record of an erratic burster in guinea pig jejunum

The intracellular studies have not detected a counterpart of the steady burster neurons. This may be because ganglion cells of the myenteric plexus are highly variable in size and shape, and it is unlikely that all neurons, especially the smaller ones, are sampled in intracellular studies.

The discharge patterns of erratic bursters are characterized by irregular inter-burst intervals and by periodic conversion to continuous discharge of

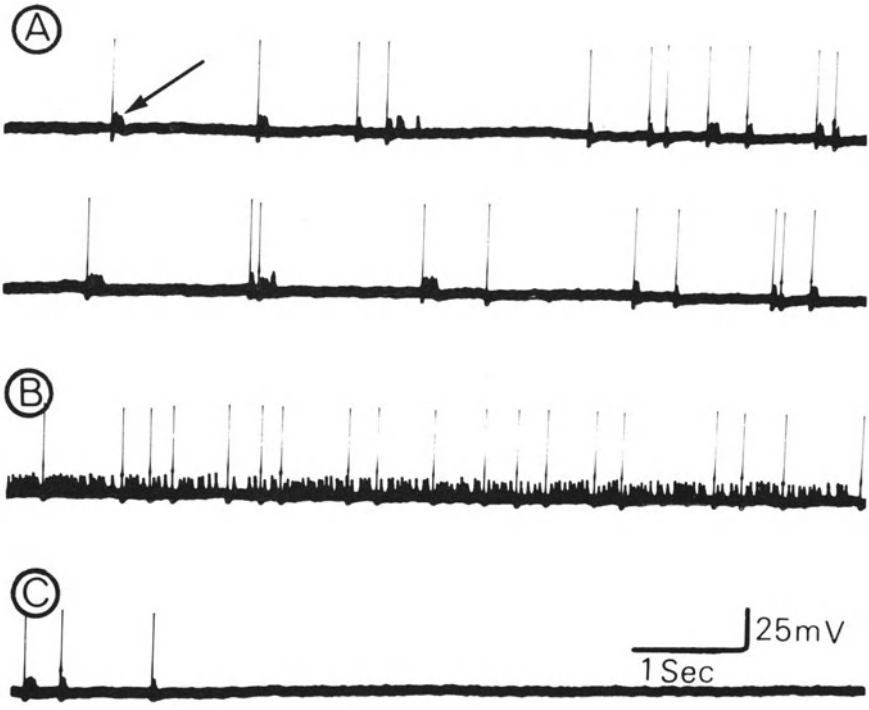

Figure 32.5 Intracellular record of an erratic burster in Auerbach's plexus of guinea pig jejunum. (A) Continuous record of bursts of EPSPs (arrow) accompanied by spikes. (B) Same neuron after conversion from bursts of EPSPs to continuous synaptic activity. (C) Onset of long duration silent period of cyclical discharge pattern of the cell

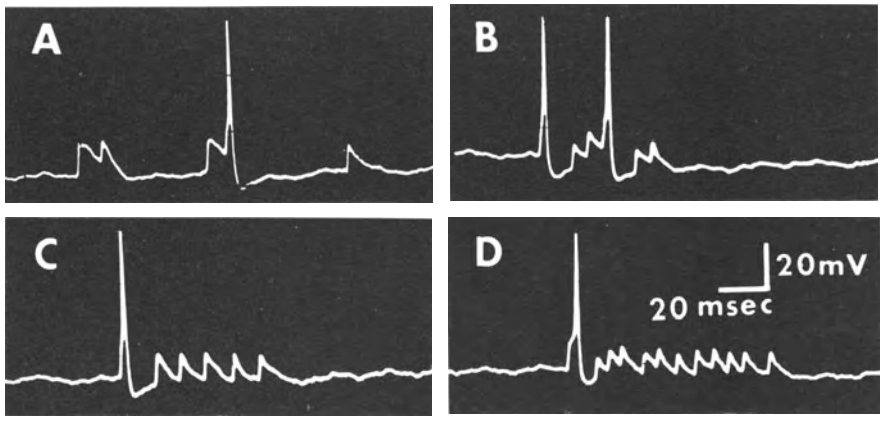

Figure 32.6 Intracellular record with expanded time base of spontaneous EPSPs and spikes of the same erratic burster as Figure 32.5. (A–B) EPSPs and spikes during same time period as Figure 32.5B. (C–D) Bursts of EPSPs during same time period as Figure 32.5A

either single spikes or spike doublets (Figure 32.4B). The irregularity of the inter-burst intervals of some erratic bursters is repeated systematically in cyclical patterns[6]. In these cells, each cycle of activity consists of the following sequential changes in spike discharge: (1) a relatively long duration silent period; (2) a series of bursts at regular intervals; (3) progressive decrease in successive inter-burst intervals; (4) continuous discharge of spikes[6]. The discharge of the erratic bursters appears to be dependent upon synaptic input to the cell because it is blocked in solutions with elevated magnesium[11].

The intracellular counterpart of the erratic burster is illustrated in Figures 32.5 and 32.6. Intracellular recording reveals that synaptic input to these neurons consists of cycles in which bursts of excitatory postsynaptic potentials (EPSPs) later convert to continuous trains of EPSPs followed by long duration silent intervals during which there is no synaptic input (Figure 32.5C).

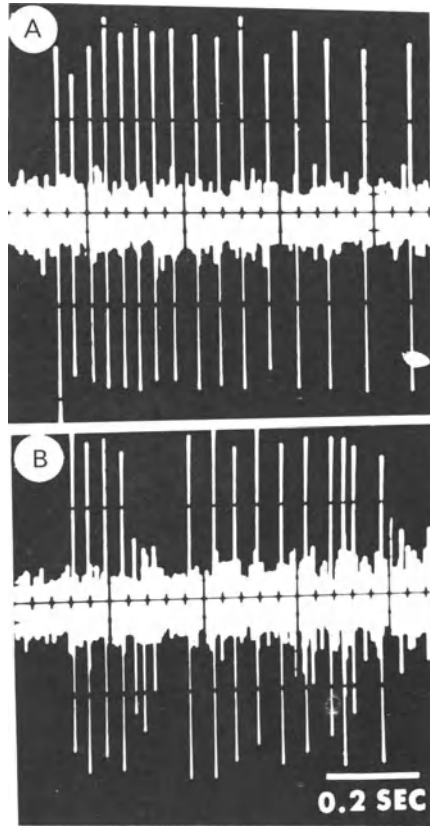

Figure 32.7 Extracellular record of inhibitory interaction between two myenteric neurons in cat jejunum. (A) Single burst of spikes without inhibitory discharge of second neuron. (B) Same burst unit, discharge of second neuron occurred after fourth spike of burst and inhibited discharge of the burst-type unit

Inhibitory neuronal interactions

Multi-unit extracellular records often contain indications of inhibitory interactions between two myenteric neurons[2,14]. In the example of Figure 32.7, about one-third of the bursts of large amplitude spikes were accompanied by action potentials of a second neuron, the discharge of which seemed to inhibit firing of the burst-type cell.

Intracellular recording has provided evidence of a synaptic basis for the inhibitory interactions observed on extracellular recordings. These synaptic events consist of both spontaneous and stimulus-evoked inhibitory postsynaptic potentials (IPSPs) in myenteric neurons. Figure 32.8A shows spon-

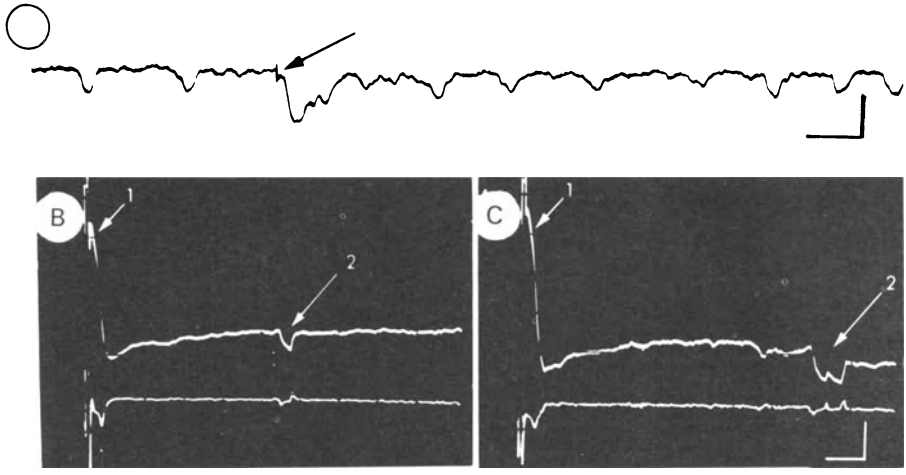

Figure 32.8 Intracellular records of spontaneous and stimulus-evoked IPSPs in myenteric neurons of guinea-pig duodenum. (A) Spontaneous IPSPs and hyperpolarizing response to a single stimulation of a fibre tract entering the ganglion (arrow). (B–C) Stimulation of fibre tract evoked a spike (arrow No. 1) followed by a hyperpolarizing potential (arrow No. 2). Upper trace, trans-membrane voltage; lower trace, dV/dt. Calibration: time-scale for *A*, 1 s and for *B–C*, 10 ms; vertical calibration for *A*, 20 mV and for *B–C*, 20 mV and 60 V/s

taneous hyperpolarizing potentials that were interpreted to be IPSPs because the polarity of the potentials reversed when the membrane potential was experimentally hyperpolarized to values greater than the estimated equilibrium potential for potassium and chloride ions[12]. Single electrical shocks applied to the interganglionic connectives elicited hyperpolarizing responses in some of the myenteric neurons (Figures 32.8B and 32.8C) and repetitive stimulation produced prolonged hyperpolarization in these cells. The stimulus-evoked IPSPs had exceptionally long latencies of 50–148 msec, and occurred in cells in which depolarizing current pulses elicited spikes and in which extracellular electrical stimulation at other points on the ganglion elicited EPSPs.

Acknowledgements

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Discussion

H. L. Stockley: (UK) Could these prolonged spike-bursts which follow electrical stimulation be correlated with contractions which are seen following electrical stimulation, i.e. after contractions or rebound contractions?

J. D. Wood: (USA) I don't know; we have no direct evidence. But it can be assumed that the sustained discharge at the tonic-type enteric neurones is transformed into either prolonged excitation or inhibition at the effector.

A. Bennett: (UK) Prostaglandins might be substances that sensitize the neurones. Have you studied the effect of indomethacin on responses to stimulation? And what does ATP do to the responses?

Wood: We have yet to test these substances on the enteric neurones.

J. Gonella: (France) Have you any idea on the surface of the receptive field? In other words, is it located to the ganglion itself or does it extend to the surrounding smooth muscle?

Wood: The receptive fields at the mechanoreceptors seem to be localised to the ganglia and to be smaller than 500 μm in diameter.

J. Van Nueten: (Belgium) Do you see peptides, belonging to the group of the so-called endorphins, as possible candidates for the endogenous substances, released during repeated stimulation of the neurons?

Wood: We have not tested these substances. I have been vague about the identity of the endogenous substance that activates the tonic-type enteric neurons because much of our data is still in raw form. However, all of our evidence suggests that serotonin is the substance.

J. S. Davison: (UK) The one feature of these otherwise elegant experiments which concerns me is the disruption to the plexus that occurs in setting up the preparation. Have you any plans for modifying the preparation in order to preserve the integrity of the plexuses?

Wood: You are certainly correct. It would be much more physiological if connections between the myenteric and submucous plexuses were maintained. However, in order to visualize the neurons, a thin preparation that permits utilization of transmitted light optics is necessary. We are now converting to reflected light, differential contrast optics which will circumvent this problem.

33

The genesis of the myoelectrical activity in the circular muscle layer from the cat colon: a pharmacological analysis *(Abstract)*

J. CHRISTENSEN, S. ANURAS AND C. ARTHUR

The electromyogram (EMG) of the circular muscle layer of cat colon contains slow waves (SW) phase-locked to spread orad in the proximal colon. Also, prolonged bursts of electrical spikes migrate aborad at intervals of 1–6 min, the migrating spike-bursts (MSB). The hypothesis was examined that these two independent events are controlled by activity of intramural nerves. Strips of muscle, 1 × 10 cm, were cut in the long axis of the colon and mounted in a bath that allows superfusion at 6 ml/min with Krebs solution (aerated with 95% O_2–5% CO_2 at 37 ± 1 °C), while eight monopolar glass pore electrodes, applied 1 cm apart to the circular muscle layer, detected the EMG. Superfusions with various concentrations of drugs were preceded and followed by control periods. Records were read for slow-wave frequency (SWF) at all eight sites. Phase-lock of SW was measured as the coefficient of variation of slow-wave frequency (SWCoV) among the eight electrode sites. Migrating spike-bursts were measured to determine frequency (MSBF) and percentage of total time they occupied (MSB%). Tetrodotoxin (10^{-6} M) had no effect on SWF, SWCoV and MSBF, but it raised MSB% from 40.3 to 81.7 ($p < 0.05$). Lidocaine (5×10^{-4} M) raised MSB% from 29.0 to 75.5 ($p < 0.05$). Phenoxybenzamine (10^{-5} M) and propranolol (10^{-6} M) did not affect MSB% ($p < 0.05$). Atropine (10^{-6} M) and physostigmine (10^{-7} M) had no effect ($p < 0.05$) on SWF, SWCoV, MSBF and MSB%. Acetylcholine (up to 10^{-6} M), methacholine (up to 10^{-7} M) and carbachol (up to 10^{-8} M) had no effect ($p < 0.05$) on SWF, SWCoV, MSBF and MSB%. At higher concentrations of these three cholinergic agents, SW and MSB were replaced by random irregular electrical transients.

CONCLUSIONS

SW are not controlled by intrinsic nerves; MSB represent intermittent escape of the muscle from tonic activity of non-adrenergic inhibitory nerves.

Discussion

B. N. Catchpole: (Australia) The duration of the spike-bursts recorded at different points along your muscle strips seemed to vary. Was this so, and if so why? Did the various drugs you used affect the variability of these bursts?

J. Christensen: (USA) Yes, the duration of the migrating spike-burst is different from one region to another, but is quite constant over time at any one site. I do not know why. The effect of drugs is proportionately the same at all levels.

S. K. Sarna: (Canada) Is there any relationship between the direction of propagation of spike-bursts and phase-lock direction among control waves? Can they occur in opposite direction and, if so, how?

Christensen: There seems to be no relationship between the migrating spike-bursts and the slow waves. They can, and often do, spread in opposite directions. I do not know how this occurs, but it indicates to me that the two phenomena have quite different controls, representing the operation of unrelated physiological systems.

J. S. Davison: (UK) In an earlier discussion on the inhibition of migrating complexes by distension, I proposed that the 'volume' detectors concerned may in fact be 'tension' receptors which would therefore also respond to active contractions. I would like to suggest an alternative hypothesis to the one you propose. Rather than there being periodic release of tonic inhibition, I would like to suggest that there is periodic turning on of inhibition by these same mechanoreceptors. If, as you suggest, the migrating spike-bursts are associated with contractions then these same mechanoreceptors could detect this and then reflexly excite the inhibitory neurons. Have you any comments on this hypothesis?

Christensen: I think that it is an attractive idea; one that I had not thought of. Of course, since the mucosa has been removed in these preparations, such receptors are not mucosal in location, but they may be in the muscle layers.

J. D. Wood: (USA) I would like to also comment on Joe Davison's question. Firstly, the mucosa has been removed, the lumen is empty and there are no overt stimuli to activate sensory receptors; consequently, the onset of the MMC appears to be spontaneous, not evoked. Secondly, if mechanoreceptors are involved in the MMC, these must be responding to contractile activity of the musculature. Thirdly, if it is accepted that the musculature through which the MMC propagates is a functional electrical syncytium, then tonic neuronal inhibition is the most probable mechanism to account for unidirectional, as opposed to bi-directional, spread of excitation from the site of activation within the syncytium.

34

In vitro studies of the electrical activity of the longitudinal and circular muscle layers of the human colon

D. KIRK AND H. L. DUTHIE

Interest in the myoelectrical activity of the colon *in vivo*[1] has emphasized that relatively little is known of the electrophysiology of human colonic smooth muscle[2]. This paper describes some of the findings from a study of isolated smooth muscle strips cut from surgically resected specimens of colon.

MATERIAL AND METHODS

Sixty specimens of resected colon were obtained for study. The indication for resection was colonic or rectal carcinoma in fifty-three, diverticular disease in four and ulcerative colitis in three. A segment of bowel a few centimetres in length was cut from one or both ends of the surgical specimen immediately after its removal, and placed into Krebs solution. Unless it was planned to study it within a short time, the whole segment was stored at 4 °C for a period not exceeding 24 h. Muscle strips measuring 1.5 × 0.2 × 0.1 cm were cut immediately before use. Taenial strips were cut from the serosal surface, and circular muscle strips from the mucosal aspect of the intertaenial regions, after removal of mucosa and submucosa.

The strips were mounted individually in a perfused tissue bath at 37 °C. Monopolar extracellular recordings were made with glass pore electrodes[3], using a silver/silver chloride coil as reference electrode. The output was amplified 100-fold by a purpose-built amplifier. Intracellular recordings were made with 3 M KCl filled micropipettes, impedance 25–50 mΩ, the output of which was taken by short leads to a high-impedance differential amplifier (Fenlow A D 55). One end of the muscle strip was attached to a strain gauge for isometric recording of tension.

A modified Krebs solution of the following composition was used: Na^+ 151 mM, K^+ 4.7, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 142, HCO_3^- 16.3, $H_2PO_4^-$ 1.4, SO_4^{2-} 1.2, dextrose 7.1. The solution was equilibrated with 95% O_2 and 5% CO_2. The following drugs were used: acetylcholine perchlorate (ACh), atropine sulphate, hexamethonium bromide, indomethacin, physostigmine sulphate, tetrodotoxin (TTX). Where relevant, concentrations given in the text are those of the base.

RESULTS

Taenia coli

Eighty-five strips of taenia were studied. Spontaneous activity was present in fifty-four. Activity was dominated by spike action potentials which occurred in a regular rhythm, with a mean rate of 22 ± 5 (SD) counts per minute (c.p.m.). Contraction of the muscle only occurred in the presence of spikes. In twenty-nine strips activity was continuous. In twenty-five, intermittent periods of spike activity occurred, each associated with a tetanic contraction of the muscle, an increment of tension occurring with each spike (Figure 34.1). In eleven of these intermittently active preparations, slow potentials, in the form of sinusoidal oscillations, occupied all or part of the period between contractions. Intracellular recording has confirmed that the spikes in the extracellular record correspond to typical action potentials.

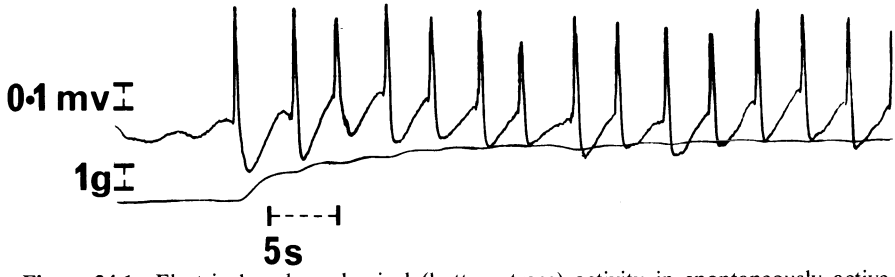

Figure 34.1 Electrical and mechanical (bottom trace) activity in spontaneously active taenia

The effect of acetylcholine was tested in twelve strips. In all cases a stimulatory effect was noted. In strips showing intermittent activity, ACh in low concentrations (10^{-7}–10^{-6} g/ml) produces continuous activity with little or no effect on the actual spike rate. ACh (10^{-5} g/ml) accelerates spike rate progressively causing a sustained contracture. Similar contractures are seen with physostigmine (10^{-6} g/ml). Eight of the thirty-one strips in which spontaneous activity was absent also were treated with ACh. Six responded with regular spikes. The minimum concentration required to stimulate these six strips varied from 10^{-7} to 5×10^{-5} g/ml, and in all cases accompanying mechanical activity was weak. The action of ACh was inhibited by atropine

(10^{-5} g/ml) but not hexamethonium 10^{-5} g/ml. Some continuously active strips, in response to atropine alone, developed an intermittent pattern of activity, an effect which could also be produced by treatment with tetrodotoxin (10^{-7} g/ml).

Indomethacin (10^{-7} g/ml) was applied to six strips of taenia. There was a small reduction in spike rate, the significance of which is uncertain. No stimulation of taenia by indomethacin was observed.

Circular muscle

Twenty-six strips of circular muscle were studied. Of these only five showed spontaneous activity. In two this took the form of periods of regular spikes, in a pattern resembling taenia. The other three demonstrated spontaneously the type of activity seen in most circular muscle strips only in response to stimulation with ACh.

Of the remaining twenty-one strips, nineteen responded to ACh (10^{-5} g/ml), two with regular runs of spikes, similar to those seen in taenia, the remaining seventeen with an entirely different form of activity. Irregular short bursts of spikes occurred, each burst being followed by a single contraction (Figure 34.2).The pattern of activity did not alter as the concentration of ACh was increased. The duration of the individual bursts of spikes varied, and they occurred at rates of between one per minute and twelve per minute. The increase in tension which followed each burst of spikes relaxed completely before the start of the next one. Tetanic contractions were rarely seen in circular muscle.

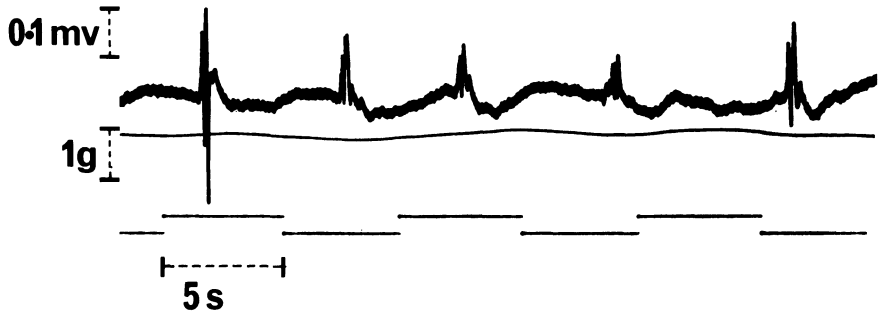

Figure 34.2 Response of circular muscle to stimulation with acetylcholine

Strips of circular muscle, which had responded to ACh by producing isolated bursts of spikes, were treated with indomethacin (10^{-7} g/ml). After 10–15 min, activity appeared in all strips without further introduction of ACh. This activity closely resembled that seen in the same strip in response to ACh and persisted after withdrawal of the indomethacin (Figure 34.3).

After treatment with TTX (10^{-7} g/ml) the action of ACh in circular muscle

was different. Instead of isolated bursts of spikes, regular spikes with tetanic contractions occurred, in a pattern which resembled that seen in taenia.

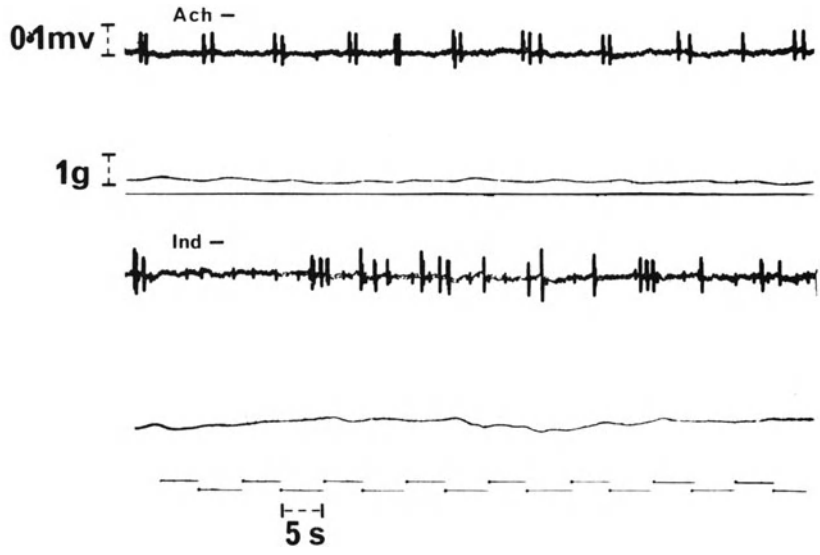

Figure 34.3 Response of circular muscle to indomethacin. Top: response of strip to acetylcholine. Bottom: behaviour of strip after 15 min treatment with indomethacin

DISCUSSION

The majority of strips of taenia showed spontaneous activity. Whether its absence in the remainder was due to damage during surgery or whether it is of physiological significance cannot be determined. Many of the inactive strips responded to stimulation with ACh. However, the failure of atropine and TTX to abolish spontaneous activity suggests it to be myogenic, rather than the result of intrinsic parasympathetic neural activity. The intrinsic nerves may stimulate the normally intermittently active taenial strips in some cases, producing continuous activity, as is demonstrated by the response of such strips to atropine and TTX. Such an effect has previously been reported in the guinea pig jejunum[4]. It is therefore likely that the intrinsic myogenic activity of taenia is one of intermittent periods of electrical activity, associated with tetanic contractions of the muscle, a pattern noted in previous studies *in vitro*[5,6]. It also correlates with the myoelectrical recordings from the colon *in vivo*, in which activity also is intermittent[1].

Each taenial strip has a constant, regular rhythm. The slow potentials seen in approximately half of the intermittently active preparations have been described in a previous study[6]. They are similar to the slow waves seen in the small intestine of the cat, and as such would provide the basis for the regular rhythm seen in the taenial strips. We are unable to account for their absence

in recordings from some strips, the behaviour of which is in other respects similar.

Circular muscle behaves in a manner different from that of taenia. The absence of spontaneous activity has been noted before[6], and in this respect the human colon resembles the small intestine of the rabbit and cat[7], but it differs from the cat colon, in which the circular muscle layer is spontaneously active and generates slow waves[3]. Indomethacin, in the concentration used, is a specific inhibitor of prostaglandin synthesis[8]. Prostaglandins of the E group are known to relax the circular muscle of the colon[9,10]. The action of indomethacin on circular muscle is unlikely to be due to direct stimulation, since the effect takes some 15 min to appear, and persists after withdrawal of the drug. In addition, indomethacin does not stimulate taenial strips. It is suggested that the absence of spontaneous activity in circular muscle is due to inhibition by intrinsic prostaglandins.

When stimulated with ACh, or after treatment with indomethacin, the activity of circular muscle differs from that of taenia. The latter produces sustained tetanic contractions, while contractions of the circular layer are short and isolated. This adaptation of circular muscle appears to be due to an inhibitory neural influence, since after treatment with TTX, the response to ACh is one of tetanic contraction, similar to that seen in taenia.

The behaviour of taenial and circular muscle strips in the present study is consistent with the thesis that in the intact bowel, activity arises in the taenia, possibly controlled by slow waves. Evidence for this has also been deduced from studies of intact bowel segments[6]. If this is true, the human colon differs from that of the cat in which slow waves are generated in the circular muscle[3].

Table 34.1 Activity of colonic muscle *in vitro* and *in vivo*

In vitro	
Taenia	Spontaneous activity. Myogenic. Regular spikes. Slow waves in some strips. One rhythm per strip 22 ± 5 cpm. Intermittent activity. Tetanic contractions.
Circular	Not spontaneously active. Response to ACh with spike-bursts 1–12/min. Isolated contractions.
In vivo[1]	
	Slow waves. Spikes rare. Two rhythms. 3 cpm and 10 cpm. Intermittent activity.

The significance of the electrical and mechanical behaviour of the stimulated circular muscle to the function of the intact colon has yet to be determined (Table 34.1). However, it has been found that two different rhythms are present in the human colon *in vivo*[1], and no basis for this can be found

from the behaviour of the taenial strips alone, in which only one rhythm was found. It may be that the different behaviours of the two layers of colonic muscle are reflected *in vivo* by different electrical rhythms, which occur in association with different types of colonic movements, in which contraction of one or other of the muscle layers dominates. In making such a comparison of *in vitro* studies with the behaviour of the intact colon, significant differences in activity are seen. The rhythms differ in rate; that of taenia *in vitro* being twice the faster of the two rhythms recorded from the normal colon *in vivo*. Recordings *in vitro* are dominated by action potentials, which are rare *in vivo* where the main electrical events are sinusoidal slow waves. A final extrapolation from *in vitro* to *in vivo* activity will have to account for these differences.

Acknowledgements

We would like to thank B. H. Brown and R. H. Smallwood of Sheffield Area Medical Physics Department for their advice on electronic equipment and P. Robinson for much of its construction. F. D. Naylor and Mrs Jennie Clarke provided valuable technical assistance.

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Discussion

J. Christensen (USA): Could you tell us more about the preparation of the tissues? I ask because we have observed that the electromyogram of the cat colon seems to be very sensitive to hypoxia, chill, anaesthetics and other kinds of abuse.

D. Kirk (UK): All specimens of human colonic tissue have inevitably been 'abused' by the surgeon and anaesthetist before they come into our hands! This may account for a degree of variability in our results, and does produce the danger both that artifacts due to this abuse can occur and, secondly, that unexpected or unusual results could be rejected as the result of surgical trauma, when they might be of physiological significance.

H. L. Stockley (UK): (1) Has PGE_2 in low concentrations been tested on circular muscle strips to see if it counteracts the effects of indomethacin, as has been found on mechanical activity in human stomach and small intestine? This would increase your information on the specificity of action of indomethacin which is not a very selective inhibitor of PG synthetase. (2) Do you have any evidence that a PG might act by the non-adrenergic neurotransmitter, or do you think it more likely that there are two separate mechanisms of tonic inhibition in the circular muscle?

Kirk: (1) We have not tested the effect of PGE_2 on our strips. (2) I would not like to give a conclusive answer. The effect of indomethacin is different from TTX, suggesting the neural inhibition is distinct from prostaglandins. In a few strips, we have found that guanethidine does not mimic TTX, suggesting the inhibition to be due to non-adrenergic nerves.

J. D. Wood (USA): Your results indicate that the taenia coli are much more sensitive to acetylcholine than the circular muscle layer. How do you interpret this?

Kirk: This has been observed in tissue from other species. It is an impression only, but the sensitivity of the circular layer to ACh seems greater after TTX. The difference may be a further aspect of the neural inhibition of the circular layer.

A. Bennett (UK): May I first make a comment, mainly in reply to Dr Wood's question. We do not find substantial differences in sensitivities of the circular and longitudinal muscle of human colon, although in some animals, such as guinea pig and rat, the circular muscle of the small intestine is very poorly sensitive to acetylcholine. Next a point to Dr Christensen since this is relevant to the paper he just presented. The different effects of prostaglandin E compounds on the longitudinal and circular muscles of the gut has been known for many years, and the differential effect of indomethacin on these two muscle layers has been known also for a few years. Is it possible that the inhibition of migrating electrical complexes in the circular muscle of cat intestine is due to prostaglandin release?

Christensen: You may be right. We have not examined the possibility.

Section VII
Humoral Factors

35

Human lower oesophageal sphincter (LES) response to submaximal and maximal effective doses of synthetic human big gastrin (G-34) and gastrin I (G-17) *(Abstract)*

D. M. JENSEN, R. W. McCALLUM AND J. H. WALSH

Gastrin has been proposed as one of the regulators of human lower oesophageal sphincter pressure (LESP). G-17 in pharmacologic doses significantly increases LESP in man. We compared the effects of maximal and submaximal doses of synthetic G-34 and G-17 on LESP in five normal men. Subjects received, on separate days, rapid intravenous injections of 12.5, 25, and 100 pmol/kg of G-34, G-17 or saline. Serum gastrin levels were determined by radioimmunoassay. The mean LESP calculated for the 10 min period before each injection was considered the basal pressure. The mean change in LESP from basal was determined for continuous 2 min intervals after G-34 injection and 1 min intervals after G-17. Results: Maximal LESP increases occurred 2–6 min after G-34 injections and 1–3 min after G-17 injections. Peak responses to equimolar doses of G-34 and G-17 were similar, but the responses to G-34 were more prolonged.

The mean ($\pm$SE) changes in LESP from basal (mmHg) after saline bolus were: 0.1 $\pm$ 0.5 (2–6 min) and 0.5 $\pm$ 0.4 (14–16 min). The average half-time

Table 35.1 Mean (S + SE) Change in LESP from basal (mmHg)

Dose (pmol/kg)	2–6 *min*	14–16 *min*	1–3 *min*	14–15 *min*
12.5	7.5 $\pm$ 1.3	3.8 $\pm$ 0.7	7.8 $\pm$ 1.3	−0.7 $\pm$ 0.3
25	11.8 $\pm$ 0.6	10.3 $\pm$ 2.1	13.7 $\pm$ 1.0	−0.4 $\pm$ 0.3
100	17.7 $\pm$ 0.4	12.7 $\pm$ 0.3	21.5 $\pm$ 1.9	4.0 $\pm$ 0.6

of G-34 was estimated as 58 min. The serum concentrations of G-34 required to produce half maximal stimulation of LESP (300–400 fmol/ml) were well above the physiological range of G-34 responses to a protein meal (20–50 fmol/ml). We conclude that: (1) G-34 and G-17 both cause stimulation of LES contraction; (2) LES response to G-34 is more prolonged, consistent with the longer half time of G-34; (3) G-34 by itself it not likely to be a physiological regulator of LESP in man.

Discussion

J. H. Szurszewski: (USA) Did you try topical application of G-17 or G-34?

R. W. McCallum: (USA) No.

J. S. Davison: (UK) I have long been convinced that by itself gastrin is unlikely to be a physiological regulator of LESP, and this view is clearly confirmed by this study. However, it is important to consider possible interactions either with other hormones or with nerves. One such interaction between gastrin and ACh on the parietal cell is well established. One consequence of this interaction is that sub-threshold doses of gastrin become effective when combined with cholinergic excitation. This same interaction can be seen in smooth muscle preparations such as the ileum (Figure 1). Here the 'twitch' response to transmural

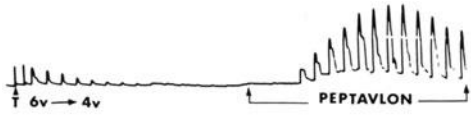

Figure 1

stimulation is enormously potentiated by a dose of pentagastrin which was previously without effect. These were isotonic contractions, but isometric recordings show the same result. It is premature to dismiss gastrin as a physiological regulator until the possibility of such interactions have been excluded.

McCallum: Again I would have to conclude that the cholinergic background remains constant and hence would have been included in the results of the present study. In addition, it has been found that cholinergic stimulation with, e.g., bethanechol, has no effect on the magnitude of the gastrin dose response of the LES in man, and that atropine has no effect on the gastrin dose–response of the LES in man.

E. E. Daniel: (Canada) The notion that very low ineffective levels of G-34 may be increasing LESP by potentiation responses to vagal or other nerves has frequently been put forward. If vagal activity was occurring at all times, this notion requires that the interaction be different before and after a meal. There is no evidence for this. If vagal activity to LES is assumed to be increased after a meal, this notion suggests that expression of G-34 effects will be inhibited by inhibition of vagal excitation. I know no evidence of this; but it could easily be tested by determining LESP changes to G-34 in the presence or absence of vagal stimulation (by meals) or by determining the sensitivity to LESP to G-34 with or without atropine. Would you comment?

McCallum: I would agree with you, Dr Daniel, in that the vagal activity would be constant and present at all times. Atropine has no effect on stimulation of LESP by G-17 and I would assume that a similar result would be operative for G-34.

G. Charbon: (Netherlands) Which is the decisive factor in stimulation with gastrin? (1) the change of concentration, or (2) the amount presented to the receptors per unit of time? In pharmacology it is assumed that only rarely is the change of concentration crucial, e.g. stimulation with potassium ions; mostly it would be the amount presented per unit of time. How does gastrin act in this respect?

McCallum: After a protein meal the major form of gastrin release is G-34 (making up 75% of the total gastrin) and this increase is 30–60 min after a meal. At this time LESP is also increasing. In our study with G-34 we also simulated the prolonged exposure to gastrin by measuring sphincter pressure after G-34 injection for 45 min. This degree of potential exposure of the receptor in the LES to gastrin would be adequate.

D. O. Castell: (USA) The use of bolus injections may be potentially confusing since the transient high serum gastrin levels may have poor relationship to tissue receptor site levels. In previous studies from our laboratory using slow infusion rates of heptadecapeptide gastrin we did show significant increases in LESP with serum gastrin levels well within a physiologic range.

McCallum: G-34 has a disappearance half-time of approximately 45 min. We studied serum gastrin concentrations of G-34 at 30 min when concentrations were relatively stable. We have since followed up this study with another study where G-34 was infused at the rate of 100 pmol/kg/h. Again the delta gastrin G-34 necessary for a threshold increase in LESPs was 90–100 fmoles/ml – above the delta G-34 response to a meal of 31 fmoles/ml.

A. Bennett: (UK) You properly said that G-34 is unlikely to be the single factor in controlling LESP, but it is important to infuse both gastrins to study their importance. I take issue with Dr Daniels' argument that if there is a background release of ACh it will be constant. As a result of feeding many pathways are brought into play, and gastrins might interact with them as part of a physiological control mechanism.

McCallum: There is some suggestion from acid stimulation studies in dogs that the effect of G-34 with a background infusion of G-17 may be more potent than when G-34 is given alone. The interaction of both gastrins (G-17 and G-34) together will no doubt be studied in order to evaluate this question of potentiation. Present indications are that the gastrin concentration required to increase sphincter pressure will still be outside the physiological range.

36

The effects of thyrocalcitonin on pentagastrin-induced contraction of the lower oesophageal sphincter in normals and in patients with achalasia *(Abstract)*

J. DEBAT, D. COUTURIER, C. ROZE AND C. DEBRAY

Thyrocalcitonin (TCT) is known to decrease gastric secretion in man through a mechanism involving probably both a direct effect on the secretory cells and an inhibition of gastrin release. Pentagastrin (PG) increases the pressure in the lower oesophageal sphincter (LES). These experiments were devised to investigate a possible antagonism between TCT and PG on the LES pressure (LESP).

METHODS

Fifteen control subjects with normal oesophageal motility and fifteen patients with untreated achalasia were studied. A four-tube, laberal hole pressure probe assembly was used, infused with water at 1 ml/min. One of the tube tips was maintained in the LES throughout the whole measurement period. Pressure determinations were done every minute and expressed relative to the gastric expiratory pressure. After the basal level was obtained, a venous bolus injection (1 min) of PG (0.125 or 0.25 μg/kg) was administered. Fifteen minutes later began a venous infusion of synthetic salmon TCT (1 or 2 U/kg/h); after a further 10 min another bolus injection of PG was administered over the TCT infusion.

RESULTS

(a) Basal pressures were 24.9 $\pm$ 2.38 in controls and 44.9 $\pm$ 3.22 cm water in achalasia patients; (b) TCT alone displayed no significant effect on basal

pressure in either group; (c) pressure increased to 33.8 $\pm$ 7.76 in controls and to 85.2 $\pm$ 10.9 cm water in achalasia patients, within 3 min after injecting 0.25 μg/kg PG; (d) the increase in LESP induced by PG was completely suppressed by TCT (1 U/kg/h) in controls; it remained unchanged in achalasia patients, even when doubling the dose of TCT to 2 U/kg/h.

CONCLUSIONS

The motility disturbances observed in achalasia are generally thought to be related to oesophageal smooth muscle denervation. The results reported here are consistent with the hypothesis of TCT acting on the LES through an inhibitory nervous pathway, which would be lost or deficient in achalasia.

Discussion

I. Taylor (UK): Were you satisfied that the fifteen patients were suffering from achalasia? How did you make the diagnosis?

D. Couturier (France): The diagnosis was suggested from clinical and radiological considerations and established with a manometric investigation. In all patients we observed dysperistaltism of the corpus of the oesophagus, high resting pressure in the LES, and achalasia of the sphincter.

A. Bennett (UK): Calcitonin inhibits gastric acid secretion. Does this influence the response of the sphincter?

Couturier: Calcitonin only decreases basal gastric acid secretion 20–40% in long-lasting experiments. We do not measure gastric acid secretion in the present study. I think that the change in secretion may be very small during the 20 min infusion of calcitonin.

M. A. Cook (Canada): Calcitonin is a potent hypocalcaemic hormone. Did you measure the plasma calcium concentration in your subjects following administration of calcitonin and, if so, did it change?

Couturier: In some subjects we measured plasma calcium concentrations; as in other studies using the same doses of calcitonin we did not observe any significant change in calcium concentration.

R. W. McCallum (USA): The other hormones that inhibit acid secretion will, in bolus doses, decrease sphincter pressure. I wonder why you did not use bolus doses of thyrocalcitonin in order to evaluate the question of whether thyrocalcitonin will decrease resting LESP? The serum calcium in your study did not change and I am concerned that your infusion dose of thyrocalcitonin may not have been adequate.

Couturier: It was generally considered that a slow continuous infusion of exogenous hormone is a better procedure to investigate an eventual action. When this study was undertaken the doses of 1 and 2 U MRC/kg/h were often used in the studies on gastric and small bowel secretion. We know now that the doses used here are strong and probably unphysiological.

37

Comparison of the biological activity of pentagastrin, G-17 and G-34 on canine antral motility and intracellular electrical activity *(Abstract)*

K. G. MORGAN, V. L. W. GO AND J. H. SZURSZEWSKI

The intracellular microelectrode technique was used to determine the effects of pentagastrin, G-17 (synthetic human gastrin I) and G-34 (natural human G-34 I) on the electrical activity of single cells of the circular layer of canine antral muscles. Mechanical activity of the cells was simultaneously monitored by attaching a transducer to measure tension in the direction of the long axis of the circular fibres. The preparation measured approximately 2 × 7 mm. In this tissue, the action potential consists of an upstroke potential followed by a plateau potential. Changes in the strength of contraction are related to changes in the size of the plateau potential. All three forms of gastrin increased the action potential frequency and the amplitude and duration of the action potential plateau. Similarly, the frequency and force of contractions were increased. Dose–response curves for the effects on electrical activity were determined for single cells. The mean ED_{50} for the effect of pentagastrin on plateau amplitude was $5 \times 10^{-12} \pm 1.5 \times 10^{-12}$ M ($n = 4$) and on frequency was $5.5 \times 10^{-10} \pm 2.5 \times 10^{-10}$ M ($n = 3$). G-17 was found to be slightly less potent, with ED_{50} for plateau amplitude and frequency being $3.9 \times 10^{-11} \pm 2.1 \times 10^{-11}$ M ($n = 4$) and $2.0 \times 10^{-9} \pm 0.4 \times 10^{-9}$ M ($n = 3$), respectively. This difference in potency was confirmed by determining the dose–response curves for both agents in a single cell. Although there is a difference in potency, the comparison of both agents in a single cell indicates that the efficacies are similar. For both G-17 and pentagastrin, the effects on the size of the action potential plateau occurred in a lower concentration range than the effect on frequency. The effects on the plateau occur within the

range of concentrations of gastrin present in the blood after a meal, whereas the effects on frequency are probably relevant only with respect to conditions of hypergastrinaemia. A sufficient quantity of G-34 was available for a single dose (3×10^{-10} M) but not for an entire dose–response curve. This dose was effective in increasing the size of the action potential plateau, action potential frequency, and the frequency and force of contraction. The effects of this dose were compared with the dose–response curve for G-17 obtained from the same cell. The results indicate that G-34 may have a greater biological activity than G-17 both on electrical and mechanical activity. We conclude that both G-17 and G-34 have physiological actions on canine gastric antral motility.

Acknowledgement

This work was supported by USPHS NIH AM 17238.

Discussion

E. E. Daniel: (Canada) After atropine ± hexamethonium, responses of the canine antrum to intravenous or intra-arterial pentagastrin are inhibited, suggesting that there is a receptor involving release of acetylcholine. Also there is no* inotropic response to pentagastrin after appropriate cholinergic blockade. Thus there must be another receptor involving nerves to pentagastrin in the stomach. This does not eliminate the conclusions about the effectiveness of gastrins in a muscle receptor *in vitro*, but raise the question of which receptors are involved *in vivo*. Please comment.

J. H. Szurszewski: (USA) Our studies have been concerned with the receptors for gastrin located on the postsynaptic cell – the smooth muscle cell. That there are such receptors is evident from the data presented. We too find receptors for gastrin on cholinergic nerves or their terminals because atropine and tetrodotoxin reduce the peak maximum response to about 15%. Recently, Strunz and Grossman have confirmed *in vivo* our own observations. Strunz and Grossman's studies were done in the unanaesthetized dog. However, in our studies, and the one mentioned, the primary effect of gastrin appears to be on the antral circular smooth muscle cell. Our own studies do not exclude the possibility that receptors for gastrin may also be located on sites in addition to those receptors located on the antral circular smooth muscle cell.

J. S. Davison: (UK) The full physiological significance of such responses ultimately will depend upon their interaction with other mechanisms, nervous or hormonal. Have you examined any interactions between the gastrins and any other hormones, neurotransmitters or cholinergic nerve stimulation?

Szurszewski: Yes. We have previously described the combined action of pentagastrin and acetylcholine on the longitudinal muscle of the canine antrum. In this muscle acetylcholine increased the size of the plateau potentiated and the strength of contraction and pentagastrin increased the frequency of contraction. Presently we are investigating electrical and motor activities when two or more gastrointestinal hormones are present together.

R. W. McCallum: (USA) What do you anticipate your data would show when applied to humans? Present information based on camera (gamma-camera) technique for gastric emptying of a meal, using a semi-solid meal and physiologic infusions of G-17, do not show any effect on gastric emptying rate.

Szurszewski: Our results obtained from human stomach *in vitro* – which I did not have time to present – indicate that pentagastrin and gastrin have much the same effects as those just described for canine antral muscle.

* See Cook *et al.* (1974). *Proceedings of the Fourth International Symposium on Gastrointestinal Motility.* (Vancouver: Mitchell Press).

I would not want to comment on gastric emptying, for the correlation between type and nature of motor contraction induced by neurotransmitters and hormones and gastric emptying is not understood.

J. A. J. Schuurkes (Holland): I would like to add some *in vivo* data that confirm your statement that contractile force is far more sensitive to pentagastrin than is contractile frequency. We measured antral contractile activity in the anaesthetized dog with strain gauges and made a dose–response curve with intravenous bolus injections of pentagastrin. The effective dose to obtain half maximal response for contractile force was 128 ng/kg. To obtain half maximal response for contractile frequency a five times higher dose was needed; that is 630 ng/kg.

Szurszewski: Thank you.

38

Direct measurement of pyloric diameter and tone in man and their response to cholecystokinin

J. F. MUNK, R. M. GANNAWAY, M. HOARE AND A. G. JOHNSON

There is increasing evidence that gastric ulceration is the result of an incompetent pyloric sphincter allowing reflux into the stomach[1,2]. Duodenal contents, containing bile and other substances, lower gastric mucosal resistance and the resulting gastritis, more common in the antrum, predisposes to gastric ulceration[3,4]. The normal pyloric sphincter not only has some effect on the gastric emptying of liquids and the selection of particulate size of solids, but also prevents duodenogastric reflux. Why does an apparently normal pylorus sometimes allow reflux to occur? To prevent reflux the timing of pyloric closure is important, as any process which upsets this dynamic sequence of events in the antropyloric and duodenal regions causes an alteration in the rate of gastric emptying and/or duodenal reflux[5].

Local, hormonal or neural factors may affect the motility of this region. Fisher and Cohen[6] have described pyloric dysfunction in gastric ulcer patients when cholecystokinin and secretin failed to increase the pyloric pressure when compared with normal subjects. They suggested that this lack of response could explain why the pyloric sphincter allows reflux. This paper describes a method of direct endoscopic measurement of the pyloric diameter and the assessment of pyloric tone and their response to exogenous cholecystokinin.

METHODS

A Fogarty balloon catheter is calibrated by inflating the balloon with a standard volume of air. Concentric rings are drawn around the proximal part of this balloon and the diameter of each ring is measured with a micrometer (Figure 38.1).

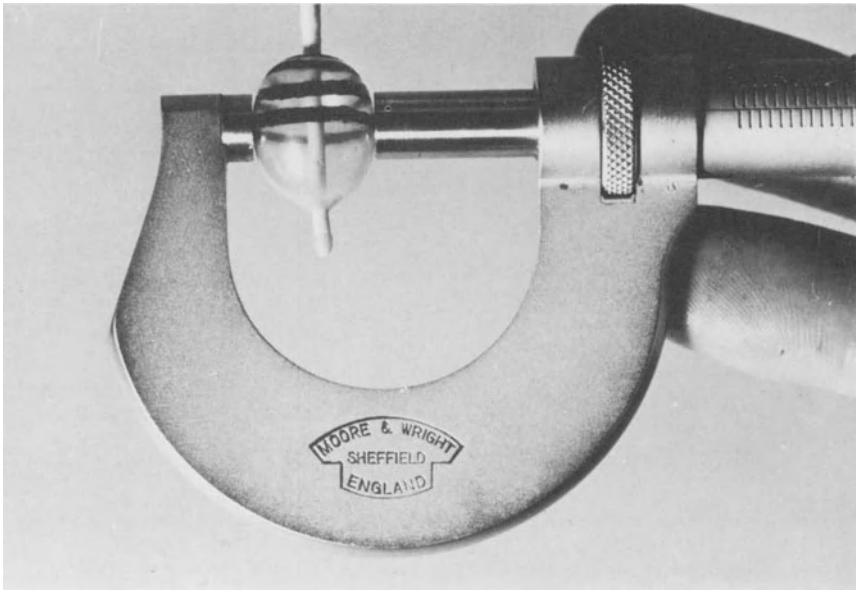

Figure 38.1 This photograph shows a micrometer measuring the diameter of the rings drawn on an inflated balloon of a Fogarty balloon catheter

Consenting patients are fasted overnight and intravenous diazepam only is administered prior to endoscopy. Initial gastric inflation is minimized, and is just sufficient to visualize the pylorus clearly. Rarely is further air insufflation needed during each study. The deflated Fogarty balloon catheter is passed into the duodenum through the biopsy channel of the fibre-optic endoscope. The same standard volume of air is used to inflate the balloon. Between antral contractions the inflated balloon is withdrawn against the pyloric sphincter and two observers judge the number of rings visible through the pylorus (Figure 38.2). Only those subjects with rhythmic antral contractions are studied, to ensure that the motility has not been disturbed by the procedure. Duodenal contractions are recorded by the balloon while it is in the duodenal bulb. The endoscope was not introduced through the pyloric sphincter at any stage during the study, and we found that diazepam did not interfere with the rhythm of antral contractions.

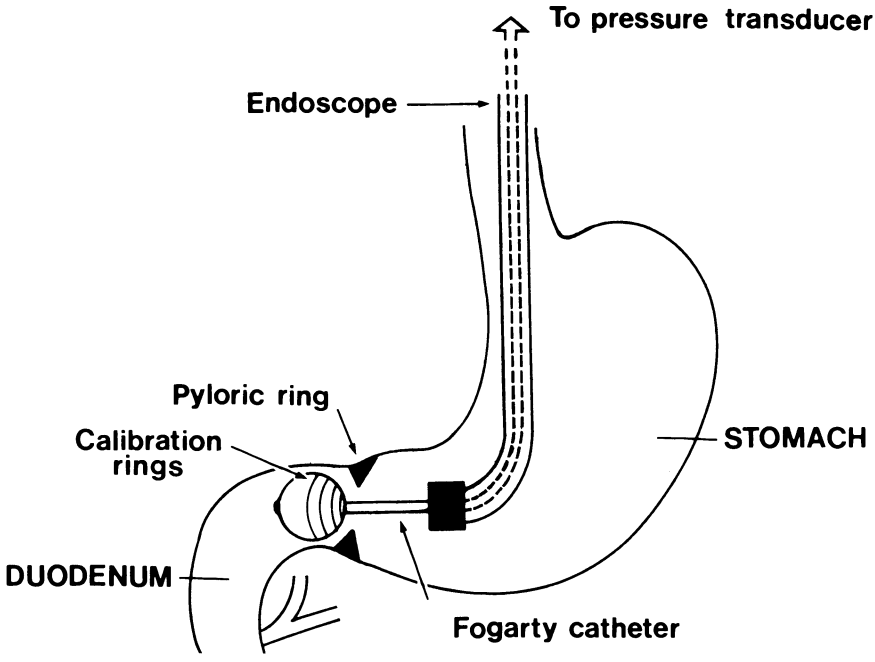

Figure 38.2 This schematic representation shows the inflated balloon of the Fogarty balloon catheter in the duodenum. The concentric rings can be seen through the endoscope

The tone, or resistance to stretch, of the pyloric sphincter is measured by withdrawing the balloon through the sphincter into the stomach. The lumen of the balloon is connected to a pressure transducer and simultaneous pressure changes can be visualized on an oscilloscope and ultraviolet recorder. After three basal measurements of diameter and pressure have been made cholecystokinin (GIH, Karolinska), 1 IDU/kg, is injected intravenously over 2 min and pyloric diameter and pressure measurements are recorded each minute for 10 min. Control injections of normal saline administered to patients in each group showed no alteration in response. Diagnostic endoscopy is then performed, usually with the later addition of atropine.

RESULTS

We have studied twenty-nine patients, comprising five with no gastroduodenal disease, nine with gastric ulceration, nine with gastritis and six with duodenal ulceration (Table 38.1). Patients with gastric and duodenal ulceration were included only if an active ulcer crater was seen and if biopsies, taken in the case of gastric ulcers, demonstrated no malignancy. Patients with merely scarring, stenosis or deformity were not included. Gastritis was usually confirmed by biopsy, and intestinal metaplasia was also present in

five of the nine patients with gastritis. Patients regarded as having no gastroduodenal disease were those with no abnormality after endoscopy, a normal barium meal and cholecystogram where indicated.

Table 38.1 Details of the Patients Studied

Group	*Number*	*Mean age* (years)
Normal	5	35
Gastric Ulcer	9	61
Gastritis	9	52
Duodenal ulcer	6	41
	29	

Basal pyloric diameter

We found that gastric ulcer patients had a significantly larger resting pyloric diameter 16.0 $\pm$8 mm (mean $\pm$ 2 SD) than the duodenal ulcer patients, 6.4 $\pm$ 9 mm ($p < 0.05$, Mann–Whitney **U** test). We also found that gastric

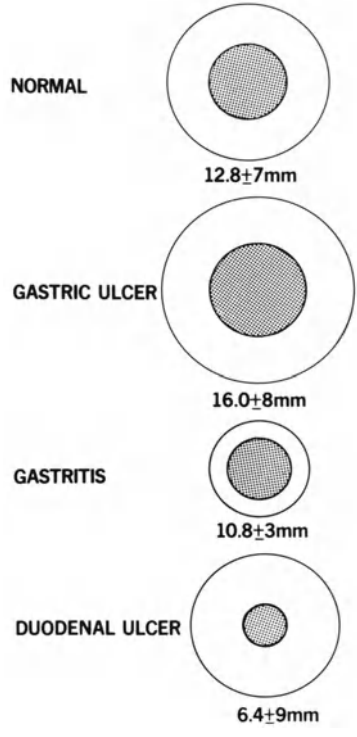

Figure 38.3 Comparison of the resting pyloric diameters ($\pm$ SD)

ulcer patients had a larger resting pyloric diameter than normal patients, but this was not significant (Figure 38.3).

Response to cholecystokinin

Twenty-five of the twenty-nine patients were given cholecystokinin (CCK). The change in diameter in response to CCK occurred within 2–3 min after the injection, and the response was significantly greater in gastric ulcer patients than in normals, patients with duodenal ulceration and those with gastritis (Figure 38.4). In only one subject, a duodenal ulcer patient, was the pylorus tightly closed prior to administration of CCK and this was not included in assessing response.

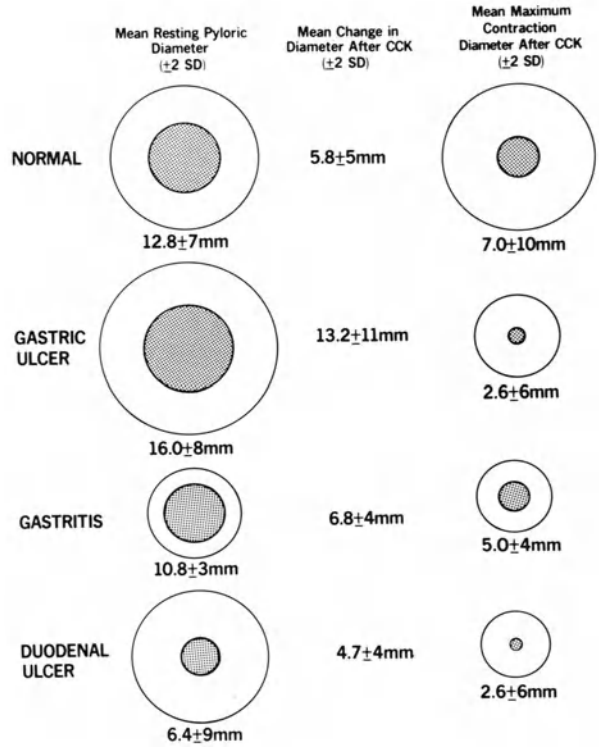

Figure 38.4 Comparison of the resting pyloric diameters before and after CCK (1 IDU/kg.) In particular note the change after CCK

Pull-through pressures

Pull-through pressures were inversely proportional to the diameter of the pylorus, and were only recordable if the diameter of the pyloric sphincter

was at least 1 mm smaller than the maximum balloon diameter. As might be expected the basal pull-through pressure in duodenal ulcer patients was the highest, gastric ulcer patients the lowest, and that of normals between these two.

When the pylorus contracted in response to CCK the pull-through pressure showed an inverse relationship to the diameter of the contracted pylorus. The rise in pressure confirmed that the change in diameter was not due to mucosal prolapse or redundancy. In five of eight gastric ulcer patients no pressure rise was recorded even when the balloon diameter ranged between 11 and 13 mm (Figure 38.5).

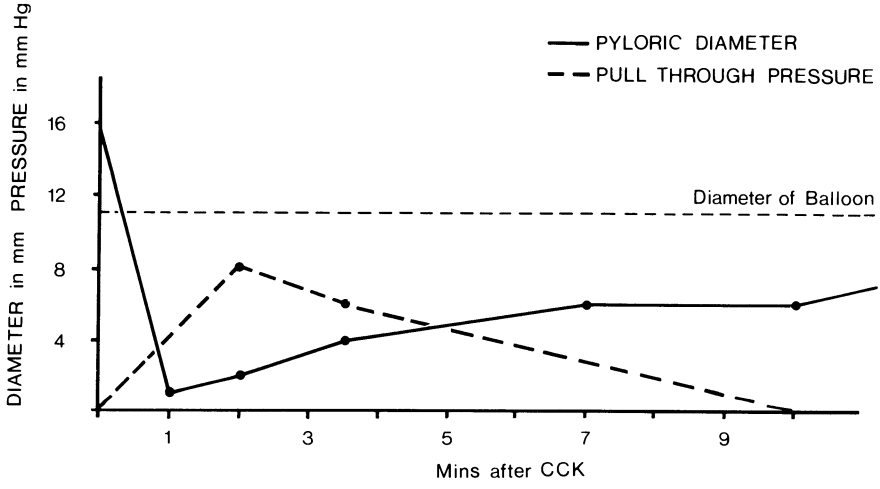

Figure 38.5 Comparison of the resting and stimulated pyloric diameters with pull-through pressures at intervals after the administration of CCK in a male gastric ulcer patient. The resting pyloric diameter was greater than the diameter of the balloon and did not show any pressure rise. When the pylorus contracted there was a pressure rise, and when the effects of the CCK waned the pylorus relaxed and the pressure decreased

Reflux

Reflux was seen in nearly all patients with gastric ulcer and gastritis, and only in one normal and one duodenal ulcer patient (Table 38.2). Gastric ulcer and gastritis patients showed significantly more reflux that duodenal ulcer patients ($p < 0.05$, Fishers Exact Probability test). Reflux occurred in three of the nine gastric ulcer patients prior to the administration of CCK, and in four afterwards. In none of these four patients did the pylorus close completely, and reflux occurred after the maximum response of the CCK. It is suggested that reflux occurs more frequently in gastric ulcer patients during the fasting basal state when compared to the other groups; but no definite conclusion can be gained as the number of patients studied was small.

There was no correlation in individual subjects in any group between the incidence of reflux and pyloric closure.

Table 38.2 The incidence of reflux in each of the groups studied. The incidence of reflux was significantly greater in the gastric ulcer and gastritis patients when compared with the duodenal ulcer patients

Group	*Number*	*Incidence of Reflux*
Normal	5	1
Gastric ulcer	9	7*
Gastritis	9	7*
Duodenal ulcer	6	1*

* $p < 0.05$

DISCUSSION

Our results show that the resting (relaxed between antral contractions) pyloric diameter in gastric ulcer patients is greater and shows a greater response to exogenous CCK when compared to normal patients, and patients with duodenal ulceration and gastritis. We found that no pressure increase occurred if the pyloric diameter was greater than the diameter of the recording device – in this case a balloon. Fisher and Cohen (1973)[4] recorded a rise in pressure when a 5.4 mm diameter fluid-filled catheter assembly was withdrawn through the pyloric sphincter in gastric ulcer patients in the right lateral decubitus position. To record a pressure rise this assembly must be: (i) withdrawn through an aperture smaller than the assembly; (ii) impinge against the side wall of the pylorus; or (iii) be withdrawn at an angle to the pylorus. The pressure rise on Fisher and Cohen's recording is present over a distance of 2–2.5 cm with the gastroduodenal mucosal junction in the mid-third. Our patients were studied in the supine position, and the pyloric ring and the state of the antrum was directly visualized at endoscopy. No component of the thickened terminal antrum was included in these measurements.

We agree with Valenzuela and Difilippi[7] that the resting pyloric pressures in normal patients are greater than those in gastric ulcer patients but less than those in duodenal ulcer patients. We found that the mean resting pyloric diameter of normal patients, lying in the supine position was 12 mm and Kaye *et al.*[8], using a 5.4 mm diameter pressure recording assembly, and also a miniature pressure transducer, found no high-pressure zone in the normal pylorus, with the patient in the supine position. Atkinson *et al.*[9] used balloon catheters and found that the pyloric channel offered little or no resistance to being stretched to a diameter of 7–12 mm. Our results are in agreement with these findings.

Valenzuela and Defilippi[7] and Fisher and Cohen[6] found that the pyloric pressure in gastric ulcer patients did not alter after infusion with HCl into

the duodenum. It was assumed that HCl caused the release of endogenous CCK and secretin. However Fisher and Cohen administered secretin and CCK, which showed a similar lack of response. They administered CCK, 2 IDU/kg/h, by continuous intravenous infusion, whereas we used a bolus injection of 1 IDU/kg intravenously over 2 min. These doses are different, but unlikely to explain the different findings.

Our results show that the resting pyloric diameter is larger in gastric ulcer patients when compared with patients with duodenal ulcer. The response to CCK in gastric ulcer patients is dramatic. Pyloric reflux does not appear to be due to a failure of contraction in response to CCK. Only a completely closed pylorus will prevent reflux, and for gastric emptying to occur the pylorus must be open for a large part of the antral cycle. The key to prevention of reflux is the timing of pyloric closure in relation to duodenal contraction. CCK may narrow the resting diameter to make the closure more complete or of longer duration during the antroduodenal cycle.

Acknowledgements

We are indebted to Sisters Monk and Ilyffe for their assistance, and to Dr K. McCrae for his statistical advice in the preparation of this paper.

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Discussion

A. Dubois: (USA) I am not sure how pyloric sphincter measurements correlate with gastric emptying. However, in contrast to the difference you detected between duodenal and gastric ulcer, we found that they react in the same fashion to another peptide, pentagastrin. In fact, whereas pentagastrin decreases fractional emptying in normal subjects, it increases fractional emptying both in gastric ulcer and duodenal ulcer patients. We have not tested the effect of CCK as yet.

J. Munk: (UK) During these studies we have not used pentagastrin. It would be very interesting to study the effects of both CCK and pentagastrin in duodenal and gastric ulcer patients. Our duodenal and gastric ulcer patients responded to CCK in a similar way but the response was greater in gastric ulcer.

R. Earlam: (UK) There is considerable controversy as to whether a high-pressure zone exists at the pylorus. This may be due to variations of technique in relation to the position of the patient. In what position were your patients and did you notice any changes in diameter in different positions?

Munk: All patients were studied in the supine position during the procedure. We did not vary this protocol, so we have not had the opportunity to observe any change in the diameter of the pylorus in various positions.

H. J. Ehrlein: (Germany) To understand the mechanism of duodenal reflux it is important to know in which period of digestion reflux mainly occurs. Do you know if reflux occurs in human patients mainly during the postprandial period or in the empty stomach during the interdigestive period?

Munk: All our patients were fasted overnight and no studies were performed without the patient fasting. There is evidence that reflux occurs both in the fasting and postprandial situations (Rhodes[2]; Capper[1]).

R. Bass: (USA) I am a little concerned that the area under study is different among the normal controls and those people with pathology. Could the different response between normal and diseased patients be due to: (a) looking at different areas of the terminal antrum and pylorus? (b) different groups of patients demonstrating a different sensitivity to CCK?

Munk: At endoscopy we could see that CCK produced antral inhibition and the antrum became flask-shaped and there could be no mistake in identifying the pylorus (see Figure 38.2). Our data do not enable us to interpret whether different groups demonstrate varying sensitivities.

A. M. Connell: (USA) Endoscopy in itself has marked effects on gastrointestinal motility. Have you been able to assess the influence of the endoscope? As a corollary, were there any differences in the details of the procedure used?

Munk: Three measurements of the resting diameter were made between

antral contractions, and these were made over several minutes. Each diameter did not vary within 1 mm in the great majority of patients. At all times measurements were taken only when antral contractions were present and these were rhythmic. Because of the decreased apprehension and the inhibition of retching we thought that the diazepam was beneficial. We did not observe that the endoscope had any effect on the study.

N. Painter: (UK) It is generally accepted clinically that gastric ulcers are associated with gastric retention. They heal after drainage of the stomach. Yet your gastric ulcer patients had the most widely open pylorus. Have you any comment to make on this?

Munk: Gastric ulcers are associated with delayed gastric emptying, but emptying does not depend on the diameter of the pylorus. It is the strength of the duodenal contractions, and also the strength of the antral contractions in association with the degree of linkage between the antrum and duodenum, that is important.

A. L. Blum: (Switzerland) In contrast to gastric ulcer, the pylorus in duodenal ulcer is not round but invariably deformed. How did you measure the diameter in these cases? We measured pyloric size before and after the passage of an endoscope; it was nearly twice as large after the passage. Did the same thing happen when you pulled your balloon catheter through the pylorus? Would it not be more meaningful to perform these studies in patients with healed ulcers where the oedema at the pylorus is minimal? Did you exclude patients with scars at the pylorus?

Munk: The majority of cases showed no deformity. However, there were cases in which the pylorus was assymetric and even oval. If the diameter was larger than the balloon the balloon was manipulated to one side and the remaining diameter was compared with the diameter of the balloon. It was possible to 'guess' the remaining diameter. If the diameter was smaller than the balloon it was possible to estimate the greatest diameter. Where the diameter was oval the greatest diameter was measured. We did not study any patients with pyloric channel and pre-pyloric ulcers. Also we found that the duodenal ulcer patients did not exhibit a great degree of oedema, which would have interfered with our studies. We did not insert the endoscope through the pylorus, and the balloon was only inserted through the pylorus between antral contractions; the three basal measurements did not vary to any great degree.

W. J. Hogan: (USA) I am still concerned over the introduction of air during the endoscopic procedure. Do the authors know how much air they actually used in each study? Do they know what graded changes in air insufflation may do to pyloric diameter and configuration? Generally, air insufflation is necessary to continue pyloric visualization during endoscopy.

Munk: We kept the amount of air used to a minimum, and after the initial observation of the pylorus rarely was any further air used in each study. We did not measure the total amount of air used in each study. We do not know what graded changes occur but there was minimal variation in the basal diameters over several minutes when these measurements were taken.

J. Christensen: (USA) Was there a signficant difference in the resting diameter between gastric ulcer patients and normal subjects? Did you see any change in the resting diameter with healing in the gastric ulcer patients?

Munk: The mean resting diameter in gastric ulcer patients was greater than normal subjects but there was no significant difference. We only studied nine gastric ulcer patients and five normal subjects. These numbers were small and if the numbers were greater, then this may be significant. We did not repeat our studies on these gastric ulcer patients to note any changes after healing.

DISCUSSION

S. Cohen: (USA) Fisher has suggested that the pyloric abnormality in response to CCK in patients with gastric ulcer is reversible. Is it possible that endoscopy reversed this abnormality to CCK response? Jokingly, perhaps endoscopy is a good treatment for abnormal sphincter responses in gastric ulcer.

Munk: We noted that during endoscopy the rhythmic antral contractions were not altered. Also it must be noted that the doses of CCK that we used were 1 IDU/kg intravenously over 2 min, whereas Fisher used 2 IDU/kg/h by infusion. Also acidification of the antrum may have caused the inhibition of gastrin release when the effects of healing were studied.

K. Kelly: (USA) Was the smaller diameter of the pylorus in duodenal ulcer compared to controls due to fibrosis and stenosis, or due to a greater pyloric tone?

Munk: We think that this smaller diameter of the pylorus in duodenal ulcer patients is due to an increased tone. Patients with scarring or deformity, observed at endoscopy, were excluded from the study, as also were patients with pyloric channel and pre-pyloric ulcers. Our duodenal ulcer patients had early ulcers. Patients with fibrosis would be expected to show little alteration in diameter, but this did not occur.

39

The relationship between gastric inhibitory peptide and right colon electromechanical activity after feeding *(Abstract)*

L. F. SILLIN, R. E. CONDON, W. J. SCHULTE, J. H. WOODS, P. BASS AND V. W. L. GO

After feeding, colon motor activity increases. Instead of a neurally mediated 'gastrocolic reflex', the increase in colon contractions more properly should be termed a 'gastrocolic response'.

Four preconditioned, stump-tailed monkeys had paired extraluminal strain gauge transducers and bipolar electrodes placed 5 cm orad to the ileocaecal valve, on the caecum, 3 cm aborad to the ileocaecal valve, and 7 cm aborad to the hepatic flexure to record electrical potentials and muscle contractions. Colon mechanical activity and electrical potentials were recorded continuously from 30 min prior to 90 min after stimulation. Recordings were made during fasting intervals and in response to feeding (150 g monkey chow); saline (150 ml by nasogastric tube), and $MgSO_4$ (0.1 g/kg in 150 ml H_2O by nasogastric tube) in a randomized sequence. Serum gastrin (G), cholecystokinin (CCK) and gastric inhibitory peptide (GIP) were measured prior to and 15, 30, 60 and 90 min after stimulation.

Stomach to caecum transit time in these animals is approximately 90 min. After feeding, right colon electromechanical activity increased within 30 min (3.3 ± 1.5 to 7.0 ± 1.0 contractions/6 min; $p < 0.01$). Similar responses were demonstrated in the caecum and transverse colon. Saline and $MgSO_4$ did not increase colon activity.

Serum GIP increased within 15 min of feeding compared with fasting (224 ± 55 to 1556 ± 406 pg/ml; $p < 0.05$) and was maximal at 90 min (424 ± 238 to 3055 ± 569 pg/ml; $p < 0.01$). No changes in G or CCK were seen after stimulation.

Increased colon electromechanical activity post-feeding appears not to be neurally mediated (30 min response delay) nor due to release of G or CCK (no increase in serum concentration). The association of elevated serum GIP, temporally related to increased colon motor activity, suggests that release of GIP in response to feeding may mediate the 'gastrocolic response'.

Discussion

H. J. Ehrlein: (Germany) A comment on the response in rabbits: in fasted rabbits caecal motility is increased immediately feeding begins. When the stomach is inflated with a balloon, caecal motility again is immediately increased. Therefore I think that in rabbits stimulation of the large intestine by feeding is a complex mechanism starting with pharyngocaecal and gastrocaecal reflexes, and then probably prolonged by hormones. If you have seen no immediate response in monkeys during feeding, there must be species differences.

L. F. Sillin: (USA) We saw no marked early response in our animals. I agree there must be a species difference between the monkey and the rabbit.

H. Pescatori: (Italy) Do you not think that you should have measured the different substances possibly responsible for gastrocolic response after you had varied the composition of meal (in terms of lipids, glycides and proteins)?

Sillin: I do not think that it would be an interesting study to look at the effects of various dietary components on this response.

A. L. Blum: (Switzerland) My own gastrocolic response starts immediately after eating. The early reports on 'gastrocolic reflexes' also describe such a time-relationship. How can you reconcile this with your late response?

Sillin: The 'classic gastrocolic reflex' after meals is well known to us all. As I previously mentioned, we feel that the proximal portion of the colon may be a functionally different organ than the distal colon. It is the distal colon and rectosigmoid which provides for the sensations we feel.

W. J. Snape, Jr.: (USA) This was a very nice study. There seems to be some difference between your studies in the proximal colon of the stump-tailed monkey and our findings in the distal colon of man. We have shown that the postprandial stimulation of colonic spike and motor activity in man is immediate and is temporally related to an increase in serum gastrin. Also we could reproduce this colonic response with continuous infusion of gastrin, and we can block the meal-related stimulation of colonic motility with an anticholinergic.

Sillin: We suspect that there are significant differences between the proximal colon and the distal colon.

J. Christensen: (USA) How have you excluded the possibility that the increased contractions of the colon make the GIP levels go up?

Sillin: The rise in GIP distinctly precedes the rise in right colonic activity. However, further experiments are planned to determine whether or not your suggestion is indeed the case.

Y. Ruckebusch: (France) Are there not an early and then a late response of the ascending colon in the stump-tailed monkey? Both these responses are recorded in dogs, pigs, etc.

Sillin: We do see a rise in activity at 15 min which may represent your

suggested bimodal response. However, our method of analysis for this report may blur this.

A. R. Cooke: (USA) I do not see a causal relationship between the GIP levels and the motility. Have you administered exogenous GIP to determine if it can mimic the motor activity you see?

Sillin: We make no claim of having proved a causal relationship; merely that GIP must be considered as a candidate hormone for mediating this response. We have obtained GIP from Dr Brown in Vancouver and our GIP administration data are very preliminary at the present.

D. L. Wingate: (UK) (1) The absence of gastrin or CCK response is disturbing. How reliable are the assays? (2) Your data do show a rise (nearly double basal levels) at 15 min and we would guess that further experiments will bring this to significant levels – therefore your response might not be as delayed as you think. (3) Have you looked at insulin?

Sillin: (1) The motor activity of the ascending colon in response to the experimental states and the gastrin and CCK levels show no change. Rosatto and others have demonstrated a rise in serum gastrin levels in response to feeding and antral distension in Rhesus monkeys. Even if there is a defect in the gastrin analysis the 'wet' meal (saline) given should produce sufficient antral distension to release gastrin, and this stimulus did not produce a rise in the proximal colonic activity. $MgSO_4$ is a known CCK releaser and this also failed to stimulate a colonic response. Therefore, even if the assay is not sensitive to this particular species the releasing stimuli are present to produce the hormone release and no colonic response was seen. (2) These animals are not the only ones we have studied in this fashion and the results are consistent with these. (3) Not yet.

H. G. Beger: (Germany) First a comment: gastrointestinal hormone measurements in animal experiments, as well as in human studies with blood sampling in a peripheral vein, may not reflect the actual events in the release of intestinal hormones. The gastrointestinal hormones first enter the splanchnic blood circulation, and therefore one has to consider in such mechanisms as dilution in portal blood, sequestration in the liver and inactivation by the liver. Did you measure GIP, gastrin and CCK also in the portal vein blood? If not, you cannot say there is no change in gastrin or CCK after stimulation.

Sillin: We did not measure hormone levels in portal blood. As I stated in answer to another question (Dr Wingate), we saw no rise in activity in response to stimuli known to release either gastrin or CCK, and so it seems unlikely that these hormones mediate the observed response to the dry feeding.

Section VIII
Colonic Control

40
Colonic motility

J. CHRISTENSEN

I do not intend, in the few minutes I have, to try to review all the information of recent years bearing on the matter of motility in the colon. There is not enough time. Similarly, I cannot presume to summarize a large body of data to give you an overall picture of colonic motility. There is not enough information. Thus, I find myself in a strange position, with not enough time to give you all the information, and not enough information to give you a complete picture. But this characterizes the current state of the subject. I can only use the available time to point out to you some of the problems that have so hampered the study of motility in the colon.

I would like to begin by quoting the words of a citizen of our host country, Scotland. R. C. Garry, a physiologist from Glasgow, wrote a long review of this subject in 1934[1]. His opening paragraphs neatly summarize the early history of the matter:

> In the latter half of the nineteenth and in the beginning of the twentieth century the problems of gastro-intestinal physiology in general, and of the physiology of the large bowel in particular, attracted many workers. This phase of experimentation on lower animals culminated in the work of Langley and Anderson, of Bayliss and Starling, of Elliott and Barclay-Smith, and, above all, of Cannon.
>
> The apparent finality of the results obtained discouraged further investigation, and the crystallization of the findings in text-book dogma tended to mask the discrepancies and obscurities so obvious on examination of the original work.
>
> In addition, the introduction of the opaque meal surrendered this field of investigation to clinical workers, so that practically all modern knowledge of the behavior of the large bowel is due to work on man. Information gained from investigation of clinical materials is difficult to evaluate,

is often scanty, and is, at all times, at the mercy of the capriciousness of disease processes and of injury.

Garry believed then, 43 years ago, that there was an immediate need for an improved understanding of colonic physiology. I am not sure that that improved understanding came about, however, for one reads Garry's review now with the feeling that one is reading a contemporary author.

To my mind, the greatest problem of a general nature that has held back this area of study is the problem of species variation. Most writers allude to this problem, but the existing reviews often fail to make clear the degree of the variation. Figure 40.1, from Garry's review, shows examples of the anatomic variation. Observe that the dog colon, to your right, is what is sometimes described as a 'simple tube'. (Though it is certainly a tube, I think it is far from simple). This sort of colon is often said to be typical of the carnivore. Garry says: '. . . the longitudinal coat is a uniform powerful muscle layer. The cecum is vestigial, and the ileocolic junction is a straight tube'. In herbivores, as exemplified by the rabbit colon shown in Figure 40.1, the colon shows great complexity of structure, with a large sacculated caecum,

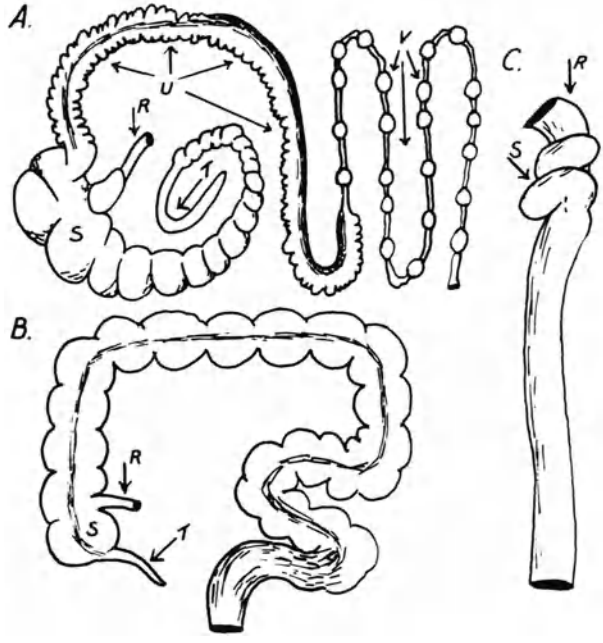

Fig. 1. The large bowel. A. In the rabbit. *R.*, ileum; *S.*, cecum; *T.*, appendix; *U.*, proximal colon; *V.*, distal colon. B. In man. *R.*, ilum; *S.*, cecum; *T.*, appendix. C. In the dog. *R.*, ileum; *S.*, cecum.

Figure 40.1 Garry's illustration of the anatomy of the colon. (From Reference 1)

a sacculated ascending colon, with a narrower distal colon that has a uniform muscle coat that is devoid of sacculation but that often has subsidiary loops. Figure 40.2, taken from a textbook of veterinary anatomy[2], shows examples of the colons of other species. The herbivore colon is further exemplified by the colons of the cow and horse, both herbivores. In omnivores, continues Garry, '. . . the colon lies structurally between the extremes of the carnivorous and herbivorous types'. Garry, and most other writers on the subject, taught that the 'simple tube', a short colon with the longitudinal muscle coat distributed uniformly in the circumference, is the pattern for the carnivore colon, while in the herbivores the colon has become elongated, sacculated, and taeniated and equipped with a long caecum, all as an adaptation to the vegetable diet. If the pig is an omnivore, as I believe it is in the wild, then its colon, shown in the lower left, looks far more like the herbivore model than the carnivore. Yet the colon of the rat, another omnivore, looks very much like that of the dog, a carnivore. Elliott and Barclay-Smith describe it as having '. . . no secondary curvatures . . . [with] . . . the outline of the note of interrogation'. That is, the rat colon is a 'simple tube'[3]. The colon of man, another omnivore, is a sacculated colon, right down to the level of the sigmoid colon, but it is not elongated and it does not have a particularly large caecum. It doesn't resemble either extreme postulated by Garry.

This brings me to make two points; *first*, there is such enormous structural variation in the colon that one makes physiological extrapolations among species with considerable uneasiness. Where there is such great anatomical variation, it seems reasonable to suppose that there is great physiological variation also. And *second*, I believe the correlation made by Garry (and virtually all other writers) between morphology and diet is, to a degree, fallacious: there are too many exceptions; too many cases that do not seem to conform to the rule. In any event, the correlation, if it exists, seems to me to be pointless: it doesn't tell us very much about colonic motility.

Still, despite the great morphologic variation that exists among mammalian colons, the colons do have some features in common. The colon has a developmental identity among species as revealed by embryology, and the general function of the organ is common to all species. Thus, there must be some physiological identities that exist among all mammalian species in respect to colonic motility. Can any such functional generalizations be stated? That is, I believe the major problem that has held back our understanding of colonic motility: we have not found enough physiological or functional generalizations on which to base a theory.

This may be, in part, because we have not read our own literature. For some such generalizations were, indeed, put forth a very long time ago by Elliott and Barclay-Smith who wrote, in 1904, a paper from the country across the border, in Cambridge[3]. They wrote of studies they did on the gross anatomy and the contractions of the colon in the cat, rat, guinea pig, rabbit, dog, ferret and hedgehog. Their methods were simple, their observations seem

Figure 40.2a Dog intestines

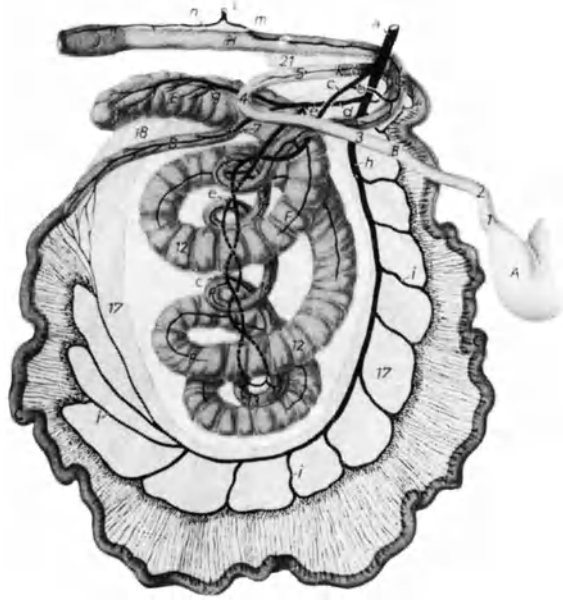

Figure 40.2b Pig intestines

Figure 40.2 Domestic animal intestines. (From Reference 2). In these four schematic drawings the following symbols apply to the colon: D, ileum; E, caecum; F,F′, ascending colon; G, transverse colon; H, descending colon; J, rectum; K, anus

Figure 40.2c Ox intestines

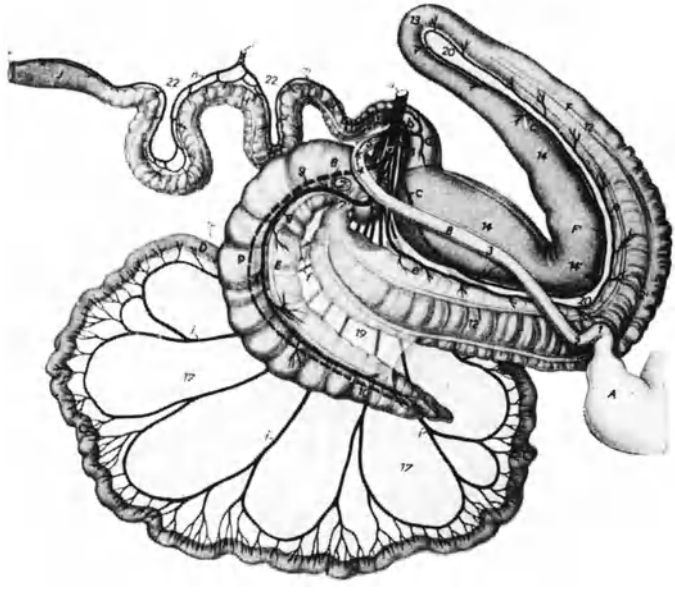

Figure 40.2d Horse intestines

careful, their conclusions seem firm. I have time only to describe the experiments in brief summary. In general, the viscera of anaesthetized and pithed animals were floated in a bath of warm saline or Ringer's solution, and the contractions were observed directly. The colon was distended by '. . . driving thick yellow gruel – prepared by mixing pea-flour with water until the whole was of precisely the same consistency as that of the food in the ileum – through an incision made in the ileum . . .'. In this way the authors could observe the contractions induced by distension of the colon and the flows induced by the contractions.

From their studies, they first concluded that a revision in terminology was in order. They criticized the use of the terms ascending, transverse and descending colon on the grounds that the implied different corresponding functions do not exist. Instead, they preferred, on the basis of the kinds of movements they observed, the terms *proximal*, *intermediate* and *distal* colons, the terminological distinctions being based on functional distinctions. The physiological generalizations they made are stated in the last page of their paper:

1. Antiperistalsis is a characteristic feature of the muscular activity of the colon. The regressive current of material so produced explains the need of a strong ileo-colic sphincter, and is to be correlated with the development of the caecum.
2. Such antiperistalsis consists merely of moving rings of constriction originating in the circular muscle, and not abolished by nicotine. It is not a coordinated movement of contraction preceded by relaxation. A true peristalsis has not been observed to move reversely.
3. The sacral visceral nerves do not control the caecum. Generally, their territory is limited to the final part of the colon, wherein they cause both circular and longitudinal muscles to contract.
4. The inferior mesenteric or sympathetic nerves carry inhibitory impulses to the caecum and to the whole of the colon.
5. The colon tends to show a division into three regions of different activities, the proximal, intermediate and distal. These are completely distinct in the herbivorous mammal. Antiperistalsis is then confined to the first division: the sacral visceral nerves control only the last.

They saw antiperistalsis in the most proximal part of the colon in all the species they studied: herbivores, carnivores and omnivores. This was described in the cat as a 'succession of slowly travelling rings of constriction, which originate at a point about 7 cm beyond the caecal apex and moving at a rate of 1 or 2 mm a second with an interval between their troughs of about 1 cm'. This pattern of movement defines the segment that they called the proximal part of the colon.

They also described what they called 'coordinated peristalsis', a movement that was dependent on the muscle and nerve machinery in the wall of the bowel. They saw this generally with distension in the lower half of the colon.

This was described as a contraction proximal to the distended level and relaxation distal to it, the two cooperating to drive a distending balloon downward. It was seen to be the sole movement of the intermediate colon.

They recognized, also, a third division: the most distal part of the colon. In this region, they observed that propulsive contractions were produced by stimulation of the sacral nerves. In this region the sacral visceral nerves cause both muscle coats to contract.

While I accept that Elliott and Barclay-Smith did indeed put forth some true generalizations about colonic motility in 1904, it is clear that that was not recognized by many authorities between then and now. I will therefore use the rest of my time to defend my belief in the truth of one of their generalizations.

It should be no surprise to us that antiperistalsis is a prominent feature of motility in the proximal colon. It has been recognized for a long time that bowel content tends to reside for long periods of time in the proximal colon. This has been demonstrated more recently also in studies of the transit of radioactive tracers through the gastrointestinal tract. Also, several investigators who are here today have observed that radiopaque markers taken by mouth many hours apart are retained and mixed in the proximal colon so that they emerge in the stool in random order, an order unrelated to the order of their ingestion.

This prolonged residence of content in the proximal colon should suggest some special pattern of movement in this region designed to bring it about. Others besides Elliott and Barclay-Smith saw antiperistalsis. Cannon[4] saw it as well in the isolated cat colon. He had a theory to explain it: he postulated that a contraction ring of the proximal colon distends or stretches the colon walls adjacent to that ring. Such distension then elicits reflexly a wave of contraction that moves along the colon through progressive excitation of a reflex response to stretch. Such waves pass both ways. When, however, the colon content is solid, the movement of the ring is impeded because the wall is stretched less. This is why the peristalsis of the proximal colon is only cephalad when solid stool is present in the distal colon. Cannon attributed the moving character of antiperistalsis of the proximal colon to reflexes.

I believe that the true basis for antiperistalsis in the proximal colon lies, instead, in the electrical slow waves which have been studied in the cat colon in recent years. In Figure 40.3 you see a record of slow waves from the proximal colon of the cat in which there is a pause in the cycle, revealing the fact that slow waves are propagated cephalad. The basis for this is revealed in Figure 40.4 where you see the gradient in intrinsic frequency of the slow waves all along the colon. When a ring of the colon just below the ileocaecal junction is separated from the rest of the colon, the frequency of slow waves drops to about half that when it is in continuity[5]. Such a gradient is, of course, the reverse of that which is seen in the small intestine. Thus, if the slow wave gradient in the colon establishes the direction of peristalsis in the

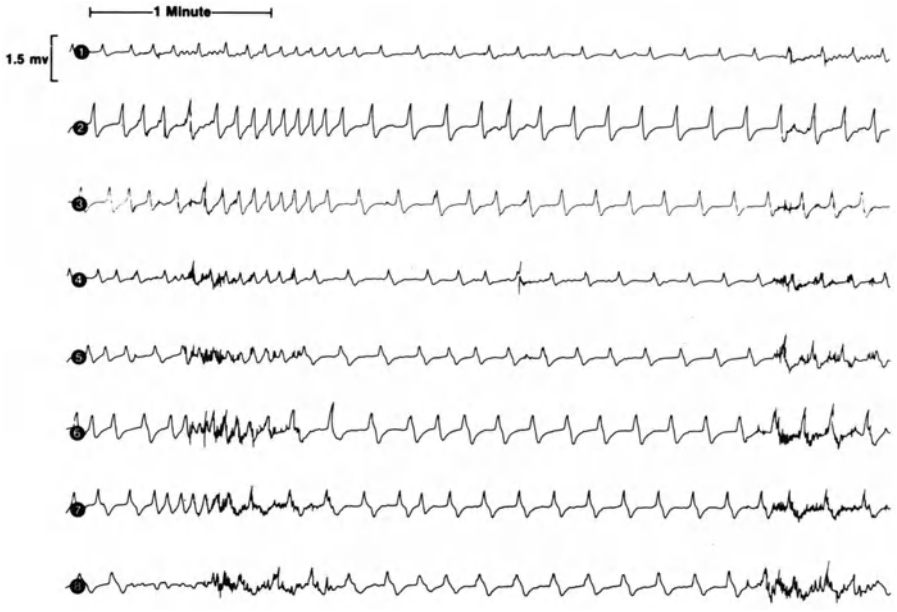

Figure 40.3 A record of the electromyogram of the circular muscle layer of the cat colon taken *in vitro* from a muscle strip 10 cm long (about half the length of the colon) from the region of the mesenteric insertion. Numbers at the left indicate the positions of the eight electrodes: electrode 1 is about 1 cm below the level of the ileocaecal junction. The other electrodes are 1 cm apart. The record from each site shows electrical slow waves. At the left of the record there is a period when the slow wave briefly accelerated. When records from the eight separate sites are compared, it can be seen that this irregularity began at or about electrode site 7 and was propagated toward electrode site 1, that is, cephalad

same way that it does in the small intestine, that peristalsis would have to be directed cephalad, as 'antiperistalsis'.

Elliott and Barclay-Smith stated another generalization that has to do with the variations in colonic structure and function. Let me quote their paper further:

> The backward current produced by antiperistalsis in the proximal colon, being refused passage into the ileum by the ileocolic sphincter, is responsible for the development of the caecum. Caecum and proximal colon may interact, as in the rat, for the continual churning of the whole of the contained food. Or the caecum may enlarge continually, as in the rabbit, so as to provide a great reservoir' . . . . Or again, the proximal colon may gain in capacity, as in the guinea pig, with an especial development of its churning activities and perhaps a lessened tendency to antiperistalsis.

That is, they proposed that antiperistalsis itself alters the morphology of the colon by producing elongation. This elongation could be directed mainly

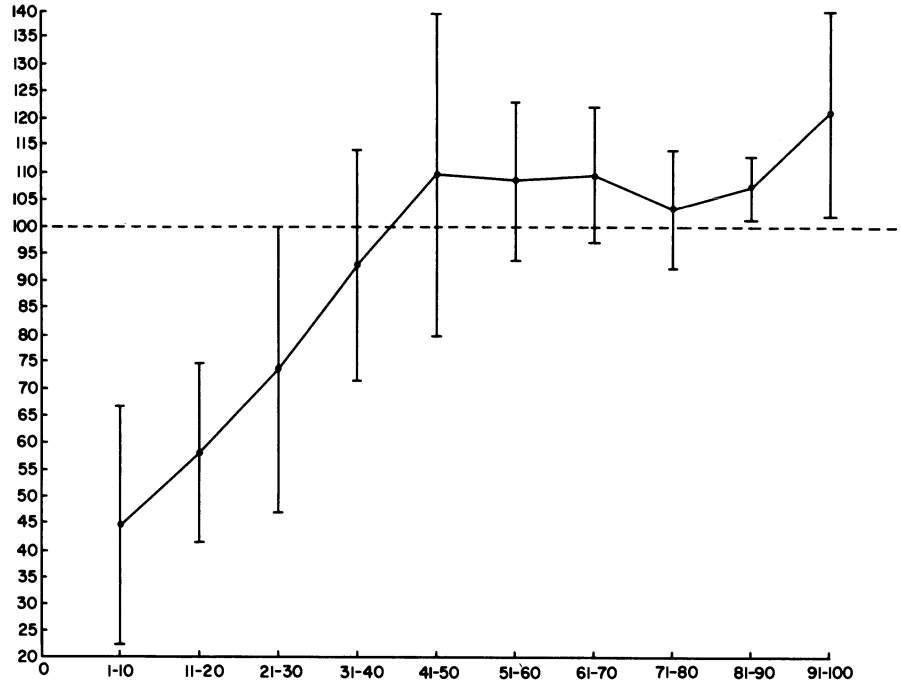

Figure 40.4 The frequency of slow waves all along the cat colon *in vitro*. The whole colon was mounted *in vitro* and slow waves recorded simultaneously from sixteen sites. The colon was then divided into ten rings of equal width and records were continued. The frequency of the slow waves decreased in the proximal half of the colon and showed little change in the distal half. Here the abscissa is marked in 10% segments of total colon length. The ordinate indicates the ratio of slow-wave frequency after the division of the colon into rings to that before, multiplied by 100. The dashed line indicates the 100% percentile. Observe that in the first 10% of the colon, slow-wave frequency diminished to 45% of that before the cut; at more distal sites, the degree of reduction was progressively less, up to the 50th percentile of colonic length. (Data from Reference 5)

towards development of the caecum. Alternatively, it could be mainly downstream from the ileocolic junction. That is, evolution could tend both towards elongation of the colon itself and towards elongation of the caecum (Figure 40.5). Thus, the caecum, and perhaps the proximal colon as well, may be composed of muscle, in some species, that develops later and so is different from that of the rest of the colon, being a consequence of the evolutionary elongation of the colon. We have recently surveyed a variety of animals, looking at caecum and proximal colon for electrical slow waves. In the opossum, slow waves are readily recorded in the colon just distal to the ileocaecal junction, but they are not to be found in the caecum. This is true of the cat and dog as well. We do not find slow waves in the most proximal regions of the colon of the rhesus, baboon, rabbit or rat; I do not say that they are not there: I say only that we do not find them. Thus, it could be that

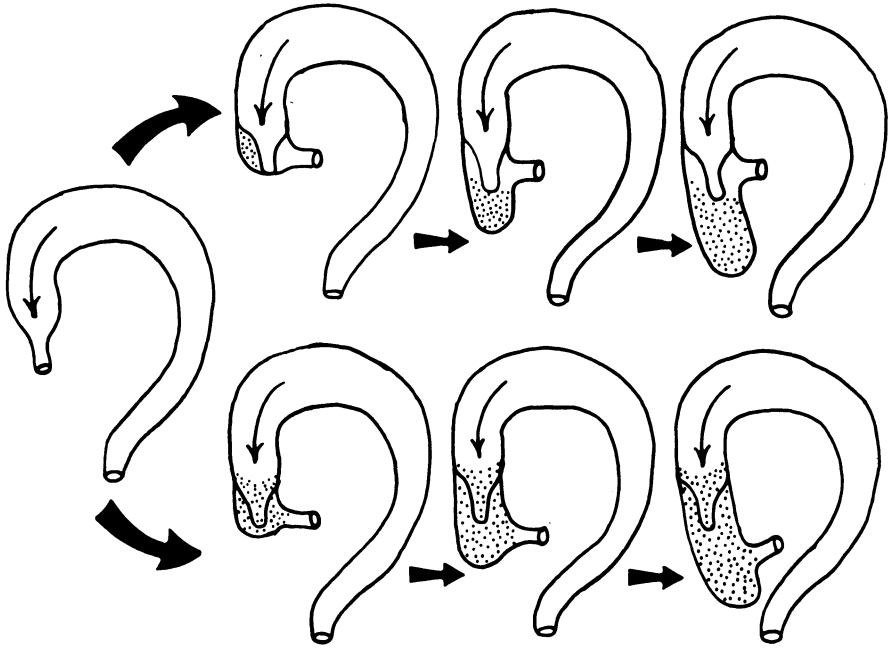

Figure 40.5 A sketch of the possible ways the colon has elongated physiologically. From a primordial state (left), the cephalad migration of contractions, or antiperistalsis (arrow), may tend to cause evolutionary elongation of the proximal colon. This elongation may have been chiefly aimed to develop the caecum; that is, the ileocolic junction may not have been pushed away from the zone of antiperistalsis, as in the top line of drawings. Alternatively, the elongation may have occurred downstream from the ileocolic junction so that that junction was pushed away from the zone of antiperistalsis, as in the bottom line of drawings. In either case, there is an *added* length of the colon indicated by the stippling.

the proximal colon of the dog, cat and opossum is functionally analogous to the middle or distal parts of the colon of some other species; it could be that the caecum of the opossum, where slow waves are not found, is analogous to the ascending colon of the other species. The matter can only be settled by a systematic exploration of the whole colon in a variety of species with heterogeneous anatomy.

I would like to leave, then, with the hope that I have impressed you with one caveat and one generalization:

First, the very great anatomic variations that exist in the colon among species make it seem very likely that there are great physiological variations as well, and so broad generalizations about colonic physiology are to be looked upon with great suspicion until there is abundant evidence on the matter.

Secondly, there is evidence to indicate that there are three or more regions of the colon that act very differently from one another.

If you accept these points, then you will recognize the extraordinary complexity of colonic motility. We are, many of us, physicians, and so we are

especially interested in the human colon. To quote Truelove in 1966[6], 'So far as human colonic motility is concerned, the whole subject has come into a state of flux.' Truelove went on to anticipate substantial advances in the understanding of human colonic function. I suspect that he is still anticipating them. I anticipate them too, but it seems to me that we have a very long way to go!

Acknowledgements

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41

Cellular ion concentrations and electrical control activity in various regions of the colon

K. SCHULZE AND J. CHRISTENSEN

The electrolyte content of smooth muscle from proximal oesophagus is different from that of distal oesophagus in the opossum. The striking gradient of tissue potassium found along the smooth muscle segment must be due to a gradient in cellular potassium concentration, since there is no evidence of a gradient in the extracellular space[1,2]. Regional differences in cellular electrolyte concentrations have also been found in cat small bowel, and they have been correlated with regional variations in myoelectrical activity[3–5]. We measured electrolyte content and extracellular space in the muscle coat of the opossum colon in an attempt to correlate differences with the electrical events in this organ[6].

METHODS

Opossums of both sexes, weighing 2.0–3.5 kg, were anaesthetized by intraperitoneal injection of sodium pentobarbital, 50 mg/kg. The colon was freed from the mesentery and terminal ileum and opened lengthwise along the mesenteric border. The colonic mucosa was carefully cleaned of all faeces by rinsing in successive containers of aerated Krebs solution. It was then transferred to an organ bath with fresh Krebs solution at 36–38 °C and gassed continuously with 95% O_2–5% CO_2. The Krebs solution contained 15 mMol glucose and (in mEq/l): sodium 139, potassium 4.6, calcium 5.0, magnesium 2.2, chloride 125, bicarbonate 22; phosphate 3.5; sulphate 2.3.

The entire colon from the caecal pole to the cut end of the distal sigmoid was then marked at six equidistant sites. Segment 1 corresponded to caecum,

segment 3 to mid-transverse colon, segment 6 to rectosigmoid junction. Preliminary studies had shown gradients of potassium and sodium content along the length of the colon in specimens in which the mucosa had been left intact. All subsequent studies were done on strips of smooth muscle from which the mucosa had been removed by sharp dissection at the submucosal level. Strip weights ranged from 50 to 150 mg. Strips lost water during a period of recovery from cutting, an observation that had also been made in oesophageal muscle[1]. Colonic muscle handled this way shows stable electrical signals after about 30 min for several hours. Before analysis, strips were therefore equilibrated in Krebs solution for 1 h after cutting.

Either inulin or sorbitol, labelled with ^{14}C, was mixed into the Krebs solution and strips were incubated for 1 h after the initial equilibration. For extraction of sodium, potassium and inulin or sorbitol, strips were then blotted, weighed and transferred to individual tubes containing a known volume of 0.1N HNO_3. Tubes were shaken vigorously overnight at room temperature. Preliminary studies showed that this procedure extracts these substances completely, but not calcium and magnesium. For extraction of calcium and magnesium, strips were ashed in individual crucibles at 550 °C during 4 h. The white ash was extracted in 1 ml of concentrated HCl, 2 ml HNO_3 and 7 ml distilled water, by mild heating. Appropriate dilutions of the respective extraction fluids were made in acid-rinsed glassware. The sodium, potassium, magnesium and calcium content of aliquots was measured by atomic absorption spectrophotometry (Perkin–Elmer 303). Aliquots of incubation and extraction fluid were also pipetted into vials with 15 ml of scintillation fluids (ACS, Amersham–Searle). The ^{14}C-content was measured in a liquid scintillation system (Beckman, LS–250).

The ion content of the muscle tissue was expressed in mEq/kg of wet weight for sodium and potassium, and in mMol/kg for magnesium and calcium. The volume in millilitres occupied by labelled inulin or sorbitol was calculated per kilogram wet weight. Intracellular sodium and potassium (mMol per litre of cell water) was calculated according to the formula of Boyle and Conway *et al.*[7]. The ratio of intracellular water to dry weight in μl/mg was called 'cell volume'. Data were treated with standard statistical methods, values expressed as means $\pm$ SE, and Student's *t* test used to determine significance ($p < 0.05$).

RESULTS

Tissue electrolyte content

The tissue potassium content was lower in the proximal and distal parts of the colon than in its mid-portion. Thus, values obtained at segment 3 were significantly higher than those at segments 1 and 5 (see Table 41.1). An opposite trend was observed for tissue sodium content. It was high at either end and low in the middle of the colon (see Table 41.1). The tissue magnesium

Table 41.1 Extracellular space and electrolyte content in various segments of the opossum colon

Segment	*1**	*2*	*3*	*4*	*5*	*6*
K^+ (N = 10)	28† ± 5	39 ± 6	49 ± 3	47 ± 5	38† ± 3	43 ± 5
Na^+ (N = 10) (in mEq/kg)	93† ± 4	90 ± 6	77 ± 6	80 ± 3	81 ± 3	89† ± 7
Mg^{2+} + (N = 5)	5.2 ± 0.4	5.0 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	5.2 ± 0.4	4.9 ± 0.5
Ca^{2+} + (N = 3) (in mMol/kg)	2.9 ± 0.5	2.6 ± 0.5	3.1 ± 0.5	3.6 ± 0.7	3.7 ± 0.5	2.1 ± 0.2
Inulin space (N = 8) (in ml/kg)	200 ± 17	210 ± 23	180 ± 15	190 ± 18	180 ± 12	180 ± 16

Values are means ± SE.
* Numbers refer to six equally long colonic segments from the caecum (*1*) to the rectosigmoid junction (*6*).
† Values are significantly different from segment 3.

content was uniform along the colon, whereas the calcium content was much lower at the rectosigmoid junction than in the remaining colon (see Table 41.1).

Tissue extracellular space

The extracellular space when determined by inulin was 190 ± 10 ml/kg for the entire colon. It was rather uniform throughout the colon (see Table 41.1). Likewise, no significant difference among various segments was found, when sorbitol was used as a space marker. The mean sorbitol space in four animals was 440 ± 32 ml/kg. The 'cell volume' was 4.2 ± 0.2 μl/mg and uniform throughout the colon. (Data not shown).

Intracellular sodium and potassium concentrations

Intracellular potassium concentrations were significantly higher in mid-colon than in proximal and distal colon, when calculated on the basis of mean inulin space. When calculations were based on sorbitol space, differences were even greater (see Figure 41.1). Intracellular sodium concentrations are also given in Figure 41.1, and show a trend that is opposite to that of the cellular potassium.

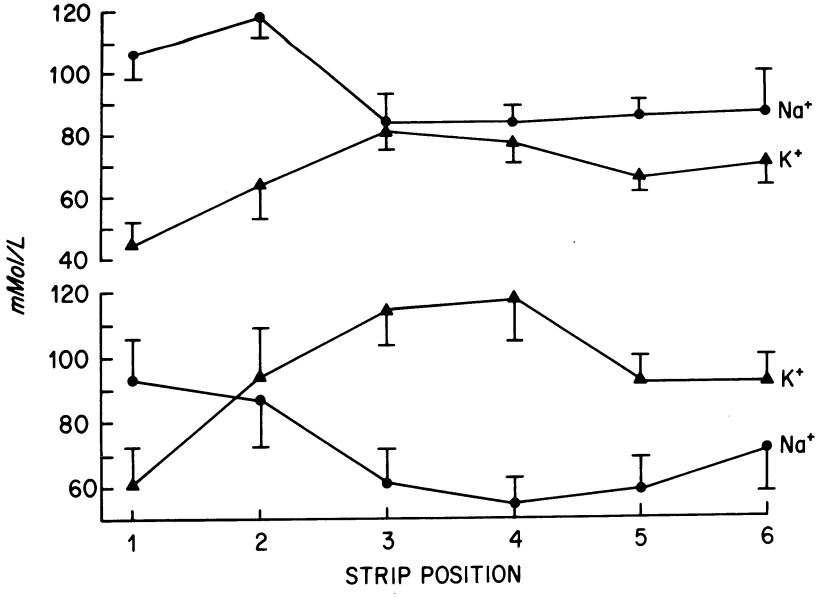

Figure 41.1 Intracellular ion concentrations. Strip position 1 refers to the caecal segment, strip position 6 to the segment at the rectosigmoid junction. Bars indicate 1 SE. In A, calculations have been based on space measurements with inulin; in B on measurements with sorbitol

DISCUSSION

The observed variations of ion content in muscle from various segments of opossum are best explained by differences of cellular ion concentrations along the colon. Cellular ion concentrations affect membrane potential and other electrical phenomena of smooth muscle[1–5]. These variations must therefore be compared to variations of electrical and mechanical characteristics along the colon. Links between chemical, electrical and mechanical properties of gastrointestinal smooth muscle have also been postulated in small bowel and oesophagus[1–5].

Various factors influence measurements of electrolytes and extracellular space in smooth muscle. For example, cellular damage from cutting results in influx of sodium and water into the cell and efflux of potassium. Partial reversal of alterations occurs if tissues are allowed to recover in physiological salt solution[1]. The thickness of the muscle coat and its adherence to the mucosa vary along the colon. Different degrees of muscular damage and artificial variations in the electrolyte content could result. It is unlikely that different degrees of damage account for the variations of electrolyte content observed in our studies. For example, on electrical recordings, there is no evidence of faster deterioration of the proximal and distal colon as compared to the mid-colon. Also, 'cell volume' was rather consistent throughout the entire length of colon; if cells were damaged consequent swelling would result in a larger 'cell volume'. Furthermore, it is unlikely that mucosal removal itself was responsible, because a gradient has been demonstrated with the mucosa intact. Measurements of extracellular space with different space markers show no significant difference along the colon, whether measured by a large molecule such as inulin or by a small one such as sorbitol. The ideal space marker for this tissue is not known, and calculations of the intracellular ion concentrations must be considered preliminary at present[8,9]. However, our studies clearly show important gradients in intracellular ion concentrations regardless of the type of space marker, since inulin and sorbitol may be considered as prototypes for large or small molecules respectively. We found at times that sorbitol distributed into a larger space than sodium. Therefore, we prefer to use the inulin space in this tissue.

Quantitative data on electrical activity in the opossum colon are presently available only for the frequency of slow waves. The frequency of this electrical control activity is zero in the caecum, and rises to between 4.7 and 5.3 cpm in mid and distal colon[6]. Generally, areas with low potassium and high sodium appear to be areas with less frequent or absent slow waves; whereas areas with high intracellular potassium are areas of high, intrinsic frequency of slow waves. Previous *in vitro* studies have shown ionic changes of extracellular fluid to affect properties of the electrical signals generated by colonic smooth muscle[10]. Further studies are needed to determine any correlations between ion concentration inside colonic smooth muscle cells and their electrical signals.

Acknowledgements

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Discussion

J. D. Wood: (USA) Your data suggest that the ionic pumps at the musculature in the mid-region of the colon are most effective in maintaining Na^+–K^+ gradients. Have you tested this by applying Na–K ATPase blocking drugs?

K. S. Schulze: (USA) Not yet.

A. Bennett: (UK) To what extent are your results due to the fact that the tissue is put in an artificial bathing solution; and how do the results vary with time?

Schulze: I am not sure. The electrolyte composition of the bathing solution was similar to that of opossum serum. Cells lost water and sodium and gained potassium during the first hour of incubation at the same rate in all colonic segments. Thus there was no difference in the trauma done to the various segments, but we cannot be sure how completely colonic smooth muscle recovers from the initial insult. All data published show relatively high cellular concentrations of sodium in the colon. Whether this is because this is *in vivo*, or whether a great degree of trauma leads to a high sodium uptake of cells, I am unable to answer.

E. E. Daniel: (Canada) If you wish to correlate electrolyte concentrations in cells with frequency of intrinsic activity, you would like to measure the electrolytes in the cells determining this frequency. These cells are not the entire muscle layer, but may be only a few cells. Thus it seems unlikely that you can expect to find any correlation using electrolytes in the entire wall. Do you agree?

Schulze: I do agree. We do not know which muscle cells or even which muscle layer is responsible for the generation of electrical potentials in opossum colon. We were unable to separate the muscle layers. It is worth noting that Dr D. D. Job did, in 1972, a study similar to ours in the small bowel of cat. In that study he showed differences of electrolyte content, not only along the length of the small bowel but also between the circular and longitudinal muscle layer.

42

Electrophysiological control of motility in canine colon

T. Y. EL-SHARKAWY

Electrical slow-wave activity has been recorded from the colon of cats[1–4], rabbits[5–9] and man[7–14], but the described characteristics of this activity have been inconsistent. In the cat, the slow waves were reported to be omnipresent in the entire colon, while in the rabbit they are omnipresent only in the proximal colon and occur only intermittently in the distal colon. In man, they were reported to occur only intermittently by Taylor *et al.*[12], and Snape *et al.*[13], but were omnipresent in the recordings of Provenzale *et al.*[10] and Bardakjian *et al.*[14]. In the cat their *in vivo* frequency is 4–6 cycles per minute (cpm). In man, Taylor *et al.*[12] described two types of slow wave activity: a slow rhythm with a frequency of 3–4 cpm and a fast rhythm of 6–12 cpm. Bardakjian *et al.*[14] using Fast Fourier Transform frequency analysis reported that human colonic slow waves occur at a fundamental frequency of 3.5 cpm. The longitudinal and circumferential coupling of colonic slow waves was studied in the cat, and to a lesser extent in man. In cats the slow waves are phase-locked along the circumferential axis 93–97% of the time and appear to spread either clockwise or counterclockwise or split at an apparent velocity of 8–10 mm/s. [3] In the longitudinal axis slow waves were phase-locked only 67% of the time and during that time the spread was orad 8.7%, caudad 3.5% and split 87.9% of the time[2]. In man Bardakjian *et al.*[14] reported that there was no consistent pattern of phase-locking in either the circular or longitudinal direction.

The purpose of the present report is to discuss some preliminary observations we have obtained in a study of the myogenic control mechanisms of colonic motility in dogs.

MATERIALS AND METHODS

The present experiments were conducted on canine colon both *in vitro* and *in vivo*.

In vitro experiments

Under pentobarbital anaesthesia (30 mg/kg intravenously), the abdomen was opened by a midline incision. The colon was tied off at both the ileocaecal junction and the most distal end and removed immediately after clamping the blood supply. The colon was then opened flat along the mesenteric border, and the contents removed, taking care to prevent contact of the faecal matter with the muscle coat. The preparation was then stretched to its *in situ* length and pinned flat to the wax floor of a dissecting dish containing oxygenated Tyrode solution. Transverse strips (5 mm wide) were then cut out at the desired locations along the colon, the mucosa was removed and silk threads tied to both ends of each strip. Four such strips were placed with the circular muscle facing upward in the recording chamber containing oxygenated Tyrode solution at 37 °C; one thread tied to a screw micrometer and the other to a strain gauge transducer (Grass FT.03) to record mechanical activity. A monopolar suction electrode[15,16] was placed on the circular muscle layer of each strip to record the electrical activity. The mechanical and electrical activities of the four strips were recorded on a Hewlett Packard (7858A) direct-writing ink recorder.

The composition (in millimoles/litre) of the Tyrode solution used was: NaCl, 133.19; KCl, 4.7; $CaCl_2$, 1.92; $MgSO_4$, 0.78; NaH_2PO_4, 1.17; $NaHCO_3$, 18.57; glucose, 11.5; it was equilibrated with a 95% O_2–5% CO_2 gas mixture.

In vivo experiments

After opening the abdomen of the anaesthetized dog, the colon was exposed and monopolar suction electrodes were placed on the serosal surface of the colon at the desired locations. The electrical activity detected by the suction electrodes was graphed on a Hewlett Packard direct-writing ink recorder.

RESULTS AND DISCUSSIONS

Strips of canine colonic smooth muscle exhibited spontaneous contractile activity of the circular muscle layer as shown in Figure 42.1. This activity consisted of weak phasic contractions which occurred at a regular frequency of 4.5–6 cpm. Occasionally some of these contractions became much stronger and when strong enough the muscle usually failed to relax completely before the following contraction started, giving the general appearance of a slow rise in baseline tension with superimposed phasic contractions. It is tempting to compare these contractions to the motor patterns recorded *in vivo* by Templeton and Lawson[17]. It is possible that the weak contractions are analogous to Type I waves, the stronger contractions to Type II waves and

the failure of complete relaxation between strong contractions may be responsible for Type III waves.

The spontaneous electrical activity recorded from the circular muscle of such strips is shown in Figure 42.2. The three panels in this figure represent the activity of three strips taken from the proximal colon, 3, 6 and 9 cm distal

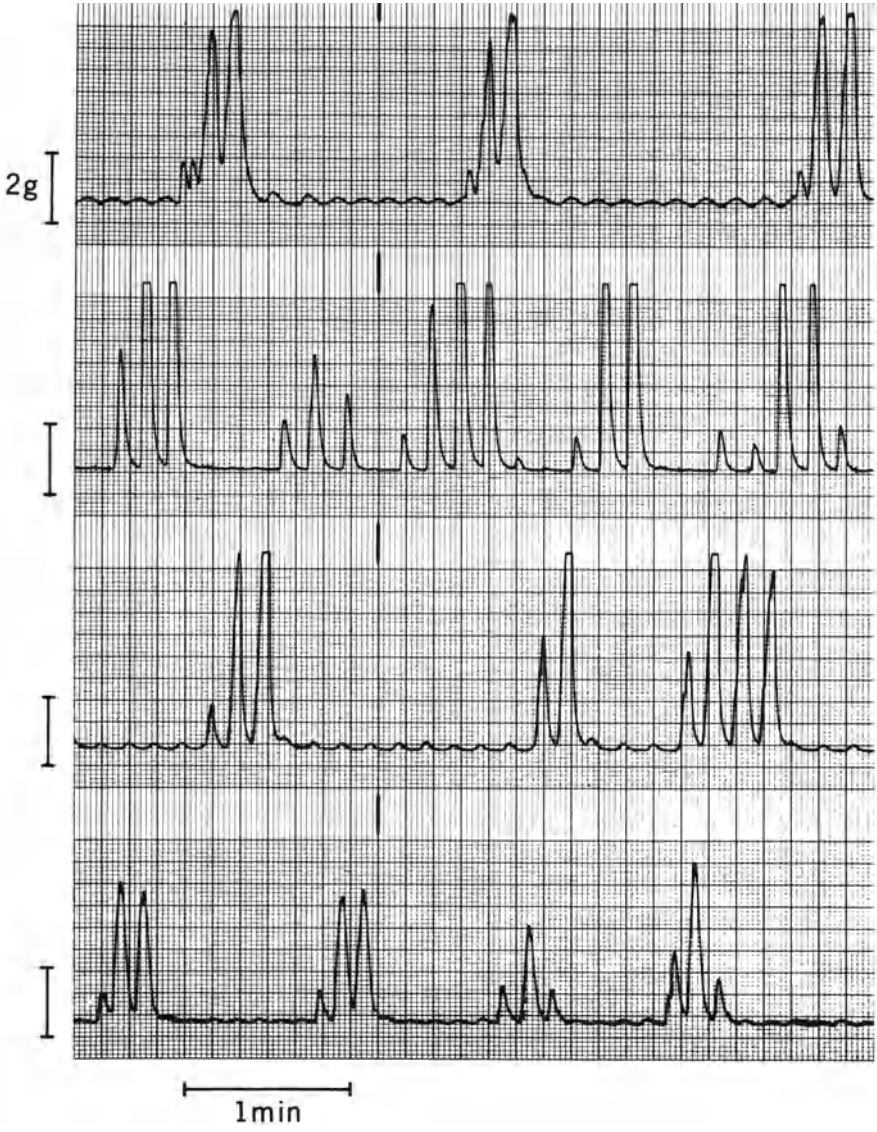

Figure 42.1 Spontaneous mechanical activity of the circular muscle layer of transverse strips of canine colonic muscle coat

to the ileocaecal junction. Slow waves occurred regularly at a frequency of 4.1–6.2 cpm. As recorded by the suction electrode, the colonic slow wave had an amplitude of 1–5 mV and consisted of a relatively rapid depolarization followed by a plateau lasting 3–5 s which was terminated by a slow repolarization. The interval between successive slow waves was almost always isopotential. It is to be emphasized at this point that, although suction electrodes record monophasic potentials of the same configuration as those recorded by intracellular microelectrodes, they are not the electrodes of choice in analysing the quantitative aspects of cellular electrical activity.

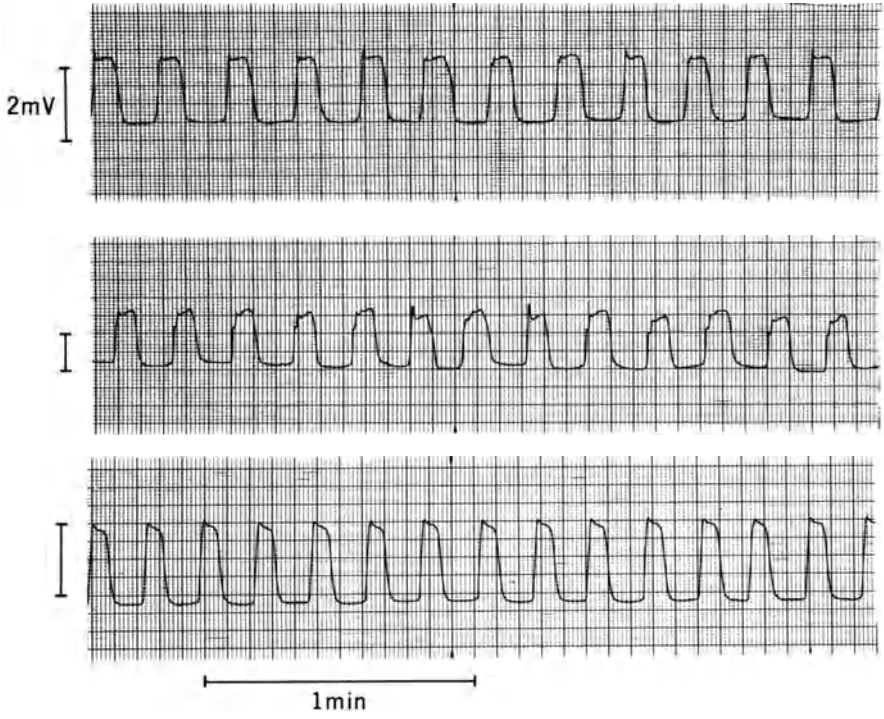

Figure 42.2 Spontaneous electrical activity of the circular smooth muscle of canine proximal colon as recorded by suction electrodes. Recording was made from the circular muscle side of strips of the muscle coat removed 3, 6 and 9 cm distal to the ileocaecal junction

Colonic slow-wave activity is not restricted to the proximal colon but can be recorded from any part of this organ. Figure 42.3 shows the slow waves of three strips removed from the colon of another dog 13, 10 and 7 cm proximal to the anal sphincter. The slow waves recorded from these strips represent the activity of the mid– and distal colon. Like its counterpart in the cat colon, and unlike that of rabbit distal colon, the slow-wave activity of dog colon is omnipresent as far as can be judged from the present

experiments. It could be continuously recorded in experiments lasting up to 8 h *in vitro* and 5 h *in vivo* (see below).

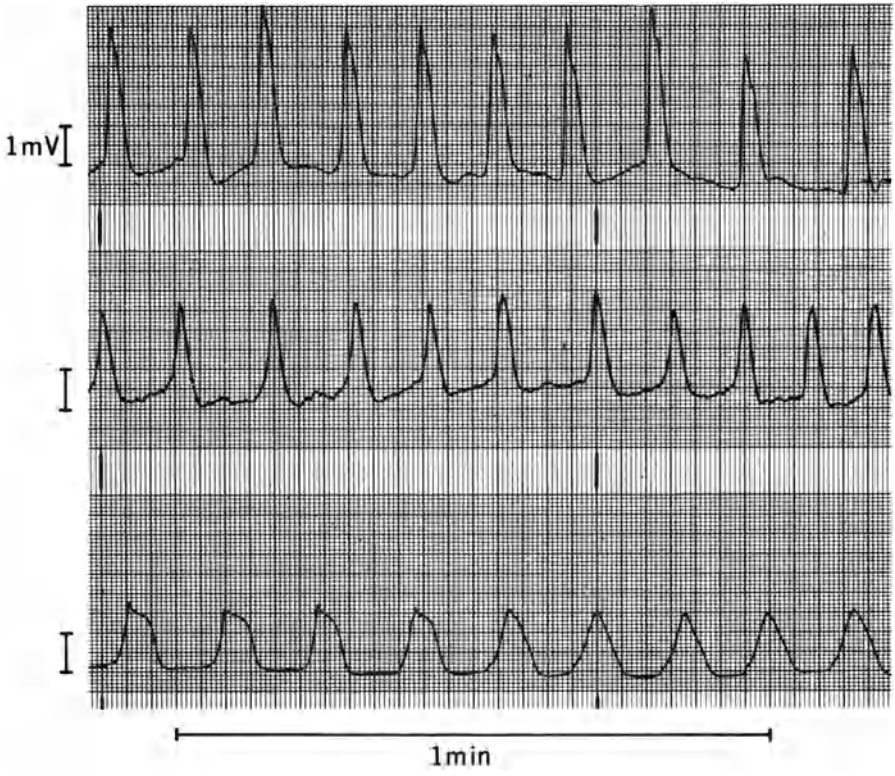

Figure 42.3 The slow-wave activity recorded from the circular muscle of strips taken from the mid- and distal colon, 13, 10 and 7 cm proximal to the anal sphincter

The slow-wave frequency recorded from any particular strip in canine colon was fairly constant, and varied only within narrow limits; e.g., between 4.2 and 5.9 cpm. We have not been able to detect more than one type of slow-wave activity, a fast and a slow rhythm, as has been reported for the human colon[12]. On rare occasions, however, the records revealed two populations of cells under the recording electrodes, the slow waves of one falling in and out of phase with those of the other (bottom panel in Figure 42.4). When phase lag between the two populations exceeded the slow-wave duration, the slow waves of each population occurred during the interslow-wave periods of the other, and the frequency appeared as though it had doubled (second panel from top in Figure 42.4). The two populations were easily distinguished by the relative amplitudes of the slow waves. It is possible that desynchronization of slow-wave activity of this type may account for

the multiple frequency peaks detected in the analysis of slow-wave activity of the canine colon described by Bowes *et al.* on p. 251 of this volume. It is also possible that this phenomenon might explain the existence of slow and fast rhythms of slow-wave activity in the human colon.

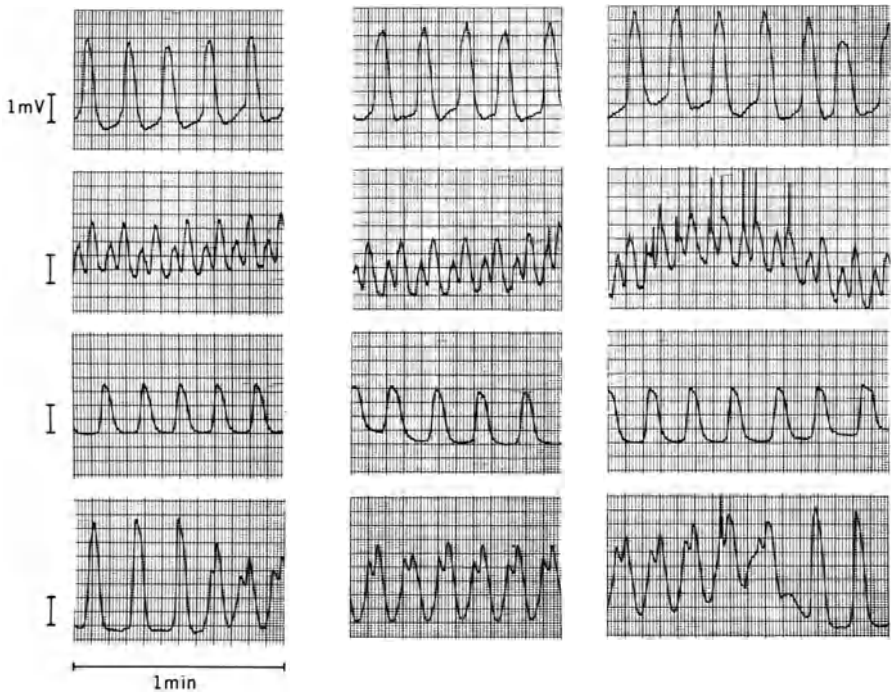

Figure 42.4 Desynchronization of colonic slow waves. The electrical activity recorded simultaneously from four strips of the proximal colon is shown in the four panels, the centre and right-hand panels represent the activities 5 and 15 min after the records in the left-hand panels. Notice that while the first and third panels exhibited synchronous activity, the second and fourth panels show desynchronized slow waves; the electrodes detect two populations of cells exhibiting slow waves. The slow waves of the two populations fall in and out of phase in the fourth panel. In the second panel they are permanently out of phase and the phase-lag is such that the slow waves of one population occur during the interslow-wave period of the other, giving the impression that the slow-wave frequency is twice that of the synchronized strips. The two populations can be distinguished by the relative amplitudes of the slow waves

The slow waves of the canine, like their counterparts in the cat colon, are myogenic. They result from inherent properties of the smooth muscle cells since they persisted unaltered after nerve blockage by tetrodotoxin (1.5×10^{-6} M), ganglionic blockade by hexamethonium (1×10^{-3} M), cholinergic muscarinic blockade by atropine (2×10^{-6} M) and adrenergic blockade by propranolol (2×10^{-6} M) and phentolamine (2×10^{-6} M).

The finding that the slow waves occur at the same frequency as the phasic contractions of the circular muscle illustrated in Figure 42.1 suggested that

the two activities are related. Figure 42.5 illustrates this relationship. When the muscle strips were mechanically quiescent, the slow waves were the only type of electrical activity recorded. During periods of phasic mechanical activity, another type of electrical activity, consisting of bursts of one to several spikes, occurred. The spike discharges occurred only on the peak depolarizations of the slow waves, and each wave with its associated burst of spikes led to a phasic contraction. The force of contraction was related to the intensity of spike discharge associated with the slow-wave. In actively contracting strips, atropine ($1 - 2 \times 10^{-6}$ M) or isopropyl noradrenaline (1×10^{-6} M) abolished the spike discharges and contractions without altering the slow-wave activity. In these respects colonic slow waves appear to determine the occurrence of spike discharges in a manner similar to their counterparts in the stomach[18–21] and small intestine[19,22,23] and we conclude that they represent the myogenic control system of colonic smooth muscle in the dog.

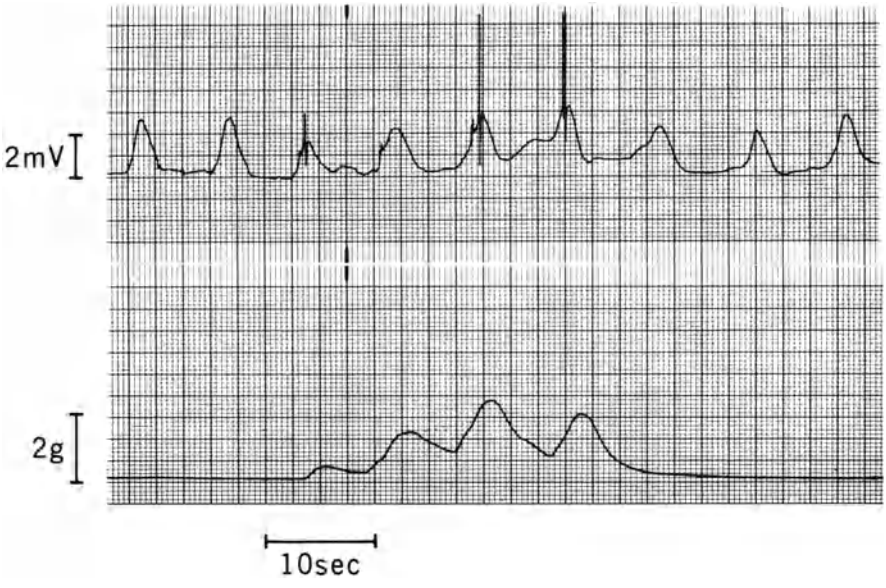

Figure 42.5 Relation between electrical and mechanical activities in colonic circular smooth muscle strips. Top and bottom tracings represent, respectively, the electrical and mechanical activities of the circular muscle of a strip of the muscle coat of canine colon

To study the coupling of slow waves in the longitudinal and circumferential axes of the colon we recorded the electrical activity simultaneously from multiple sites in the dog colon *in vivo*. Figure 42.6 illustrates the activity recorded from four suction electrodes placed on the serosal surface of the colon 9, 11, 12 and 13.5 cm distal to the ileocaecal junction and a fifth electrode placed 1 cm circumferentially to the fourth electrode. Regular

slow-wave activity was recorded from all five electrodes. The frequency of the slow waves was exactly the same in all five locations. They occurred simultaneously or almost so in the circumferential direction (compare panels 4 and 5 in Figure 42.6) and had a fixed temporal relationship to each other in the longitudinal direction. These relationships persisted for the entire length of the experiment (about 4 h). These findings indicate that the slow waves of the dog colon can be recorded from the longitudinal muscle layer of the intact colon *in vivo* and that they may be coupled and phase-locked in both the longitudinal and circumferential axes. The direction of phase-lag in the longitudinal axis could not be established from the experiments we have done so far.

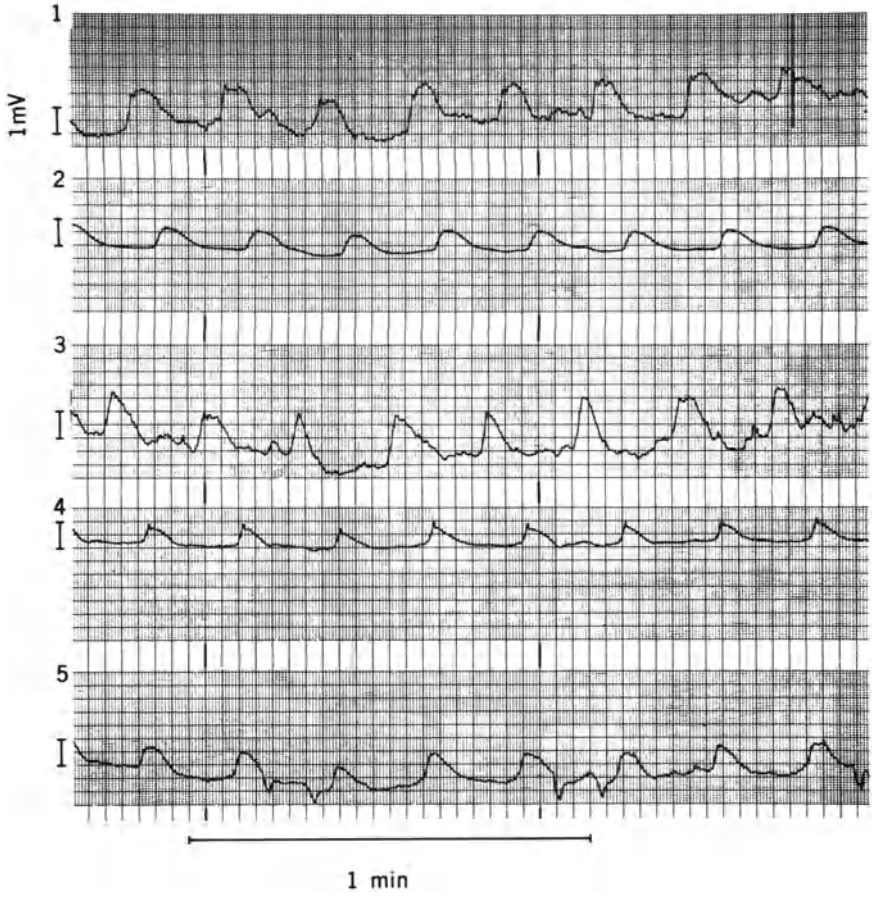

Figure 42.6 Coupling of slow-wave activity of canine colon *in vivo*. The tracings represent simultaneous recordings from suction electrodes placed at different sites on the colon. Panels, 1, 2, 3 and 4 show the activities of four electrodes placed along the longitudinal axis 9, 11, 12 and 13.5 cm distal to the ileocaecal valve, while panel 5 represents the activity of an electrode placed 1 cm circumferentially to the fourth electrode

CONCLUSION

The smooth muscle layers of the dog colon exhibit rhythmic oscillations in the membrane potential (slow waves) resembling the slow-wave activity of the small intestine. Colonic slow waves are omnipresent and can be recorded at all times from any region of the colon. Their frequency is 4.1–6.2 cpm and exhibits little variability at any particular level of the colon. They are myogenic since they were unaffected by neural and ganglionic blockade as well as blockade of cholinergic and adrenergic muscle receptors. *In vivo*, the slow waves appear to be coupled and phase-locked such that they occur almost simultaneously in the circumferential axis and exhibit phase-lag in the longitudinal axis. The direction of phase-lag is yet to be determined. These slow waves, like those of the small intestine, appear to control the excitability of the smooth muscle cell membrane such that spikes can occur only at their peak depolarizations. Slow waves by themselves cannot induce contractions. Each slow wave exhibiting a spike burst at its peak depolarization initiates a phasic contraction.

Thus colonic slow waves can be designated as the electrical control activity of colonic smooth muscle. The cellular characteristics of this activity, its ionic mechanisms and the details of its coupling remain to be examined.

Acknowledgements

The author gratefully acknowledges the skilful technical assistance of Mrs. Wilma MacDonald, the expert typing of Miss Laurie White and Mrs Sharon Dunlop, and the constructive suggestions of Dr N. E. Diamant. The work was supported by Research Grant MA3353 from the Medical Research Council of Canada and the Elsie Watt Research Fund. Dr T. Y. El-Sharkawy is a Scholar of the Medical Research Council of Canada.

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Discussion

M. Wienbeck: (W. Germany) Your mechanical record also exhibited contractions recurring at a very slow rate (about 1 cpm or more). Are they comparable to the migrating spike burst Dr Christensen has described? In which direction were they propagated?

T. Y. El-Sharkawy (Canada) Bursts of contractile activity recurring every 1–3 min were best observed in isolated segments of the colonic muscle coat in which both the electrical and mechanical activities were recorded simultaneously from three or four sites. These bursts of contractions were associated with bursts of spike activity occurring at the peak of several consecutive slow waves. These contractions and the associated spike burst migrate distally. In these respects they resemble the migrating spike bursts recorded from the cat colon by Dr Christensen, but they differ in the fact that in the dog colon the spike bursts occur only on the peak depolarization of the slow waves, whereas in the cat Dr Christensen suggested that the spikes occurred during any phase of the slow wave cycle.

D. Kirk: (UK) (1) Have you recorded from the longitudinal muscle *in vitro*? (2) Have you recorded from isolated longitudinal preparations?

El-Sharkawy: The answer to your first question is yes. We have recorded from the longitudinal muscle layer of isolated strips as well as segments of the colonic muscle coat which contained both muscle layers. Slow-wave activity can be recorded from this layer which is indistinguishable from that recorded from the circular muscle layer. During periods of mechanical activity spike discharges occur, superimposed on the slow-wave depolarizations. The answer to the second question is no; we have not as yet attempted to record from isolated longitudinal layer.

C. F. Code: (USA) In your *in vitro* recordings you mentioned that the electrodes were placed in the circular layer of the wall; did you ever separate the longitudinal from the circular muscle and continue your recordings, or take recordings from the longitudinal fibres?

El-Sharkawy: We have not yet recorded from isolated longitudinal fibres but our future experiments involve a study of the origin of colonic slow waves in which we will record the activity of isolated 'pure' longitudinal and isolated 'pure' circular muscle fibres. The separation of the two muscle layers does not seem to be difficult.

J. Gonella: (France) You have said that the electrical activity persists after alpha- or beta-blockers, but did you observe any increase in spike activity after adrenergic blockers? If it is the case, do you think that there could exist a more or less permanent release of noradrenaline which could depress the activity of intramural nerve cells?

El-Sharkawy: I have observed an increase in spike activity following beta-adrenergic blockade by propranolol in some but not all experiments. Alpha-

adrenergic blockade did not appear to influence the spike activity, but as a matter of fact we have not looked at that carefully since we were studying the effects of adrenergic blockade on slow-wave activity. These results do not really allow me to address the question of whether tonic release of noradrenaline occurs in colonic smooth muscle. If such release occurs, it will most likely suppress the activity of myenteric neurons.

I. Taylor: (UK) Have you any data on *in vivo* chronic preparation? Do you recognize as many action potentials *in vivo* as *in vitro?*

El-Sharkawy: We have recorded the electrical activity of the colon of conscious dogs with chronically implanted bipolar electrodes. The activity recorded from these animals with such electrodes cannot easily be analysed visually. We plan to analyse this activity by the Fast Fourier frequency analysis technique. As to the frequency of action potentials or spike activity, we record more spike activity in the chronic *in vivo* experiments than in the acute *in vivo* experiments. I believe that the anaesthesia used in acute experiments (pentobarbital, 30 mg/kg intravenously) somehow suppresses the spike activity without significantly interfering with the slow-wave activity.

43

Nycthemeral variation of ileocolic myoelectrical activity in the cat

M. WIENBECK, H. JANSSEN AND G. KREUZPAINTNER

Gastrointestinal motility is usually studied for rather short time-periods. Therefore cyclic variations of long duration are easily missed. It is but recently that a periodic type of activity recurring every 1–3 h within the small intestine of many species has received appropriate attention[1,2]. Periodic changes in intestinal motility of even longer duration are still poorly understood.

In previous studies in the colon of the cat we had demonstrated a circadian rhythm of the myoelectrical activity suggesting corresponding variations in colonic motility[3]. The purpose of the present study was to investigate the effects of the time of day upon the terminal ileum of the cat and to correlate it to the activity of the proximal colon.

MATERIAL AND METHODS

Six healthy cats weighing 2–3.4 kg were used. We implanted four to eight bipolar silver needle electrodes[4] on the antemesenteric side of the terminal ileum and proximal colon. Inter-electrode distances were 12 mm. During electromyographic recording the cats were housed in a box open along one side, 48 × 30 × 21 cm in size, thus allowing them to move and to lay down. Thin flexible electrical wires led to a plug in the neck of the animals, and from there to a direct writing polygraph.

Electrical records were taken from the fully conscious animals 1–5 months post-operatively. After a 12 h fast, recording began by turns in the morning or in the evening. During the following 24 h experiments the animals were

fed 100 g of canned food and 80 ml of diluted milk. The time of feeding was randomized in such a way that each hour of the day was represented once within twelve experiments. The intervals between meals were 12 h. The laboratory was illuminated by daylight from 6 a.m. to 6 p.m.; during the night the room was dark except for the very weak illumination of the recording pens.

We evaluated activity time relative to total time (in per cent) at 60 min intervals in the terminal ileum and in the ascending colon. Each slow-wave cycle bearing spikes was thought to represent activity time. The counting did not take into consideration the variation in the number of spikes superimposed on slow-wave cycles.

We tested differences in activity time between the ileum and the colon for significance by Student's *t* test for paired data and differences within time by the standard *t* test.

RESULTS

Types of electrical activity

The following electrical signals were recorded on both sides of the ileocolic

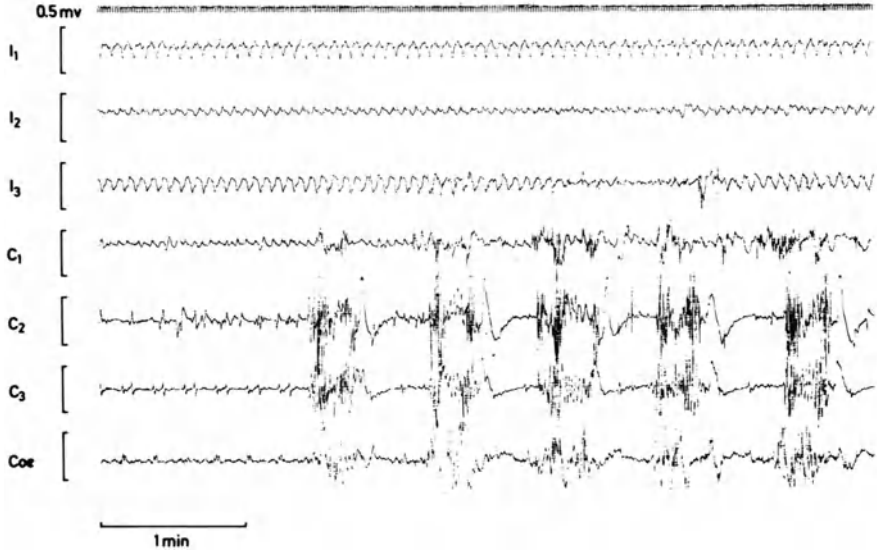

Figure 43.1 Electromyogram from the terminal ileum and proximal colon of the cat. The three electrodes in the terminal ileum (I_1–I_3) pick up regular slow waves and a few spikes in I_3. The tracing from the first electrode in the proximal colon (C_1) shows low amplitude fast-type slow waves (17.5 cpm), whereas the slow waves from the other electrodes of the ascending colon (C_2–C_3) and of the caecum are much slower (4.8 cpm). In addition, the electromyogram from the proximal colon exhibits a minute rhythm-like activity with periodic recurrence of spike bursts and oscillating potentials

junction: spikes, slow waves and propagated myoelectric complexes (Figure 43.1). Only in the colon were two additional types of electrical activity encountered; these were oscillating potentials varying in frequency between 28 and 47 cycles per minute (cpm) and a minute rhythm-like activity consisting of spike bursts and oscillations recurring at 41–98 s intervals.

Slow waves were continuously present 24 h a day. The other signals occurred only intermittently. Mean slow-wave frequency was 12.48 $\pm$ 0.96 (SD) cpm in the terminal ileum and 5.07 $\pm$ 0.51 cpm in the proximal colon. Intermittently we recorded a second type of apparent slow-wave activity from electrodes adjacent to the ileocolic junction. The frequency of these faster slow waves was 18.31 $\pm$ 1.19 cpm.

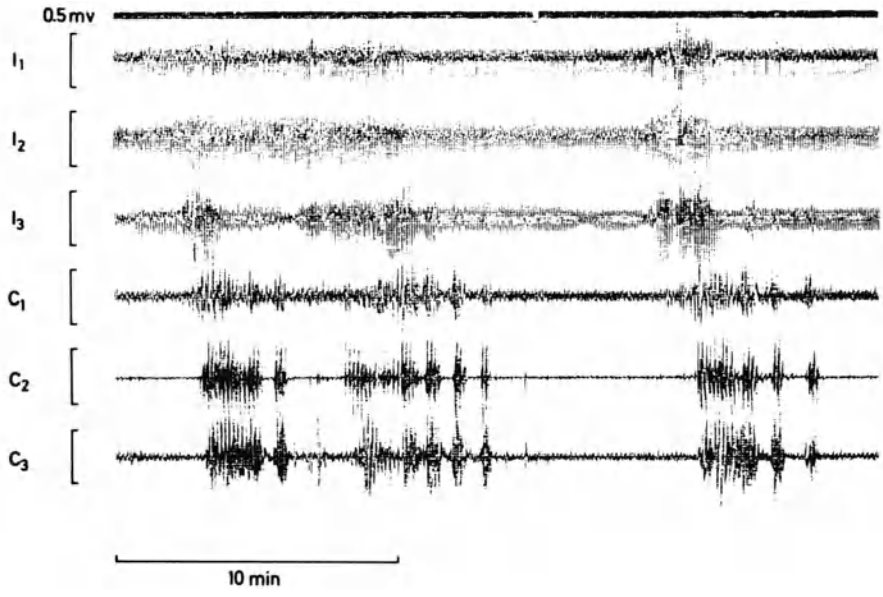

Figure 43.2 Electromyogram from the terminal ileum (I_1–I_3) and ascending colon (C_1–C_3) of the cat. Three propagated spike complexes pass at irregular intervals (5 and 12 min) from the terminal ileum across the ileocolic junction to the proximal colon, where they get subdivided into a minute-rhythmic activity

Spikes occurred either at random, during periods of more or less continuous activity, or spike bursts formed an integral part of activity complexes being propagated along the terminal ileum into the colon or originating in the colon. (Figure 43.2). These activity complexes recurred at intervals of 2 min to 2 h. Although the complexes were more prevalent when the animals were fasted, they did not exhibit the uniformity of appearance and the regularity of recurrence which is known from the interdigestive migrating complex in dogs and other species.

Effect of time of day

Slow waves

Changes in time did not have any apparent effect on slow-wave activity. Slow-wave frequency was the same during the day and at night, both in the terminal ileum and in the proximal colon. Also, the occurrence and disappearance of the faster type of slow-wave activity close to the ileo-colic junction did not show any relationship to the nycthemeral cycle.

Spike activity

By contrast, spike activity varied to a great extent (Figure 43.3).

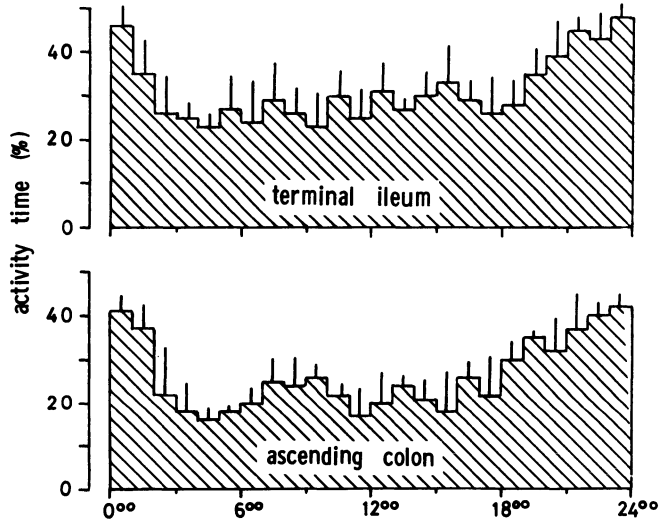

Figure 43.3 Histogram representing the myolectrical activity time (in per cent) during the nycthemeral cycle in the terminal ileum and ascending colon of the unanaesthetized cat. The data are collected from twelve experiments (means); the standard error of means is indicated by vertical lines

(*a*) *In the terminal ileum*, calculated activity time attained a maximum of 48% of total time during the hour before midnight. Then it dropped rather suddenly to a minimum of 23% of total time at 5 a.m. which was significantly ($p < 0.01$) lower than the activity at midnight. The occurrence of spikes levelled off during the daytime; the mean activity time was then 28%. Only in the evening was there a gradual but definite increase in spike activity which reached values between 40% and 50% after 9 p.m.

(*b*) *In the proximal colon*, a very similar slow cyclic variation became apparent. A significant decrease ($p < 0.01$) of spike activity in the early morning hours was followed by a period of relative quiescence between 4 and 6 a.m. when activity time was only 16% of total time. Then there was a tendency for a

brief increase in spiking between 7 and 10 a.m. In the evening, activity time rose again and climaxed between 11 p.m. and 12 midnight when it was 42% of total time.

(*c*) *A comparison of spike activity* did not show any significant difference between tested areas. The diurnal changes of activity time, i.e. the sudden drop in the early morning and the gradual increase towards midnight, exhibited the same pattern in the terminal ileum and proximal colon.

DISCUSSION

Diurnal variations are well known from endocrine and other functions of the human and animal organism[5–7]. In the gastrointestinal tract, circadian patterns have been studied mainly in their effects upon secretory and absorptive processes[3,8]. Gastrointestinal motility has received little attention.

A nycthemeral cycle has been observed in the motility of the rat small intestine[9] and of the rabbit caecum[10]. Previous studies had demonstrated that the myoelectrical activity of the cat colon also exhibits a circadian rhythm[3], and this is confirmed by the present investigation. In both studies the occurrence of spike discharges in the proximal colon increased towards midnight and remained rather low during daytime.

Changes in colonic motility could be restricted to the large intestine or could involve the small intestine in addition to the colon. The present study suggests that nycthemeral variations affect both parts of the gut. The underlying control mechanisms may act either on the small intestine, and thence affect the proximal colon secondarily, or they may act on the terminal ileum and proximal colon simultaneously. A retrograde action of colonic motility on the terminal ileum is unlikely[11]. The close link in the circadian myoelectrical activity across the ileocolic junction which was not demonstrable between the proximal and distal colon[3] favours the idea of a primary variation of small intestinal activity affecting the ascending colon secondarily.

The diurnal oscillator responsible for this variation is unknown. Five factors have to be taken into consideration:

1. Food intake;
2. Changes in the absorptive and secreting function of the gastrointestinal tract;
3. Central nervous influences;
4. Hormonal effects;
5. Intrinsic mechanisms in the ileal and colonic muscle itself, e.g. circadian variations in the intracellular electrolyte composition[12].

Of these, only food intake can be ruled out. The randomized timing of feeding should compensate for the well-known effects[11] of food intake. As far as the other four factors are concerned, the present observation does not

allow us to draw any conclusions. Therefore, discussion remains speculative at present.

CONCLUSIONS

From our observations at the ileocolic junction of unanaesthetized cats we conclude:

1. Spike activity and hence contractile activity of the feline terminal ileum and ascending colon are clearly influenced by the time of day.
2. Food intake is not likely to be responsible for this diurnal variation.
3. The resemblance of the circadian myoelectrical pattern between the terminal ileum and proximal colon suggests a closer link in the motility of these two parts of the gut than between the ascending and descending colon.
4. The mechanism controlling nycthemeral variations in intestinal motility has still to be determined.

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Discussion

C. A. Lanfranchi: (Italy) Have you recorded EEG? The question is related to the fact that it is already demonstrated that cyclic variations of the spike activity in the duodenum of man depend on EEG modifications.

M. Wienbeck: (W. Germany) We did not record EEG. The state of consciousness certainly is one of the mechanisms possibly being responsible for the observed nycthemeral variations in intestinal motility.

D. L. Wingate: (UK) We have found clear evidence of a circadian rhythm in small intestinal spike activity in three out of four 24 h fasting studies in chronic dogs. In two studies, there was obvious diminution of intermittent spike activity *at night*: in the third, the timing was different, and in the fourth there was no rhythm. These preliminary results show changes in the small intestine opposite to those you have shown for the colon.

Wienbeck: I am very glad that your observations in the dog lend support to the existence of a circadian rhythm in the intestinal motility. Differences in the day and night cycle between the dog and cat could well be due to species differences.

T. G. Parks: (N. Ireland) It is true that the proximal and distal segments are more alike structurally than is the case of the terminal ileum. On the other hand, the terminal ileum and proximal colon are both embryologically mid-gut structures with a similar blood supply and nerve supply, thus differing from the distal colon. Could it be that the circadian rhythm which you describe for the terminal ileum and proximal colon is related to the neuronal controlling mechanisms for mid-gut structures which differ from those controlling the hind-gut.

Wienbeck: A common neuronal controlling mechanism for the terminal ileum and proximal colon appears to be the most logical explanation for our observation. But, since we did not study this specifically, we cannot add any more data to support this hypothesis.

Y. Ruckebusch: (France) Do you think that the time of feeding was really randomized. An hypothesis may be that during daytime the level of catecholamines is higher than during night-time. Circadian patterns in the occurrence of the myoelectrical complexes are abolished after splanchnicectomy.

Wienbeck: The time of feeding was in fact randomized during the experiments, but not before. Catecholamines could be responsible for the observed nycthemeral variations, but it is difficult to reconcile the previously observed differences between the proximal and distal colon with this hypothesis.

B. N. Catchpole: (Australia) I would suggest our next host department organize a study of the disturbed colonic function amongst members coming from the various parts of the world, with their consequently disturbed circadian rhythms.

Weinbeck: This is an excellent idea. We only have to agree on the experimental design.

44

The identification of an electrically silent zone at the ileocaecocolic junction *(Abstract)*

T. W. BALFOUR AND J. D. HARDCASTLE

Our preliminary studies of the myoelectrical activity recorded from the canine ileocaecocolic junction were reported to the Fifth International Symposium[1]. At that time we were able to identify a regular slow wave – (13–15 cycles per minute (cpm)) – in the terminal ileum and an intermittent slow wave (4–6 cpm) in the proximal colon. Since then, further chronic studies in unanaesthetized dogs, using surgically implanted serosal bipolar silver/silver chloride electrodes, have persistently demonstrated, in addition to the above two basic rhythms, a narrow band of electrical silence at the anatomical junction between ileum and colon. An analogous zone of electrical silence had already been described at the gastroduodenal junction[2].

In the second part of this study we demonstrate that the silent zone can also be identified in isolated perfused preparations[3] of the canine ileocaecocolic region, in which all extrinsic nerves have been divided during the surgical isolation.

Our myoelectrical records are presented in detail[4], both from the chronic unanaesthetized dog and from the isolated perfused preparation. The effects of infusing gastrointestinal hormones are also described – pentagastrin (0.02 μg/kg^1/min) and cholecystokinin (1 U/kg/min) evoked action potentials, particularly at the terminal ileal electrode; secretin (1 U/kg/min) and glucagon (10 μg/kg^1/min) produced an 'arrhythmia' of both ileal and colonic slow waves. The silent zone remained resistant to such hormonal stimulation.

Electrical models, simulating both ileal and colonic patterns, should be prepared to test the hypothesis that mixing of the two electrical patterns could, under certain conditions, produce electrical 'silence'.

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Discussion

A. Bortoff: (USA) In order to ensure that the area you are recording from at the ileocaecal junction is truly 'silent', have you tried altering the arrangement of your bipolar electrodes or recording monopolarly? If activity at the junction is transversely synchronized you might not detect electrical activity with transversely orientated electrodes.

T. W. Balfour: (UK) If the junctional bipolar electrode is placed longitudinally (and not transversely as in this study), then an ileal type of slow wave is obtained. I have not tried monopolar electrodes.

M. Pescatori: (Italy) Did you try to elicit a motor response in your'silent zone' by inflating proximally a balloon in the lumen of the ileum (by a peristaltic reflex)?

Balfour: I have recorded a zone of elevated intraluminal pressure (using perfused tubes) at the ileocolic junction of the dog. This zone is much wider (1–2 cm) than my 'silent zone'. I have not tried the effect of simultaneous myoelectrical recording from the junctional zone and proximal balloon distension of the ileum.

E. E. Daniel: (Canada) What is the structure of the region you describe as electrically silent? Is there muscle continuity, or nervous continuity?

Balfour: Under the light microscope there is a thickened ring of circular muscle which merges into the muscle coat of the colon. There does not appear to be any band of connective tissue which could possibly explain the electrical silence. I have not studied the question of nervous continuity.

R. E. Condon: (USA) Records obtained in fasting monkeys with either bipolar or monopolar electrodes placed on distal ileum and colon adjacent to the ileocaecal valve, demonstrate no locking or other association between electrical events in the ileum and the colon. Incidentally, shown in these records are also slow waves in the caecum and both orad and aborad phase-locking of electrical spikes in the proximal colon. Apparently our monkeys are more like Mr Balfour's dogs than Dr Wienbeck's cats.

Balfour: What happens if you put an electrode on the anatomical ileocolic junction?

Condon: We have no information regarding a possible silent zone in the ileocaecal valve.

Balfour: Quite apart from my controversial zone of electrical silence, my recordings demonstrate an insulation of the ileal slow wave from the intermittent colonic slow wave. Yet, Dr Wienbeck has already demonstrated in the preceding paper that (in cats at least) spike activity can be propagated from the terminal ileum into the proximal colon.

Section IX

Clinical Aspects of Gastrointestinal Motility

45

Idiopathic intestinal pseudo-obstruction syndromes – a clinicopathological study of six adults *(Abstract)*

M. D. SCHUFFLER, C. E. POPE II, T. D. BIRD, M. C. LOWE, A. COOK AND S. M. SUMI

Six patients with idiopathic intestinal pseudo-obstruction (IIP) underwent radiographic, manometric, and pathological studies. Two forms of IIP were discerned. In one form, occurring in a brother and sister, IIP was the presenting manifestation of a previously unrecognized neurological disease characterized by gait ataxia; small, irregular, poorly reactive pupils; dysarthria; absent deep tendon reflexes; adhidrosis; pupillary denervation hypersensitivity; and inappropriate blood pressure responses to phenylephrine, the Valsalva manoeuvre and upright posture. Radiographically there were chaotic, spontaneous contractions of the oesophagus, dilatation of the proximal small intestine, and extensive colonic diverticulosis. Manometrically, there was oesophageal aperistalsis, multiple spontaneous contractions, and positive Mecholyl tests. Post-mortem examinations showed degenerated myenteric plexuses containing quantitatively reduced numbers of neurons and nerve fibres in the oesophagus, small intestine, and colon. Many neurons contained an eosinophilic intranuclear inclusion which by electron microscopy was a dense array of non-viral, non-membrane-bounded fibrils. Intestinal smooth muscle was normal. Central nervous system neurons had cytoplasmic degenerative changes and the neurons of the brain, spinal cord, dorsal root and coeliac plexus ganglia contained intranuclear eosinophilic inclusions.

Four patients without neurological disease or scleroderma had a second form of IIP. Radiographically, the oesophagus was atonic, there was marked widening and hypomotility of the small intestine and colon, and diverticula

were absent. Manometrically, oesophageal peristalsis was absent in all, there were no spontaneous contractions, and the Mecholyl response was negative in one patient tested. One of these patients and two family members of a second had bladder smooth muscle dysfunction. Light and electron microscopic studies of intestine and bladder showed smooth muscle degeneration. The myenteric plexus was normal. The disease in two of these patients was transmitted as an autosomal dominant trait.

CONCLUSIONS

IIP is a heterogenous syndrome which may be caused by degeneration of either the myenteric plexus or smooth muscle.

Discussion

A. M. Connell: (USA) (1) What is the opinion of neurologists and virologists about this condition? (2) Since we are now recognizing specific, definable conditions with intestinal pseudo-obstruction, what is the status of the term idiopathic intestinal pseudo-obstruction?

M. D. Schuffler: (USA) (1) They have never seen this neurologic syndrome or inclusions before. They have no idea what the inclusions represent. By histochemistry, the inclusions do not contain DNA or RNA so they are not likely to be viral. (2) The term should be used to describe a family of different diseases. However, as each entity within the syndrome gets characterized, it should be given a specific designation.

E. E. Daniel: (Canada) This beautiful study shows clearly one type of IIP which seems to involve neurological damage, and another type which you relate to smooth muscle degeneration. However, we have studied a patient and her brother who do not have any evidence of neuropathy but have severe IIP without extensive smooth muscle damage. The patient's intestinal muscle lacked electrical control activity except during MMCs, but strips of muscle *in vitro* contracted to stretch and to nerve stimulation. Thus these patients may have another variety of IIP which is myogenic in origin but does not result from smooth muscle degeneration. Possibly they represent an earlier stage of your myopathic type.

Schuffler: I agree that there are probably a variety of pathogenic mechanisms which can produce the clinical syndrome of IIP. This might involve either smooth muscle or the nervous connections of the bowel, or combinations of both. There might or might not be identifiable pathological changes. The two forms of IIP which I have described are only two diseases within the spectrum of IIP. Certainly other forms of IIP are waiting to be fully characterized by clinical, radiographic, genetic, pathological and physiological techniques.

B. N. Catchpole: (Australia) Has the possibility of this being due to a 'slow' virus disease been excluded?

Schuffler: We have determined by histochemical stains that the internuclear inclusion does not contain DNA or RNA. The possibility of a 'slow' virus disease therefore seems unlikely.

C. E. Code: (USA) (1) The symptoms of your two patients with neuropathic variety of this disease suggest neural involvement in the central nervous system Was this examined?

(2) Have your neuropathological consultants seen the inclusion bodies you showed in other neurological conditions?

Schuffler: (1) Yes. Both patients had intranuclear inclusions, identical to those found in the intestine, within neurons and supporting cells of the brain, spinal cord, dorsal root ganglia, and coeliac ganglion. (2) These intranuclear inclusions have not previously been seen by my neuro-

pathology collaborators. They have not previously been reported in the literature.

D. O. Castell: (USA) Did you perform the same careful quantitative studies on the myenteric neurons in the patients previously described by you with the myopathic form of IIP?

Schuffler: Yes, but I could hardly do this in the one patient in whom I had the opportunity to cut my own serial sections. In that patient, previously reported in the August 1977 issue of *Gastroentrology*, myenteric neurons were present in normal numbers.

W. J. Snape, Jr.: (USA) You showed abnormal oesophageal peristalsis both in the syndrome with altered myenteric plexuses and in the myopathic syndrome. Since orderly peristalsis seems to be under intrinsic neural control, why is not the oesophageal motility peristaltic and of low amplitude in the myopathic syndrome; or possibly there is also a lesion in the intrinsic neural system, in the patients with the myopathic syndrome?

Schuffler: I cannot answer this directly because I have not had the opportunity of studying the morphology of the oesophagus in the myopathic syndrome. I cannot rule out the possibility that there is a neural lesion in the oesophagus.

46

Activity of derivatives of tyrosine on gastrointestinal motility

P. SENIN, F. MAKOVEC, A. L. ROVATI AND P. L. CASULA

The common aim of our previous studies was to single out and isolate molecular structures pharmacologically active in the pathology of the stomach and duodenum.

The purpose of the present study was to choose an amino acid on which to induce such molecular changes as to obtain a non-atropine-like antispasmodic activity on the smooth muscle of the gastrointestinal system.

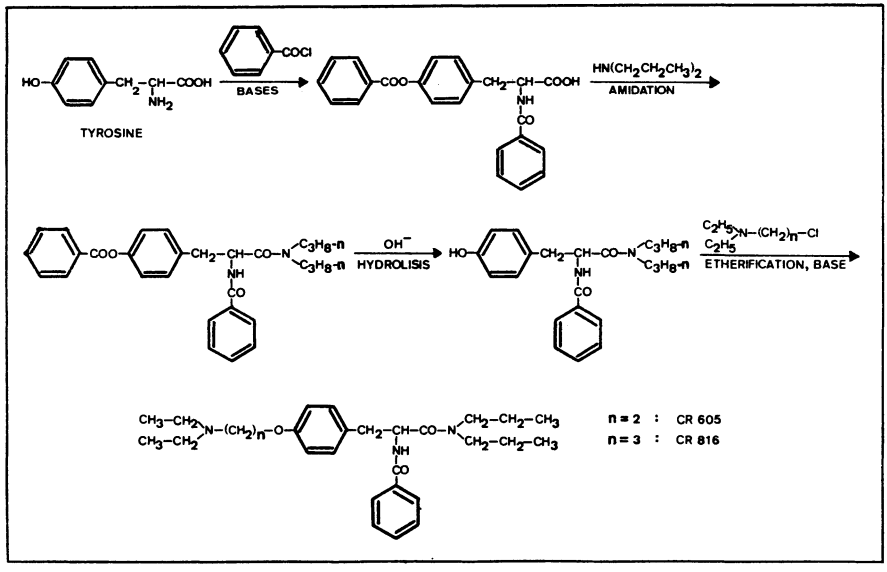

Figure 46.1 Scheme of chemical synthesis of CR 605 and CR 816

Based on a consideration of molecular pharmacology, which is too long to be discussed here, the most suitable amino acid for these purposes was identified in tyrosine. We synthesized about 600 original molecules and intermediates.

Among these synthesized compounds, the most interesting molecules were found to be those bearing the mark CR 605 and CR 816 respectively. Figure 46.1 schematizes our method of synthesis.

The relationships between chemical structure and pharmacological activity of this series of tyrosine derivatives will be discussed in detail in a future paper. However, the antispasmodic activity is bound to the presence in R_1 of a basic group, whereas the substituents in R_2 and R_3 regulate the amount and rate between toxicity and pharmacological activity in the various compounds.

SPECIFIC PHARMACOLOGY

In vivo pharmacology

The pharmacological evaluation of these compounds was started in Italy in our laboratories and is still in progress in Italy and in other laboratories in West Germany, Switzerland and the USA. These evaluations concern the *in vitro* and *in vivo* activity on the gastrointestinal tract, biliary and urinary pathways and on uterine muscle. As comparison we chose two papaverine-like drugs (flavoxate and papaverine itself) and an atropine-like agent largely employed in therapeutics, hyoscine butyl bromide.

Intestinal transit in the mouse

One of the evaluations of the antispasmodic activity on the gastrointestinal system is shown in Table 46.1 where the values are expressed as ED_{50} in mg/kg on the intestinal transit in the mouse. A study of this table shows greater activity for CR 605 and CR 816 after intraperitoreal administration than papaverine, an activity similar (CR 605) or greater (CR 816) than flavoxate, and slightly lower than hyoscine butyl bromide, which is an atropine-like agent and has, therefore, a greater local and systemic toxicity.

Table 46.1 Activity of tyropramide and mepramide on intestinal transit in rat

	ED_{50}	
Drug	(mg/kg; intraperitoneally	(mg/kg *per os*)
CR 605	14.3 (10.5–18.2)	100 (73.0–127.0)
CR 816	11.3 (7.6–15.0)	66.6 (46.1–87.1)
Papaverine	32.5 (25.4–39.6)	Inactive
Hyoscine butyl bromide	10.3 (7.2–13.4)	50 (35.6–64.4)
Flavoxate	14.5 (9.9–19.1)	Inactive

More clear-cut is the difference after oral administration, since CR 605 and CR 816, among the papaverine-like compounds, are the only active ones, whilst hyoscine butyl bromide is active at toxic levels.

Perfusion of the rat stomach *in vivo*

In order to evaluate the whole activity of tyropramide (CR 605) and mepramide (CR 816) on gastric muscle and pyloric sphincter, a test was performed which enabled us to quantify the pharmacologic effects induced by the substances under study on this type of muscle. This test was performed perfusing the stomach of anaesthetized rats at 37 °C with saline solution and collecting the liquid flowing out from the pylorus in the first hour. The drug being tested is injected intavenously and the collection procedures are repeated during the following 60 min. The pharmacological effect is deduced from the difference between the volume of fluid collected in the first and second hour. Using this method, it is possible to evaluate at the same time both the relaxant action of the drug under normal conditions and an incidental effect antagonizing the contraction induced by a spasmogenic agent: in this specific case morphine.

CR 816 exerts a powerful action relaxing the smooth muscle of the stomach and pylorus (Table 46.2); this activity is greater than that of papaverine and cannot be compared with that of flavoxate, which has instead a spasmogenic action. CR 605 under these conditions exerts no relaxant activity, therefore it does not modify any parameter of normal gastric motility.

Table 46.2 *In vivo* perfusion of rat stomach – activity of CR 816 and CR 605: comparison with other drugs

Drug	*Dose* (mg/kg intravenous)	*0–60 min*	*1–2 h*	*Changes (% after the 1st hour*	*t*	*p*
Physiological solution	—	114.2	121.9	+ 6.51	4.55	~0.02
Papaverine	5.00	99.35	186.4	+87.6	3.86	>0.05
CR 816	1.25	94.75	174.0	+83.6	3.42	>0.05
CR 605	5.00	131.2	132.9	+ 1.2	1.25	NS
Flavoxate	5.00	113.7	42.9	−62.1	6.07	>0.01

The values are expressed as perfusion liquid millilitres in 1 h.
Each value is the average of the results in four animals.
NS = not significant.

CR 816, in confirmation of its powerful relaxant action, not only removes the spasm induced by the simultaneous administration of morphine (Table 46.3), but further acts by causing a remarkable release of the gastric and pyloric muscles; papaverine exerts a similar function, even though slightly less marked, whereas flavoxate is unable to inhibit the spasm. Particularly interesting is the action of CR 605, which inhibits only the spasmogenic effects of morphine, therefore exerting a function normalizing the tone of the smooth muscle of the stomach.

Table 46.3 *In vivo* perfusion of rat stomach – activity of CR 816 and CR 605 against spasm induced by morphine: comparison with other drugs

Drug	*Dose* (intravenous)	*0–60 min*	*1–2 h*	*Changes (% after the 1st hour*	*t*	*p*
Morphine	250 μg/kg	122.6	76.8	−37.4	4.06	>0.05
Papaverine + morphine (250 μg/kg)	5 mg/kg	91.32	137.8	+50.9	3.03	~0.05
CR 816 + morphine (250 μg/kg)	5 mg/kg	132.3	244.7	+85.1	8.85	<0.01
CR 605 + morphine (250 μg/kg)	5 mg/kg	171.3	186.6	+ 8.9	0.87	NS
Flavoxate + morphine (250 μg/kg)	5 mg/kg	161.2	62.6	−61.2	10.7	<0.01

The values are expressed as perfusion liquid millilitres in 1 h.
Each value is the average of the results in four animals.
NS = not significant.

At present some investigations are in progress to evaluate whether the different behaviour of CR 605 and CR 816 must be ascribed simply to quantitative differences of the same pharmacological effect, or to a real different mechanism of action. This type of study is particularly stimulating, in view of the small structural differences between these two molecules; and could lead to their differentiated or supplementary use in human therapeutics.

Perfusion of biliary pathways of guinea pig *in vivo*

Using a method similar to that described above, we studied the effect of tyropramide (CR 605) and mepramide (CR 816) on biliary pathways and the sphincter of Oddi.

The technique employed consists of the perfusion, under steady hydrostatic pressure, of the biliary pathways of the anaesthetized guinea pig and in the collection of the perfusion fluid as it flows into the duodenum through the sphincter of Oddi. As above, the effect of the substances under study is deduced from the difference between the volume of fluid outflowing in the first hour (i.e. under normal physiological conditions) and that following the administration of the drug.

Since the perfusion fluid has to pass along all the biliary pathways and through the sphincter of Oddi, it may be thought that the fluid flow is correlated with the muscular tone of the involved channels and with their propulsive capacity, as well as with the tone of the sphincter. Also in this instance, the experiment was carried out in the presence or absence of morphine, employed as a spasmogenic agent.

In the absence of the spasmogenic agent (Table 46.4), CR 816 exerts a marked relaxant effect on the biliary ways and the sphincter of Oddi, even though in dose levels greater than those found effective on gastric muscle. Papaverine, hyoscine butyl bromide and CR 605 show no statistically significant action.

Table 46.4 *In vivo* perfusion of guinea pig biliary pathways – activity of CR 605 and CR 816: comparison with papaverine and hyoscine butyl bromide

Drug	*Dose* (mg/kg intravenously)	*0–60 min*	*1–2 h*	*Changes % after the 1st hour*	*t*	*p*
Physiological solution	—	55.23	51.72	− 6.3	1.53	NS
Papaverine	5	77.30	86.20	+11.5	0.97	NS
CR 816	5	43.95	61.75	+40.5	3.17	~0.05
CR 605	5	46.60	56.60	+10.0	1.27	NS
Hyoscine butyl bromide	5	48.20	49.40	+ 2.5	0.86	NS

The values are expressed as perfusion liquid millilitres in 1 h.
Each value is the average of the results in four animals.
NS = not significant.

With simultaneous administration of morphine (Table 46.5), CR 816 removes the spasm and is capable of exerting further relaxant action; CR 605 inhibits the action of morphine by returning the muscular state to normality, whereas papaverine is unable to counteract the induced spasm.

Table 46.5 *In vivo* perfusion of guinea pig biliary pathways – activity of CR 605 and CR 816 against spasm induced by morphine: comparison with papaverine

Drug	*Dose* (intravenous)	*0–60 min*	*1–2 h*	*Changes (% after the 1st hour*	*t*	*p*
Morphine	250 μg/kg	53.9	25.1	−53.3	5.18	<0.02
Papaverine + morphine (250 μg/kg)	5 mg/kg	82.3	57.1	−30.6	6.39	<0.01
CR 816 + morphine (250 μg/kg)	5 mg/kg	62.7	81.7	+30.3	3.16	~0.05
CR 605 + morphine (250 μg/kg)	5 mg/kg	50.8	58.3	+15.0	1.37	NS

The values are expressed as perfusion liquid millilitres in 1 h.
Each value is the average of the results in four animals.
NS = not significant.

Pharmacology *in vitro*

In vitro tests were carried out on the isolated stomach of rat and guinea pig,

on the isolated ileum of guinea pig, ascending colon of rat; and in the urogenital tract, on the female rat uterus and on the ascending ureter of rat.

Isolated rat stomach

On the isolated rat stomach the threshold of response of CR 605 in inhibiting the electric stimulus according to Patton's method is about 10γ/ml, whereas for CR 816 this type of activity is still under study.

Isolated guinea pig stomach

In the isolated guinea pig stomach the ability to inhibit the vagal and transmural stimulus was evaluated. In both cases CR 605 is found to be less active than hyoscine butyl bromide, which is, however, a typical anticholinergic agent. Also in this test data pertaining to the activity of CR 816 are at present lacking.

Guinea pig ileum and ascending colon of rat

The values of mepramide (CR 816) and tyropramide (CR 605), inhibiting the contractions induced by various spasmogenic agents respectively on the guinea pig ileum and on the rat ascending colon, are shown in Tables 46.6 and 46.7). The values are expressed as ED_{50}, i.e. the concentration of drug in mg/ml capable of reducing the induced contractions by 50%. In order to facilitate the evaluation, we showed also the activity of specific antagonists in absolute value (mg/ml) and as index of activity (in brackets).

Table 46.6 Activity of CR 605 and CR 816 in inhibiting the spasm induced by different agents on *in vitro* isolated guinea pig ileum

	Antispasmodic activity – ED_{50} (mg/ml)		
Drug	*Serotonin*	*Spasm induced by Histamine*	*$BaCl_2$*
Papaverine	—	—	6.6×10^{-6}(1)
Mepyramine	—	$\sim 10^{-11}$(1)	—
Methysergide	5.4×10^{-7}(1)	—	—
CR 605	7.6×10^{-7}(−1.4)	—	3.6×10^{-6}(+2)
CR 816	5.2×10^{-6}(−10.4)	$\sim 10^{-6}$(−10^5)	3.3×10^{-6}(+2)

Table 46.7 Activity of CR 605 and CR 816 in inhibiting the spasm induced by different agents on *in vitro* ascending rat colon

	Antispasmodic activity – ED_{50} (mg/ml)		
Drug	*Serotonin*	*Spasm induced by $BaCl_2$*	*Acetylcholine*
Papaverine	—	3.6×10^{-6}(1)	8.5×10^{-5}(1)
Methysergide	1.34×10^{-7}(1)	—	—
CR 605	—	6.2×10^{-7}(+6)	8.9×10^{-6}(+10)
CR 816	7.6×10^{-6}(−56.7)	2.5×10^{-6}(+1.4	5.8×10^{-6}(+14)

CR 605 and CR 816 are more active than papaverine itself in inhibiting the spasm induced by barium chloride on the guinea-pig ileum (Table 46.7), whereas the high anti-serotonin effect of CR 605 is particularly interesting; in fact this activity is nearly the same as that of methysergide, one of the most powerful known anti-serotonin agents. The same applies to the rat ascending colon, on which tyropramide (CR 605) and mepramide (CR 816) are markedly more active than papaverine in inhibiting the spasm induced by both $BaCl_2$ and acetylcholine.

These differences are particularly remarkable for CR 605 against barium chloride (about six times more active) and for both agents against acetylcholine (CR 605 ten times more active and CR 816 about fifteen times more active).

GENERAL PHARMACOLOGY

Other experimental tests, performed to outline accurately the pharmacological properties of these compounds, demonstrated that both are devoid of mydriatic activity (therefore, are not anticholinergic agents); do not stimulate or inhibit salivary and gastric secretions, whereas they do exert in the rat a slight hypotension with respiratory stimulation (after intravenous administration) and a certain vasodilatation; however, less than that produced by papaverine.

TOXICOLOGY

Acute toxicity

The values of LD_{50} estimated for tyropramide and mepramide on various animals, and by various routes of administration, are shown in Table 46.8. Acute toxicity is remarkable by parenteral route, but in both cases markedly

Table 46.8 Acute toxicity of CR 605 and CR 816 in different animals and by different administration routes

		Acute toxicity as LD_{50} (mg/kg) *Administration route*			
Drug	*Animal*	i.v.	s.c.	i.p.	*per os*
CR 816	Rat	34.0 (24.8–43.1)	>250 —	77.0 (63.9–90.0)	430 (347–513)
	Mouse	23.5 (19.8–27.2)	160 (139–180)	84.0 (70.9–97.1)	285 (244–325)
CR 605	Rat	51.8 (45.4–59.1)	430 (331–559)	140 (131–150)	800 (672–952)
	Mouse	40.5 (36.6–47.4)	245 (225–267)	120 (95–151)	550 (478–632)

i.v. = intravenous; s.c. = subcutaneous; i.p. = intraperitoneal
Range in parentheses

decreases after oral administration. This is particularly important since, differing from other papaverine-like agents and papaverine itself, tyropramide and mepramide both exert a marked anti-spasmodic activity when given *per os*.

Chronic toxicity

With tyropramide (CR 605) chronic toxicity tests at 1, 3 and 6 months in the rat and dog showed no difference from the usual for this type of toxicity; the same is true for teratogenesis, which gave negative results in two animal species (rat and rabbit). These same experiments are planned or in progress for mepramide (CR 816).

CLINICS

For CR 605 the tolerability tests in humans were concluded successfully and therefore a total of thirteen clinical trials were started in Italy, Germany and France. The following were the pharmaceutical forms developed and employed in the trials:

(a) ampoules for intramuscular and/or intravenous use, containing 50 mg CR 605 in 5 ml;
(b) tablets of 100 mg;
(c) suppositories of 200 mg.

The following pharmaceutical forms are foreseen:

(a) ampoules for intramuscular and/or intravenous use, containing 25 mg CR 816 in 5 ml;
(b) tablets of 25 and 50 mg;
(c) suppositories of 50 and 100 mg.

For tyropramide (CR 605) the preliminary results of double-blind trials already evaluated may be divided into two groups:

1. Intramuscular or intravenous administration a single time in acute pains (colics);
2. Repeated administration in the event of spasms of the urinary tract and gastroenteric system.

In clinical trials on fifty-five patients the regression of pain with time was evaluated in comparison with a test agent, hyoscine butyl bromide. Pain intensity was evaluated by a score from 0 (no pain) to 4 (peak intensity). In a large percentage of patients the pain disappeared after intramuscular administration within the peak time of 40 min, as reported in Figure 46.2. Statistical analysis demonstrates that tyropramide in clinical practice is significantly more active than hyoscine butyl bromide. The long-term evaluation *per os*

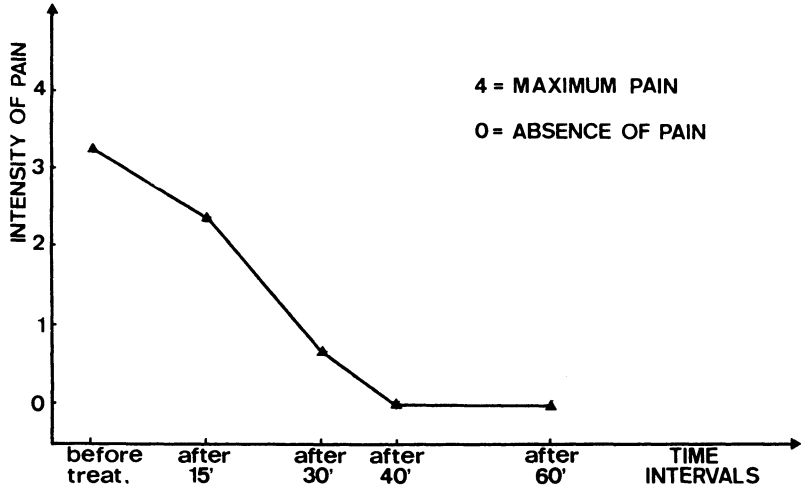

Figure 46.2 Average intensity of pain in patients treated with CR 605 (tyropramide) 1 ampoule intramuscularly

was completed recently in a German clinic. The results obtained, at present under statistical evaluation, reveal that the compound is very active in removing pains in the urinary and gastrointestinal tracts, especially when given *per os* and by rectal route. A complete evaluation of the therapeutic results in clinical practice requires a greater amount of data, and therefore, awaits the completion of the trials at present in progress.

To conclude, we may state now that in the treatment of acute and chronic symptoms due to smooth muscle spasm in humans, tyropramide (CR 605) has the following interesting features:

(a) prompt activity *per os* and parenterally;
(b) particular activity *per os* or by rectal route, (an unusual characteristic in antispasmodic agents);
(c) therapeutic activity which tends to modify the pathologic parameters without changing the normal body function.

The clinical data concerning CR 816 are not yet available.

CONCLUSIONS

The results obtained in the *in vitro* and *in vivo* trials of two new tyrosine derivatives (CR 605 and CR 816) with antispasmodic activity are reported.

The experimental results show that these agents are more active than papaverine and have a more complex mode of action, which is still under study.

Both agents are very active also *per os*, and their therapeutic index suggested their clinical evaluation in acute and chronic syndromes due to smooth muscle spasm.

The preliminary clinical results confirm the usefuleness of these two compounds due to their high antispasmodic activity and indirectly due to their analgesic activity in acute pains (biliary and renal colics) and also in chronic pains of the gastrointestinal and urogenital systems.

Discussion

A. L. Blum: (Switzerland) According to your comments, five clinical trials with your drugs have already been completed. Could you please tell us the results of these trials?

P. Senin: (Italy) I do not have these data with me but I can send you the information.

47

Sources of inaccuracy in measurement of sphincter pressures using perfused catheters

D. J. FROMMER

Lower oesophageal sphincter pressure is one of the most frequently studied parameters in clinical investigations of gastrointestinal motility. Perfused catheters are usually used, but there are wide differences in normal mean values quoted for resting pressures at the lower oesophageal sphincter, e.g. 12.0 mmHg[1] to 31 mmHg[2]. The majority of papers quote mean values in the range of 18–25 mmHg. Such variations may be due to the different techniques and apparatus used, and the present study was designed to determine the effect of these on sphincter pressures recorded using sphincters at known pressures.

METHODS AND MATERIALS

Pressures were measured by a Statham P23 transducer connected to a Devices M19 recorder having a 3552 pre-amplifier set at zero damping. Full scale deflection was set at 50 or 25 mmHg, whichever was most appropriate, the sensitivity having been checked by use of a sphygmomanometer. The transducer was connected to a 100 cm length of polyvinylchloride (pvc) tubing with a terminal 20 cm nylon catheter, outside diameter 3.24 mm, bore 2.44 mm, with a side hole 1.25 mm diameter situated 10 cm from the end. The catheter was passed through an excised human lower oesophagus with a child's sphygmomanometer cuff, 3.0 cm wide, wrapped around it in the region of the lower oesophageal sphincter. The cuff was connected to a sphygmomanometer and the cuff blown up until the pressure in it was 10, 25 or 35 mmHg.

The following variations in technique were tested:

Flow-rates

Water was perfused along the catheter at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 ml/min. Flow-rates were checked by collections into measuring cylinders over 5–15 min intervals.

Pull-through and station techniques

The catheter was pulled through the compressed lower oesophageal sphincter at approximately 1 cm/s, ten times in succession. The catheter was then pulled through the sphincter very slowly so that when the maximal pressure was registered, catheter movement was stopped for approximately 20 s. This 'station' manoeuvre was repeated ten times. Mean values and standard deviations were calculated from ten observations. Student's *t* test was used for tests of significance.

High (pump) and low (gravity) compliance systems

The high compliance system consisted of a new Harvard syringe pump, model 936, with plastic 50 ml Terumo syringes connected to the PVC tubing and the transducer via a Y-connector. In the low compliance system a plastic bag filled with water was suspended 163 cm above the level of both the transducer and the sphincter and provided fluid for perfusing the catheter at a pressure equivalent to 120 mmHg. The bag was connected to the pvc tubing by an intravenous giving set and number 25 needle entering the side arm of the 3-way tap. The other two arms of the tap communicated simultaneously with the transducer and the tubing. The compliance of each system was measured by the method of Dodds *et al.*[3]. The catheter and tubing were perfused at the desired rate, then the catheter was suddenly clamped close to and proximal to the side hole with a pair of surgical forceps. The time taken for the pressure to rise 50 mm was determined, and from that the rate of rise in mmHg/s was calculated. The rate of pressure rise (i.e. compliance) of the two systems was similar up to and including 2 ml/min. Above 2 ml/min the rate of pressure rise increased sharply in the gravity system, showing very low compliance, whereas there was very little increase in pressure rise with the pump system.

Side hole size

Three nylon catheters were sealed at one end and one was connected to the pvc tubing. They had side holes of either 0.68, 0.95 or 1.25 mm diameter. Each was used with the pump system, both station and pull-through techniques, and a sphincter pressure of 25 mmHg.

RESULTS

Flow-rates

Under all conditions recorded pressures rose with increasing flow-rates. In the majority of cases the maximal increase in recorded pressure occurred at low flow-rates and the pressures reached a plateau by 5 ml/min. The values at 5 ml/min were generally below the sphincter pressure, being between 83.0 and 106.0% of it. However, the pressures recorded at the lowest flow-rate, 0.5 ml/min, were very inaccurate, being only 15–70% of the sphincter pressure.

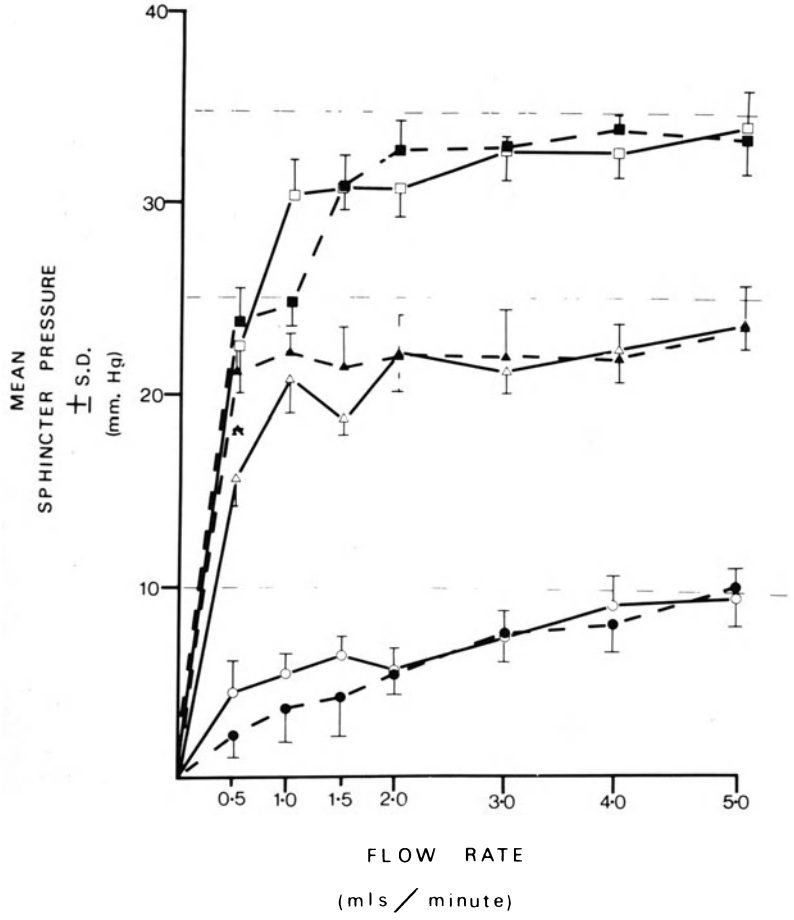

Figure 47.1 Recorded sphincter pressures using station technique with pump or gravity systems. Applied cuff pressures of 10, 25 and 35 mmHg shown by dashed horizontal lines. Gravity, – – –; pump, ———

* Indicates significant difference between results of the two systems.

Station and pull-through techniques

Using the station technique (Figure 47.1) the recorded pressures rose to close to the sphincter pressures when a flow rate of 5 ml was reached. With the 25 mmHg and 35 mmHg sphincter pressures the recorded pressures at 2 ml/min did not differ significantly from those at 5 ml/min. However, recorded pressures using the sphincter at 10 mmHg gave a curve approximating to a straight line. Using the station technique both gravity and pump systems gave very similar results.

With the pull-through technique (Figure 47.2) the recorded pressures rose

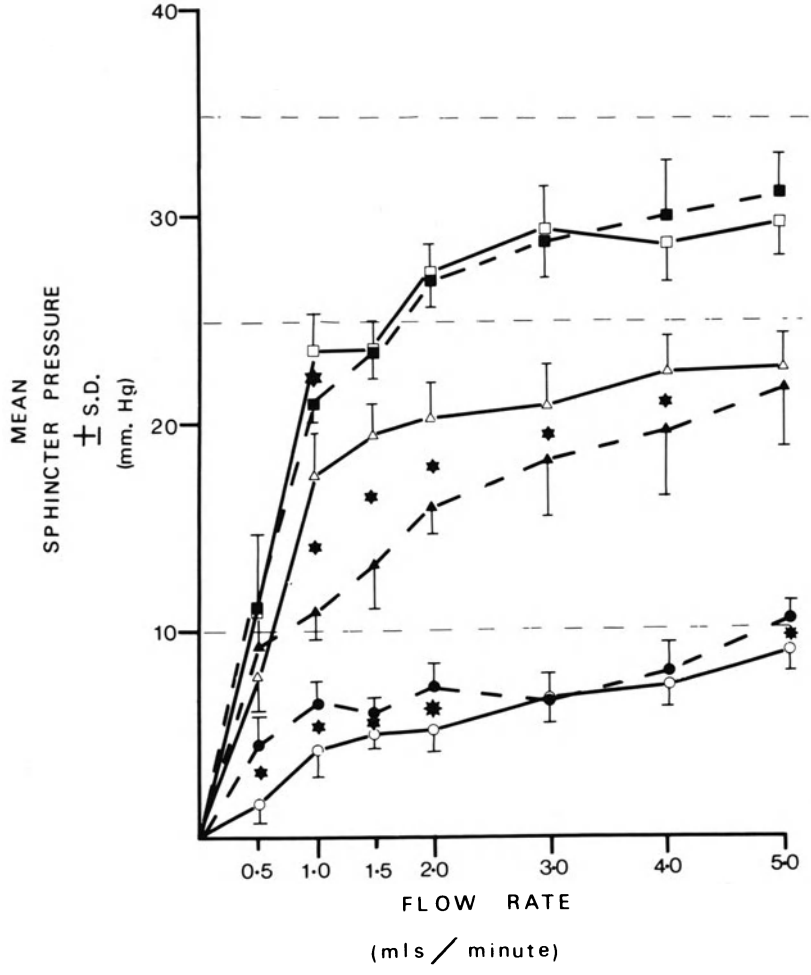

Figure 47.2 Recorded sphincter pressures using pull-through technique. Gravity, ———; pump – – –

more gradually with increasing flow-rate and were generally lower than the equivalent with the station technique. This tendency was most marked with high sphincter pressures and low flow-rates; e.g., at a sphincter pressure of 35 mmHg and 0.5 ml/min, from a gravity system, the station technique gave a mean value of 24.10 $\pm$ 1.91 mmHg, whereas the pull-through technique gave a value of only 11.40 $\pm$ 2.99 mmHg ($p < 0.001$) (Figure 47.3).

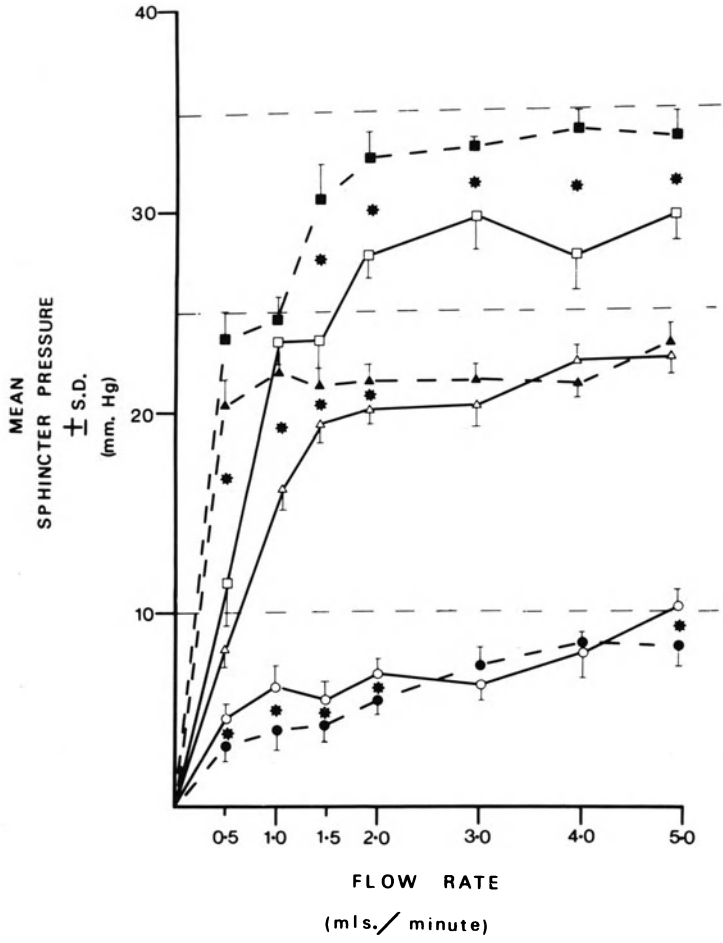

Figure 47.3 Recorded sphincter pressures using gravity system. Pull-through technique, ———; station technique, – – –

Using the pump assembly the increase in recorded sphincter pressure of station technique over pull-through was significant at the 5% level in most cases (Figure 47.4). This was less often true with the gravity system (Figure 47.3) and at the lower flow-rates with the sphincter pressure of 10 mmHg, the

pull-through technique gave significantly higher values ($p < 0.05$) than the station technique. However, at 5 ml/min the mean difference in recorded pressures using the two techniques was only 1.9 mmHg, or 8.9% of the pressure obtained by using the station technique.

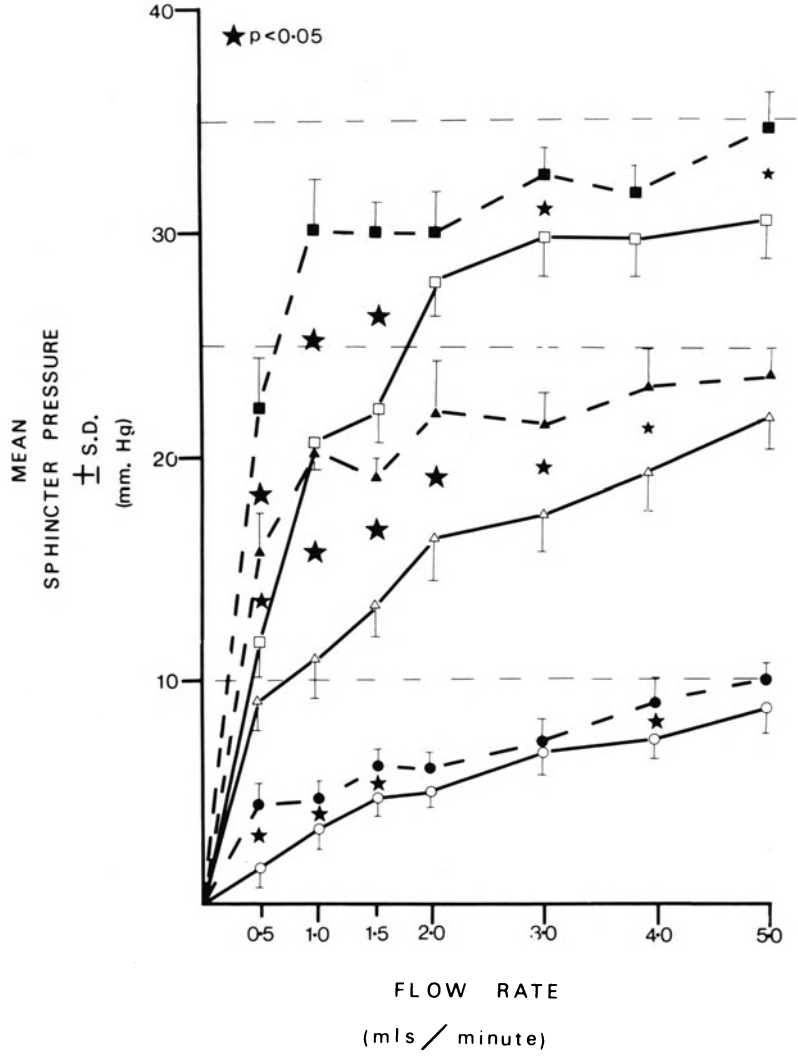

Figure 47.4 Recorded sphincter pressures using pump system. Pull-through technique, ———; station technique, – – –

Low (gravity) and high (pump) compliance systems (Figures 47.3 and 47.4)

The recorded values with both systems were very similar, especially when used with the station technique (Figure 47.1) and values close to those at 5 ml/min

were obtained at 2 ml/min with the sphincter pressures at 25 and 35 mmHg. However, using the pull-through technique (Figure 47.2) the gravity system gave significantly higher values than the pump for the sphincter at 25 mmHg, but the reverse was true for the sphincter at 10 mmHg.

Side holes of different diameters (Figures 47.5 and 47.6)

Using the pump and the sphincter at 25 mmHg the smallest hole (0.68 mm diameter) gave values above the sphincter pressure, whereas the larger holes gave values below the sphincter pressures. This was true using both station and pull-through techniques, but with the former technique (Figure 47.5) the effect of using the smallest hole was so marked that at a flow-rate of only

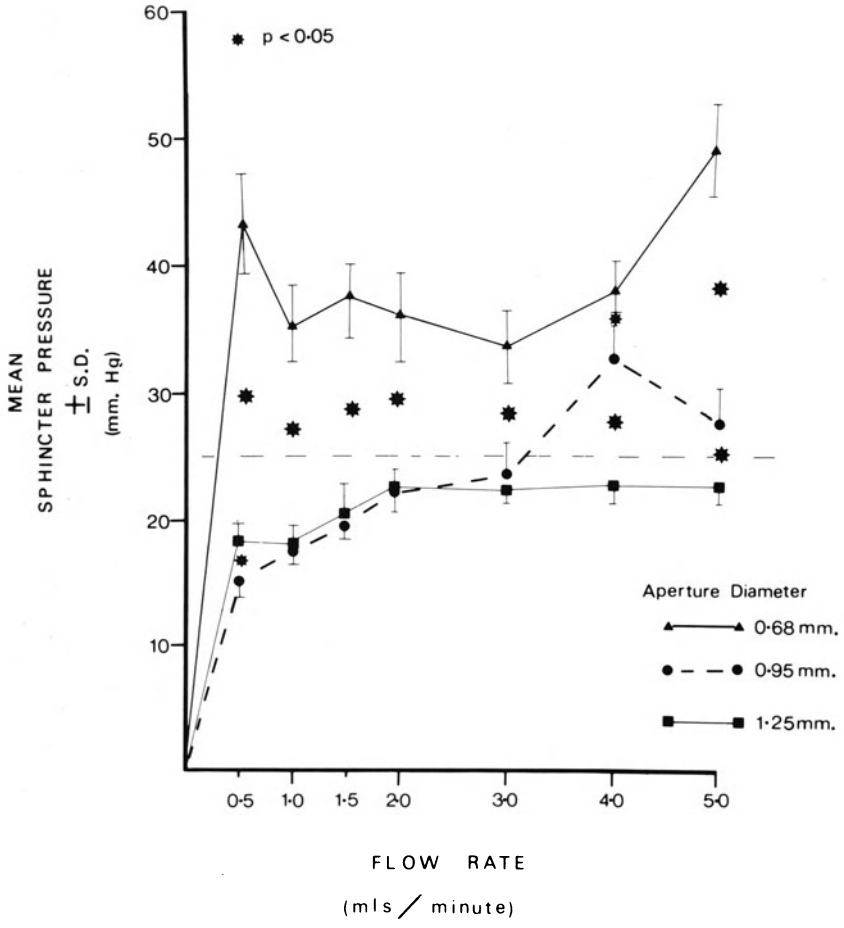

Figure 47.5 Recorded sphincter pressures using a sphincter at 25 mmHg, the pump system, tubing with side holes of different sizes and the station technique

0.5 ml/min the mean pressure recorded was 44.7 $\pm$ 4.7 mmHg. At 5 ml/min the mean pressure was 51.4 $\pm$ 6.6 mmHg. These values were very significantly raised ($p < 0.005$ in all but one case) over values for the two larger holes. With the pull-through technique (Figure 47.6) the values with the smallest hole were below 25 mmHg until a flow-rate of 2 ml/min was reached, and then the values remained relatively constant at about 28.4 mmHg. With this technique most of the values for the different sized holes were significantly different from one another.

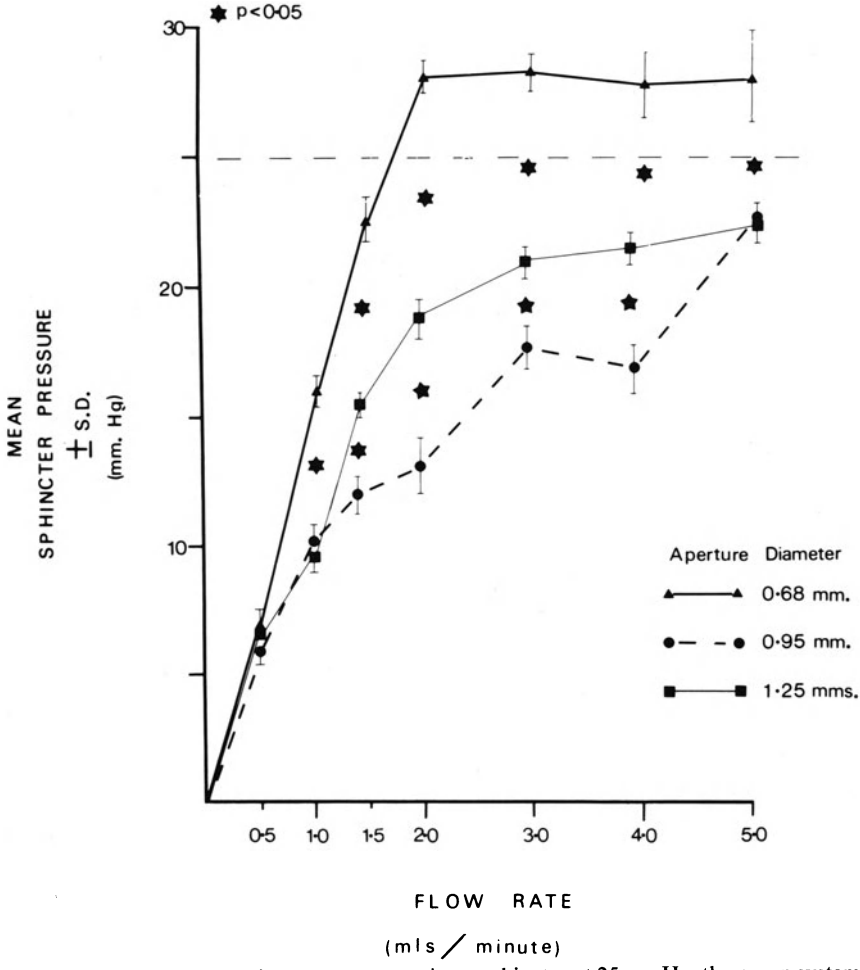

Figure 47.6 Recorded sphincter pressures using a sphincter at 25 mmHg, the pump system, tubing with side holes of different sizes and the pull-through technique

DISCUSSION

These results show that the most marked and consistent factor affecting

recorded sphincter pressure was the rate with which the catheter was perfused. Using a flow-rate of 0.5 ml/min the recorded pressures were gross underestimates. Although in most cases the recorded pressure at 2 ml/min was close to that at 5 ml/min there was usually a rise in pressure between the two flow-rates. In a few cases, not recorded on the graphs, pressures continued to increase between 5 and 10 ml/min. It was only at low sphincter pressures (10 mmHg) that the recorded pressures reached the sphincter pressure, but as the recorded pressure rises with flow-rate it would be anticipated that recorded and sphincter pressures, however great, would coincide if a sufficiently high flow-rate is used. At 5 ml/min flow-rate the recorded value is about 90% of true sphincter pressure and is acceptable, but the degree of inaccuracy will vary with sphincter pressure.

Relatively little work has been done on the influence of flow rate on recorded sphincter pressures. Rinaldo and Levey[4] found that, using the pull-through technique, there were often significantly higher pressures in the oesophageal sphincters with increasing flow-rates over the range 0.297–2.24ml/min. In an oesophageal model with pressure changes similar to those affecting a catheter passing through a sphincter, Pope[5] noted that recorded pressures rose progressively and markedly as perfusion rates increased from 0.48 to 2.40 ml/min; they felt that this phenomenon was of significance in recording peristaltic waves but not for sphincter pressures. Waldeck[6], using a pull-through technique on an oesophageal model, stated that 4–5 ml/min was the lowest perfusion rate giving 'an exact pressure transmission', but no data were given.

It may be of significance that the mean lower oesophageal sphincter pressure in normal subjects was lowest, viz. 12.0 mmHg, in the study with the lowest perfusion rate (0.123 ml/min; Hall *et al.*[1]) and highest in the study by Osborne *et al.*[2], i.e. 31 mmHg, where a perfusion rate of 6.0 ml/min was used. Intermediate pressure and perfusion rates were given in other studies. However, it is likely that other factors are important.

The pull-through technique usually gave lower recorded pressures than the station technique, and this tendency was most marked at low flow-rates and medium and high sphincter pressures where the former technique gave values of about 50% of that found using the latter one. However, the value obtained at 5 ml/min perfusion rate using the pull-through technique were about 91% of those found with the station technique, and would be regarded as fairly satisfactory, especially in view of the greater ease and smaller observer error[7] in measuring the sphincter pressure in subjects with the former technique. However, Dodds *et al.*[7] found that the pull-through technique gave values about 15% higher than the station technique.

The pump assembly gave surprisingly low rates of pressure increase on occlusion of the catheter. The high compliance that this demonstrates may be due to the pump (as suggested by the work of Dodds *et al.*[3]), but the same authors showed that diminishing leakage in the syringes by greasing them

diminished the compliance of the system. It is possible that the plastic syringes with rubber-tipped plungers that were used contributed substantially to the compliance. The gravity system had a high compliance only in the range 3–5 ml/min and it was at this range of flow-rate that the difference in results between the two systems was least. It was not surprising that there was no difference using the two systems with the station technique, but the lack of any clear-cut difference in results between the two with the pull-through technique was unexpected. Although measurements with the 25 mmHg sphincter showed that the gravity system gave higher values, results with the 10 and 35 mmHg sphincters did not show a similar trend. The advantages of using the gravity system at 5 ml/min flow-rate over the pump system therefore lies in other factors; e.g., cheapness, ability to run for up to 200 min continuously without need for reloading syringes, and the (theoretical) advantage of such a low compliance system in accurately measuring peristaltic waves in the body of the oesophagus.

There have been no studies on the effect of size of side holes on recorded pressures, but these results suggest that varying the size of the hole in the range used does not make a substantial difference to the accuracy of the measured pressure, provided a high perfusion rate and a pull-through technique is used. However, the values recorded for the 0.68 mm diameter hole under these circumstances were significantly higher ($p < 0.001$) than those with the larger holes, and this tendency was markedly increased when the station technique was employed, even at the lowest flow rates. It is likely that this phenomenon is due to mucosa becoming stuck in the side hole and that the high pressures generated (36–51 mmHg for a 25 mmHg sphincter) are due to the pressures required to dislodge the mucosa rather than being sphincter distension pressures. It would be expected that this phenomenon would be less marked in the pull-through technique where the movement of the catheter would dislodge any mucosa, and this was found to be the case. The 1.25 mm diameter hole gave somewhat more accurate values than the 0.95 mm diameter hole, and is therefore to be preferred. Most authors appear to use a hole diameter of at least 1 mm.

Acknowledgement

Thanks are due to Mr Arthur White for able technical assistance.

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Discussion

A. M. Connell: (USA) Balloons (small) have difficulties: (1) manufacture; (2) possibility of rupture; (3) air is compressible, and they therefore need low volume. They do have some advantages, although we must remember all the disadvantages above.

D. Frommer: (Australia) I agree.

C. E. Pope: (USA) You are measuring the pressure at which fluid escapes from the catheter. In my experience, the results are not reproducible if fresh excised human tissue is not used. How did you obtain your specimens?

Frommer: I used freshly excised specimens. However, I found that reproducible results were obtainable with formalin-fixed specimens but only at 25 and 35 mmHg pressures. The fixed specimens did not allow pressures of 10 mmHg to be obtained.

J. T. Farrar: (USA) There are many difficulties in getting reproducible results. Should we be using fluid- or air-filled balloons to measure the pressure, as suggested by Code many years ago?

Frommer: Balloons have their own difficulties, i.e. what size, rotting of rubber, punctures, etc. However, the message that I want to get across with the paper I have presented is to test whichever method is used by the use of sphincters of known pressures and noting the accuracy of the pressures recorded. The inaccuracies found, and any corrections made for them, should be mentioned in any papers published.

W. J. Dodds: (USA) (1) The data show that low *v.* high compliance systems have not been compared. You have used two high compliance systems; therefore the data show only a low rise in pressure. (2) We have shown that reproducible results can be obtained with low flow-rates and we should not reject studies with such flows.

Frommer: I do not think that the compliance of the gravity feed system of 150 mmHg found with a flow-rate of 5 ml/min could be described as high compliance; it should accurately reproduce all pressure rises in the body of the oesophagus, as your own data have shown, and should be more than adequate for the low pressures of the lower oesophageal sphincters. However, it is interesting that in your latest paper you demonstrated similar compliances with your hydraulic-capillary system and a pvc catheter of 2.0 mm internal diameter to the values obtained with the gravity perfusion and a pvc catheter of 2.44 mm internal diameter. It seems probable that the compliance of the systems I used could be improved with narrower bore and thicker walled tubing. I agree that studies with low flow-rates should not necessarily be rejected, but they should be validated by studies such as yours.

48
Food sensitivity in reflux oesophagitis

S. F. PRICE, K. W. SMITHSON AND D. O. CASTELL

Patients with symptomatic oesophagitis frequently note the association between heartburn and the ingestion of certain foods including citrus juices, spicy foods, coffee, chocolate and fatty foods. Although this phenomenon has been well recognized[1], the responsible mechanism remains obscure. Numerous recent studies have shown an effect of some foods on the lower oesophageal sphincter (normally abbreviated as LES). It has been proposed that foods such as fat[2], alcohol[3] and chocolate[4] may cause heartburn through their ability to reduce sphincter pressure with resulting acid reflux[5]. Studies of other foods such as orange juice and tomato products (a common base in spicy foods) have failed to reveal sustained decreases in LES pressure[4]. The effect of coffee remains controversial; recent studies have suggested both resultant increases and decreases in sphincter pressure[6,7].

It was our purpose to evaluate the mechanism by which heartburn is produced following the ingestion of orange juice, spicy tomato drink and coffee.

METHODS

Patients

The study was performed on twenty-one patients (sixteen male and five female) 21–81 years of age, referred to the gastroenterology clinic for evaluation of chest pain. A careful description of the patient's symptoms, a detailed food questionnaire, a medical history, a physical examination, and a statement of informed consent were compiled prior to inclusion in the study. Typical heartburn was defined as a substernal sensation, radiating orad, more frequent after meals and brought on by bending or lying down.

Modified Bernstein test

The infusion procedure was patterned after the original acid challenge of Bernstein[8]. After an 8 h fast, the patient was placed in a sitting position and an opaque nasogastric tube was advanced into the stomach and the gastric contents aspirated. The tube was then withdrawn to a position 30 cm from the nares. The free end of the tube was connected via a series of valves to bottles containing saline, O.1 N HCl, and the other three test solutions. Bottles were covered, and neither patient nor investigator were aware of the substance being infused. All solutions were given at room temperature at a rate of 6–7 cc/min and the sequence randomized from patient to patient.

After 10 min of saline administration, a technician began infusion of either one of the test substances or the 0.1 N HCl, and continued for a period of 30 min or until a positive test was obtained. The test was considered positive if the patient's chest pain was reproduced and could be rapidly relieved by saline infusion. Evidence of pain was volunteered by the patient and interpreted by the interviewer. In equivocal cases the patient was re-challenged. The test was considered negative if the patient experienced no pain during the 30 min period. Patients with pain prior to the onset of the study, pain caused by the initial saline infusion, or unremitting pain were excluded from the study.

The 0.1 N HCl solution was prepared by the hospital pharmacy. The saline, used as a control, was obtained from hospital intravenous solution stock. The orange juice (Minute Maid frozen concentrate), coffee (Taster's Choice freeze dried), and tomato drink (Tabasco Bloody Mary Mix) were prepared according to manufacturer's instructions.

Statistics were performed according to conventional chi-square calculations.

RESULTS

Twenty-one patients underwent infusion of coffee, orange juice, and tomato drink in addition to 0.1 N HCl infusion. Five of these patients had a negative acid perfusion test and all five were insensitive to all 3 food substances (Tables 48.1–48.3).

Sixteen of the patients were acid-positive. As shown in Table 48.1, fifteen of these patients were also sensitive to orange juice, with the chi-square value of 12.2 indicating a highly significant ($p < 0.001$) association between oesophageal acid sensitivity and sensitivity to intra-oesophageal orange juice. Fifteen of the sixteen acid-positive patients also showed symptoms during intra-oesophageal tomato drink infusion (Table 48.2). Again, a chi-square value of 12.2 indicates a highly significant association ($p < 0.001$) between these two variables. Finally, the association between acid sensitivity and sensitivity to intra-oesophageal coffee infusions was also highly significant

(chi-square = 6.8; $p < 0.01$). Thirteen of the sixteen acid-sensitive patients were sensitive to coffee (Table 48.3).

Table 48.1 Relationships between sensitivity to intra-oesophageal acid and orange juice

		Orange juice	
		+	−
Acid	+	15	1
Perfusion	−	0	5

Chi-square = 12.2; $p < 0.001$

Table 48.2 Relationship between sensitivity to intra-oesophageal acid and tomato drink

		"Spicy" Tomato Drink	
		+	−
Acid	+	15	1
Perfusion	−	0	5

Chi-square = 12.2; $p < 0.001$

Table 48.3 Relationship between sensitivity to intra-oesophageal acid and coffee

		Coffee	
		+	−
Acid	+	13	3
Perfusion	−	0	5

Chi-square = 6.8; $p < 0.01$

DISCUSSION

Patients frequently complain of symptoms precipitated by the ingestion of particular foods; however, with the exception of the disaccharidase deficiencies and coeliac sprue, cause-and-effect relationships have been difficult to document. Nonetheless, the phenomenon seems particularly well entrenched, especially in literature concerning reflux oesophagitis. In addition, patients with decreased lower oesophageal sphincter (LES) pressures have been shown to have a greater number of food intolerances than controls[9]. Our study, and

previous ones[9], show an increased frequency of food intolerance among patients with clinical evidence of oesophagitis.

Many of the food substances implicated as heartburn producers, ethanol, peppermint, fat, etc., have been shown to decrease LES pressure, and subsequent reflux of acidic contents has been postulated as the cause of pain. Studies on the effects of orange juice and tomato products, the substances most incriminated by patients in this study, have failed to reveal decreases in LES pressure. Coffee, the third substance tested, has been shown in one recent study to actually increase LES pressure[6]. It has been suggested, therefore, that such foods may cause heartburn by direct irritation of the oesophageal mucosa analogous to the effects of acid or bile salts[10]. The present study confirms this prediction and demonstrates that when these three food substances are instilled into the distal oesophagus of Bernstein-positive (acid-sensitive) individuals, these patients became symptomatic. In contrast, patients who were Bernstein-negative were largely insensitive to the food solutions.

No attempt was made to isolate the major irritant or irritants contained in these seemingly unrelated food substances, although their pHs (orange juice: 3.7; tomato drink: 4.1; coffee: 5.2) were only slightly below that of the saline control solution (pH 5.2). This suggests that the sensitivity is not accounted for by acidity alone. These data support the accepted clinical recommendation that specific irritating food substances should be avoided in patients with symptomatic reflux oesophagitis.

Acknowledgement

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Discussion

J. Behar: (USA) In our experience, patients with reflux oesophagitis complain of substernal burning pain after the ingestion of the foods that you have studied. How do you explain your observations that your patients complain of substernal pain during the infusion?

D. O. Castell: (USA) As you well know, the symptoms attributed to reflux oesophagitis are quite variable. I think our studies tell us that many patients probably experience pain with ingestion of foods that may be irritating to the inflamed oesophagus. Our studies, however, do not provide any evidence against the possibility that these or other substances may not also promote gastro-oesophageal reflux and be associated with symptoms occurring later.

J. T. Farrar: (USA) It was an excellent paper. Have you infused bile? You know that Hendrix and his colleagues have presented evidence that heartburn may be due to bile. The timing of heartburn in many cases would be consistent with the reflux of bile.

Castell: This is an excellent question. In fact we initially planned to study the effect of bile (the 'bile Bernstein' if you will), but found it very difficult to regularly obtain sufficient quantities of bile from the patients. I have a strong suspicion that patients who are acid-sensitive will also be bile-sensitive.

C. F. Code: (USA) Did you test any alcoholic beverages? The dry martini appears to be a major offender. What is your experience?

Castell: My clinical experience seems to indicate that many patients with chronic heartburn experience discomfort when ingesting alcohol. We, however, have not objectively studied this question in the manner described in our present experiments.

W. Silber: (S. Africa) Did your cases have endoscopic evidence of oesophagitis? As you know, patients can experience heartburn without evidence of oesophagitis. This is because heartburn can be precipitated by local spasm.

Castell: All of the cases in this study did not have oesophagoscopy performed, since the criteria for entry were only a history felt to be consistent with gastro-oesophageal reflux, with the acid perfusion (Bernstein) test then used as the criterion for acid sensitivity.

R. Earlam: (UK) I am pleased to see specific food substances tested on the lower oesophageal mucosa. My interpretation of your results is that the use of 0.1 N HCl in the Bernstein test is the correct solution for differentiating sensitive from insensitive mucosae. Would you agree?

Castell: Yes. I think our studies indicate that HCl with a pH below 2.0 is important to identify the sensitive oesophagus.

A. L. Blum: (Switzerland) Theoretically, reflux of acid could occur while you perfuse the oesophagus with neutral fluid, turning this fluid acidic again. Did you exclude this possibility by measuring intra-oesophageal pH while

performing the perfusion, or have you other evidence for a neutral intra-oesophageal pH during perfusion.

Castell: We did not measure intra-oesophageal pH during these studies. However, orange juice and spicy tomato are fairly good buffers, and may reasonably be expected to maintain their pH during these studies.

49

Motor response of the human oesophagus to different intraluminal pHs

E. CORAZZIARI, C. POZZESSERE, S. DANI, F. ANZINI AND A. TORSOLI

The main function of the oesophagus is to transport contents from the hypopharynx to the stomach and at the same time to avoid gastro-oesophageal reflux. The oesophagus also transports back to the stomach gastric contents occasionally refluxed into the oesophagus. Gastric acid reflux has been shown to occur in asymptomatic subjects in resting conditions[1,2]. The contact time between acid gastric contents and oesophageal mucosa is related to the frequency of reflux and to the capacity of the oesophagus to clear its contents; secondary peristalsis being the most important mechanism of oesophageal clearing.

Intraluminal distension is the stimulus for eliciting secondary peristalsis[3–6]. This situation would normally occur when a bolus is left in the oesophageal body after an ineffective primary peristalsis, or when gastric contents reflux into the oesophagus.

To our knowledge no information is available on the effect of intraluminal pH variation on oesophageal secondary peristalsis. The aim of the present study was to investigate the effect of intraluminal distension with liquid boluses of different pH on non-post-deglutitory motor activity in the lower oesophageal body.

METHODS

Fourteen subjects (eight males and six females; mean age 43 years; range 24–74 years) in good health and without gastrointestinal disorders, were investigated. Each gave informed consent. Before the investigation, acid

clearing test, Bernstein test, acid reflux test, oesophageal motility and lower esophageal sphincter pressure were shown to be within normal limits.

Three water-filled polyethylene catheters, 0.8 mm internal diameter, were used to transmit intraluminal pressures to Elema–Schönander external transducers. Manometric variations were then recorded on a multichannel Hellige polygraph. The catheters had side openings 1.2 mm in diameter arranged to measure intraluminal pressures at three points, 5 cm apart. The pressure recording tubes were perfused with distilled water at a constant rate of 0.5 ml/min with a Brown syringe pump. An additional polyvinyl catheter, 1.8 mm internal diameter, with multiple orifices over its distal 4 cm was assembled with the manometric catheters so that its tip was located 1 cm proximal to the uppermost manometric orifice. A gastric pH probe (Beckman 39042) was assembled with the polyethylene catheters with its tip located at the level of the middle manometric orifice. A standard reference lead (Beckman 39168) was held in contact with two fingers immersed in KCl-saturated solution[7].

HCl 0.1 N in the amounts of 0.5, 2.3, 5.4, 8.7 and 14.7 ml was added to 10 ml portions of barium sulphate to obtain suspensions at pH 6, 5, 4, 3 and 2 respectively. NaOH 0.1 N in the amount of 0.13 ml was added to 10 ml of barium sulphate suspension to obtain a suspension at pH 7. The same specific gravity of 1.170 for all the suspensions was achieved by adding distilled water as required. Titratable acid was 0.002, 0.009, 0.21, 0.028 and 0.05 mEq/ml in perfusates with pH 6, 5, 4, 3 and 2 respectively. Titratable acid content was measured by titrating a 10 ml sample of perfusate against 0.2 N NaOH with a microburette to pH 7 as measured by glass electrode (Radiometer, Copenhagen, Denmark).

All subjects were studied in the supine position after overnight fasting. The assembly was passed through the mouth and positioned with all the manometric orifices in the stomach. It was then withdrawn by 0.5 cm interval pull-throughs until the distal manometric orifice was located 1 cm proximal to the lower oesophageal sphincter. The assembly was then tightly anchored to the subject's cheek. The suspensions were perfused through the additional polyvinyl catheter at a rate of 1 ml/s until an oesophageal motor response was elicited. The acid suspensions were perfused in decreasing order of pH and each of them was preceded by the administration of the neutral suspension. Before and after each perfusate administration, the catheter was flushed with 10 ml of distilled water and the patient asked to swallow repetitively until the intraoesophageal pH returned to basal values, as recorded by the glass electrode, and no residual contrast medium was detected in the oesophagus at fluoroscopy. Before each administration the perfusate was thoroughly stirred and the subject asked to refrain from swallowing. If the subject happened to swallow during the perfusion time, the administration was interrupted, the catheter washed, the oesophagus cleared as previously described and the test resumed perfusing the neutral suspension. The volume of each suspension

required to evoke an oesophageal motor response was read to the nearest ml and has been expressed as percentage variation as compared to the mean of the control values of the neutral suspension.

Paired Student's *t* test and regression analysis were used for statistical evaluation of data.

RESULTS

Oesophageal motor response, either secondary peristalsis or tertiary contractions, was elicited during each stimulation test. Seventy stimulation tests with neutral suspension gave rise to secondary peristalsis on forty-one occasions (58.6%) and to tertiary contractions on twenty-nine (41.4%). Seventy stimulation tests with acid suspensions gave rise to secondary peristalsis on thirty-seven occasions (52.8%) and to tertiary contractions on thirty-three (47.2%). No correlation could be found between the type of motor response and either the pH value of the perfusate or the sequential order of perfusate administration. Except for one subject, rather constant

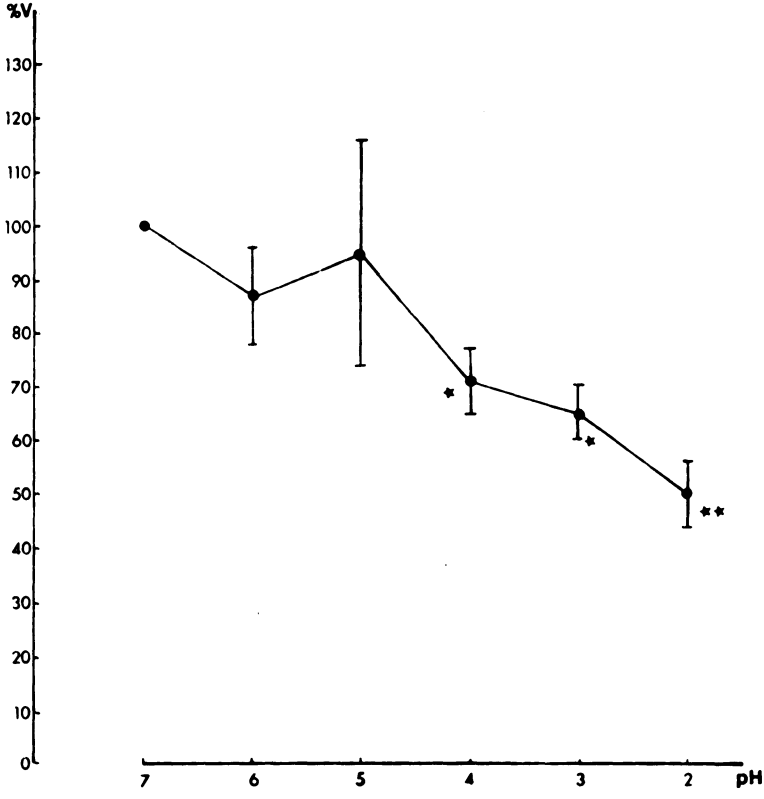

Figure 49.1 Volumes of perfusate, expressed as percentage variation of the neutral suspension, eliciting secondary peristalsis at pH 7–2. M ± SEM; $*p < 0.05$; $**p < 0.01$

volumes of neutral perfusate were needed to elicit the peristaltic response in the same person. Great variability in the volume necessary to produce a motor response was however found from one subject to another. The average amount of neutral suspension eliciting secondary peristalsis was 16.2± 1.5 (M ± SEM) and the range varied from 44 to 4 ml. Two subjects, otherwise having regular primary peristalsis, responded only with tertiary contractions to the stimulation tests.

The volume of perfusate at pH 6 and 5, necessary to elicit a secondary peristaltic motor response, did not significantly differ from the neutral control suspension. The administration of perfusate at pH 4, 3 and 2 significantly and progressively lowered the volume necessary to stimulate secondary peristalsis (Figure 49.1).

The average amount of neutral suspension eliciting tertiary contractions was 16.0 ± 1.3 ml, and the range varied from 40 to 8 ml. Five subjects did not show tertiary contractions and responded only with secondary peristalsis.

The volumes of perfusate eliciting tertiary motor responses did not show any significant variation with suspensions at different pH (Figure 49.2).

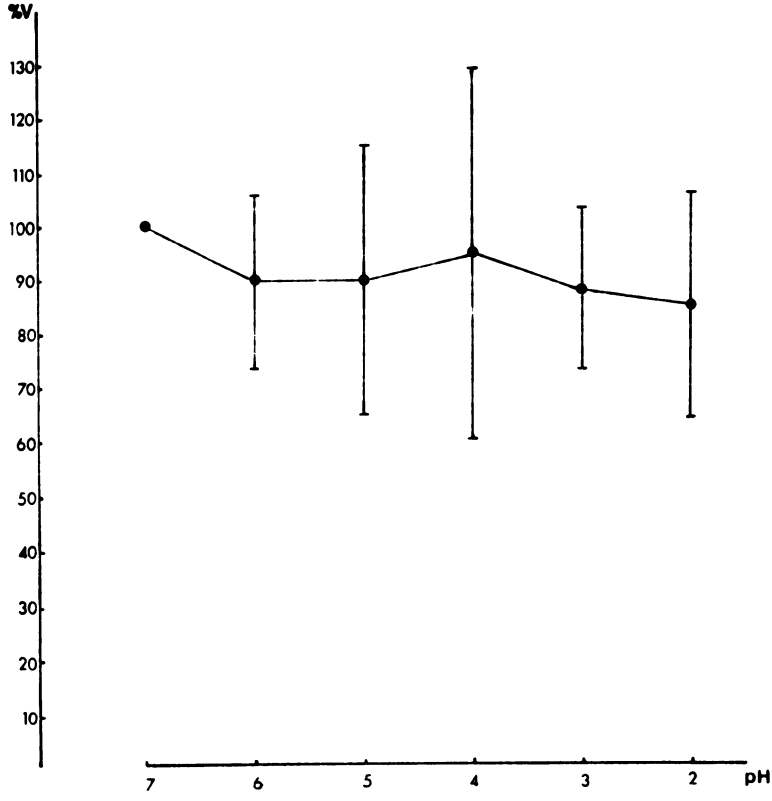

Figure 49.2 Volumes of perfusate, expressed as percentage variation of the neutral suspension, eliciting tertiary contractions at pH 7–2. M ± SEM

DISCUSSION

Meltzer and Auer[8] first reported that distending the rabbit oesophagus with air or fluid produced a sequence of peristaltic waves not preceded by deglutition. Secondary peristalsis during intra-oesophageal distension with air, fluid[3–5] and balloon inflation[6] has been described in man. Our data confirm that distension of the lower oesophageal body with a liquid bolus elicits two forms of motor activity: namely secondary peristalsis and tertiary contractions in about the same ratio as previously reported[5]. No explanation is available for the fact that the oesophagus responds to the same stimulus with two different motor patterns in an apparently random order. It is not possible to exclude that one reason may lie in the experimental condition itself. The intubation of the oesophagus, the prolonged supine position and the voluntary restraint from swallowing (even if for a short period of time) might interfere with the integrated mechanism of peristalsis. The volumes of neutral perfusate necessary to stimulate oesophageal motor response, particularly secondary peristalsis, appeared to be constant in the same subject while differing greatly from one subject to another. The large variation in volumes necessary to elicit motor responses in different subjects could be ascribed to different oesophageal sensitivity to intraluminal distension or to spreading of the perfusate within the oesophageal lumen, or different lengths of stimulated oesophagus.

In the literature no information is available on whether intra-oesophageal pH affects motor responses induced by intra-oesophageal distension. This study indicates that the threshold of intraluminal distension for eliciting secondary peristalsis is significantly lowered by an intra-oesophageal pH of 4 or less. A similar pH-related decrease of the volume needed to elicit peristaltic motor response has been previously described in human colon[9]. On the contrary tertiary motor activity was not affected by intra-oesophageal pH values in the range investigated.

These results indicate that the control mechanism of secondary peristalsis is regulated by both intraluminal volume and pH, yet they do not give information on whether the response to low pH values is a function of the length of acidified oesophagus. It seems reasonable to speculate that the oesophagus is provided with a mechanism sensitive to intraluminal pH and capable of reducing the contact time between the mucosa and low pH boluses. This mechanism might be effective in preventing prolonged contact time between refluxed gastric acid content and oesophageal mucosa.

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Discussion

F. Brown: (USA) Did any of your subjects experience oesophageal symptoms associated with the motor events you elicited?

E. Corazziari: (Italy) No patient experienced oesophageal symptoms during the eliciting of motor responses.

R. W. McCallum: (USA) Since you did not have a sensor in the lower oesophageal sphincter, how could you be certain of differentiating secondary peristalsis from tertiary contractions?

Corazziari: Tertiary contractions appeared as simultaneous waves at the level of the three recording orifices. Secondary peristalsis appeared as co-ordinated waves in temporal segmental pattern while recorded by the three recording orifices. I do not see any reason for obtaining additional information from the lower oesophageal sphincter to identify such clear-cut differences.

Section X
Gastric Emptying and Antroduodenal Control

50

The role of the antral pacesetter potential in canine gastric emptying of solids

R. A. HINDER AND K. A. KELLY

The distal portion of the canine stomach has a major role in gastric emptying of solids. The antrum and pylorus sense the size of the particles of food in gastric chyme and allow only those less than about 1 mm in diameter to pass into the duodenum[1]. Larger particles are retained in the stomach, where antral peristaltic contractions thoroughly mix and grind them and gradually break them into fragments small enough to be discharged into the duodenum[2].

The frequency and direction of antral peristalsis are determined by the gastric pacesetter potential[3]. The pacesetter potential is generated regularly at a frequency of about 5 cycles per minute (cpm) by the gastric pacemaker located in the mid-corpus of the stomach along the greater curve[4]. The pacesetter potential sweeps distally from the corporal pacemaker to the antrum, phasing the onset of action potentials, and hence of peristaltic contractions, as it goes.

It follows, then, that the rate of gastric trituration and the speed of gastric emptying of solids ought to be dependent on the frequency of the antral pacesetter potential.

We tested this hypothesis in dogs that had undergone a circumferential myomectomy of the gastric corpus. Gastric trituration and the rate of gastric emptying of digestible and indigestible solids were assessed in these dogs at the slow frequency of the pacesetter potential found in the antrum after the myomectomy, as well as when the antral frequency was restored to normal by electrical pacing.

MATERIAL AND METHODS

Electrical activity was recorded and the antrum paced using chronically implanted silver electrodes[5]. Gastric emptying of a digestible solid (radioactive liver) was measured using a duodenal perfusion system that quantitated the rate of appearance of radioactivity in the duodenum[6], while gastric emptying of indigestible solids (radiopaque plastic spheres) was assessed fluoroscopically[7].

Preparation of materials

Electrodes

Monopolar and bipolar electrodes, similar to those used in our laboratory in the past, were constructed from silver wire[5]. The diameter of the wire was 1 mm, and each electrode projected about 1 mm from an acrylic plastic disc. Bipolar electrodes were positioned 5 mm apart on the disc. The shafts of the electrodes were insulated with teflon paint, but their tips were exposed and chlorided electrolytically. Each electrode was soldered to an insulated copper wire and the junction was encased within, and insulated by, the disc. The copper wires led from the discs to a connector that was positioned in a stainless steel cannula.

Radioactive calf liver

^{57}Co Cyanocobalamin (0.4 μCi/kg) was injected intramuscularly into 75–155 kg calves. Twenty-four hours later the animals were killed and the livers excised. The livers were diced into 1 cm cubes and frozen in plastic bags in 50 g lots. The mean number of gamma emissions per g of liver over 5 min was determined by counting the gamma emissions in twenty to thirty randomly chosen cubes of liver. The raw liver was thawed to room temperature (22°C) prior to its administration to the dogs.

Plastic spheres

Plastic spheres, 7 mm in diameter, were made radiopaque by rolling them in epoxy glue and then in powdered $BaSO_4$. The specific gravity of the coated spheres was about 1.02.

Preparation of the experimental animals

Four mongrel dogs were subjected to coeliotomy under sodium pentobarbital anaesthesia and using a sterile technique. A 1 cm wide circumferential band of the entire gastric tunica muscularis was removed at a site about 6 cm proximal to the pylorus, thus separating the antrum from the corporal pacemaker[8]. Care was taken not to injure the branches of the vagal nerves

in the lesser omentum adjacent to the site of the myomectomy. A well-vascularized piece of greater omentum was sutured into the defect created by the myomectomy to prevent apposition of the proximal and distal cut edges of the tunica muscularis in the post-operative period.

A bipolar electrode for stimulation and four monopolar electrodes for chronic recording of electrical activity were sewn to the anterior serosal surface of the stomach, midway between the greater and lesser curves. The bipolar electrode was placed distal to the myomectomy, at a site 5 cm proximal to the pylons. Two monopolas electrodes were placed proximal to the myomectomy at sites 8 and 7 cm from the pylorus, and two were placed distal to the myomectomy at sites 3 and 2 cm from the pylorus. The cannula to which the electrodes were attached was positioned in, and sewn to, the left anterior abdominal wall.

Two polyvinyl chloride (pvc) catheters, one for perfusion and one for aspiration, were then implanted into the duodenal lumen through a small enterotomy made 10 cm distal to the pylorus. The tip of the perfusion catheter was placed 10 cm distal to the enterotomy, and the tip of the aspiration catheter lay 20 cm distal to the enterotomy. The opposite ends of the catheters were brought out to two Luer-lock connectors embedded in a cannula which was implanted in the right anterior abdominal wall. When not in use the connectors were kept tightly capped.

The dogs were allowed 3 weeks to recover from the operation before the tests of gastric emptying were commenced.

Tests of gastric emptying

No pacing

The animals were starved of food but not water for at least 24 h, after which they were placed standing and fully conscious in loose-fitting canvas slings held by an adjustable steel frame. Electrical recordings were begun by attaching leads from the monopolar electrodes to alternating current amplifiers and direct-writing, rectilinear pen recorders (Brush, Mark 260). The amplifiers had a time constant of 1 s and the recorders a paper speed of 1 mm/s.

An infusion of [^{14}C] polyethylene glycol (mol. wt. $\sim$ 4000; sp. activity, 2 mCi/g) was next begun through the proximal duodenal catheter at 2.4 ml/min and continued for 1 h, so as to achieve steady-state conditions in the duodenum.

The dogs were then fed 50 g of radioactive liver and forty radiopaque spheres by manually placing both the liver and spheres far back in the oropharynx to avoid chewing. While the electric recordings and duodenal infusion were continued, 2 ml samples of duodenal content were aspirated from the distal duodenal catheter every 10 min for the ensuing 4 h. The number of plastic spheres emptied from the stomach was also determined by radiological screening at hourly intervals during the test.

The test was repeated four to six times in each animal, but only one test per day was performed on any particular animal.

Analysis of electrical recordings. The occurrence and rhythm of the cycles of the pacesetter potential in the antrum and corpus was determined, and the mean frequency assessed by counting the cycles over a 5 min interval both before feeding and every hour after feeding. The direction of propagation of the cycles was also noted, as was the occurrence of action potentials superimposed on them.

Analysis of duodenal samples. The samples aspirated from the duodenum were decoloured in sunlight for 24 h after which 15 ml of scintillation fluid was added. The number of beta emissions (^{14}C) occurring in each specimen during a 5 min interval was measured in a liquid scintillation system (Beckman, Model LS-3150T). The gamma emissions (^{57}Co) were similarly measured in a gamma counter (Beckman, Gamma 310). These data were stored on punched tapes, and, following corrections for isotope decay, quench and activation of the scintillation fluid by the gamma emissions, the rate of gastric emptying of the liver was calculated. The amount of gastric marker (Q) passing through the duodenum in time t was determined using the formula:

$$Q = V_P \frac{^{14}CPEG_P}{^{14}CPEG_D} M_D$$

where V_P = the volume of duodenal perfusate given in time t; $^{14}CPEG_P$ and $^{14}CPEG_D$ are the $^{14}CPEG$ concentrations in the duodenal perfusate and aspirate respectively; M_D is the concentration of ^{57}Co in the aspirate.

The method also allowed for the determination of the volume of fluid (F) passing through the duodenum in each time period by using the formula:

$$F = V_P \frac{^{14}CPEG_P}{^{14}CPEG_D} - V_P$$

Tests during pacing

The gastric emptying tests were again repeated in each dog, except that on these occasions the antrum was paced by stimulating the proximal bipolar electrode with square-wave, electrical pulses. Pacing was begun 10–15 min before the ingestion of the liver and spheres, and was continued until the end of each experiment. The test meal was not administered until it was clear, from the electrical recordings, that the antrum was being paced at the desired site with each stimulus. The strength of the stimuli was 4 mA and the duration 100 ms, while the frequency was set to drive the pacesetter potential to its maximum driven frequency. Minor adjustments of the frequency of stimulation were required from time to time to maintain entrainment during the experiments.

Each of the four animals had four or five tests during which the antrum was paced forward by stimulating the proximal bipolar electrode.

Statistics

A Mann–Whitney U test was applied to the data, comparing the results obtained without pacing to those obtained during pacing, using each dog as its own control.

RESULTS

All four dogs survived the operative procedure and remained healthy for the duration of the study. Minor sepsis occurred around some of the cannulae.

No pacing

Electrical activity

The mean frequency of the pacesetter potential in the fasting dogs after myomectomy was much slower in the antrum than it was in the corpus. The corporal frequency ranged from 4.3 cpm to 4.7 cpm among the four dogs (overall mean, 4.6 cpm), while the antral frequency ranged from 1.0 to 2.2 cpm (overall mean, 1.7 cpm). The direction of propagation of the cycles was aborad, both in the corpus and in the antrum (Figure 50.1). These patterns of the pacesetter potential did not change appreciably over the 4–6 months of the experiment, and were similar to those found by us in past experiments[8].

When action potentials occurred in the stomach proximal to the myomectomy they were generally also observed in the distal stomach. On some occasions when bursts of action potentials appeared in the corpus and antrum, the frequency of the pacesetter potential distal to the myomectomy often temporarily increased (Figure 50.2).

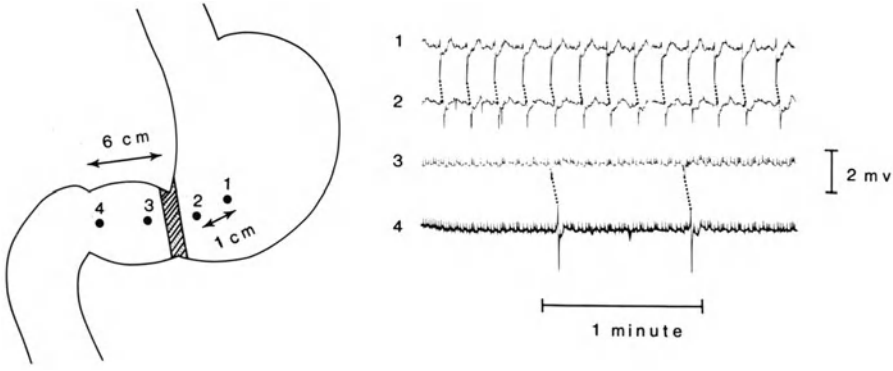

Figure 50.1 Electrical recordings from monopolar electrodes 1 to 4 (large dots) in dog with distal corporal myomectomy (shaded area). Frequency of pacesetter potential in antrum is slower than in corpus, but direction of propagation of the pacesetter potential (dotted lines) is aboral in both areas

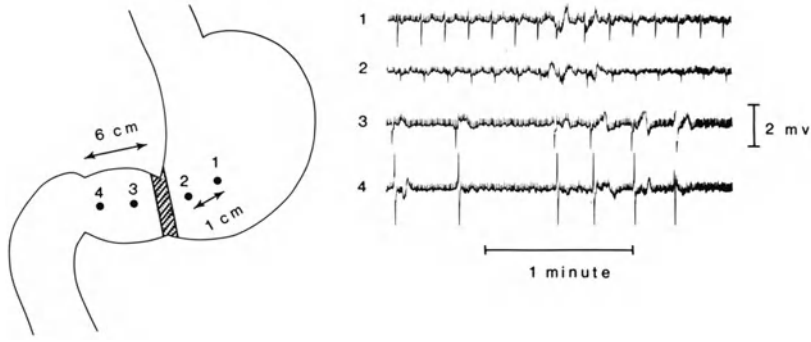

Figure 50.2 The frequency of the antral pacesetter potential after myomectomy increases when bursts of action potentials sweep with the pacesetter potential through corpus and antrum

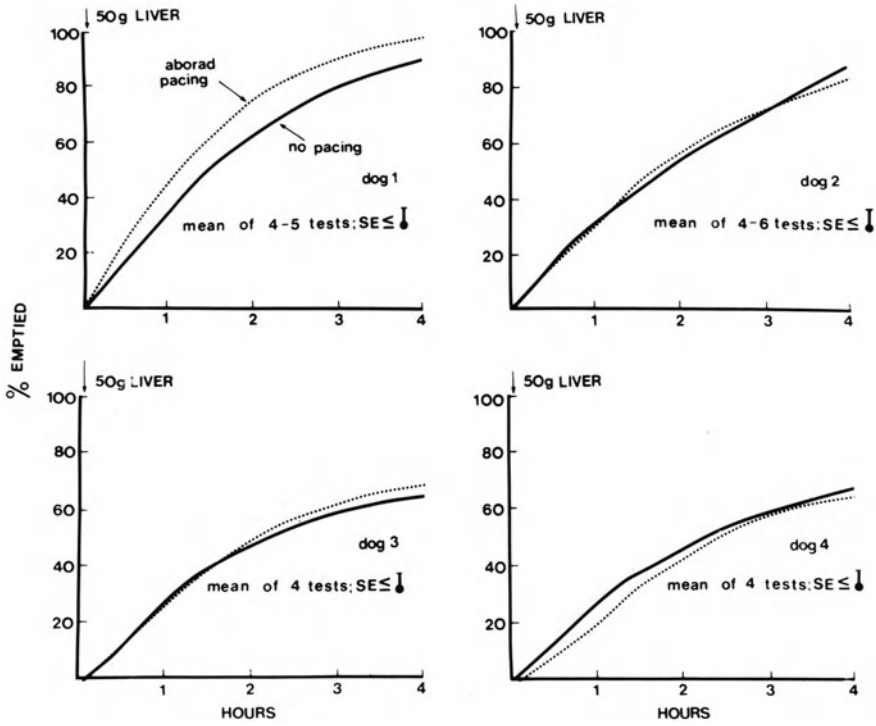

Figure 50.3 Mean cumulative percentage liver emptied in four dogs with no pacing or aboard pacing after myomectomy

Feeding caused little change in the frequency of either the corporal or antral pacesetter potential. The frequency 1 h after eating ranged from 4.4 to 5.0 cpm in the corpus over the four dogs (overall mean, 4.7 cpm) and ranged from 1.4 to 2.3 cpm in the antrum (overall mean, 1.9 cpm).

Gastric emptying

The liver emptied from the stomach in a steady, linear pattern during the first $2\frac{1}{2}$ h after its ingestion, following which the cumulative amount emptied approached a horizontal asymptote (Figure 50.3). By 4 h, 62–97% of the liver had left the stomach.

In contrast to the liver, only a few spheres were emptied in the 4 h after their ingestion. The spheres were propelled into the distal antrum by the aborally moving antral peristaltic wave, but were blocked from passing into the duodenum at the pylorus. Trapped in the distal antrum, the spheres were ground together and finally retropelled backwards as the advancing peristaltic wave passed over them. Only a few spheres were discharged from the stomach during the same interval that most of the liver was emptied (Table 50.1).

Table 50.1 Pacing and gastric emptying of spheres

	Mean percentage of spheres emptied (SE)							
	Before pacing (hours after ingestion)				*During antral pacing* (hours after ingestion)			
Dog	1	2	3	4	1	2	3	4
1	0.6(0.3)	0.6(0.3)	1.8(1.6)	4.2(4.0)	0	2.6(1.5)	7.2(3.0)*	25.2(7.0)*
2	0	0	0	0.3(0.3)	0	0	0.4(0.4)	2.2(0.9)*
3	0	4.3(1.4)	12.5(3.4)	18.3(6.5)	3.3(1.7)	14.5(3.0)	25.3(5.5)*	30.8(4.0)
4	0	0	0.3(0.3)	0.8(0.5)	0	0	0	0

* Values differ from corresponding values before pacing; $p < 0.05$

Duodenal flow

About 150 ml of fluid flowed through the duodenum during each of the four hours the dogs were studied after the meal (Table 50.2).

During pacing

Electrical activity

Entrainment of the antral pacesetter potential was achieved consistently when pacing via the proximal antral bipolar electrode (Figure 50.4). Pacesetter potentials generated by stimulation of the proximal electrode propagated through the antrum in an aborad direction. The maximum driven frequency obtained by pacing was 3.0–6.7 cpm (mean, 5 cpm). Antral pacing did not change the frequency or direction of propagation, of the pacesetter potential in the corpus.

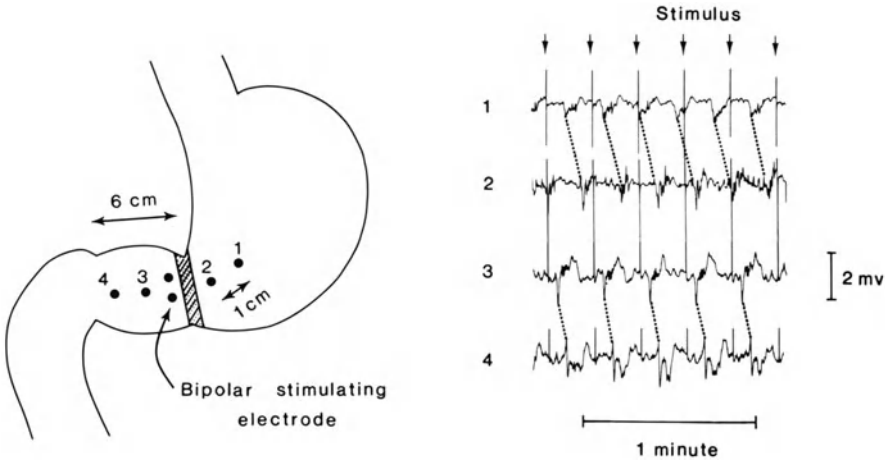

Figure 50.4 Electrical stimulation of the proximal antrum after myomectomy at about four stimuli/min (arrows). Each stimulus initiates a distally propagating pacesetter potential in the antrum (dotted lines), but the corporal pacesetter potential is unaffected

Gastric emptying

Pacing the antrum did not alter the rate of emptying of the liver compared to when no pacing was employed (Figure 50.3). In contrast pacing speeded slightly the emptying of the spheres in three of four dogs (Table 50.1).

Duodenal flow

The volume of fluid flowing through the duodenum was the same without antral pacing as with antral pacing (Table 50.2).

Table 50.2 Pacing and flow of duodenal fluid

Pacing	*Mean ml* (SE) *per hour**
None ($n = 19$)	142(6)
Proximal antrum ($n = 17$)	153(9)

* Values do not differ from one another; $p > 0.05$

DISCUSSION

Our experiments show that marked changes in the frequency of the antral pacesetter potential do not appreciably alter the rate of gastric emptying of digestible solids. The rate of emptying of liver in all four of our dogs with a slow frequency of the antral pacesetter potential after corporal myomectomy was not speeded by increasing the frequency of the pacesetter potential. Moreover, the rate of emptying of liver in both experimental conditions was nearly identical to that found by us in experiments on dogs with intact stomachs, as reported elsewhere[6].

The maintenance of gastric trituration of digestible solids after the myomectomy may have been in part due to the fact that the slow post-myomectomy frequency in the antrum did temporarily speed up on occasions when bursts of action potentials appeared. Such periods undoubtedly aided trituration by temporarily increasing the frequency of peristalsis. Also, the net slowing of the frequency of antral peristalsis that did occur with myomectomy may have been offset by an increase in the strength of each peristaltic contraction. Lastly, factors other than antral peristalsis must have a role in gastric trituration. Chemical digestion by gastric juice, for example, may be able to compensate for poor mechanical dispersion due to impaired antral peristalsis.

Once thoroughly triturated, digestible solids would pass into the liquid phase of the gastric content and be emptied with the liquid phase[6]. Slow antral peristalsis at this point would not be expected to greatly alter the pattern of emptying, since the emptying of gastric liquids is mainly regulated by the steady, sustained contractions of the proximal stomach[9,10]. The proximal stomach was intact in our dogs.

The maintenance of gastric continence for spheres after myomectomy shows that continence for solids is not dependent solely on the frequency of the antral pacesetter potential, hence of antral peristalsis. Marked slowing in the frequency did not greatly disturb continence. However, pacing the antral pacesetter potential at a frequency of 4–5 cpm did slightly disturb gastric continence for spheres and speed their rate of emptying in some of our dogs. Moreover the rate of emptying of spheres during pacing with myomectomy was also faster than it is in dogs with intact stomachs[10]. The exact mechanism by which pacing disturbed continence and speeded emptying of spheres is not known.

The maximum driven frequency obtained by pacing the antrum of our dogs with corporal myomectomy was less than expected. In the intact stomach it is possible to pace the gastric pacesetter potential up to frequencies as fast as 8 cpm[5]. Why it was not possible to pace the antrum, chronically electrically isolated from the natural pacemaker, to these faster frequencies is not known.

Acknowledgements

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Discussion

C. F. Code: (USA) Did you observe fluroscopically the action of the antrum during prolonged pacing (hours)?. We have noted a disassociation between the abnormal direction of pacing and the motor action of the stomach and small bowel during prolonged (hours and weeks) abnormal pacing.

R. A. Hinder: (S. Africa) The spheres were observed fluoroscopically to be propelled, triturated and retropelled during action potentials. In the pacing experiments no obvious differences in the grinding action of the antrum were observed compared to the action of normal stomachs.

C. J. Stoddard: (UK) How do you account for the increase in frequency of the waves distal to the myomectomy when activity potentials are present in the proximal stomach? If you think this is vagally mediated can you substantiate this theory by evidence from vagal section experiments?

Hinder: The increase in frequency of the pacesetter potential during bursts of action potentials may have been a vagal effect, a hormonal effect, may be due to the mechanical effect on the antrum of action potentials or be due to some other unknown factor. We are at present studying the effect of vagotomy on the phenomenon.

H. L. Duthie: (UK) I would suggest that it might be an advantage to increase the size of the meal from 50 g of liver in order to provide a greater stress on the emptying capacity of the stomach. This might show up differences hidden in the present protocol.

Hinder: Our small meal volume certainly did not greatly distend the stomach and so it is possible that a bigger meal volume might give different results.

E. E. Daniel: (Canada) Your rates of emptying of liver seem slow, and this may have resulted from your operative procedures. Could you comment?

Hinder: Great care was taken, in doing the myomectomy, not to damage the nerves of Latarjet. The pattern and rate of emptying of the liver was similar to that found in a separate set of experiments carried out in dogs with intact stomachs. It therefore seems as if the operative procedure did not greatly influence the emptying rate of the liver.

51

Myoelectrical activity and histology at duodenal anastomoses

G. W. SCOTT, A. C. BROUGHTON, M. G. LORD, R. HENDERSON AND E. E. DANIEL

The coupling of myogenic electrical control activity (slow waves, pacesetter potentials) in the longitudinal muscle plays a major role in the coordination of contractions along segments of the intestines. Electrical coupling presumably requires the presence of low-resistance pathways between adjacent muscle cells, such that the oscillating electrical control activity in one group of muscle cells can influence the occurrence of control activity in neighbouring groups of muscle cells. This enables 'pulling' to occur, in which an oscillator has the ability to elevate the frequency of electrical control activity arising from an adjacent oscillator of lower intrinsic frequency. A high degree of coupling can produce entrainment or 'phase-locking' of electrical control activity, in which the frequency of electrical control activity along a segment is pulled up to that of the oscillator with the highest intrinsic frequency. This characteristically occurs in the duodenum. Coupling is abolished at the site of surgical transection of the intestine, resulting in a fall in the frequency of electrical control activity recorded from the intestine distal to the site of transection. Theoretically, anastomosis of the transected intestine could produce recoupling of electrical control activity if low-resistance pathways are re-established by close apposition of muscle cells. However, recoupling might not occur if the method of constructing the anastomosis or the degenerative and repair processes in the wound prevent close apposition of muscle cells, or if coupling requires the involvement of specialized cellular junctional elements that are absent after transection and anastomosis. Code and Szurszewski[1] found that transection and anastomosis produced a definite and

pronounced reduction in the frequency of pacesetter potentials (electrical control activity) in the segment of duodenum distal to the transection–anastomosis and that this persisted throughout the period of observation, from 2 weeks to 3 months. Akwari *et al.*[2] noted that duodenal pacesetter potentials (electrical control activity), which could be entrained by external electric pacing, were not propagated across sites of duodenal transection and anastomosis. On the other hand Atanassova *et al.*[3] believe that the electrical control activity becomes fully coordinated in the stomach after transection and anastomosis, and they appear to have indicated that the regeneration of smooth muscle cells can lead to recoupling of electrical control activity in the transected–anastomosed duodenum. In the present study the coupling of electrical control activity and histological changes in the wounds were examined immediately and up to 5 days after transection and layer-to-layer or inverting anastomoses of the canine duodenum.

METHOD

Tests were performed upon seventeen healthy mongrel dogs weighing 14–20 kg, that were fasted for 24 h, anaesthetized with sodium pentobarbital, and had their core body temperatures maintained at 38 °C using a heating blanket. Five silver-wire bipolar electrodes were inserted subserosally, 2 cm apart, along the duodenum as shown in Figure 51.1. The abdominal wound was closed, and after the duodenum was allowed to warm for 30 min to body temperature, duodenal electrical control activity was recorded for 20 min on a multichannel recorder (Beckman Dynograph, time constant 0.1 s, high-frequency cut-off above 30 Hz, paper speed 10 mm/s, rectilinear pens with accurate alignment). The abdomen was then reopened and the duodenum was transected between electrodes 3 and 4 as shown in Figure 51.1. A sheet of insulating plastic was inserted between the cut ends of duodenum, to prevent

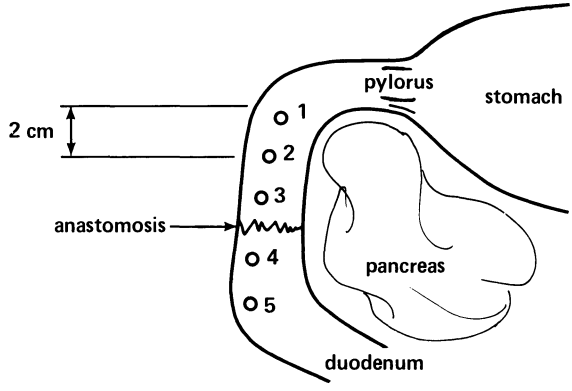

Figure 51.1 The location of bipolar recording electrodes and the location of the transection and anastomosis

their contact with each other; the abdomen was closed for another warm-up period, and the electrical control activity was recorded. The dogs were then randomly split into two groups, in which nine dogs underwent a layer-to-layer and eight dogs underwent inverting anastomosis of the transected duodenum. The layer-to-layer anastomoses were meticulously performed to ensure accurate realignment and approximation of the muscle layers, using interrupted silk sutures that anchored the submucosal layer (Gambee technique). The inverting anastomoses, using interrupted silk sutures, brought the serosa of the cut ends into contact but prevented apposition of the muscle layers. The electrical control activity was then recorded after another warm-up period.

As shown in Figure 51.2, the tests were terminated at this stage in four of the dogs with inverting anastomoses. The remaining four dogs with inverting anastomoses, and five of the dogs with layer-to-layer anastomoses, underwent retransection at the anastomosis followed by a warm-up period and recording of electrical activity. In the remaining four dogs with layer-to-layer anastomoses the duodenal electrodes were removed, the dogs were allowed to recover, and the electrodes were reinserted under anaesthesia for subsequent final recordings after 24 h (two dogs), 3 days (one dog), and 5 days (one dog). At the conclusion of the tests in each dog the duodenum was fixed by intra-arterial perfusion with phosphate-buffered glutaraldehyde and the anastomo-

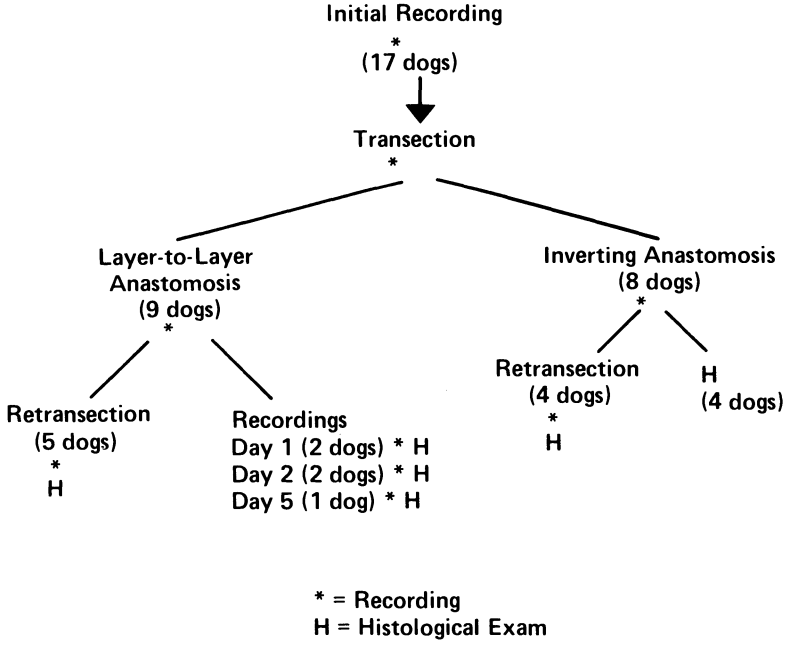

Figure 51.2 Experimental design showing the number of dogs, the operations performed upon them, and the number of recording periods used for analysis

tic zone was excised for light- and electron-microscopic examination. The specimens for electron microscopy were post-fixed in osmium tetroxide, embedded in a known orientation, thick-sectioned to determine the area to be examined, then thin-sectioned and stained with uranyl acetate and lead citrate as previously described by Henderson *et al.*[4].

The 20 min recordings of duodenal electrical control activity were examined to determine whether phase-locking was present, using the criteria of Sarna *et al.*[5], and the frequencies of electrical control activity at electrodes 3 and 4 (proximal and distal to the site of transection–anastomosis) were measured and compared using Student's *t* test to determine whether coupling was present.

RESULTS

Electrical control activity

Prior to transection the frequency of electrical control activity was identical to all recording sites along the duodenum, creating a frequency plateau as previously described by others[5,6]. The electrical control activity was phase-locked (entrained), indicating a high degree of electrical coupling. Transection created two frequency plateaus, one proximal and the other distal to the transection, as shown in the recording in Figure 51.3. Electrical control activity remained phase-locked within each of these segments of duodenum, but there was a significant reduction in the frequency of electrical control activity in the segment distal to the transection, as shown in Figure 51.4. This indicated that the two segments were electrically uncoupled.

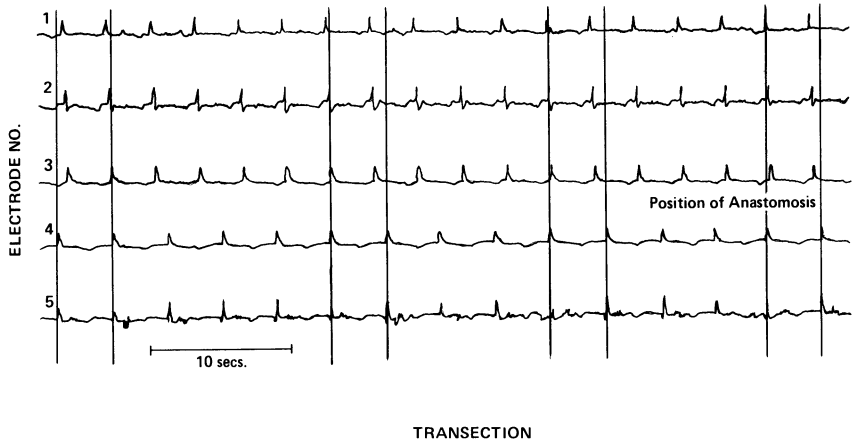

Figure 51.3 Recording of electrical control activity after duodenal transection. Note that waves from electrodes 1, 2, and 3 are phase-locked and that waves from electrodes 4 and 5 are phase-locked but that waves from 3 and 4 are uncoupled, creating proximal and distal frequency plateaus

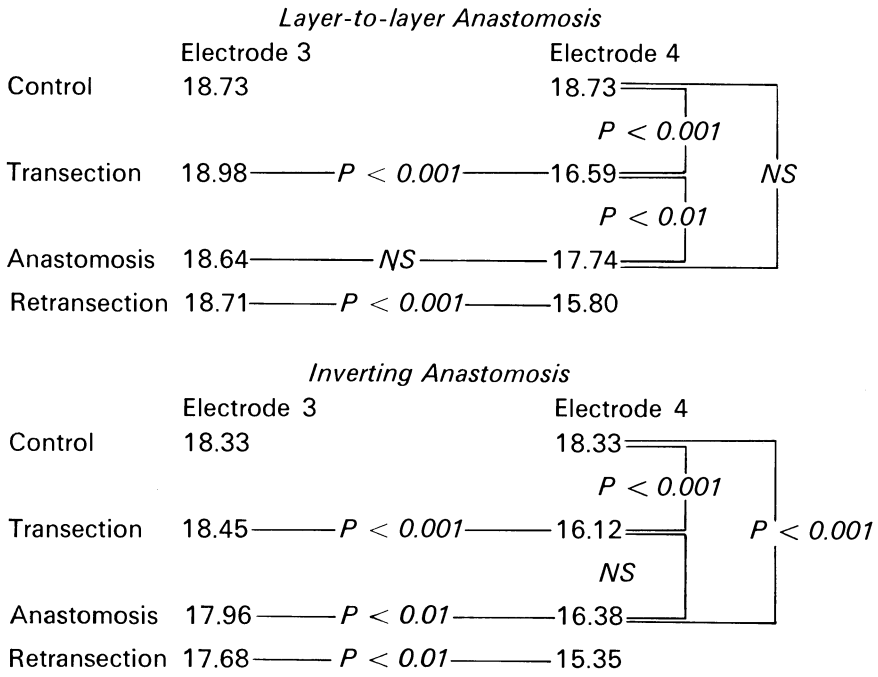

Figure 51.4 The mean frequencies of electrical control activity waves, in cycles per minute, proximal (electrode 3) and distal (electrode 4) to the site of transection and anastomosis, calculated from each 20 min recording period, together with the degrees of significance where appropriate. NS = not statistically significant

Following accurate layer-to-layer anastomosis there was a significant rise in the frequency of electrical control activity in the segment of duodenum distal to the anastomosis, so that the difference between the frequencies of electrical control activity proximal (electrode 3) and distal (electrode 4) to the anastomosis became non-significant. Phase-locking of electrical control activity also occurred intermittently in all of the dogs that underwent layer-to-layer anastomosis, and it was concluded that recoupling had occurred at the anastomosis. An example is given in Figure 51.5. When the dogs that had layer-to-layer anastomosis were re-tested 1, 3 and 5 days later, it was found that there was no evidence of coupling of electrical control activity across the anastomosis; phase-locking was not present and there was a significant difference in the frequencies of electrical control activity between electrodes 3 and 4. This occurred in all four dogs that were re-tested, and Figure 51.6 is a typical example. Similar evidence of uncoupling occurred in the five dogs in which the layered anastomoses were retransected.

Following inverting anastomosis, in which the muscle layers were not in contact, there was no significant change in the frequency of electrical control

activity in the segment distal to the anastomosis. Figure 51.4 shows a reduction in the mean frequency of electrical control activity proximal to the anastomosis, but this was non-significant. It was concluded that recoupling of electrical control activity had not occurred after inverting anastomosis.

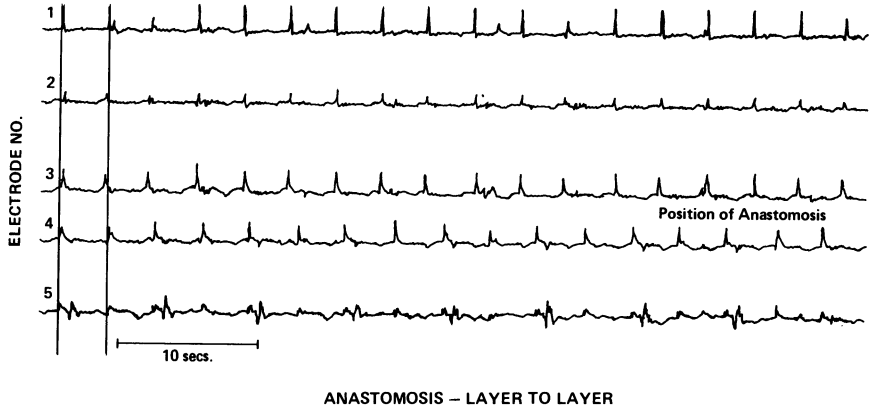

Figure 51.5 Electrical control activity after layer-to-layer anastomosis. There is a high degree of coupling between waves from electrodes 3 and 4

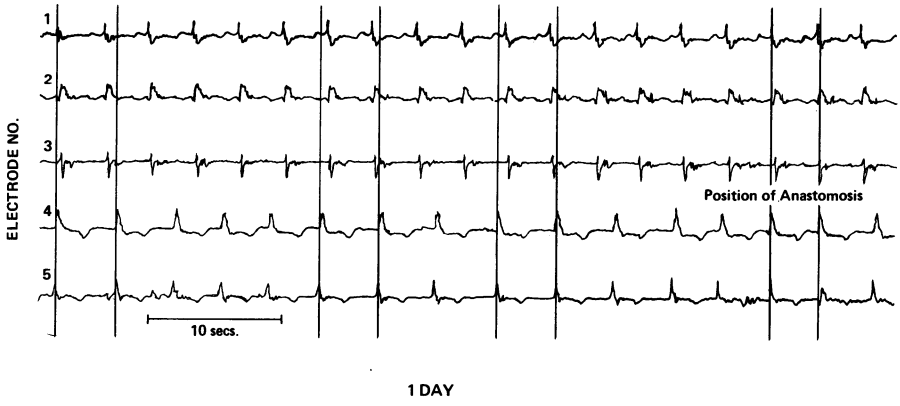

Figure 51.6 Electrical control activity 24 h after layer-to-layer anastomosis. The waves from electrodes 3 and 4 are uncoupled

Histology

Light microscopy showed that the accurate alignment of muscle layers was maintained in layer-to-layer anastomoses, but that a zone of inflammatory infiltrate and fibrinous exudate developed within 24 h, and that this separated the previously approximated cut ends of muscle. Electron microscopy showed

progressive deterioration of muscle cells and of their apposition to each other. The changes were variable from one region to another around the anastomosis. At 24 h muscle from some areas would appear normal, whereas at

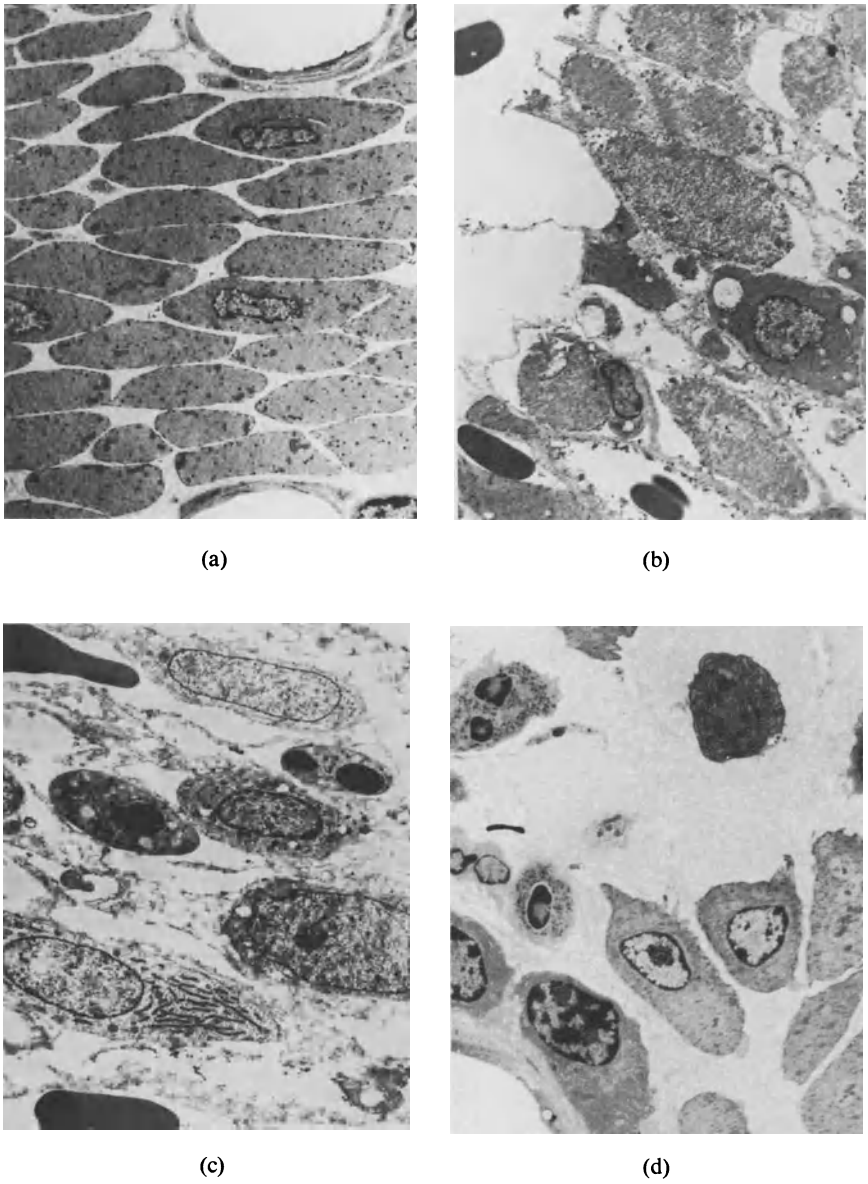

Figure 51.7 Electron microscopic appearance of the anastomotic region (a) prior to transection, (b) after 24 h, (c) after 3 days, (d) after 5 days. Magnification $\times 5000$. See text for description

other areas it would show marked degeneration. Figure 51.7a shows normal circular muscle. Note the close spacing of cells, and several gap junctions (nexuses). Figure 51.7b shows circular muscle at 24 h; two cells appear to be normal apart from disruption of mitochondria, but the remaining cells are in various stages of degeneration, some with cytoplasm pulling away from the cell membrane and others with only the cell membrane and intercellular material remaining, producing a 'lacy' pattern. There is patchy degeneration of muscle cells and a typical infiltration of red cells. At 3 days the lacy pattern of degeneration was still present but some muscle cells appeared to be regenerating. Cells were widely separated, compared to the normal, by an infiltrate of polymorphonuclear leucocytes and red cells and by what appeared to be oedema. Figure 51.7c, at 3 days, shows muscle cells with loss of actin filaments, increased rough endoplasmic reticulum and free ribosomes, and nuclei with decreased marginal chromatin and prominent nucleoli. By 5 days the lacy pattern of degeneration had disappeared, muscle and connective tissue cells showed prominent rough endoplasmic reticulum and nucleoli, but the muscle cells were widely separated, as shown in Figure 51.7d.

DISCUSSION

The results of this study indicate that electrical control activity, uncoupled at the site of duodenal transection, can become recoupled immediately after accurate layer-to-layer anastomosis. This recoupling is temporary, lasting 24 h, and it appears from the light- and electron-microscopic findings that the uncoupling that follows recoupling could be due to degeneration of muscle cells and separation of viable muscle cells by an inflammatory infiltrate and exudate within the anastomotic region. The failure to detect any evidence of recoupling at inverting anastomoses reinforces the view that muscle cell-to-cell apposition is necessary for electrical coupling to occur. It is generally accepted that nexuses provide pathways of low electrical resistance between muscle cells, and that they are the structural basis for electrical coupling. This certainly appears to be true in circular intestinal muscle, where nexuses are abundant, but coupling also occurs in certain muscles where nexuses are notably absent. For example, Henderson *et al.*[4] were unable to find nexuses in canine duodenal longitudinal muscle. Daniel *et al.*[7] have recently reviewed the question of whether nexuses are necessary for coupling, and have presented data which suggest that the failure to demonstrate nexuses in certain smooth muscles such as longitudinal muscle is not due to the method of preparing specimens or a lability of nexuses. In the present study it is unlikely that cell-to-cell contacts, providing for low-resistance transmission between excitable cells, could have developed immediately within the anastomotic region. The coupling may have occurred by way of extracellular current flow and/or by the formation of low series resistance bridges of inactive damaged cells which coupled active regions.

The damage leading to low series resistance should also lead to leakage of current into the extracellular space; thus, these two possible mechanisms may have operated concurrently. This study, while not denying the role of nexuses within the circular muscle of intact intestine, supports the concept that nexuses are not essential for coupling of electrical control activity.

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Discussion

N. E. Diamant: (Canada) At times following transection, frequency below the transection will only drop slowly over several hours and frequency will fluctuate up and down over short periods before a lower frequency is constantly established. Have you repeated your experiments increasing frequency and comparing records for 'phase-lock' or 'coupling' over a long period, e.g. 4–5 h after transection and without reanastomosis?

G. W. Scott: (Canada) Although we were aware of your observation regarding a delay before the frequency falls after transection, we did not encounter a delay. We have not taken recordings over a prolonged period.

A. Bortoff. (USA) Would you care to speculate on the mechanism of transmission in the transected and layer-to-layer anastomosed preparations?

Scott The coupling may have occurred by way of extracellular current flow and/or the formation of low resistance bridges of damaged cells which coupled active regions.

J. H. Szurszewski: (USA) Years ago Ladd Prosser showed that simple apposition of two cut segments of cat intestine coupled the slow-wave activity in the two segments. They postulated that electron coupling was responsible for the coupling. Perhaps this occurs in your muscle-to-muscle anastomosis before infiltration takes place.

E. Atanassova: (Bulgaria) Thank you for supporting our work showing smooth muscle cell regeneration in the transected duodenum with layer-to-layer anastomosis. I think the phase-unlocked slow waves are so obvious because of the short time-interval after transection in their experiments (5 days). Later after surgery smooth muscle cell regeneration ensures very long periods with phase-locked slow waves. As the regeneration is not complete there are phase-unlocked slow waves also.

52

Characterization of the feline pyloric sphincter by neural and hormonal stimulation *(Abstract)*

J. BEHAR, P. BIANCANI AND M. P. ZABINSKI

The response of the gastroduodenal (GD) junction to vagal and hormonal stimulation was studied in anaesthetized, adult cats. GD pressures were measured with constantly perfused manometric catheters.

(A) The mechanical characteristics of the GD junction were defined by determining pressure–diameter curves using a gradual pull-through with single motility catheters attached to plexi-glass 'olives' of increasing diameter, from 2.5 to 7.5 mm. Pyloric sphincter pressures were higher than antral and duodenal pressures at all diameters tested. The smallest probe (OD (outside diameter) = 2.5 mm) stretched the pylorus by only 8%, suggesting the existence of a high-pressure zone at pyloric closure.

(B) The remainder of this study was performed with a catheter assembly consisting of a hollow tube (OD = 5 mm) and seven small tubes (each one with OD = 1.3 mm) with a final OD of 6.05 mm. These dimensions were selected because they stretched the pyloric muscles near conditions of maximal contractility. The catheter assembly was anchored by inserting a thin metallic pin so that the side openings remained fixed to the same site throughout the experimental studies. The mean basal pyloric pressure was 17.0 $\pm$ 0.9 mmHg. Electrical stimulation of either peripheral vagus nerve caused a fall in pressures in the pyloric sphincter and contraction in the antrum and duodenum; pyloric pressures fell by 7.5 $\pm$ 0.6 mmHg. Electrical stimulation of both central ends of the vagus nerve induced contraction of the pyloric sphincter in only twenty of thirty-six animals studied; pyloric pressures increased by 6.1 $\pm$ 0.8 mmHg. The genesis of basal pyloric tone was studied by using atropine and hexamethonium (alone or in combination), propranolol,

phentolamine, and tetrodotoxin. With the exception of phentolamine, none of these pharmacological antagonists affected basal pyloric tone. Phentolamine decreased pyloric pressures from 15.8 ± 1.0 to 11.4 ± 1.0 mmHg ($p < 0.001$). Pyloric relaxation during vagal stimulation was not affected by phentolamine, propranolol, and atropine. However, hexamethonium alone or in combination with atropine partially inhibited, and tetrodotoxin completely abolished, this relaxation. Pyloric sphincter contraction was completely abolished by a combination of hexamethonium and atropine, by phentolamine, and by tetrodotoxin. It was not influenced by atropine or propranolol. The octapeptide of cholecystokinin caused a fall in baseline pressures in the majority of the animals. In a few animals, however, it caused either a decrease in basal pressures with short contractions, or contractions alone. The pyloric sphincter did not respond to pentagastrin in the majority of the animals. However, in eight animals, it caused a slight rise in basal pressures with a brief burst of contractions.

These results are consistent with the existence of a sphincter at the gastroduodenal junction that behaves differently from the adjacent duodenal and antral segments.

Discussion

S. Cohen: (USA) There is no question that there is indeed a pyloric sphincter. However, the pressure is low and is difficult to measure. In the present study, a large-diameter probe was used. Sphincter relaxation was only 40% at this larger diameter probe. The higher pressure achieved with this large probe may be producing too great a degree of stretch. Pyloric sphincter relaxation in man is 100%.

J. Behar: (USA) The lack of full relaxation may indeed be due to the diameter of the manometric catheter. However, the pylorus may have considerable passive tension and full relaxation may not occur even in the absence of the probe.

A. Bennett: (UK) The use of the term 'sphincter' requires care. For example, in man this region should probably be called the pyloric ring, and numerous species differences exist. One definition of a 'sphincter' is a ring of circular muscle which normally closes the lumen and is contracted by α-adrenoreceptor stimulation. Does this apply to the cat?

Behar: The gastroduodenal junction may meet your criteria of 'sphincter'. The smallest probe (2.5 mm in diameter) revealed a high pressure zone; it also relaxes during vagal stimulation and contracts in response to phenylephrine.

H. S. Ormsbee: (USA) In the dog efferent vagal stimulation produced contractions of the antrum and pylorus. Following administration of atropine the pylorus can be observed to relax. I would like to ask first, did you observe pyloric contractile activity, and second, how long did relaxation of the cat pylorus last with vagal stimulation?

Behar: We observed rebound pyloric contraction following relaxation. The duration of pyloric relaxation was similar to that of the lower oesophageal sphincter of about 3–6 s.

A. G. Johnson: (UK) The relaxation after cholecystokinin, instead of the contraction we observed in conscious humans, may be due to an anaesthetic. In dogs, cholecystokinin stimulates the antrum in anaesthetized animals but relaxes it in conscious animals. This was reported some years ago from Scandinavia and we have found the same; but the mechanism is obscure. Were your animals anaesthetized?

Behar: We have not studied conscious animals. Our different results could certainly be explained by the effect of anaesthesia or perhaps by species differences.

53

The pressure relationship in the gastric body, antrum and duodenum during gastric (abomasal) emptying in the calf

F. R. BELL

Gastric emptying has been studied by a wide variety of technical methods over many years, but there is still much equivocation as to the separate and co-ordinate action of the gastric body, antrum, pylorus and duodenum to generate the forces necessary to transfer gastric chyme to the duodenum. Information up to 1968 has been reviewed by Code and Carlson[1] and Hunt and Knox[2], and subsequently by Cooke and Stemper[3].

In previous work on gastric emptying a single physical factor such as volume change, muscular activity measured by electromyography or deformation of a strain gauge, or radiography has been used. The results of these methods of study are often difficult to interpret because the data obtained are often marred by inherent artifacts. An improvement in understanding is more likely to be obtained when a range of methods are brought together which can measure the various physical phenomena activated synchronously during gastric emptying, so they can be considered simultaneously. Furthermore it is necessary to control the composition of the gastric chyme within reasonable limits during the course of the experiment.

We have investigated the function of the abomasal part of the stomach of the pre-ruminant calf and shown it to be the homologue of the simple stomach[4]. We have also shown that when duodenal receptors are activated, neural and hormonal responses occur which, through excitatory and inhibitory mechanisms, control the contractility of gastric smooth muscle[5]. The experiments reported here have been conducted on the abomasal moiety of the stomach of the minimally restrained, conscious, pre-ruminant calf with the objective of assessing the interaction of abomasal body and antrum in

transferring gastric chyme to the duodenum by attempting to meet the criteria mentioned above.

METHODS

In all experiments the stomach, which had been evacuated of its contents, was washed out and a standard test meal of 1.5 l. isotonic sodium chloride introduced. Smooth muscle activity at various loci in the stomach was recorded from fine wires insulated except where they were sewn into the muscular layers of the stomach[4]. Muscular activity was sampled from at least four sites in the body of the stomach, two in the antrum and one in the proximal duodenum. Pressure measurements within the viscus were made from water-filled, open-tipped, non-distensible tubes of small volume introduced into the body, antrum and duodenum through somewhat larger catheters which had been placed in defined positions verified by radiograph. When necessary the test meal was made radiopaque by barium sulphate; the osmolality of the test meal being unaffected. The outflow of radiopaque test meal from the stomach was monitored by image-intensified fluoroscopy and recorded on video tape. A continuous commentary and timing of events on the tape, and suitable timing on the polygraphs, permitted all measurements to be coordinated. Comparable experiments were made in the same calf so that difficulties that arise from intra-subject variation of electrode placement, and so on, were eliminated. Experiments were conducted on a number of occasions on four calves and closely comparable results were obtained.

RESULTS

In the calf a test meal of isotonic sodium bicarbonate solution has been shown to be stimulatory and to produce a positive feedback with evacuation of most of a 1.5 l. test meal in about 45 min and by contrast a test meal of 0.06 M HCl is almost wholly retained during inhibition of gastric emptying[6]. The positive feedback produced by alkali in the duodenum is associated with maximal activity of the electromyogram (e.m.g.) whereas negative feedback initiated by acid is associated with inhibition of the action potential component of the e.m.g.[4]. In the experiments described here alkali and acid were used separately as test meals and their general effect, apart from the records made on video tape, are shown in Figures 53.1 and 53.2.

It is noticeable that in Figure 53.2, where emptying is minimal, the e.m.g. is much reduced or abolished, and the pressure records from body, antrum and duodenum deviate only by a few centimetres of water from the resting level. Occasionally the duodenum shows a sharp rise of pressure to 100 cm H_2O, but this is dissociated from any pressure change in the stomach. On the other hand when the calf blared the increased thoracic pressure was

conveyed simultaneously to all three channels. Micturition and defaecation did not produce any noticeable pressure change on the intragastric record.

When gastric emptying was maximal with an alkaline meal there was also no increase in pressure for a period of about 15 min except for a phasic

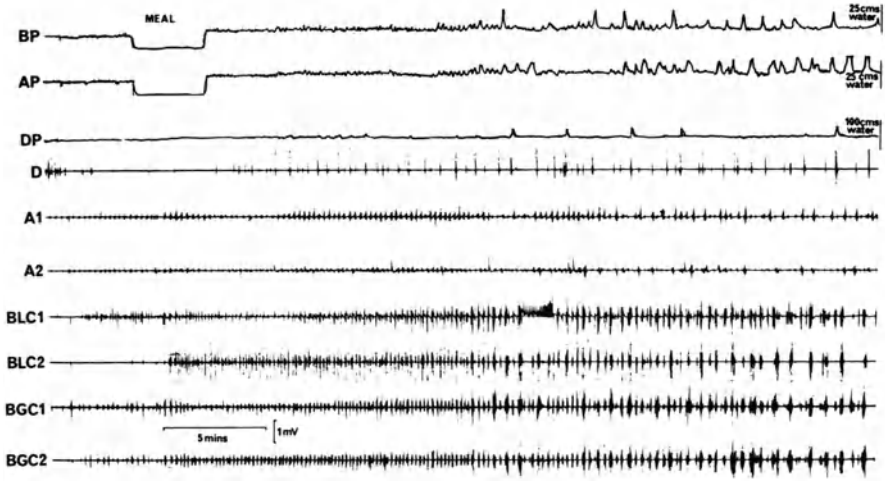


Figure 53.1 Intragastric measurements made during emptying of 1.5 l isotonic $NaHCO_3$. Intraluminal pressure recorded from gastric body (BP) distal antrum (AP), and proximal duodenum (DP). Electromyograms from antrum 2 cm (A1) and 7 cm (A2) anterior to pylorus, body lesser curvature near incisura (BLC1) and 7 cm anterior to incisura (BLC2) and complementary placing on greater curvature (BGC1 and BGC2). The duodenal e.m.g. was recorded 5 cm distal to the pylorus (D)

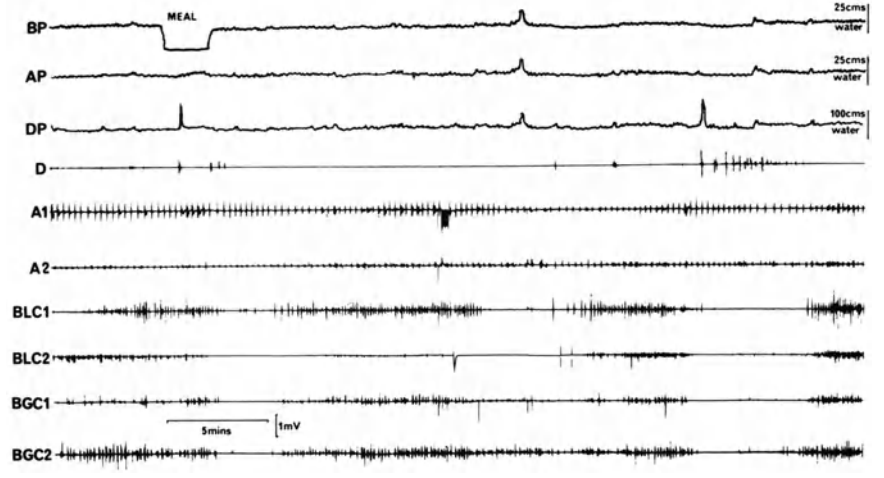


Figure 53.2 Intragastric measurements when meal of 0.06 M HCl was instilled into abomasum. The notation of records is the same as Figure 53.1

rise of 5 cmH_2O synchronized with the antral e.m.g. When the alkaline meal emptied more rapidly unsynchronized, phasic pressure-changes of up to 15 cmH_2O occurred in both body and antrum. Fluoroscopy showed these pressure changes to be associated with filling of the distal segment of the antrum. At the same time the e.m.g. in the body showed marked muscular activity. During the time of rapid gastric emptying, regular e.m.g. activity was recorded from the duodenum with occasional sharp rises of pressure.

The body and the antrum of the abomasum were readily distinguishable radiographically by the constriction produced by the incisura gastrica (Figure 53.3). Changes in contour of the stomach and outflow of gastric

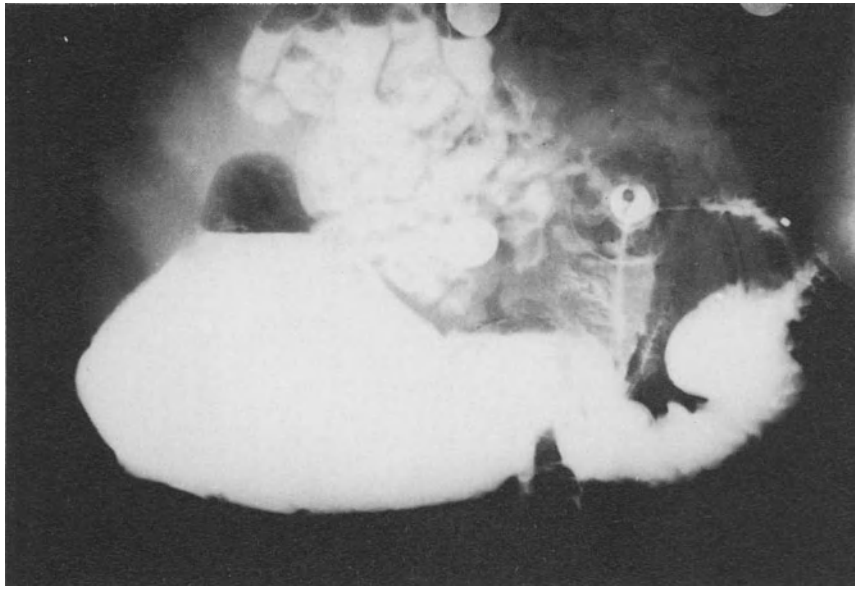
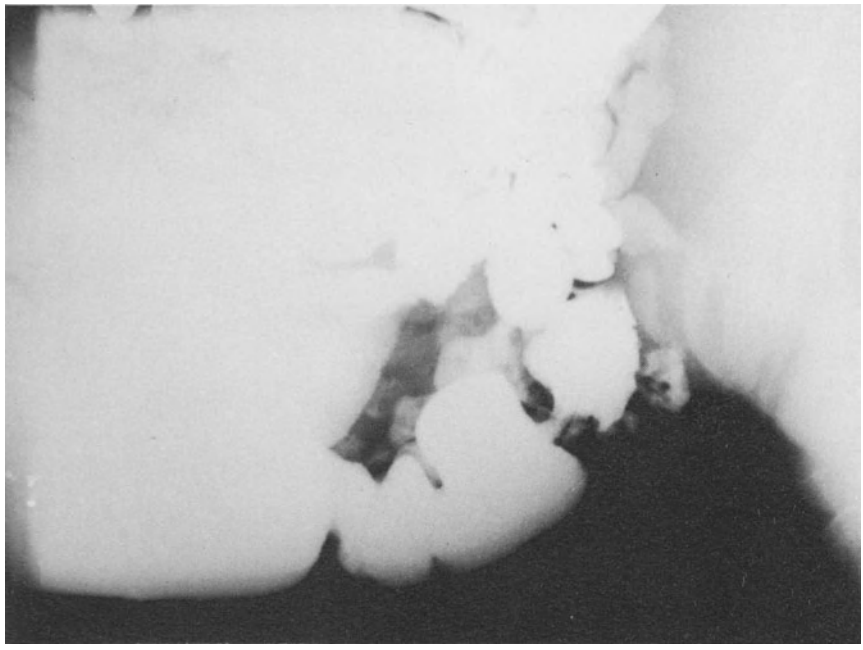


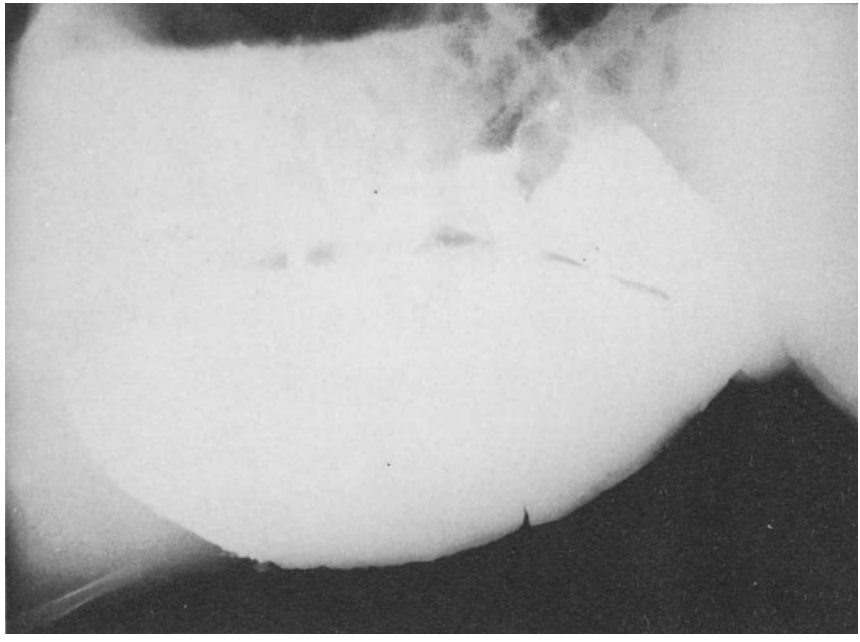
Figure 53.3 Lateral radiograph of abomasum following the instillation of 1.5 l radiopaque isotonic $NaHCO_3$ via gastric cannula. The demarcation of body and antrum is clearly seen at the incisura gastrica

chyme (the intragastric test meal plus gastric secretions) can be visualized fluoroscopically and recorded on video tape. The activity of body and antrum is quite distinct. The body shows very little change in shape when viewed fluoroscopically, and there is no obvious peristaltic movement or segmentation. By contrast the antrum when emptying rapidly shows characteristic segmentation with the terminal segment antrum adjacent to the pylorus being most prominent (Figure 51.4a).

During propulsive discharge of fluid from the terminal segment to the duodenum retropulsive movement of fluid to the penultimate antral segment was occasionally seen, but propulsion and retropulsion of liquid between



(a)



(b)

Figure 53.4 (a) Antral segmentation which occurs when an alkaline test meal is being emptied. The terminal antral segment is clearly seen. (b) When an acid meal is instilled then segmentation does not occur and the antrum is distended

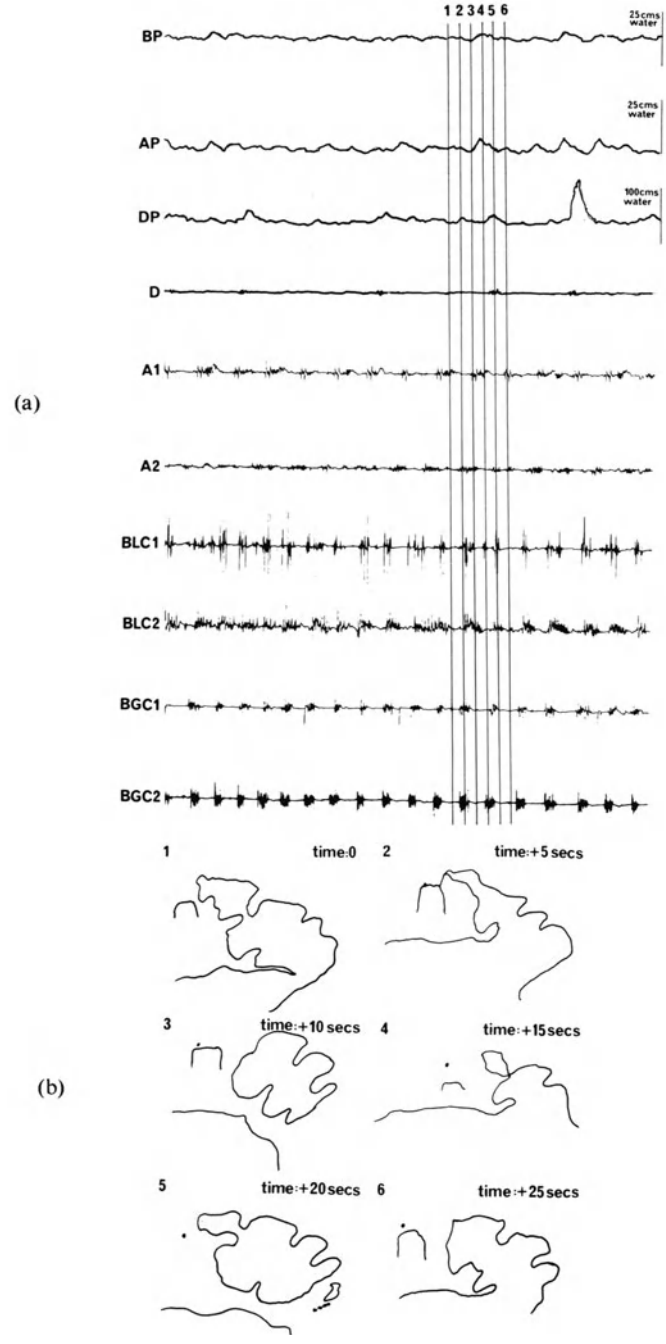


Figure 53.5 (a) Records made with faster time base; the interval made between the vertical lines numbered 1–6 is 5 s and they coincide with numbered videotape traces in Figure 53.5b showing transference of radiopaque meal from terminal antral segment to duodenum

other antral segments was usual. Retropulsive movement of fluid from the duodenum to the antrum did not occur, except rarely in those experiments where a catheter was placed in the antrum from the duodenum, through the pylorus.

Fluoroscopic examination gives a visual impression of a marked rise in pressure in the terminal antral segment as it fills and contracts to transfer its contents to the duodenum, but examination of the pressure records show that the maximal pressure was only 15 cmH_2O (Figure 53.5). The production of spasm of the distal antral segment which transfers fluid to the duodenum does not appear to be directly associated with the basic electrical rhythm of the antrum, although small rises of pressure (around 5 cmH_2O) in the antrum occur coincidentally with each muscular action. During segmentation of the antrum there is a great deal of propulsion and retropulsion between the segments, and it is likely that communication with the body occurs in a similar fashion. During this marked activity intragastric pressure changes are not greater than 10 cmH_2O and are often much less.

When gastric emptying is inhibited by an acid meal antral segmentation is abolished, so that the body and antrum form a non-segmental, distended viscus with the incisura gastrica between body and antrum seen only as a small notch of the greater curvature (Figure 53.4b). During an acid test meal, however, small volumes of gastric contents are passed to the duodenum, for radiopaque material can be seen in the intestine. During the periodic emptying of small amounts of an acid meal recordable action potentials always occur in the antral e.m.g. complexes, but segmentation of antrum does not occur. The whole of the antrum, however, is filled and distended when an acid meal is instilled into the stomach, and fluoroscopy at this time suggests a non-active, flaccid viscus.

DISCUSSION AND CONCLUSIONS

Abomasal emptying is effected by the development of a low pressure head of about 10 cmH_2O in the body. The gastric body is a non-pulsatile organ, but when distended muscular activity develops, especially when the duodenum is alkalinized so that the sustained pressure moves liquids from gastric body into the antrum. When the antrum is distended it develops vigorous segmentation associated with marked increase in e.m.g. activity, especially when the duodenum is alkalinized. In segmentation of the antrum the distal terminal segment is isolated as a pouch from the penultimate segment so that on contraction 5–50 ml volumes of liquid are transferred through the pylorus to the duodenum. This 'systolic' contraction, however, generates only a low pressure of less than 20 cmH_2O. Antral segmentation may be independent of extrinsic innervation since after vagotomy small volumes of chyme move to the duodenum[7]. When the duodenum is acidified then muscular activity is inhibited in both body and antrum so that they remain flaccid and transfer of

fluid to the duodenum ceases. This inhibition of gastric efflux following acidification of the duodenum is maintained even when the stomach is grossly distended and after vagotomy, and there is evidence that it is brought hormonally[8].

The process of transfer of liquid meals from the stomach to the duodenum is apparently controlled by receptors in the duodenum which mediate variation in activity of the gastric smooth muscle to increase intragastric pressure by a few cmH_2O so that the antrum is filled from the body. Segmentation of the antrum charges the terminal antral segment for discharge into the duodenum under low pressure (15–20 cmH_2O). The volume of the terminal antral segment depends upon the volume of material in the body which, by changes in tone controlled by receptors in the duodenum, varies the volume in the antrum so that the volume reaching the terminal antral segment also varies. The whole process of fluid transfer from the body is maintained at low pressure, and the gradient between body, antrum and duodenum is very small probably 10 cmH_2O or less.

Acknowledgement

This work was supported by the Agricultural Research Council.

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Discussion

A. R. Cooke: (USA) In our studies, presented at the 5th International Symposium on Gastrointestinal Motility, we measured pressures as well as volume emptied to correlate both parameters. Have you tried to do this in your studies?

F. R. Bell: (UK) No, we have only measured volume outputs and antral pressure separately.

54

Transformation of the slow potential rhythm of the fundus of the stomach after functional loading following Billroth I gastrectomy

E. ATANASSOVA, Z. JURUKOVA AND E. DRAGANOVA

The mechanical activity of the fundus of the intact stomach is characterized by contractile waves having two types of frequency: in some cases 3 waves per minute, in other cases 5 waves per minute[1]. Correspondingly the electrical activity of its muscle wall is represented by slow waves, the frequency of which is in 1:2 ratio to the frequency of antral waves. After feeding the rhythm of the fundic slow waves increases to reach the slow potential rhythm of the antrum[2].

Since the functional loading of the fundus increases after removal of the antrum, we have used this model and have studied the changes occurring in the electrical activity of the fundus and in its muscle wall structure.

MATERIALS AND METHODS

Chronic experiments were carried out on ten dogs weighing 10–25 kg. The distal two-thirds of the stomach were removed and the remnant joined to the duodenum (Billroth I) under aseptic conditions and chloralose anaesthesia. In two animals the resection was made 2 cm distal to the boundary between the fundus and the corpus. Silver, bipolar, ball-shaped electrodes[3] were implanted subserosally in the fundic muscle wall. The positions are shown on each illustration. The electrical activity of the fundic muscle wall was recorded on a VEB Berlin electroencephalograph at a paper speed of 4 mm/s with a time-constant of 0.3 s.

Tissue from the fundic wall adjacent to the anastomosis was taken for histological study from four dogs at the end of the fifth month after surgery.

Four dogs in which no gastric resection was performed served as controls. Histological sections 3 μm thick, stained with haematoxylin–eosin were used for karyological studies: (1) determination of the mean number of smooth muscle cells per 1000 μm^2 area of the longitudinal layer of the muscle wall; (2) determinations of the mean area of the smooth muscle cell nuclei from microphotographs. All quantitative data were analysed statistically. Ultrathin sections of the fundic wall were examined with electron microscope, Hitachi IIA.

RESULTS

The animals were divided into two groups according to the electrical pattern of the fundus after gastrectomy.

In the first group, as early as the end of the first week after surgery, constantly appearing slow waves with a frequency of 4.5 cycles per minute (cpm)

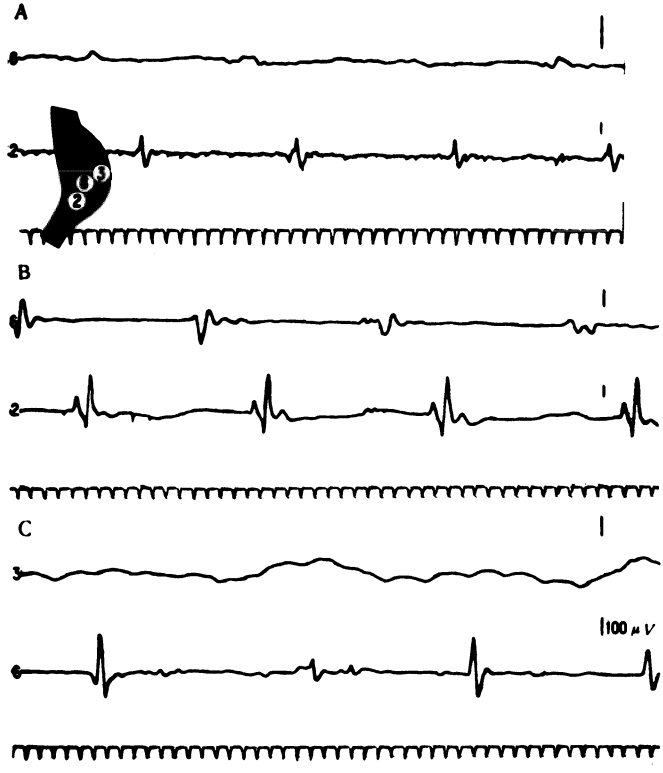

Figure 54.1 Transformation of the fundic slow-wave rhythm into a rhythm similar to that of the intact stomach in the animals of the first group. A: imposed rhythm of about 4.5 cpm in the distal part of the fundus (7th day after surgery); B: considerable increase in the slow-wave amplitude in the distal and medial fundic regions; C: very slow changes in the fundic region near the fornix (5th month after surgery)

occurred in the fundic area near the anastomosis (Figure 54.1A). In the third week after resection, low-amplitude slow waves with the same frequency were recorded from the medial area of the fundus (Figure 54.1B). Later the amplitude of these waves increased to 100–150 μV, reaching 400 μV during the fifth month after surgery. Moreover, in the upper part of the fundus, towards the fornix, very slow, low-amplitude potential changes began to occur (Figure 54.2C).

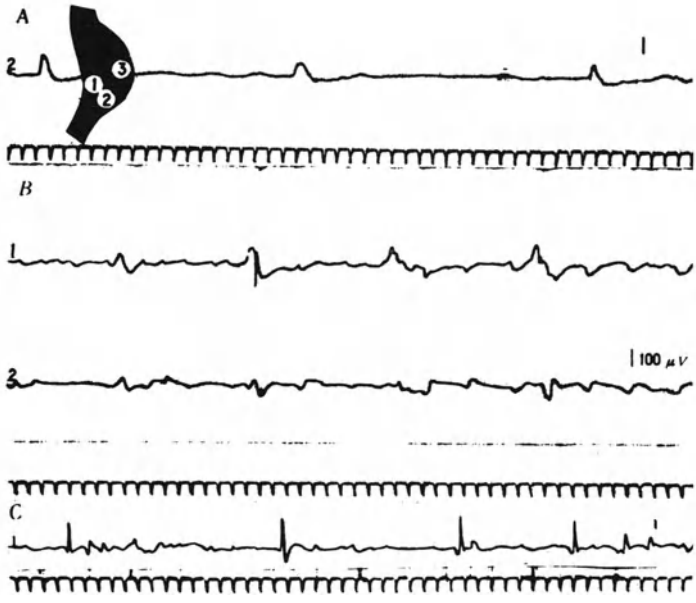

Figure 54.2 Transformation of the fundic slow-wave rhythm into a faster one in the animals of the second group. A: rhythm characteristic of the fundic slow waves (4th day after surgery); B: consecutive slow waves from the distal part of the fundus (11th day after surgery); C: high amplitude of the fundic slow waves 2½ months after surgery

Different phenomena were observed in the animals of the second group. No electrical activity of the fundus muscle wall was recorded on the second day after resection in the course of 1 h recording. On the fourth day after surgery slow, low-amplitude waves appeared from time to time in the record from the fundic area near the anastomosis, sometimes following one another with a frequency of 2–3 cpm (Figure 54.2A). The number of these waves increased subsequently and at about the fifteenth day after surgery the slow waves near the anastomosis occurred more frequently with a rhythm of 4.5–5 cpm, whereby the amplitude of each subsequent wave was higher than that of the previous one (Figure 54.2B). Gradually, towards the end of the the first month after resection, slow waves with the rhythm of the intact stomach (around 5 cpm) were recorded from the middle region of the fundus.

The slow-wave amplitude increased long after surgery: it was about 50–70 μV soon after surgery and became 150–200 μV during the third month (Figure 54.2C).

Feeding intensified the fundic electrical activity. In the first few minutes after feeding, several slow waves might reach a frequency of 5–7 cpm. Then a rhythm of 4–5 cpm was established for a short time. Later this rhythm became slower. Consecutive appearance of slow waves with a rhythm of about 4.5 cpm was observed more frequently in the fourth and fifth month after surgery. However, this was not a constant rhythm of slow waves in the fundus as is the case in the antrum.

Since we thought the rapid and lasting change of the fundic rhythm in the animals of the first group might be due to a part of the pacemaker area of the stomach remaining within the limits of the fundus after resection, we operated on two dogs in such a way that the line of resection passed 2 cm distal to the boundary between fundus and corpus. In these two dogs, as early as the fourth day after surgery, a frequency of about 5 cpm was recorded from the distal region of the fundus (Figure 54.3A), while on the eleventh day such a rhythm was already established in the medial region of the fundus (Figure 54.3B).

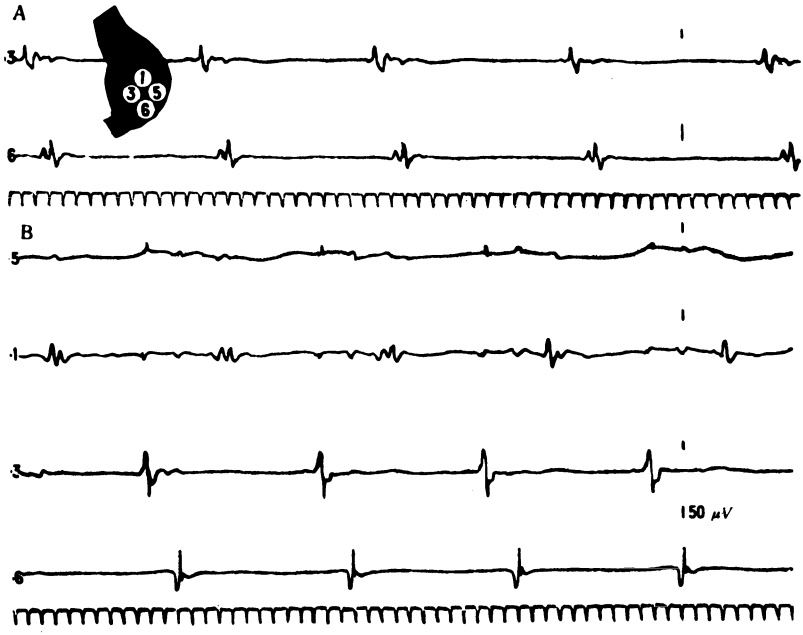

Figure 54.3 Fast transformation of the slow-wave rhythm from the fundus in animals with resection 2 cm below the boundary between the fundus and corpus. A: imposition of a frequency of about 5 cpm in the distal part of the fundus (4th day after surgery); B: switching over of the slow waves from the medial part of the fundus to the faster rhythm of the distal fundus (12th day after surgery)

The higher frequency of slow waves and the increase of the slow wave amplitude were followed by the appearance of spike potentials. In the animals of the second group the spike potentials had low amplitude and low frequency. In some cases the slow-wave frequency decreased to the rhythm characteristic of the fundus, i.e. about 2 cpm (Figure 54.4A). In the animals of the first group, spike potentials appeared earlier in the already established faster rhythm, and had a higher amplitude and frequency (Figure 54.4B). In particular, bursts of high-frequency and high-amplitude spike potentials were often recorded from the medial and distal region of the fundus in the animals with resection 2 cm below the boundary between the fundus and corpus (Figure 54.4C).

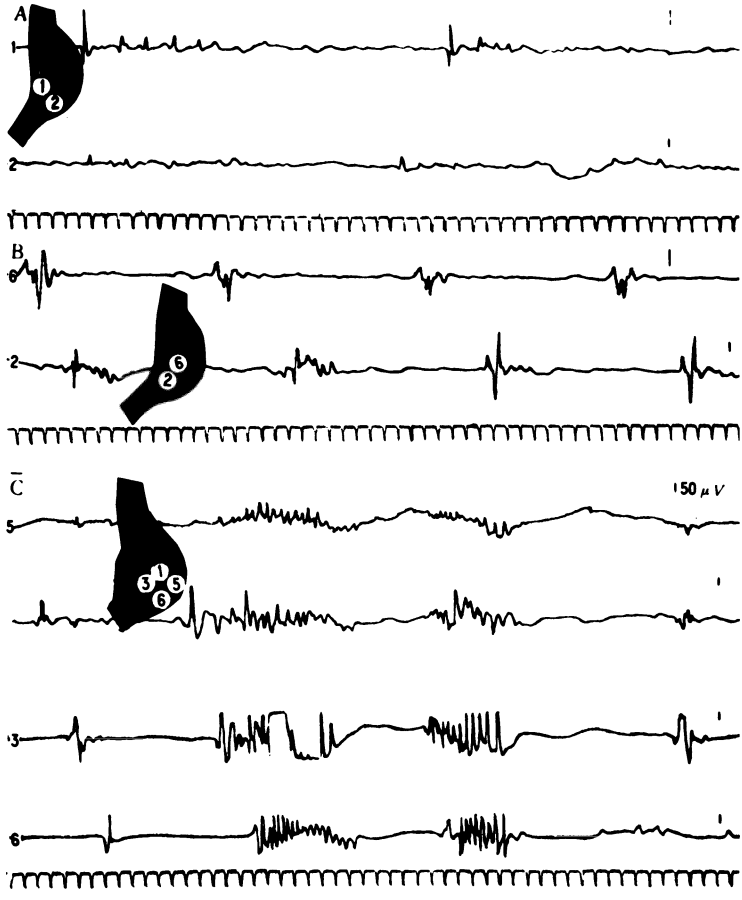

Figure 54.4 Spike activity recorded from the fundic muscle wall after resection. A: in the animals of the second group; B: in the animals of the first group; C: in the animals with resection of the stomach 2 cm distally

Naked-eye examination of the fundus 5 months after surgery showed a marked thickening of the muscle wall, particularly pronounced in the region near the anastomosis. The histological study of the muscle wall of the fundus near the anastomosis suggested that the thickening was chiefly due to an increase in size, i.e. hypertrophy, of the smooth muscle cells. This was supported by the results of the quantitative karyological studies. Counting the smooth muscle cell nuclei per unit area of the fundus longitudinal layer revealed a significant reduction in the mean nuclear number in the experimental animals as compared with the controls (Figure 54.5) (Table 54.1). This reduction was a result of an augmentation in the contractile cell mass and a subsequent rarification of the nuclei. The karyometric data also supported such an

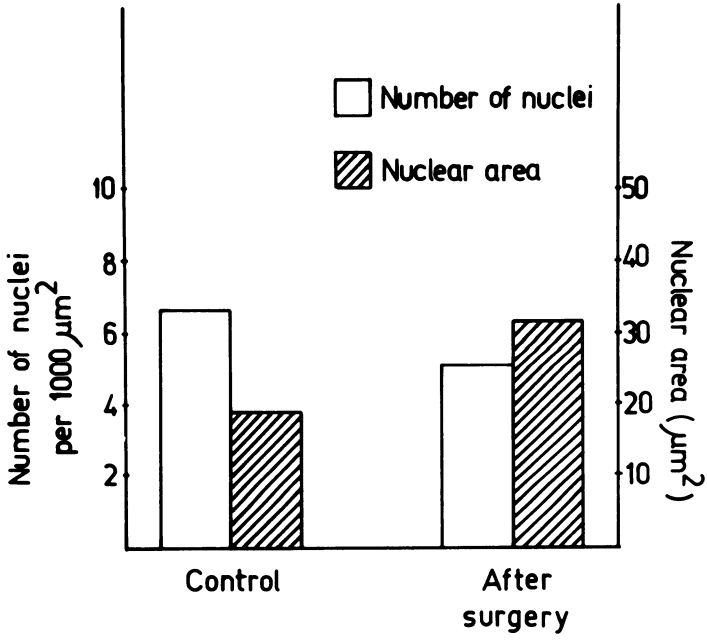

Figure 54.5 Data on the number of cell nuclei per 1000 μm^2 area of fundic longitudinal muscle layer and on the nuclear area (in μm^2) of fundic smooth muscle cells

Table 54.1 Data on the number of cell nuclei per 1000 μm^2 of fundic longitudinal muscle layer and on the nuclear area (in μm^2) of fundic smooth muscle cells

Groups	*Number of cell nuclei per* 1000 μm^2 *area*	*p*	*Nuclear area of fundic smooth muscle cells*	*p*
Control	6.63 ± 0.94	—	19.04 ± 1.94	—
After surgery	5.10 ± 0.40	<0.2	31.40 ± 3.27	<0.01

Mean values ± standard error; *p* = level of statistical significance

assumption. They showed a significant increase in the mean nuclear area of the smooth muscle cells from the fundus longitudinal layer near the anastomosis (Figure 54.5). The mean nuclear area exceeded that of the control by 64.91 %. This finding favours the concept of smooth muscle cell hypertrophy which is known to be associated with an increase in nuclear volume[4]. Single smooth muscle cells undergoing mitosis were observed in the longitudinal and circular layers of the hypertrophic fundus. Since in normal conditions mitotic division of the stomach smooth muscle cells does not occur, this finding indicated that part of the increase in the thickness of the muscular coat was accounted for by an increase in the number of smooth muscle cells; i.e. by hyperplasia of the smooth muscle cells.

Ultrastructurally the hypertrophic smooth muscle cells of the fundus were characterized by abundant organelle-rich cytoplasm. The hyperplasia of myofilaments and granular endoplasmic reticulum was particularly pronounced. Smooth sarcoplasmic reticulum appeared also to be more prominent than in the control muscle cells. The electronmicroscopic study revealed very few, if any, nexuses in either layer of the muscle wall.

DISCUSSION

The results of this study indicate the possibility that the slow electrical rhythm of the fundus of a resected stomach can be transformed permanently, i.e. it becomes identical to the rhythm of the antrum of the intact stomach. The cause of this transformation is increased functional loading. Obviously the function, which is usually characteristic of the antral part of the stomach, is assumed by the fundic area close to the anastomosis. Therefore, in this area, originally a constant rise in the slow-wave frequency was observed, the frequency being identical to that of the slow potential of the antrum of the intact stomach. This has a favourable influence on the faster transformation of the slow-wave frequency of the medial region of the fundus as well. Thus, the distribution of the frequency of the fundic slow electrical pattern begins to resemble that of an intact stomach: the distal and medial fundic regions have the rhythm of the antrum and corpus, while the activity of the region close to the fornix resembles the activity of the fundus of the intact stomach.

The higher frequency of the fundic slow waves is accompanied by a gradual increase in their amplitude. The considerable rise in the slow-wave amplitude (about a ten-fold increase), might be considered as a manifestation of synchronized activity of a still greater number of smooth muscle cells. The synchronization was particularly facilitated in the two dogs in which part of the pacemaker area of the stomach was left attached to the fundus. According to data in the literature, the stomach region with highest slow potential frequency is found along the greater curvature, in the upper third of the stomach corpus[5,6]. In the animals of the first group this area is most probably slightly wider. Perhaps the pacemaker cells remaining in the distal

region of the fundus after two-thirds stomach resection are the cause for the rapid imposing of faster slow-wave rhythm in the distal part of the fundus. In the animals of the second group, the fundic slow-wave rhythm never became constant as in the intact stomach and in the animals of the first group. Evidently, even a small part of the pacemaker area may guarantee permanent transition of the fundic rhythm to the rhythm of the intact stomach. This observation was supported by the fast transformation of the fundic slow-wave rhythm in the animals with resection at 2 cm below the boundary between the fundus and corpus.

The changes in the electrical activity of the fundus after partial resection of the stomach are determined by structural changes in its muscle wall. The increase in the thickness of the muscular coat of the fundus is due to both hypertrophy and hyperplasia of the smooth muscle cells.

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Section XI
Coordination in the Oesophagus

55

Vagal control of lower oesophageal sphincter function

S. COHEN, J. J. KRAVITZ AND W. J. SNAPE, JR

The purpose of this study is to evaluate the function of different portions of the vagus nerves on lower oesophageal sphincter (normally abbreviated as LES) function in the opossum. Studies were performed during vagal section or electrical vagal stimulation of the cervical, thoracic and abdominal portions of the vagus nerves.

METHODS

Studies were performed on adult opossums (*Didelphis virginiana*) of either sex weighing from 2 to 5 kg. Each animal was fasted for 12–16 h before the study and anaesthetized with 50 mg/kg sodium pentobarbital. Supplemental doses of anaesthetic were administered via a catheter located in the left femoral vein.

Oesophageal manometry was performed using three open-tipped catheters, continuously infused with distilled water at a rate of 1.2 ml/min. Intraluminal pressure was transmitted to external transducers (Statham P231a), with outputs graphed on a Beckman rectilinear ink-writing recorder. After all orifices were in the stomach, the tube assembly was withdrawn at 0.5 cm intervals, and pressures were recorded at each level for a 1-min period. Upon completion of the pull-through, the distal catheter opening was positioned to record maximal LES pressure and the recording assembly anchored at both the mouth and the stomach using small paper pins. Pressures are expressed using gastric pressure as the zero reference. All pressures were read at end-expiration. Gastric pressure was checked periodically. A belt pneumograph over the larynx was used to monitor swallowing.

Swallowing (primary peristalsis) was induced by gently touching the cricoid cartilage. Secondary peristalsis was elicited by inflating a balloon with 6 ml of air for 5 s and then deflating. The balloon was located in the smooth muscle region of the oesophagus slightly above the proximal recording site. Visual inspection of the balloon placement at autopsy confirmed the smooth muscle location. LES pressure was measured for 3 min prior to each swallow and the mean pressure (average of LES pressure at 1, 2 and 3 min) used for determining the percentage of sphincter relaxation. The percentage by which LES pressure decreased during swallowing was calculated as:

$$\frac{(\text{resting LES pressure} - \text{nadir of LES pressure during swallowing})}{\text{resting LES pressure}} \times 100$$

Control values for the resting LES pressure and the percentage of sphincter relaxation were obtained only after insertion of the venous catheter and exposure of the vagus nerves. Preliminary studies indicated the isolation procedure had no effect on any of the parameters measured. The vagi were exposed bilaterally in the neck, behind and between the carotid artery and the jugular vein. Each nerve was secured with two loops of thread, and gently raised for better exposure.

Each animal was placed on a respirator prior to the vagotomy and maintained in this fashion for the course of the experiment.

The vagus nerves were sectioned or stimulated in the neck, thorax, or abdomen. Nerves were sectioned or stimulated in random order. Abdominal vagal studies were performed on the vagi as they traversed the diaphragmatic hiatus. The site of section or stimulation was proximal to the LES, which is below the diaphragm in the opossum. In the thoracic studies, the vagi were sectioned or stimulated at two sites. The upper thoracic vagotomy was performed 1.2–2.5 cm above the atria at the level of the skeletal muscle portion of the oesophagus. Lower thoracic vagotomy was done at the middle of the left ventricle (mid-thorax) or below, which was at the level of the smooth muscle portion of the oesophagus.

RESULTS

Table 55.1 shows the LES responses to swallowing or oesophageal balloon distension. Neither right nor left cervical vagotomy significantly affected LES relaxation. Bilateral vagotomy eliminated all LES responses to swallowing. The LES response to balloon distension was unaltered by vagotomy, unilateral or bilateral[1].

Figure 55.1 shows the results after bilateral abdominal or cervical vagotomy with stimulation of the peripheral end of the sectioned nerve. During stimulation of the peripheral end of the cervical vagus nerve, there was a frequency-related decrease in LES pressure, with a maximum response of 93.5 $\pm$ 2.5%.

Table 55.1 Effect of unilateral and bilateral cervical vagotomy on lower oesophageal sphincter function

	Swallowing (1°) (% relaxation)	*Distension (2°) (% relaxation)*
Control	99.2 ± 0.4	79.2 ± 2.8
Right vagus	96.2 ± 0.9	82.3 ± 1.4
Left vagus	97.1 ± 1.2	81.9 ± 4.9
Bilateral	0.0 ± 0.0	78.6 ± 5.2

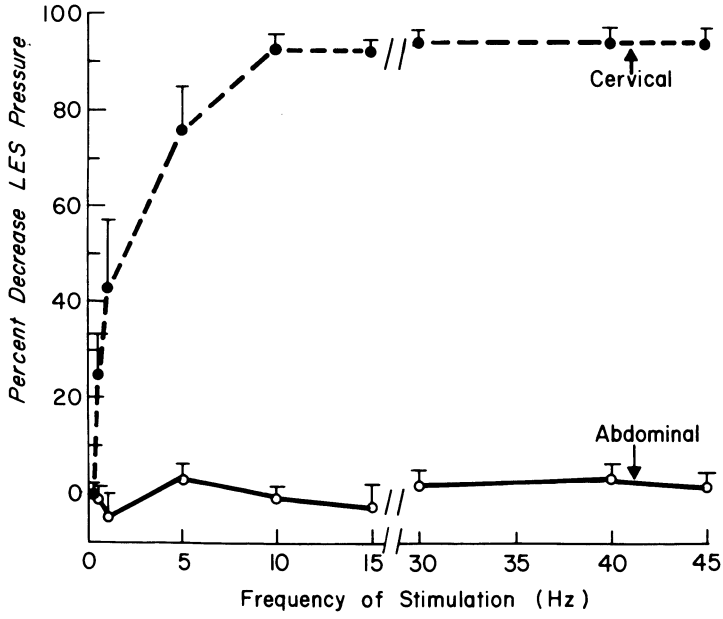

Figure 55.1 Effect of bilateral abdominal or cervical vagotomy with stimulation of the peripheral end of the sectioned nerve. Square wave pulses at 10 V, 1 ms duration and 4-s train duration were applied at a frequency range of 0.25–50 Hz. Studies were done in eight animals. Cervical vagal stimulation produced a frequency-bound decrease in the lower oesophageal sphincter (LES) pressure, but abdominal vagal stimulation did not alter the LES pressure. (Reproduced by permission of *Gastroenterology*, **71**, 999–1003)

Stimulation of the peripheral end of the abdominal portion of the vagus did not affect LES pressure over a wide frequency range. Likewise, abdominal vagotomy (bilateral) did not alter LES relaxation in response to swallowing, balloon distension or cervical vagal stimulation[2].

The site of vagal innervation of the oesophagus within the thorax was next evaluated. Figure 55.2 shows the LES relaxation response to cervical vagal stimulation, as compared to stimulation of the upper or lower thoracic vagus and small vagal branches. A complete frequency-response curve at 5 V shows similar reductions in LES pressure when the vagus is stimulated in the cervical

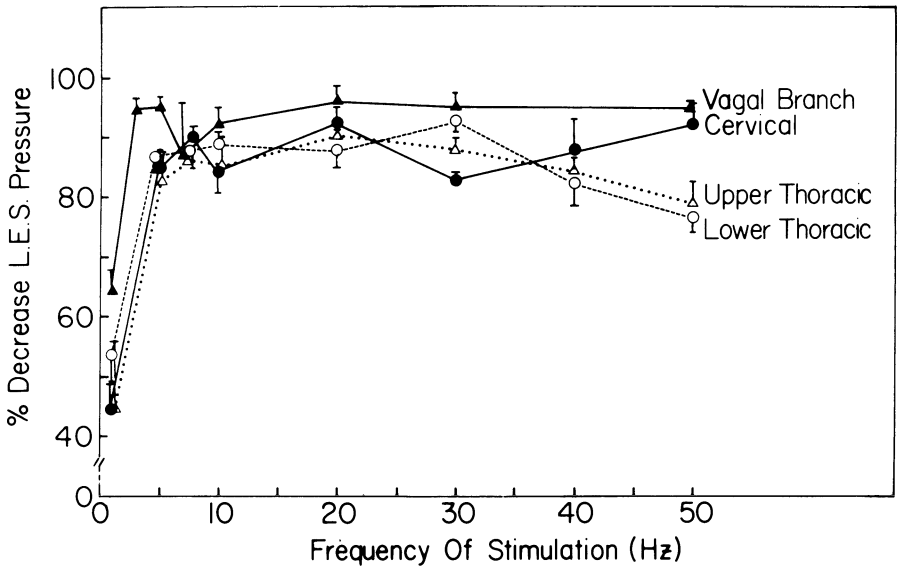

Figure 55.2 Effect of electrical stimulation of the distal end of the sectioned vagus or vagal branch upon LES relaxation. Stimulation was at 5 V over the entire frequency range. Stimulation of the vagus nerve, regardless of the level, or the vagal branch gave similar reductions in sphincter pressure

region or within either the upper or lower thorax. Stimulation of a small vagal branch to the oesophagus in the mid- to lower thorax also elicited a similar reduction in LES pressure. Thus, electrical vagal stimulation produced LES relaxation when stimulated at the cervical and thoracic levels, but not when stimulated at the abdominal level.

Although thoracic vagal stimulation produced LES relaxation, it was unclear how vagal section in the thorax would affect LES relaxation in response to swallowing. Figure 55.3 shows the LES relaxation response as a percentage of basal pressure during induced swallowing after either unilateral or bilateral cervical or thoracic vagotomy (upper or lower). Unilateral vagotomy at any level did not alter LES relaxation. Bilateral cervical or bilateral upper thoracic vagotomy abolished LES relaxation during swallowing. However, after bilateral lower thoracic vagotomy, the sphincteric relaxation response to swallowing remained intact.

DISCUSSION

Table 55.2 summarizes all studies on LES relaxation following vagal section, vagal electrical stimulation, or oesophageal balloon distension. The vagal innervation required for the physiological LES response to swallowing reaches the oesophagus at several sites below the upper thorax. Section of

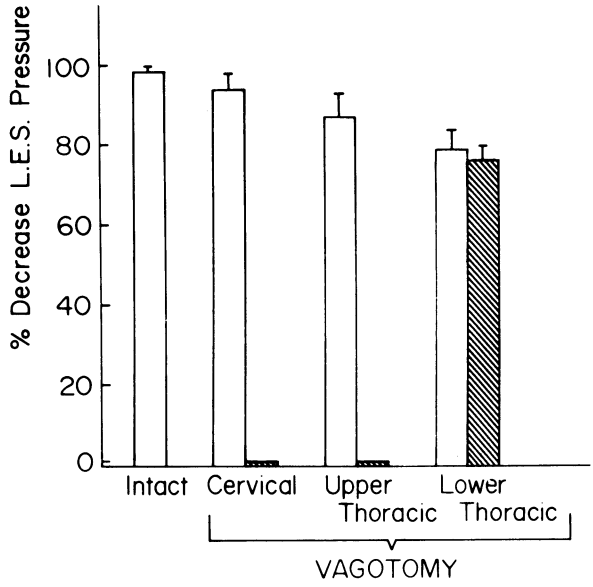

Figure 55.3 Effect of unilateral (open bar) or biiateral (cross-hatched bar) vagotomy upon LES relaxation during swallowing. Bilateral cervical or upper thoracic vagotomy abolished sphincter relaxation

Table 55.2 Effect of vagal section, vagal electrical stimulation and oesophageal balloon distension on lower oesophageal sphincter relaxation

	Swallow after vagal section	*Vagal electrical stimulation*	*Oesophageal balloon distension**
Cervical	0	+	+
Upper thoracic	0	+	+
Lower thoracic	+	+	+
Abdominal	+	0	+

0 = No LES relaxation
+ = Intact LES relaxation
* Balloon distension in mid-oesophagus following vagal section at each level, as noted.

both vagi in the neck, or above the atria, abolishes LES relaxation in response to swallowing. Only one intact vagus is required for this response. Section of both vagi in the lower thorax or in the abdomen does not impair LES relaxation in response to swallowing.

Electrical vagal stimulation induces LES relaxation when the stimulus is delivered within the neck or at any level in the thorax. Abdominal vagal stimulation (proximal to the LES) does not produce LES relaxation. These observations suggest that the vagal fibre necessary for the LES relaxation response reach the oesophagus below the upper thoracic cut, but also at many levels between this site and the diaphragm. Sufficient vagal innervation

necessary for LES relaxation in response to swallowing has already reached the oesophagus proximal to the site of lower thoracic vagotomy (level of mid-ventricle).

The LES response to balloon distension occurs independent of all extrinsic vagal innervation, regardless of the site of denervation. The distension response, therefore, depends entirely upon intrinsic neural pathways or myogenic responses.

In summary, the vagal innervation of the oesophagus is responsible for the initiation of primary peristalsis and its associated LES relaxation. These responses may be initiated by electrical vagal stimulation in the absence of any vagal pathways to the brain. The vagal innervation for LES relaxation reaches the oesophagus at multiple levels in the upper and lower thorax. Vagal innervation required for peristalsis is present only in the upper thorax. Secondary peristalsis occurs independent of vagal innervation. The contribution of the extrinsic vagal mechanism (primary) and the intrinsic non-vagal (secondary) mechanism in the transport of a bolus to the stomach is not known.

Acknowledgements

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The authors wish to thank Mrs Fe Green and Mrs Elizabeth Houseal for expert technical assistance, and Miss Mary Carroll for secretarial assistance.

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Discussion

B. N. Catchpole: (Australia) Many nerve fibres join the vagi as they pass down the neck. Do the effects on the oesophagus of stimulation and denervation of the right vagus differ from those of the left vagus?

S. Cohen: (USA) We could discern no differences between right and left vagal stimulation. Therefore, we grouped these data together.

J. Christensen: (USA) What was the pulse width and what was train length in your stimuli? You saw a rise in LES pressure after bilateral thoracic vagotomy. How do you interpret this observation?

Cohen: The pulse was 1 ms duration with a pulse duration of 4 s. We did not interpret this observation. It is important to indicate that sphincter pressure is somewhat reduced following thoracotomy. This was a prolonged response to unknown mechanism. Antagonists were not tested to determine the mechanism of sphincter pressure increases following bilateral upper or lower thoracic vagotomy. An acute increase in pressure following cervical vagotomy is antagonized by phentolamine or atropine. We are not certain if this is a similar mechanism of response.

M. Pescatori: (Italy) Did you find any difference in amplitude and velocity of propulsion between spontaneous and electrostimulated peristalsis?

Cohen: We could show no difference in amplitude or velocity.

J. M. D. Janssens: (Belgium) As far as your fourth conclusion is concerned, we have done experiments in rhesus monkeys the other way round; cutting the intramural instead of the extramural innervation. A transection of the smooth muscle oesophagus was performed together with a procedure for bolus deviation, in such a way that the two parts of the oesophagus were no longer connected to each other except via the extramural innervation. And when a bolus was instilled into the distal oesophagus, the secondary peristaltic contraction started in the oesophageal segment above the level of transection. So, at least in the rhesus monkey, secondary peristalsis in the smooth muscle oesophagus can be regulated via extramural nerves, as the intramural system has been cut before. Could you agree with our idea that there exist two pathways, one which is sufficient to bring about peristalsis, and that it remains to be determined which pathway is used in physiological conditions?

Cohen: We agree that multiple pathways may be present. The actual mechanism that is active during bolus transport is unknown. We did not do studies during transection or bolus deviation. We simply showed that balloon distension induces sphincter relaxation and peristalsis in the absence of extrinsic vagal innervation. Therefore, a completely intrinsic pathway is present.

N. E. Diamant: (Canada) (1) Where did you blow up the balloon to induce LES relaxation: in the smooth or striated muscle portions? (2) Do you think the pathway for balloon-induced LES relaxation is all intramural whether the balloon is distended in smooth or striated muscle oesophagus, or is there a control pathway especially for the striated muscle portion?

Cohen: (1) The balloon was within the smooth muscle region of the oesophagus. (2) We have no data on skeletal muscle balloon distension.

C. Roman (France) I would like to make a few brief comments: First, in the cat and the monkey it is very difficult, if not impossible, to trigger oesophageal peristalsis by stimulating the vagus nerve. Second, what you call secondary peristalsis after severing the two vagi is in fact a tertiary or autonomous peristalsis due to the activity of myenteric cells. According to Fleltzer's definition (1898), the secondary peristalsis was a reflex-propagated contraction involving extrinsic vagal loops (sensory and motor fibres). Indeed, we have demonstrated in conscious animals (sheep, monkey) that the vagal efferent fibres were firing during swallowing and also after an oesophageal distension (secondary peristalsis). This was also true for the fibres controlling the smooth muscle oesophagus of monkey. Third, you showed that there was no effect of vagotomy on the resting tone of the LES. Since your animals are anaesthetized this is not surprising: we have seen in our preparations that a very slight anaesthesia caused the vagal spontaneous discharges to disappear.

56

The cat oesophagus: responses of the circular layer of smooth muscle from the body to electrical field stimulation

D. J. DE CARLE, D. L. TEMPLEMAN AND J. CHRISTENSEN

The oesophagus of the American opossum when isolated in an organ bath is capable of generating peristaltic contractions in response to a variety of stimuli[1]. Thus it seems likely that peristalsis is due, at least in part, to local neuromuscular control mechanisms. These local mechanisms can be elucidated by study of the responses of isolated strips of oesophageal smooth muscle to electrical field stimulation and drugs. We have previously described the responses in American opossum oesophageal circular smooth muscle[2]. It has been concluded that the dominant response in that species is a twitch contraction occurring after the end of the stimulus train, the off-response, and that it is mediated by a non-adrenergic, non-cholinergic neurogenic mechanism. It has been shown that, in the cat[3] and Australian possum[4] there is an on-response, which is a twitch contraction occurring at the beginning of the stimulus train; it has been suggested that this may be an important local control mechanism. It has also been suggested that in the cat and Australian possum the off-response is cholinergically mediated[5]. We studied responses in cat oesophageal circular smooth muscle and compared them to responses seen in oesophageal smooth muscle from the American opossum.

METHODS

Mature adult cats and American opossums of either sex were anaesthetized with intraperitoneal pentobarbital and the stomach and oesophagus were removed *en bloc*. The specimens were opened along the greater curve of the

stomach, the mucosa removed by sharp dissection and transverse strips, 2 × 20 mm, cut from the smooth muscle segment of the oesophagus. In the cat there is only a short segment of smooth muscle, and it flairs prior to joining the stomach; so great care was needed to ensure that the strips were cut in the long axis of the circular smooth muscle bundles. In the cat oesophagus it is likely that some longitudinal muscle was cut obliquely rather than across the long axis of the longitudinal muscle. Strips cut in the long axis of the oesophagus, and representing the activity of longitudinal muscle, were prepared for some studies.

The strips were suspended in a tissue bath, which has previously been described in detail[6] and superfused with Kreb's solution of composition sodium, 138.5 mM; potassium, 4.6 mM; calcium, 2.5 mM; magnesium, 1.2 mM; chloride, 125.0 mM; phosphate, 1.2 mM; and bicarbonate, 21.9 mM gassed with 95% O_2, 5% CO_2 and warmed to 37 °C. The strips were stretched to about 150% of initial length and their tension recorded continuously with force/displacement transducers (Grass FT-03) connected to a strip chart recorder (Beckman type RM Dynograph). Electrical field stimulation in the form of trains of rectangular pulses was delivered through platinum ring electrodes from a constant current stimulator. Using 3 s trains of 1 ms pulses at 10 Hz current strength was varied from 5 to 500 mA. Using current strength of 30 mA the frequency was varied from 2 Hz to 60 Hz. Using 3 s trains of 30 mA pulses at 10 Hz the strips were stimulated every 30 s for 3 h.

In other experiments drugs were added to the superfusion fluid and the responses to electrical field stimulation assessed in the presence of various concentrations of those drugs. The drugs used were tetrodotoxin 10^{-8}–10^{-6} M, atropine sulphate 10^{-9}–10^{-7} M, physostigmine 10^{-9}–10^{-6} M, hemicholinium 10^{-5} M and choline 10^{-5} M.

3 s trains of 1 ms 30 mA pulses at 10 Hz were used and repeated every 30 s. Amplitude and duration of responses were measured visually after stable values were achieved, and the results expressed in absolute values or as a percentage of the control responses.

RESULTS

Cat circular smooth muscle showed both on-responses and off-responses to electrical field stimulation. Both responses increased in amplitude with increasing current strength but both were maximal with pulses of 30 mA. On-responses showed little variation over a wide range of frequencies but were maximal at 25 Hz. Off-responses were maximal at 10 Hz and diminished rapidly at higher frequencies (Figure 56.1). On-responses diminished in amplitude on repeated electrical field stimulation falling to 24% of control values after 25 min and had disappeared by 90 min. Off-responses remained constant for up to 3 h (Figure 56.2).

It was impossible to obtain stable values after prolonged exposure to

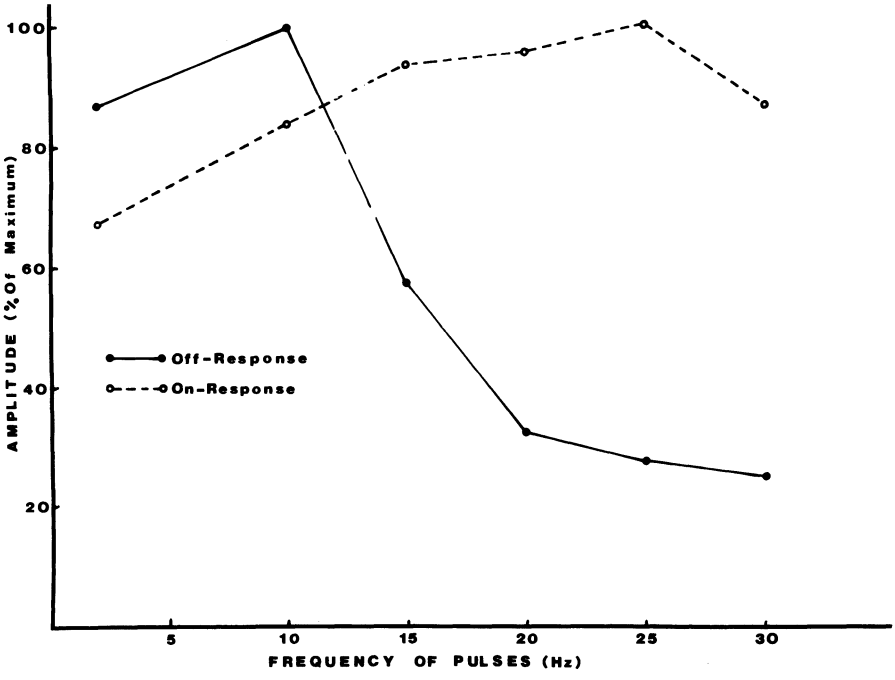

Figure 56.1 The amplitude of cat responses expressed as a percentage of the maximum response seen and plotted against frequency of pulse train in Hz. Each point represents the mean observations of eight strips. The off-response is maximal at 10 Hz, while the on-response is maximal at 25 Hz

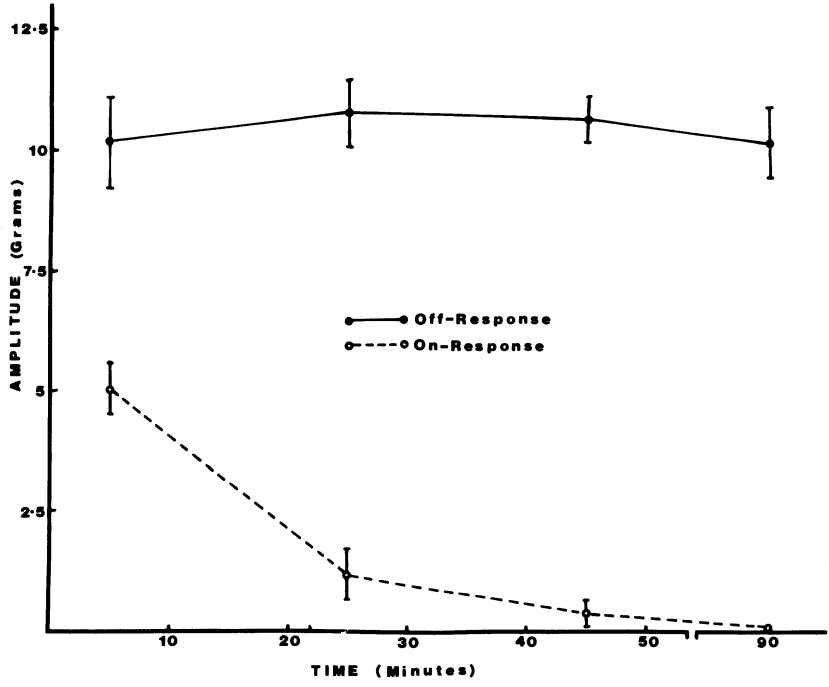

Figure 56.2 The amplitude of cat responses in grams plotted against the time from initial stimulation. The strips were stimulated with 3 s trains every 30 s. Each point represents the mean ± SE of observation on twenty-seven strips. The off-response remains constant while the on-response diminishes after repeated stimulation

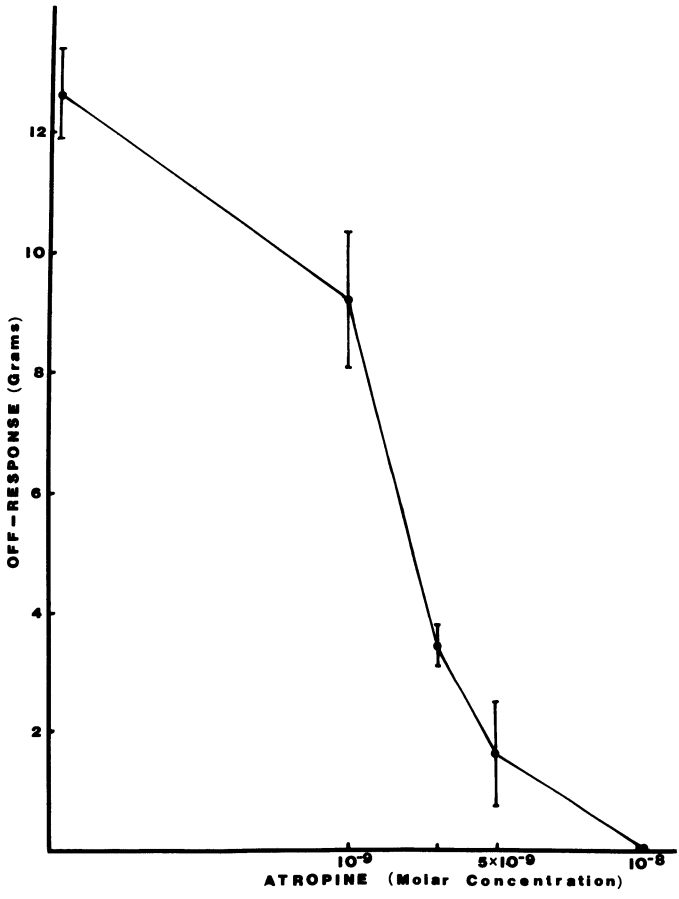

Figure 56.3 The amplitude of cat off-responses in grams plotted against molar concentration of atropine. Each point represents the mean ± SE of observations on ten strips. It can be seen that the off-response is reduced by atropine and abolished by a concentration of 10^{-8} M

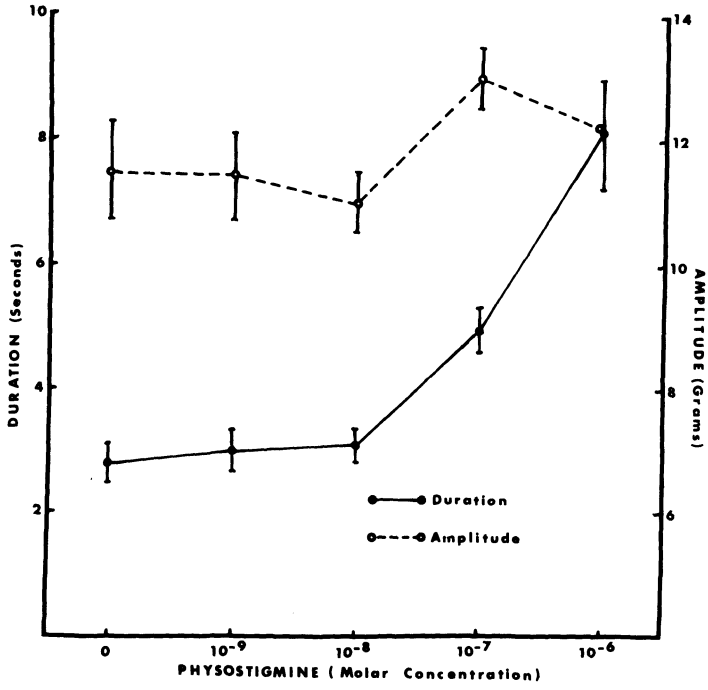

Figure 56.4 The amplitude in grams and the duration in seconds of cat off-responses plotted against the molar concentration of physostigmine. Each point represents the mean ± SE of observation on sixteen strips. The amplitude of responses did not alter significantly. The duration of contraction significantly increased

drugs because cat on-responses diminished on repeated electrical field stimulation; however cat on-responses did not appear to be altered by either tetrodotoxin 10^{-6} M or atropine 10^{-6} M.

Both cat and opossum off-responses were abolished by tetrodotoxin 10^{-6} M. Atropine caused a dose-dependent decrease in the amplitude of cat off-responses and they were completely abolished by atropine 10^{-8} M (Figure 56.3). Opossum off-responses were not altered by atropine.

Physostigmine caused a dose-dependent increase in the duration of off-responses in cat oesophageal smooth muscle, but in the concentrations tested did not alter the amplitude of cat off-responses (Figure 56.4). Opossum off-responses were not altered by physostigmine.

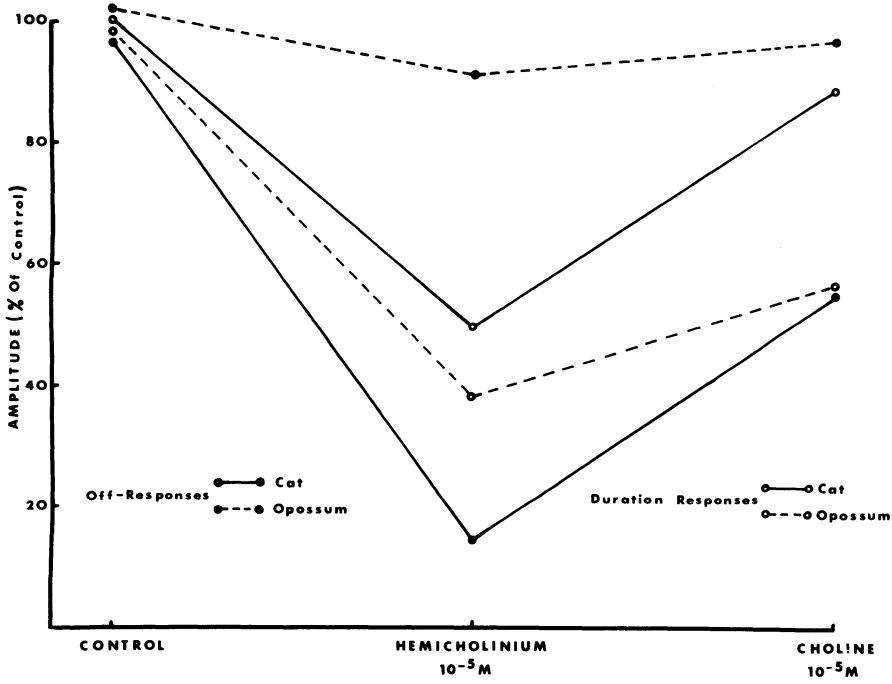

Figure 56.5 The amplitude of off-responses and duration responses in oesophageal smooth muscle from cat and opossum expressed as a percentage of control responses. The results shown for hemicholinium 10^{-5} M were obtained after 20 min of repeated electrical field stimulation in the presence of that substance while the results for choline 10^{-5} M were obtained 20 min after hemicholinium had been washed out and replaced with choline. Off-responses in the cat, and duration responses in both species, were significantly depressed by hemicholinium while off-responses in the opossum were unchanged

Hemicholinium, 10^{-5} M, caused the amplitude of off-responses in the cat to fall to 14% of control values. The hemicholinium-induced fall in off-response amplitude was significantly but not completely reversed by choline, 10^{-5} M (Figure 56.5). Longitudinal strips of oesophageal smooth muscle

from both cat and opossum showed prolonged contractions (duration responses) throughout each train of pulses. The duration responses in both species were diminished in amplitude by hemicholinium (Figure 56.5). Off-responses in opossum smooth muscle were not altered by hemicholinium.

DISCUSSION

The local control mechanisms within the wall of the oesophagus probably play an important role in oesophageal peristalsis. Detailed information is available about responses in the American opossum, a species which has an oesophagus consisting mainly of smooth muscle like that of man. In the experiments reported here we studied the responses in the cat, which differs anatomically from man and the opossum and may provide information about species variation in neuromuscular control mechanisms.

Cat oesophageal circular smooth muscle shows both on-responses and off-responses. The on-responses appear to be myogenic because they are resistant to tetrodotoxin, an agent which abolishes nerve conduction. The stimulus characteristics required for optimal generation on-responses differ from those required for generation of off-responses.

We have shown that cat off-responses are abolished by atropine and increased by physostigmine. This suggests that they are mediated by cholinergic nerves and thus differ from off-responses in opossum oesophageal smooth muscle. It is possible that opossum off-responses are also mediated by cholinergic nerves but that the sites of action of acetylcholine are inaccessible to atropine and physostigmine. The resistance of the opossum off-responses to hemicholinium, an agent which interferes with acetylcholine synthesis, confirms that it is not cholinergically mediated. The cat off-responses is depressed by hemicholinium, this being further evidence that the cat off-response is mediated by a cholinergic nerve. Hemicholinium blocked the response to electrical field stimulation in longitudinal strips. These responses are cholinergically mediated.

It has been shown that electrical field stimulation of cat oesophageal circular smooth muscle is associated with nerve-mediated smooth muscle hyperpolarization[7]. This occurs during the stimulus and precedes the off-responses, suggesting a central role for an inhibitory nerve in the off-response. The cholinergic nerve which is involved in the off-response may be inhibitory and responsible for the hyperpolarization which precedes the off-response. However cholinergic nerves are usually excitatory. A second, more likely explanation of our findings is that two nerves are involved; an excitatory cholinergic nerve and an inhibitory nerve. The off-response is not affected by alpha or beta adrenergic-blocking agents or by dopaminergic-blocking[6] agents, suggesting that the inhibitory nerve is non-adrenergic in type. The precise mechanism by which the off-response is produced is still not clear.

Acknowledgement

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Discussion

J. H. Szurszewski (USA): Why does the amplitude of contraction due to direct muscle stimulation decrease and become abolished as a function of time?

J. de Carle (Australia): Dr Schultze has pointed out that muscle electrolyte concentrations are disturbed by dissection and they subsequently return to normal. Perhaps this disturbance makes the muscle more susceptible to direct stimulation.

S. Cohen (USA): I would suggest that the 'off'-response is indeed a rebound excitation but the amplitude of this response can be altered by cholinergic factors.

de Carle: You may well be right. One has to postulate that the rebound phenomenon does not generate any tension in the cat without the cooperation of a cholinergic nerve.

J. D. Wood (USA): The off-response has often been interpreted to be postinhibitory rebound excitation that follows activation of non-adrenergic inhibitory neurons. Are you disregarding this as an interpretation of your results?

de Carle: No. I still think that the mechanism you describe is most likely one for the off-response. I am not sure how the cholinergic nerve, so clearly necessary for that response in the cat, fits in to the scheme of things.

A. Bennett (UK): What direction were the stimulating electrodes?

de Carle: One ring electrode either end of a longitudinally mounted strip.

Bennett: This is a bad arrangement, because in guinea pig ileum direct muscle stimulation occurs at lower pulse widths with such arrangement, compared with electrodes one either side of the muscle. Was the absence of potentiation of responses to physostigmine due to the fact that the response was already maximal? Did you test this with additions of acetylcholine to the preparation?

de Carle: No, I am sure the amplitude was already maximal and that is why the only response seen was an increase in duration of responses.

N. W. Weisbrodt (USA): Did you see any gradients in latency between the end of stimulation and the start of contraction which could be correlated with the location of the strip?

de Carle: No, not in these experiments. We have seen a latency gradient in off-responses from the cat in previous experiments. It is a very small but real gradient. It is small because the smooth muscle segment is so short.

C. Roman (France): I would like to emphasize that the responses obtained on the cat smooth muscle oesophagus seem to be somewhat different according to the technique of stimulation: by simple pulse stimulation of the vagus nerve in the living cat or on the isolated and perfused organ, we have been able to record two kinds of responses (Gonella, Niel and Roman, *J. Physiol.*, in press): excitatory responses, i.e. excitatory functioning potentials giving rise to spike potentials; inhibitory responses, i.e. inhibitory function potentials often followed by a transient depolarization which may initiate spikes. The two kinds of responses were

recorded on both muscular layers (longitudinal and circular). Excitatory responses were suppressed by atropine but not the inhibitory ones. By stimulating the vagus nerve with a series of pulses we could also get on or off responses.

H. L. Stockley: (UK) In human lower oesophageal circular muscle also there is an on-response and an off-response. The 'on' contraction is at least partly cholinergic and is followed, while stimulation continues, by an non-adrenergic inhibition. The off-response is mainly a hyoscine-resistant contraction, although there may be a small cholinergic contribution. My question refers to apparent reduction in off-response at frequencies above 15 Hz. Were the 'on'- and 'off'-responses clearly distinguishable or was the off-response possibly masked by an increase in the on-response? A further comment is that a prostaglandin (e.g. E_2) produced in increasing amounts during the first half hour might, by analogy with ileal circular muscle, account for the gradual diminution of the transitory on-response without necessarily suppressing the stronger off-response.

57

Mechanism of tetrodotoxin-insensitive relaxation of opossum lower oesophageal sphincter

E. E. DANIEL, S. SARNA AND J. CRANKSHAW

In experiments to define the Ca^{2+} requirements of lower oesophageal sphincter (LES) contraction to direct muscle depolarization, we found that tetrodotoxin (TTX) inhibited responses to pulses shorter than 2 ms, but failed to inhibit the 'on' and 'off' relaxation to 5 ms pulses or longer. As reported elsewhere in this book[1], we found that the abdominal oesophagus of the opossum initiated propagated contractions which were usually accompanied by early and delayed LES relaxation. Only the early relaxation was blocked by intra-arterial TTX. Thus, there was evidence of an inhibitory response which did not involve nerve axon-action potentials. We have examined the mechanism of this relaxation.

METHODS

Four strips of muscle (1–2 × 10–15 mm) were removed from the LES (sometimes one of these was from the body just proximal to LES) after excision of the oesophagus and stomach under anaesthesia, removal of mucosa and visual identification of thickened LES muscle. They were tied to one end of a glass tube and placed through two concentric platinum stimulating electrodes 1 cm apart. The other ends of the strips were attached to four Grass FTOC3 strain gauges. The mounted strips were placed under 1 g tension in 20 ml baths containing Kreb's Ringer solution[2] at 37 °C and oxygenated with 95% O_2–5% CO_2. Atropine SO_4 10^{-6} w/v was added in most studies but omitted in some later ones because it had no effect. Electrical stimulation was applied consecutively to each strip from a Grass S4 stimulator; parameters of 40 V, 5 Hz, 0.5 ms were chosen after preliminary study

to yield maximal relaxation. Active tension was defined as the difference between tension observed without stimulation and after maximum relaxation with field stimulation or 1 μg/ml isopropyl-norepinephrine bitartrate. These values were always similar or identical by the two techniques and amounted to 0.5 to 2.5 g in LES. In body strips, they were zero or very small.

Some tissues were fixed for electronmicroscopic study by draining the bath and refilling it with cold 2% glutaraldehyde in cacodylate buffer at pH 7.4. After fixation for at least 2 h, they were post-fixed in 1% OsO_4, embedded in Spurr resin, stained with uranyl acetate *en bloc*, sectioned silver to grey, further stained in sections with lead citrate and viewed with a Phillips 301 electron microscope[3].

Drugs and chemicals were highest grade available, black widow spider venom from *Lactrodectus mactans tredecimguttanus* was obtained through the generosity of Dr W. D. Dorian, Laboratories Merck-Frost, Quebec. It was stored at $-20\,^{\circ}C$; thawed just before use and added directly to the bath. Stock solutions of indomethacin (5 mg/ml) and prostaglandins (1 mg/ml) were made up in 70% ethanol and eicosatetrayonic acid (2.5 mg/ml) was made up in 0.5 ml of $CHCl_3$ diluted with absolute alcohol to 5 ml.

RESULTS

LES strips stimulated with 0.5 ms pulses from 5 to 10 s relaxed during stimulation and usually contracted on cessation of stimulation (Figure 57.1). Sometimes they had little or no 'off' contraction. Strips from the body responded to 0.5 ms pulses with an 'off' contraction. LES strips in all cases responded to 5 ms stimulation with an 'on' relaxation, smaller than that to 0.5 ms pulses. The 'off' responses was usually (30/45 cases) a partial contraction not back to basal tension followed by a second relaxation (Figure 57.1). In the remaining cases, the 'off' response was predominantly a contraction above basal tension followed by some relaxation below it. After TTX (10^{-6} w/v), responses to 0.5 ms pulses were abolished or markedly reduced (Figure 57.1) but those to 5 ms pulses continued. 'On' relaxations were not significantly diminished; 'off' relaxations were often enhanced or 'off' contractions to 5 ms pulses were converted to relaxation (19/20). In body strips and rarely (2/20) in LES strips, after TTX, 'on' contractions to 5 ms pulses were obtained (Figure 57.1). Longer duration pulses gave responses like those to 5 ms pulses. Our initial expectation had been that all strips would show 'on' contractions to longer duration pulses after TTX.

To determine if nervous mechanisms insensitive to TTX were involved in the 'on' and 'off' relaxation to 5 ms pulses, we treated tissues with extract of black widow spider venom. This venom is reported to selectively destroy nerve endings[4]. We used the amounts equivalent to from 0.5 to 1.5 spiders in the bath. Higher doses abolished active tension, but this was restored by by 0.1 or 1 μg/ml carbachol. All doses abolished all responses to 0.5 ms

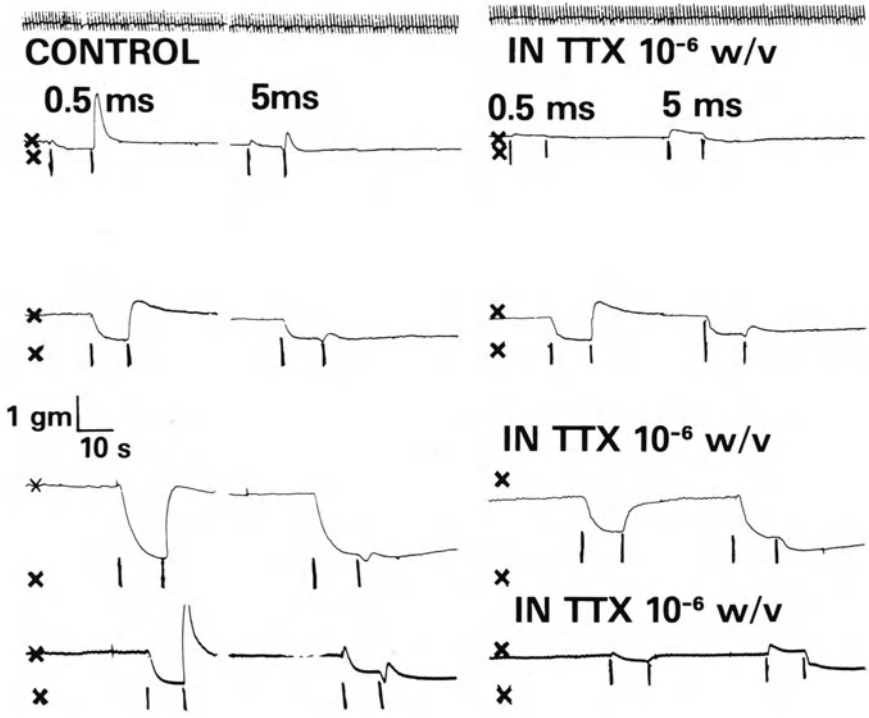

Figure 57.1 Responses of four strips of oesophageal circular muscle to electrical field stimulation at 40 V, 5 Hz and 0.5 or 5 ms for 10 s. At top body strip near LES; other strips LES. Subsequently TTX was administered in all strips but one, and field stimulation was repeated. Body strips were unable to relax and 'off' contractions were abolished by TTX, but 5 ms stimulation then caused small contractions. LES strips usually responded with 'on' relaxation and 'off' contraction to 0.5 ms stimulation and with 'on' and 'off' relaxation to 5 ms stimulation; the 'off' relaxation was of long duration. Usually the 0.5 ms stimulation was ineffective after TTX, but it was only reduced in this experiment in the third strip. Neither response to 5 ms stimulation was abolished by TTX in most cases. An exception is shown here (bottom) which was the result we expected initially; in this case, 5 ms pulses caused an 'on' contraction after TTX. Note that an 'off' relaxation still occurred. Active tension is indicated by the lower X on each record which is the level of maximal relaxation; the upper X is the starting tension. Application of field stimulation occurred between vertical bars. Calibrations at left

pulses and converted the 'on' relaxation to 5 ms pulses to an 'on' contraction (Figure 57.2). The 'off' relaxation to 5 ms pulses usually persisted. Evaluation of venom-treated strips using the electronmicroscope revealed swelling, loss of vesicles and/or disruption of all nerve varicosities (Figure 57.3). In some cases axons with neurofilaments and neurotubules remained intact. In high doses of venom, smooth muscle also show varying degrees of damage (Figure 57.3) as did the fibroblast-like interstitial cells associated with nerves (see below).

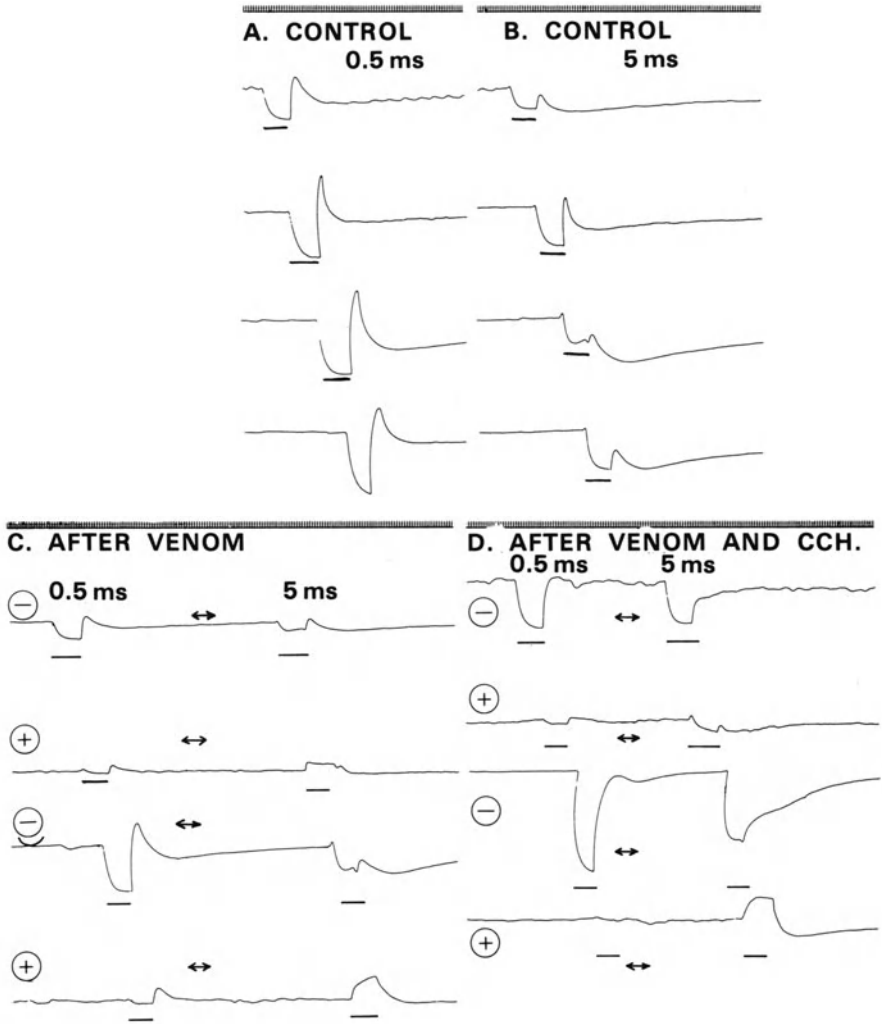

Figure 57.2 Responses of LES strips to field stimulation at 0.5 and 5 ms duration, before and after black widow spider venom (equivalent of 1.5 spiders in each) in a 20 ml bath. Two strips with (+) and two without venom (−). Venom abolished active tone but this was restored by CCH or carbachol (1 μg/ml). Initial tension indicated by arrows. Note that venom nearly abolished active tension and pulse stimulation and converted 'on' responses to 5 ms pulses to contractions in most cases: 'off' relaxations persisted. Duration of field stimulation shown by horizontal lines under each trace. Relaxation in top left record was 1 g

Release of mediator from nerve endings is usually inhibited by elevation of Mg^{2+} and lowering of Ca^{2+} in the medium[5]. In modified Kreb's solution (Mg^{2+} 5.8 mM; Ca^{2+} 0.5 mM), active tension was abolished but could be restored by carbachol. Responses to 0.5 ms pulses were abolished, and relaxations to 5 ms pulses were seriously diminished. However, interpretation

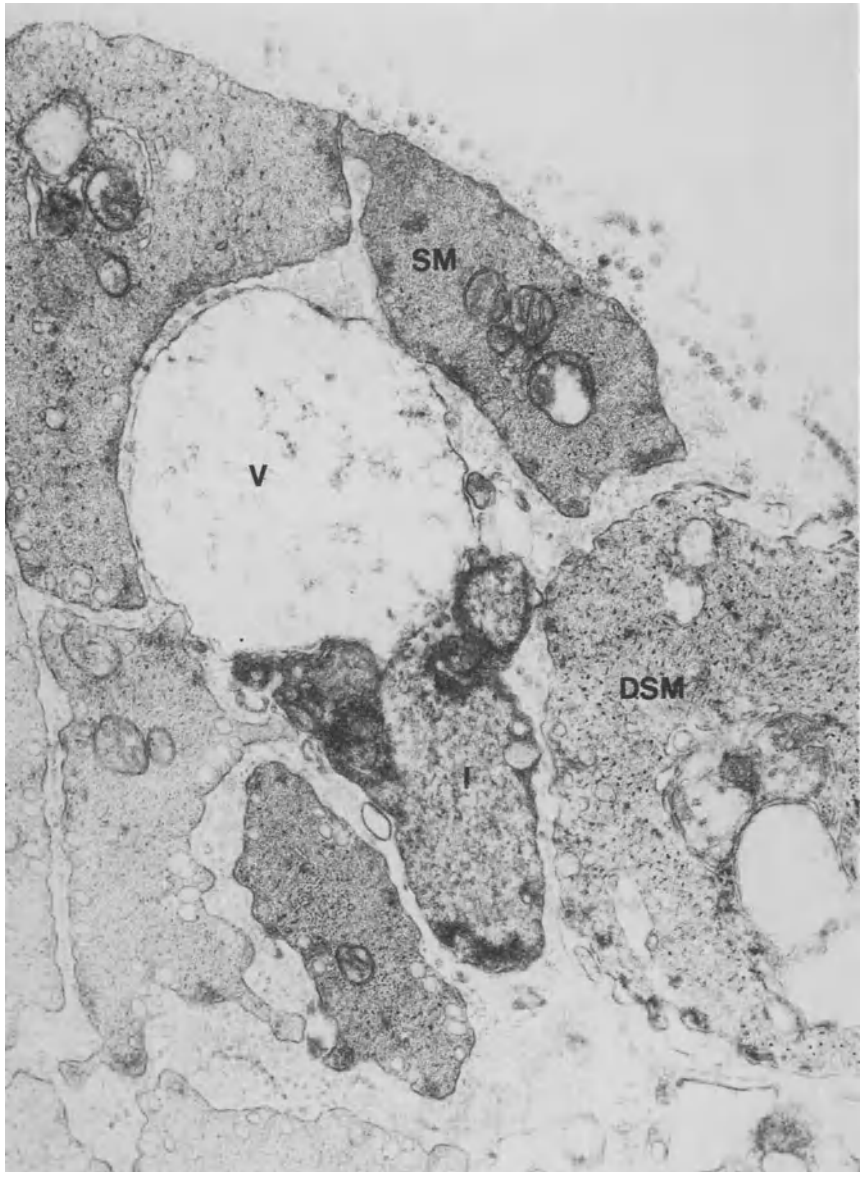

Figure 57.3 Electron micrograph of damaged nerve varicosity (V) after treatment as in Figure 57.2. Note damaged 'interstitial' cell (I) and damaged smooth muscle (DSM) and more nearly normal smooth muscle (SM); however, many muscle cells have different densities suggesting partial damage. Approximately × 30 000

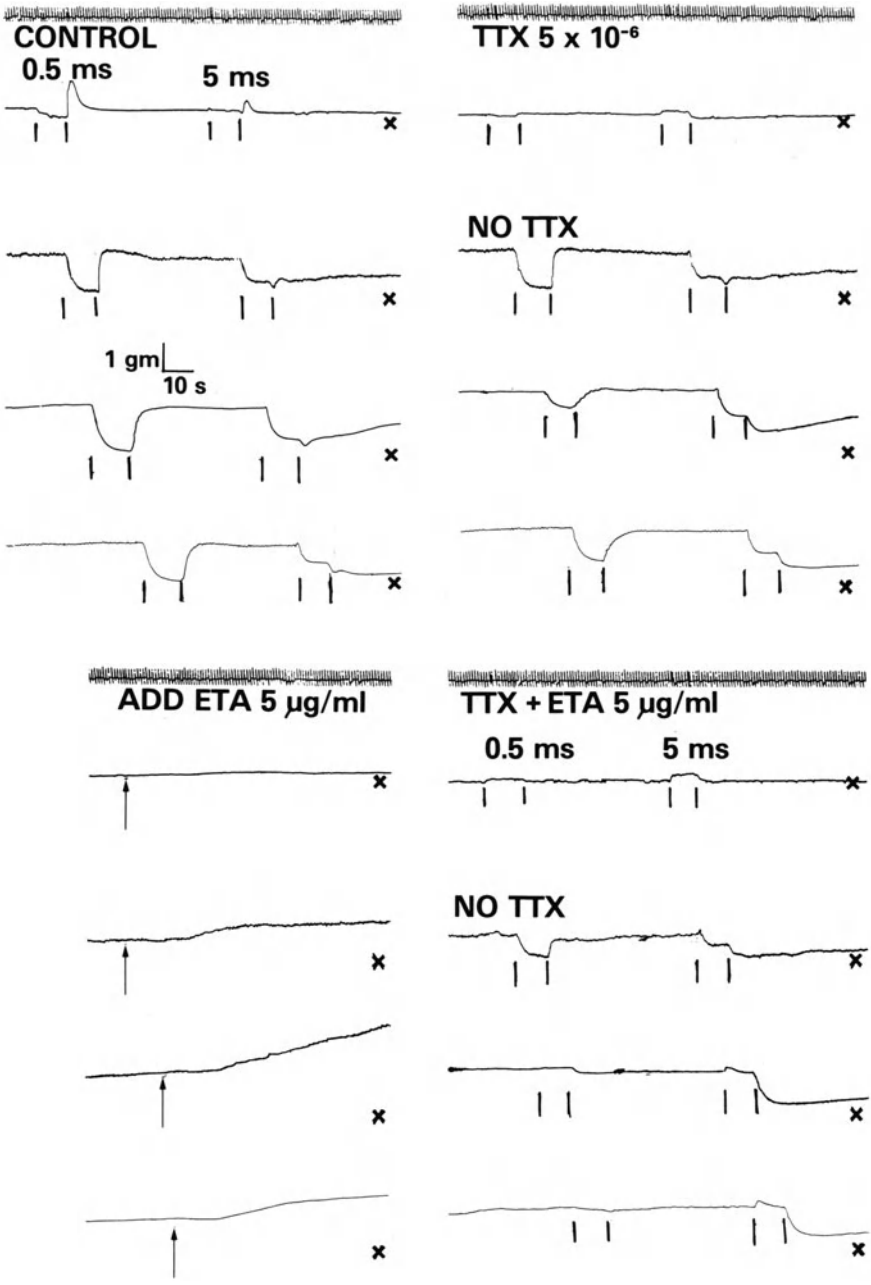

Figure 57.4 Responses of LES strips to field stimulation before and after 5, 8, 11, 14-eicosatetrayonic acid (ETA). Some strips were pre-treated with TTX which was only partially effective in the experiment. Note the contraction induced by ETA and the abolition by ETA of all 'on' relaxations (and reversal of responses to 5 ms pulses to contraction) but not 'off' relaxation to 5 ms pulses. In strips not pre-treated with TTX, ETA did not abolish 'on' or 'off' relaxations on each record which shows maximum relaxation; application of field stimulation between vertical bars below each trace. Calibrations at left

of these results was difficult because the modified medium also decreased relaxation responses to isoproterenol (1 μg/ml). We concluded from studies with venom and media with increased ratios of Mg^{2+}/Ca^{2+} that release of inhibitory mediator was involved in 'on' relaxations to both 0.5 and 5 ms pulses and in 'off' contractions. 'Off' relaxation to 5 ms pulses may have involved myogenic mechanisms since it was not abolished by venom or high Mg^{2+}/Ca^{2+} ratios.

Next we tested whether the TTX-insensitive pathway might involve prostaglandins in some way. We treated some strips with TTX; both treated and untreated were subsequently exposed to inhibitors of prostaglandin synthesis[6,7], to indomethacin (1, 2.5, 5 or 10 μg/ml) or to 5,8,11,14-eicosatetrayonic acid (1, 2.5, 5 or 10 μg/ml) or ETA. Both these agents, especially ETA, produced initial increased active tension followed in high doses by decreased active tension. In doses of 5 or 10 μg/ml, indomethacin converted TTX-insensitive 'on' relaxations to 'on' contractions and decreased the 'on' relaxation to 0.5 ms pulses. 'Off' relaxations to 5 ms pulses were usually unaffected. In several instances, these inhibited responses were restored when active tension was elevated by 5 μg/ml of PGF_2. Effects of indomethacin were reversible. Eicosatetrayonic acid has similar effects (Figure 57.4) but these were irreversible and not affected by PGF_2. We concluded that prostaglandins might be involved in active tension production by the sphincter muscle and in the TTX-insensitive transmission of the stimulation of 5 ms pulses to the nerve varicosities from which inhibitory mediator was released. There could also be some role of prostaglandins in axon-mediated transmission to nerve endings, since larger doses of both inhibitors of prostaglandin synthesis abolished relaxation to 0.5 ms pulses.

Next we considered what possible transmission pathway could exist to nerve varicosities which was not TTX-sensitive and required longer duration pulses to be effective. Earlier we observed[8,9], that in the small intestine there were gap junction contacts between circular muscle cells and interstitial cells, which we called 'hybrid cells' because of their structural similarities both to smooth muscle and to glial cells. These cells were intimately apposed to nerves. Similar relationships were observed in the opossum oesophagus (Figure 57.5). Cells which usually contained numerous small mitochondria, few or no actin filaments, membrane caveolae, and much endoplasmic reticulum were found to be in gap junction contact with circular muscle cells within muscle bundles and to be intimately related to nerve varicosities and to glial cells. These cells should be depolarized during longer duration current pulses since these pulses are capable of depolarizing smooth muscle to which they are presumably coupled by a low resistance contact. Smooth muscle cells are normally not excited by 0.5 ms pulses because of their longer time constants (around 100 ms). Therefore, this structural arrangement could lead to depolarization of the 'interstitial' cells which are in gap junction contact with smooth muscle. If they are electrically excitable or if they respond

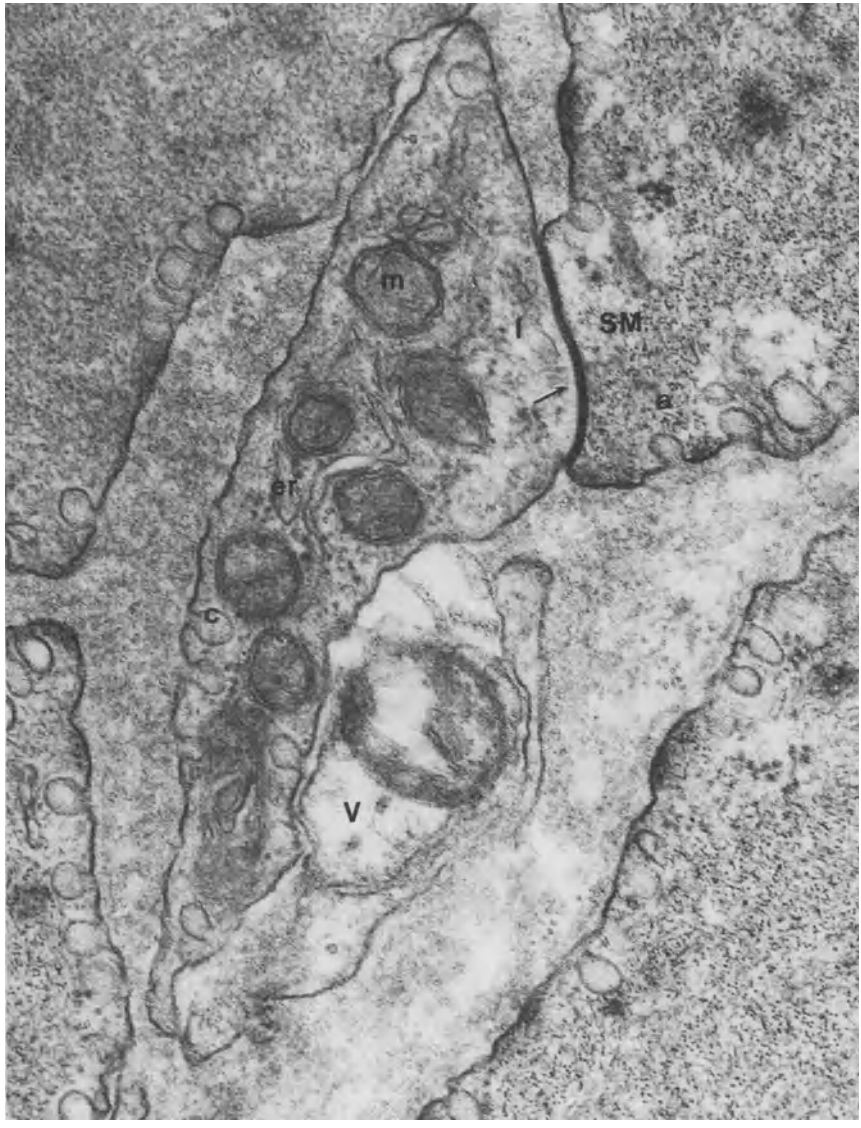

Figure 57.5 Electron micrograph of gap junction (arrow) between LES smooth muscle (SM) and 'fibroblast-like' 'interstitial' cell (I) containing small mitochondria (m), endoplasmic reticulum (er), cavaolae (c) but lacking actin filaments (a). Note its relation to nerve varicosity (V). (Approximately × 100 000)

to depolarization by release of prostaglandins, they might initiate release of mediator from nerve varicosities by electrical or chemical transmission. In the absence of TTX they might also be able to initiate axonal spikes. Figure 57.6 summarizes our hypothesis about the mode of TTX-insensitive release

of inhibitory mediator. A depolarizing applied pulse would depolarize circular smooth muscle if sufficiently long, and initiate spikes in it; this activity would be transmitted electrotonically to 'interstitial' cells. These would respond electrically or chemically in such a way as to release mediator from apposed nerve varicosities and excite axons.

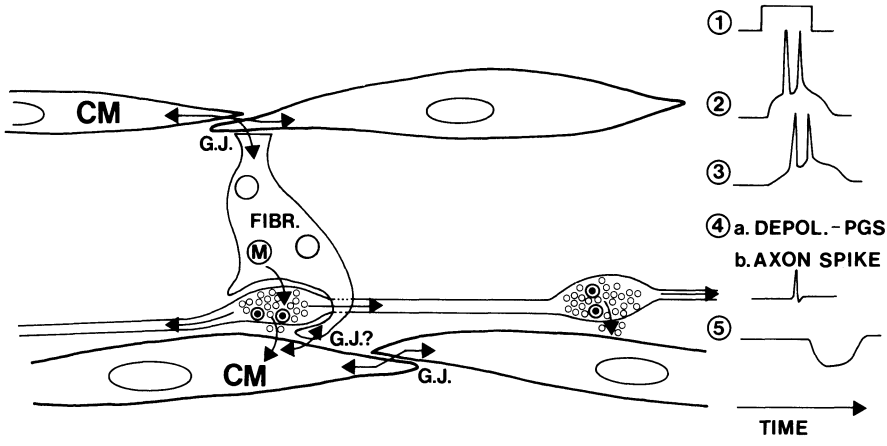

Figure 57.6 Diagram of hypothesis to explain TTX-insensitive relaxation from 5 ms pulse durations. *On left* oesophageal circular muscle (CM) in contact by way of gap junctions (G.J.) with fibroblast-like 'interstitial' cell (FIBR), which in turn is apposed to nerve varicosity with synaptic vesicles. When activated, this releases inhibitory mediator onto nearby circular muscle and may set up an axonal action potential. *On right*, the presumed electrical and/or chemical events associated with the structures at left which occur in response to a pulse of field stimulation.

DISCUSSION

These results clearly establish that long duration depolarizing pulses can initiate release of inhibitory mediator by a mechanism insensitive to TTX and involving prostaglandin synthesis. We have proposed speculatively that an ultrastructural arrangement involving gap junctions between circular muscle and an 'interstitial' fibroblast-like cell which is apposed to nerves may participate in this TTX-insensitive pathway muscle of the gut[8,9,10]. Testing this hypothesis may prove difficult.

Whatever its mechanism, the physiological role of this TTX-insensitive mechanism is intriguing. First, there is a high probability that it is operative whenever circular muscle contractions are initiated. The duration of smooth muscle action potentials should suffice to activate it. Thus, any local contraction of circular muscle might be limited by a concomitant release of inhibitory mediator. If axons as well as varicosities are excited (in the absence of TTX), then the inhibition could be transmitted distally, so that a circular muscle contraction would automatically excite distal inhibition irrespective of mechanoreceptors, and possibly not require transmission within myenteric

ganglia, at least for a short distance. In regions of the gut or other smooth muscle organs containing cholinergic neurons, this type of arrangement could account for TTX-insensitive atropine-sensitive excitation. We are aware of no such cases.

In the absence of direct evidence nothing other than convention so far requires us to assume that the inhibitory mediator is released directly from nerve varicosities rather than secondarily from other associated structures such as the 'interstitial' cell. Operation of a mechanism of release from this cell would require that short pulses release nerve mediator, possibly acetylcholine to act on the 'interstitial' cell to release inhibitory mediator. In addition, release by stimulation of muscle with longer pulses would involve electrical current spread into the 'interstitial' cell and spider venom would then eliminate relaxation by damaging this cell as well as nerves. We have discussed evidence for and against the general possibility of secondary release of inhibitory mediator elsewhere[11].

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Discussion

A. R. Cooke: (USA) Your model includes the release of a non-adrenergic non-cholinergic mediator from inhibitory nerves. The responses you obtained to exogenously added ATP were, however, variable. Do you have any comment, therefore, on the identity of the inhibitory mediator or any explanation for the variability of the responses to ATP?

E. E. Daniel: (Canada) Since responses to ATP did not always consist of relaxation when field stimulated released non-adrenergic inhibition mediator caused relaxation, I doubt ATP is the mediator. Furthermore, the amounts of ATP required to relax LES strips were very large. However, one can think up explanations for these paradoxes (rapid breakdown of exogenous ATP, action of exogenous ATP on other receptors than those on which nerve released ATP acts, etc); thus they do not rule out that ATP is mediator.

J. D. Wood: (USA) Regarding the presence of TTX-resistant action potentials in enteric ganglion cells: tetrodotoxin-resistant calcium spikes occur in the ganglion cell somas, but we have not observed TTX resistance in the processes of these cells.

Daniel: I appreciate this comment because it helps resolve the possibility of TTX-insensitive neurons as an explanation of some of our results.

A. Bennett: (UK) There is so much to comment on in your presentation that it is possible to pick on only a few points. You suggest that the prostaglandin is a modulator rather than a mediator. In that case you would expect that the response in the presence of indomethacin would be restored by the appropriate prostaglandin. This would probably not be prostaglandin E, which you used.

Daniel: You are certainly correct that the prostaglandin involved in release of non-adrenergic inhibitory mediator is not likely to be PGE, since very large amounts were required to affect these tissues. Also it could not overcome the effects of PG synthesis inhibitors. The same applies to PGE_2 and $PGF_{2\alpha}$, except that the latter could sometimes restore relaxations to 0.5 and 5 ms stimulation after indomethacin. Thus one of the unstable intermediates in the PG cascade is a more likely candidate.

58

Analysis of the electrical activity of the canine gastro-oesophageal sphincter in terms of overlapping pulse sequences *(Abstract)*

D. MAYNARD, P. A. THOMAS AND R. J. EARLAM

Basic electrical activity has been recorded from the canine gastro-oesophageal sphincter[1,2]. In the proximal half there are superimposed fast action potentials which occur with contractions, but in the distal part there is only a basic activity. Using EEG analysis expertise, sinusoidal activity was visible, so a detailed computerized analysis of basic electrical activity in the distal part of the sphincter was made using recently developed techniques[3–5]. If a sequence of pulses (for example those produced by membrane depolarizations) occurs in such a way that successive pulses overlap each other in time, then the summated wave-form can be approximated by a sinusoidal function. The sinusoid begins at OH_z at a phase of plus or minus 90°, rises to some upper frequency limit whilst attenuating in amplitude, and then returns to OH_z increasing in amplitude. The computerized analysis method used in this study detects such sinusoids[6]. When applied to the basic electrical activity of the canine gastro-oesophageal sphincter, recorded in a unipolar derivation, such sinusoids occur at regular intervals. If interpreted in terms of pulse sequences they indicated that several sequences are overlapping at any one moment, such that one begins before another ends. Under normal recording conditions DC terms are removed. However, the analysis technique can test the effect of re-inserting a DC term. This shows that, if the signal does consist of pulse sequences, then these have a specific polarity.

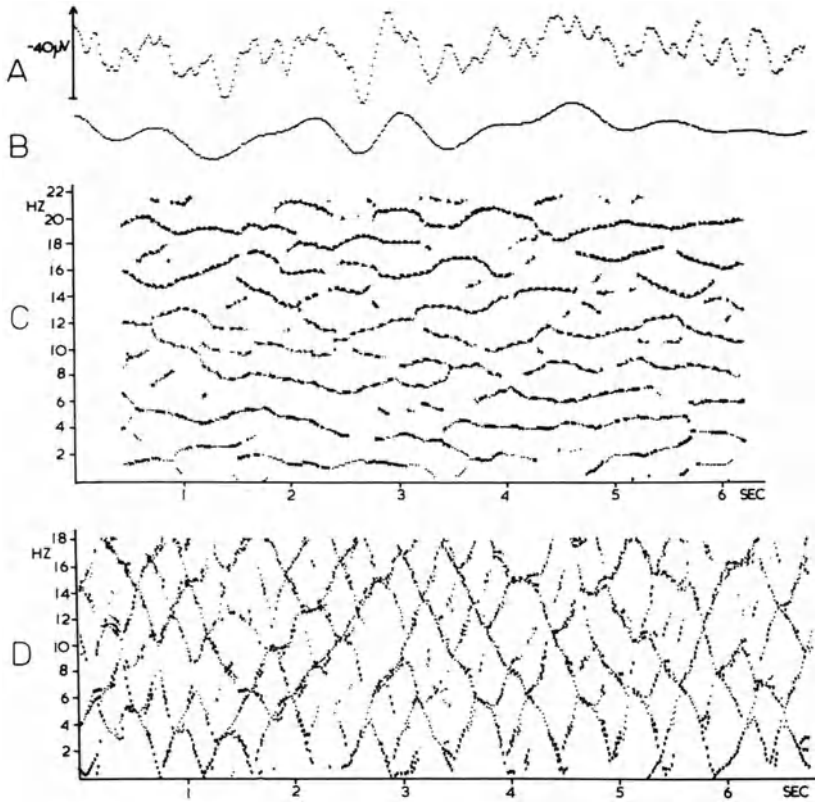

Figure 58.1 (A) A sample of gastro-oesophageal sphincteric activity. (B) The low frequency content of (A). (C) Analysis of (A) in terms of narrow band sinusoids. (D) Analysis of (A) in terms of rapidly changing frequency sinusoids. The low frequency content shown in (B) has been removed before analysis because it has different characteristics. A negative DC level has been added and tracks of sinusoids below 1.5 Hz are interpolations. The waves appear to move positively from a negative baseline

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Discussion

J. Gonella: (France) Did you try to do any nerve stimulations to modify the basal activity?

R. Earlam: (UK) No. Our policy has been to try and find what basic frequencies exist before putting in any stimuli; but obviously this should eventually be done.

C. E. Pope: (USA) What happens to the analysis at the sphincter signal when pharmacological agents (atropine, black widow spider venom) are added to the sphincter?

Earlam: We have only analysed the effect of gastrin and glucagon. Glucagon does not alter the baseline but gastrin increases the high frequency content.

D. Linkens: (UK) There is a major difference between the method of Fourier analysis and the method proposed in this paper. Fourier analysis has a mathematical foundation which shows that any periodic data can be uniquely described as the summation of a number of sinusoidal components. In the method of summation of components with varying frequency and amplitude no such foundation exists, and the problem of non-uniqueness is formidable.

59

The myogenic control of oesophageal peristalsis and LES relaxation and interaction between myogenic and neural control systems *(Abstract)*

S. K. SARNA, E. E. DANIEL AND W. E. WATERFALL

The smooth muscle portion of oesophagus of anaesthetized opossum showed bidirectionally propagated contractions (recorded by extraluminal strain gauges) in response to direct muscle stimulation (20–40 V, 100–400 ms). In addition, when this propagated contraction arrived at the lower oesophageal sphincter (commonly abbreviated LES), it relaxed. The relaxation was sometimes accompanied by either a superimposed, but shorter-lasting, contraction or an after-contraction. Neither the bidirectionally propagated contractions nor the LES relaxation and contraction were blocked by atropine (100 μg/kg intravenously), hexamethonium (10 mg/kg intravenously) or tetrodotoxin (TTX; 20–40 μg intra-arterially; perfusing only the distal 3–5 cm of oesophagus), whereas 'off-responses' to balloon stimulus and vagal stimulation were blocked by TTX. Propagated contractions due to induced swallows, and the accompanying LES relaxation, were also not blocked by any of the above drugs, which showed that the myogenic control system in the smooth muscle oesophagus is independently capable of producing oesophageal peristalsis, and that it can excite the LES to produce relaxation. The myogenic control of peristalsis can, however, be modulated by the extrinsic and the intrinsic nerves. Balloon inflation (10–15 cc) or vagal stimulation (10–25 Hz, 0.5–5 ms, 20–40 V) can inhibit the initiation and/or propagation of direct muscle stimulation responses. Likewise, distal balloon inflation can also inhibit proximal balloon 'off-responses', but not vice versa. The muscle response was not refractory to the previous occurrence of either the balloon or the vagal 'off-responses', in the sense that it could be superimposed on these

responses. Balloon inflation inhibited vagal 'off-response' while vagal stimulation inhibited balloon 'off-response'. The myogenic control system in the oesophagus may operate by producing a smooth muscle depolarization whose propagation is a muscular phenomenon and can be inhibited by hyperpolarization of the muscles, but not by depolarizations from 'off-responses'. The propagation of depolarization may behave like a chain of bidirectionally coupled 'one-shot' oscillators. The mechanism of this depolarization in producing LES relaxation is not clear, but does not require nerve action potentials.

Acknowledgement

This work was supported by the Medical Research Council of Canada.

Discussion

H. S. Ormsbee: (USA) Is it possible that the presence of the two LES relaxations which you observed is affected by fundic muscle contractions which mask one continuous LES relaxation?

S. K. Sarna: (Canada) We implanted our fourth strain gauge on the fundus to compare LES and fundic responses. There was no correspondence between the LES and fundic responses.

J. Christensen: (USA) If the strain gauge on the LES is not precisely perpendicular to the longitudinal muscle layer, it might be extended by longitudinal muscle contraction and so cause an apparent relaxation. Can you reassure us that this did not occur?

Sarna: Of course, we cannot be sure that the strain gauge will always be exactly perpendicular to the longitudinal muscle layers, but the deviation from it should not exceed a few degrees. In such a case the component of force of longitudinal muscle contraction along the axis of strain gauge would be negligible.

J. D. Wood: (USA) I would suggest caution regarding your statement of assumption that the enteric neurons are non-functional 20 min after circulatory stasis in your animals. We can demonstrate by direct recording from the neurons that the neurons are highly resistant to hypoxia. Resting potentials, input resistance and spike discharge are unaffected after 2.75 h at pO_2 of 4 mmHg.

Sarna: That may be true, but extrinsic neurons seem to be non-functional since responses to vagal stimulation were absent and intrinsic neurons seem to be non-functional since responses to balloon stimulation were absent. Also, in many cases, there had been administration of TTX, atropine and hexamethonium prior to death. So I do not think they were contributing to responses at that time – unless by mechanisms other than propagation of axonal action potentials.

Section XII
Lower Oesophageal Sphincter Control

60

Comparison of mechanical characteristics of the lower oesophageal sphincter and pyloric sphincter *(Abstract)*

P. BIANCANI, M. P. ZABINSKI, M. D. KERSTEIN AND J. BEHAR

Adult cats of either sex were anaesthetized with ketamine hydrochloride and the gastro-oesophageal and gastroduodenal junctions exposed. The *in vivo* lower oesophageal sphincter (usually abbreviated as LES) and pyloric sphincter (PS) were identified with pressure-measuring probes having a perfused side opening adjacent to a metal plug obstructing the distal tip. With the catheter's side opening located at the point of high pressure a pin was placed on the outer surface under fluoroscopy to coincide with the proximal end of the metal plug. In this manner, the precise location of the side opening was identified on the outer surface and a suture was used to mark the location of the LES and PS high pressure points. The oesophagus, stomach, and duodenum were removed together and pinned on a wax block and stretched to their respective *in vivo* lengths. Consecutive rings were cut from the gastro-oesophageal and pyloric regions with blades held in a block, 1.7 mm apart. The rings were suspended between two platinum hooks in a muscle bath. The lower hook was rigidly attached to the bottom of the muscle bath. The upper hook was attached to a force transducer (UC-2 cell Statham Instruments) which was mounted on a micrometer stage (Edmund Scientific Co.). The stretch applied to the rings was measured by the distance between the hooks. After mounting, the rings were allowed to equilibrate for 30 min in Tyrode solution at 37 °C.

After the initial period of equilibration in Tyrode solution, the forces developed by the rings were recorded (basal force). This solution was then replaced by Tyrode solution to which potassium chloride (140 mM) had been added. This solution depolarized the muscle causing a sustained contraction

(total force). After 15 min in KCl solution, it was replaced by calcium-free Tyrode solution containing 5 mM EDTA which binds extracellular free calcium causing the muscle to relax (passive force). The force developed by contraction of the muscle (active force) is the difference between total and passive forces. This sequence was repeated at various lengths of stretch to obtain force–length curves.

With increasing levels of stretch, the active force developed by each ring increased at first, peaked at the length of maximum active force (MAF) development, and subsequently decreased.

The LES ring identified by the suture exhibits the steepest basal force–length curve. This ring also exhibits the highest active and total force–length curves with potassium chloride stimulation; but its passive force–length curve does not differ from those of adjacent oesophageal rings.

The MAF for consecutive rings from the gastro-oesophageal junction are compared in Figure 60.1. The passive forces are shown at specific length of stretch (28 mm) which corresponds to the length of MAF development for the LES ring. At MAF, the strip corresponding to the LES high pressure point exhibits the MAF. Its passive force is the same as for the adjacent rings.

The PS ring, identified by the suture, exhibits the highest passive, basal, and total forces at all levels of stretch when compared with adjacent rings. The length of MAF is lower in the pylorus than in the antrum and duodenum.

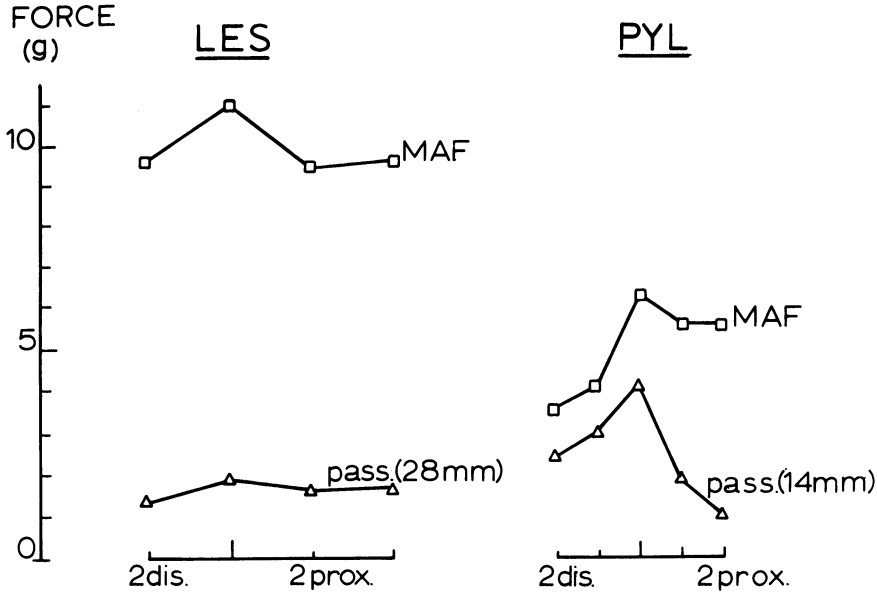

Figure 60.1 The left panel shows the maximum active force (MAF) developed by adjacent rings from the LES region. The passive forces at 28 mm stretch are shown by the lower curve. The right panel shows the MAF developed by adjacent rings from the pyloric region. The passive forces at 14 mm stretch are shown by the lower curve.

The MAF of the PS ring is higher than the MAF of the duodenal rings, but not significantly higher than the antral rings (Figure 60.1). The passive force at a specific length of stretch (14 mm) which corresponds to the MAF development for the pyloric ring is also shown in Figure 60.1, and is highest for the pyloric ring.

It is concluded that in the LES region no differences in passive force are observed and the high total force observed in the LES ring is due to a higher active force than that of adjacent rings. In contrast, the higher total force of the pyloric ring is due to higher passive force which may be caused by the presence of a stricture rather than to higher active forces.

Acknowledgement

This research was supported by NIH Grant RO1 AM 16021.

Discussion

J. H. Szurszewski (USA): (1) What is the difference between basal tension and passive tension? (2) Would it not have been better to express your data as grams-force/gram tissue?

P. Biancani (USA): (1) None in some striated muscles. In the LES and pyloric regions I have called basal forces those recorded in Tyrode solution and have called passive forces those recorded in EDTA, in the assumption that EDTA relaxes the muscles and abolishes all active components. (2) Stress–strain relationships of these tissues are being worked up.

A. G. Johnson (UK): What point did you take as the pyloric ring? How long was the high-pressure area at the pylorus?

Biancani To average data in such a way that rings from the same location may be averaged for different animals, we took rings with the highest length–tension curve in Tyrode solution, called them pyloric rings and averaged them. Other rings were averaged according to the distance from the pyloric ring so taken. Anuras *et al.* have shown that in the pyloric region there is a strip that in Tyrode solution exhibits a higher length–tension curve than all the others, and that this strip relaxes upon electrical stimulation. In our study such a strip proved to be either the one identified by the suture as the high-pressure point, or the one immediately proximal to it, both for the pylorus and the LES. No attempt was made to measure the length of the pylorus in this study. The length of the LES high-pressure zone was measured (13–14 mm). The length of the cat pyloric high-pressure zone was estimated in Chapter 52. It is approximately 2 mm.

S. Cohen (USA): I am concerned that your passive tension differs from what other investigators call passive tension. You have artificially abolished myogenic function with EDTA. How much stretch is present at L_0?

Biancani: To record passive tension muscle activity should be abolished. In original work in striated muscles 'passive' and 'basal' tension coincide, since the striated frog sartorius exhibits no active component unless electrically stimulated. In tonic smooth muscles, such as the LES, some tonus is present when the muscle is kept in Tyrode (basal state). This tonus is at least in part abolished when, upon electrical stimulation, the muscle relaxes. However, there is no way to determine how much of the force measured in Tyrode solution is due to tonic contraction of the muscle, and how much is due to passive resistance to stretch exerted by the fibrous element present. Therefore the muscle should be relaxed as fully as possible by abolishing all active contractile components in order to determine the passive components. To do so we have used EDTA, which inhibits muscle contraction without, hopefully, affecting collagen fibres and other passive elements present. Maximum active force is developed by the LES at a stretch of approximately 1.4 times the *in vivo* length.

E. E. Daniel: (Canada) Your results do not suggest that the pyloric terminal region, which you call a sphincter, has a higher active tension *in vivo*, but that the chief difference between it and adjacent antral muscle is a greater passive tension to a given length owing to a narrower diameter. I have always believed that a 'sphincter' should have, as part of its definition, the initiation of active tension 'at rest' which closed the lumen. Do you feel that this definition can be applied to the gastroduodenal junction? If not, do you feel that we should change the definition of a sphincter?

Biancani: Your comment is correct in that we have not shown relaxation in this particular work and that the ultimate strength, that is the maximum active force of the pyloric ring, is not much different from the antrum. However, Chapter 52 shows the presence in this region of an inhibitory innervation. These data are in agreement with previous work by Anuras *et al.* in the same species. Moreover, a high-pressure zone seems to exist *in vivo*, in this region, at all diameters tested. So it would seem that a 'pyloric sphincter' exists although its musculature is a fairly weak one. Whether we should call it a sphincter or something else I really cannot say. I call it a sphincter mostly for convenience since its functional properties (presence of a pressure barrier and relaxation) seem to be like those of other structures that are called sphincters, for instance the LES.

J. Behar: (USA) In response to Dr Daniel's comments, I think we should not impose our biases on structures like sphincters that we do not fully understand. What is important about the gastroduodenal junction is that it behaves differently from those of the antrum and duodenum.

J. Christensen: (USA) I think we should give up the term 'sphincter' and call these regions the oesophagogastric and gastroduodenal junctions. I have been training myself to do so, not entirely successfully!

61

The role of the duodenum in the regulation of lower oesophageal sphincter pressure (LESP) in the dog

G. LEPSIEN, H. R. KOELZ, H. F. WEISER, A. L. BLUM AND R. SIEWERT

The ingestion of a meat meal causes an increase of lower oesophageal sphincter pressure (usually abbreviated as LESP)[1]. The mechanism of this rise is unknown. In a previous study it has been shown that in the dog the antrum does not mediate the postprandial rise of LESP[2]. In the present study the role of the small bowel is investigated. It is shown that intestinal contents play an important role in the postprandial rise of LESP.

METHODS

Experiments were performed in six mongrel dogs weighing 15–20 kg.

Surgical procedure

Surgical procedures were performed under general anaesthesia. The dogs were equipped with a Komarov type oesophagostomy. A partial proximal gastric vagotomy was performed. Sphincter region, gastric fundus and antrum were not denervated. The duodenum was separated from the pylorus and from the proximal jejunum. A 10 cm segment of proximal jejunum was also isolated. The proximal end of the duodenum was anastomosed isoperistaltically end-to-end with the jejunal segment which in turn was led out through the abdominal wall in order to form a mucocutaneous fistula. The distal end of the duodenum was anastomosed Roux-en-Y with the mid-jejunum. The pylorus was anastomosed end-to-end with the free end of the proximal jejunum; thus food instilled into the mucocutaneous fistula traversed the duodenum and

the lower jejunum without entering the stomach, while food instilled into the stomach through the Komarov fistula entered the jejunum directly without traversing the duodenum. Experiments were started 4 weeks after the last operation.

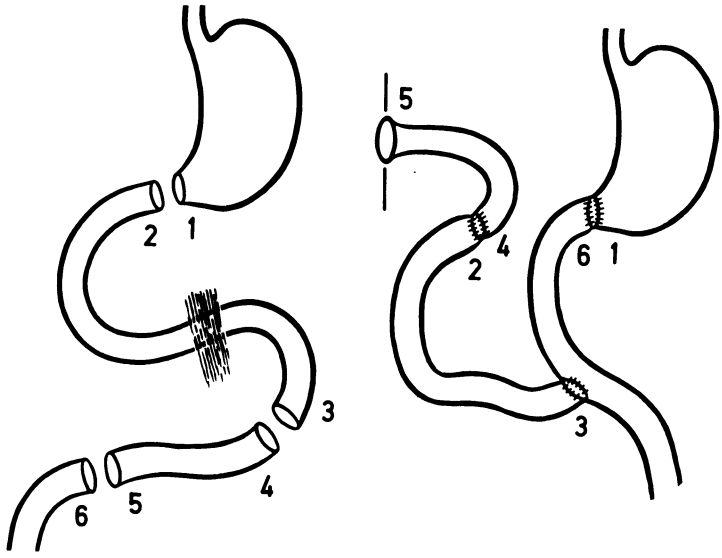

Figure 61.1 Surgical preparation; proximal gastric vagotomy and Komarov type oesophagostomy are not shown

Manometric technique

LESP was measured in unrestrained, trained dogs using an 'ultrarapid pull-through technique' as described in a previous publication[2]. A perfused (3 ml/min) polyvinyl catheter with an outer diameter of 4 mm, equipped with four lateral recording orifices at the same level, was inserted via the Komarov oesophagostomy into the stomach. Every 15 s, a 6 cm withdrawal and pushback manoeuvre was performed, each yielding a profile of LESP) In order to record oesophageal peristalsis, a second perfused (0.5 ml/min) polyvinyl tube with an outer diameter of 2 mm was glued to the main tube with its recording orifice lying 4 cm proximal to the orifices of the main catheter. The recording unit consisted of Statham transducers (model P 23 dB) and a Hellige recorder. For calculations, all pressure profiles were taken except those which coincided with a swallow and those which occurred 15 s prior, during or 15 s after a fundic pressure wave.

Duodenal instillation

Immediately before the instillation, a urinary catheter (Folatex balloon

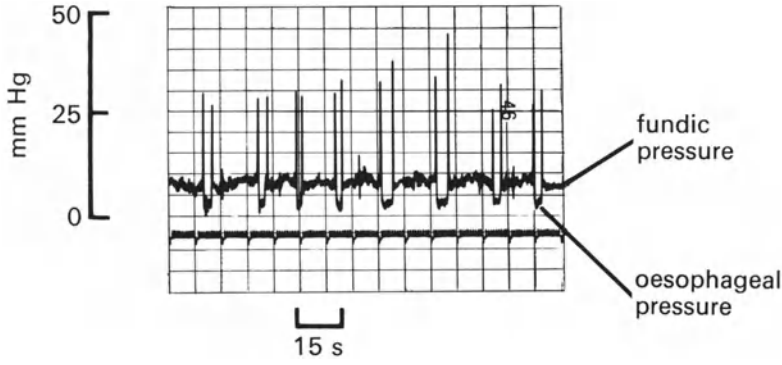

Figure 61.2 Typical profiles of LESP obtained by rapid push–pull technique in a dog

catheter, Ch. 16, Eschmann, England) was inserted into the proximal duodenum via the mucocutaneous fistula. The catheter was connected to a Harvard infusion pump (model H 975). During 10 min, 100 ml of 20% peptone solution (Liebig AG, Basle, Switzerland; 1.6 osmol, pH 5.7, Na 203 mM, K 350 mM, Cl 135 mM) were instilled. Then the catheter was removed.

Gastric instillation

A polyvinyl catheter with an outer diameter of 5 mm was inserted into the stomach via the oesophagostomy. Instillation of 100 ml peptone solution or 100 ml NaCl was performed as in duodenal instillation experiments.

Determination of serum gastrin

For aspiration of blood samples, a 21-gauge butterfly needle was inserted into a leg vein and kept open by heparin solution (Liquemin, Roche, 10 U/ml). Serum gastrin was determined radioimmunologically[3].

For balloon dilation in the duodenum, a catheter with a large-bore latex balloon was introduced into the duodenum under fluoroscopic control. The balloon was placed in the mid-position of the duodenum. In some experiments the inner surface of the balloon was coated with urografin solution. The position of the unit was controlled by image intensifier and fluoroscopy. The catheter was connected to a barostat and pressure was increased stepwise up to a maximal pressure of 30 cmH_2O.

Calculations

When not specified otherwise, each series of experiments was performed three times in each dog. For calculation of LESP, one mean value per dog was

used. This was calculated by taking the mean of the three corresponding 5 min periods, each consisting of forty pressure profiles. Statistical significance was evaluated by the Student's *t* test.

RESULTS

Figure 61.3 shows LESP changes before and after perfusion of 100 ml of 20% peptone solution or of 0.9 NaCl solution into the duodenum with peptone, LESP rises from 20.1 ± 1.7 (SE) mmHg to a maximum of 41.0 ± 2.1 (SE). The rise starts 15–20 min following completion of the peptone perfusion and lasts for at least 30 min. Perfusion of saline solution does not raise LESP. Figure 61.4 shows LESP after instillation of a bolus of 100 ml of 20% peptone solution into the stomach. LESP rises from a mean basal pressure of 18.0 ± 1.4 (SE) to 28.4 ± 2.3 (SE) mmHg. The rise of LESP following intraduodenal perfusion is greater than following intragastric instillation at 60, 65, 70, 75, 80, 85 and 90 min respectively (p from <0.05 to <0.001).

Figure 61.5 shows the effect of balloon dilation in the duodenum on LESP. With pressures in the balloon up to 30 cmH_2O, LESP did not change. Fluoroscopic examination did not reveal duodenal dilatation during balloon inflation.

Figures 61.6 and 61.7 show the serum gastrin levels. During duodenal perfusion of peptone, serum gastrin rises from 54.2 ± 7.6 (SE) pg/ml to

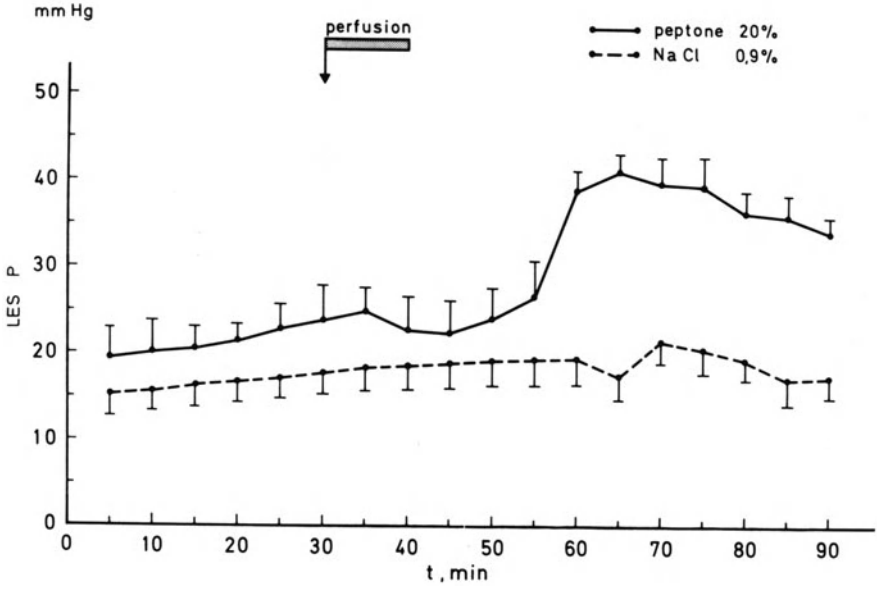

Figure 61.3 LESP before and after perfusion of 100 ml of 20% peptone solution or 0.9% NaCl solution into the duodenum (X ± SE, three experiments in each of six dogs). The differences are significant at 40, 50, 60 and 70 min ($p < 0.001$), 80 and 90 min ($p < 0.0001$)

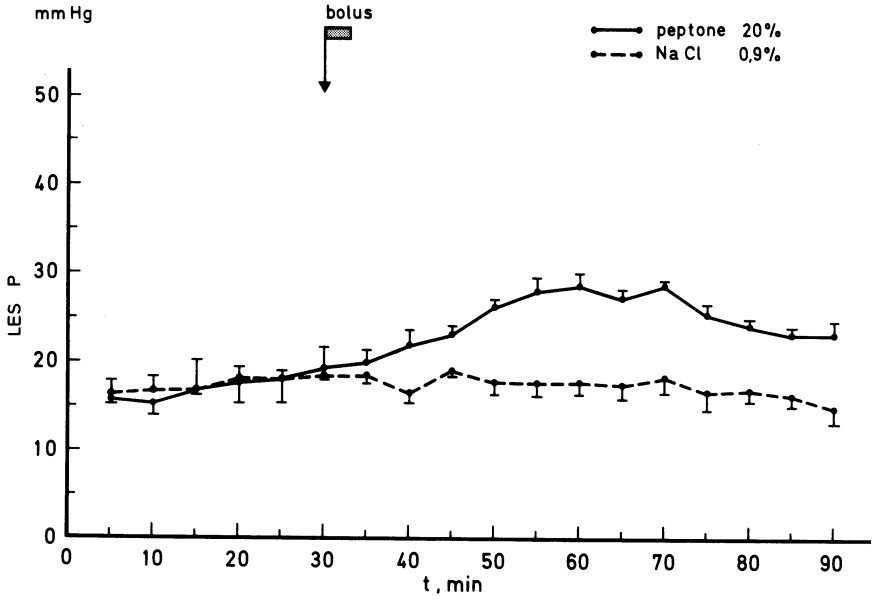

Figure 61.4 LESP before and after bolus application of 100 ml of 20% peptone solution or 0.9% NaCl solution into the stomach ($X \pm$ SE, three experiments in each of four dogs). The differences are significant at 60 min ($p < 0.05$), 70 min ($p < 0.01$), 80 min ($p < 0.002$) and 90 min ($p < 0.001$)

81.4 $\pm$ 30.0 (SE) pg/ml ($p > 0.05$). Following bolus application of peptone into the stomach the serum gastrin level rises from 79.3 $\pm$ 19.7 (SE) pg/ml to 193.2 $\pm$ 28.5 (SE) pg/ml after 60 min ($p < 0.01$). Saline solution by either route does not affect IRG levels.

Figures 61.8 and 61.9 show intraluminal pH values. In the duodenum perfusion of 100 ml of 20% peptone solution lowers pH from 7.3 $\pm$ 0.2 (SD) to 5.3 $\pm$ 0.4 (SD) within 5 min. During this acidification LESP remains unchanged. While pH returns to 7, LESP starts to rise. Instillation of peptone into the stomach raises intragastric pH from 2.0 $\pm$ 0.4 (SD) to 4.5 $\pm$ 0.9 (SD). This effect begins within 5 min of the bolus administration and lasts for 20 min. LESP starts to rise during this period.

DISCUSSION

Peptone traversing the canine duodenum raises LESP more than peptone traversing the stomach. The long-lasting and pronounced rise of LESP following duodenal perfusion does not bear a temporal relationship with the short and small acidification of the duodenum by peptone and is not due to dilation of the duodenum. It is unlikely that infusion of peptone into the duodenum produces a pressure increase within the duodenum. Furthermore

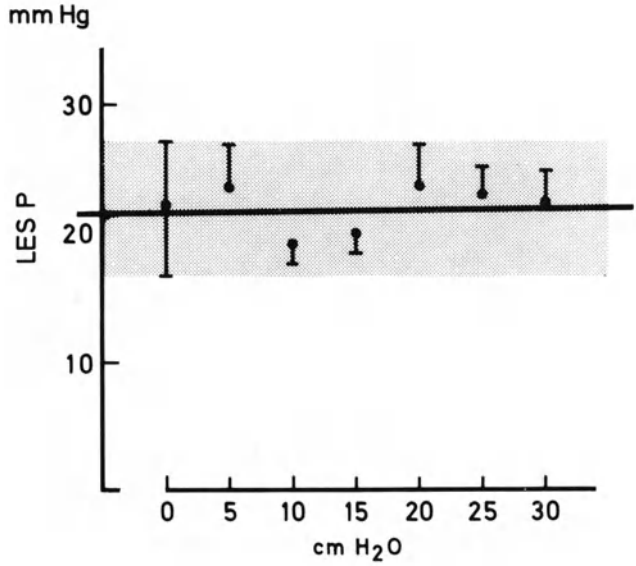

Figure 61.5 LESP before and during stepwise balloon dilation of the duodenum. Each pressure was maintained for 15 min. The x axis shows balloon pressure in cmH_2O. The shaded area represents basal pressure ($X \pm$ SE, one series of dilations in each of four dogs)

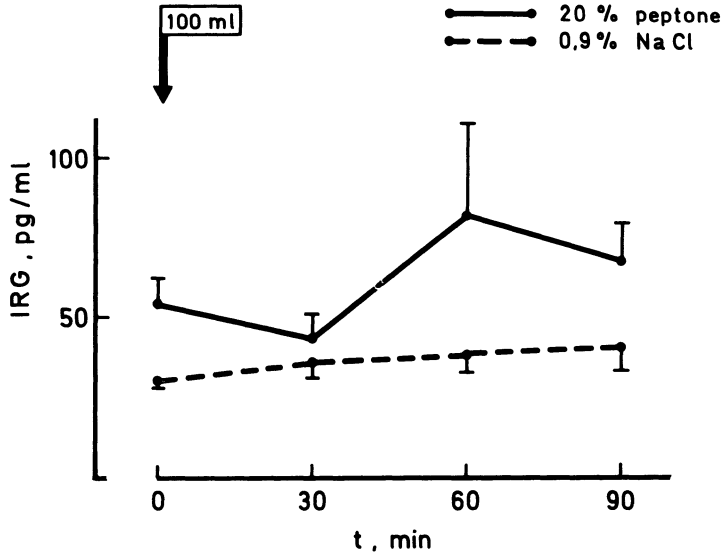

Figure 61.6 Serum gastrin (IRG) levels ($X \pm$ SE) before and after duodenal perfusion of peptone and NaCl. Experiments were performed as outlined in Figure 61.3. The differences between peptone and NaCl application are statistically not significant

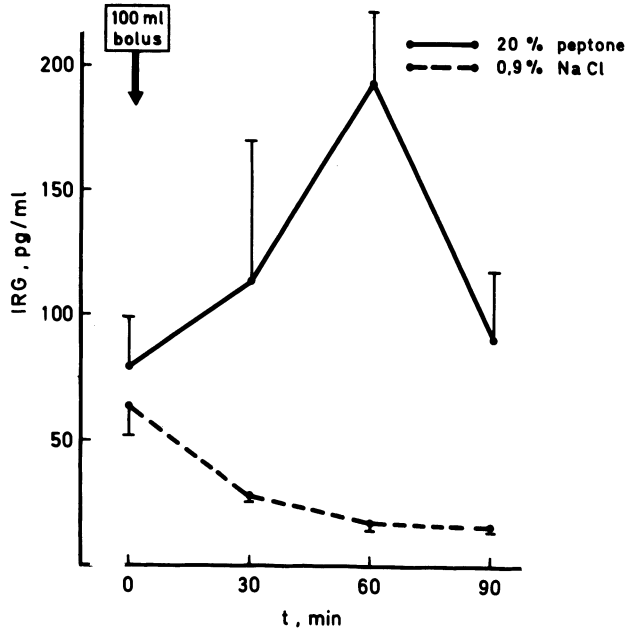

Figure 61.7 Serum gastrin (IRG) levels ($X \pm$ SE) before and after bolus application of peptone solution or NaCl solution into the stomach. An arrow indicates a bolus injection of 100 ml into the stomach. Experiments were performed as outlined in Figure 61.4. The differences between peptone and NaCl applications are significant at 60 min ($p < 0.05$) and 90 min ($p < 0.025$)

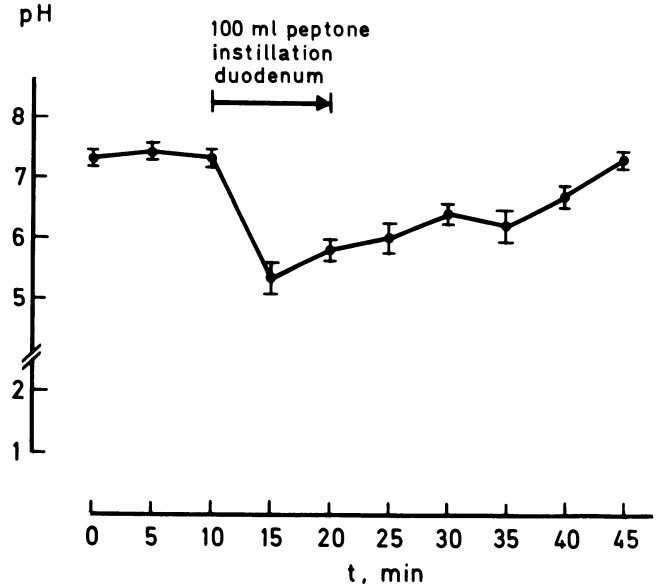

Figure 61.8 Intraduodenal pH-values before and after peptone perfusion ($X \pm$ SD). Experiments were performed as outlined in Figure 61.3

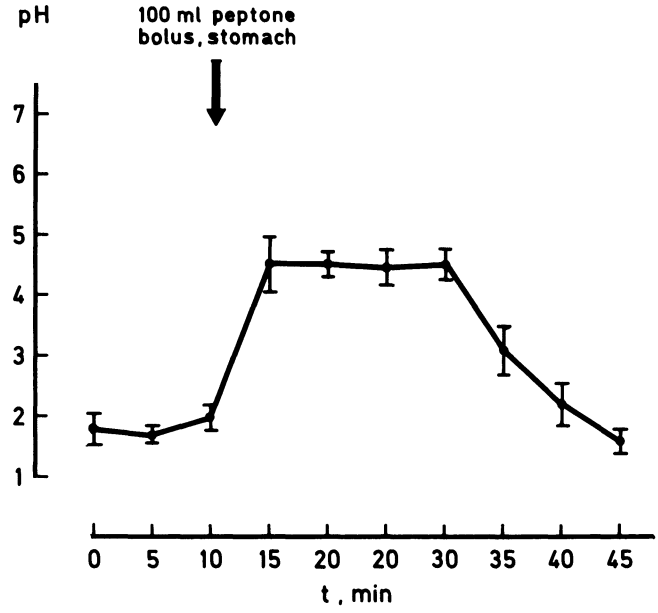

Figure 61.9 Intragastric pH-values before and after bolus application of 100 ml of peptone into the stomach ($X \pm$ SD). Experiments were performed as outlined in Figure 61.4

saline infusion into the duodenum does not raise LESP, and dilatation of the duodenum with a balloon has no effect on LESP either. A direct mechanical effect of peptone solution on the duodenal wall can therefore be excluded.

Since in a previous study the peptone-stimulated antrum had no effect on LESP, and since in the present study the gastric corpus has been vagally denervated, a role of the stomach in the postprandial rise of LESP is unlikely. The jejunum and the more distal part of the intestine might raise LESP but their effect is smaller than the effect of the duodenum since bypassing of the duodenum by gastric instillation of peptone is shown to lead to a much smaller rise in LESP than duodenal perfusion.

The mechanism by which the duodenum transmits its signal to the LES is at present unknown. Both hormonal and neural effects are possible. The time interval of 15–20 min between the completion of duodenal perfusion and the rise of LESP speaks rather in favour of hormones. Since serum G-17 gastrin levels show only a minor and statistically non-significant rise during duodenal perfusion, and since a much more marked rise of serum gastrin during peptone perfusion of the stomach is not associated with a rise of LESP, G-17 gastrin is not likely to be the mediator of duodenal stimulation by peptone.

It is likely that the human LES is regulated by mechanisms similar to the sphincter of the dog. Patients with a Billroth I stomach resection (gastro-duodenostomy) show a postprandial increase of LESP which is similar to the

postprandial increase of healthy controls. Patients with a Billroth II stomach resection (gastrojejunostomy) do not show such an increase of postprandial LESP[4].

Acknowledgement

This work was supported by Swiss National Foundation, grant Nr 3.298.074.

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Discussion

W. Silber: (S. Africa) I have listened to the fascinating papers on the oesophagogastric junction and am leaving Edinburgh most confused on this subject. Twenty-five years ago when we postulated that the LES was the most important factor in LES competence it was ridiculed. Today we are having the LES of the dog, cat, opossum, etc. investigated with pharmacological doses of as many hormones and drugs as are coming onto the market. What is happening in man? Can one imagine that the human LES is being stimulated and inhibited continuously by these substances?

G. Lepsien: (Germany) In the present study the stimulus is a meal and not a pharmacological dose of an exogenously administered hormone. The teleological meaning of the increase of LESP in response to a meal has not been examined in our study. It is likely that continuous postprandial stimulation of LESP prevents the regurgitation of chyme.

A. Bennett: (UK) First of all, you measured G-17, so you cannot exclude an action of G-34 on the LES. Second, there was a trend for G-17 to increase in the blood following duodenal administration of peptone. You do not give the probability value, and are prepared to disregard these data. Probability values greater than 0.05 do not mean that effects do not occur.

Lepsien: We agree with this comment. We have not completely excluded the role of gastrin, but we think that a major role is unlikely because a marked increase of serum gastrin during gastric instillation precedes a minor increase of LESP, while a questionable increase of gastrin during duodenal perfusion is followed by a major increase of LESP.

R. W. McCallum: (USA) I would like to congratulate you on your study. We came to a similar conclusion studying the effect of alkali on LESP. We found that in normal subjects and Billroth I patients alkali ingestion resulted in an increase in sphincter pressure, but not in Billroth II patients. Hence I think the duodenum is playing a role in influencing LES and that gastrin in these situations is not playing any role.

Lepsien: We do not think that a role of gastrin has been completely excluded in our experiments.

D. L. Wingate: (UK) This is an elegant study, but the fact that a chemical stimulus is specific does not rule out humoral factors: peptide release is controlled by specific chemical factors. The effect could be due to motilin: the dog is not a good model to answer this question because (a) the factors causing motilin release in the dog are not clear, and (b) motilin is difficult to assay in the dog. Did you try to measure motilin?

Lepsien: Motilin has not be assayed in our experiments.

D. O. Castell: (USA) I would like to congratulate the investigators for designing a model to study effects which many of us have been interested in for years. We have been well aware that administration of substances into the

stomach may well evoke very definite responses from the duodenum. I think, however, that these studies do not eliminate gastrin as accounting for a portion of the LES response to protein, but rather indicate that gastrin is not the sole contributor to this response. This conclusion is supported by the manuscript of Bybee *et al.* using Somatostatin, reported elsewhere in this volume (Chapter 64).

Lepsien: We agree with this comment.

62

Clinical and experimental studies on the effect of nifedipine on smooth muscle of the oesophagus and LES

H. F. WEISER, G. LEPSIEN, K. GOLENHOFEN AND R. SIEWERT

In *in vitro* experiments on isolated circular strips of gastrointestinal smooth musculature it has been possible, through Ca^{2+}-antagonists such as nifedipine and sodium nitroprusside to obtain a selective blockade of Ca^{2+}-activation system and, in this way, a decrease in muscle tone[1-5]. The present study aims to test whether the Ca^{2+}-antagonist nifedipine, used up to now as a coronary drug, has a relaxing effect on the smooth muscle of the tubular oesophagus and the LES, and whether the therapeutic use of nifedipine is possible in the treatment of spastic or hypotonic motility disorders of the oesophagus and the lower oesophageal sphincter (usually abbreviated as LES).

METHODS

The strength development of circular muscle strips in the LES of twelve mongrel dogs was recorded before and after the application of nifedipine in a conventional organ bath by means of mechano-electric transducers. Nervous effects were eliminated by 10^{-6} mol/l tetrodotoxin (TTX). In each case the preparation was 20 mm in length, the strip section 1.5 mm^2 and the muscle tone 15 mN (9.8 mN = 1 P).

The effect of 20 mg of nifedipine was examined *in vivo* on the LES of eight mongrel dogs with Kamarov oesophagostomy over a period of 60 min. Pressure recording was carried out on unanaesthetized, trained and unsedated animals using a modified rapid pull-through method, the so-called twin manometry. A continuously perfused catheter was withdrawn and reinserted four times per minute manually through the LES pressure zone.

Calculations were made every 5 min, based on forty individual values per

unit time[6]. Twenty minutes after the application of nifedipine an additional dose of 3 μg/kg bodyweight pentagastrin was administered intravenously and the sphincter pressures recorded over a further 40 min. The same eight dogs served as controls.

In order to find out the optimum effect of nifedipine on humans, the drug was administered orally to six healthy volunteers (four female, two male), average age 24.8 years, using in each case four resting pressures of 10, 20 and 30 mg in random order. The pressure recording was carried out using the rapid pull-through manometry, described by Waldeck, in intervals of 5 min over a total period of 60 min[7].

In order to check the possible therapeutic effect of nifedipine, the LES resting pressure and distal oesophageal motility was measured in twelve patients suffering from achalasia who had already been confirmed by X-ray and three-point manometry, and also in three patients suffering from manometrically confirmed diffuse oesophageal spasm. The LES pressures in the achalasia patients were also recorded by rapid pull-through manometry. Next four LES resting pressures were taken from each patient. Following oral administration of 20 mg of nifedipine the LES pressure variations were then recorded over a further 120 min at 5 min intervals. The examination of the patients suffering from diffuse oesophageal spasm was carried out by three-point manometry. Amplitude, frequency and contraction pattern were continuously recorded 15 min before and also 60 min after administration of 20 mg of nifedipine.

RESULTS

Figure 62.1 shows the effect of 10^{-6} mol/l nifedipine on the spontaneous and also the acetylcholine (ACh)-induced muscle activity of circular muscle strips from the LES. The twelve experiments showed on average that 10^{-6} mol/l nifedipine produced a significant decrease in spontaneous muscle activity from 0.95 ± 0.7 mN/mm^2 to 0.04 ± 0.03 mN/mm^2 ($p < 0.05$) and also a maximal lowering of muscle activity stimulated by 10^{-7}g/ml ACh from 51.9 ± 9.7 mN/mm^2 to 4.9 ± 0.9 mN/mm^2 ($p < 0.001$).

Figure 62.2 shows the effect of 20 mg of nifedipine on the LES resting pressure of eight mongrel dogs, and also the LES pressure after the additional intravenous dose of 3.0 μg/kg body weight pentagastrin. The application of nifedipine led after 25 min to a significant decrease in pressure from 18.5 ± 1.8 mmHg to a minimum of 8.2 ± 0.9 mmHg ($p < 0.001$). A comparison with the control group shows that the decrease in pressure induced by nifedipine cannot be reversed by pentagastrin stimulation.

The quantitative effect of nifedipine on the LES resting pressure of six healthy volunteers is shown in Figure 62.3. Oral administration of 10, 20 and 30 mg of nifedipine leads to a decrease of the mean LES resting pressure, depending on the size of the dose from 26.8 ± 3.8 mmHg to 7.2 ± 1.9 mmHg.

Figure 62.4 shows the time-related effect of 20 mg of nifedipine on the LES resting pressure of six healthy subjects. After oral administration of nifedipine a significant decrease takes place within 15 min of the mean resting pressure from 30.0 $\pm$ 5.4 mmHg to 8.3 $\pm$ 1.1 mmHg ($p < 0.01$) and lasts for the whole experimental period of 60 min.

Figure 62.5 shows the effect of 20 mg of nifedipine on twelve patients suffering from achalasia. After 25 min it causes a significant decrease in the raised LES resting pressure from 45.5 $\pm$ 2.6 mmHg to 14.5 $\pm$ 0.4 mmHg ($p < 0.001$). In comparison with the effect on healthy subjects, a slightly delayed onset occurs in achalasia patients while the effect lasts the same time.

In the first pilot experiments on three patients with diffuse oesophageal spasm, application of 20 mg of nifedipine led after only 5 min to a decrease of contraction frequency from 2.7 to 0.75 contractions per minute over the whole 60 min measurement period (Figure 62.6). Neither amplitude nor pattern of contractions were effected by nifedipine.

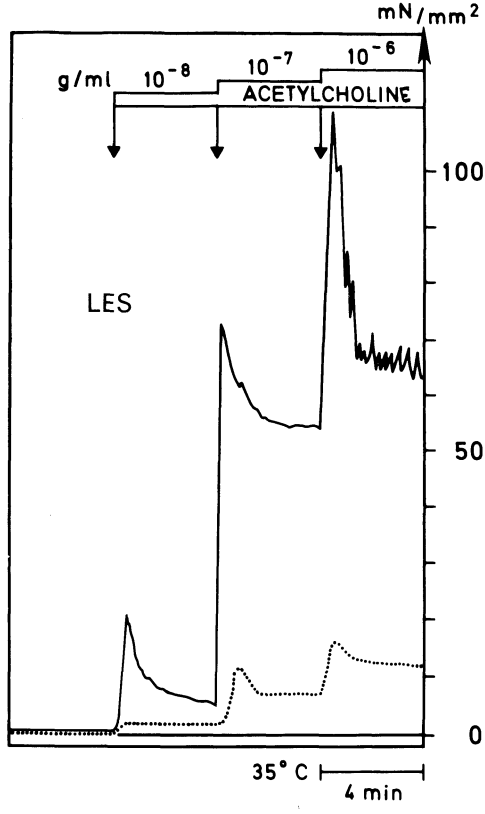

Figure 62.1 Reaction of isolated LES circular muscle strips to acetylcholine (ACh) (10^{-8}–10^{-6} g/ml ACh) without (unbroken line) and with nifedipine (10^{-6} mol/l; broken line) (dog; $n = 6$)

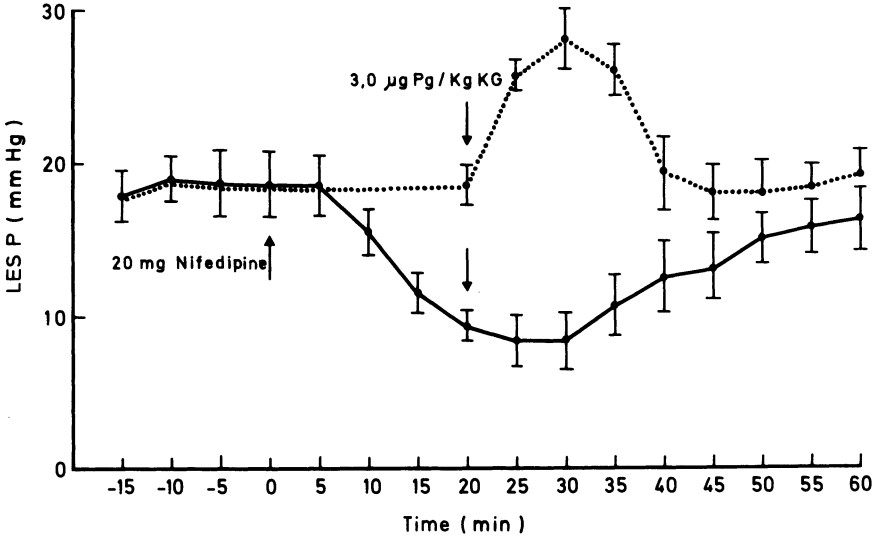

Figure 62.2 The influence of nifedipine (20 mg) on the LES before and after application of pentagastrin (3.0 μg/kg; unbroken line) in comparison to the controls (broken line) having no nifedipine. The values of 20, 25, 30, 35 and 40 min are significant ($X \pm$ SE) (dog; $n = 8$)

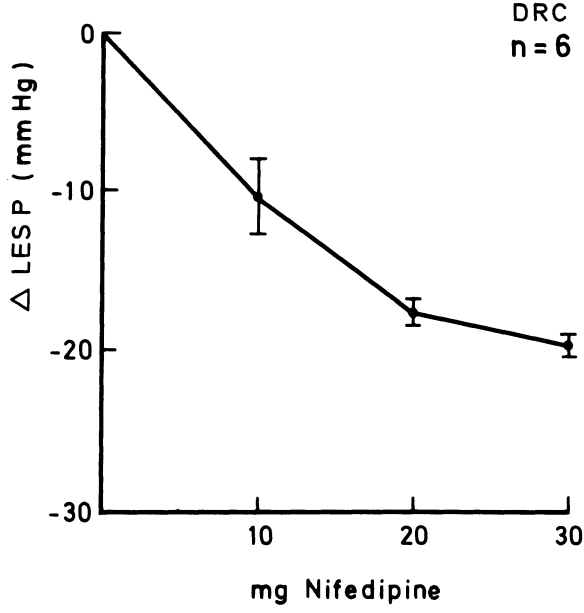

Figure 62.3 Dose–response curve from oral doses of nifedipine on the human LES ($X \pm$ SE)

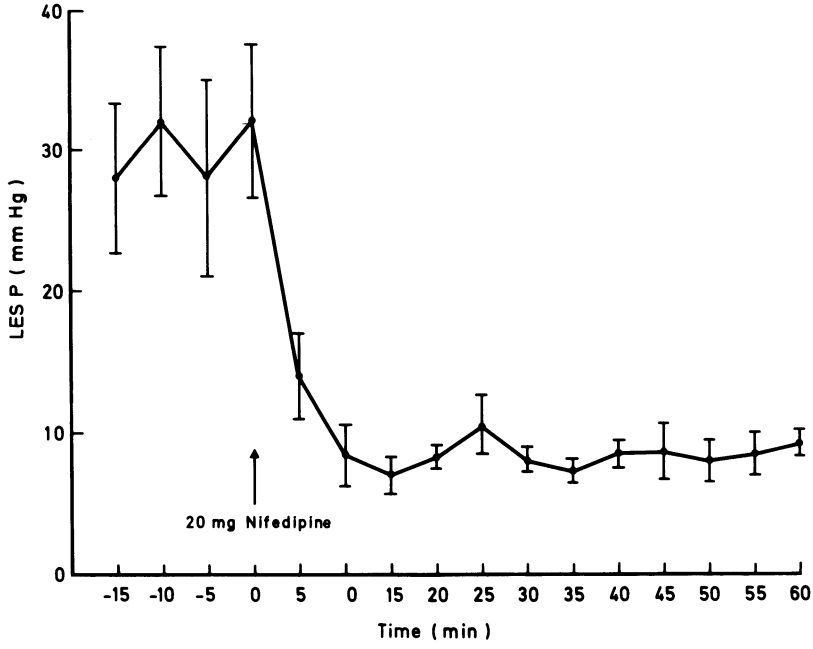

Figure 62.4 The influence of nifedipine (20 mg orally) on the LES resting pressure of six healthy volunteers ($X \pm$ SE) (controls; $n = 6$)

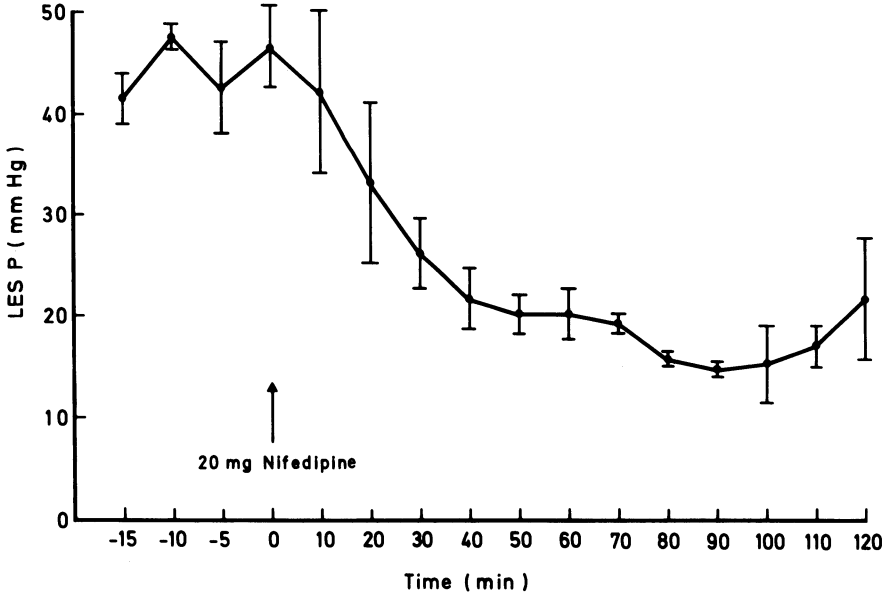

Figure 62.5 The influence of nifedipine (20 mg orally) on the LES resting pressure of twelve patients suffering from achalasia, diagnosed by X-ray examination and manometry ($X \pm$ SE) (achalasia: $n = 12$)

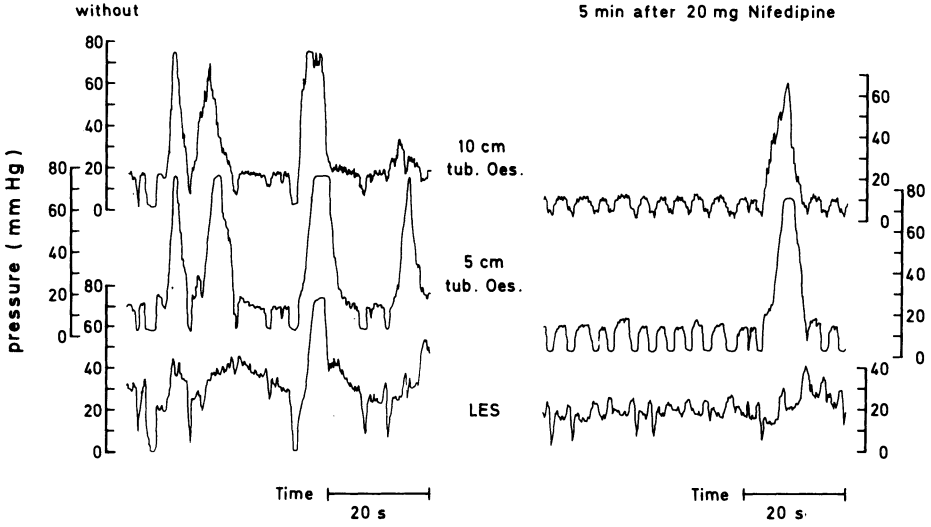

Figure 62.6 The influence of nifedipine (20 mg orally) on the frequency of oesophageal contractions of three patients suffering from diffuse oesophageal spasm ($n = 3$)

DISCUSSION

In vitro tests of oesophageal and stomach motility in dogs have led us to believe that oesophageal musculature, in spite of its more tonic activity, is particularly sensitive to nifedipine[8,9]. These results indicated the possibility that nifedipine, used up to now as a coronary drug, might also be effective as a spasmolytic[10]. In addition to the *in vitro* findings our own investigations have shown the effect of nifedipine on dogs *in vivo* and on humans. After 20 mg of nifedipine both dogs and humans show a LES pressure reduction of nearly 70%.

This effect continues for at least 60 min. A similar effect has also been noted for glucagon and atropine which led to a similar pressure reduction in dog and man[11]. Since the Ca^{2+}-antagonist nifedipine has a direct effect on cell function, it is understandable that pentagastrin stimulation does not lead to any increased tone in the LES. The maximal effect of nifedipine is reached with a dose of 30 mg. In order to prevent side-effects as a result of overdose we chose a dosage of 20 mg, which is about the same as that considered effective for coronary therapy. The long-lasting and noticeably relaxing effect of nifedipine opens new possibilities in the treatment of spastic or hypermotile diseases of the oesophagus. As we have been able to demonstrate on twelve patients with achalasia, 20 mg of nifedipine administered orally leads within 25–40 min to a 2–4 h period of relative well-being. Manometry registered a lowering of the pathologically raised LES resting pressure from 45.5 ± 2.6 mmHg to 14.5 ± 0.4 mmHg. The relaxation of the LES on swallowing remained, however, unaffected.

A similar effect for glucagon in achalasia has also been described[12]. Nifedipine can be used for conservative therapy. Eight patients treated with 3 × 20 mg of nifedipine were subjectively free of symptoms over a 6 month observation period.

Clinical practice will have to show whether it represents an alternative to the proven methods of treatment, namely pneumatic dilation and myotomy. A further indication for the use of nifedipine could be in cases of diffuse oesophageal spasm. It has been confirmed that intravenous application of spasmolytics can have a beneficial effect on the typically spastic retrosternal pain in this disease[13]. No orally effective spasmolytic has yet been discovered. In tests on three patients with diffuse oesophageal spasm it was found possible, by oral administration of 20 mg of nifedipine, to bring about a decrease in mean frequency of segmental oesophageal contractions of 72.2% together with a subjective sense of well-being lasting 2–4 h. Amplitude and pattern of contractions remained, however, unaffected. In recent studies long-term nitrate treatment has also been found to be of therapeutic value in diffuse oesophageal spasm[14].

In spite of these findings, the question of the clinical value of nifedipine, or of long-term nitrate treatment in cases of diffuse oesophageal spasm, must remain an open one.

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Discussion

R. W. McCallum (USA): You mention in your abstract that nifedipine decreases oesophageal peristaltic activity. Therefore it may not be of value in achalasia because while decreasing LES pressure it may also decrease what little motor function there is left in the body of the oesophagus of the patient with achalasia, hence impairing swallowing.

H. F. Weiser (Germany): We just started a double-blind study with nifedipine. During achalasia the oesophageal emptying is primarily dependent on gravity, and not on the segmental and insufficient oesophageal wall contractions. The emptying can only be improved by a reduction of the resistance in the LES. This is also the principle of all measures up to now at our disposal (distension, myotomy).

E. E. Daniel (Canada): This drug is believed to be a 'calcium-current' antagonist. Thus it will inhibit any tissue which is excited by an inward Ca^{2+} current, including heart, many smooth muscles, some nerves, etc.; thus it is probably not selective for LES tension. Did you study its action on the swallow-induced relaxation of LES, on peristalsis, etc?

Weiser: The swallow-induced relaxation is not influenced. By reducing the resting pressure, the opening ability of the cardia is improved, despite unchanged relaxation. In the studies presented, no measurements of the peristalsis were carried out, for methodological reasons.

W. Silber (S. Africa): In my series of 150 cases of achalasia, only 6% have high sphincter pressures. The disease is primarily one involving the body of the oesophagus and the patient swallows by gravity. I think the drug is a dangerous one. Reflux can also be disastrous.

Weiser: In our seventy-two patients studied, fifty-one had an increased resting pressure in the LES, so that at least for this proportion of the patients an improvement by nifedipine is to be expected, and can be proven. Referring to the swallow-induced relaxation cf. above.

C. F. Code (USA): It seems to me that this may be quite a dangerous drug to use continuously; the LES pressure may remain so low that instead of diffuse spasm the patients may develop reflux oesophagitis. What is your experience?

Weiser: Up to now it has not been proven that a reduction of the resting pressure in the LES is the sole cause of a gastro-oesophageal reflux. Even if it comes to a gastro-oesophageal reflux, consequences in the sense of a reflux oesophagitis are not to be expected, while sustaining the peristalsis in the tubular oesophagus. Up to now a reflux oesophagitis in patients treated this way has not been examined.

D. O. Castell (USA): What are the side-effects of this drug on the other calcium-dependent cells in the body?

Weiser: No side-effects were studied in patients treated up to now with the coronary therapeutic agent in this dose of the medicament.

C. E. Pope (USA): Even though amplitude of waves on the body at the oesophagus is not reduced in diffuse spasm, is pain relieved?

Weiser: A satisfactory manometric method to register the peristalsis quantitatively is not yet available. Therefore, even in our studies, only qualitative evidence is possible. According to that method, the amplitude of the peristaltic wave is not influenced. This leads, however, to a retrogression of the tertiary contractions. Our clinical results show that the patients treated with nifedipine are without complaints.

S. Cohen: (USA) Any drug that lowers sphincter pressure may improve oesophageal emptying of barium. However, this effect can only be seen while the patient is upright. Without gravity, the oesophagus does not empty in achalasia.

63

The upper oesophageal sphincter during vomiting, eructation and distension of the cardia: an electromyographic study in the unanaesthetized dog

H. MONGES, J. SALDUCCI AND B. NAUDY

In the papers presented during the 4th and 5th International Symposia on Gastrointestinal Motility, we studied the electrical activity of the stomach and duodenum[1] and of the diaphragm and particularly the muscle fibres surrounding the oesophageal hiatus[2] during vomiting.

The work presented here is essentially an electromyographic study of the upper oesophageal sphincter (commonly abbreviated as UES) during vomiting. Our objective was to determine whether the UES remains closed during the retching phase, as usually assumed[3–5], and only opens during the expulsive phase or vomiting itself. Moreover, in the dogs prepared for this study of vomiting, we also studied the behaviour of the UES during eructation and during distension of the cardia caused by the withdrawal of an inflated balloon from the stomach into the oesophagus.

METHODS

This work involved ten adult mongrel dogs weighing 15–20 kg each.

Preparation

The dogs were operated under general anaesthesia (Nembutal: 20 mg/kg). A median cervicotomy was performed along 8 cm from the upper edge of the thyroid cartilage, and bipolar electrodes were chronically implanted into the

muscle layers of the UES (cricopharyngeal muscle) and into the first 4–5 cm of the cervical oesophagus (i.e. 2–5 cm from the lower edge of the sphincter). Each bipolar electrode was formed by the stripped ends of two 125 μm diameter stainless steel wires fixed 2 mm apart into the muscle fibres along the transverse axis of the organ. In most cases the electrodes were implanted in the left side of the sphincter and cervical oesophagus; more rarely in the right side. In a few animals a similar bipolar electrode was also implanted into the mylohyoideus muscle. The electrode leads were brought out through the skin in the dorsal region.

Experimental procedure

Experiments were conducted from the 7th to the 30th post-operative days. Prior to each recording session a bipolar electrode was implanted into the rectus abdominis muscle and a belt pneumograph was secured around the thorax. In some cases (study of eructation) the endoluminal pressure was recorded from the thoracic oesophagus by means of two water-filled fused polyvinyl catheters (ID: 1.4 mm; OD: 2.5 mm) whose open tips were placed 5 cm apart. Electrical activity, pneumogram and pressures were recorded using a multichannel ink projection type polygraph (Elema Schönander, Sweden). During the recording sessions the animals stood quietly and unrestrained.

Vomiting was induced by a subcutaneous 5–10 mg injection of apomorphine in an animal which had been fed a normal meal prior to the experiment.

Eructation was induced by insufflation of 150–200 ml of air into the stomach through a tube which was then quickly withdrawn. Eructation occurred during the following few minutes.

Distension of the cardia was induced as follows: a small latex balloon secured to the tip of a catheter was introduced, deflated, through the mouth into the stomach, then inflated with 15–20 ml of air and withdrawn from the stomach into the oesophagus. The inflated balloon measured 3–4 cm in length and 2 cm in diameter.

RESULTS

The electromyogram (EMG) of the UES under basal conditions was characterized by a continuous spike discharge, often with higher amplitudes recorded at each inspiration.

Vomiting

The vomiting sequence comprises two distinct phases: the initial retching phase and the expulsive phase or vomiting itself. The retching phase is characterized by a series of violent rhythmic abdomino-thoracic contractions.

During the expulsive phase, the animal thrusts its head forward, opens its mouth wide and contracts its abdominal and diaphragm muscles throughout the forceful ejection of gastric material through the mouth.

During the retching phase (Figures 63.1, 63.2)
At each retching pulse, shown on the rectus abdominis muscle EMG by a strong burst of fast spikes, the UES electrical activity was completely inhibited for about 0.6 s. Between each retching pulse a strong discharge of spike activity was recorded from the UES; this activity ceased at the onset of the succeeding retching pulse. The mean duration of the inter-retch spike potential discharge was about 0.6 s for the initial retching pulses; for the final pulses the discharges were generally shorter and of lower amplitude. Between each retching pulse, together with the strong spike discharge recorded from

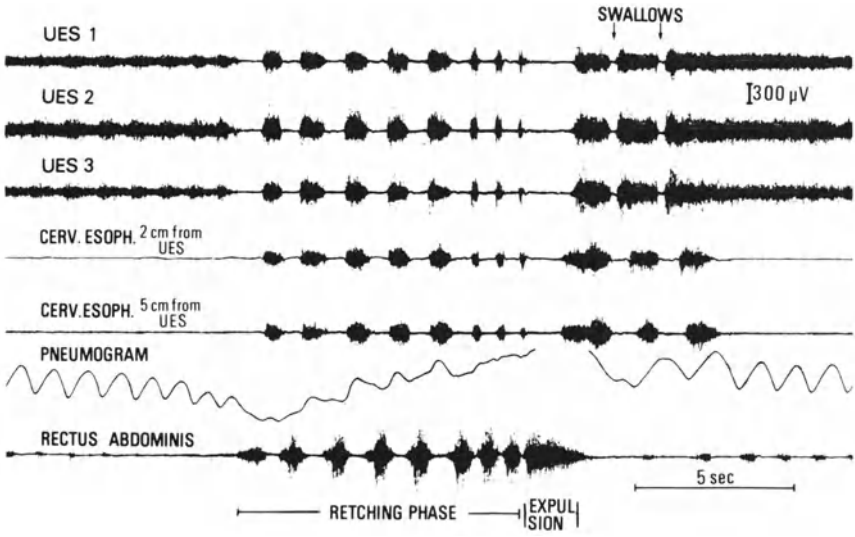

Figure 63.1 *Vomiting*. The EMGs of the upper oesophageal sphincter (UES 1, UES 2, UES 3) are recorded by closely spaced electrodes: the uppermost electrode (UES 1) was situated 7–8 mm above the lowest (UES 3). On the pneumogram, downward deflections indicate the inspiration, as on the following figures.

During the retching phase, at each retching pulse (shown on the rectus abdominis muscle EMG by a burst of spike potentials) the UES electrical activity ceases. Between the retching pulses a strong spike burst is recorded from the UES and almost simultaneously from both levels of the cervical oesophagus (cerv. oesoph.). These discharges become much weaker between the final retching pulses.

During the expulsive phase (expulsion), shown on the rectus abdominis muscle EMG by a prolonged spike potential discharge, the UES electrical activity ceases for a longer interval than during the retching pulses. After the expulsion a very strong spike discharge is recorded from the UES and the cervical oesophagus, beginning in the latter slightly before the onset of the UES discharge.

The vomiting is followed by two swallows shown by a brief inhibition of the UES electrical activity, and on the cervical oesophagus EMGs by a sequential spike potential discharge.

the UES, a major burst of fast spiking activity was recorded by the electrodes implanted in the first few centimetres of the cervical oesophagus. The UES and cervical oesophagus activity appeared simultaneous, although when recorded at higher paper speed the onset of the UES burst preceded that of the cervical oesophagus by a fraction of a second. As with the UES activity, the spike discharges from the cervical oesophagus were weaker between the final retching pulses than at the onset.

During the expulsive phase (Figures 63.1, 63.2)

UES electrical activity was inhibited over a longer period (0.9–2.5 s and up to 3.5 s in some cases). This inhibition occurred from the beginning of the sustained abdominal contraction accompanying the expulsion, which was characterized by a very strong burst of spiking activity from the rectus abdominis muscle. The inhibition of UES electrical activity lasted approximately as long as the abdominal contraction, and was followed by a recovery of UES activity, characterized by a strong spike discharge indicating the closure of the sphincter. A spike potential discharge, occurring slightly before the closure of the UES or – in the event of prolonged inhibition of the UES electrical activity – preceding the closure by up to 1–1.5 s (Figure 63.2) was recorded simultaneously by electrodes situated at various levels in the cervical oesophagus. This discharge became stronger during the period of

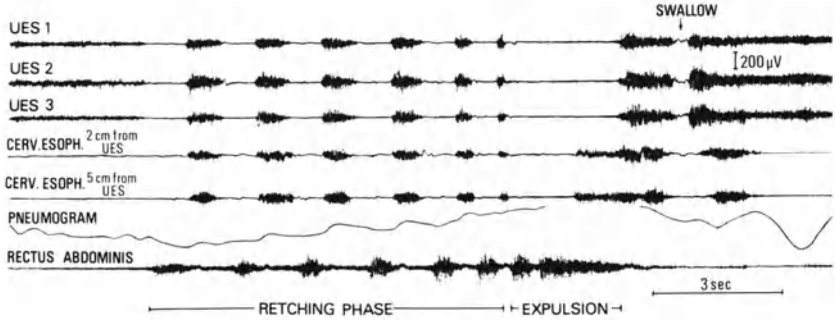

Figure 63.2 *Vomiting*. The position of the electrodes implanted in the upper oesophageal sphincter (UES 1, UES 2, UES 3) is the same as in Figure 63.1.

UES electrical activity is inhibited during each retching pulse (shown on the rectus abdominis EMG by a burst of spike potentials); a strong spike potential discharge is recorded between retching pulses from the UES and the cervical oesophagus (cerv. oesoph.). This recording made at high paper speed shows that the spike discharge from the cervical oesophagus begins slightly after the onset of the discharge recorded from the UES. The spiking activity from both the UES and the cervical oesophagus becomes weaker between the final retching pulses. The UES activity is inhibited for a long period (2.5 s) during the expulsive phase (expulsion). A spike potential discharge beginning 1 s before sphincter closure is recorded almost simultaneously from two different levels in the cervical oesophagus, and its amplitude increases with the closure of the UES.

The vomiting is followed by a swallow shown by a brief inhibition of the UES electrical activity and a peristaltic contraction of the cervical oesophagus.

sphincter closure. Vomiting was generally followed by one or more deglutitions.

Eructation (Figure 63.3)

UES activity was completely inhibited for 0.7–2 s during eructation. This inhibition began simultaneously with the abdominal contraction accompanying eructation as soon as the air entered the thoracic oesophagus, as shown on the pressure recordings. A small burst of fast spikes was recorded from the cervical oesophagus while the UES activity was inhibited. This inhibition was followed by a return to basal UES electrical conditions.

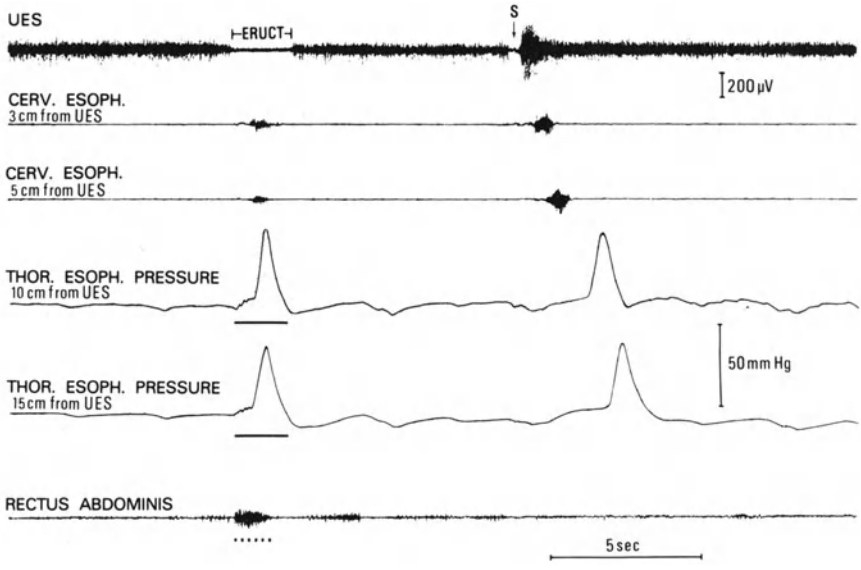

Figure 63.3 *Eructation.* The eructation (Eruct.) is accompanied by a 2 s inhibition of upper oesophageal sphincter electrical activity beginning at the onset of the rectus abdominis muscle contraction (dotted line) which accompanies eructation and the onset of the pressure wave produced by the air passing through the thoracic oesophagus marked by a solid line under both pressure tracings (thor. oesoph.). A short spike burst was recorded in the cervical oesophagus (cerv. oesoph.) during the UES inhibition period.

A swallow (S) occurs 8 s after the eructation, as shown by a brief inhibition of the UES activity, a peristaltic contraction recorded on both EMGs from the cervical oesophagus and the pressure recordings from the thoracic oesophagus.

Distension of the cardia (Figure 63.4)

The sudden withdrawal from the stomach into the oesophagus of a balloon inflated with 15–20 ml of air regularly produced complete inhibition of UES electrical activity as the balloon passed through the cardia. This inhibition lasted 0.7–2 s, after which the UES electrical activity returned to normal

basal levels. The UES electrical activity was not affected when the balloon was allowed to return into the stomach, nor when it was withdrawn slowly. The inhibition was caused only by the sudden withdrawal of the balloon.

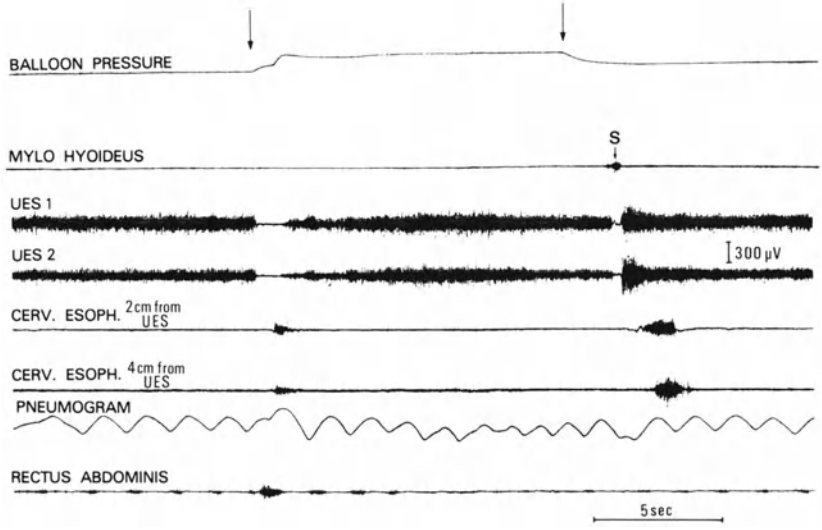

Figure 63.4 *Distension of the cardia.* Upper oesophageal sphincter electrical activity (UES 1, UES 2) was recorded by means of two closely spaced electrodes (UES 1, UES 2).

The balloon (15 ml of air) is first held in the stomach, then quickly withdrawn into the oesophagus (first arrow) and held stationary 7–8 cm above the cardia. It is then released and returns to the stomach (second arrow).

UES electrical activity ceases at the instant the balloon passes upwards through the cardia (first arrow) and remains inhibited for 1.1 s; during this inhibition a small burst of spiking activity is simultaneously recorded from two different levels in the cervical oesophagus (cerv. oesoph.). When the balloon returns to the stomach (second arrow), UES electrical activity is unaffected as the balloon passes through the cardia.

A swallow (S) occurs 1 s later as shown by a burst of spike potentials from the mylohyoideus muscle, a brief inhibition of the UES electrical activity and a peristaltic contraction of the cervical oesophagus.

DISCUSSION

Vomiting

During the retching phase, as shown by X-ray data[3,6,7], gastric material rises into the oesophagus at each retching pulse and returns passively into the stomach between pulses. These X-ray data had suggested that the UES remains closed during retching, thus preventing the gastric material from reaching the pharynx and the mouth[3–5]. Our work clearly shows, on the contrary, that the UES relaxes with each retching pulse and is strongly closed between pulses: UES electrical activity is interrupted during each retching pulse, while a strong burst of spike potentials is recorded between each pulse,

becoming weaker for the final pulses prior to expulsion. Between retching pulses, simultaneously with the sphincter closure, the cervical oesophagus contracts, as shown by the activity recorded by electrodes implanted 2–5 cm below the lower portion of the UES. No electrodes were implanted more than 5 cm below the sphincter, so that it is impossible to specify whether below this point the oesophagus is also contracted. In a previous study[2] in which electrodes were implanted in the lower thoracic oesophagus, no spike discharges were recorded during the retching phase.

During the expulsive phase, UES relaxation is more prolonged than during retching pulses, sometimes lasting up to 3.5 s. It is probable that the UES remains relaxed after the gastric material has been ejected from the mouth; as during retching pulses, it remains relaxed throughout the abdominal muscle contraction. Whereas after each retching pulse the cervical oesophagus contraction begins simultaneously with or shortly after the onset of the UES contraction, during expulsion the cervical oesophagus contraction begins prior to the closure of the UES, particularly if the UES relaxation was of long duration. UES relaxation during the expulsive phase appears to be unrelated to the rise of gastric content in the oesophagus, but instead, as during retching, to be the result of central nervous system phenomena.

Eructation

The UES is relaxed for a relatively long time during eructation. The small spike burst recorded from the cervical oesophagus while the UES is relaxed is probably due to the passage of air. In an earlier study[2] similar activity was regularly recorded with electrodes in the lower thoracic oesophagus. Unlike retching and expulsion, in which UES closure is recorded by a strong spike discharge, the sphincter closure following eructation is characterized by a resumption of basal electrical activity similar to that which preceded the eructation. It is not accompanied by contraction of the cervical oesophagus as after retching pulses and expulsion.

Distension of the cardia

The UES relaxation, triggered by sudden distension of the cardia by withdrawing an inflated balloon from the stomach into the oesophagus, is undoubtedly of reflex origin. As previously mentioned, this relaxation occurs only during sudden distension of the cardia, and is not observed under gradual distension.

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Discussion

J. D. Wood: (USA) Do you have any knowledge of the reflex pathways involved in the relaxation response of the UES to pulling the balloon into the cardia? Does the neural pathway involve circuits within the myenteric plexus, vagal reflexes, or a combination of the two?

J. Salducci: (France) We have not done any experiments concerning this subject; but we have seen, in acute experiments, that the transient drop of the intraluminal pressure when the cardiac balloon is withdrawn proves that this opening of the cardia is active, and it is only after the opening of the cardia that the sphincteric UES is inhibited, probably involving vagal reflexes.

C. F. Code: (USA) In your experience, can human beings relax their UES at will without swallowing?

Salducci: Yes; it is usually noticed on laryngectomized patients who learn to relax their UES for introducing air into the oesophagus and then to expel it, so as to have an oesophageal voice.

C. Roman: (France) In answer to Dr Code's remarks, I can add the two following points: (1) The UES is under the control of a cortical area so it is easy to conceive the voluntary control of this sphincter in man. (2) As for the voluntary control of LES, Dr Monges told me that he knew a man in Marseille who could swallow living frogs and fishes and was then able to regurgitate at will either a fish or a frog!

W. Silber: (S. Africa) To substantiate what Dr Salducci has stated about the UES in laryngectomized people, it is quite true they learn to relax the UES in order to swallow air; i.e. voluntary aerophagists. Sometimes they complain of abdominal discomfort. The air is both in the oesophagus and stomach.

64

Somatostatin inhibition of LESP elevation caused by glycine: support for a role of endogenous gastrin in physiological responses of the sphincter

D. E. BYBEE, F. C. BROWN, P. GEORGES AND O. CASTELL

The control of the lower oesophageal sphincter (usually abbreviated as LES) appears to be a result of the modulation of three basic systems. These are the intrinsic properties of the sphincteric smooth muscle, the neural pathways to the sphincter, and gastrointestinal hormones[1]. The role of gastrointestinal hormones in the physiology of the LES dates back to the observation that instillation of HCl into the stomach decreased the LES pressure and administration of alkali produces an increase in pressure[2]. These observations were followed by the demonstration that exogenous pentagastrin had a pressor effect on the LES and led to the suggestion that endogenous gastrin played a role in the regulation of the LES. Subsequent studies have demonstrated that most gastrointestinal hormones have a pharmacological effect on the LES. The hormones gastrin[2], motilin[3], and substance P[4] increase LES pressure whereas glucagon[5], cholecystokinin[6], secretin, VIP[7], and GIP[8] decrease sphincter pressure. These studies have led to a great deal of controversy over the physiological role of these hormones in the regulation of the LES.

Somatostatin, or growth hormone release inhibiting hormone (GHRIH), has been shown to diffusely inhibit the release of peripheral and pituitary hormones. The peripheral hormones whose release is inhibited by GHRIH include insulin[9], glucagon[9], gastrin[10], motilin[11], VIP[12], and secretin[13]. Since somatostatin blocks the release of several gastrointestinal hormones, including those known to have a pharmacological effect on the LES, we have utilized this agent as a method to determine the physiological role, if any, of the

GHRIH-suppressible hormones on the LES. We report the effect of GHRIH infusion on basal LES tone, on the response of the LES to exogenous pentagastrin, and on the response of the LES to intragastric alkali and glycine.

METHODS

All studies were performed in conscious, restrained adult male baboons (*Papio anubis*). The animals were anaesthetized with ketamine HCl (7.5 mg/kg intramuscularly), placed in restraint chairs, and allowed to awaken for 120 min to minimize anaesthetic-induced decrease in LES tone[14].

LES pressures were measured by the use of water-filled polyvinyl catheters (1.4 mm ID) connected to external transducers (Hewlett Packard 1280 Series). The pressure recording assembly was arranged as a fixed unit containing two catheters, each with a side orifice 1.2 mm in diameter, spaced 5 cm apart, joined to a nasogastric sump tube. Each recording catheter was infused with distilled water at a constant rate (0.8 ml/min) by a syringe infusion pump (Harvard Apparatus Model No. 933). The characteristics of the system were such that occlusion of the catheter orifices gave a pressure rise rate in excess of 80 mmHg/s. The tubing was passed through the nares into the stomach and slowly withdrawn across the LES until the distal orifice was positioned at the point of maximal sphincter pressure just distal to the pressure inversion point. The position of the recording catheter was confirmed at frequent intervals (no greater than every 4 min) by identification of the pressure inversion point. LES pressure was recorded in mmHg at mid-inspiration with gastric fundal pressure as the zero point.

The effect of GHRIH on basal LES pressure was studied using an infusion rate of 2 μg/min. The LES response curves to exogenous pentagastrin given as a rapid intravenous bolus dose of 1.6 μg/kg was recorded during saline and GHRIH (2 μg/min) infusion.

The responses of the LES to intragastric instillation of 100 ml of 0.1 N NaOH (pH 12.5) and of 200 ml of glycine (1.5% w/v pH 6.1) were evaluated during separate infusions of saline and GHRIH (2 μg/min). The Student's *t* test for paired results was used to evaluate statistical significance.

RESULTS

The results of our studies are summarized in Figure 64.1. The infusion of GHRIH at the rate of 3 μg/min did not alter the basal LES pressure of 21 $\pm$ 4 mmHg (mean $\pm$ SEM). The response of the LES to the 1.6 μg/kg dose of intravenous pentagastrin was 48 $\pm$ 6 mmHg. The pressure response (51 $\pm$ 4 mmHg) to this same pentagastrin dose during GHRIH infusion (2 μg/kg) was not significantly different.

The sphincteric response to the intragastric instillation of alkali was an increase from the basal sphincter pressure of 27.6 $\pm$ 0.7 mmHg to a level of

51 ± 2.1 mmHg. GHRIH did not alter the basal pressure (32.3 ± 3 mmHg) but did completely abolish the pressor response to the alkali ($p < 0.05$).

The intragastric instillation of a glycine solution resulted in an increase in LES pressure from 22.4 ± 2.5 mmHg to a peak pressure of 42 ± 3 mmHg. During GHRIH infusion, the basal pressure did not change, but the pressure increase seen during saline infusion was completely abolished ($p < 0.05$).

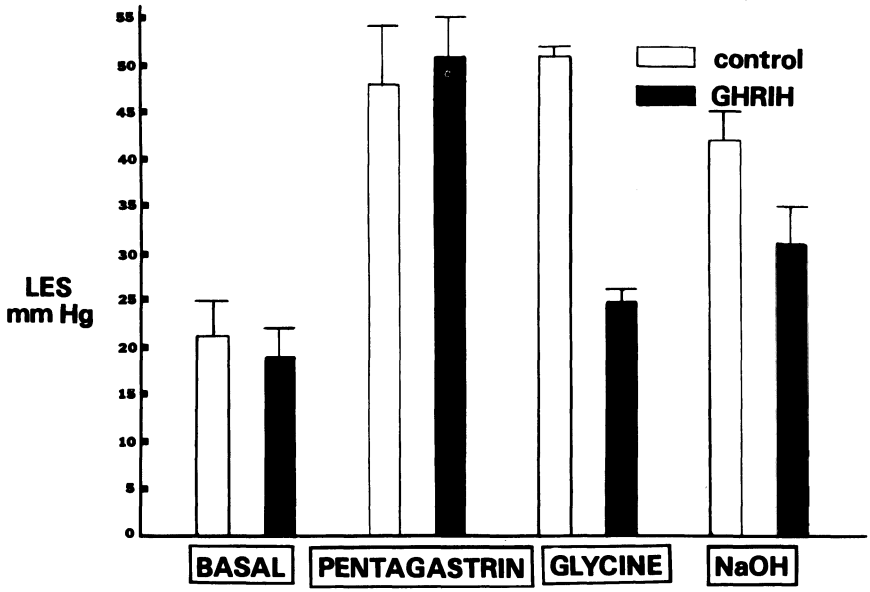

Figure 64.1 Effect of somatostatin infusion (2 μg/min) on basal LES pressure and the LES response to pentagastrin infusion and alkali and glycine instillation. Control studies are open bars, somatostatin studies are shown as hatched bars. The vertical lines indicate SEM

DISCUSSION

Somatostatin, a diffuse inhibitor of hormone release, was found not to alter the basal lower oesophageal sphincter pressure when given as a constant infusion at a dose documented to have a pronounced effect upon hormone release[9–11]. This observation suggests that a somatostatin-suppressible hormone or hormones do not have a measurable role in the maintenance of basal LES tone. In addition, GHRIH infusion did not alter the sphincteric response to an exogenous bolus dose of pentagastrin, suggesting that GHRIH does not alter the peripheral or direct effect of this hormone on the sphincteric muscle. However, GHRIH did abolish the sphincteric response to the intragastric instillation of alkali and of glycine, two gastric secretagogues, suggesting that this pressor response is mediated through a somatostatin-suppressible mechanism which is most probably hormonal in nature.

Our study suggests that hormonal mechanisms do not contribute to the

maintenance of basal lower oesophageal sphincteric tone but may have a role in the response of the sphincter to other stimuli. Further, the mechanism of this response does not involve peripheral interaction with hormones and their receptors, but rather by the suppression of hormone release in response to appropriate stimuli. With the development of new techniques, it should ultimately be possible to sort out the hormone or hormones involved in the physiology of the LES.

Acknowledgement

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Discussion

S. Cohen: (USA) It is becoming disturbing to me that to date all substances that have been studied act upon the LES. All of the hormones, histamine and serotonin act on the sphincter. Is the sphincter a delicately balanced organ regulated by many factors, or is it simply a non-specific receptor to all substances? This is a key question that should be answered in the near future.

F. C. Brown: (USA) The question of what is physiological and what is pharmacological in the control of the LES has been the subject of much debate in the literature, and at symposia such as this one. The current level of our sophistication makes separation of these phenomena difficult. We shall all await the answer to this key question.

R. W. McCallum: (USA) Somatostatin has been called, by the endocrinologists, 'hormonal cement', because it inhibits so many hormones – specifically cholecystokinin, secretin and VIP, gastrin. It has been clearly shown in man that alkali and glycine do not release gastrin, so I think that the somatostatin inhibition of the LESP is related to inhibition of another duodenal agent, as has previously been suggested in this book (p. 561). I would suggest that phenylalanine and tryptophan be used in man as gastrin-releasing agents, but unfortunately these would also release cholecystokinin which would also be inhibited by somatostatin. In summary, it is very difficult to draw any conclusions from somatostatin experiments regarding the role of serum gastrin. Also your meal of glycine and alkali induced only a 24 pg/ml rise in gastrin, and I suggest this was due to antral pH changes. The significant difference in integrated gastrin response was mainly due to the fall in serum gastrin after somatostatin.

Brown: We agree with your statement about the difficulty in drawing conclusions about the role of specific hormones such as gastrin from our study. We do feel that our data indicate that a hormonal (or at least a somatostatin-suppressible) mechanism is responsible for the sphincteric response to alkali, glycine and distension. The exact role of gastrin in this system remains doubtful.

Section XIII
Regulatory Factors in Gastrointestinal Motility

65

Sphincter of Oddi motor activity in man: a zone of unique, phasic high-pressure contractions *(Abstract)*

W. J. HOGAN, W. J. DODDS, J. E. GEENEN, R. D. SCHAFFER, A. C. STEWART AND R. C. ARNDORFER

Manometric pressure measurements have been recorded from the common bile duct (CBD) and duodenum (D) in man at time of endoscopic retrograde cholangiopancreatography (ERCP) but scant information is available about the pressure characteristics of the sphincter of Oddi (SO). During ERCP in twelve patients without evidence of periampullary disease, pressure measurements were obtained from the CBD, SO and D using a high-fidelity infusion system with negligible compliance. After cannulation of the CBD with a side-hole catheter (0.8 mm ID) the catheter was connected to a pneumo-hydraulic capillary system which infused water at 0.25 ml/min. CBD and SO pressures were recorded by incremental 1.0 mm catheter withdrawals. A second catheter, attached to the endoscope, continuously recorded duodenal pressure. SO pressure responses were determined to intravenous pulse injections of glucagon (0.4 mg) and secretin (1 μg/kg).

RESULTS

Mean mid-respiratory CBD pressure was 30 $\pm$ 1.95 mmHg; baseline SO pressure was 37 $\pm$ 1.6 mmHg; and mean D pressure was 17.5 $\pm$ 1.5 mmHg. In each patient, hitherto undescribed phasic contractions were recorded from a segment of the SO approximately 5–10 mm in length. These unique phasic contractions, not present in either the D or the CBD, occurred regularly at three to six per minute (4.4 $\pm$ 0.3 SEM). Their peak amplitude measured 107 $\pm$ 17 mmHg and duration 4.7 $\pm$ 0.2 s. When the manometric catheter was infused by a standard pump-syringe infusion system featuring undue

compliance, the SO high-pressure oscillations were either greatly damped or, in many instances, obscured completely. In eight patients, intravenous pulse-glucagon significantly ($p < 0.001$) reduced the SO pressure peaks to 54 ± 12 mmHg and wave-frequency to 1.6/s, but caused minimal decrease in the basal SO pressure. In four patients, secretin caused a similar trend.

CONCLUSIONS

1. Using a high-fidelity recording system, unique high-pressure phasic contractions, occurring at three to six per minute, have been recorded from the SO region.
2. The frequency and amplitude of these contractions are reduced by pharmacological doses of glucagon and secretin.
3. The relationship of the SO contractions to duodenal BER and CBD emptying remains to be determined.

66

Antagonism by metoclopramide of some gastrointestinal effects of secretin

P. R. BLOWER, J. D. FLACK AND M. D. DAY

Metoclopramide is an anti-emetic drug which has gastrointestinal stimulant effects in laboratory animals[1] and man[2]. It has dopamine receptor blocking activity[3] but its mechanism of action on gastrointestinal motility has not been fully elucidated.

During our investigations of the pharmacology of this drug, we have reported[4] its ability to antagonize the direct or indirect agonist effects of secretin at cardiovascular dopamine receptors in the rat. In view of these findings, the opposite effects of metoclopramide and secretin on motility, and recent postulations[5,6] suggesting the presence of inhibitory dopamine receptors in the gut, we have further examined the effects of these two drugs on gut motility.

METHODS

Gastric motility of conscious rats

Male Wistar rats, weighing between 250 and 700 g, were anaesthetized with sodium pentobarbitone (60 mg/kg) and chronic gastric fistulae were surgically implanted using aseptic techniques, as previously described[7]. The discs or washers which secured the cannula to the stomach and abdominal wall were constructed from polyethylene, as was the tube. The fistulae, which were implanted in the rumen of the stomach approximately 1 cm from the corpus–rumen junction, were brought out through the flank of the animal and the tube was sealed with rubber plugs taken from 1 ml disposable plastic syringes. To prevent gnawing of the polyethylene cannula a 2 cm length of stainless steel tube was bonded to the outside of the cannula using cyano-

acrylate ester adhesive (Loctite I.S.-12). The rats were not used for at least 2 weeks after the operation, to ensure complete wound healing.

Intragastric pressure was measured in these conscious chronic fistula rats in the following manner: after overnight fasting, rats were placed in restraining cages and the stomach of each rat was gently lavaged with warm tap water to remove traces of food still in the stomach. A water-filled catheter was placed in the fistula through a suitable polyethylene bung ensuring an air-tight seal. The other end of the catheter was connected to a Bell and Howell pressure transducer (type 4–422–0001) and the recording of intragastric pressure displayed on a Devices MX2 or MX4 pen recorder. The preamplifier of the recorder was set to monitor pressure changes in the range 0–25 mmHg using a −3dB damping top cut at 1 Hz. An index of gastric motility was obtained by summing the magnitude of all increases in gastric pressure of 1.25 mmHg or greater in each 10-min period and multiplying this total figure by the number of such pressure changes within the 10-min period. For the sake of convenience this final figure, which represented the total height and frequency of all practically measurable pressure changes within a 10-min period, was divided by the (arbitrary) figure of 1000 to provide more easily manageable results.

Animals with gastric fistulae of proven patency were further prepared by the aseptic implantation of chronic intravenous or intraduodenal cannulae under sodium pentobarbitone anaesthesia (60 mg/kg intraperitoneally). Duodenal cannulae were constructed using lengths of Portex nylon cannulae (White, OD 1.34 mm) onto the tip of which had been shrunk two small rings (1 mm apart) of polyolefin heat-shrinkable plastic sleeving (ID 1.6 mm). After exposing the duodenum by a right flank incision, the tip of the sterilized cannula was placed through a small incision into the lumen of the duodenum approximately 1.5 cm below the pylorus. The cannula tip was held in place by a purse-string suture (1.5 metric Mersilk 0000 gauge) tied through the wall of the duodenum and between the two polyolefin rings onto the nylon cannula. The cannula was brought through the abdominal wall and under the skin to the back of the neck, where a short length of tubing issued through the skin and was sealed with a small pin to prevent leakage. This method was found to be satisfactory for periods of up to 3 weeks, following which time the rats sometimes managed to pull the duodenal cannula out and had to be killed. Post-mortem examination of all animals in which the cannulae were superficially intact at the time of death, confirmed that the tip was still correctly positioned within the duodenum.

Chronic intravenous cannulae were implanted under anaesthesia in the left jugular vein. The vein was dissected free and cannulated with a Portex nylon intravenous cannula (Blue OD 0.75 mm) filled with 0.96% sodium chloride solution containing heparin (50 iu/ml). The cannula was taken under the skin to the back of the neck where a short length of tubing issued through the skin and was sealed with a pin to prevent leakage.

The effects of intravenously administered secretin, intraduodenally administered hydrochloric acid, and subcutaneously administered metoclopramide on gastric motility have been examined.

Drugs were freshly prepared in 0.96% sodium chloride solution for intravenous and subcutaneous administration and in distilled water for intraduodenal administration.

Infusions of drugs intravenously or intraduodenally were made using a Palmer slow injection apparatus.

In vitro experiments

Dunkin–Hartley guinea pigs of either sex were killed by cervical dislocation and were exsanguinated. Sections of distal (but not terminal) ileum were removed, washed free of contents and mounted in 10 ml tissue baths containing McEwens[8] solution at 37 °C and bubbled with a 95% oxygen, 5% carbon dioxide gas mixture. The resting load was adjusted to 1 g, and contractions of the tissue were recorded auxotonically using a Devices force transducer (type 4151) and light spring, and displayed on a Devices MX2 pen recorder. The tissues were washed at 2-min intervals and left to equilibrate for 45 min before the experiments commenced.

The tissue bath was 'washed' by overflow using pre-warmed McEwens solution. Drugs were freshly prepared and administered in volumes of 0.5 ml or less. The drug dilutions were made up in McEwens solution. Administration of 0.75 ml of McEwens solution or less had no effect on the tissue.

The effects of metoclopramide and secretin on responses of the isolated ileum to doses of acetylcholine were determined.

Tests for significance were carried out using the paired 't' test.

Drugs

O-Acetylcholine bromide (BDH Chemicals Ltd.); hydrochloric acid: N (BDH Chemicals Ltd.); metoclopramide hydrochloride (Beecham Pharmaceuticals); secretin (Porcine, Grade II: Sigma Chemical Co.).

RESULTS

The effect of secretin and metoclopramide on gastric motility in the conscious rat

Bolus intravenous injections of secretin were found to decrease gastric motility, but the effects were variable and short-lasting (5–10 min), therefore intravenous infusions were used.

The minimum reliable effective dose of secretin which reduced gastric motility was found to be 0.0003 U/kg/min. However, doses of 0.001 U/kg/min

were commonly employed, in order to be well above the 'threshold' dose of secretin required to reduce gastric motility to near zero levels. The effects of secretin on motility were maintained for as long as the infusion lasted (up to $2\frac{1}{2}$ h in control experiments) whilst in control experiments intravenous infusions of saline were not found to modify motility.

Doses of 3 or 10 mg/kg metoclopramide administered by subcutaneous injection were found to increase gastric motility despite the simultaneous infusion of secretin. Prior experiments in our laboratories (B. McRitchie, unpublished observations) have shown that the effects of metoclopramide on gastric motility in the conscious rat are not dose-dependent and that an 'all-or-none' type of response occurs. However, during infusions of secretin, the stimulant effects of metoclopramide were limited to approximately 20 min duration, whereas in control experiments the effects of metoclopramide frequently lasted for several hours. The effects of secretin and metoclopramide on gastric motility in a typical experiment are shown in Figure 66.1.

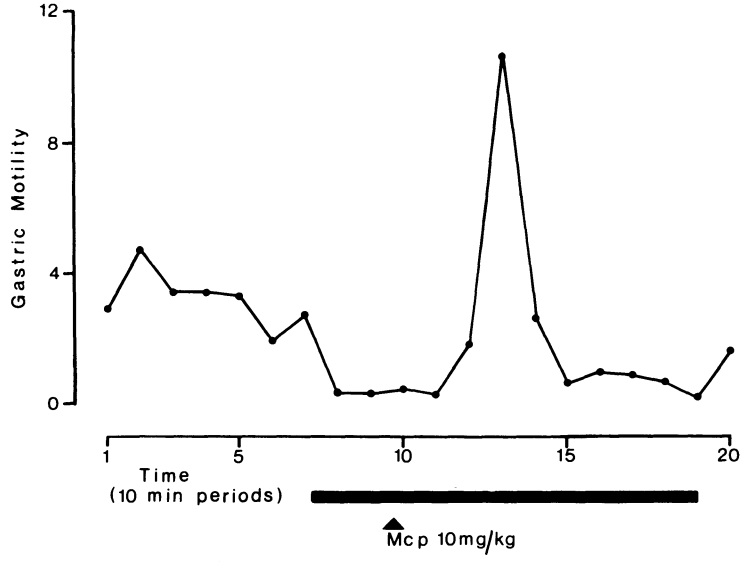

Figure 66.1 The effects of secretin and metoclopramide on the gastric motility of a conscious rat. Bar shows duration of intravenous infusion (0.001 U/kg/min) of secretin. The dose of metoclopramide (Mcp) was given subcutaneously and increased motility despite the secretin infusion

The effects of intraduodenally infused hydrochloric acid on gastric motility

The intraduodenal injection of 2 ml/kg of 50 mM hydrochloric acid (pH 1.37) resulted in an abrupt reduction in gastric motility. This effect was however

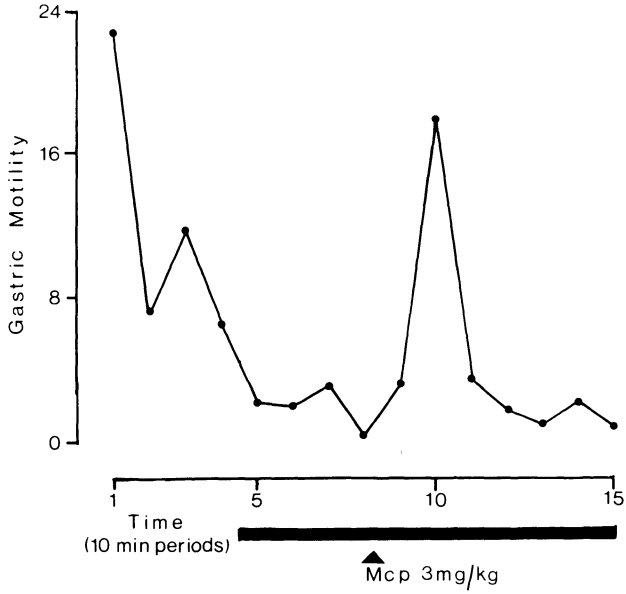

Figure 66.2 The effects of intraduodenally infused hydrochloric acid and subcutaneously administered metoclopramide on the gastric motility of a conscious rat. Bar shows duration of infusion (0.105 ml/min) of 50 mM hydrochloric acid infusion

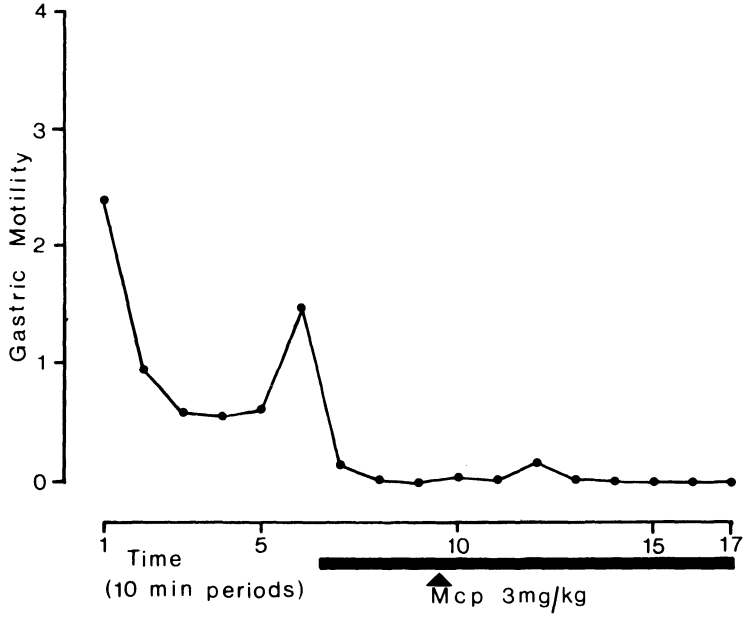

Figure 66.3 The effects of intraduodenally infused hydrochloric acid (50 mM) and subcutaneously administered metoclopramide on the gastric motility of a conscious rat. Bar shows duration of infusion (0.105 ml/min) of 50 mM hydrochloric acid. Metoclopramide (Mcp) did not increase motility which had been totally inhibited by acid

short-lived, lasting between 5 and 10 min. By infusing 50 mM hydrochloric acid into the duodenum, much longer-lasting reductions in gastric motility were obtained which lasted for the duration of the infusion. An infusion rate of 0.105 ml/min was found to be satisfactory to reduce motility. Infusion of 0.105 ml/min distilled water did not modify gastric motility. In five out of seven experiments metoclopramide (3 or 10 mg/kg subcutaneously) was found to greatly increase gastric motility, despite the continuing infusion of acid into the duodenum. A typical experiment is shown in Figure 66.2. In the other two experiments metoclopramide did not increase motility after this had been reduced by acid infusion. The effectiveness of the acid in reducing gastric motility varied between animals, and it is noteworthy that both the rats in which metoclopramide was ineffective were extremely sensitive to the acid (for example, see Figure 66.3). It would appear that, provided gastric motility was not abolished, then metoclopramide was effective in preventing the reduced gastric motility resulting from duodenal acidification.

The effects of secretin and metoclopramide on the responses of guinea pig isolated ileum to acetylcholine

In previous experiments (Blower, unpublished) it has been observed that incubation of sections of guinea pig ileum with metoclopramide (1 μg/ml) for periods of 10 min resulted in significant ($p < 0.01$, paired t test) potentiation of responses to only low concentrations of acetylcholine, when compared

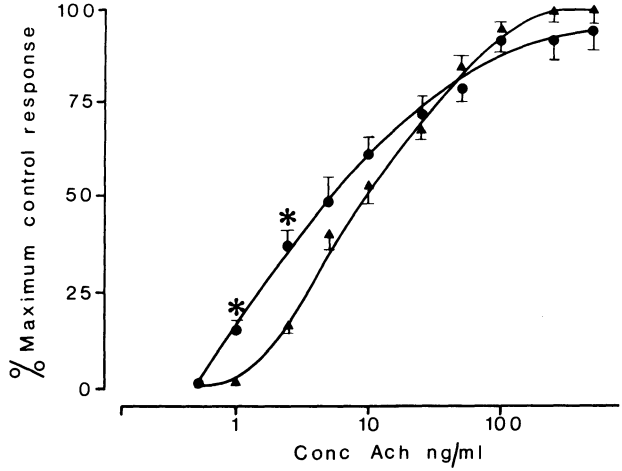

Figure 66.4 The effects of metoclopramide (1 μg/ml) on responses of guinea pig isolated ileum to acetylcholine (Ach). Triangles show 'control' responses to acetylcholine and the circles show responses in the presence of metoclopramide. Responses, which are the mean ($\pm$ SEM) of six experiments, are shown as a percentage of maximum controls. Asterisks denote significant (paired t test, $p < 0.01$) difference from controls. Concentrations of acetylcholine are expressed on a logarithmic scale

to control responses (see Figure 66.4). These results were used as a basis on which to examine the effects of secretin on isolated gut.

Concentrations of less than 50 mU/ml of secretin had no effect on the responses of the ileum to varying concentrations of acetylcholine. However, incubation of the tissue for 2 min with concentrations of 50 mU/ml (12.5 ng/ml) secretin elicited a significant depression of responses to only low concentrations of acetylcholine. After incubation of the tissue for 10 min with metoclopramide (1 μg/ml) and for 2 min with secretin (50 mU/ml), the responses to low concentrations of acetylcholine were enhanced. No depressant effects of secretin were observed in metoclopramide-pretreated tissues, nor was the potentiating action of metoclopramide reduced or enhanced by the presence of secretin (Figure 66.5). After washing the tissue to remove

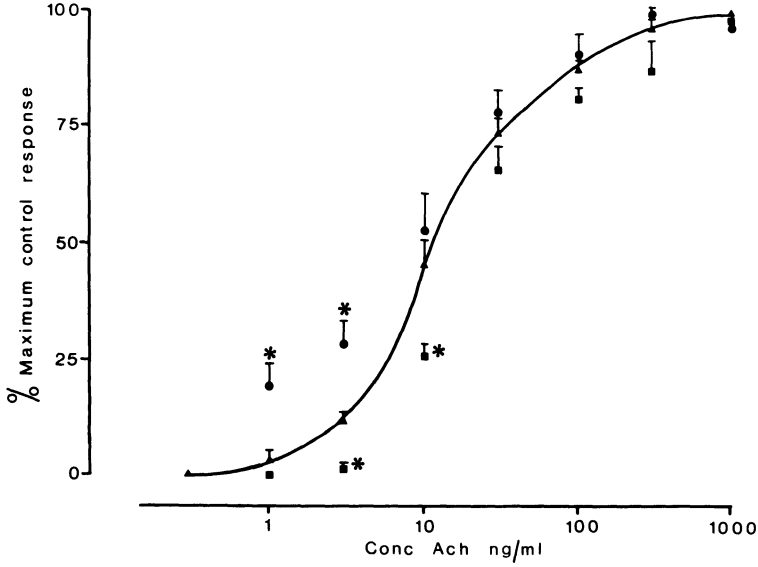

Figure 66.5 The effects of secretin (50 mU/ml) and a combination of secretin (50 mU/ml) and metoclopramide (1 μg/ml) on responses of guinea pig isolated ileum to acetylcholine (Ach). Triangles show 'control' responses to acetylcholine, squares show responses in the presence of secretin (50 mU/ml), and circles show responses in the presence of secretin (50 mU/ml) and metoclopramide (1 μg/ml). Responses, which are the mean ($\pm$ SEM) of four experiments, are expressed as a percentage of maximum controls. Asterisks denote significant (paired t test, $p < 0.01$) difference from controls. Concentrations of acetylcholine are expressed on a logarithmic scale

metoclopramide, secretin was once more observed to depress the magnitude of responses to only low concentrations of acetylcholine. The results of these experiments are also shown expressed in terms of the double-reciprocal concentration response plots (Figure 66.6).

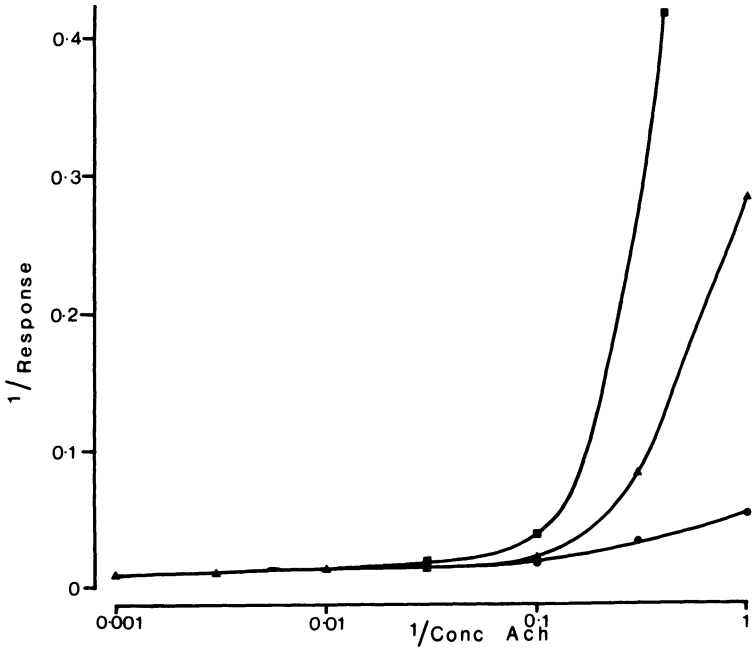

Figure 66.6 Double-reciprocal plot of concentration–response curve to acetylcholine (Ach) of data shown in previous figure (Figure 66.5). Triangles show 'control' responses to acetylcholine, squares show responses in the presence of secretin (50 mU/ml) and circles show responses in the presence of secretin (50 mU/ml) and metoclopramide (1 μg/ml). Each point is the mean of four experiments, and the abscissa is on a logarithmic scale

DISCUSSION

In the conscious fistula rat, metoclopramide was capable of stimulating gastric motility despite the inhibitory effects of simultaneous intravenous infusion of secretin or intraduodenal infusion of hydrochloric acid. Although the specificity of such an action is not known, metoclopramide is not effective in stimulating motility after the administration of some other chemicals (e.g. citrate or sucrose 'meals') which inhibit gastric motor function.

On the guinea pig isolated ileum preparation, the responses to low concentrations of acetylcholine were significantly reduced by the presence of secretin, but in the presence of both metoclopramide and secretin these responses were potentiated. The finding that metoclopramide can potentiate responses to only low concentrations of acetylcholine has previously been reported[9], but apparently not investigated in detail.

When the *in vitro* results with secretin and metoclopramide were expressed on a double-reciprocal plot it was seen that secretin enhanced the deviation from linearity of responses to low concentrations of acetylcholine whilst metoclopramide, despite the presence of secretin, corrected this deviation.

This deviation of the double-reciprocal plot to acetylcholine has been considered as a serious objection to the hypothesis[10,11] of a quantitative relationship between agonist and receptor. However, the finding that H_2-receptor antagonists can correct the deviations in linearity of these plots to histamine on the isolated ileum[12] has suggested that, in the case of histamine, the deviation results from activation of inhibitory H_2-receptors rather than reflecting true agonist–receptor interaction.

Since there are no suggestions in the literature of metoclopramide possessing H_2-receptor blocking properties, and we have failed to uncover any such actions in our laboratories (J-P. Terry, unpublished observations) the inhibitory pathway which apparently causes deviation of the double-reciprocal plot to acetylcholine would not appear to be histaminergic.

In view of our previous observations of the opposing actions of secretin and metoclopramide at cardiovascular dopamine receptors, we conclude that these results support, but do not prove, the existence of inhibitory dopamine receptors in the gut which limit the sensitivity of the smooth muscle to low concentrations of agonists.

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Discussion

D. L. Wingate: (UK) We have studied the effects of metoclopramide and secretin separately on the myoelectrical activity of fasting conscious dogs. In general, we found that metoclopramide stimulates, and secretin depresses, activity; but our doses are very different. We need more secretin, but much less metoclopramide, to get an effect. Can you comment on this?

P. Blower: (UK) We have used the conscious rat model for some time and have found that the effect of metoclopramide on gastric motility is of an 'all-or-none' type. Although the threshold active dose of metoclopramide is about 0.5 mg/kg subcutaneously, we routinely use 3–10 mg/kg subcutaneously in untreated rats, to be sure of evoking a response. Thus the dose of metoclopramide used in these rats after treatment with secretin or hydrochloric acid was no greater than that required for untreated rats.

R. W. McCallum: (USA) There are a number of interactions in man of metoclopramide and L-dopa where you could substitute secretin for L-dopa, e.g. metoclopramide is a potent stimulant of prolactin in man, and L-dopa inhibits this; L-dopa decreases gastric emptying whereas metoclopramide overcomes this; and metoclopramide increases lower oesophageal sphincter pressure and there is evidence of L-dopa inhibition of this rise in sphincter pressure.

Blower: I quite agree that it would be very interesting to try secretin on other, more fully established, dopaminergic systems and we intend to carry out such studies. One possible problem, however, is that our evidence at present (based on cardiovascular dopamine receptor studies) points towards secretin having an indirect rather than a direct dopamine agonist effect. It may act through release of dopamine, and this is another aspect which we hope to clarify.

J. DeCarle: (Australia) Since you are interested in dopaminergic receptors, have you tried applying dopamine to your preparation?

Blower: We have used dopamine, but in view of its α- and β-adrenoreceptor stimulant properties we pre-treated the tissue with a combination of phentolamine and propranolol. This combination caused considerable stimulation of spontaneous activity of the tissue by itself, and although dopamine had no inhibitory effect on the spontaneous activity its possible effects on responses to low concentrations of acetylcholine could not be determined because of spontaneous activity masking such responses.

E. E. Daniel: (Canada) Have you tried selective antagonists to dopamine to test your hypothesis of inhibitory dopamine receptors decreasing sensitivity to low doses of acetylcholine?

Blower: The problem here is one of specificity. Phenothiazines have antihistamine and α-adrenoreceptor blocking actions. Haloperidol, which

we have tried, has an α-adrenoreceptor blocking action and causes such stimulation of spontaneous activity that contractile responses to low concentrations of acetylcholine are masked. Pimozide, of course, is a potent atropinic drug, and it blocks acetylcholine responses. As far as I am aware, metoclopramide is more specific than many other dopamine antagonists.

67

Effects of bile salts and prostaglandins on colonic motility in the rabbit

J. DIANE FALCONER, A. N. SMITH AND M. A. EASTWOOD

Following the ingestion of food there is an increase in motor activity throughout the gastrointestinal tract, originally attributed to a neural mechanism by Pavlov in 1910[1]. The persistence of the phenomenon following the destruction of the spinal cord raises the possibility that a humoral mechanism might be involved. The intravenous injection of gastrin has been shown to stimulate small bowel and colonic motility. Similarly cholecystokinin has been shown to increase the intestinal transit rate[2]. Both gastrin which has a choleretic action and cholecystokinin which empties the gall bladder could exert these effects through the agency of bile, which has been shown to stimulate intestinal motor activity[3]. Exaggerated colonic motility is a feature of cholerrhoeic enteropathy in patients in whom the output of bile acids is markedly elevated. Part of this response could be due to the secretory and the exudative effects known to be produced by bile salts from the colonic mucosa[4].

METHODS

In order to elucidate whether there is a direct action of bile acids on the motility of the colon a series of animal experiments were designed. These took the form of acute experiments in rabbits anaesthetized with a mixture of halothane and nitrous oxide. The abdomen was opened and the terminal ileum tied to prevent the further passage of bile into the colon. A pressure-recording tube was passed into the rectum to 15 cm. Motility was recorded from two sites 5 cm apart, at 15 and 10 cm from the anal verge. The results are expressed as a colonic motility index (MI), obtained by taking the mean of the peak heights in mmHg and multiplying it by the percentage of time of

wave activity. In the tests, 5 ml boluses of bile acids, sodium glycocholate and sodium deoxycholate were instilled into the bowel, in concentrations ranging from 3 to 30 mM respectively.

These are the main bile acids present in the rabbit gastrointestinal tract and represent primary and secondary bile acids respectively. In a separate series of experiments prostaglandin E_2 was infused into the sigmoid colon under similar conditions.

Comparisons of the action of bile acids were made with detergents of three main types, and with control volumes of normal saline and of water. Experiments were also performed *in vitro* on isolated rabbit large and small intestine. Tissue was suspended in a glass organ bath filled with warmed Tyrode solution (37 °C) and aerated with 95% O_2 + 5% CO_2. Isotonic contractions were recorded from the longitudinal and from the circular muscle layers of the rabbit gut respectively. Any mechanical displacement produced by the gut was recorded on a potentiometer via a mechanical transducer. The tissue was allowed to equilibrate for an hour before the application of any drug agents to the Tyrode solution; the tissue was then primed with the cholinergic drug carbachol until a reproducible dose–response relationship was established. Six-minute cycles were used for the addition and wash-out of various drugs under test.

RESULTS

The effects of sodium glycocholate (Na GC) infused into the sigmoid colon of the anaesthetized rabbit

Pre-infusion and post-infusion motility indexes were calculated after the administration of varying concentrations (9–24 mM) of the primary bile acid sodium glycocholate. From the results in Table 67.1, it is seen that sodium glycocholate produced only minimal changes in the motility of the sigmoid colon. The changes in the motility indexes did not differ significantly from those produced either by distilled deionized water or isotonic saline.

Table 67.1 Infusion of 5 ml Na GC into the sigmoid colon of the rabbit

Concentration (mM)	*Pre-infusion MI*	*Post-infusion MI*	*Change in MI*
9	363	446	83
15	113	98	−15
15	248	269	21
24	205	229	24
24	101	109	8
Mean	206 ± 107	230 ± 142	24 ± 36
	5 ml water and isotonic saline (n = 6)		
Mean	84 ± 46	86 ± 57	2 ± 37

Not significant

The effects of sodium deoxycholate (Na DC) infused into the sigmoid colon of the anaesthetized rabbit

The post-infusion motility indexes resulting from the infusion of 5 ml of Na DC (3–30 mM) were significantly different from the pre-infusion motility indexes ($p < 0.001$). The changes in motility indexes produced by Na DC (mean 285 $\pm$ 211) were significantly different from those produced by control infusions of distilled deionized water or isotonic saline (mean 2 $\pm$ 37, $p < 0.01$). In every experiment, infusion of Na DC produced an increase in the motility index. The results of these experiments are shown in Table 67.2. The motor effect of Na DC occurred in 5.4 $\pm$ 2.9 min and had a duration of 2–3 h.

Table 67.2 Infusion of 5 ml Na DC into the sigmoid colon of the rabbit

Concentration (mM)	*Pre-infusion MI*	*Post-infusion MI*	*Change in MI*
3 ($n = 1$)	129	182	53
6 ($n = 4$)	313 $\pm$ 136	433 $\pm$ 211	120 $\pm$ 97
9 ($n = 1$)	152	185	32
12 ($n = 1$)	79	197	118
18 ($n = 1$)	234	354	120
24 ($n = 24$)	135 $\pm$ 104	488 $\pm$ 205	353 $\pm$ 205
30 ($n = 1$)	398	522	124
Mean	166 $\pm$ 123	451 $\pm$ 206	285 $\pm$ 211
Onset time: 1–10 min; mean 5.4 $\pm$ 2.9 min Duration: 2–3 h			
	5 ml water and isotonic saline ($n = 6$)		
Mean	84 $\pm$ 46	86 $\pm$ 57	2 $\pm$ 37
			$p < 0.01$

Effects of Na DC on the activity of isolated rabbit intestinal tissue

The effect of Na DC was tested on the spontaneous activity and on a cholinergic stimulus given to the rabbit intestine. The results are summarized in Table 67.3. Carbachol almost uniformly contracted jejunal and colonic tissue, and acted in a like manner on both longitudinal and circular muscle. Na DC concentration 2×10^{-7} mM, relaxed the longitudinal muscle of the jejunum, and produced no effect on colonic tissue, whether longitudinal or circular muscle. Furthermore, it produced a partial inhibition of the carbachol response when applied to both jejunal and colonic muscle. This is seen in Figure 67.1 where the contractile effect of 8×10^{-9} moles of carbachol was reduced by 19.2×10^{-6} moles of Na DC. Following the administration of the Na DC, it required four successive administrations of carbachol before there was complete return of the contractile effect of this substance. Na DC was therefore without a direct motor action on the isolated rabbit colon.

Table 67.3 Effect of carbachol and Na DC on the activity of isolated rabbit tissue

Muscle	*Carbachol* 10^{-10}—10^{-11} mM		*Na DC* 2×10^{-7} mM	*Na DC + carbachol*	
Jejunum ($n = 15$) Longitudinal muscle	13 ↑	2–	4 ↓	4*	
Jejunum ($n = 2$) Circular muscle	2 ↑				
Colon ($n = 26$) Longitudinal muscle	24 ↑	2–	10–	8*	2–
Colon ($n = 3$) Circular muscle	3 ↑		3–	2*	1–

↑ = Contraction; ↓ = relaxation; – = no effect; * = partial inhibition of carbachol

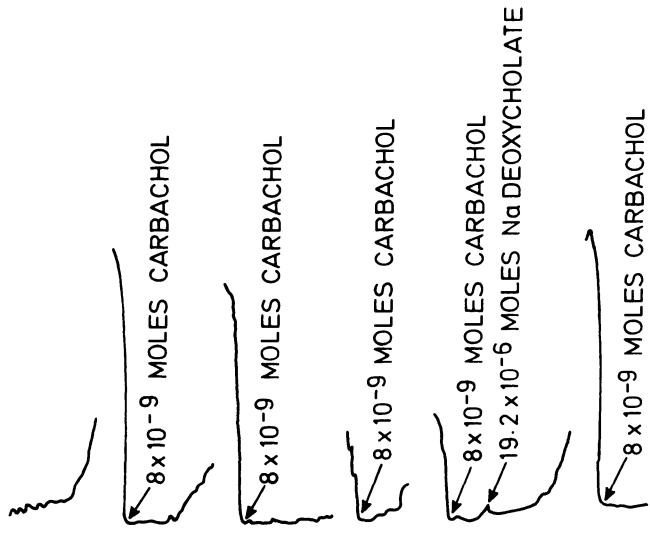

Figure 67.1 Isotonic contractions produced by 8×10^{-9} moles of carbachol are compared both prior to and following the addition of 19.2×10^{-6} moles of Na DC to the organ bath. The contractile action of carbachol is partially abolished, returning to its initial level after three subsequent administrations. (Trace reads from right to left)

Nevertheless, it had been shown to be capable of stimulating the *in vivo* preparation. This suggested the possible intermediary release of an active agent in the wall of the bowel *in vivo*. Since Na DC actively antagonized cholinergic activity, it did not appear likely that the transmitter substance for this effect would be acetylcholine. Prostaglandins have been shown to be present in the intestinal tissue of many species. In view of the evidence that prostaglandins of the E series are capable of exhibiting a stimulatory action on isolated intestinal tissue of the guinea pig, rat and human[5,6], together with the theory that prostaglandins may play a role in maintaining smooth muscle tone of the rabbit isolated jejunum[7], it seemed possible that a prostaglandin could be

the intermediary agent necessary for Na DC to produce stimulation of the rabbit colon *in vivo*.

The action of prostaglandin E_2 on the rabbit sigmoid colon was subsequently examined.

The effect of prostaglandin E_2 (PGE_2) infused into the sigmoid colon of the anaesthetized rabbit

Prostaglandin E_2 was infused into the lumen of the colon in the range 400–800 μg, and produced a marked increase of the motility index. The changes in MI produced by PGE_2 (mean 458 $\pm$ 238) differed significantly from those produced by control administrations of water and isotonic saline (mean 2 $\pm$ 37, $p < 0.001$) (Table 67.4). The onset time was 1–5 min, with a mean of 2.5 $\pm$ 1–8 min, and the average duration was 2 h.

Both PGE_2 and Na DC had a stimulatory action of long duration when infused into the sigmoid colon of the anaesthetized rabbit. The possibility that Na DC may act via the release of PGs was examined using the prostaglandin synthesis inhibitor, indomethacin. The action of Na DC was compared when given both before and after the intravenous administration of indomethacin (2 mg/kg) in individual rabbits.

Table 67.4 Infusion of PGE_2 into the sigmoid colon of the rabbit

Dose infused (μg)	*Pre-infusion MI*	*Post-infusion MI*	*Change in MI*
400	0	450	450
400	0	214	214
800	0	380	380
800	206	680	474
800	28	354	326
800	137	1042	905
Mean	62 $\pm$ 88	520 $\pm$ 298	458 $\pm$ 238
Onset time: 1–5 min; mean 2.5 $\pm$ 1.8 min Duration: 2 h			
	Water and isotonic saline ($n = 6$)		
Mean	84 $\pm$ 46	86 $\pm$ 57	2 $\pm$ 37
			$p < 0.001$

Pressure recordings before and after infusion of 24 mM Na DC into the sigmoid colon of the anaesthetized rabbit in relationship to indomethacin administration

Figure 67.2 shows a typical motility record from an animal whose basal motor activity (A) was stimulated by the administration of Na DC. The intravenous administration of indomethacin (B) in a dose of 2 mg/kg markedly reduced the effect of a further infusion of Na DC. Pre-treatment with

indomethacin therefore diminishes the stimulatory effect of the main faecal bile acid of the rabbit. This suggests that the stimulatory action of Na DC may be due to the release of prostaglandins in the wall of the colon.

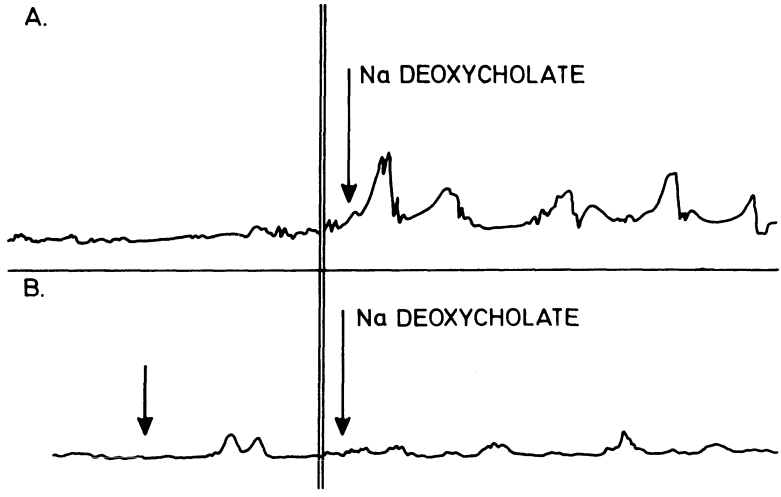

Figure 67.2 Intracolonic pressure recording from an anaesthetized rabbit showing: (A) basal pressure altered at arrow by the infusion of 5 ml of 24 mM Na DC into the sigmoid colon, producing stimulation of colonic motility; (B) after the return of basal conditions, indomethacin (2 mg/kg) was administered intravenously at arrow on the left-hand side, followed by a repeat infusion of Na DC as above. The motor stimulation was markedly reduced

Effects of detergents on colonic motility

In view of the action of bile acids as detergents, and to determine what proportion of the above motor effects were related to this physical property, comparisons were made of the action of Na DC and the anionic, cationic and non-ionic surfactants listed in Table 67.5. All increased the motility index, with an onset time of 2–4 min and a duration of 1–2 h. The changes in MI produced by these surfactants (mean 132 $\pm$ 85) differed significantly from those produced by control solutions of water and isotonic saline (mean 2 $\pm$ 37, $p < 0.001$).

The stimulant effect of one of these, namely Tween 80, was examined and shown to be markedly reduced in animals which had been pre-treated with intravenous indomethacin (2 mg/kg).

DISCUSSION

The primary bile acid, Na GC, which is not normally present in the colon of

Table 67.5 Infusion of surfactants and control solutions into the sigmoid colon of the rabbit

Infusion	*Mean pre-infusion MI*	*Mean post-infusion MI*	*Mean change in MI*
Surfactants ($n = 7$) Anionic, Cationic and Non-ionic*	120 ± 87	252 ± 124	132 ± 85
Controls ($n = 6$) (Water and isotonic saline)	86 ± 46	84 ± 46	2 ± 37
			$p < 0.001$

Onset time: 2–4 min; duration 1–2 h

* Anionic surfactant – Na-n-Octanoate (24 mM); cationic surfactant – cetylpyridinium chloride (24 mM); non-ionic surfactant – Tween 80 (1% solution)

the rabbit, was without effect when infused into the sigmoid colon of the anaesthetized rabbit.

In comparison, the secondary bile acid, Na DC, which is the main bile acid found to be present in the colon of normal rabbits (mean ($n = 10$) 0.119 ± 0.072 mMoles) stimulated colonic motility when infused into the sigmoid colon of anaesthetized rabbits. The infusion of 5 ml of 24 mM Na DC resulted in the instillation of 0.120 mMoles Na DC in the sigmoid colon, which would have been equivalent to the entire bile acid content of the large bowel entering the sigmoid colon.

However, when examined on *in vitro* preparations, Na DC did not have any effect on the spontaneous activity of the isolated colon, but did exhibit a partial inhibition of the contractile response produced by a cholinergic stimulus.

The disparity between the *in vivo* and *in vitro* effects suggested the existence and release by Na DC of a possible intermediary factor *in vivo*. PGE_2 was examined as a possible candidate for this role.

Intraluminal infusions of PGE_2 were found to produce a comparable stimulatory action on the motor activity of the sigmoid colon of the anaesthetized rabbit, with a latency and duration of action similar to that of Na DC.

The stimulatory action of Na DC was shown to be reduced after pretreatment of each animal with intravenous indomethacin which is known to block the synthesis of prostaglandins[8–10].

Intravenous indomethacin had no such effect on the stimulatory response following the infusion of exogenous PGE_2 into the sigmoid colon; so it is unlikely that indomethacin was having any direct effect on the muscle.

As indomethacin itself was without apparent effect on the pre-infusion resting motility, it was thought that this reduction in the increase of the motility response to Na DC was due to indomethacin preventing the response to Na DC, and not by acting via a reduction in the pre-infusion resting motility.

These results suggest the possible release, by Na DC, of prostaglandins *in vivo*, resulting in an increased motility of the sigmoid colon.

Whatever the precise mechanism of action of Na DC in eliciting this motility response, the possibility that the response was due to its surfactant properties was investigated.

When either of the surfactants, Na-n-octanoate (anionic), cetylpyridinium chloride (cationic) or Tween 80 (non-ionic), were infused into the sigmoid colon of the anaesthetized rabbit, an increase in colonic motility occurred with a latency and duration of action similar to that of Na DC. In addition the motility response produced by Tween 80 was reduced in animals which had been pre-treated with indomethacin, suggesting that this compound also exerts its action via prostaglandin release.

CONCLUSIONS

From the results it would appear that the secondary bile acid, Na DC, is capable of increasing the motility index of the rabbit sigmoid colon *in vivo* and that this effect may be due to its surfactant properties. These studies suggest that its mechanism of action may be via the secondary release of prostaglandins in the wall of the colon. A similar mechanism is postulated for the action of Tween 80.

Acknowledgement

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Discussion

C. F. Code: (USA) Did you measure the secretory response of the colon to the bile? Increased volume of contents may have been the cause of increased motility of the colon after the introduction of bile. I would not have expected bile to have a direct effect on the smooth muscle.

M. A. Eastwood: (UK) It occurred to me that an increased volume of colonic secretion might be a factor in the stimulation of colonic activity. Thus we tested cumulative volumes of H_2O and saline infused into the sigmoid, as controls, in the range 5–75 ml; there was no significant change in the motility response. It was because of the possibility of the motor response being provoked by an intermediary, not by bile itself, that we took up the relationship of the bile 'effect' and possible release of prostaglandins.

R. W. McCallum: (USA) Schoenfield and Coyne have shown that the secretory effect of bile salts on colonic mucosa (adenyl cyclase mediated) can be inhibited by propranolol. Have you tried to block the colonic motility in your study with propranolol?

Eastwood: Propranolol was mainly administered by us after the blocking action of indomethacin on the bile acid response had been established. Under these conditions, propranolol should reverse the blocking effect of indomethacin if this was an adrenergic effect. Propranolol did not directly inhibit the bile acid response.

C. E. Pope: (USA) 24 mM bile salt is a rather damaging concentration. Were there histologic changes in the colon, and were they absent in the colon whose motility was decreased by indomethacin?

Eastwood: There are histological changes in the colon but the degree was variable, whether or not indomethacin was given.

M. Pescatori: (Italy) Did you observe which was the type of motor activity mainly increased by bile salts *in vivo*, whether propulsive or segmental?

Eastwood: The mean effect appeared to be segmental, though not exclusively so. For example, some propulsive waves were followed by spontaneous defaecation.

E. E. Daniel: (Canada) Indomethacin does not act specifically by inhibition of PG synthesis. Do you have any evidence that it was selective or specific? Did it inhibit responses to prostigmine, prostoglandins, acetylcholine, etc?

Eastwood: Exogenous prostglandin infused into the sigmoid increased motility both in the presence and absence of indomethacin. This implies that the slight reduction in the motility response when the bile acids are infused into the sigmoid in the presence of indomethacin was due to indomethacin acting as a PG synthesis inhibitor rather than a direct inhibitory action on the muscle. We accept that this is not foolproof, however.

A. M. Connell: (USA) Did your last slide (Figure 67.2) imply that indomethacin inhibited the colonic response to prostigmine?

Eastwood: Indomethacin also reduced the response to prostigmine; the change in the bile acid responses after indomethacin may in part be effected through a change in basal tone, which may be cholinergic and/or prostaglandin-mediated.

A. Bennett: (UK) In reply to Dr Daniel's query, there is considerable evidence that prostaglandins can modulate responses to cholinergic nerve stimulation, thus increasing them. This could explain why indomethacin reduced the motor effects of anticholinesterase.

68

Evidence suggesting prostaglandins mediate lower oesophageal sphincter incompetence associated with inflammation

F. BROWN, B. BECK, R. FLETCHER, D. CASTELL AND G. EASTWOOD

At this meeting 2 years ago we showed that oesophagitis could be created in cats by infusing hydrochloric acid into the animal's lower oesophagus. This oesophagitis markedly decreased the resting pressure of the lower oesophageal sphincter (normally abbreviated to LES). Furthermore the response of the inflamed LES to various pharmacological stimuli has been described[1]. This paper reports results of our attempts to prevent the oesophagitis with indomethacin.

METHODS

Oesophageal manometry

Two groups of five cats each were studied. The cats were anaesthetized with 15–20 mg/kg of intramuscular ketamine HCl. A two-lumen oesophageal motility catheter marked at 1 cm intervals was passed through a hole in a wooden bite block into the stomach. The catheters were perfused with water via a Harvard pump at 1.0 ml/min. Sphincter pressure was measured during a slow pull-through. After three pull-throughs the highest pressure was selected as the basal LES pressure. An injection of 100 μg/kg edrophonium was given intravenously. Three more pull-throughs were made between 30 s and 5 min after the injection. Again the highest pressure was selected as the maximum response to edrophonium. All tracings were coded and read in a blind fashion. The motility catheter was removed and placed adjacent to a Quinton multi-purpose biopsy tube. The biopsy capsule was then passed to a point

2 cm above the manometrically defined LES and a suction oesophageal biopsy was obtained.

Study design

Both LES pressures and oesophageal biopsies were obtained on each cat as a baseline (Day 0). On days 1–4, all cats received a 0.1 N HCl infusion into the oesophagus 5 cm above the LES at 1 ml/min as described previously[1]. One group of cats received indomethacin 75 μg/kg intravenously twice daily on days 1–4. Basal LES pressures, response to edrophonium, and oesophageal biopsies were obtained again on day 5 in the same manner as on day 0. All of the biopsies were coded and read by one of us (G.E.) in a blind fashion. Statistical analysis was accomplished by application of Student's t test for paired data.

RESULTS

Since our previous studies had shown the LES response to edrophonium to be markedly decreased 1 day after the end of the infusion period[1], we elected to accentuate any protective effect of indomethacin by measuring both basal LES pressure and response to edrophonium. Figure 68.1 shows the mean LES pressures before (day 0) and after (day 5) the oesophageal acid infusion in both study groups. There was no difference between basal LES pressure or response to edrophonium in the two groups of cats on day 0. By day 5 mean basal LES pressure fell from 28 to 11 mmHg and the response to edrophonium decreased from 62 to 15 mmHg in the control group. In contrast, there was no significant change between LES pressures before (day 0) and after (day 5) the acid infusion in the group of cats treated with indomethacin, including both basal pressure and response to edrophonium. The differences between both basal LES pressure and response to edrophonium between the two groups on day 5 is significant ($p < 0.05$).

In the control group all oesophageal biopsies from day 5 showed submucosal oedema and neutrophilic infiltrate identical to that described in the previous report[1]. However all the biopsies from day 5 in the indomethacin-treated group were normal, showing none of the oedema or inflammatory cells.

DISCUSSION

Heartburn is a common clinical symptom frequently associated with signs of oesophageal inflammation and decreased LES pressure[2]. The inflammation is presumably caused by reflux of gastric acid into the oesophagus, but the cause of the decreased LES pressure is unknown. Our previous work with the cat model of oesophagitis showed that a normal feline LES could be damaged

by intra-oesophageal acid infusion. Mean basal LES pressure fell by an average of about 60%[1]. The mediator of the fall in pressure was unknown. Previous work[1] showed that the inflamed LES responded normally to bethanechol, a direct smooth muscle stimulant, and the lower oesophageal smooth muscle was histologically normal. Thus a direct effect of acid on smooth muscle seemed unlikely. It has been shown in man[3] and the opposum[4] that intravenous infusion of prostaglandin E can cause marked decreases in LES pressure. We hypothesized that prostaglandins accumulated in the inflamed lower oesophagus and decreased LES pressure. Because indomethacin has been shown to inhibit prostaglandin synthesis[5], we treated one group of cats with a very low dose of indomethacin to minimize its other anti-inflammatory effects. As might be expected, the oesophageal biopsies showed that the indomethacin, a potent anti-inflammatory agent, prevented the acute inflammatory infiltrate found in the control animals. However, as is shown in Figure 68.1, indomethacin also prevented the fall in LES pressure, both basally and in response to cholinergic stimulation. Although this evidence is hardly a rigorous proof of the involvement of prostaglandins in oesophagitis, it does suggest that they play a role in the LES hypotension commonly found in patients with reflux oesophagitis.

In conclusion, we have confirmed the previous observation that oesophagitis could be created in the cat by the simple technique of infusion of acid into

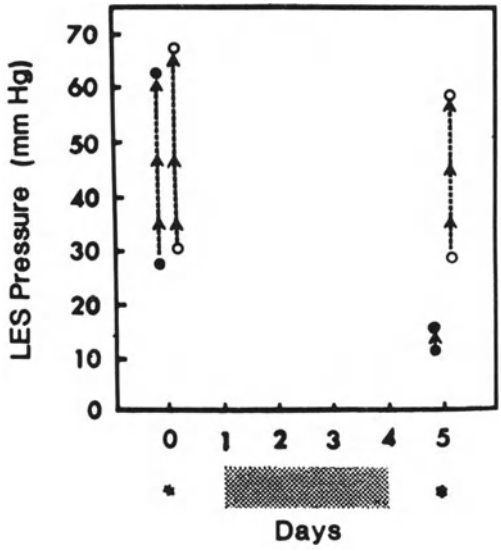

Figure 68.1 Basal and edrophonium-stimulated LES pressures are shown before (Day 0) and after (Day 5) the acid infusion. The period of acid infusion is indicated by the black rectangle. The lower values of each pair are basal LES pressures. The higher values of each pair are the maximum pressures produced by 100 μg/kg intravenous edrophonium. Control animals are shown in solid circles. indomethacin-treated animals are shown in open circles

the oesophagus, and that this oesophagitis was associated with a decrease in sphincter pressure and an attenuated response to edrophonium. Indomethacin therapy prevented the acid-induced inflammatory infiltrate and also preserved the normal function of LES smooth muscle. A role for prostaglandins in this model was suggested by the ability of a very low dose of indomethacin to prevent the adverse effect of inflammation on the LES.

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Discussion

W. Silber: (S. Africa) What is the status of the mucous glands in the cat oesophagus? This may be important because in man the most significant factor preventing oesophagitis is the presence or absence of the mucous gland. In the neonate and in the geriatric patient (in both groups oesophagitis may be severe) the mucous glands are deficient. The status of the cat oesophagus may be an important mitigating factor.

B. Beck: (USA) We have not investigated the mucous glands in the inflamed cat oesophagus.

A. G. Johnson: (UK) Have you tried the effect of indomethacin *after* inflammation has been produced? Clinically, one would have to be cautious of using it in established oesophagitis because of the risk of bleeding, which certainly occurs in patients with gastritis.

Beck: We are currently evaluating the effect of indomethacin in healing established oesophagitis, and results are not available.

R. W. McCallum: (USA) It is known that prostaglandins are released non-specifically by inflammatory tissue. Since indomethacin increases LES pressure anyway, this study could be explained on this basis alone – not necessarily involving prostaglandin inhibition. A method to overcome this confusion would have been indicated to use metoclopramide or bethanechol as control agents, which are not thought to inhibit prostaglandin synthesis, and then see if sphincter integrity was preserved with these agents. As it stands now, without prostaglandin levels in the oesophageal mucosa or the oesophagitis milieu, it is only an interesting hypothesis. In addition, oral 16, 16-dimethyl prostaglandin E_2 in doses up to 1.5 μg/kg will not decrease LES pressure in man.

Beck: We feel that the effect of indomethacin on the LES is of very little significance. The acid was infused directly into the oesophagus 5 cm above the LES. Also LES pressures on day 5 were obtained almost 20 h after the last dose of indomethacin was given. As for the inactivity of the oral prostaglandin, this does not worry me. This seems an unphysiological way to administer the agent and intravenous prostaglandin E is capable of markedly decreasing LES pressures.

K. Kelly: (USA) Another control you might consider would be to infuse acid into the stomach, rather than into the oesophagus.

Beck: We have infused 0.1 N HCl into the stomach of several cats and infused normal saline into the oesophagus, and have seen no consistent effect on either LES pressure or oesophageal histology.

A. Bennett: (UK) The finding that indomethacin increases the lower oesophageal sphincter tone does not necessarily indicate a non-specific effect of the drug. At the present time it seems reasonable to argue that prostaglandin release within the sphincter normally tends to relax it, and that increased prostaglandin release relaxes the sphincter further. The

importance of the finding with indomethacin in conscious human subjects is that this is the only evidence of its type that prostaglandins normally contribute to motility within the gut. Since damage to tissues readily releases prostaglandins, effects observed *in vitro* or in anaesthetized animals may not reflect a physiological mechanism.

69
Hydrogen ions inhibit jejunal flow

R. W. SUMMERS

The objectives of the study being reported were:

(a) To study simultaneously the flow of solutions, the myoelectrical and mechanical activity in the jejunum of conscious dogs.
(b) To test the hypothesis that intraluminal acid solutions inhibit intestinal flow by stimulation of intestinal motility.

METHODS

Materials

We studied eleven dogs weighing 18–20 kg. A laparotomy was done under anaesthesia and an inlet Thomas cannula was placed in the jejunum, 10–15 cm beyond the ligament of Treitz. An outlet Thomas cannula was placed 45 cm caudad to the inlet cannula. The loop of intestine between the cannulas is called the study segment. Bipolar silver electrodes were sutured to the serosal surface at each of three sites: a pre-segmental electrode 10 cm proximal to the inlet cannula, a segmental electrode midway between the cannulas, and a post-segmental electrode 10 cm distal to the outlet cannula. A coil of silver wire, tunnelled under the peritoneum, served as a reference electrode. A strain gauge was sutured adjacent to the segmental electrode in several dogs[1]. The axis of the gauge was oriented to record circular muscle contractions. The lead wires were brought through the abdominal wall by a stab wound, tunnelled subcutaneously to the interscapular region, and terminated in a Cannon plug. The dogs were allowed to recover for at least 7 days before studies began.

Procedures

The dogs were fasted at least 15 h so that the study segment would not

contain food residue. They stood in harnesses in which holes were cut for the cannulas so that the level of the cannulas would remain constant during experiments. Prior to the study, a specially designed insert was then placed in the inlet cannula as shown in Figure 69.1. The balloon of the orally-directed Foley catheter prevented entry of secretions into the study segment, but distended it only minimally. The balloon also prevented reflux of perfusate into the pre-study segment. The inner catheter provided drainage of proximal secretions. The aborally directed catheter provided entry for the perfusate.

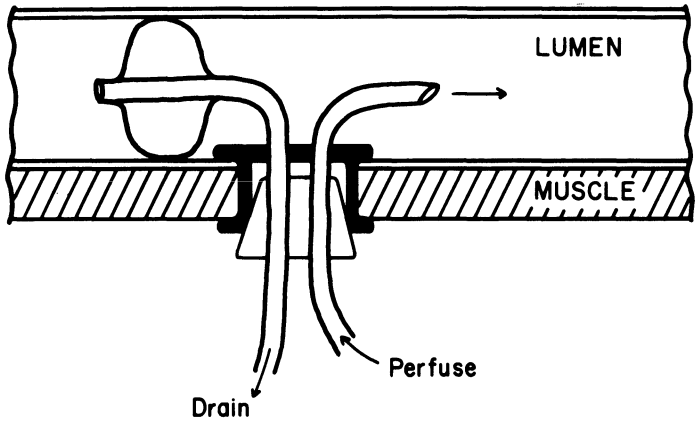

Figure 69.1 Diagram showing insert placed in inlet Thomas cannula 10 cm distal to the ligament of Treitz. The inflated balloon of the 16 Fr. Foley catheter is oral and the straight catheter aboral.

The inflow water pressure could be adjusted before the experiment but was kept constant during the experiment by a gravity flow perfusion system (Figure 69.2). The perfusion reservoir was constantly replenished with perfusate from a measured supply reservoir, and an overflow tube maintained a constant height and pressure head. The inflow rate was defined as the volume difference in the supply reservoir measured at 5-min intervals. A circulating heating pump warmed the perfusate to 37 °C via a water jacket. The perfusate drained from the outlet cannula.

The electrical and contractile activity of the small intestine was monitored using a Beckman R 411 Dynograph with combined voltage-force couplers (No. 9853A). For electrical recordings, the high-frequency filter was set at 30 Hz.

The migratory myoelectric complex (MMC) was usually well established at the beginning of each experiment (Figure 69.3). One hundred calories of an elemental diet (Vivonex), infused into the outlet cannula, usually intert rupted the MMC. The height of the perfusion reservoir was adjusted above the inflow cannula in order to produce inflow rates of the control solution

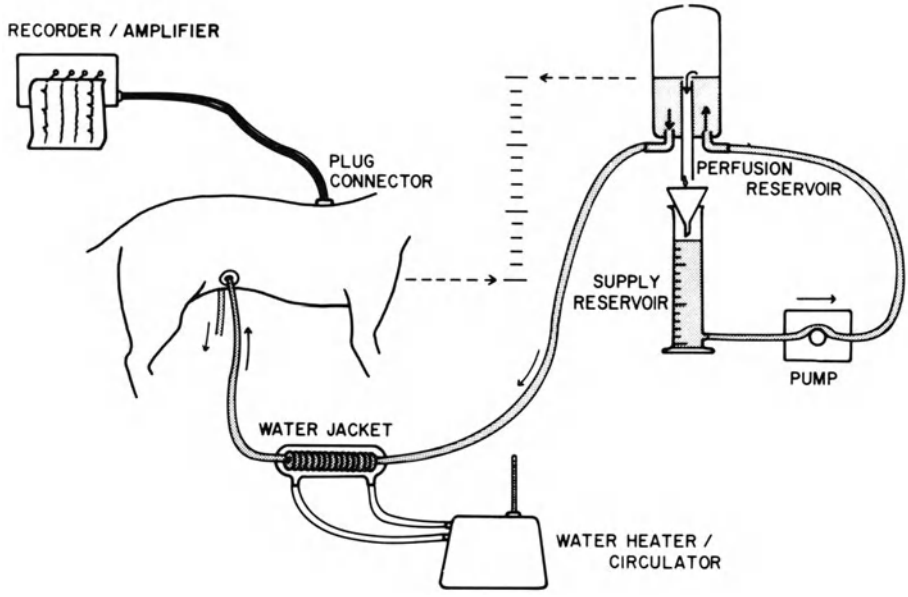

Figure 69.2 Schematic diagram of gravity flow perfusion system. The heater and water jacket warm the perfusate to 37 °C. The graduations indicate the height of the column above the inlet cannula

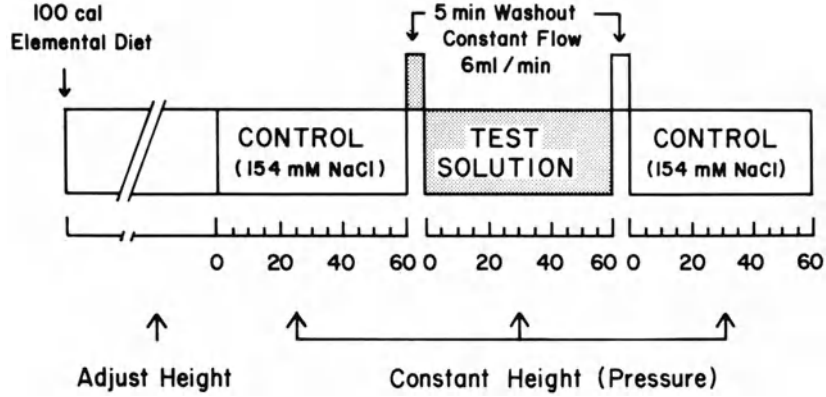

Figure 69.3 Study design. The elemental diet enteres the distal cannula. The height of the perfusion reservoir is adjusted over a 15–30-min period to produce a flow into the inlet cannula of about 6 ml/min of 154 mM NaCl. Each solution is perfused at that same height for 60 min periods with two exceptions. The last two periods are preceded by a 5-min washout using a constant flow pump at 6 ml/min. At 5-min intervals the volume inflow, the number of spike bursts, and the sum of the amplitudes of the contractions are measured.

of 4–8 ml/min. The height varied from 12 to 18 cmH_2O from day to day, but a constant height was maintained throughout the remainder of the experiment.

The control 154 mM NaCl solution was perfused for 1 h followed by a

washout period during which the subsequent test solution was perfused by a pump at a constant volume of 6 ml/min for 5 min. The washout by pump was necessary to assure that the subsequent solution entered the study segment; inflow rates were sometimes very low by gravity flow. The test solution was then allowed to flow at the same pressure as the initial control solution for 1 h. The control solution was then perfused, first by pumping at a constant rate of 6 ml/min for 5 min, then by the gravity flow system at the initial height (pressure).

Solutions

We chose 154 mM NaCl as the control solution for all of the studies. The test solutions were 90 mM NaCl, 50 mM NaCl, 0.1 N HCl, 0.025 N HCl and 0.025 N HCl plus 130 mM NaCl. Osmolalities of the perfusion solutions were checked with an Osmette Osmometer by freezing point depression.

Analyses and statistics

The slow-wave frequency did not change significantly from control with any of the test solutions. Spike bursts were counted if there were two or more rapid transients above and below the baseline and if the amplitude was greater than 20% of full-scale deflection. The sum of the spike bursts occurring in each 5-min period was recorded. The strain gauges were calibrated prior to surgical implantation according to the method of Jacob[2]. Each elevation of the baseline of more than 5 g not accounted for by artifact was measured and cumulated for each 5-min period by a simple map mileage marker as the motility index. The mean of the 5-min values from each 1 h test period was subtracted from the mean of the 5-min values from the corresponding initial 1 h control period. A mean change from control was calculated for each test solution for flow-rates, spike burst frequency and motility index. We used the unpaired t test to compare solutions.

RESULTS

At constant pressure the inflow rate of 154 mM NaCl gradually rose over the 3-h period of study (Figure 69.4). This was associated with a slight, but not significant, increase in the frequency of spike bursts recorded from the pre-segmental, segmental, and post-segmental electrodes. When 90 mM NaCl was substituted for saline during the second hour, the mean inflow rate remained more constant. The comparison of mean change in inflow from control for 154 mM NaCl and 90 mM NaCl was not significant ($p = 0.52$).

With 0.1 N HCl as the test solution, we observed a marked reduction in flow-rate (Figure 69.5). In several experiments, the inflow stopped completely during the perfusion. After washout of the acid with saline, there was a gradual return of the flow-rates to control levels.

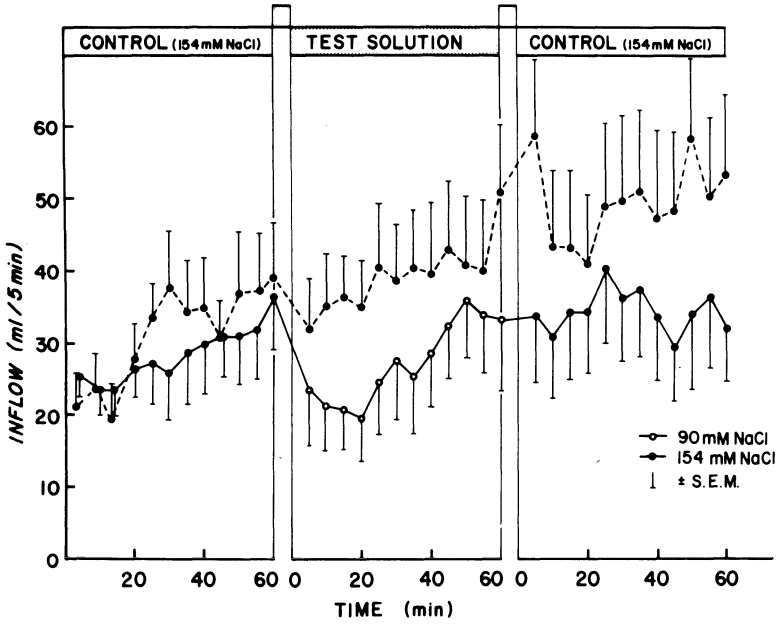

Figure 69.4 Inflow rates (± standard error) with 90 mM NaCl and 154 mM NaCl before and after perfusion with control solution (154 mM NaCl). The inflow rate is the volume of perfusate entering the jejunal study segment at 5-min intervals

There was a highly significant change in the mean inflow rates from control values (Figure 69.6) with 0.1 N HCl (−59%) when compared to 90 mM NaCl (−5%). The frequency of spike bursts in the segment did increase with both 90 mM NaCl (+39%) and 0.1 N HCl (+18%). However, the frequency did not increase as much with acid as it did with saline, and the difference was not significant ($p = 0.25$). Similar findings were observed in the pre- and post-segmental electrodes. In contrast, large differences were observed in the contractile activity of the segment. The motility index associated with 90 mM NaCl was 76% greater than control but it was 495% larger than control with 0.1 N HCl ($p < 0.025$).

The 0.025 N HCl solution flowed more slowly into the segment than control (−37%), however so did the NaCl solution of the same osmolality (−28% in Figure 69.7). Since we could not separate an effect of low osmolality from an effect of low hydrogen ion concentration, we compared the 154 mM NaCl solution and a 0.025 N HCl solution made isotonic by the addition of NaCl. The difference in the change in inflow rates from control was marked ($p < 0.025$).

DISCUSSION

Gregory utilized Thiry-Vella loops to observe the flow patterns of acid and

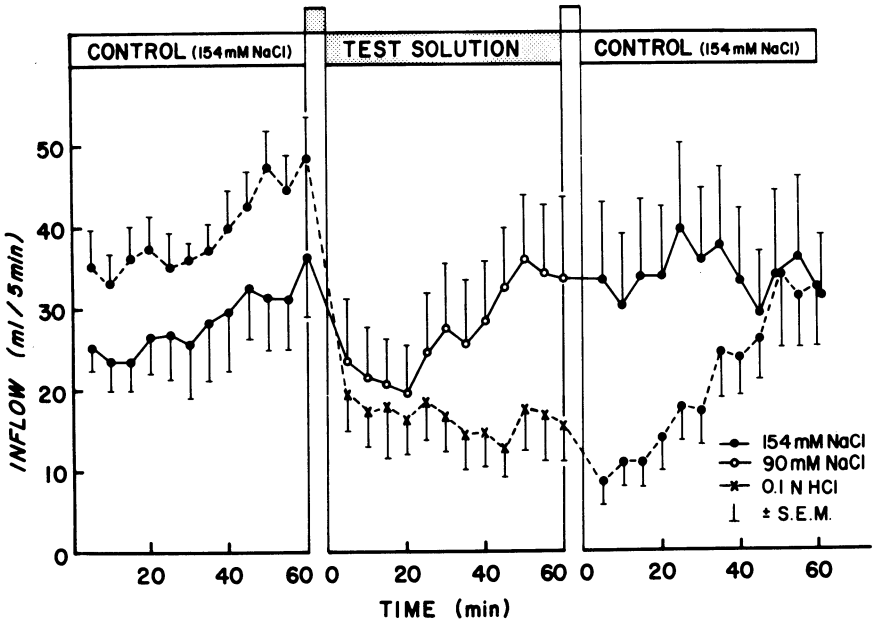

Figure 69.5 Inflow rates (± SEM) with 90 mM NaCl and 0.1 N HCl before and after perfusion with control solution

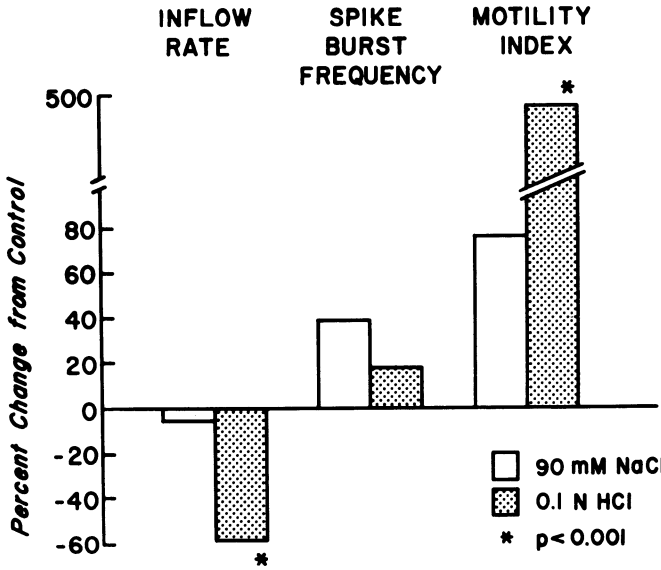

Figure 69.6 Percentage change of inflow rate, spike burst frequency, and motility index during test solution perfusion from control perfusion. Unpaired *t* test used for statistical comparisons between actual values, not percentages as shown

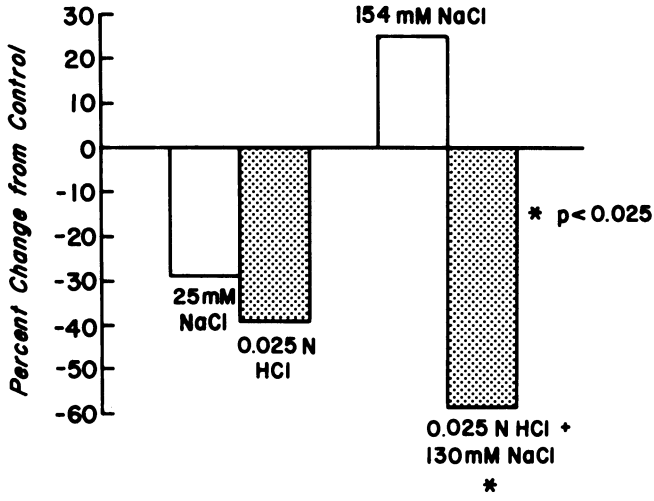

Figure 69.7 Percentage change of inflow rates during test solution perfusion from control perfusion. Same statistical method as in Figure 69.6

neutral solutions[3]. His preparation required transection of the intestine, the mucosa is known to change with time and the function of the loop deteriorates even if fed. Nakayama made similar observations in transected denervated segments of anaesthetized dogs[4]. The model being described has several advantages in the study of intestinal flow. It does not interrupt the continuity of the intestine. The nervous innervation remains intact and electrical and mechanical measurements can be made repeatedly with the animals fully awake. Stretch of the wall is an unlikely explanation for any of the changes seen, because of the gravity flow perfusion system. However, the use of a harness is essential for the flow measurements in order to maintain a constant height difference between the perfusion reservoir and the inlet cannula.

Acid solutions flowed into the jejunum much more slowly than neutral salt solutions of equal osmolality. This finding confirms the findings of Gregory[3] and Nakayama[4] using different models. Acid inhibition of flow seems to be analogous to the regulation of gastric emptying by intraduodenal acid solutions. Much experimental evidence exists to support the postulate of Hunt[5] that acid receptors exist in the small intestine which regulate gastric emptying, probably through nervous reflexes. However, there has been little information regarding autoregulation of flow in the small intestine. We found the retardation in flow to be associated with an increase in contractile activity, but were unable to detect any significant increase in the frequency of spike bursts. Therefore, the increased resistance to flow is at least in part due to stronger and not more frequent contractions. Since we only recorded circular muscle activity at one point, we were unable to detect any change in longitudinal muscle contractions or changes in patterns of contraction such

as reverse peristalsis. The change in flow and motility has been cited as an example of the 'intrinsic mucosal reflex' which elicits contractions above and inhibition of contractions below the site of stimulation[4]. Our studies were not designed to look for the mechanism of the change in motility, but it could be due either to intrinsic reflexes or to release of hormones or both. If this phenomenon exists in the duodenum, it is possible that similar changes in motility may also contribute to the slowing of gastric emptying by increasing duodenal resistance to flow. This concept was earlier suggested by Weisbrodt *et al.*[6] and more recently supported by Kelly and Code[7]. Kent *et al.* observed delay in gastric emptying and small intestinal propulsion after intestinal injury had been induced in rats by several agents such as 0.1 N HCl[8]. They did not find gastric retention or slow intestinal transit in the parabiotic partners of injured rats, thus arguing against a humoral mechanism.

Acknowledgements

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Discussion

A. Bennett: (UK) I don't suppose you have studied lower segments of the bowel, but it is appropriate to study acid in these regions. The pH in the colon may fall to pH 3.5 in diarrhoea, and a similar pH can be reached with lactulose used to treat constipation. Because of this we studied acidification of guinea pig small and large intestine and found stimulation of propulsion. Such an effect might even contribute to diarrhoea in the Zollinger–Ellison syndrome, although your data argue against this.

R. W. Summers: (USA) We plan to study other regions but have at this time only studied the jejunum.

E. E. Daniel: (Canada) (1) Was there a dissociation of spiking from contractile index; i.e. were there more spike bursts associated with the larger contractions which presumably produced your increased motility index? (2) Did you test the effect of flow-rate on the response of the intestine to acid?

Summers: (1) I only counted the number of spike bursts per unit of time, but would agree that the number of spikes will probably be related to the amplitude of the contractions. (2) We used a constant-pressure rather than a constant-flow perfusion system so that flow would be the variable. However, we looked at pressure–flow relationship before starting this study. Higher pressures were required to produce equivalent flow rates with acid in comparison with the salt solutions.

C. F. Code: (USA) Did you measure changes in volume of the intestinal contents as a result of infusing acid?

Summers: In several studies we looked at the change in phenol red concentration. When flow-rates were very low or zero with acid of course, nothing could be measured. With higher flow-rates there was very little change in volume; less than 10%. The change in volume therefore does not explain the changes in flow induced by acid.

F. R. Bell: (UK) Two comments: (1) since the body temperature of the dog is 39 °C, this would be a better perfusion temperature; (2) a similar study in sheep has produced a similar result, thus there seems to be a species similarity.

Section XIV
Workshops

70

Gastroduodenal contractile activity in fed and fasted unrestrained rats

X. B. PASCAUD, M. J. H. GENTON AND P. BASS

Since the early 1960s, when the extraluminal force transducer technique was first developed[1], most experimental studies with these units have been done in dogs[2,3] and some in cats[4], monkeys[5,6] and recently, in pigs[7]. Only one study using a silicone crystal semiconductor element has been attempted in rats[8]. In this study the transducer lacked temperature compensation needed for monitoring tone[9] and was described only as a single recording unit implanted on the jejunum of anaesthetized rats.

Apart from an extensive work dealing with the electrical spiking activity in chronic rats[10], data on the gut contractile activity in unrestrained rats are lacking. The purposes of this chapter are (a) to describe the technique used to manufacture the miniaturized strain-gauge transducers, and (b) to define the main characteristics of the gastroduodenal motor activity in the unanaesthetized and unrestrained rat.

MATERIALS AND METHODS

Sensor unit

The extraluminal contractile force strain-gauge microtransducers used in this study were constructed from the principles published by Jacoby *et al.*[1] (the details of the procedure of construction have been submitted for publication to the *American Journal of Physiology*). Briefly, the contractile transducer consisted of two strain-gauges (EA–06–031–EC–350 option E – Micro Measurements, Inc., Rolulus, Michigan – abbreviated MM) bonded back to back with M-Bond 610 adhesive (MM). Then the unit was cut to precisely

4 × 1.5 mm. Three one-strand Teflon-insulated copper wires, 25 cm length (Leico Industries, Inc., 250 W 57th Street, New York, NY 10019) were inserted into a 0.3 mm ID, 0.64 mm OD silicone tubing (602–105 – Dow Corning Corp., Medical Products Midland, Mich.; abbreviated DC). Then, under a zoom stereo microscope (Olympus – Japan), it was soldered together with a Teflon-isolated silver wire to form two arms of a Wheatstone bridge of 350 Ω each. The solder junctions were then waterproofed with a thin layer of M–Coat–D (MM), and the sensor unit was encapsulated in silicone adhesive (CAF 3 – Rhodorsil, Rhône Poulenc, France) and sandwiched

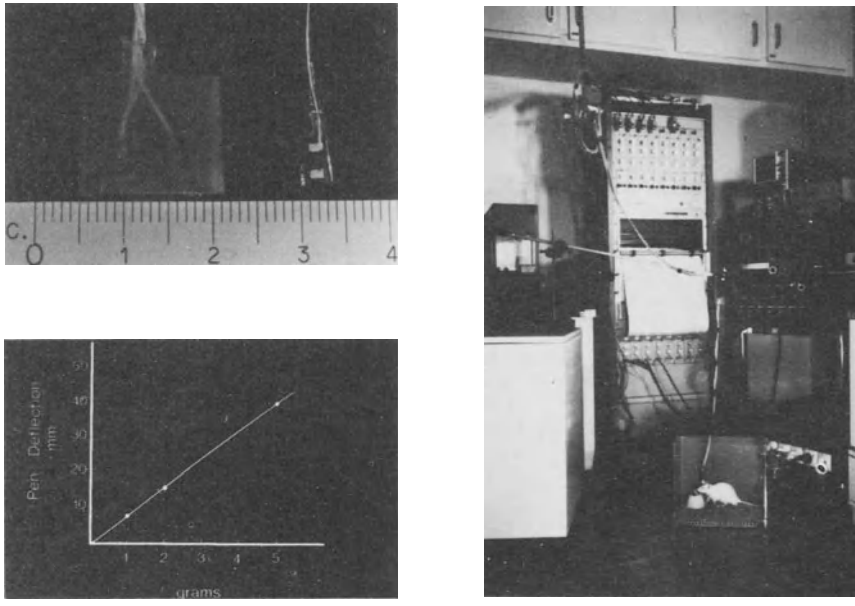

Figure 70.1 Techniques of recording gastrointestinal motility in the chronic unrestrained rat. *Upper left*: comparison of the conventional (1) and miniaturized transducer. *Lower left*: calibration curve obtained for forty transducers; bars represent standard deviation; each transducer was linear. *Right*: unanaesthetized rat, polygraph and tape-recorder in recording situation. See text for further explanation

between two 0.005 in. thick Silastic sheeting (DC). The whole unit was then placed in a plaster mould to produce a curved transducer with a uniform thickness of 1 mm, an ID of 6.5 mm; it was cured and then trimmed to 5 × 3 mm (Figure 70.1). Next the lead wires from the sensor were soldered into a 15-contact female Cannon plug (MD1–15SL1, ITT, Cannon Electric. Inc., Los Angeles, Ca.). Finally each transducer was calibrated before implantation for 1–5 g of tension, and their response was linear within the range in which they were to be used (Figure 70.1). The sterilization before implantation was performed by immersing the whole unit in a 1% Cetrimide solution (Cetavlon – ICI Pharma, France).

Animal preparation

Seven male SD/CD rats (Charles River – Elboeuf, France), averaging 300 g body-weight on the day of operation, were prepared under aseptic conditions, with the animals anaesthetized by intraperitoneal administration of pentobarbital sodium 50 mg/kg (Nembutal – Abbott Laboratories, North Chicago, Ill.), and premedicated with atropine sulphate 50 μg/kg – intraperitoneally (Fluka AG, Buchs, Switzerland). A median laparotomy, as well as a longitudinal median incision of the skin on the skull, was made. Then a Teflon trochar made with a one-end-bevelled Teflon tubing, 150 mm length, 8 mm ID (Code 08 499 08 Prolabo – Paris, France), and containing the sensor units, was passed subcutaneously down the back on the left side, and brought through the left rectus muscle and the peritoneum into the abdominal cavity. The transducers were carefully sutured upon the serosa of the fundus (Fnd), antrum (Ant), proximal (PD) and distal (DD) duodenum, using 5/O Tevdek Deknatel (T. Leclerc – Paris, France), and the midline closed. Then the connector was fixed upon the skull bone by embedding it with dental cement (Texton SS-White – Philadelphia, Pa.) which was secured by tiny screws previously implanted into the bone[11]. A 5-day period was then allowed for recovery from surgery before starting the experiments.

Animal recording

For recording (Figure 70.1), the connector mounted in the head of the rat was joined through a twelve-channel revolving connector (SFENA, Vélizy – Villacoublay, France) to a strain-gauge coupler (Type 9803 – Beckmann) of a polygraph (Type S II, eight channels rectilinear, Dynograph recorder – Beckmann Instruments, Inc.). The bridge received its excitation voltage of 6 V from the polygraph and the output was recorded simultaneously on the polygraph and a seven-channel tape recorder (Analog 7 – Phillips, Eindhoven, Netherlands) for further computer analysis of the signal. For test purposes, the animal was placed in a free-moving situation inside a plexiglass cage with a wire bottom to avoid coprophagy, and had free access to food pellets and water.

Three recording sessions were performed over a 3-day period which was scheduled as follows: after an overnight fast the rat was connected to the Dynograph and placed in its cage with free access to water. Seven hours' continuous recordings were made simultaneously on the polygraph and the tape-recorder. The first day, after 30 min basal period and at time zero of the experiment, the rat had free access to its regular chow pellet (Code A-0-3 from UAR, France) for 1 h. After this time the quantity of food eaten was weighed. Then the rat was fasted until the end of the 3-day session. During the two following days, two 7-h recordings were obtained at the same time in the morning and in the same way, except that no food, but water, was given.

Data analysis

The quantitative analysis of the data (Compagnie Internationale de Services en Informatique – Saclay, France) was performed on a 8.400 Computer after digital transformation of the analogue signal by a 8.900 Hybrid Calculator (Electronique-Associés, rue Ginoux, Paris). The program written in FORTRAN, integrate the signal with a sampling frequency of four per second, in view of computing the following parameters: the amplitude (grams) duration (seconds) and area (g/s) of each wave, the number of waves per time-interval and the quiescent period (seconds) between waves for the same time-intervals. All this information was stored and averaged for the time of analysis, and an index of motility was calculated as the mean surface of activity per time unit using the formula: Index $= \Sigma S/T$, where ΣS is the sum of the area of the waves during the time T of analysis. Each value is given as the mean $\pm$ standard deviation (m $\pm$ SD).

RESULTS

The transducers and the animals

In all instances, the transducers were well tolerated by the animals, which continued to gain weight after surgery. No septic problem arose either at the location of the connector, along the wires, or inside the abdominal cavity. At autopsy, surprisingly, only a few adhesions to the transducers and the wires with the viscera were seen. The recordings were quite free of artifacts even when the rat was moving, drinking or eating, and no baseline drift during the recordings was observed.

Meal and postprandial gastroduodenal motor pattern

During the 1-h meal-time when the rats had free access to food, they ate 5.2 $\pm$ 1.8 g (m $\pm$ SD) of their regular chow pellet. In all instances, one very specific burst of activity in the antrum appeared just when the rat started eating (Figure 70.2). This pattern was characterized by a fairly constant number of contractions ($n = 17 \pm 1$), with a maximum amplitude (7.49 $\pm$ 0.60 g), and a regular duration (195 $\pm$ 20 s). These values and the corresponding motility index (2240 $\pm$ 130) were higher than those registered in the remainder of the experiment. Meanwhile the motor activities of the fundus and duodenum gradually increased until reaching the typical pattern of the fed state. This pattern (Figure 70.3) was established for the next 75 $\pm$ 12 min and was characterized by a sustained train of medium-height contractions appearing both in the fundus and the antrum, with a mean quiescent period between contraction of about 8 s. In the duodenum, little bursts of four to five waves

of weak contractions could be observed, with a mean duration of 5.6 ± 2.0 s. These bursts seemed to correlate with one antral contraction.

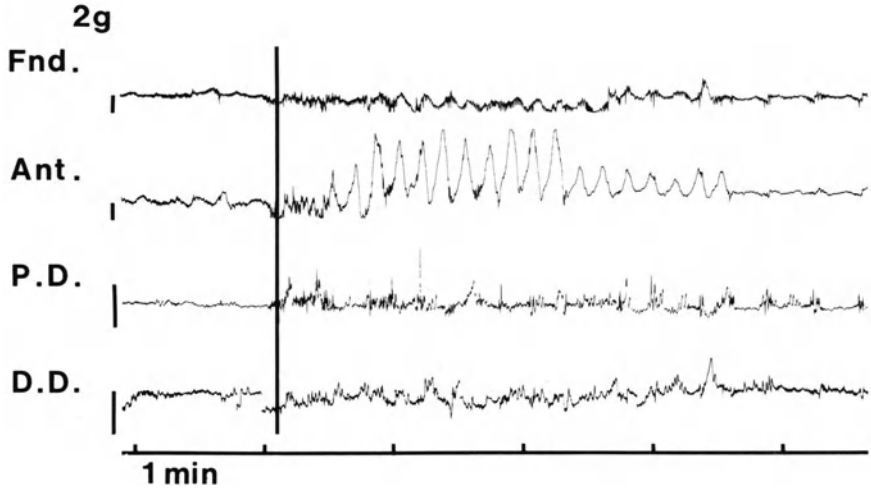

Figure 70.2 Gastric and duodenal contractile activity when the 18-h fasted rat is just beginning to eat. Note the typical burst pattern of maximal contractions in the antrum. At the vertical line the rat is placing the first pellet of food in its mouth. See text for further explanation. Fnd = fundus; Ant = antrum; PD = proximal duodenum and DD = distal duodenum. Vertical bars are 2 g calibration

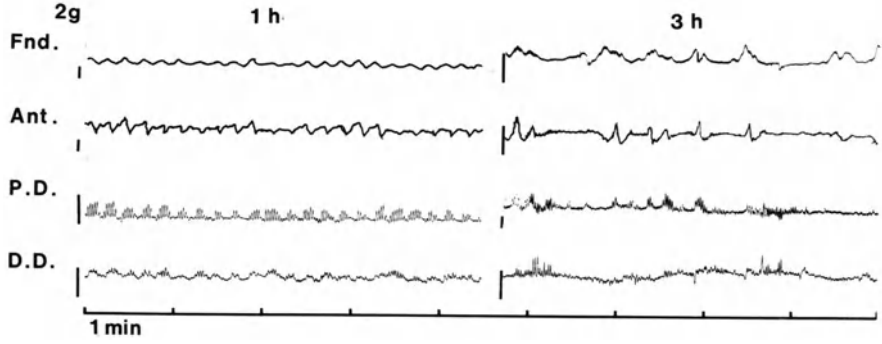

Figure 70.3 Gastric and duodenal digestive pattern 1 h and 3 h postprandial in rats. Note at 1 h the continuous waves of medium-height contractions in the stomach and the little bursts of four or five contractions in the duodenum; at 3 h the bursts of four or five waves in the antrum and the larger duration (ten to fifteen waves) of the duodenal bursts. Symbols as in Figure 70.2

The evolution with time of the fasted pattern

Approximately $1\frac{1}{2}$ h after feeding, the motor activity began to convert to a pattern characteristic of the fasting state. In the fundus and the antrum the

contractions decreased progressively in number and, in the antrum, they bunched together gradually to form little bursts of three to five waves (Figure 70.3); the frequency of the waves, their amplitude and indeed the related index were also decreasing with time (Table 10.1). After a 24-h fasting, all these values, except those of the fundus, were again decreased. At this time, the typical pattern of the fasted gut was observed. In the stomach it is characterized by very few and low amplitude contractions appearing at random, and a marked increase in the duration of the quiescent period.

Table 70.1 Computer analysis of the gastroduodenal patterns in the fed and fasted unrestrained rat

	0–1st hour	*4th–5th hour*	*7th–8th hour*	*24th–25th hour*
Fundus				
MI	248 ± 58	120 ± 49	50 ± 49	51 ± 56
Amp.	0.80 ± 0.34	0.62 ± 0.36	0.47 ± 0.39	0.56 ± 0.36
Freq.	3.68 ± 1.0	2.47 ± 1.10	2.03 ± 0.80	1.22 ± 0.90
Antrum				
MI	271 ± 108	150 ± 70	90 ± 33	51 ± 37
Amp.	1.41 ± 1.04	1.09 ± 0.77	0.46 ± 0.28	0.42 ± 0.26
Freq.	4.9 ± 3.3	3.7 ± 2.5	2.6 ± 1.8	1.9 ± 2.6
Proximal duodenum				
MI	125 ± 38	93 ± 36	44 ± 23	38 ± 22
Amp.	0.34 ± 0.24	0.31 ± 0.19	0.17 ± 0.12	0.19 ± 0.11
Freq.	32.0 ± 6.9	22.6 ± 5.8	24.8 ± 3.1	24.1 ± 9.1
Distal duodenum				
MI	89 ± 33	63 ± 33	39 ± 21	30 ± 23
Amp.	0.24 ± 0.20	0.21 ± 0.16	0.19 ± 0.10	0.23 ± 0.14
Freq.	21.3 ± 4.6	16.7 ± 1.4	23.4 ± 4.2	14.9 ± 1.6

The values are the mean ± SD of eighteen experiments in seven rats. Each parameter is calculated from a 1-h period at each time from the beginning of the meal

MI = motility index × 10^3; Amp. = amplitude in grams; Freq. = frequency in contractions/min

Simultaneously the duodenum began to generate bursts lasting more than 1 min. At 24 h it was characterized by bursts lasting 2.82 ± 0.43 min, and composed of ninety waves of contractions with a mean frequency of 38.6 ± 1.33 waves/min. The mean duration between two consecutive bursts was 4.96 ± 2.47 min. The average duration of the entire cycle (burst + quiescent period) was 9.60 ± 2.37 min. During both the fed and fasted state the duration of each contraction was fairly constant: 8.24 ± 3.54 s for the fundus; 8.74 ± 3.50 s for the antrum and 1.68 ± 0.50 s for the duodenum.

Finally there appears to be a relationship between the sleep–awake cycle of the rat and the motor pattern. During most of the two 7-h-fasted recording periods, the rat seemed to be sleepy in a corner of its cage. Periodically the

animal awakened and moved within the cage, seemingly searching for food. At the time a 'fed' pattern appeared (Figure 70.5). Both phenomena were so concomitant that it was impossible to differentiate between cause and effect. For these reasons, these two related events suggest hunger behaviour of the animal.

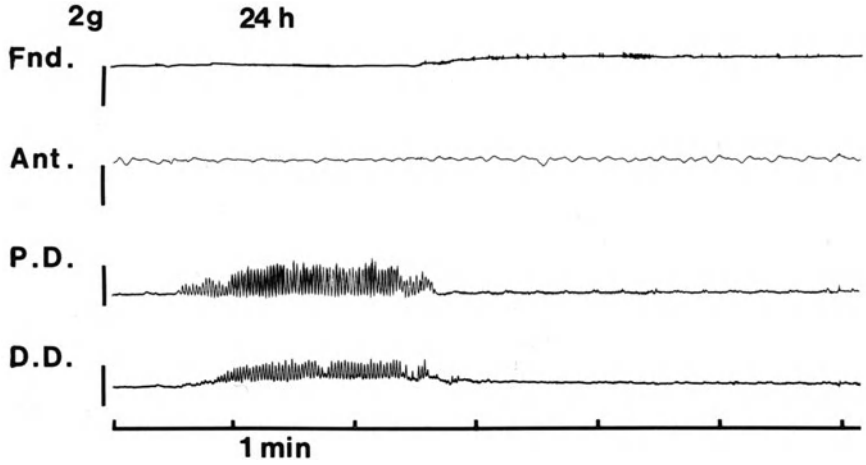

Figure 70.4 Gastric and duodenal contractile activity in a 24-h fasted rat. Note the nearly complete quiescence in the stomach and a typical interdigestive burst in the duodenum. Symbols as in Figure 70.2

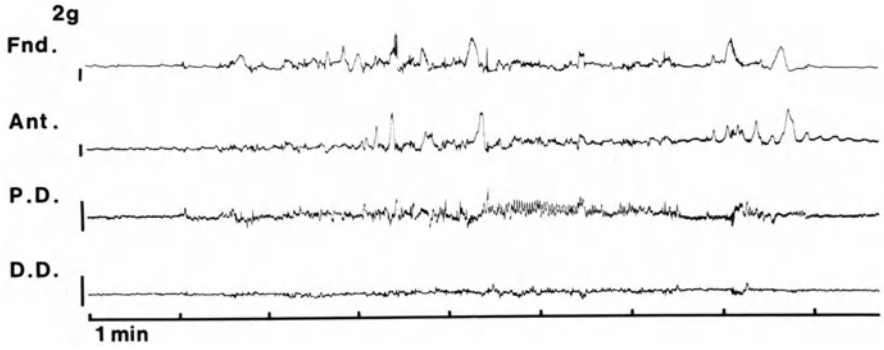

Figure 70.5 The 'fed'-like pattern in the 24-h-fasted rat. Compare with Figures 70.3 and 70.4. Note the train of irregular and mainly strong contractions in the stomach, and the little bursts (< 60 s) in the duodenum. See text for further explanation. Symbols as in Figure 70.2

DISCUSSION

The main advantage of the sensor unit constructed for this study in comparison with other methods described in rats[9,10] is that it can be used in

unanaesthetized, free-moving animals. This was possible because the transducer consists of half a Wheatstone bridge insensitive to temperature changes. This gives a stable baseline which permits the recording of both phasic and tonal contractile activity. Furthermore, as can be seen in Figures 70.2–70.5, the recordings obtained are relatively free from disturbing artifacts, regardless of the body movements of the animals. Finally the transducer is sensitive and it responds in a linear manner. The lack of recording artifact permitted the computer analysis of our data. This type of analysis has already been used for several years on our dogs' motility results.

A fasted pattern of quiescent and burst activity and continuous fed pattern were recorded in the rat. Similar fasted and fed states have been described in dogs[2,3], cats[4], monkeys[5,6] and pigs[7]. As in dogs and pigs[1,7] the fasted pattern always disappeared as soon as the animal ate. There appears to be a close relationship between motor patterns recorded in the rat and the electric pattern described by Ruckebusch *et al.*[10] about the electrical spiking activity in chronic rats. The ability of eating to initiate an antral burst of activity has been reported in pigs[7] and monkeys[5], but is not seen in the dog in which maximal bursting of activity is seen during fasting[2,3]. Another difference is the gradual increase of the number and amplitude of contractions appearing in the fundus at the time the rat has eaten, instead of the so-called receptive relaxation described in dogs[3]. This could be explained if we consider the presence in rats of the rumen, which is more expandable than the fundus and presumably works as a reservoir; a function which is performed by the body of the stomach in other species.

Concerning the stomach, such a pattern of three to five contractions happening between the immediate digestive and the late interdigestive phases has already been described as the intermediate pattern in dogs[3]. After 1-h feeding in rats the interdigestive pattern settled down between the 4th and the 6th hours postprandial. It is characterized in the stomach by a decrease in the number of contractions, and in the duodenum by a very regular rhythm of large bursts followed by quiescent periods. The mean duration of the whole cycle was about 10 min in the duodenum of all the seven rats tested. As with dogs and pigs[2,3,7] the fasted pattern above always disappeared as soon as the animal ate. Some similar motor parameters of the jejunum of the anaesthetized rat has already been reported by Scott and Summers[8]. The slight differences between our data is probably due to their use of the acute animal, and above all the different areas of the bowel studied. Such a loss of activity along the gut has also been described in sheep, dogs and rabbits[12].

Acknowledgements

The authors are most indebted to Mrs Ruth Bass who initiated one of us (X.B.P.) a few years ago into the difficulties of the strain-gauge technique.

They are also grateful to Mr Marcillac from Vishay (France) for his valuable advice in manufacturing the miniaturized transducers; they also appreciated the able assistance of Mrs Danchin in carefully preparing the manuscript.

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71

Calibration of strain gauge force transducers and quantification of gastric motility

J. A. J. SCHUURKES, E. J. VAN DER SCHEE,
J. L. GRASHUIS AND G. R. A. CHARBON

Although strain gauge force transducers are now widely used to measure gut motility, little attention has been paid to the development of a proper calibration method. The first aim of this study is to compare our newly developed calibration method with the one described by Jacoby *et al.*[1] and with that of Reinke *et al.*[2].

Comparing results from different motility studies with strain gauges is not only made difficult by different calibration methods, but also by a wide variety in the choice of motility parameters to be quantified. The second aim of our study, therefore, is to select an adequate method for the quantification of gastric motility.

METHODS

Force transducer

Strain gauge (Micro-Measurements, Romulus, Michigan, USA, type SA-03-090DH-350) force transducers without a CuBe-strip were constructed as described by Bass and Wiley[3]. The same transducers were used in each of the three calibration methods.

Calibration

Method I

Jacoby *et al.*[1] clamped the transducer at one end in a horizontal position

(Figure 71.1(I)). Weights were hung on the free end of the transducer. The weight (F) was plotted against transducer output (volts).

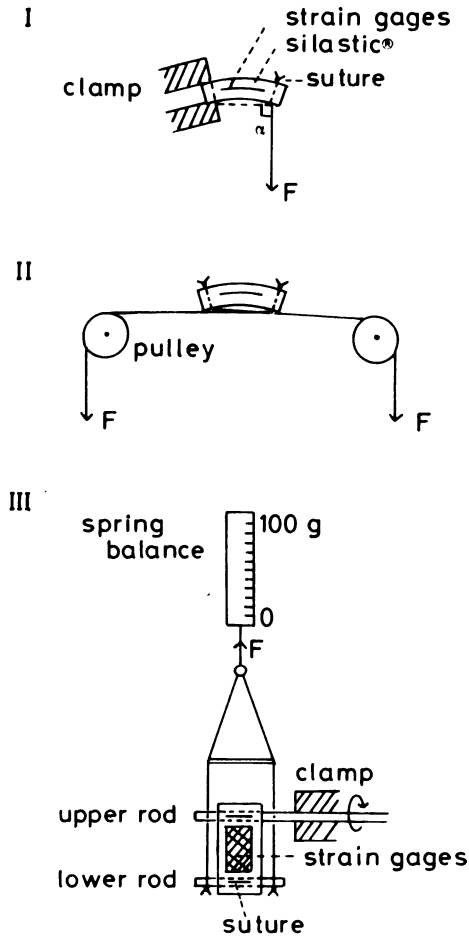

Figure 71.1 Calibration methods; for explanation see text

Method II

Reinke *et al.*[2] used a counterweight instead of a clamp (Figure 71.1(II)). They placed the transducer in a horizontal position and fixed the lead wire to a small supporting rod. Threads were passed through each end of the transducer, in the middle between the suture holes, and led over a small pulley opposite that transducer end. Reinke plotted the sum of the weights (2F) on both sides of the transducer against the calibration values (volts). Since the counterweight (like the clamp in method I) only exerts the reaction force, his scale on the abscissa should be halved.

Method III

The transducer is sutured to two small rods (Figure 71.1(III)). The transducer is put in a vertical position; the upper rod is fixed in a clamp. Two threads are fixed to the lower rod and led friction-free through holes in the upper rod to a spring balance, from which the exerted force can be directly read. The lead wire was supported. The bridge was balanced with a tension of 10 g on the threads and the transducer, to make sure that in the initial situation the threads were not hanging slack (initial tension in method II: 4 g). Force was exerted on the transducer by moving the spring balance upwards. To align the exerted force and the transducer under strain, the upper rod could be rotated. We plotted voltage against the exerted force in grams and calculated the regression line (least sum of squares). Details of this method are intended to be published elsewhere. Additionally this method allows us to decide how far strain gauge measurements may be considered isometric measurements, by measuring the distance between the two rods.

Quantification of gastric motility

To assess gastric motility, two transducers were sutured in transverse direction on the gastric corpus and antrum of thirteen fasted, pentobarbital-anaesthetized mongrel dogs. Gastric motility was stimulated by intravenous bolus injections of pentagastrin (Peptavlon®) at intervals of 2 min in doses doubling from 1 to 8192 ng/kg. Recordings were made on a polygraph (Schwarzer) and on magnetic tape (Ampex) for off-line analysis. Recordings were analysed for each experiment after each injection for (1) maximal change of tone; i.e. baseline (g); (2) maximal amplitude of the contractile wave (g); and (3) contractile frequency (contractions/min), calculated from the time-interval between contractions. For antral force and frequency, mean regression lines (least sum of squares) were calculated.

RESULTS

Force transducer calibration

Method I (Figure 71.1 (*I*))

The slope of the calibration line (V/g) appeared to depend on the angle α between the pulling force and the transducer ((slope at $\alpha = 90°$): (slope at $\alpha = 60°$) = 1:1.4). The pulling force should be operating on the transducer at the same angle as *in vivo* when the muscle wall of the gut contracts ($\alpha = 0°$). This excludes method I; in methods II and III this condition is fulfilled.

Methods II and III (Figure 71.2)

Gauge signals were normalized to 2.5 V at 40 g force in method III. With this amplification the transducers were calibrated with Reinke's method

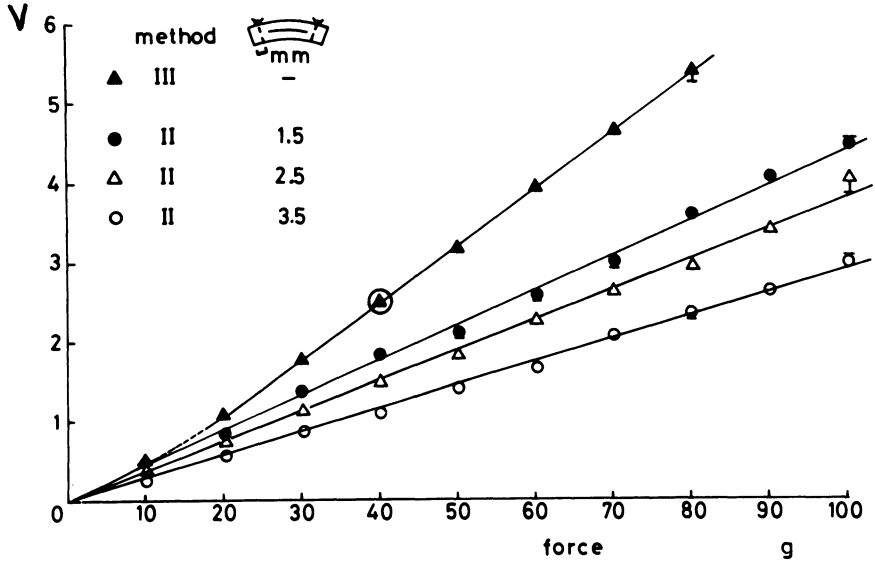

Figure 71.2 Mean calibration regression line (▲) for three transducers (spring balance method III, three replications per force for each transducer) and mean calibration regression lines for a transducer (Reinke's method II, three replications per force for each transducer) with short (●; 1.5 mm), medium (△; 2.5 mm) and long (○; 3.5 mm) transducer ends beyond suture holes. In both methods the same amplification was used. (40 g force ~ 2.5 V in method III (○)). A SEM (⊥) smaller than the symbol is not indicated

(II). For each transducer the same force gave lower voltage values with Reinke's method than it did with method III, indicating some hindrance in method II. The difference appeared to run parallel with the length of the Silastic transducer end beyond the suture holes, 1.5; 2.5 and 3.5 mm respectively. The longer this end, the lower the voltage value obtained with Reinke's method. The length of the transducer end was linear with relative obstruction (Figure 71.3). Extrapolating to an end length of 0 mm results in only 8% obstruction (not statistically different from 0% obstruction). The origin for this obstruction is found in the fact that in Reinke's method the bending of the transducer is hindered because the transducer ends are resting on the threads that transfer the pulling force (Figure 71.4 (1)). A transducer with smaller ends (Figure 71.4 (2)) is hindered less by the pulling threads.

We thus prefer method III because: (1) the direction of the pulling force is identical with the direction of the force exerted on an implanted transducer by the gut; and (2) this method avoids Reinke's obstruction by leading the pulling threads beside the transducer (Figure 71.4 (3)) instead of under the transducer. If this obstruction is of importance when the transducer is sutured to the gut, it can be reduced by reducing the length of the transducer end.

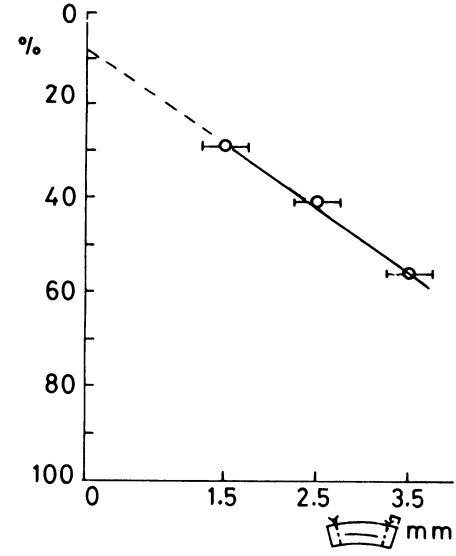

Figure 71.3 Regression line of transducer end length (three transducers) against relative obstruction in method II (spring balance value minus Reinke's value, divided by the spring balance value, at 40 g force). Horizontal bars indicate measuring accuracy. SEM of replications was smaller than the symbol used

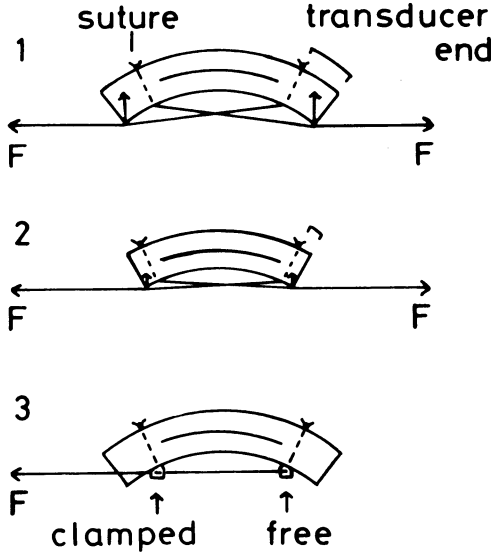

Figure 71.4 Obstruction in Reinke's method II depends on the length of the transducer end. (1) Transducer with long ends and (2) with short ends in Reinke's method. (3) Spring balance method. For further explanation see text

Quantification of gastric motility

Representative tracing from transducers on antrum and corpus during pentagastrin stimulation are shown in Figure 71.5. By injecting pentagastrin in graded doses from sub-threshold to maximally effective, a complete dose–response curve was obtained within a period of 30 min. Corpus tone, corpus and antral contractile amplitude and frequency increased.

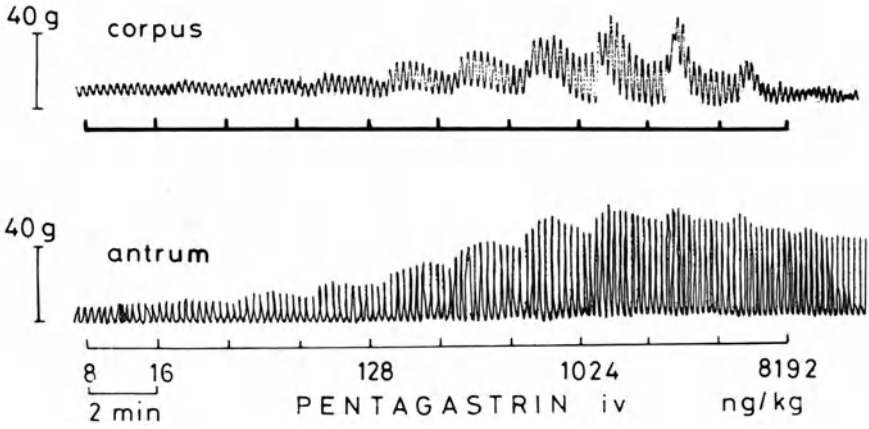

Figure 71.5 Representative force transducer recordings of motor activity of the corpus and antrum of a dog's stomach

Antral contractile force responded at lower doses than contractile frequency: contractile force; $^{2}\log ED_{50} = 6.9 \pm 0.4$; $ED_{50} \sim 128$ ng/kg and contractile frequency; $^{2}\log ED_{50} = 9.3 \pm 0.3$; $ED_{50} \sim 630$ ng/kg. Frequency increased gradually (Figure 71.6 (1), decreasing the interval between contractions) whereas contractile force reached a maximum within 1 min after injection (Figure 71.6 (2)). This distinction of force from frequency response to pentagastrin (*in vitro* shown by Morgan *et al.*, 1977(4)) emphasizes that these parameters should be quantified separately. The motility index as used by Jacoby and Marshall[15] (Table 71.1) would conceal the distinction between the responses of the parameters. An increasing value for this motility index can be caused by increasing frequency, by increasing amplitude or by frequency and force both changing. The Jacoby and Marshall index is a rough approximation but essentially the same as the index of Sugawara *et al.*[6] (Table 71.1). The mean motility index of Cooke and Kottemann[7] (Table 71.1) is a measure of the mean maximal amplitude of contractile force. It gives no information about the contractile frequency. Misiewicz *et al.*[8] and Kwong *et al.*[9] (Table 71.1) defined a motility index that is the mean force in t min and consequently depends on all parameters of motor activity: change of tone, change of amplitude, duration of the individual contraction and frequency of contractions.

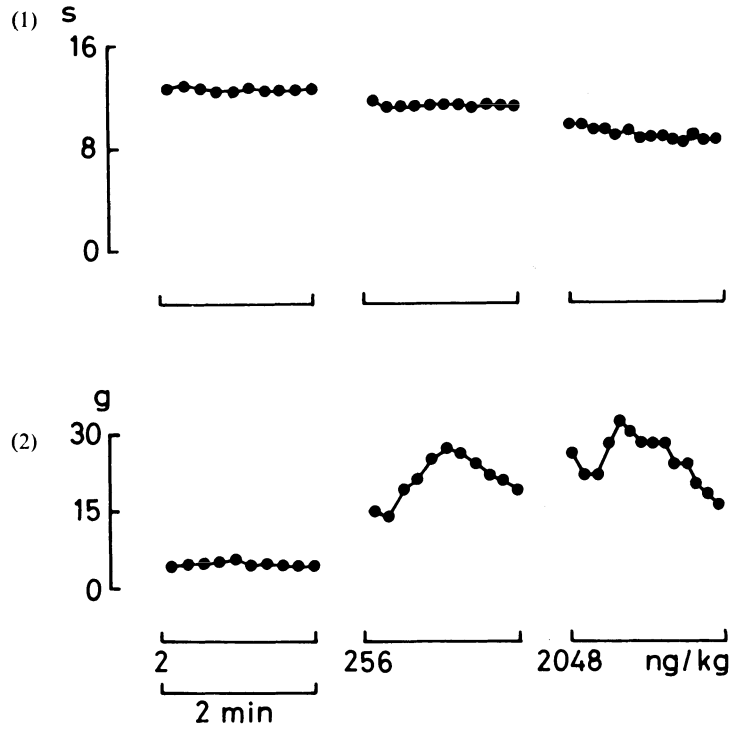

Figure 71.6 (1) Interval between consecutive contractions; and (2) amplitude of the contractile force of individual contractions of transverse antral musculature between two injections for three intravenous doses of pentagastrin

Table 71.1 Motility Index (MI)

Jacoby, H. I. and Marshall, C. H.[5] Ludwick, J. R., Wiley, J. N. and Bass, P.[10] Walker, G. D., Stewart, J. J. and Bass, P.[11] Ormsbee, H. S. and Bass, P.[12]	$(N_1 \times 1) + (N_2 \times 2) + (N_3 \times 4) + (N_4 \times 8)$*
Sugawara, K., Isaza, J. and Woodward, E. R.[6] Stemper, T. J. and Cooke, A. R.[13]	Σ amplitude in t min
Cooke, A. R. and Kottemann, W. J.[7]	mean MI $= \dfrac{\Sigma \text{ amplitude in } t \text{ min}}{\text{number of contractions in } t \text{ min}}$
Misiewicz, J. J., Holdstock, D. J. and Waller, S. L.[8] Kwong, N. K., Brown, B. H., Whittaker, G. E. and Duthie, H. L.[9]	$\dfrac{\Sigma \text{ (amplitude} \times \text{duration) in } t \text{ min}}{t \text{ min}}$

* N_1 = the number of contractions in t min within the force range from 5–10 g. N_2: 10–20 g. N_3: 20–40 g. N_4: 40–80 g.

Thus we propose to quantify each parameter individually, because of the distinct behaviour of different parameters of motility. The use of a motility index conceals information and suggests that gut motility can be captured in one number.

Acknowledgements

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72

Pressure and motility studies in clinical practice

W. SILBER

Studies in gastrointestinal motility commenced in 1803, but balloon kymography of the oesophagus specifically is over 80 years old. Much of the original work was performed by Cannon. Compared with some other fields of medicine, knowledge of gastrointestinal motor functions and dysfunctions has accumulated slowly.

Interest and progress in this field has increased over the past 25 years, chiefly because of the development of new techniques and the appreciation of new concepts. In spite of this, Bayliss and Starling's statement in 1899 'that on no subject in physiology do we meet with so many discrepancies of fact and opinion as in that of physiology of the intestinal movement', holds true today.

The whole question of gastrointestinal motility has been plagued with problems of methodology and semantics, and I have experience of this over the past two decades; yet we have been most productive in this sphere.

METHOD OF INVESTIGATION

Various techniques have been used over the years, each with its own possible advantages and limitations. Technical skills are improving all the time, but do we necessarily have to discard the precise results obtained with our present methods in spite of, as yet, many unanswered problems? What are we looking for? Personally I have developed a technique utilizing sophisticated instrumentation which will define the nature of the functional impairment and form a useful baseline in assessing the natural history of the particular patient. I would submit that the major area in which it has played the most positive role is oesophagology.

OESOPHAGUS

This again is a dynamic structure peculiar in its anatomy and physiology, and very prone to dysfunction. The data obtainable from manometric studies refer to the function and reaction of the cricopharyngeal and inferior oesophageal sphincters as well as to that of the body of the oesophagus.

These studies, together with pH and PD measurements and pharmacological investigations, have definitely allowed for precise diagnosis, application of specific therapy and provided for excellent follow-up assessment.

The importance of manometric studies *per se* cannot be over-stressed in the diagnosis of chest pain, dysphagia, hiatal hernia complex, collagen diseases and all the motor dysfunctions, e.g. achalasia, spasms, involvement of striated or non-striated muscles.

In my experience of over 5000 cases of benign oesophageal disease I can categorically state that of all methods available today the single most positive method is the use of manometry. It is, however, important to stress that this must be correlated with other conventional investigations. It is with the universal acceptance and utilization of these investigations that consistent diagnostic evaluation will be achieved.

STOMACH

The knowledge gained from the study in this uncharted ground of gastric motility is chiefly physiological, but it can play an important role in the elucidation of the post-vagotomy and dumping syndromes. The function of the pylorus in relation to peptic ulcer disease and alkali oesophagitis is most important to evaluate.

SMALL INTESTINE

These studies, using a telemetering capsule, have not appreciably assisted in the precise diagnosis of disease processes, but they are important in the understanding of the physiology; this has been discussed specially in relation to myoelectrical responses in the first chapters of this book.

COLON

Connell has maintained that studies of colonic motility have contributed most in the area of concepts, rather than diagnostic procedures. The contractions of the colon are complex, not absolutely understood and its significance remains to be investigated. We have seen in earlier chapters many aspects of these controversies especially in relation to diverticular disease; discussion of these aspects continues.

RECTUM AND ANAL CANAL

The mechanism of defaecation and the state of continence have been completely assessed by means of manometric studies. It is of particular value in the diagnosis of aganglionic segments associated with Hirschsprung's disease and its post-operative follow-up.

BILE DUCT

Manometric studies of the bile duct and sphincter of Oddi have assumed a most important role with the introduction of endoscopic retrograde cholangiopancreatography. This may well play a role in the diagnosis of dyskinesia of this region.

CONCLUSION

In spite of all the difficulties experienced, it can be stated categorically that progress in these studies, together with the ancillary techniques, has been made; especially so in the study of benign oesophageal diseases.

73
Relaxation oscillators

S. K. SARNA

Oscillators are devices that have a continuous and rhythmic output. These devices may be electronic circuits, computer programs, mathematical equations or biological systems. Some of the familiar examples of biological oscillators are cardiac electrical activity, bladder emptying, sleep cycle, menstrual cycle in women and gastrointestinal electrical control activity (ECA).

Basically there are two types of oscillators:

1 Linear Oscillators
2 Nonlinear Oscillators.

Linear oscillators are those that can mathematically be described by linear differential equations; e.g. the sinusoidal oscillators can be described by the differential equation

$$\ddot{x} + \omega^2 x = 0$$

where: x is the output variable;
$\ddot{x}$ is the second derivative of x;
ω is the frequency of oscillation in rad/s. Frequency of oscillation in cycles/s $= \omega/2\pi$. Frequency of oscillations in cycles/min $= 30\ \omega/\pi$.

This is called a linear differential equation because the coefficients of x (the output) or its derivatives are constants and are not a function of x itself.

Analytical solutions of linear differential equations are readily available, and for this reason engineers and scientists like to use them in modelling wherever possible. However, there is hardly any phenomenon in nature that can be truly described by a linear differential equation. Often one has to

resort to non-linear differential equations to represent these phenomena sufficiently accurately to allow valid models to be studied.

Relaxation oscillators, which are a type of non-linear oscillator, have been successfully used in the modelling of several biological phenomena. These oscillators are represented by non-linear differential equations. A well-known and widely used relaxation oscillator is the Van der Pol oscillator[1]. It is represented by the equation:

$$\ddot{x} - k(1 - x^2)\dot{x} + \omega^2 x = 0$$

where: x is output variable;

$\dot{x}$ and $\ddot{x}$ are the first and second derivatives of x;

k is a constant determining the deviation of Van der Pol oscillator from a simple sinusoidal oscillator, for $k = 0$ a Van der Pol oscillator becomes a simple sinusoidal oscillator, ω along with k determines the frequency of oscillation.

Perhaps the most important characteristic of relaxation oscillators that distinguishes them from other types of non-linear oscillators, and which makes them most useful for representing biological oscillations, is that although these oscillators have an intrinsic frequency of their own, when they are coupled* with other oscillators (that is, the output of one is fed to the other and vice-versa), their frequencies are changed without appreciable changes in their wave-forms.

In the situation that on coupling, the two oscillators acquire the same frequency, the oscillators are said to be *phase-locked*† and the phenomenon is known as *frequency entrainment* (Figure 73.1). Whether or not two oscillators that are coupled will entrain depends upon the difference between their intrinsic frequencies and the strength of coupling between them. The smaller the intrinsic frequency difference between two oscillators, the smaller is the amount of coupling that will be required to entrain them. It should be noted that if the intrinsic frequency difference is too large entrainment may not occur even with a coupling factor of 1. In general there is a limit to which a relaxation oscillator can be driven above its intrinsic frequency. This is due to the refractory properties of relaxation oscillators (that is, after an oscillation has been completed these oscillators enter an absolutely refractory state during which no external stimulus, however strong, can initiate another oscillation). After this phase the oscillator enters a relatively refractory state, during which refractoriness decreases with advancement in the cycle (that

* Coupling, in simple terms, means a portion of the output of one oscillator is being fed to the other.

† In a rigid sense phase-locking means that the phase-lag between two waves will remain fixed. This does not happen in biological oscillators, since their frequencies and other parameters are not fixed as in electronic oscillators but tend to vary a little with time. For biological oscillations, therefore, two waves are defined to be phase-locked over a period of time if the maximum deviation in phase between them does not exceed 360° from the initial phase difference over that period of time.

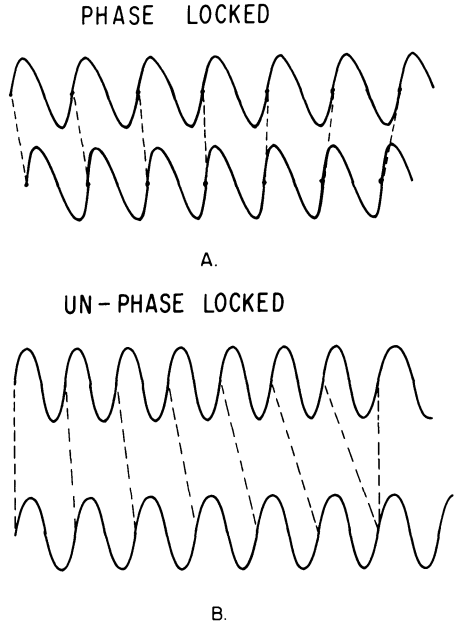

Figure 73.1 (a) Two waves that are phase-locked. Although there is variation in phase between different cycles, it does not exceed 360° from the initial phase-difference. (b) Two waves that are phase-unlocked. Here the lower waves falls 360° out of phase with the upper one and thus loses one complete cycle, compared with it

is, as it gets closer and closer to its natural time of oscillation, a smaller external stimulus is required to initiate a premature oscillation).

There are three possibilities regarding the frequency of entrainment when two oscillators with different intrinsic frequencies are coupled[3,4]. The first is that the frequency of entrainment may be somewhere in between the intrinsic frequencies of two oscillators (for example, in the case of computer models of Van der Pol oscillators[5,6]). The second possibility is that the frequency of entrainment is the same as that of the higher intrinsic frequency oscillator or even higher than this[7,8]. The second type of behaviour is observed both in the small intestinal and the gastric relaxation oscillators. Lastly, the entrained frequency may be equal to, or lower than, the frequency of oscillation of the lower intrinsic frequency oscillator. Each characteristic of relaxation oscillators can be obtained by suitable modifications of the Van der Pol equation. In case of frequency entrainment the oscillator with lower intrinsic frequency will always have phase-lag with respect to the higher intrinsic frequency oscillator (that is, the oscillations of the lower intrinsic frequency oscillator will begin with some time-lag after the beginning of oscillation of the higher intrinsic frequency oscillator). Phase-locking is thus an important phenomenon which can set fixed spatial and temporal relationships between two oscillators if they are representing different regions of an organ. In the gastro-

intestinal tract, for example, this can set up spatial and temporal relationships between control potentials at different sites, which in turn control the occurrence of electrical response activity (ERA) and hence contractions. This gives rise to peristaltic contractions.

If the coupling strength between two oscillators is not strong enough for entrainment, the frequencies of oscillation of the two oscillators may become different from their intrinsic frequencies but not equal (for example, if two Van der Pol oscillators are coupled with an insufficient coupling factor the frequency of the higher intrinsic frequency oscillator may be lowered, while that of the lower intrinsic frequency oscillator may be raised but they will not be entrained). The coupled oscillators in this state are said to be *phase-unlocked* and the phenomenon is called *frequency pulling* (Figure 73.1).

Let us now look at the application of relaxation oscillators to model the gastric ECA[8]. Figure 73.2 shows this activity recorded from six electrodes

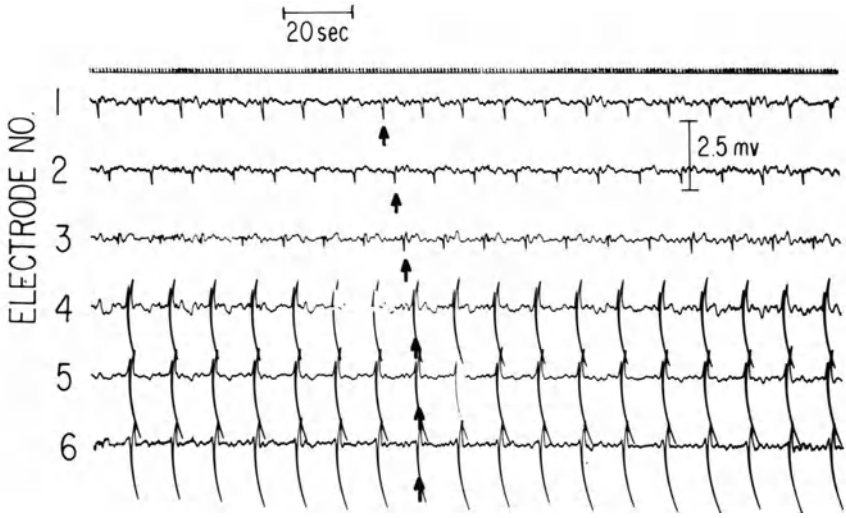

Figure 73.2 Electrical control waves recorded from six electrodes implanted on greater curvature. Distances of electrodes 6 to 1 from pylorus were 0.4, 2.2, 4.0, 6.3, 8.0 and 10.0 cm respectively. Arrows show the corresponding control potentials at different sites. Note that the phase-lag per centimetre reduces distally[8]

implanted in an anaesthetized dog along the greater curvature; electrode 1 being most proximal and 6 being most distal. It is clear that the direction of phase-lag among control potentials is aboral, as shown by arrows. If electrodes are implanted around the circumference, a small phase-lag is observed in that direction as well.

Let us briefly compare other possible models for these activities with the relaxation oscillator model. One could visualize this pattern of activity as resulting from a single oscillator in the proximal stomach, the control poten-

tial (oscillations) of which are conducted distally along the stomach, as would happen if one end of an axon is made to fire action potentials on a rhythmic basis. The possibility of this can be ruled out in the stomach, since if myotomy is done between the sites of electrodes 1 and 2, a control wave still persists at the latter site but with a slightly lower frequency. One can also rule out the possibility that all sites have independent and uncoupled oscillators all at the same intrinsic frequency, because biological oscillations are inherently unstable in frequency (unlike electronic oscillators and computer models) in which case a fixed phase-relationship like the one observed in Figure 73.2 could not be sustained over a period of time.

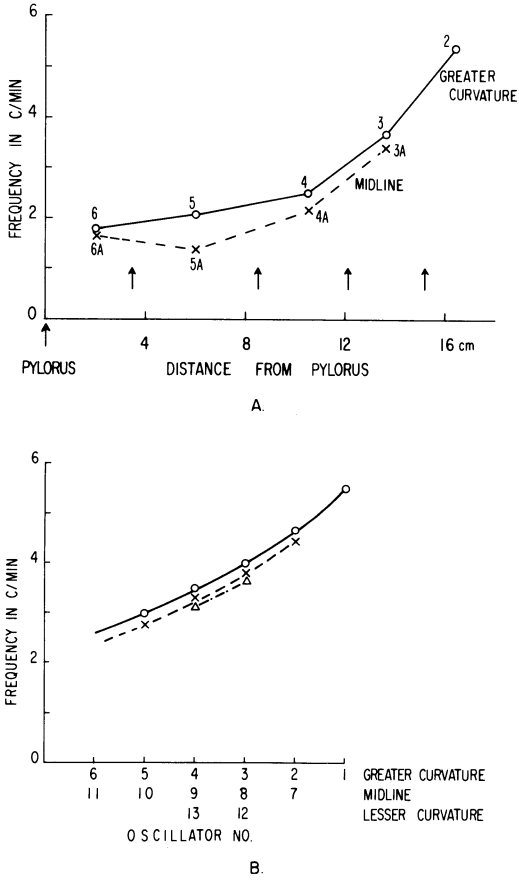

Figure 73.3 (a) The intrinsic frequency gradients along the greater curvature (——) and on the midline (– – – –) of the dog stomach. The sites of circumferential cuts are shown by arrows. There was a longitudinal cut running parallel to greater curvature and 2–3 cm from it. Numbers 1–5 refer to electrodes as in Figure 73.2. Numbers 1(a)–4(a) are corresponding electrodes on the midline.[8] (b) Intrinsic frequency gradients along the greater curvature (—), along the midline (– – –), and along the lesser curvature (—.—.—.) used in the gastric ECA model[8]

If the stomach is divided into several small segments by making longitudinal and circular cuts in the muscle layers, each isolated segment continues to show oscillations, but now they are all at different frequencies. If the frequency in each segment is considered to be the intrinsic frequency of that region then the intrinsic frequencies reduce distally and vary slightly from greater curvature to lesser curvature (Figure 73.3 (a)). The intrinsic frequency of the most proximal region near the greater curvature is nearly the same as that of the intact stomach. What this means is that each small region in the stomach is an independent oscillator, but when the stomach is intact (that is, when these oscillators are coupled), the highest intrinsic frequency oscillator located in the corpus near the greater curvature entrains the frequencies of all other oscillators to that of its own. This can happen if the oscillators are of the relaxation type as discussed above. The highest intrinsic frequency region entrains its neighbouring region to its own frequency, which in turn entrains the next distal and lateral regions to the same frequency, and so on until the entire stomach acquires the frequency of the highest intrinsic frequency oscillator. Thus the gastric ECA behaves like an array of bidirectionally coupled relaxation oscillators.

In the model each relaxation oscillator was represented by the following set of equations

$$x = k(a_1 y + a_2 x + a_3 x^2 + a_4 x^3 + I_n)$$

$$y = -1/k(b_1 y + b_2 x + b_3 x^2 + b_4 x^3 - b_0)$$

where: x is the output variable

y is another state variable

I_n is the resultant input to this oscillator from all neighbouring oscillators,

$k_1, a_1 \ldots a_4, b_0 \ldots b_4$ are constants which determine the characteristics of the oscillator and its intrinsic frequency.

These constants in each of the oscillators were adjusted to obtain an intrinsic frequency gradient similar to that seen in dog stomach (Figure 73.3 (b)). Figure 73.4 (a) shows the model of gastric ECA when the stomach is divided into several small segments. Figure 73.4 (b) shows the model of the intact stomach when these oscillators are bidirectionally coupled. Figure 73.5 shows the intrinsic frequencies of oscillators 1 to 6. But when these oscillators are coupled as in Figure 73.4 (b), they all acquire a frequency very close to that of oscillator 1 (Figure 73.6). This was also observed in the stomach.

The phenomenon of frequency pulling in the dog stomach and in its model are shown in Figure 73.7 and 73.8. In the dog stomach a partial cut, extending to 50% of the circumference, was made between the sites of electrodes 1 and 2. This reduced the coupled factor between those two sites to less than that required for entrainment. Hence control waves across the cut were no longer phase-locked, but the frequency of control waves distal to the cut was higher

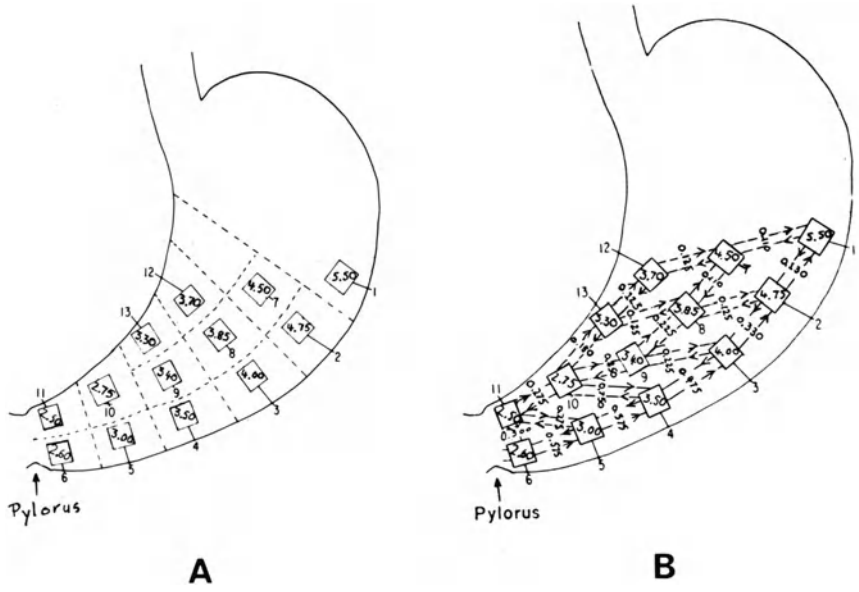

Figure 73.4 (a) Model of gastric ECA when the stomach is divided into several small segments. Numbers inside the boxes show the intrinsic frequencies, while those outside indicate the oscillator numbers. Broken lines show the cuts in gastric muscle layers to isolate it into several segments.[8] (b) The same model as above but of the intact stomach. All the oscillators have now been bidirectionally coupled with their neighbouring oscillators. Numbers along the coupling level shows the coupling factors[8]

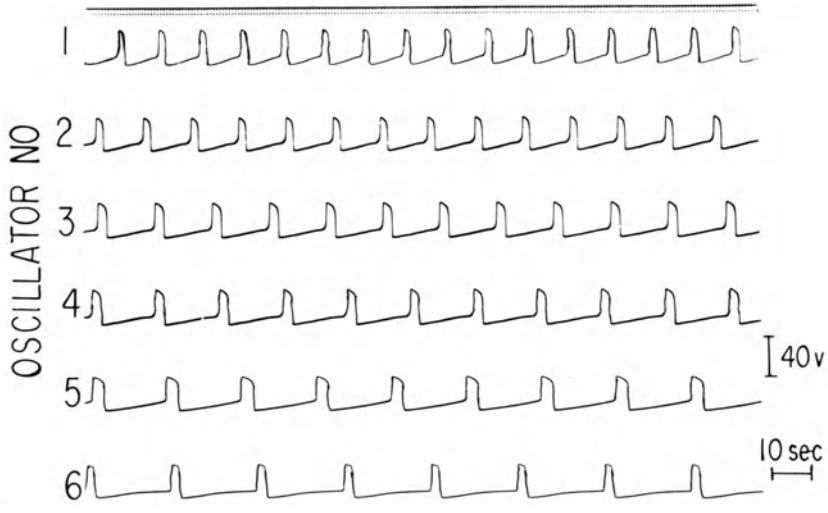

Figure 73.5 Outputs of oscillators 1–6 when they are uncoupled, showing their intrinsic frequencies[8]

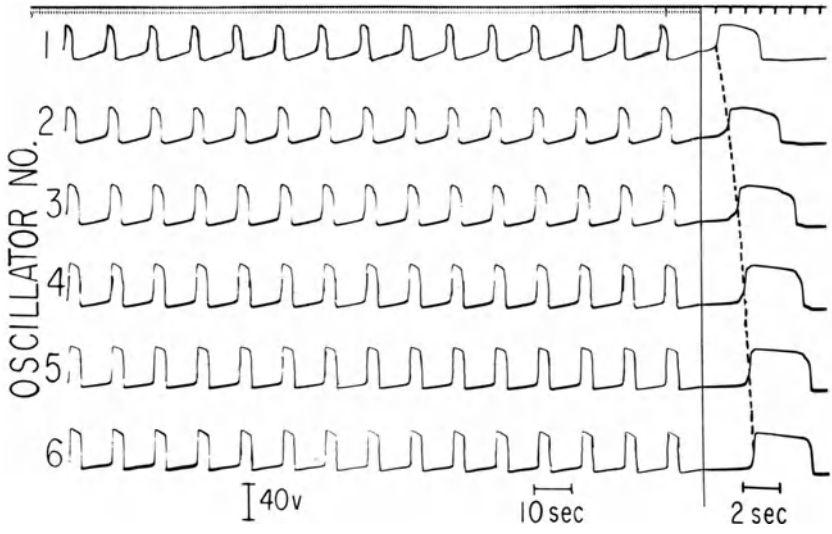

Figure 73.6 Outputs of oscillators 1–6 when they are bidirectionally coupled as in Figure 73.4(b), simulating the frequencies of the intact stomach[8]

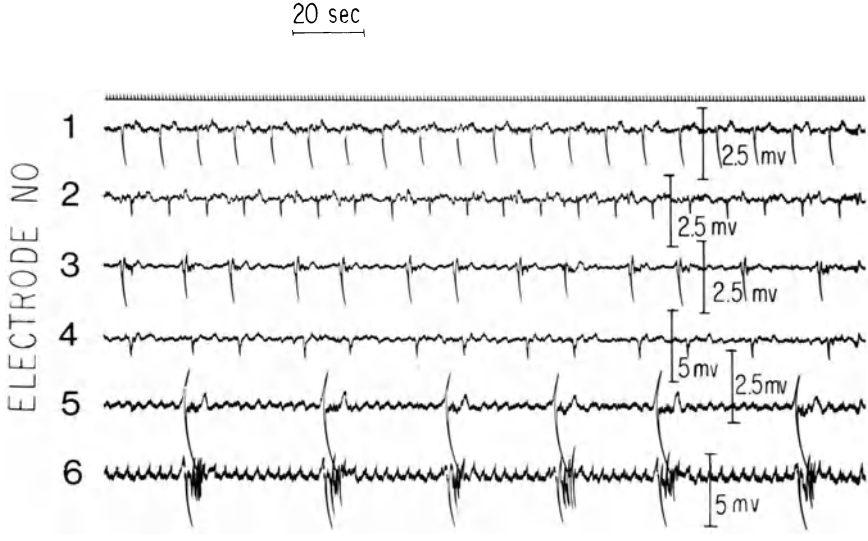

Figure 73.7 Control waves recorded from six electrodes implanted near greater curvature. A complete circumferential cut (3.0 cm from pylorus) existed between electrodes 4 and 5, and a partial (50%) circumferential cut (8.2 cm from pylorus) existed between electrodes 2 and 3. Note frequency pulling of control waves recorded at electrodes 3 and 4. Control waves at electrodes 5 and 6 are at their intrinsic frequency value[9]

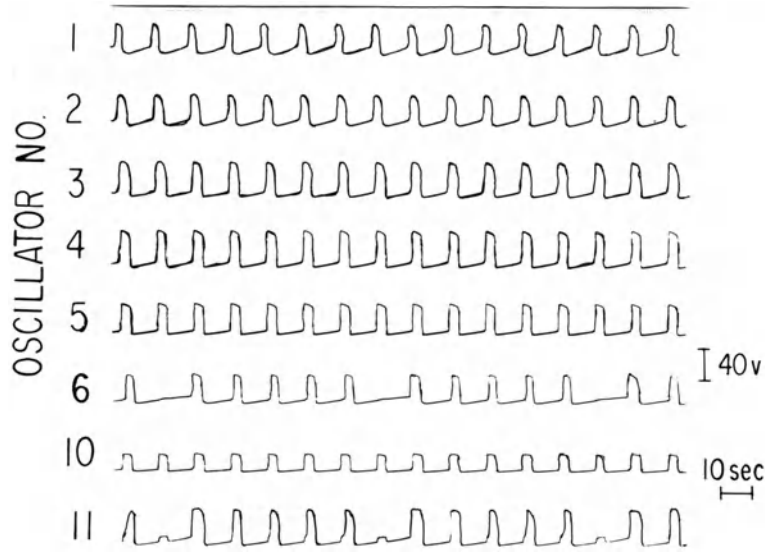

Figure 73.8 Recording showing the phenomenon of frequency pulling in oscillators 6 and 11. A simulated cut by setting coupling factor to 0 existed between oscillators 5–6 and 5–11. See Figure 73.4(b) for arrangement of oscillators

than the intrinsic frequency in that region. There were some faster cycles when the higher intrinsic frequency oscillators could drive distal ones, because their oscillations appeared at an appropriate phase of the lower frequency oscillation; and there were some slower cycles when the higher intrinsic frequency oscillators could not drive the lower intrinsic frequency oscillators. This phenomenon of frequency pulling was simulated in the computer model by reducing the coupling factor between oscillators 5 and 6, and, 10 and 11 (Figure 73.8).

In the dog stomach the phase lag per centimetre reduced distally. In terms of velocity of propagation of ring of contractions, this can be taken to mean that the propagation velocity increases distally. Thus the phenomenon was also present in the computer model (Figure 73.6); the inset at the right-hand side shows oscillations with a faster paper speed, and it shows that phase-lag/unit decrease in frequency reduces distally.

The coupling factors among oscillators were determined empirically to get the desired phase-lag pattern and entrainment among all oscillators in the intact state. But their relative magnitudes were similar to those determined in dogs by doing a variety of partial cuts[9] which indicated that the coupling was strong along the greater curvature and increased distally. The coupling was strong in all axes throughout the distal 4–6 cm of stomach, and it was weaker near the lesser curvature in the stomach proximal to this region.

Thus the array of bidirectionally coupled relaxation oscillators is an

appropriate model of gastric ECA[8]. Simple but adequate models of small intestinal ECA, consisting of a chain of coupled relaxation oscillators, can be used because little or no phase-lag is observed along the circumference[5–7]. In this case a radial segment can be considered as one oscillator. These models and those of colonic ECA[10] have been discussed in detail in other chapters of this book.

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74

Human colonic modelling and multiple solutions in non-linear oscillators

D. A. LINKENS AND S. P. DARTARDINA

The modelling of slow waves in the stomach and small intestine, using chains and arrays of coupled non-linear oscillators, has been demonstrated in previous papers in this Workshop section. The existence of a single stable rhythm with relatively small changes in amplitude, apart from the 'waxing and waning' phenomenon in the lower part of the duodenum, has encouraged the use of nonlinear oscillator dynamics of the classic van der Pol type. The different nature of slow-wave behaviour in the human colon has prompted modelling investigations using modifications to the normal van der Pol dynamic and a study of multi-mode phenomena in coupled oscillators.

Monopolar recordings from the human colon using filters having frequency response 0.02–1 kHz have indicated four basic patterns in the electrical activity. These are: (a) periods of electrical silence; (b) lower frequency rhythm in the region of 0.05 Hz; (c) high-frequency rhythm in the region of 0.1–0.2 Hz; (d) occasional periods of simultaneous occurrence of both lower and higher frequencies[1]. It is to answer the question whether the concept of a single population of coupled oscillators can account for such multiple phenomena that this paper is concerned.

SINGLE OSCILLATOR DYNAMICS

The conventional van der Pol dynamic has the form

$$\ddot{x} - \varepsilon(a^2 - x^2)\dot{x} + \omega^2 x = 0 \qquad (1)$$

and a single stable oscillation of amplitude a and frequency mainly determined by ω if ε, the wave-shape factor, is not too large. The zero condition

is unstable and hence oscillations are self-starting. The nonlinear term is equivalent to a cubic law conductance term in an electronic circuit implementation of this equation.

Van der Pol investigated many different non-linear characteristics, and showed that the following equation gives zero as a stable condition[2]:

$$\ddot{x} + \varepsilon(b - cx^2 + dx^4)\dot{x} + \omega^2 x = 0 \qquad (2)$$

With a suitable choice of the constants b, c and d a stable oscillation is also obtained. Such an equation includes a non-linear characteristic having a fifth power term with a typical shape shown in Figure 74.1. The wave-shape generated by equation (2) is indistinguishable by eye from that of equation (1); but there is an essential difference that both the zero state and the oscillation are stable conditions which can be excited by disturbances to the model.

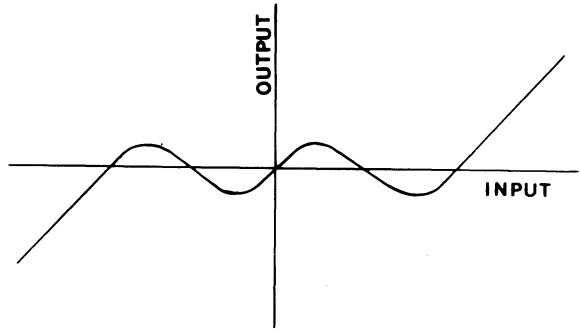

Figure 74.1 Non-linear conductance characteristic represented by the bracketed terms in equation (2)

SINGLE MODES IN COUPLED OSCILLATORS

When non-linear oscillators are coupled together the phenomenon of entrainment occurs. The value of entrainment frequency and the number of modes depends not only on the strength of coupling but also on the points of interconnection between oscillators. It is valuable to relate the dynamic equations with an equivalent circuit representation of the model. Figure 74.2 is an equivalent circuit for two coupled oscillators each having non-linear conductance and with capacitive, resistive and inductive coupling components. The dynamic equation for one of these oscillators would be:

$$\ddot{x}_1 + \varepsilon(b - cx_1^2 + dx_1^4)\dot{x}_1 + \omega^2 x_1 = \lambda_C \ddot{x}_2 + \lambda_R \dot{x}_2 + \lambda_L x_2 \qquad (3)$$

where λ_C, λ_R and λ_L represent capacitive, resistive and inductive coupling terms.

In terms of the equivalent circuit it can be shown analytically[3] that two stable oscillation frequencies can be obtained with either capacitive or in-

ductive coupling, and only one stable mode with resistive coupling. One mode corresponds to a nearly in-phase condition with an entrainment frequency approximately equal to the average of the uncoupled intrinsic frequencies. The other mode has a nearly anti-phase condition and gives a higher than intrinsic frequency for inductive coupling, and lower than intrinsic frequency for capacitive coupling. While these analytical results were obtained for almost sinusoidal oscillations, the same general effects occur for more non-linear wave-forms.

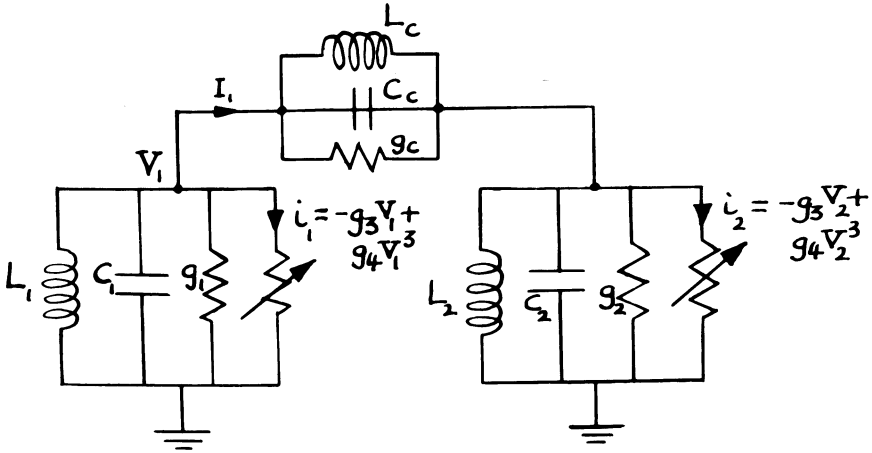

Figure 74.2 Equivalent circuit of two van der Pol oscillators coupled with a parallel RLC network

When a chain of oscillators of the van der Pol type is considered, further modes become possible. It has been shown analytically and experimentally that many modes are possible if the chain has its ends short-circuited to earth. In the case of a chain with open ends and an even number of oscillators the only two modes which are stable are the in-phase and anti-phase conditions already referred to for either the capacitive or inductive coupling conditions[4].

Analytical work has also been done on two-dimensional arrays of coupled van der Pol oscillators giving similar phenomena to those for a chain connection[5]. Although most of the analysis has been done on systems without a frequency gradient, it has been possible to predict phase shifts in chains having a frequency gradient[6].

It has thus been demonstrated so far that a model comprising coupled van der Pol oscillators having fifth-power non-linearity can reproduce conditions of zero activity and two stable frequencies whose values will depend both on the intrinsic frequencies and on the type and strength of coupling. The feasibility of having both frequencies present simultaneously will now be considered.

MULTI-MODES IN COUPLED OSCILLATORS

Simultaneous occurrence of two frequencies has been observed both visually and via FFT analysis in the human rectosigmoid[7]. It also occurs occasionally more proximally in the human colon and Figure 74.3 shows frequencies of about 0.03 and 0.2 Hz present in both monopolar and bipolar recordings from a transverse colonic recording.

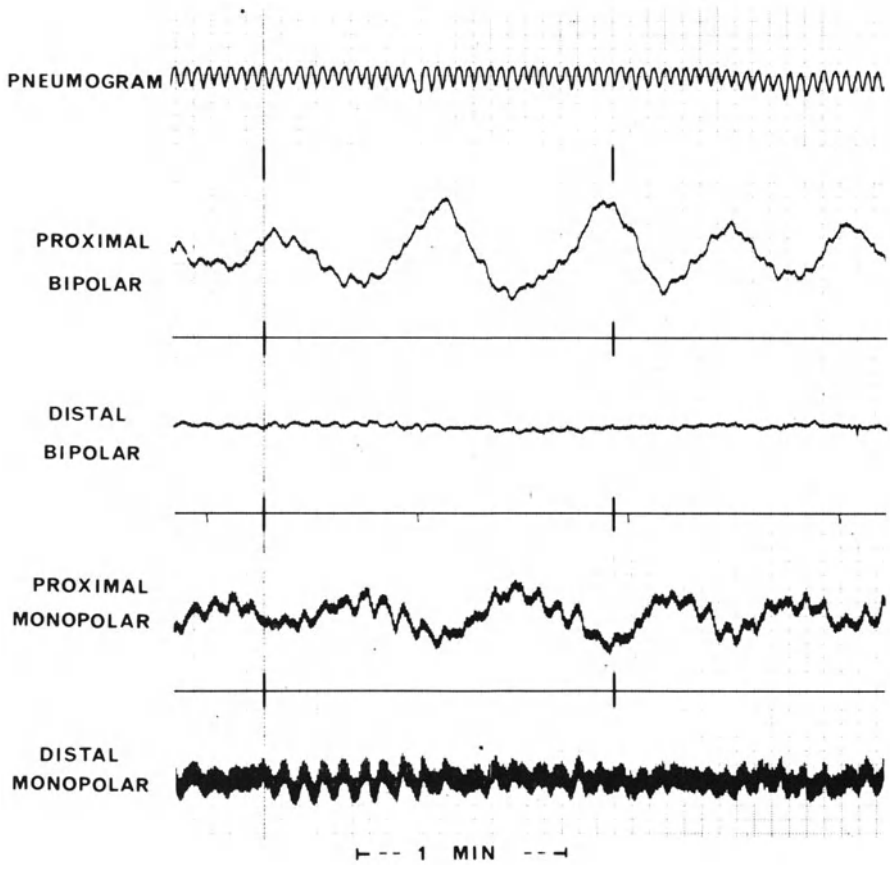

Figure 74.3 Monopolar and bipolar recordings from a human transverse colon showing simultaneous occurrence of two rhythms

The possible occurrence of these so-called multi-modes has been studied analytically in electrical circuit theory recently, although there appears to have been no direct motivation for such work. It has been shown that two frequencies can be present in certain types of chain and array connections, but that three frequencies are almost impossible under all conditions. For an open-ended chain, which is of most interest in colonic modelling, double

modes are possible using conventional van der Pol dynamics[4]. The frequencies contained in this multi-mode do not, however, correspond to the single-mode frequencies and this does not correlate with the data recorded from the human colon. A further point is that multi-modes are not possible if there are only two oscillators coupled together.

It has been found recently that two or more coupled van der Pol oscillators with fifth-power non-linearity can give a double-mode whose individual frequencies equal the single-modes. The range of parameters for which this double-mode is feasible have been predicted analytically and confirmed via analogue computer simulation[8]. These results have also been confirmed using an electronic implementation with a straight-line approximation to Figure 74.1. The output from two oscillators is shown in Figure 74.4, in which it can be seen that the higher-frequency mode is basically anti-phase, and the lower frequency is nearly in-phase, The coupling in this case was inductive and gave a ratio of the two frequencies of about 2:1.

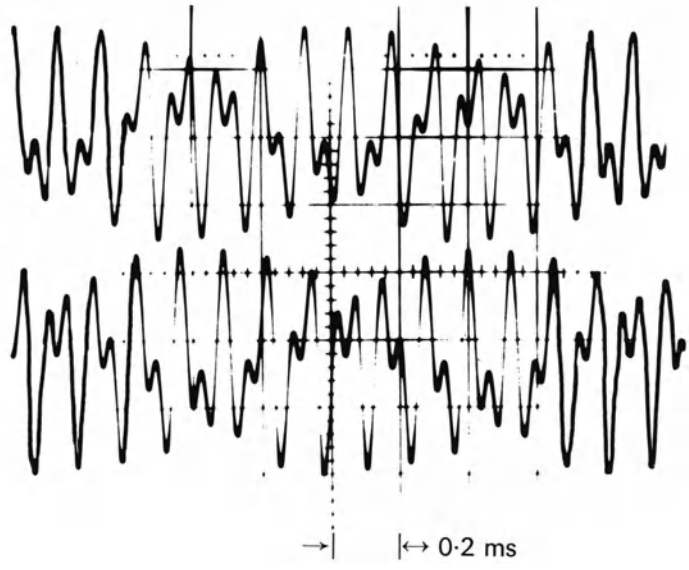

Figure 74.4 Simultaneous occurrence of two oscillations in an electronic implementation of two coupled modified van der Pol oscillators

CONCLUSIONS

The attainment of a clear picture of behavioural pattern for the slow waves in the human large bowel is considerably more difficult than for the stomach and small intestine. Stable, single-frequency oscillations present all the time are replaced by considerable changes in amplitude and frequency and even

the possibility of several frequencies present simultaneously. A major modelling question is whether the concept of linked non-linear oscillators can account for such phenomena. It is concluded both from analytical and simulation studies that such behavioural patterns do not negate the concept of linked oscillators for digestive tract modelling.

It has been shown that small modifications to the non-linear conductance term in van der Pol's equation can give zero activity as a genuine stable condition. Furthermore, such modified oscillators, when coupled together with either inductive or capacitive components, not only give two stable frequency single-modes, but also the simultaneous occurrence of these two modes. It is emphasized that this double-mode is not possible with the more conventional van der Pol dynamics.

The considerable cross-fertilization between gut data acquisition, mathematics and electrical circuit theory is of interest, since some of the analytical results mentioned in this chapter were obtained before any obvious practical application or experimental results were available.

Although the modelling referred to in this chapter has been based on van der Pol-type equations, it should be noted that other oscillator dynamics can also be studied experimentally. Such dynamics seldom offer much hope of analytical study, but they may be closer to physiological notation and experimental measurement. An example of this is the use of the Hodgkin–Huxley model originally developed for squid axon, but modified for other tissue such as cardiac pacemakers, and Purkinje fibres. A model based on the Hodgkin–Huxley equations for the oscillator dynamics has shown the phenomena of frequency entrainment and two single-frequency modes[9,10]. Such simulations on a digital computer are lengthy, and a model comprising eight oscillators implemented using an electronic approximation to the Hodgkin–Huxley equations has recently been studied[11]. This model retains the concepts of ionic conductance and membrane capacitance whilst having four adjustment parameters which determine the frequency and wave-shape of each oscillator. This model also produces a double-frequency condition when coupled inductively.

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Index