Old Herborn University Seminar Monograph

9. GASTRO-INTESTINAL MOTILITY

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GENERAL INTRODUCTION TO THE NINTH OLD HERBORN UNIVERSITY SEMINAR: GASTRO-INTESTINAL MOTILITY

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SUMMARY

Gastro-intestinal (GI) motility represents a complex, multifactorial interplay between factors deriving from the host, the food and the microbial flora. The intention is to break down food products into absorbable compounds and to excrete nonabsorbable compounds, bacteria, cell debris, etc.

The motility pattern differs markedly between the fasting and the postprandial state and is also influenced by the microbial status in various GI compartments. Direction and rate of flow of luminal contents depend on the force and frequency of stationary and propagated contractions.

This symposium is focused on three topics: 1. The motility itself; 2. The interplay between motility and the microbial flora; and 3. The influence of some antimicrobial agents on motility.

GI motility can be measured by many different methods. Pro's and con's related to these methods will also be discussed.

INTRODUCTION

The scope of the 9. Old Herborn University Seminar is defined by three task keywords: i) Motility, ii) Microflora, and iii) Methods. Hopefully, these task keywords will be looked upon from another 3M-point of view, i.e. at a i) Macroscopic, ii) Microscopic, and iii) Molecular level.

MOTILITY

According to Dorlands Illustrated Medical Dictionary, the term motility is defined as "the ability to move spontaneously". Motility is a basic prerequisite for all macroorganisms with a gastrointestinal tract. However, in spite of the simple definition given above, a close to 100 years old statement still holds true:

"On no subject in physiology do we meet with so many discrepancies of facts and opinions as in that of the physiology of the gastro-intestinal movements" (*Bayliss* and *Starling*, 1899).

Obviously, the discrepancies are caused by the complexity of the simple problem; how to move substances from mouth to anus? Any oral intake of any substance triggers a complex interplay between motility, secretion and absorp-

tion along the entire gastro-intestinal (GI) tract. Under physiological conditions, these processes are regulated by intrinsic and extrinsic nerves, and by a long series of polypeptide hormones. Principally, the same mechanisms are responsible for the coordination of GI motor and transport events during fasting. In fact, the events during fasting are even more complicated than during digestion. Gastric and intestinal motor and secretory functions are cyclically activated by intrinsic biological rhythms of enteric origin operating like coupled oscillators (*Wingate*, 1983). Thus, during fasting as well as during digestion, the GI tract acts as a functional unit in which the subunits oesophagus, stomach, duodenum, jejunum, ileum and colon are coordinated by stimulatory and inhibitory nervous and humoral mechanisms (*Ruppin*, 1985).

In an attempt to simplify this complex interplay, I will first focus upon some basic physiological cornerstones. The motor functions of the GI tract include mixing of dietary compounds with all kinds of secretory products from the body itself, followed by a propulsion of the content aborally (caudally).

Some parts of the GI tract are divided from the rest of the tract by sphincter mechanisms, so is the stomach and the large intestine. Sphincter mechanisms may also regulate the in-flow of endogenous secretion, as Sphincter Oddi.

The speed by which the content is passing through the various compartments is varying considerably. To give some perspective: transit through oesophagus is measured in seconds, whereas transit through the stomach is measured in minutes, through the small intestine in hours and through the colon in days.

The amount of content which is processed through the GI "pipeline" is also varying considerably. To give another perspective: it might be more than 10 litres a day through the duodenum, 1-2 litres through the very distal part of ileum and less than 500 ml or grams, through the rectum

The motility differs considerably throughout the GI tract, and in brief, it is as follows:

The oesophagus

The body of the oesophagus is normally relaxed and contracts in response to swallowing (primary peristalsis) or distension (secondary peristalsis). At the distal end of the oesophagus there is a sphincter mechanism, separating the positive gastric pressure from the negative oesophageal pressure. After initiation of either a primary or a secondary peristaltic wave there is a fall in lower oesophageal sphincter pressure which precedes the arrival of the peristaltic wave. Agents as gastrin, acetylcholine serotonin, prostaglandin F2-alpha and alpha-adrenergic agents increase the lower oesophageal sphincter pressure whereas secretin, cholecystokinin, vasoactive intestinal peptide (VIP), prostaglandin E1 and E2 and beta-adrenergic agents reduce the pressure.

The stomach

The stomach is a very muscular organ in which the food undergoes mixing and initial digestion. Peristaltic contraction waves sweep from the cardia to the duodenum. The stomach pumps its contents into the duodenum, the pump mechanisms comprises the distal portion of the gastric antrum, the pylorus and possibly the duodenal bulb. The mechanisms behind gastric motility will be described in greater details by the next speaker.

The small intestine

Contractions in the small intestine serve at least three different purposes. First, dietary products are mixed with the secretions of stomach, pancreas, biliary tract and small intestine. Second, the digestive products are brought into

intimate contact with the absorptive surface of the small intestine. Last, but not least, propulsive movements eliminate all kinds of nonabsorbable products, cell detritus and bacteria. The mechanisms behind small intestinal motility will be highlighted by the second speaker to come.

The large intestine

The motor functions include mixing, storage and slow propulsion of colonic content caudally, and a rapid, strong propulsion of content during defecation. Under physiological conditions, it is of importance that the colon does not initiate too rapid a transit. Colonic mixing and transit are mediated by several different types of contractions, and they seem to be under three levels of neural control: enteric, autonomic and central. These topics will be highlighted by the third lecture to follow.

MICROBES

The mere fact that germfree (GF) animals live as long as their conventional (CONV) counterparts does not implicate that presence of a gastrointestinal microflora has little, if anything, to do with gastro-intestinal motility. For scientists working with GF rats and mice, it is an everyday experience that the germfree GI tract demonstrates less spontaneous muscular contractions than their conventional counterparts. The enlargement of caecum, found in most species of germfree animals, still remains the main riddle of GF life.

A slower intestinal transit time in GF animals compared to their conventional counterparts was reported by several investigators almost 30 years ago (*Abrams* and *Bishop*, 1967; *Gustafsson* and *Norman*, 1969; *Ducluzeau* et al., 1970). Using the marker polyethylene glycol, *Heneghan* and *Mittelbronn* (1981) showed a reduced transit time in GF rats and dogs. They stressed the fact that the enlarged caecum in GF animals may represent a highly variable trap for a test meal, sometimes trapping large quantities, and other times small amounts.

The observation that caecal contents of GF rats and mice may contain a musculo-active substance (MAS) was also made almost 30 years ago (*Gordon*, 1967), and *Bruckner* (1997) describes the end of that story in this volume.

Utilising modern technology, *Caenepeel* and co-workers (1986; 1989) found less frequent migrating motor complexes (MMCs) in the small intestine of GF Fisher rats, when compared with the CONV counterparts, Caenepeel's findings have been extended by *Husebey* and co-workers (1990; 1992; 1994). These experiments show that the intestinal microflora represents a major stimulus for cyclic and aboral propagation of MMCs in the rat small intestine. Consequently, the resident microflora is a main factor in regulating fasting MMCs.

Rather few investigations are dealing with possible differences in colonic motor activities in GF and CONV animals. Several years ago, we investigated the caecum wall of GF and CONV rats with respect to the content of and sensitivity to some biologically active amines (*Strandberg* et al., 1966). The concentration of noradrenaline, l-adrenaline, dopamine, serotonin, acetylcholine and histamine was found to be of the same order of magnitude in both types of rats. Strips of the caecum wall from conventional rats exhibited regular spontaneous muscle contractions, whereas in germfree rats, such an activ-

Table 1: Total volume of endocrine immunoreactive cells in the gastro-intestinal tract of germfree rats

Immunoreactive cells	Fundus	Antrum	Jejunum	Ileum	Colon
Serotonin	↑	→	→	↑	↑
Motilin	∅	∅	→	↑	∅
Somatostatin	→	→	→	→	→
Neurotiensin	∅	∅	→	→	∅
Glucagon/glicentin	n.d.	n.d.	→	→	→
Gastrin	n.d.	↑	∅	∅	∅
Chromogranin A	→	n.d.	n.d.	n.d.	n.d.

↑ : increased
→ : no change
∅ : not supposed to be present
n.d. : not done

ity was never seen. In both GF and CONV rats, their type of a single contraction of the caecum strip as a response to the administered amines was similar except for serotonin. However, the threshold dose was generally higher in the GF rats than in the CONV rats, and the difference was most pronounced for acetylcholine. The caecum strip of a GF rat reacted to serotonin by contraction, initially rapid, then slowly proceeding to a maximum at about 2 minutes. The strips from the CONV rats reacted by rapid contractions.

The effects of age and microbial status upon muscular activity in caecum strips have also been evaluated (*Gustafsson* et al., 1970). Strips from 3-week-old GF and CONV rats showed the same - and low - muscular sensitivity to biogenic amines and had an absence of spontaneous activity. However, 8 weeks later, i.e. at 11 weeks of age, strips from CONV rats had the same high sensitivity and spontaneous muscular activity as in older CONV rats, whereas the strips from the GF rats reacted as those at 3 weeks of age. When young GF animals were conventionalized, i.e. were given aliquots of faecal suspension from CONV rats both by oral and rectal administration, their strips were after 8 weeks as sensitive to amines and reacted with the same spontaneous muscular activity as CONV rats of the same age. However, if one-year-old GF rats were conventionalized, no alterations in sensitivity or spontaneous activity were found after 8 weeks. The mechanism(s) behind this microbial, age-dependent "priming" of caecum motility remain(s) unknown - and may deserve some comments. *In utero*, the intestinal movements of the foetus are thought to be low. The intestine is filled with meconium, to be excreted after birth. If an excretion of meconium is not established some few days after birth, a search for reasons for this delay has to be undertaken - and most often, it is found to be a mechanical one. The establishment of GI motility - as well as other intestinal functions - is initiated and regulated by several, intrinsic as well as extrinsic, factors. The most dominating extrinsic factors are obviously food and establishment of an intestinal flora. Human milk contains high concentrations of several neuropeptides which can be absorbed in an

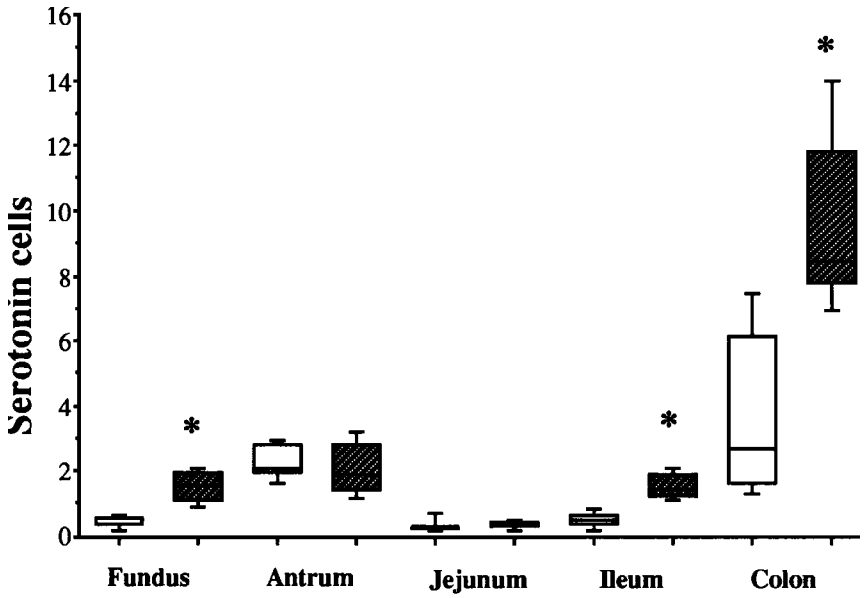

Figure 1: Total volume of serotonin immunoreactive cells in the gastro-intestinal mucosa of conventional (□) and germfree (▨) rats (*: $p<0.05$).

intact molecular form (*Werner* et al., 1985). It has also been found that formula-fed infants have higher basal levels of motilin than breast-fed infants (*Lucas* et al., 1980). It is well known that the intestinal flora in formula-fed infants differed markedly from the flora found in breast-fed infants. It is tempting to speculate that variations in diet may create variations in the microflora, thereby creating variations in the flora-associated influences upon GI peptides and motility.

We have recently investigated the volumes of endocrine cells in the GI tract of GF and CONV animals (*Alam*, 1995; *Uribe* et al., 1994), and some of our data is given in Table 1 and Figure 1. Previously it was known that considerable amounts of serotonin are present in the gut lumen (*Ahlman* et al., 1981) and that several microbial species can produce serotonin *in vitro* (*Karlsson* et al., 1988). If this occurs also *in vivo*, it will contribute to the luminal concentration of serotonin. It might then be reasonable to assume that a reduced availability of this amine, as in GF conditions, could trigger endogenous regulatory mechanisms to give rise to the hyperplasia or increased activity of serotonin-producing cells.

It is well known that endocrine cells producing serotonin may also produce motilin (*Polak* et al., 1975). Therefore the increase in motilin in GF rats might be secondary to the increase in serotonin. Another explanation might be that the increase in motilin represents a physiological up-regulation of intestinal motility in GF rats. However, as underlined before, the molecular mechanisms being the reduced GI motility in GF rats compared to their CONV counterparts are unknown (*Midtvedt*, 1989).

The clinical observation that antibiotic therapy may cause marked alterations in GI motility (from vomiting to diarrhoea) is well known indeed. In part, these alterations are due to alterations in the resident microbial flora.

However, it is also well established that several types of antibiotics have an inhibitory effect on smooth muscle contractility *in vitro*. (*Popovici* et al., 1965; *Paradelis*, 1981; *Koeda* et al., 1982). In the last decade, most attention has been paid to the macrolide group of antibiotics and effects upon GI motility. The findings by *Klika* and *Goodman* (1982) that erythromycin acted directly, i.e. not via the microbial flora, upon the motility, prompted a long series of investigations concerning the influence of antibiotics upon intestinal motility. (For reviews see: *Midtvedt* 1989; *Midtvedt* and *Greenwood* 1994; *Midtvedt* and *Greenwood*, 1995).

METHODS

The ways of observing GI motility includes a long series of different methods, from naked-eye inspection, transit time, x-ray examination, ultrasonic investigation, internal pressure curves, electromyography, etc. The following papers will discus the pro's and con's about some of the clinical and experimental methods currently in use.

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METHODS FOR STUDYING GASTRO-INTESTINAL MOTILITY: STOMACH

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INTRODUCTION

Standardized methods for measurement of gastric motor functions are required for correct diagnosis of disordered gastric emptying, as the diagnostic value of subjective abdominal and epigastric symptoms and physical findings is poor.

The methods that are used clinically can be separated into three categories:

- Measurement of gastric emptying
- Measurement of antroduodenal luminal pressures
- Measurement of gastric electric activity

Scintigraphic measurement of gastric emptying is presently the only of these methods that has proven clinical value. Other methods of luminal pressure measurements and electric activity mainly have a research potential but may add to the diagnostic value of radionuclide measurements of emptying.

MEASUREMENT OF GASTRIC EMPTYING

Scintigraphic technique

Scintigraphic measurement of gastric emptying is a convenient non-invasive method for evaluation of gastric emptying. The method permits concomitant measurements of solid as well as liquid meals. However, the diagnostic value seems greater for the emptying of solid gastric contents than for liquids (*Horowitz* et al., 1985). The radionuclide mostly used as marker is ^{99m}Tc because of its low cost, easy accessibility and convenient physical half-life. ^{99m}Tc labelled macroaggregated albumin is easily incorporated into liquid or solid meals, preferably containing egg albumin to which the marker substance can bind. We have used an egg omelette with added flavour to achieve a truly solid meal of 250 g corresponding to 310 kcal, to which 10-12 MBq of ^{99m}Tc is added. The omelette is cooked in a microwave oven and is tolerated by most patients. Alternatively, pancakes can be used, which is preferred by children, but may not be used in studies of diabetic patients, and require extra-ordinary utensils for the radioactive cooking.

The test meal is eaten within 10 min together with 150 ml of liquid and thereafter registration is started using anterior-posterior and posterior-anterior projections for 1-min periods at intervals of 5 min until the lag phase is passed, followed by projections at 10-min intervals until 120 min.

The gamma camera is linked to a computer system which enables registration of abdominal radioactivity. The stomach is recognized by its characteristic shape and the region of interest is outlined (Figure 1). The counts within this region reflect the amount of food

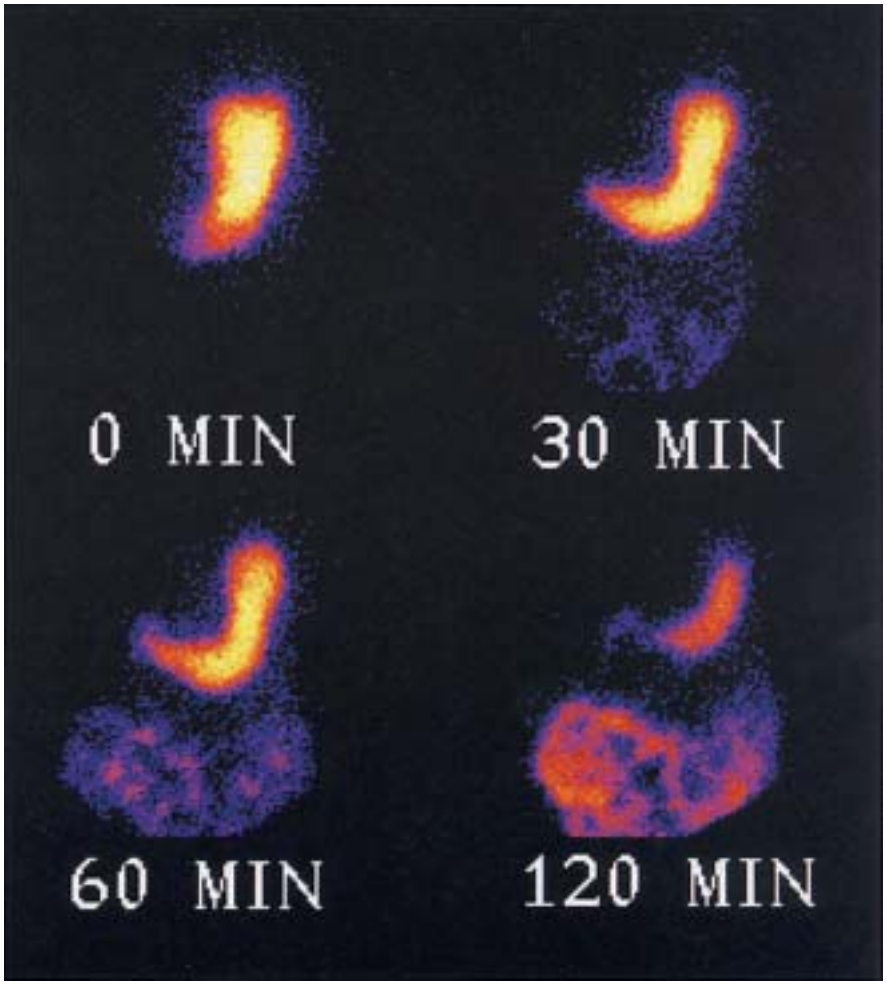

Figure 1: Scintigraphic registration of gastric emptying using a 12 MBq ^{99m}Tc-labelled omelette. Scintiscans taken at 0, 30, 60 and 120 min after food intake.

retained in the stomach. The gastric radioactivity registered by anterior and posterior projections, are multiplied and the square root of the value is taken to achieve the geometric centre of the region-of-interest. The computed values are plotted against time to determine the rate of gastric emptying (*Collins* et al., 1983).

Gastric emptying of liquid contents may be measured simultaneously with the emptying of solids, because liquids are already taken with the test meal. In such cases, ^{111}In-DTPA is added to the liquid marker. The gamma camera system separately detects the two nuclides of different energies (*Horowitz* et al., 1985).

The use of large field-of-view gamma cameras minimizes technical errors and may permit simultaneous measurements of small intestinal transit (*Read*, 1989).

Measurement of solid meals are more

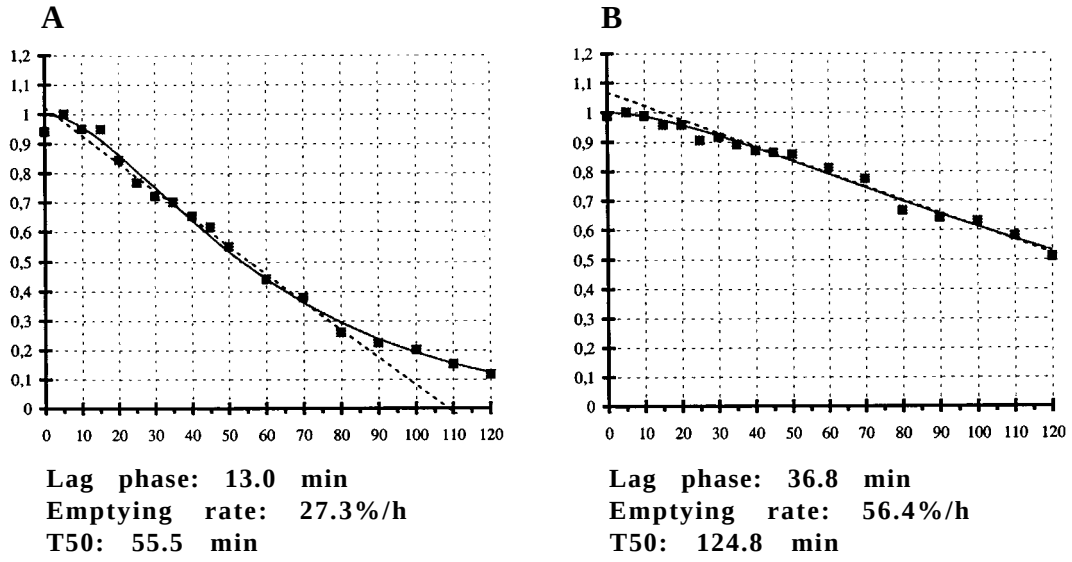

Figure 2: Illustrations showing the gastric emptying process in a healthy male (A) and female (B). Indices for the gastric emptying is given below the graphs.

sensitive than measurements of liquids in the detection of abnormal gastric emptying. Therefore, if a single tracer should be employed a solid marker is preferred (*Malmud* and *Fisher*, 1981).

Measurements of gastric emptying of solids usually demonstrate a characteristic appearance with a lag phase followed by a linear emptying phase. The mathematic evaluation of gastric emptying includes the lag phase period which we defined until 90% retention (min), as well as the post-lag linear rate of gastric emptying (%/min), the half-emptying time (T50; min) and the gastric retention at 120 min (%). The gastric emptying rate during the linear emptying phase has been estimated to be about 1-2 kcal/min. Figure 2 shows the typical appearance of two gastric emptying curves in a male (A) and female (B).

On a research basis, in order to further evaluate the association between motor function of the stomach and gastric emptying, scintigraphy has been combined with manometric techniques (*Houghton* et al., 1988).

The common problems inherent with scintigraphy is that the method is expensive and time-consuming, and occupies detection equipment that has a limited availability. Also, studies with administration of radionuclides cannot be carried out during pregnancy.

The methodological problems that limit the sensitivity and specificity of isotope gastric emptying tests include in particular the movement of the radionuclide marker within the stomach, which leads to variations in the counts detected because of the different thickness of tissue between the stomach and the gamma camera for which corrections must be made (*Collins* et al., 1983). Another drawback with the method is that external scintigraphy cannot measure the volume of gastric secretion within or emptied from the stomach. This gastric secretion progressively dilutes the gastric markers and may affect the outcome of the emptying characteristics.

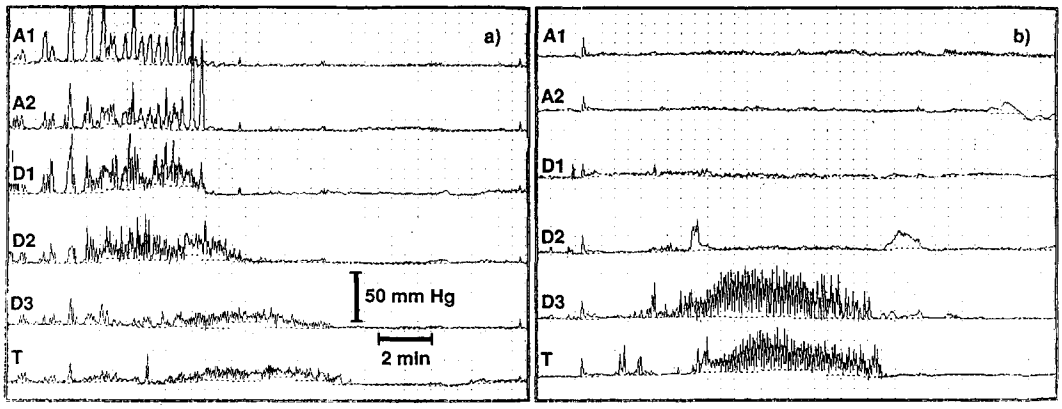

Figure 3: Recordings of antroduodenal motility in a healthy subject (a) and a patient with diabetic gastroparesis (b). Registration sites in the proximal (A1) and distal (A2) antrum, proximal (D1), middle (D2) and distal (D3) duodenum, and at the angle of Treitz (T).

Absorption kinetics of orally administered drugs

In humans there is limited absorption of drugs administered orally. The rate of appearance of the drug in plasma therefore serves as an indicator to measure the rate of gastric emptying (*Nimmo*, 1976). Paracetamol is most commonly used for detection of the gastric emptying rate because the drug can usually be analysed on a routine basis at clinical laboratories. A limitation of the method is that it only permits measurements of the liquid phase of emptying. An advantage with the method is that it can be performed in most clinical settings with availability of limited analytical equipment.

Determination of the rate of gastric emptying by measurement of plasma concentrations of intestinally absorbed paracetamol has been considered fairly inaccurate and unsatisfactory for appropriate measurements of gastric emptying. However, with the use of a suitable nutrient-liquid test meal and carefully calibrated standard curves for plasma concentrations of paracetamol, the method can be optimized. We have used paracetamol 24 mg/kg body weight, i.e. about 1.5 g, given perorally with Borgström's test meal containing 400 kcal of liquid nutrients. The absorption kinetics of paracetamol are usually evaluated as T_{max} (min), C_{max} (mmol/l) and area under curve (AUC; mmol/l.min), but may be further optimized by swapping the cumulated absorption curve for the drug which then reflects the gastric emptying rate. This mathematical operation also permits calculation of the half-emptying rate (T50; min) for the drug.

Another way of optimising the measurement of gastric emptying with this technique is to carefully coordinate the emptying process to the respective phase of the migrating motor complex (MMC) during which the drug is given. However, this may only be done on a research basis because recordings of the MMC require gastro-intestinal intubation for luminal pressure recordings of the antroduodenal region (*Medhus* et al., 1995).

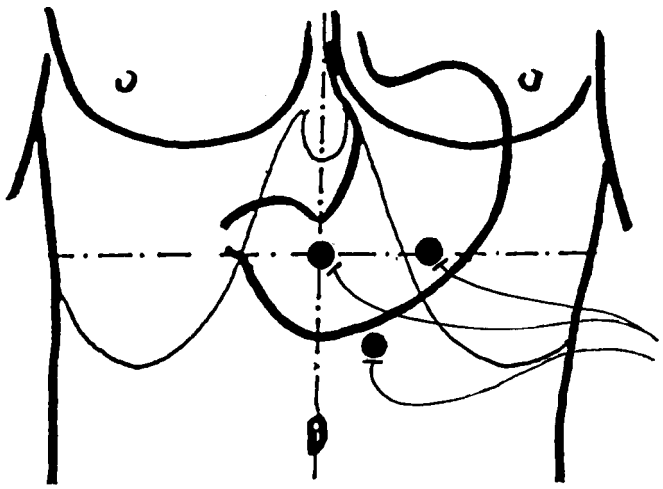

Figure 4: Placement of surface electrodes for recording of electrogastrogram.

MEASUREMENT OF ANTRODUODENAL LUMINAL PRESSURE

The technique of recording antroduodenal pressure changes aims at selectively identifying different motor activities responsible for the process of gastric emptying. Hence, motor activity of the antrum churns ingested foodstuffs and is thereby of importance for the duration of the lag phase. Thus, manometry is valuable for the detection of basic mechanisms underlying abnormal gastric emptying (*Hellström*, 1991).

Manometry is usually carried out with external pressure transducers linked to a multiple lumen manometric catheter or by miniature solid-state transducers. Manometry of the antroduodenal region can be carried out during fasting as well as after food intake. Contractions in other parts of the stomach do not evoke any pressure changes and are not possible to detect employing this method (*Fone* et al., 1990). The purpose of fasting motility studies is to evaluate the existence of MMC, and the involvement of the antral region in this migrating complex (Figure 3). Normally about 60% of MMCs start in the antrum, while 100% occur at the angle of Treitz (*Kellow* et al., 1986). Because the MMC cycle length in man varies greatly with mean values ranging from 80-120 min, prolonged studies have to be carried out to permit accurate measurements of the MMC cycle length and the characteristics of phase 3 activity (*Hellström* et al., 1991). Antroduodenal motility studies can also be carried out after food intake. This is mainly done in order to investigate the initiation of fed motility in the antrum and duodenum in response to food intake, and evaluated as an increase of motility index. The lack of an appropriate motility response after food intake is generally considered as a neurogenic disturbance of gastric motor activity, that should be associated with prolonged gastric emptying.

The problem inherent with the manometric technique is the positioning of the sensors in the antroduodenal region, since it is impossible to maintain a stable position with one pressure recording point in such mobile anatomical structures as the antrum and duodenum. Due to this fact multiple pressure sensors

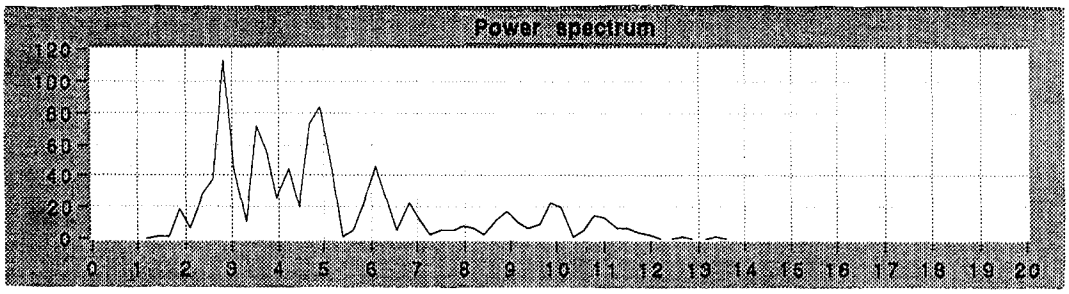

Figure 5: Distribution of electric frequencies in antral smooth muscle as registered by electrogastrography. Note the dominating frequency of 3 cycles per min.

are positioned in the stomach and duodenum. Because normal motility will push the manometric tube aborally, its position in the gastro-intestinal tract has to be continuously monitored and corrected. The position of the manometric tube can be easily determined by the characteristics of the pressure waves during phase 3 of MMC: high amplitudes at a 3 per minute-rhythm in the antrum, and low amplitudes at a 12 per minute-rhythm in the duodenum.

With the manometric tube in correct position, patients with diabetic gastroparesis have been considered to lack the antral component of the MMC and exhibit postprandial antral hypomotility (*Camilleri* and *Malagelada*, 1984). Other forms of slow gastric emptying as seen in idiopathic gastroparesis, different types of myopaties, and in anorexia nervosa may also show antral hypomotility.

Antroduodenal manometry is a clinical procedure that directly evaluates the motor components, as antral hypomotility or pylorospasm, underlying disordered gastric emptying. The main drawback for its clinical use is that the method is invasive.

MEASUREMENT OF GASTRIC ELECTRIC ACTIVITY

Electrogastrography (EGG) is made via surface electrodes attached to the skin at the positions shown in Figure 4. The obtained signal is filtered in an analogue system and thereafter converted to a digital signal that is analysed using computer software that permits overlapping of the signal and presentation of the signal as a fast Fourier analysis in a running spectrum (Figure 5). The result gives an indication of the function of the gastric pacemaker, which determines the basal electric rhythm that governs gastric contractions (*Smout* et al., 1980).

The gastric pacemaker located on the proximal part of the greater curvature generates a basal electric rhythm (slow waves) at a pace approximately 3 cycles per min. This electric rhythm governs the frequency of contractions in antral smooth muscle. It is generally considered that this pacemaker activity is not a property of the smooth muscle cells *per se*, but a function of specialized pacemaker cells, the so-called interstitial cells of Cajal. The basal electric rhythm does not produce contractions, but rather paces rapid depolarisations, i.e. spikes, which correspond to contractions of smooth muscle cells. Spiking occurs at the peak of the basal electric rhythm, and thus the normal frequency registered by EGG is 3 cycles per min.

EGG registered with a sampling frequency of 1 Hz is only capable to record

the basal electric rhythm and cannot be used to detect the occurrence of contractions in the stomach. Pathologic electrical rhythms, such as bradygastria, or tachygastria, can be detected by EGG and may be of importance for the emptying process of the stomach.

EGG has been considered mainly a research tool, but with its non-invasive technique and common handiness, it may be used also clinically to detect disordered electrical rhythms of the stomach.

ACKNOWLEDGEMENTS

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METHODS OF STUDYING GASTRO-INTESTINAL MOTILITY: LARGE INTESTINE

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SUMMARY

The colon serves to absorb fluid and electrolytes, to mix its contents, to store its contents and to empty it in a controlled fashion. Motility, absorption and secretion closely interact to fulfil these functions. Tone, phasic contractions and ano-rectal closure are the main principles of colonic motility. They are controlled by the enteric nervous system. Colonic motility differs from that in other parts of the gut in that it is very slow and irregular, coordinated activity is rare and orad movements normal.

The methods to study colonic motility are radiology which is largely abandoned, manometry with perfused catheters or ambulant with tip catheters, the use of the barostat, radioscintigraphy, marker transit, and electromyography. They all measure different aspects be it flow of colonic contents as in radioscintigraphy and marker transit or be it wall contractions as in manometry and electromyography or be it tone as in the barostat. They are therefore complimentary.

So far, only marker transit in the colon and manometry in the ano-rectum are widely clinically used.

INTRODUCTION

The colon serves to absorb fluid and electrolytes, to mix its contents in order to facilitate absorption, to store its contents and to empty it in a controlled fashion (*Wienbeck* et al., 1990). This is controlled by a very complex interplay of myogenic and neural structures, by the action of peptides and other neurotransmitters, by local mediators and by intraluminal contents be it food remnants or be it bacteria, fluid, toxins and other ingredients of faeces. The enteric nervous system of the colon closely interacts with the smooth muscle of the organ which is responsible for the wall movements. The enteric nervous system receives inputs from local afferent nerves, from the central nervous system and from interneurons. It serves to integrate and program all inputs and to transform them into outgoing signals.

Colonic motility is only one function of the organ (*Karaus* and *Wienbeck*, 1988). It closely interacts with absorption, secretion and immuno-regulatory functions of the large intestine. The mechanisms of motor action are tone, phasic contractions and the anorectal closure which prevents uncontrolled outflow of colonic contents. The phasic contractions of the colon may either be stationary and segmenting serving

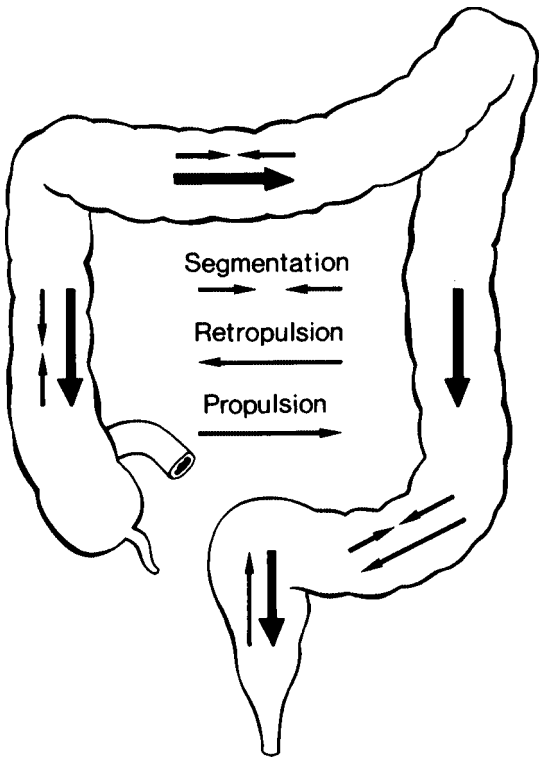

Figure 1: Schematic illustration of phasic colonic motility.

mainly to mix the contents or propagated serving to propel contents (Figure 1). In the descending colon and rectum propulsive contractions prevail whereas in the other parts segmenting contractions are predominant.

Colonic motility differs from that in other parts of the gastro-intestinal tract in several aspects:

1. Propulsion in the colon is very slow. Emptying occurs within days. Ultra-slow variations in activity may be seen, e.g. circadian rhythms (Figure 2; *Wienbeck* and *Kreuzpaintner*, 1976).
2. In addition, colonic motility appears to be rather irregular. Therefore the normal range is very wide. This has to be taken into account when abnormalities are looked for.
3. Coordinated activity, e.g. mass movements, is recognized only intermittently.
4. Retrograde (orad) movements are normal in the colon and occur quite frequently.

METHODS

A series of different methods have been introduced over decades in order to study colonic motility. Since colonic motility comprises different aspects of movements of the large intestine, the methods developed to study it pay attention either to the movements of contents within the large intestine and their characteristics which are called patterns of flow or they measure the movements

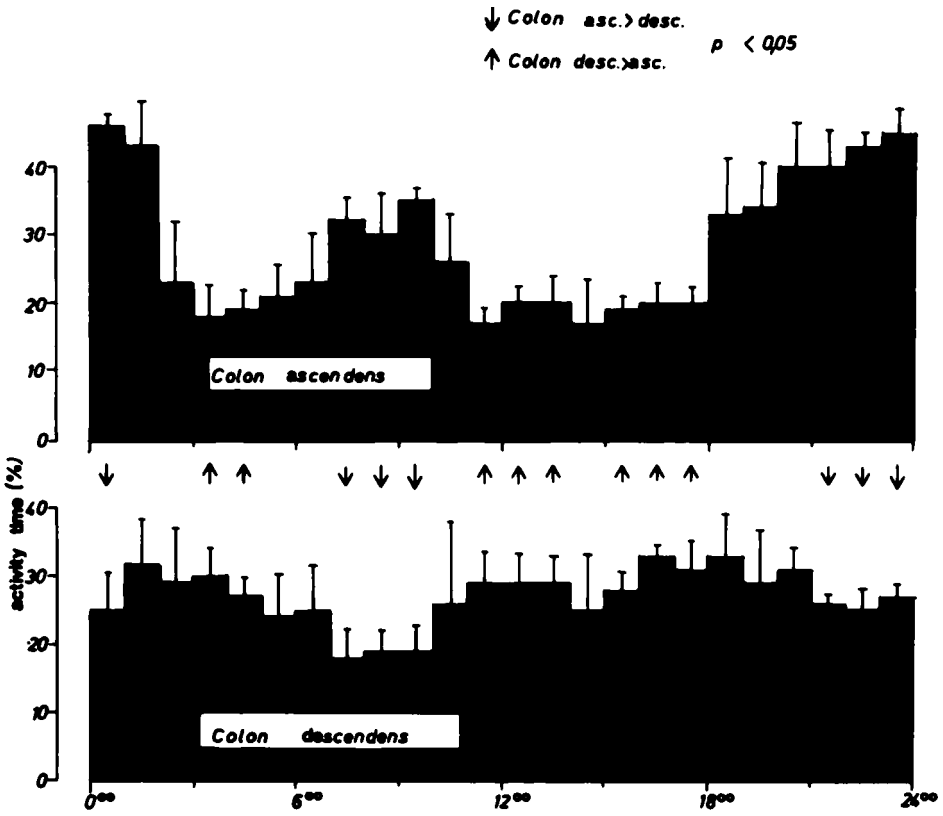

Figure 2: Circadian rhythm of colonic motility as recorded by electromyography in the cat.

of the colonic wall called motor patterns (*Karaus* and *Wienbeck*, 1991). When two methods are combined and flow and motor activity are measured at the same time, the two aspects can be correlated with each other in order to better understand colonic physiology and pathophysiology.

The well established methods for studying colonic motility are:

1. Radiological methods using contrast material previously brought into the colon.
2. Manometric methods in order to measure pressure changes within the colon and to conclude from these on wall movements of the large intestine.
3. The barostat which in its original form serves to measure volume changes of a balloon brought into the colon.
4. Radioscintigraphy after instillation of a radioactive marker into the proximal colon in order to continuously investigate movements of colonic contents.
5. Marker transit after previous ingestion of a number of radio-opaque markers and follow-up either by abdomen X-rays or X-ray films of the collected faeces.
6. Electromyography which measures the muscle activity by collecting its fast and slow signals.

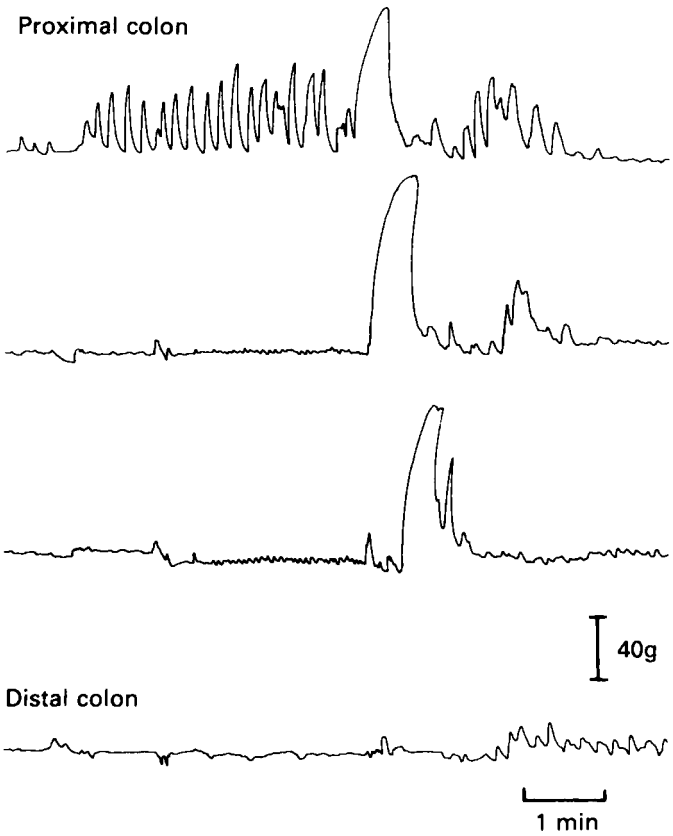

Figure 3: Giant migrating contraction in the colon ("Massenbewegung").

RESULTS (PRACTICAL USE)

The methods used to study colonic motility have received different attention and practical applicability which will be described here.

1. Radiology

Radiological methods were the first to study the movements of colonic contents. Already Holzknecht described with this method in 1909 mass movements which propelled major parts of colonic contents from the right to the left colon or even further to defecation (Figure 3). But the method is no longer used to study colonic motility because of its inherent problems which will be discussed later.

2. Manometry

Manometric methods date back about one century. At that time balloons were used to pick up pressure changes and thus record motility. The balloon catheters are largely replaced by either open-ended perfused catheters nowadays or by solid-state tip catheters. In contrast to the balloon catheters these newer catheters measure the intraluminal pressures only and not pressures exerted upon distending balloons (Figure 4). Intraluminal pressure changes largely reflect phasic wall movements whereas changes in tone rarely show up with these methods.

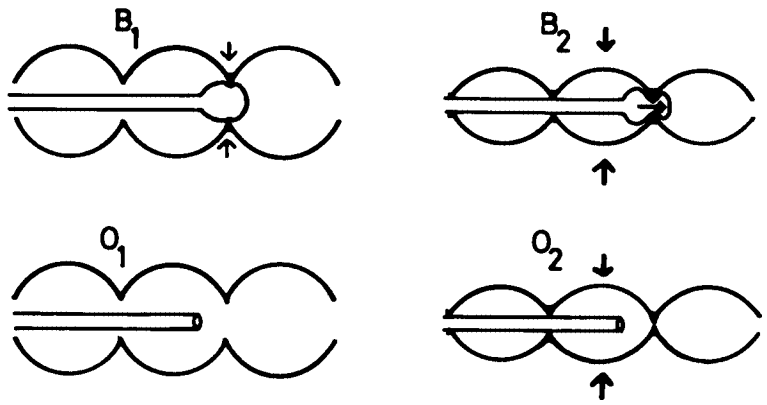

Figure 4: Recording colonic motility by balloon and perfused open-ended catheters. In this example balloon catheters show each wall contraction whereas open-ended catheters show a pressure rise only if a pressure chamber develops (O_2).

a. Stationary manometry

Stationary manometry with perfused open-ended catheters and stepwise pull back is the standard method in ano-rectal manometry which serves to analyse patients with defecation problems. The method is also used as a research tool in all parts of the colon introduction becoming more difficult the more orad the catheter is placed.

b. Ambulant manometry

Since colonic motility changes very slowly over time and motility patterns of long duration have to be taken into consideration, long-term manometry in an ambulant environment is a more physiologic way of studying colonic motility than stationary motility measured in a recumbent position. With the advent of solid-state recorders with large storage capacity ambulant manometry with tip catheters has become reality. It demonstrates that the overall motility of the proximal, but not of the distal colon increases as the large intestine fills up from the first to the second day of the study (M. Karaus, personal communication) and that in contrast to previous thinking the measured motor activity is higher in diarrhoea than in non-diarrhoeal states at least in the irritable bowel syndrome.

3. Barostat

Since the barostat measures volume changes of an inflated balloon of defined size and location, it is the method of choice to study tone in the different parts of the gastro-intestinal tract. So far, it has been used in the recto-sigmoid. The balloon if gradually inflated can also be used to test for rectal compliance, sensation and pain thresholds. So far, it is mainly a research tool, but this may change rapidly.

4. Radioscintigraphy

In contrast to the upper digestive tract where radioscintigraphy has been extensively used to measure the location of physiologic contents and their flow, the colon was neglected for a long time. Orally taken isotope markers because of their decay and overlap were not useful to study transit in the large intestine. This situation changed when radio-isotopic markers were applied locally just above the area of interest through tubes swallowed by the subjects before. The practical usefulness of this method for studies in the clinical routine has still to be demonstrated.

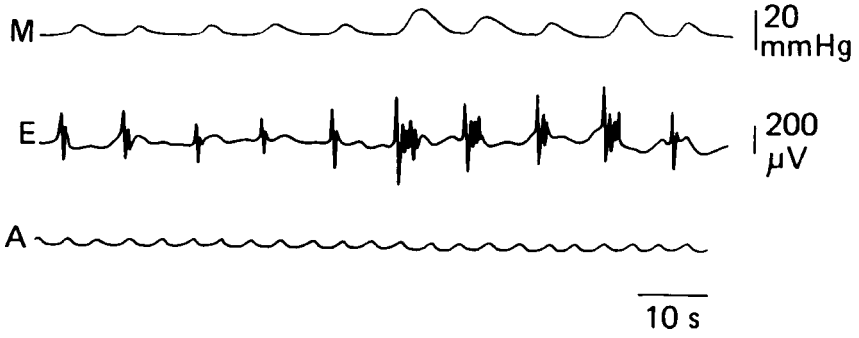

Figure 5: Electrical (E) and manometric (M) recording in the human recto-sigmoid. (A) denotes respiration. Short spike bursts superimposed on electrical slow waves are accompanied by single, low amplitude contractions.

5. Marker transit

The use of colour markers, e.g. charcoal, is an old method of studying oro-caecal transit time. But more reliable radio-opaque markers have largely replaced the colour markers as a diagnostic tool. The so-called Hinton method gave only 1 test dose of 20 markers whose appearance was tested in the stools by X-ray. Fractionated marker application over several days and an X-ray picture of the whole abdomen yields more information in that it allows for easy calculation of the differential transit times from mouth to the right, left and distal colon. Marker transit studies are the most widely used methods of studying colonic transit.

6. Electromyography

The signals which are picked up by electromyography from the colon may be differentiated into spikes, slow waves and oscillations (*Karaus* and *Wienbeck*, 1989). Spike bursts and oscillations are accompanied by muscle contractions as their mechanical counterpart whereas slow waves (synonyms: electrical control activity, pacesetter potentials) largely serve to time the occurrence of spikes and spike bursts (Figures 5 and 6). The method has been widely used in experimental animals, but apparently has lost interest in clinical studies.

DISCUSSION

All methods of recording colonic motility encounter a number of problems which restrict their use. They either measure movements of contents, i.e. radiology, radioscintigraphy and marker transit, or muscle activity, i.e. manometry, barostat and electromyography. In most studies the colon has to be prepared in order to be able to use the method which is the case in radiology, manometry, barostat and electromyography. Preparation means previous cleansing of the large intestine which sets the colon into an unphysiologic state. The problems encountered in the specific methods will be discussed.

1. Radiology

In order to visualize colonic contents not only has the colon to be cleansed, but also barium sulphate, an unphysio-

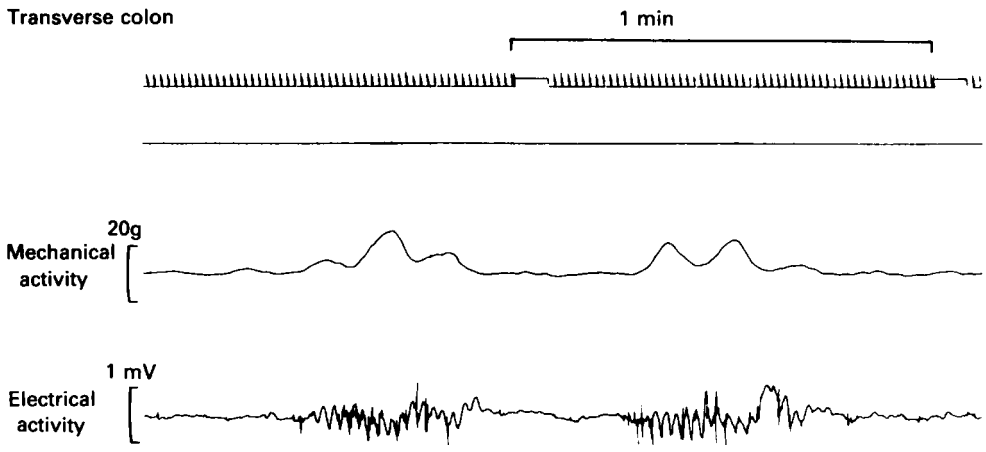

Figure 6: Electrical and mechanical activity in the feline colon. Oscillations are accompanied by sustained contractions.

logic medium, is brought into the large intestine. It is highly unlikely that this can be used to differentiate normal from abnormal colonic motility. In addition, X-ray studies mean radiation exposure which may accumulate to high amounts in the necessarily prolonged observations of colonic movements. But even then the narrow time windows possible in radiology allow only for a restricted assessment of motility in the large intestine. These restrictions are so severe that the method is abandoned nowadays.

2. Manometry

Open-ended or tip catheters pick up a wall movement only when this wall movement leads to a pressure rise. This is the case in most instances, but not in all. If a wall contraction dissipates its pressure changes in adjacent colon segments because of a lack of wall resistance here, i.e. lack of tone and phasic contractions, this contraction will not be picked up by manometry catheters if the contraction does not close exactly over the pressure tip (*Wienbeck*, 1977). Furthermore the previous emptying of the colon under study sets the organ into an unphysiologic state. This is also the case during the immobility of stationary manometry. The ambulant solid-state manometry on the other hand is prone to movement artefacts.

3. Barostat

This is a new method measuring tone, not phasic contractions. So far, it has been used only in the most distal colon. The technique is not yet standardized. Therefore the results may vary from one place to the other. Since the modern barostat is driven by a computer program minor errors may severely affect the results. The method still has other technical problems which may vary from one apparatus to the other.

4. Radioscintigraphy

In order to deliver clear results the method requires cumbersome and long lasting oral intubation. There are only few centres which have sufficient experience with the method in order to make it useful and reliable. Although there is some radiation exposure to the subject under study, this is minimal in relation to that of X-ray studies. The method deserves broader use.

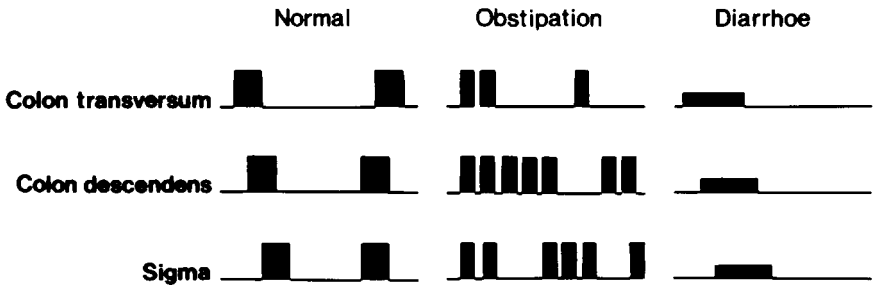

Figure 7: Schematic illustration of the myo-electrical burst activity in the colon during normal circumstances, constipation (short spike bursts prevail) and diarrhoea (migrating long spike bursts prevail).

5. Marker studies

This method is clinically useful although it delivers only coarse information on 3 parts of the colon. For practical purposes this appears to suffice. Because the method extends over several days it is preferentially done in an ambulant set-up. During this time other gastro-intestinal investigations should be postponed in order not to interfere. The radiation exposure of one flat X-ray plate of the abdomen is negligible in relation to conventional radiology of the colon.

6. Electromyography

Although this method is theoretically the clearest and most exact approach to muscle activity, the method has not attained widespread use. This is due to the difficulties in the interpretation of the records which often exhibit overlapping signals. Computer analysis may help somewhat, but not always. In addition, most spikes are lost between the location of their generation and the recording electrode. The recorded spike activity, therefore, represents only a minor part of all spike activity. The slow waves on the other hand may be covert and overlapped by other myo-electrical signals from the same region or even from other organs. Because of the necessary high degrees of amplification, movement artefacts may show up quite frequently and cause major alterations of the records. Nevertheless, as recording techniques improve rapidly and also computer analysis becomes more refined, the method may see its renaissance soon (Figure 7).

CONCLUSIONS

Although at least six methods of clinical usefulness exist in studying colonic motility, none is suitable for a detailed analysis of colonic motility in all its aspects. Each measures only certain aspects. So far, most methods are not well standardized. They are mainly used for research purposes. Only marker transit in the colon and manometry in the ano-rectum have received widespread attention and also clinical use.

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HOW IT STARTED - AND WHAT IS MAS?

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SUMMARY

The purpose of this article is to review the historical aspects of gnotobiology as it relates to gastro-intestinal and vascular smooth muscle motility. The idea of the germfree experiment was first expressed by *Pasteur* in 1885 however, he did not believe that normal germfree life was possible. About ten years elapsed before *Nuttall* and *Thierfelder* (1895a, 1895b) did their now classical experiments on germfree mammals and although few animals were derived, they noted that in the absence of the microflora "the caecum was strongly puffed up and filled to overflowing with a brown liquid which contained cheese-like coagula". This was the first reported distension of intestinal smooth muscle associated with the absence of the microflora. However, systematic studies with germfree animals did not truly begin until the late 1940s and early 1950s when Reyniers and his colleagues at the University of Notre Dame (*Reyniers* et al, 1946), Gustaffson at the University of Lund (*Gustafsson*, 1947) and Miyakawa at the University of Nagoya (*Miyakawa* et al., 1951) established academic organisations devoted to the study of germfree life. It became obvious based on these early observations that the germfree animal compared to its microflora laden counterpart exhibited numerous physiological and biochemical anomalies. The idea of a musculoactive substance (MAS) derived from the intestinal contents of germfree rats which could contribute to the observed decreased intestinal motility was first reported by *Gordon* in 1967; the events leading up to this observation and subsequent studies related to microflora associated MAS are the subject of this review.

INTRODUCTION

The early studies described nearly a century ago noted that the caecum of germfree animals was markedly enlarged and that the contents contained " a brown liquid which contained cheese-like coagula" (*Nuttall* and *Thierfelder*, 1895). Systematic studies with germfree animals did not truly begin until the late 1940s and early 1950s when Reyniers and his colleagues at the University of Notre Dame (*Reyniers* et al, 1946), Gustaffson at the University of Lund (*Gustafsson*, 1947) and Miyakawa at the University of Nagoya (*Miyakawa* et al., 1951) established academic organisations devoted to the study of germfree life. It became evident that the germfree animal exhibited numerous biochemical and physiological anomalies compared to its microflora

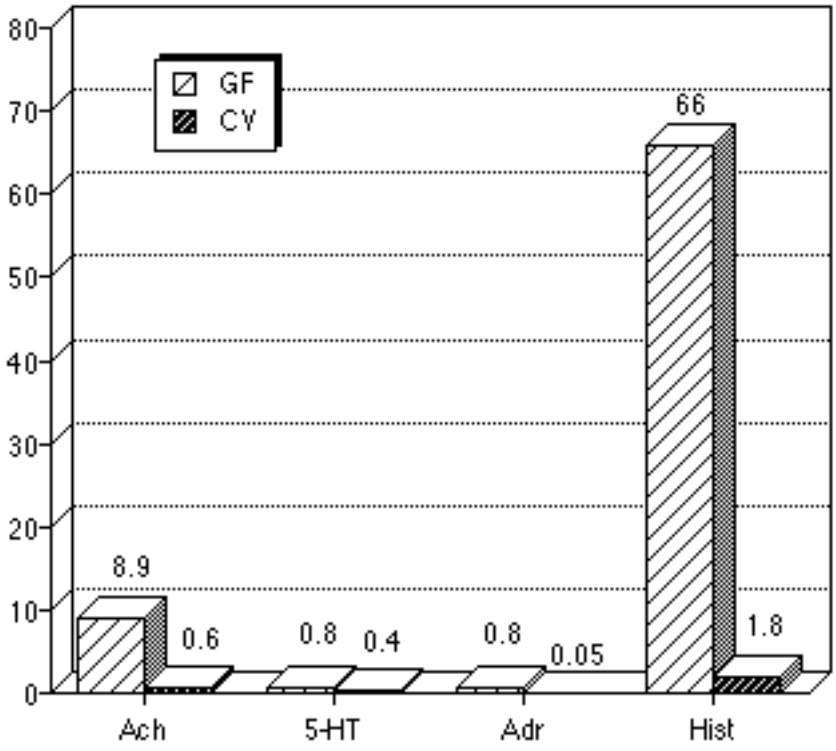

Figure 1: Caecal reactivity of germfree (GF) and conventional (CV) rats to biologically active amines (threshold dose - estimated average mg/ml bath). Modified from *Strandberg* et al., 1966.

laden counterpart. The accumulation of mucinous material in the distended lower bowels and the chronic mild diarrhoea became hallmarks of the germfree state. Although the diet may influence slightly the size of the caecum in the absence of the microflora, the germfree host has 5-10 times the caecal volume relative to the conventional counterpart. Maintaining an animal in the absence of microflora elements precipitates numerous gastro-intestinal and cardiovascular anomalies (*Gordon* and *Bruckner*, 1984). In addition to the "mild chronic diarrhoea" some of the more frequently cited physiological anomalies are 1) decreased oxygen consumption, 2) decreased cardiac output, 3) decreased regional blood flow to numerous organs and 4) decreased intestinal and vascular smooth muscle contractility.

MUSCULOACTIVE SUBSTANCE (MAS)

It was reported in the mid-nineteen sixties that the caecal contents of germfree mice and rats contained bioactive substances that were toxic when administered intraperitoneally and altered smooth muscle contractility in a number of smooth muscle preparations (*Gordon*, 1965). The amount of toxic substance(s) found in the germfree caecum was calculated to be 5-10 fold greater in amount but not in concentration compared to the microflora bearing

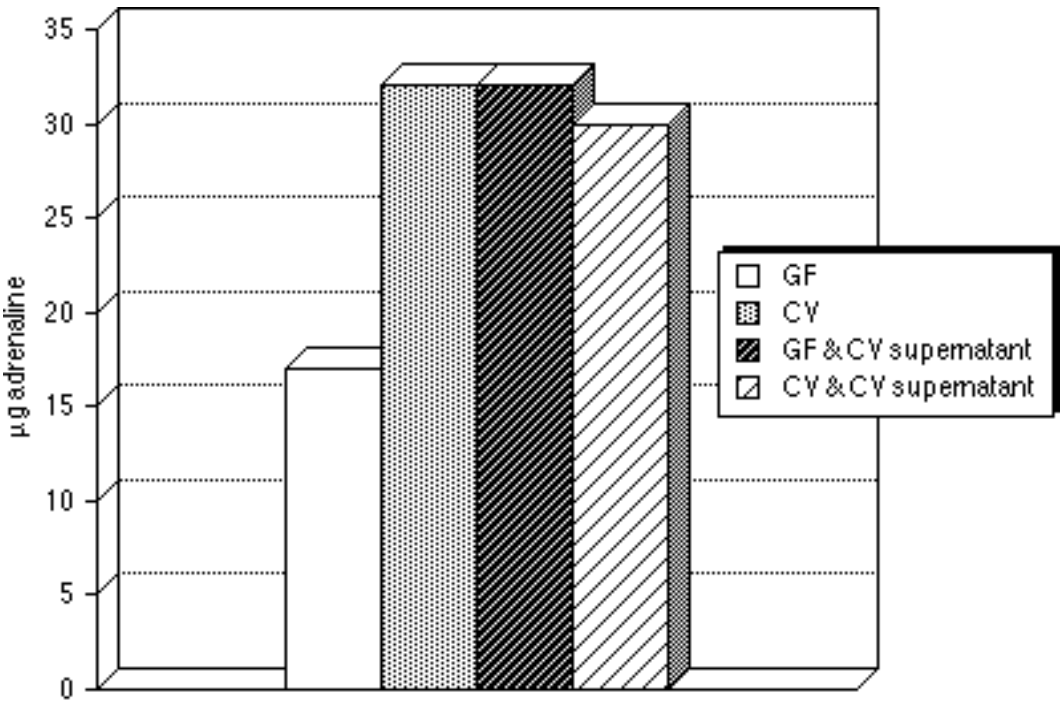

Figure 2: Adrenaline relaxation of caecal strips (GF=germfree; CV=conventional). Modified from *Staley*, 1968.

animals. These early observations suggested that these bioactive substances are endogenous to the host and that the normal intestinal microflora inactivate or reduce the amounts of these substances. The first clear indication that a reduction of caecal muscle tone occurred in the germfree animal was noted by *Strandberg* et al. (1966). It was found that the normal spontaneous caecal muscle contractions, in vitro, of conventional rats were not observed in the germfree specimens. Additionally when these caecal strips were challenged with a number of agonists (acetylcholine - Ach, serotonine - 5-HT, adrenaline - Adr, or histamine - Hist) the germfree preparations were 2-100 fold less sensitive than the muscle strips from conventional animals (Figure 1). Furthermore, *Staley* (1968) observed that a standard depressant dose of adrenaline relaxed caecal strips from germfree animals less than conventional controls (Figure 2). When the germfree caecal strips were exposed to the bacterium free conventional caecal content filtrate, they exhibited marked contractions and if further exposed to adrenaline responded with the same degree of relaxation as the conventional preparations. These observations suggested that the microflora may convert musculodepressant substances which are endogenous to the host into substances which may increase vascular tone. It appeared most likely that the hypo-responsiveness of the germfree caecum to these bioactive substances was most likely due to effects of the luminal contents, since the concentrations of bioactive amines in the caecal wall of germfree and conventional rats were similar (*Strandberg*, et al. 1966). There-

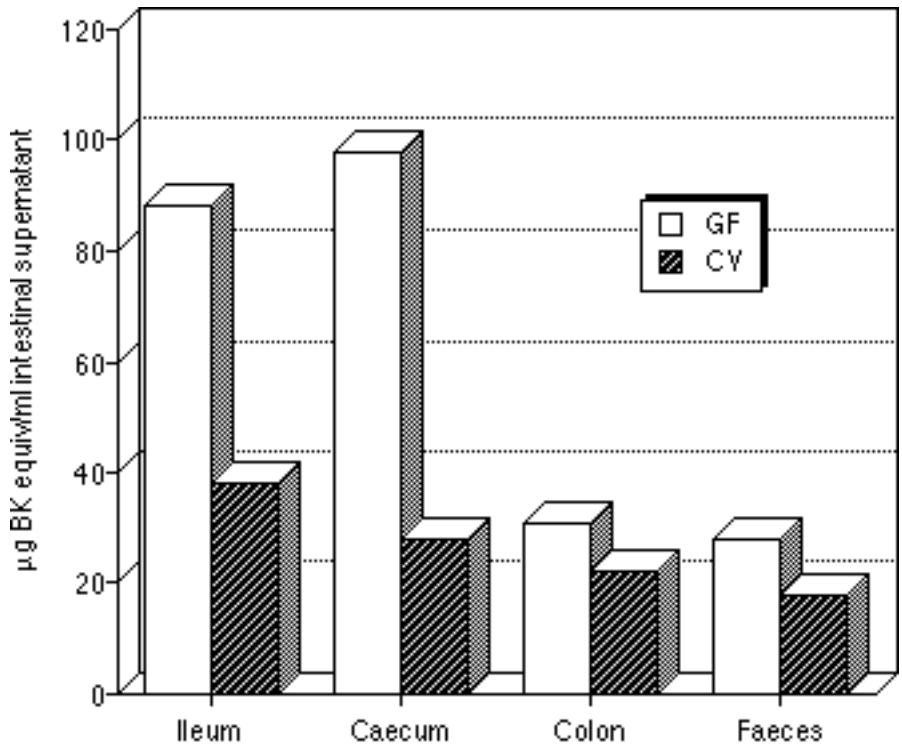

Figure 3: MAS relative concentration in the mouse gastro-intestinal tract (GF=germfree; CV=conventional). Modified from *Gordon*, 1967.

fore, the now classical studies reported by Gordon showed clearly that the toxic substances and the MAS were separate entities (*Gordon* et al., 1965). These substances were separated by ion-exchange chromatography into two pooled fractions. A fraction designated as the "terminal pool" was tested on rat uterus and guinea pig ileum in muscle bath preparations as well as cardiac output and regional blood flow to various organs in the rat. The bioactivity of the MAS as determined by uterine contractility was expressed as bradykinin equivalent units/ml of intestinal contents (Figure 3). It is evident that the germfree mouse harbours 1-3 fold more MAS in its intestinal contents than the conventional rodent; in rat caecal contents this difference is almost five fold. Mice fed various diets showed essentially the same amount of MAS in all segments of the gastro-intestinal tract. On intravenous administration of the germfree terminal pool fraction to rats a marked hypotensive effect was noted which was not observed with the conventional animal preparation (*Gordon*, 1967). These direct effects of MAS on vascular and intestinal smooth muscle appear to be blocked by specific protease inhibitors. However, an indirect effect noted by using subthreshold concentrations of MAS, which in presence of blood plasma can induce uterine contractions, cannot be blocked by protease inhibitors. The indirect effect could be inactivated by kinin degrading enzymes, indicating that the indirect effect may be related to bradykinin like substances. From these observations it was suggested that the direct MAS ef-

Table 1: Bioactivity of germfree caecal content fractions (MAS)

	Arteriole epi. refractory	Villi contraction	Blood pressure	Uterus contraction	Ileal contraction
Caecal contents	refractory	slight increase	hypo	increased	increased
Terminal pool	refractory	increased	hypo	increased	increased
Initial pool	no effect	decreased	no effect	not tested	not tested
a-pigment	refractory	increased	not tested	not tested	not tested
Amino acid	not tested	decreased	not tested	not tested	not tested

fect was due to a faecal kallikrein like substance which was capable of releasing kinin(s). Further fractionation of the "terminal pool" using anion exchange chromatography and sephadex molecular sizing yielded a compound termed a-pigment (MW 4000; *Wostmann* et al., 1973). The biologic effects of a-pigment include the following: 1) increased spontaneous contractions of the dog intestinal villi, 2) inhibition of adrenaline induced hypertension and 3) inhibition of adrenaline mediated contraction of mesenteric-precapillary arterioles. Alpha-pigment was tentatively identified as a ferritin degradation product most likely originating from desquamated epithelial cells which is known to impart adrenaline refractoriness to smooth muscles (*Gordon* and *Kokas*, 1968). Whether these MAS derived from intestinal lumenal contents can be absorbed has not been resolved and therefore the physiological regulatory role of these substances remains uncertain.

Table 1 summarizes the physiological effects of MAS.

INTESTINAL MOTILITY

The impairment of the lower bowel muscle function of germfree rodents is well established but its mechanism is insufficiently understood. Direct and indirect agents in the lumen which are capable of transferring a musculo-depressant action appear to be involved (*Dupont* et al., 1965; *Strandberg* et al., 1966; *Gordon* and *Kokas*, 1968). The impairment of gastro-intestinal function presents a serious impediment to the germfree animal's vitality ultimately resulting in cessation of peristalsis. The increased intestinal transit time can be noted early in life in both rodents and dogs (*Abrams* and *Bishop*, 1967; *Gustafsson* and *Norman*, 1969; *Heneghan* and *Mittelbronn*, 1981; *Van Elder* et al., 1988). Following caecectomy, the germfree rodent showed a slightly higher rate of intestinal transit than the caecectomized control. Furthermore the removal of the caecum has been shown to redress many of the cardiovascular anomalies of the germfree rodent. The cardiac output and refractoriness of the micro-vessel to norepinephrine approached values noted for the microflora laden counterparts (*Gordon* and *Bruckner*, 1984). The influence of the gut microflora on intestinal myoelectric activity has recently

been studied. It was found that the frequency of myoelectric complex migration was less in the germfree vs. the conventional rat. If the germfree animal was conventionalized, a microflora re-established, the frequency of the myoelectric complex migration increased within a week (*Caenepeel*, et al. 1989; *Husebey* et al, 1992). The pattern of propagation was more regular after conventionalisation but the slow wave frequency in the proximal jejunum was not increased nor the frequency of spike potentials following jejunal infusion of glucose (*Husebey* et al., 1994). The mechanism(s) responsible for the altered intestinal motility in the absence of the microflora are still not well understood. Microbial alterations of the gut milieu which may alter motility are 1) production of short chain and/or volatile fatty acids (acetic, butyric, propionic), 2) conversion of primary to secondary bile acids, 3) degradation of kalliekreins and/or kinins, 4) alterations of intestinal fatty acids which are eicosanoid precursors, 5) eicosanoid production or degradation and 5) other MAS alteration, e.g., NO, motilin. By introducing a microflora or by surgical removal of the caecum in the germfree rodent many of the physiological aberrations can be eliminated thus indicating that the large caecal pool of these vaso-active, musculo-depressant substances accumulate in the germfree state and contribute to the above described phenomenon.

CIRCULATORY ANOMALIES

Circulatory and metabolic anomalies such as reduced cardiac output, decreased regional blood flow and lower metabolic rates (oxygen consumption) have been observed in germfree animals relative to their conventional counterparts. It appears that these physiological alterations are associated with the intestinal contents since the removal of the caecum all but eliminates the differences between the germfree and conventional state (*Bruckner-Kardoss* and *Wostmann*, 1967; *Gordon* et. al., 1966; *Baez* et. al. 1973). This suggests that bioactive substances which originate from the intestinal lumen may be available for absorption and thus impart changes in vascular smooth muscle tone. It was found that in germfree or antibiotic treated animals the precapillary arterioles exhibited decreased vasomotor action and were refractory to noradrenaline compared to the conventional controls (*Baez* and *Gordon*, 1971; *Bruckner*, 1981). A peptide (α-pigment) was isolated from germfree caecal contents which on topical application to mesenteric micro-vessels imparted norepinephrine refractoriness and this same substance isolated from conventional animals showed markedly reduced activity. Furthermore, the norepinephrine refractoriness could be markedly reduced by treating antibiotic animals with Na salicylate, a prostaglandin inhibitor (*Bruckner*, 1973; 1981; See Figure 4). These substances are not eliminated by caecectomy, which normalizes many of these parameters, but seem only to be reduced in quantity and thus the systemic load appears to be diminished. Although it is not clear exactly what these substances might be the "α -pigment" which has been shown to impart epinephrine refractoriness is most likely derived from ferritin (*Gordon* and *Kokas*, 1968).

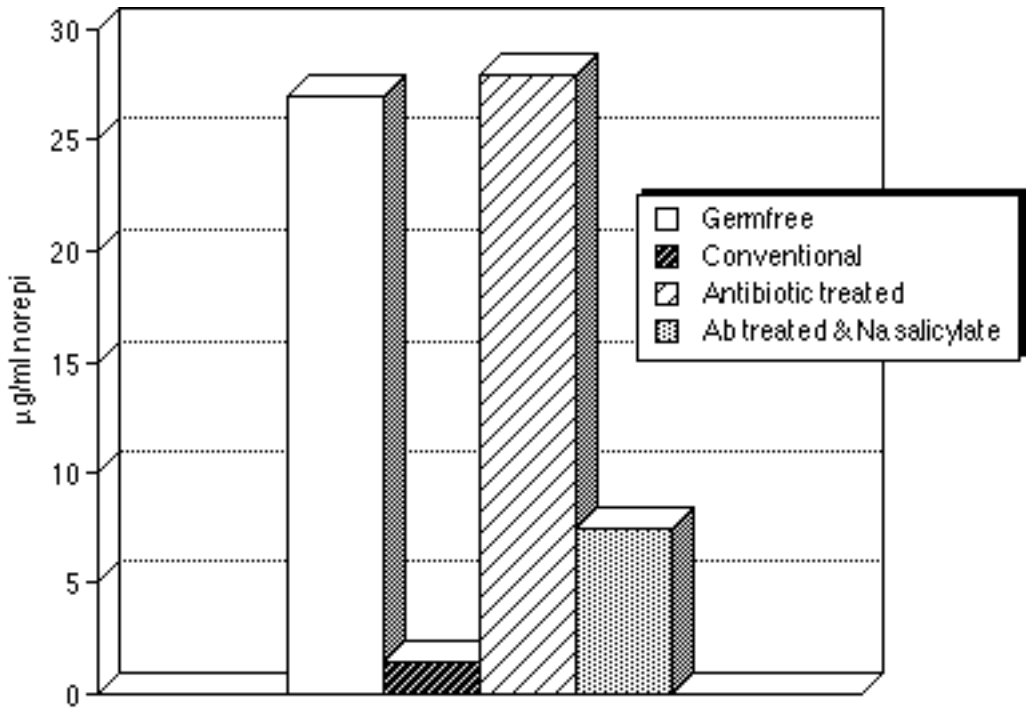

Figure 4: Arteriole norepinephrine sensitivity (mg/ml norepinephrine to elicit approximately 50% constriction). Modified from *Baez* and *Gordon*, 1971, and from *Bruckner*, 1981.

OTHER MICROFLORA RELATED MAS

Short Chain Fatty Acids (SCFA)

The intestinal microflora is capable of producing SCFA or volatile fatty acids from carbohydrate and protein substrates. The metabolism of carbohydrates generally leads to production of acetic, butyric and propionic acids while protein degradation yields primarily isobutyric or isovaleric acids (*Bugaut* and *Bentejac*, 1993). The concentration of these SCFA ranges from 1-13 mM/kg intestinal contents in the ileum-jejunum up to 50-300 mM/kg in the caecum and colon of monogastric animals. It is well known that SCFA can increase the ileal and colonic motility but appear not to alter duodeno-jejunal contractility (*Masliah* et al., 1992). Germfree animals are practically devoid of SCFA, however, small amounts were detected in the small intestine and caecum and these levels were attributed to dietary origin (*Hoverstad* and *Midtvedt*, 1986). Other physiologic effects of SCFA include stimulation of colonic blood flow (vasodilation), increasing pancreatic enzyme secretion, promoting Na and water absorption in the colon, potentiating intestinal mucosal growth, providing a preferential fuel source for the colonic mucosa and possibly lowering serum cholesterol via regulation of HMG-CoA reductase activity (*Bugaut* and *Bentejac*, 1993).

Eicosanoids

The sensitivity of gastro-intestinal smooth muscle to eicosanoids (prosta-

glandins, leukotrienes, thromboxane, prostacyclin) has been utilized for bioassay of these compounds. However, the effects of these bioactive lipids on GI smooth muscle show considerable variability depending on the type of eicosanoid, the dose, the species of the animal studied and even the muscle layer being used. For example $PGF_{2\alpha}$ contracts both longitudinal and circular gastric smooth muscle while PGE_2 relaxes circular muscle and has a variable effect on longitudinal. Prostanoids interact directly with the receptors on the membranes of gut smooth muscle cells and these receptors are not blocked by atropine, hexamethonium, mepyramine, methsergide, propranolol or phentolamine suggesting that the effects of prostanoids on gut motility are not mediated by acetylcholine, histamine, serotonin or catecholamines. These prostanoid characteristics are similar to the effects noted for the smooth muscle preparations and the effects of the caecal contents from germfree animals. As a general rule, $PGF_{2\alpha}$, prostaglandin endoperoxides and PGE_2 analogues appear to stimulate motility of most parts of the gut in most species, while the naturally occurring eicosanoids of the E series and PGI_2 are primarily inhibitory (*Moore*, 1988). Prostacyclin and PGE_2 are quantitatively the most important metabolites of arachidonic acid formed in the gut. These eicosanoids have been shown to reduce the amplitude and duration of gut slow waves, alter ion and water transport, and play a role in mucosal cytoprotection. Little is known about the effects of the microflora on eicosanoid production, however, there are reported elevations of eicosanoids associated with diarrhoea and inflammatory bowel disease which may be directly or indirectly related to microflora alterations. The absence of the gut microflora has been shown to alter dramatically essential and non-essential fatty acid metabolism (*Evrard* et al., 1964; *Demarne* et al., 1979; *Bruckner*, 1987). It has been noted that the germfree animals have 3-5 times the amounts of mono- and polyunsaturated (PUFA) endogenous intestinal and faecal fatty acids compared to their conventional counterparts. These fatty acids are normally hydrogenated or degraded by the intestinal microflora and may thus alter the availability of these fatty acids for eicosanoid synthesis. As previously mentioned the "norepinephrine refractoriness" of mesenteric micro-vessels associated with antibiotic decontamination of the gut microflora could be partly redressed by feeding the rats a prostanoid synthesis inhibitor (Figure 4). Although the mechanisms are not clear regarding the eicosanoid and microflora interactions the following events may be involved: 1) microbial degradation of PUFAs in the gut lumen thus decreasing substrate availability for eicosanoid formation, 2) microbial alteration of endogenously produced eicosanoids, e.g., PGI_2 and/or 3) kinins or α-pigment which are altered by the microflora may stimulate specific membrane phospholipase and thus influence eicosanoid homeostasis.

Motilin and Nitric Oxide

Motilin is a 22 amino acid polypeptide which has potent stimulatory effects on gastro-intestinal smooth *muscle in vivo* and *in vitro* perhaps via alteration of cytosolic calcium levels (*Higuchi* et al., 1994). Little is known about the influence of the microflora on motilin gut concentrations. Nitric oxide (NO) has been shown to be a potent vasodilator and to be involved in sodium choleate-induced fluid secretion and diarrhoea in rats (*Mascolo* et al., 1994). Although the microbial status of the rat does not seem to alter the levels of expired NO (*Persson* et al., 1994), there is virtually no information on the levels of NO in

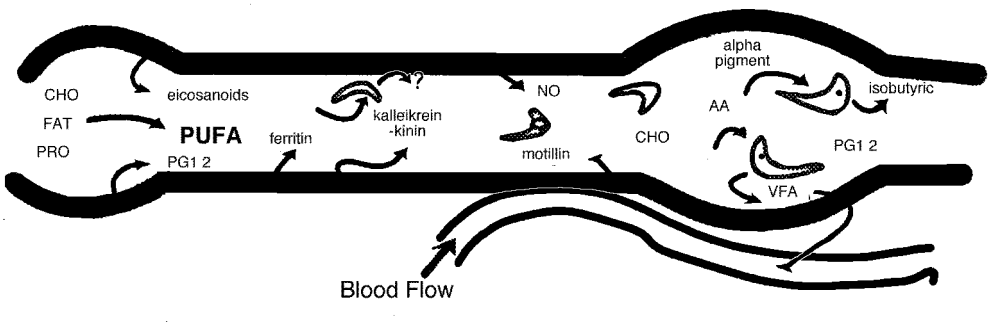

Figure 5: Microflora and intestinal bioactive substances (MAS).

the gut relative to microbial status of the host. It is evident that the gut microflora alters gastro-intestinal motility, water transport and vascular smooth muscle reactivity of the host, however, the mechanisms are far from being elucidated. Some possible interactions are depicted in Figure 5.

CONCLUSION

Gnotobiotic animals have been a valuable tool to elucidate the influence of the microflora on the physiology and biochemistry of the host animal. It is apparent that the gut microorganisms contribute to the maintenance of water and electrolyte balance, intestinal and vascular smooth muscle contractility, and to the normal physiological integrity of the GI tract. It appears that a number of the germfree anomalies are associated with the ability of the gut microflora to inactivate and/or metabolize endogenously produced substances which, if unaltered, impart vaso-depressant characteristics to intestinal and vascular smooth muscle. In the rodent these bioactive substances, e.g., kinins, α-pigment, eicosanoids, bile acids, SCFA, can produce "mild chronic diarrhoea," distension of the caecum and colon, biogenic amine refractoriness of smooth muscle and a decreased metabolic rate. By removing these MAS substances through caecectomy or by establishing a conventional gut microflora many of these anomalies can be reduced or eliminated. It is clear that many of the interactions between the intestinal microorganisms and MAS are not well understood and since these substances appear to be involved with the aetiology of many gastro-intestinal diseases, e.g., inflammatory bowel disease, diarrhoea, intestinal stasis, it is hoped that investigative efforts will be continued to elucidate the mechanisms involved.

ACKNOWLEDGEMENTS

This review of MAS is dedicated to Helmut Gordon whose pioneering efforts in the field of gnotobiology helped us to appreciate the complex interactions of the gut microflora with its symbiotic host.

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EFFECTS OF E. COLI LIPOPOLYSACCHARIDE ON GUT MOTILITY MEDIATED VIA NITRIC OXIDE PATHWAY

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SUMMARY

The interference of endotoxin (lipopolysaccharide, LPS, *E. coli* 055:B5) with motility of the small intestine, as registered with chronically implanted bipolar electrodes, was studied in rats using a radioactive Cr-51 polyethylene glycol marker for evaluation of effects on transit in the gut. Experiments were carried out with increasing doses of LPS administered luminally, intraperitoneally and intravenously, as well as after pretreatment with L-arginine analogues and steroids that inhibit the elaboration of nitric oxide (NO) by the constitutive and inducible nitric oxide synthases (NOS), respectively. Administration of LPS at doses from 20 to 160 $\mu g\ kg^{-1}$ intravenously, but not luminally or intraperitoneally, resulted in disruption of the migrating motor complex (MMC) concomitantly with a hastened small intestinal transit and diarrhoea. The effect on MMC was abolished by pretreatment of the rats with N^G-nitro-L-arginine (L-NNA), N^G-L-arginine methyl ester (L-NAME) or dexamethasone. Simultaneously, transit was normalized with L-NNA, L-NAME and dexamethasone. Provided a changed mucosal barrier in disease states promotes absorption of LPS from the intestinal lumen this may disrupt interdigestive myoelectric activity concomitant with a hastened transit and diarrhoea.

INTRODUCTION

Gram-negative bacteria are often pathogenic organisms in infections originating in the gastro-intestinal tract. In common diarrhoeal diseases different strains of *E. coli* are frequently isolated organisms. The pathophysiological effects of these infections are to a large extent due to the action of endotoxin.

Endotoxins can be isolated from the cell wall of Gram-negative bacteria. Chemically it is described as a lipopolysaccharide (LPS) with a molecular weight between 100 and 900 kD (*Gyles*, 1992). LPS produces extensive effects in man and in animals most likely through stimulation of Ca^{2+}-independent inducible nitric oxide synthase (NOS) in different cell types such as macrophages (*Rees* et al., 1990, 1990; *Leone* et al., 1991), thereby activating the nitric oxide (NO) pathway with elaboration and release of huge amounts of NO locally and systemically. The general signs of such an endotoxemia include fever, hypotension, tachycardia and reduced responses to pressor agents as well as inhibited gastric motility. Recently it has been found that after challenge with LPS, NO is produced by a Ca^{2+}-independent enzyme in macro-

phages, with profound generalized effects. Therefore, NO has been suggested to play a role in host defence mechanisms against bacteria and other invading organisms (*Moncada* et al., 1990).

Less is known about effects of LPS on small intestinal motility. Such effects could be of interest in the explanation of intestinal paralysis in connection with severe abdominal infections and after surgical procedures involving endotoxin containing organs as the large intestine.

The interdigestive migrating myoelectric complex (MMC) is a general motor pattern that can be predicted to recur at certain time intervals and therefore the MMC has been used to study effects of different agents on motility. The function of the MMC has been considered a transport mechanism, cleansing the bowel lumen of secretion, cell debris and undigested food particles (*Al-Saffar* et al., 1984, 1985).

The aim of the present study was to investigate the effects of LPS on the MMC and transit of contents in the small intestine of fasted rats. Further, the involvement of NO in the LPS-induced changes of motor activity was evaluated using L-NNA and L-NAME as inhibitors of the NO pathway, and dexamethasone as an inhibitor of inducible NOS.

MATERIALS AND METHODS

Electromyography and transit

Male Sprague-Dawley rats (ALAB, Sollentuna, Sweden), 300-350 g, were anaesthetized with pentobarbital (50 mg.kg-1; Apoteksbolaget, Umeå, Sweden). Through a midline incision three bipolar stainless steel electrodes (SS-5T, Clark Electromedical Instruments, Reading , UK) were implanted into the muscular wall of the small intestine at 5, 20 and 35 cm distal to the pylorus. The animals were provided with a catheter implanted 6 cm from the pylorus for instillation of LPS or a transit marker. All animals also had a jugular vein catheter or an intraperitoneal catheter for administration of LPS and drugs. The electrodes and catheters were tunnelled subcutaneously to exit at the back of the animals' neck. After surgery the animals were allowed to recover for at least 7 days before experiments were undertaken. During recovery the rats were trained to accept experimental conditions. Experiments were then carried out in conscious animals after a 24-h fasting period in wire-bottomed cages with free access to water. During the experiments the rats were placed in Bollman cages. The electrodes were connected to an EEG preamplifier (7P5B) operating a Grass Polygraph 7B (Grass Instruments, Quincy, MA, USA). The time constant was set at 0.015 s and the low and high cut-off frequencies were at 10 Hz and 35 Hz, respectively.

To study effects of LPS on the MMC, all experiments started with a recording of basal myoelectric activity with four propagated activity fronts over all three registration sites. After five activity fronts had passed the first electrode site, the substance to be studied was administered and an effect on the MMC pattern was analysed visually over a period of 90 min. For detailed analysis of the characteristics of activity fronts a computerized method for calculations was employed (*Hellström* et al., 1993). The main characteristic feature of myoelectric activity of the small intestine in the fasted state, the activity front, or phase 3 of the MMC, was identified as a period of at least 1 min with clearly distinguishable intense spiking activity and an amplitude at least twice that of

the preceding baseline, propagating aborally through the recording segment and followed by a period of quiescence, phase 1 of MMC. Phase 2 of MMC was characterized by irregular spiking preceding the activity front. Periods of more than 30 min with spike potentials, but no discernible cyclic activity, were considered as periods of irregular spiking activity.

To study effects on transit, the effect of LPS on the MMC was first established over a period of one hour. Then, 0.4 ml of a transit marker consisting of polyethylene glycol 4000 with 1.48 MBq $Na_2{}^{51}CrO_4$ per ml of marker solution (pH 7.2, 300 mOsm.kg-1) was instilled through the duodenal catheter over a period of 30 s.

Thirty min later the rats were killed with an overdose of pentobarbital. The abdomen was opened and after ligation of the gastroduodenal and ileo-caecal junctions, the small intestine was carefully removed in its entire length. The small intestine was divided into 10 equal segments and analysed for distribution of the radioactive marker in a gamma counter (Beckman, Fullerton, CA, USA). Intestinal transit was quantified by calculating the leading edge of the distribution of the marker in the gut.

Values are given as mean ± standard error of the mean.

Design of the study

In a first session, dose-response relationships for LPS on MMC and transit were investigated. The importance of different administration routes of LPS were studied. LPS was given at doses of 5-160 $\mu g.kg^{-1}$, each as a 0.2 ml bolus intraluminally, intraperitoneally or intravenously. As a control, saline solution (NaCl 154 $mmol.l^{-1}$) of an equal volume given the same routes of administration was used.

In a second session, the effect of LPS at a dose of 20 $\mu g.kg^{-1}$ i.v. on the MMC and transit were investigated in conjunction with different agents that to inhibit NOS pathways. The action of the NOS inhibitors L-NNA or L-NAME on the effect of LPS was studied, as was the effect of dexamethasone which inhibits the inducible form of NOS. L-NNA or L-NAME were both given at a dose of 1 $mg.kg^{-1}$ i.v. bolus plus 0.1 $mg.kg^{-1}.min^{-1}$ i.v. infusion, while dexamethasone was given at a dose of 0.25 $mg.kg^{-1}$ i.v. and 6 h later, LPS was given and the effects on MMC and transit evaluated.

Drugs and chemicals

Lipopolysaccharide, LPS, 055:B5), NG-nitro-L-arginine (L-NNA) and NG-L-arginine methyl ester (L-NAME) were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Dexamethasone was a kind gift from Merck, Sharp and Dohme (MSD, Rahway, NJ, USA). All compounds were diluted in saline before use *in vivo*, except dexamethasone which was supplied as solution.

RESULTS

Effects of lipopolysaccharide

LPS at doses of 5-160 $\mu g.kg^{-1}$ i.v. caused a dose-dependent disruption of the MMC and irregular spiking. At a dose of 20 $\mu g.kg^{-1}$ i.v. LPS inhibited MMC at all registration levels ($p<0.01$). The LPS-induced myoelectric pattern was characterized by the occurrence of successive bursts of spike potentials separated by short quiescent periods. The effect occurred 30 min after LPS administration and remained during the infusion period.

After irregular spiking was estab-

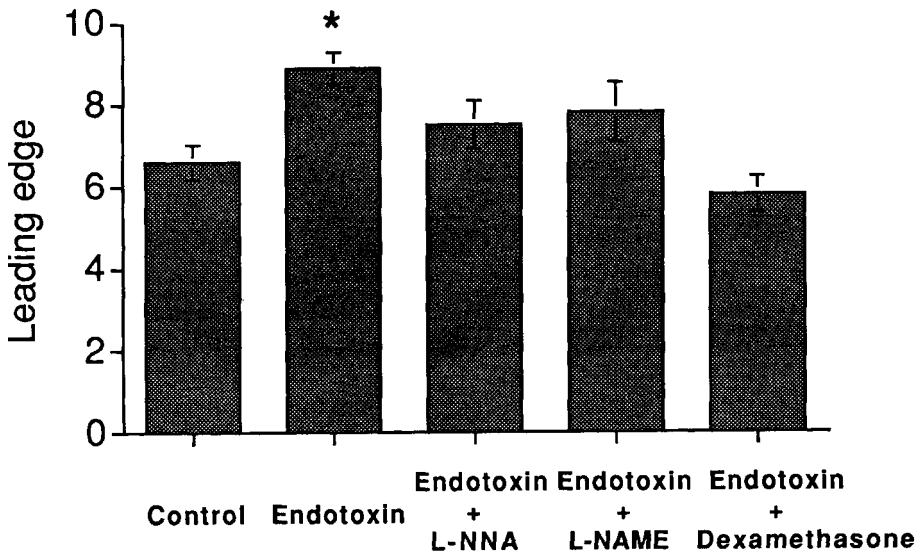

Figure 1: Stimulatory effect of lipopolysaccharide of E. coli (20 mg.kg^{-1} i.v.) on transit of the leading edge of a luminal marker, and its inhibition by the nitric oxide synthase inhibitors L-NNA or L-NAME (1 mg.kg^{-1} + 0.1 mg kg^{-1}.min^{-1}), and by dexamethasone (0.25 mg.kg^{-1}) which inhibits inducible nitric oxide synthase.
* $p<0.05$.

lished with LPS at a dose of 20 mg.kg^{-1} ($p<0.01$) the transit was hastened, as reflected by an increased propagation of the marker in the gut ($p<0.05$) (Figure 1). In all rats diarrhoea was observed ($p<0.01$).

Employing the same dose range for intraduodenal or intraperitoneal administration did not affect the MMC pattern and no diarrhoea was observed.

Inhibition of nitric oxide pathway

Inhibition of NOS with L-NNA or L-NAME inhibited the disruption of the MMC and irregular spiking induced by LPS, and normalized the distribution of the leading edge of the transit marker (Figure 1). No diarrhoea was seen in these two groups of rats.

Inhibition of inducible NOS with dexamethasone efficiently inhibited the effect of LPS on myoelectric activity and normalized the transit of the leading edge of the luminal marker (Figure 1).

Small bowel weight

The dry weight of the small intestine was determined after freeze-drying of the small bowel specimen. The relation dry/wet weight was 0.2±0.04 in control animals and 0.3±0.05 in animals treated with LPS 20 mg.kg^{-1} i.v., indicating an increase of fluid contents after LPS of about 10%.

DISCUSSION

The main finding in the present investigation was that LPS given intravenously at a dose that did not affect the general condition of the animals, induced a complete disorganisation of normal fasting motility, as measured

both directly by electromyographic recordings from chronically implanted electrodes, and indirectly by measurements of the leading edge of a radioactive luminal marker. Thus, LPS besides its great variety of other biological effects, such as inflammation, appears to be capable of affecting small intestinal motility.

A question of major concern was whether the recorded motility alteration could be mediated via an unspecific effect through inflammation, swelling and water accumulation in the tissues. This is unlikely due to the fact that the increase in dry/wet weight ratio of the tissues was only about 10%. Whether this is due to increased intraluminal accumulation of fluid, or intramural oedema could not be determined. However, preliminary data from experiments of the small intestine with extravasation of Evans blue after challenge with LPS do not show any major effect of LPS at this dose on the permeability of the small intestine . Because the increase was only 10% it does not seem likely that this accumulation of fluid could disturb the results of the transit study. Also, the method used for transit studies has been shown to be insensitive to changes in the volume of luminal contents. The distribution of radioactivity is not disturbed by cholera toxin when administered at a dose that causes a prominent increase of intestinal secretion. Finally, the hastened transit registered after LPS is in line with our results in myoelectric recordings.

The mechanism by which the LPS of *E. coli* brings about motility changes seems to involve stimulation of the nitric oxide pathway. Pretreatment of the animals with L-NNA or L-NAME, two different inhibitors of NOS, inhibited the effect of LPS on MMC and reversed the transit effect induced by LPS. Such data speak in favour of NO as a main contributor to the effect of LPS on gut motility. In fact, other studies have shown that NO is an important mediator of reflex relaxation of the stomach (*Desai* et al., 1991, 1991). Further, because also dexamethasone was able to block the effect of NO on MMC and transit, it seems that the involvement of NO in this reaction is mediated through the inducible Ca^{2+}-independent form of NOS (*Rees* et al., 1990; *Gonzalez* et al., 1992). This type of NOS is localized mainly in macrophages, and the induction of the enzyme with liberation of NO may reflect an effect of a primary host defence mechanism (*Rees* et al., 1990). The finding is interesting in view of the fact that NO has been demonstrated to exert cytotoxic actions on various organisms such as bacteria and protozoa, which are known to cause diarrhoea (*Liew* et al., 1990; *Moncada* et al., 1991; *Sherman* et al., 1991).

Since the effect of LPS was restricted to administration via the intravenous route the pathophysiological role of the endotoxin effects is less clear. We can only speculate that in disease states with a disturbed or broken mucosal barrier LPS may be absorbed from the intestinal lumen to the circulation, thereby being able to produce pathophysiological cascade effects through activation of the NO pathway.

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THE STIMULATORY INFLUENCE OF THE INTESTINAL MICROFLORA ON GASTRO-INTESTINAL MOTILITY AND MYOELECTRIC ACTIVITY OF SMALL INTESTINE

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SUMMARY

Germfree animals are useful for the study of interactions between the intestinal microflora and the host. The peculiar enlarged caecum, the main riddle of germfree mammalian life, suggested an effect on gut motility. Correspondingly, pioneer studies demonstrated slower progress of chyme through the gastro-intestinal tract of germfree animals.

The influence of the intestinal microflora on myoelectrical activity of small intestine has recently been studied in rats using germfree models. A considerable stimulatory influence on the migrating myoelectric complex, the fasting pattern of motility, has been demonstrated, whereas no major differences were observed after nutrient stimulation. The slow wave frequency remained unchanged, suggesting that the intestinal microflora exerts a modulatory effect on the enteric nervous system rather than a direct stimulatory effect on intestinal smooth muscle. The mechanism responsible for this interaction is yet unknown.

Studies on microbial modulation of gut motility are henceforth reviewed, with particular emphasis on those addressing alterations in intestinal myoelectric activity.

INTRODUCTION

The intestinal microflora activates the gastro-intestinal immune system (*Wal* et al., 1985) and promotes the release of hormones, neurotransmitters and other extracellular chemical messengers capable of modulating motility (*Roth* et al., 1982). Bacteria also secrete peptides (*Rao*, 1991) and biogenic amines (*Thompson*, 1977). Accordingly, serotonin of microbial origin has been considered responsible for symptoms associated with nematode infection (*Thompson*, 1977). Serotonin is a potent stimulator of peristalsis inducing irregular spiking in small intestine of dogs (*Ormsbee* et al., 1984) and increased frequency of migrating myoelectric complexes (MMC) in small intestine of opossum (*Coelho* et al., 1986). Microbes also produce enzymes that metabolize luminal substrates to bioactive substances such as short chain fatty acids (SCFA) (*Høverstad* et al., 1985), that may interfere with intestinal motility (*Fich* et al., 1989; *Richardson* et al., 1991).

The relationship between microbial organisms and intestinal motility has

been firmly investigated in the presence of enteropathogenic species and their enterotoxines, demonstrating the initiation of migrating action potential complexes (MAPC) and repetitive bursts of action potentials (RBAP) (*Cowles* and *Sarna*, 1990; *Mathias* and *Sninsky*, 1984). These two myoelectric patterns are considered responsible for rapid propulsion resulting in diarrhoea and for stasis, respectively (*Cowles* and *Sarna*, 1990; *Mathias* and *Sninsky*, 1984). MAPC resembles the myoelectric pattern of giant migrating contractions, that result in rapid emptying of intestinal contents and diarrhoea (*Sarna*, 1987; *Cowles* and *Sarna*, 1990).

Since *Nuttal* and *Thierfelder* (1895) managed to keep germfree guinea pigs alive and apparently germfree for more than a week, the influence of the conventional intestinal microflora on the mammalian host has been studied extensively using germfree models (*Luckey*, 1964; *Hentges*, 1983; *Savage*, 1986; *Grubb* et al., 1989). Data on the influence on gastro-intestinal motility are, however, scarce. Recent studies of intestinal myoelectric activity in germfree animal models have contributed to the understanding of how intestinal microbes modulate motility (*Caenepeel* et al., 1989; *Husebye* et al., 1991; *Husebye* et al., 1992a; *Husebye* et al., 1994; *Husebye* et al., 1995a). This review summarizes experimental data on gastro-intestinal motility in detail also with reference to related clinical studies.

EXPERIMENTAL STUDIES

Gastro-intestinal transit

The enlarged caecum is reduced to normal size after introduction of conventional intestinal microflora (*Gordon*, 1968). This early observation prompted studies on the relationship between gut microflora and intestinal motility.

Abrams and *Bishop* (1966) were the first to report delayed total gastro-intestinal transit in germfree mice. They extended this observation using a radioactive test meal and found that gastric emptying, small intestinal transit and large intestinal transit were slower in germfree mice (*Abrams* and *Bishop*, 1967). Their findings were confirmed in mice by *Ducluzeau* and co-workers (1970), and slower small intestinal transit after meal was demonstrated in germfree rats (*Gustafsson* and *Norman*, 1969; *Sacquet* et al., 1970). Evidence for slow transit has also been provided in germfree dogs (*Heneghan* and *Gordon*, 1974).

The enlarged caecum, trapping intestinal contents, partly explains the delay of total intestinal transit (*Sacquet* et al., 1970; *Heneghan* and *Mittelbronn*, 1981). Several studies have, however, confirmed that this factor does not entirely account for the slow transit in germfree animals (*Abrams* and *Bishop*, 1967; *Van Eldere* et al., 1988; *Husebye* et al., 1994). Selective measurement confined to the small intestine has demonstrated slower transit rate at this level (*Husebye* et al., 1994). After 60 min, a radioactive bolus instilled in proximal jejunum was transported 30% further distally in fasting conventional compared with germfree rats (Figure 1) (*Husebye* et al., 1994). *Iwai* et al. (1973) found that the capacity of the microflora to accelerate transit corresponded to its capacity to reduce caecal size, indicating that the same mechanism is involved in microbial enhancement of motor activity at different levels of the gastro-intestinal tract.

These studies of intestinal transit establish a pattern of slow transit through the GI tract of germfree mammals:

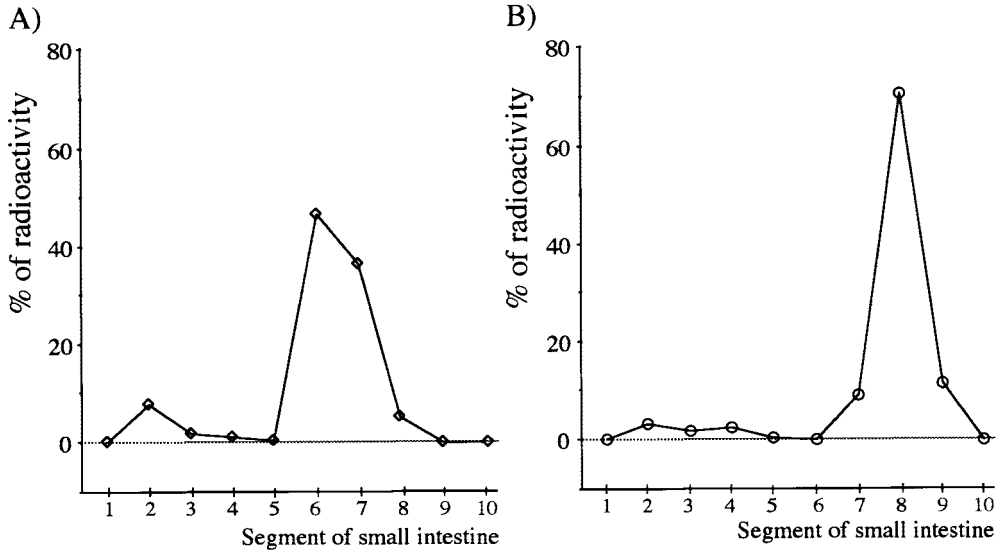

Figure 1: Linechart showing the distribution of radioactivity along the small intestine of A) a germfree and B) a conventional Sprague-Dawley rat 60 minutes after intraluminal instillation of marker ($Na_2{}^{51}CrO_4$) 15 cm distal to pylorus. The percent of total radioactivity in the small intestine is given for each 10 cm segment (From *Husebye* et al., Dig. Dis. Sci. 1994).

Within 16 hours of feeding conventional mice had passed more than 90% of the radioactivity into faeces as compared to less than 30% in their germfree counterparts ($p<0.001$) (*Abrams* and *Bishop*, 1967). The enlarged caecum contributes to a considerable degree, but transit through all segments of the gastro-intestinal tract is accelerated in the presence of normal intestinal microflora, both during fasting and after feeding.

Small intestinal myoelectric activity

Studies of transit demonstrate the final outcome of peristalsis, but do not clarify how intestinal motor activity is influenced. The technical difficulties encountered in studies of germfree animals (*Coates* et al., 1968) have hampered the progress of this work. Recently, however, experimental models have been established that allow recording of small intestinal myoelectric activity *in vivo* in germfree rats (*Caenepeel* et al., 1989; *Husebye* et al., 1992a). *Caenepeel* et al. (1989) examined germfree rats and compared with findings in gnotobiotic and conventional controls. The model established in our laboratory allows repeated recordings of myoelectric activity in rats in the germfree state and after introduction of conventional (*Husebye* et al., 1992a; *Husebye* et al., 1994) or selected microflora (*Husebye* et al., 1991; *Husebye* et al., 1995a).

Myoelectric activity is recorded from bipolar extracellular electrodes implanted in muscularis externa during laparotomy at least 5-7 days prior to experiments (*Husebye* et al., 1992a). The electrodes are tunnelled to the interscapular region to allow normal physical activity.

The small intestine exhibits two patterns of myoelectric activity: slow waves and spikes. Spikes (action potentials) usually occur in short lasting "trains" at high frequency (10-30 Hz), called spike bursts, superimposed on

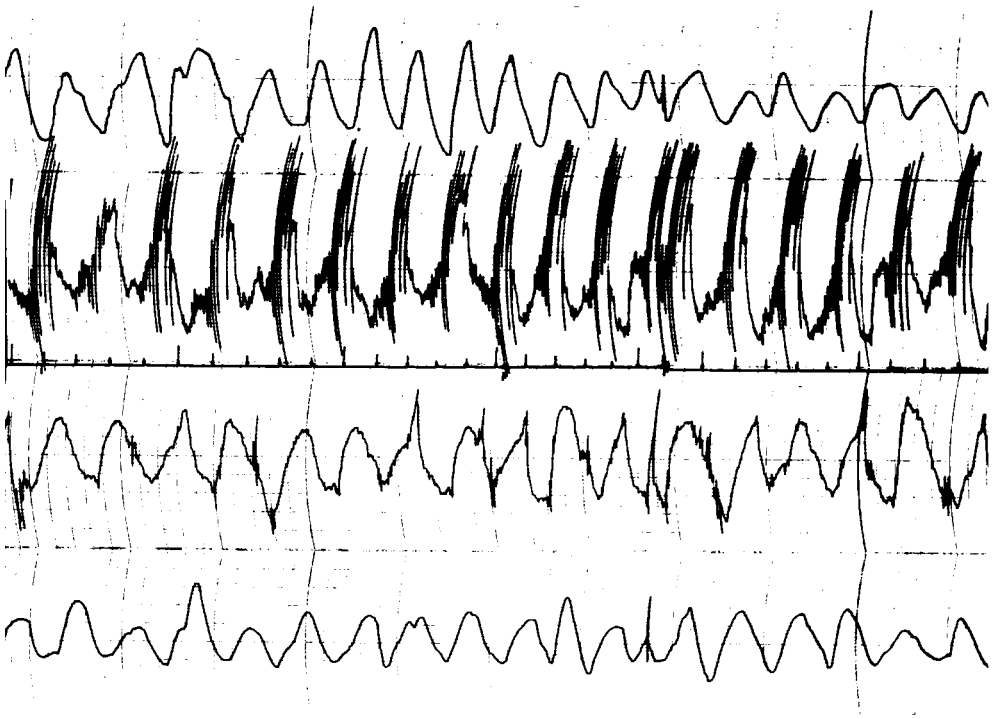

Figure 2: Fasting myoelectric activity of the small intestine of Sprague-Dawley rats recorded from jejunal electrodes 5 (first); 15; 25; 35 cm distal to the duodeno-jejunal junction. Time scale between second and third electrode with inter-marker distance of 1 sec. The first and the fourth electrode show slow wave activity without spike bursts (phase I). The second electrode shows spike bursts superimposed on every slow wave (phase III). The third electrode shows sporadic spike activity (phase II).

the plateau phase of slow waves (Figure 2).

Slow waves are the result of rhythmic depolarisations of the membrane potential of smooth muscle cells from -40 to -80 mV, and during the upstroke of slow waves cells approach threshold for action potentials.

If the amplitude of the slow wave reaches threshold the muscle cell exhibits spikes (Figure 2). The number of spikes in a burst determine the strength of the resulting contraction (*Coremans*, 1993). Accordingly, the slow wave frequency determines the maximal contractile frequency, and the frequency of spike burst determines actual contraction frequency.

Slow wave frequency

Slow wave frequency was similar in germfree rats, gnotobiotic rats colonized with a limited caecum reducing microflora (four species) and in conventional rats, amounting to about 33 cycles per min in proximal jejunum and 27 cycles per min in distal ileum (*Caenepeel* et al., 1989). Rats were of the Fischer strain. Accordingly, slow wave frequency remained unchanged one week after introduction of conventional microflora to germfree Sprague-Dawley rats at about 38 and 32 cycles per min in proximal and mid small intestine, respectively (*Husebye* et al., 1994). In this study Sprague-Dawley rats born conventional exhibited a

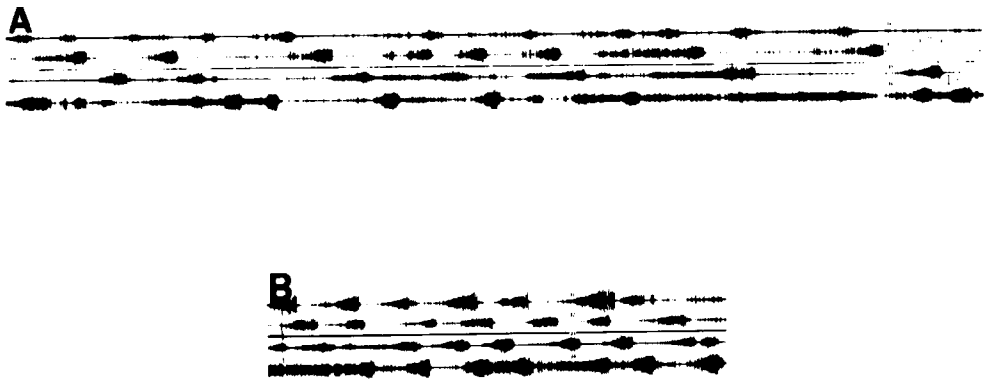

Figure 3: Fasting myoelectric recording in Sprague Dawley rat in A) the germfree state and B) one week after introduction of conventional intestinal flora. J1; J2; J3; J4 indicate jejunal electrodes 5; 15; 25; and 35 cm from the duodeno-jejunal junction. Activity fronts representing phase III of MMC are easily recognized as periods of intense spiking activity (slow waves are filtered off). (From *Husebye* et al., Dig. Dis. Sci. 1994).

higher slow wave frequency than germfree rats (43.0±0.8 vs. 38.5±1.2; mean±SE; $p<0.01$), possibly due to genetic drift (*Husebye* et al., 1994). Alternatively, it could indicate that microbial modulation of slow wave frequency is possible during the very first weeks of life, because the control rats in the study of *Caenepeel* et al. (1989), were actually ex-germfree (conventional microflora was introduced after sucking was finished).

These experiments (*Caenepeel* et al., 1989; *Husebye* et al., 1994) indicate that the intestinal microflora does not interfere with small intestinal slow wave frequency. The possible influence during the early postpartum period remains to be clarified.

Fasting pattern of spike potentials: Migrating myoelectric complex (MMC)

MMC is a cyclic migrating pattern of motility that recurs during fasting (*Szurszewski*, 1969) in most mammals (*Wingate*, 1981). In humans it can be recognized from the lower oesophagus to the distal small intestine, but it is most prominent in proximal jejunum (*Kellow* et al., 1986). This enteric rhythm usually exhibit three phases (Figure 2): Phase I when only slow waves are observed without spike bursts; phase II when spike bursts are observed on slow waves irregularly; and phase III with spike bursts on every slow wave for a few minutes (*Szurszewski*, 1969). Phase III and activity front are equivalent terms. Rats are particularly suited for studying MMC as the cycling period is only about 15 min in the conventional state (*Ruckebusch* and *Fioramonti*, 1975). The MMC period (interval between phase IIIs) shows great variability (*Kerlin* and *Phillips*, 1982), and this variability resides within individuals (humans) (*Husebye* et al., 1990).

When germfree rats were compared with gnotobiotic and conventional rats the MMC period was 20 min, 15 min and 13 min, respectively, in proximal jejunum (*Caenepeel* et al., 1989). The groups were statistically different ($p<0.05$). Slightly larger differences were found in distal ileum. Duration of phase III, spike burst frequency during phase II and propagation velocity of phase III in proximal jejunum remained

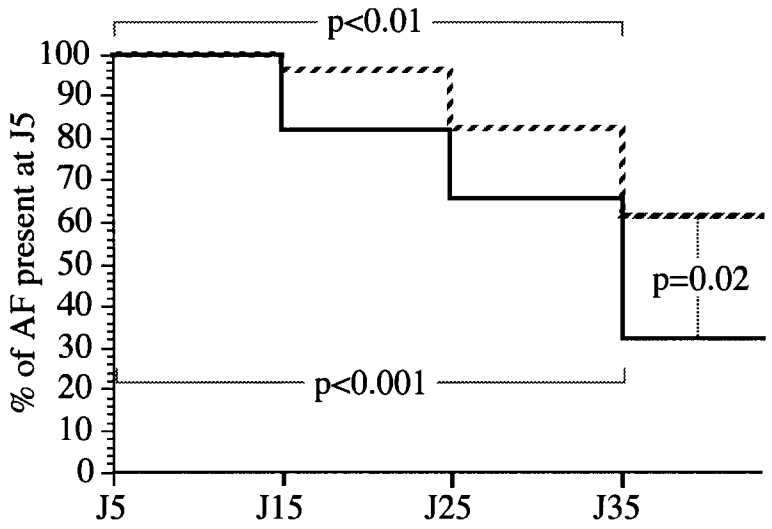

Figure 4: Plot showing how far activity fronts of MMC (AF) recorded five cm distal to the duodeno-jejunal junction (J5) propagated in the germfree state (solid line) and after conventionalisation (broken line), in six germfree Sprague-Dawley rats. Mean percentage of activity fronts that reached the respective electrode sites (J15-J35) is given. p-values for the gradient along the intestine, in the respective states, are encircled by horizontal bars, and p-value for the shift at J35 from germfree and ex-germfree state is encircled by vertical bars. The shaded areas at J35 indicate ± SEM (From *Husebye* et al., Dig. Dis. Sci. 1994).

unchanged. In distal ileum the propagation velocity was slower in germfree rats compared with conventional rats ($p<0.05$).

When germfree AGUS rats where recorded before and one week after introduction of conventional intestinal microflora (*Husebye* et al., 1992a) the MMC period was reduced from 17 to 13 min ($p<0.05$). Duration of activity fronts was slightly reduced in proximal jejunum ($p<0.05$), whereas propagation velocity remained unchanged.

With improved experimental model and germfree Sprague-Dawley rats, introduction of conventional microflora resulted in a considerable reduction in MMC period in proximal jejunum from 31 to 18 min ($p<0.01$) (*Husebye* et al., 1994) (Figure 3). Phase III propagation for at least 20 cm was required. Furthermore, a detailed analysis of periodicity and aboral spread of MMC showed that the intestinal microflora stimulated both cyclic initiation proximally and aboral propagation along the intestine. Increased aboral propagation of MMC (Figure 4) had not previously been detected because of short recording segments (*Caenepeel* et al., 1989; *Husebye* et al., 1992a). Initiation of phase III distal to the duodeno-jejunal junction was less frequent after introduction of microflora (Figure 5), probably due to shorter intervals between phase IIIs of proximal origin and their increased length of aboral propagation. The intestinal microflora thus made the pattern of MMC propagation more uniform (Figures 3 and 4). Propagation velocity of phase III was increased in mid small intestine ($p<0.01$), but not in the proximal part. Duration of phase III remained unchanged.

These studies on the MMC pattern (*Caenepeel* et al., 1989; *Husebye* et al.,

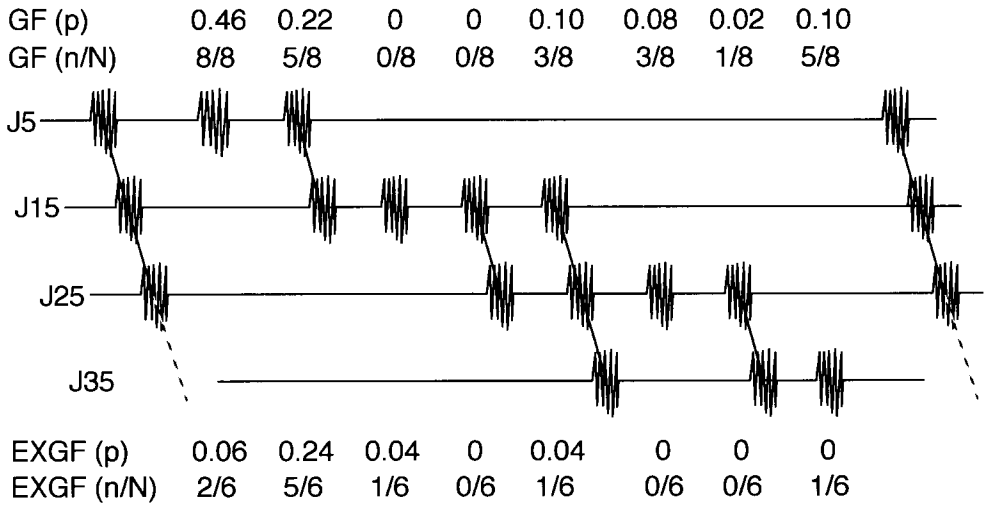

Figure 5: Schematic presentation of the possible patterns of propagation for activity fronts. GF and EXGF denotes germfree and ex-germfree state, respectively. (P) is the probability for a given pattern of propagation to occur during one complete MMC cycle, based on activity front propagating at least from jejunum at five cm past the duodeno-jejunal junction (J5) to J25. n/N gives number of rats with the particular pattern of propagation (n) and the total number of rats examined (N). (From *Husebye* et al., Dig. Dis. Sci. 1994)

1992a; *Husebye* et al., 1994) are mostly in agreement and establish a considerable stimulatory influence of the intestinal microflora on both cyclic initiation (proximal small intestine), aboral propagation and propagation velocity (mid and distal small intestine) of phase III. Shorter duration of phase III was noted in all studies, but the change was modest and statistical significant in only one study (*Husebye* et al., 1992a), suggesting a week biological effect. Difficulties in determining the exact starting point of phase III visually may have obscured these data. Moreover, MMC in rats exhibited the same high degree of variability as in humans, with predominant intra-individual variability, regardless of the presence of intestinal microflora (*Husebye* et al., 1994).

These alterations in MMC activity may explain accelerated transit through small intestine during fasting (*Husebye* et al., 1994; *Caenepeel* et al., 1989), as intestinal transport takes place mainly ahead of phase III (*Code* and *Schlegel*, 1974; *Ehrlein*, 1986; *Vantrappen* et al., 1977).

Preliminary data on the influence of bacterial species on MMC has recently been presented (*Husebye* et al., 1991; *Husebye* et al., 1995a), indicating that the ability to enhance MMC activity is confined to certain bacterial species, in accordance with previous data on reduction of the enlarged caecum (*Coates* et al., 1968). Bicontamination with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* reduced the MMC period in germfree Sprague-Dawley rats by 18% ($p<0.01$) (*Husebye* et al., 1991), and a similar response has been observed for a *Clostridium* sp. (*Husebye* et al., 1995a).

In a recent study the MMC pattern appeared to influence initial gastric emptying and postprandial pattern of motility (*Medhus* et al., 1995), suggesting that this pattern has implications beyond the fasting state. Accordingly, the

MMC pattern was found to be the most sensitive indicator of dysmotility for predicting colonisation with Gram-negative bacilli in patients (*Husebye* et al., 1995b). The MMC data reviewed here may thus have implication also for motility during the postprandial period.

Postprandial pattern of spike potentials

The influence of intestinal microflora on postprandial myoelctric activity has only been examined in one study (*Husebye* et al., 1994). A caloric liquid meal (12.5% glucose), previously shown to induce a significant postprandial response in rats (*Ruckebusch* and *Fioramonti*, 1975), was infused into proximal jejunum in the germfree state and one week after introduction of conventional microflora. The number of spike potentials was integrated at 10 min intervals. The intensity of spike potentials was similar in the germfree and ex-germfree state, but phase III returned earlier after meal infusion in presence of intestinal microflora ($p<0.05$). This finding accords with increased MMC frequency after conventionalisation and with the concept that MMC participates in the complex interplay of postprandial motility (*Medhus* et al., 1995).

Postprandial motility thus seems to depend more on nutrient challenge than on the presence or absence of intestinal microflora. Confirming studies are needed, and studies performed in phase-relation with MMC (*Medhus* et al., 1995) may provide further insight.

Other studies

Justus et al. (1983) established experimental blind loop syndrome in rats and recorded myoelectric activity. They reported increased frequency of spike bursts and MAPC, features that disappeared when the self-filling blind loop was removed surgically. Moreover, chloramphenicol reduced these myoelectric responses, and gnotobiotic rats with the same loop showed no alterations, strongly indicating that the overgrowth flora was responsible. Such microflora usually consists of species belonging to the normal intestinal microflora (*King* and *Toskes*, 1979).

Impeded clearance of the self-filling blind loop may explain initiation of the propulsive MAPC pattern, otherwise associated with the presence of enteropathogenic microbes and their enterotoxines (*Cowles* and *Sarna*, 1990; *Mathias* and *Sninsky*, 1984). Furthermore, giant migrating contractions with myoelectric similarities to MAPC also provide efficient clearance (*Sarna*, 1987). This pattern was recently reported in upper small intestine of patients with severely impaired motility and abundant bacterial overgrowth including strict anaerobic species (*Husebye* et al., 1995b). Thus, MAPC and giant migrating contractions seem to be initiated by microbes (*Cowles* and *Sarna*, 1990; *Mathias* and *Sninsky*, 1984; *Justus* et al., 1983; *Husebye* et al., 1995b) and to provide clearance in states of severe stagnation (*Justus* et al., 1983; *Husebye* et al., 1995b) and in presence of toxic microbial contents (*Cowles* and *Sarna*, 1990; *Mathias* and *Sninsky*, 1984).

When ileal loops are interposed between colon and rectum the MMC pattern is preserved (*Garavoglia* et al., 1993), in agreement with the studies of MMC in germfree rats (*Caenepeel* et al., 1989; *Husebye* et al., 1992a; *Husebye* et al., 1994). Further stimulation could hardly be anticipated as ileal loops are exposed to stationary microflora similar in composistion to colonic microflora in situ (*Smith*, 1965), and the MMC pattern is irregular and less prominent at this level (*Ruckebusch* and *Fioramonti*, 1975).

CLINICAL STUDIES

Bacterial overgrowth is associated with many clinical conditions (*King* and *Toskes*, 1979), but the possible role of dysmotility as causal factor limits the usefulness of these models for studying how microbes modulate motility (*Husebye*, 1995). Alterations in motility after BII gastric resection, truncal vagotomy and stagnant inducing surgery are thus most likely secondary to surgery.

The MAPC pattern has been reported in a patient with secretory diarrhoea of months duration, and the self limiting course of disease suggested infectious origin, even if a specific organism was not discovered (*Coremans* et al., 1987). The MAPC pattern seemed to be responsibel for diarrhoea, as in the experimental models (*Cowles* and *Sarna*, 1990; *Mathias* and *Sninsky*, 1984).

The widespread belief that lactobacilli improve peristalsis in elderly constipated patients persists, even if it remains to be settled whether this is fact or fiction (*Conway*, 1989).

Absence of gastric acid results in bacterial colonisation of the gastric reservoir (*Giannella* et al., 1972; *Allan* and *Shiner*, 1967), which seeds the small intestine. Nevertheless, healthy old people with fasting gastric hypochlorhydria and considerable Gram-positive gastric microflora (*Husebye* et al., 1992b) exhibit normal motor patterns of small intestine (*Husebye* and *Engedal*, 1992). The prevalence of propagated clustered contractions, however, was increased.

CONCLUSIONS

This overview shows that the conventional intestinal microflora stimulates myoelectric activity and motility of small intestine in animals, resulting in increased aboral propulsion. This effect is confined to selected bacterial species, but the mechanism remains to be clarified. Stimulation of the MMC pattern, but not of slow waves, suggests an effect on the enteric nervous system rather than a direct effect on intestinal smooth muscle cells. It is notable that the response persists without adaptation, and that elimination of the microflora even after years seems to restore germfree characteristics. This host response to the conventional intestinal microflora prevents further microbial colonisation of small intestine and thus serves to keep luminal conditions suitable for digestion and nutrient absorption.

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SMALL INTESTINAL MOTILITY AND BACTERIA

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INTRODUCTION

There is a close interaction between small intestinal motility and small intestinal bacteria. Normal motility patterns have an important role in the regulation of the bacterial flora of the gut. This has been clearly demonstrated by the observation that small intestinal motility disorders may lead to bacterial colonisation of the jejunum (*Vantrappen* et al., 1977; *Scott* and *Cahall*, 1982). Conversely, there is good evidence that bacteria may contribute to the development of abnormal intestinal motility patterns, which in their turn may lead to the development of gastro-intestinal symptoms (*Mathias* et al., 1976; *Burns* et al., 1978; *Caenepeel* et al., 1989; *Husebye* et al., 1994a).

This review will briefly discuss: 1) Smooth muscle contractions and their myogenic control mechanisms; 2) The enteric nervous system; 3) Normal and abnormal motility patterns; 4) The interaction between small intestinal motility and small intestinal bacteria.

SMOOTH MUSCLE CONTRACTIONS AND THEIR MYOGENIC CONTROL MECHANISMS

The smooth muscle fibres of stomach and small intestine share with the myocardium two fundamental properties. Firstly, electrically and mechanically they function like a syncytium.

The intermediate junctions are the site of mechanical coupling. At these sites actin filaments, one of the basic contractile proteins, penetrate the dense bands of adjacent cell membranes where

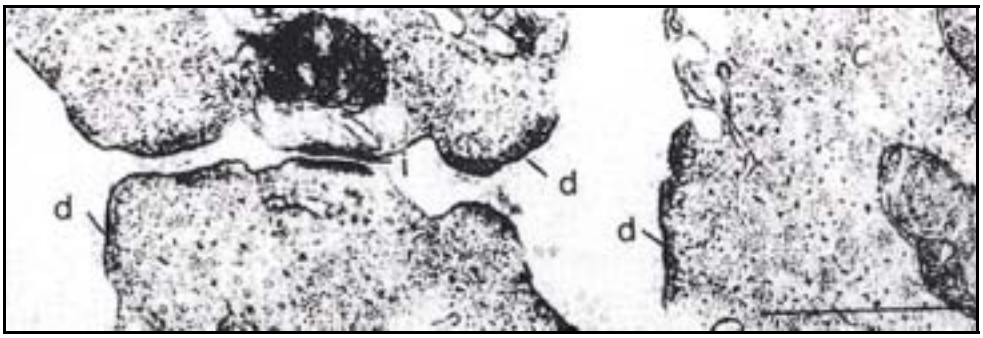

Figure 1: Intermediate junction between two smooth muscle cells: transverse section of guinea-pig *Taenia coli*. Bar: 1 mm; d: dense bands; i: intermediate junction. (From: *Gabella*, 1979).

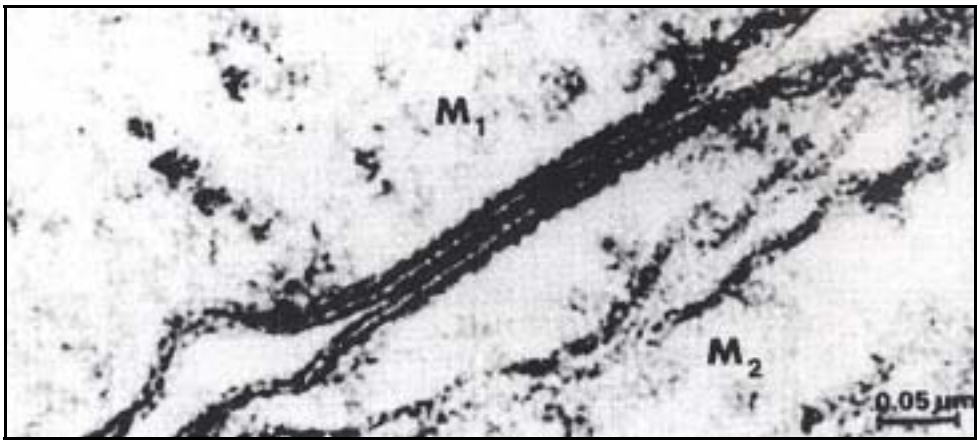

Figure 2: Gap junction between two smooth muscle cells grown in tissue culture. Magnification 155.000x.

they form a symmetrical structure that provides a direct link between the contractile units of two adjacent cells (Figure 1).

An other specialized cell membrane structure, the nexus or gap junction, is considered to be the site of electrical coupling between cells. At these junctions adjacent cells come into close proximity with only a 2-3 nm gap between them. They act as low resistance pathways for spread of electrical activity from one cell to the other (Figure 2).

The second property gastro-intestinal smooth muscles share with the myocardium is that of spontaneous rhythmic depolarisation. The resting membrane potential of 40-50 mV shows rhythmic decreases of 3-15 mV, thus giving rise to the so called slow waves.

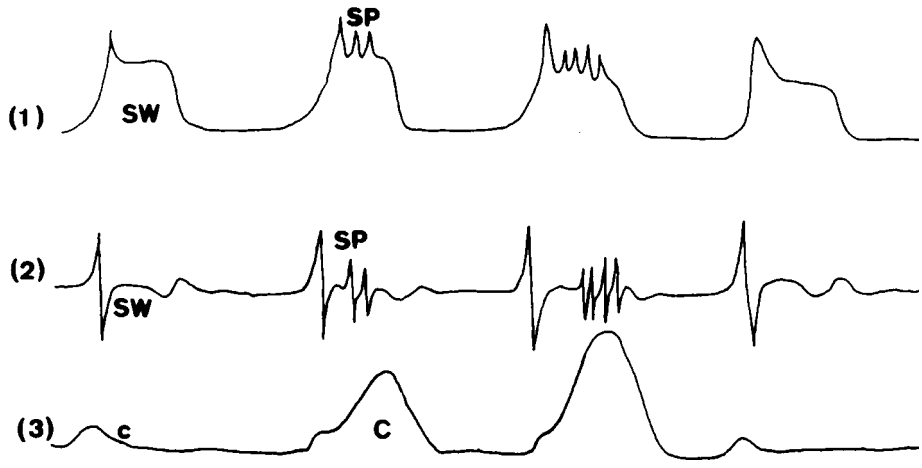

Figure 3: Schematic representation of slow waves, spikes and associated contractions. 1: intracellular recording showing slow waves (SW) with or without spikes (SP); 2: extracellular recording of the same phenomena; 3: manometric recording of contractions (C) induced by the electrical changes.

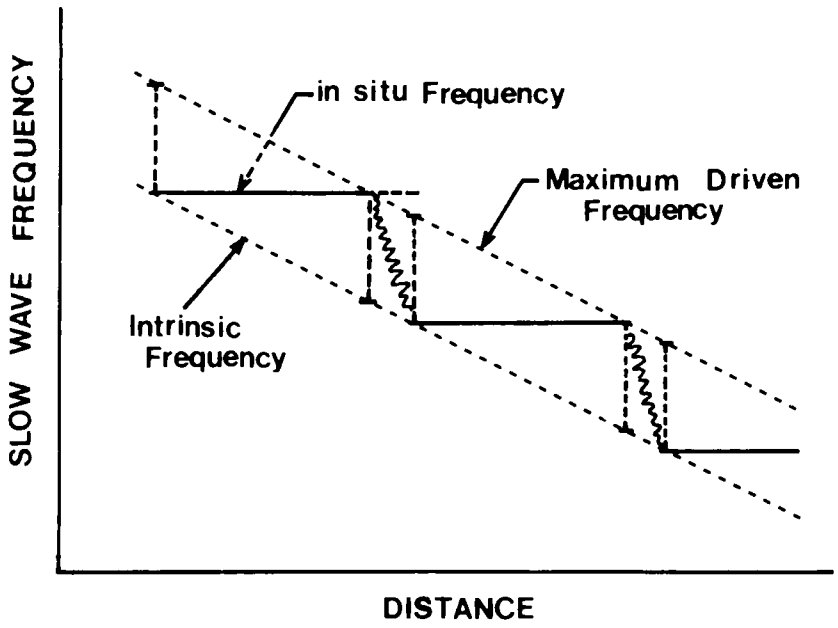

Figure 4: Schematic representation of slow wave frequency gradient in upper small intestine.

Slow waves are omnipresent (occur without interruption) but do not by themselves produce notable contractions. Contractions only develop when action potentials ("spikes") are superimposed on the plateau phase of the slow wave (Figure 3). The upper trace of Figure 3 represents an intracellular recording of electrical activity in small intestinal smooth muscle cells; the middle trace is an extracellular recording of the same phenomenon, and the lower trace shows the pressure changes produced by contractions. The strength of the contraction is related to the number of spikes.

There is an important difference between myocardial cells and intestinal smooth muscle cells. In the myocardium each depolarisation is associated with a contraction. The slow wave depolarisations of the small intestine only facilitate the contractions. As the contraction-inducing spikes only occur during a limited part of the slow wave cycle, slow waves control when and where contractions can occur.

The syncytial arrangement of smooth muscle cells and their spontaneous rhythmic depolarisations result in another resemblance between gastrointestinal smooth muscle and myocardium: the pacemaker activity.

The intrinsic rhythm of the slow waves decreases in man from 11-12/min in the duodenum to ± 8/min in the ileum. This is the so called slow wave frequency gradient. The decrease is not continuous but stepwise, giving rise to frequency plateaux. In the human small intestine the highest frequency plateau extends from the proximal duodenum to about 15-30 cm below the angle of Treitz. From there on the plateaux are shorter and more variable (*Coremans*, 1987; Figure 4).

The smooth muscle cells of the proximal duodenum have the highest intrinsic frequency and, therefore, act as a pacemaker for the more distal parts: the slow wave frequency of these more distal parts are pulled up to the fre-

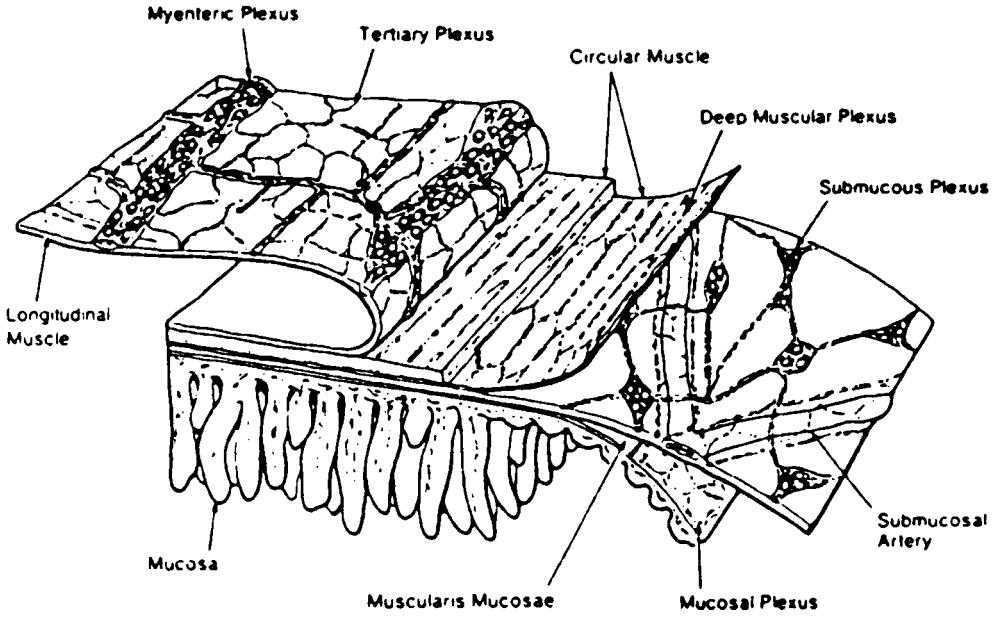

Figure 5: Diagrammatic representation of the enteric plexuses as they are seen in whole mounts of intestine (Reproduced from: *Furness* and *Costa*, 1987).

quency of the pacemaker area. When the difference in intrinsic frequency becomes too large or when the resistance to intercellular current spread increases, the more distal areas can no longer follow the slow wave frequency of the duodenal pacemaker. This area then will act as a pacemaker for more distal areas (*Bortoff*, 1976).

The small intestine, therefore, can be considered to consist of a series of functional units, each one being dominated by a pacemaker which imposes its rhythm on the segment below it.

Slow waves and pacemaker activity constitute an important myogenic control mechanism of smooth muscle contraction in that they facilitate contractions and thereby determine the normal temporal and spatial distribution of smooth muscle contractions, at least in the stomach and the small intestine. However, slow waves do not by themselves produce effective contractions.

THE ENTERIC NERVOUS SYSTEM AND NORMAL MOTILITY PATTERNS

The factors which determine the occurrence of spike potentials (and thus of contractions) are mainly neurogenic responses to stimulation of chemothermo- and mechanoreceptors in the intestinal wall and intestinal lumen. The enteric nervous system has an important role in the organisation of intestinal contractions into motility patterns.

The enteric nervous system

Figure 5 is a schematic drawing of the enteric nervous system with the myenteric plexus of Auerbach in between the longitudinal and circular muscle layers and the submucosal plexus (*Furness* and *Costa*, 1987).

The enteric nervous system is connected with the brain and the spinal cord

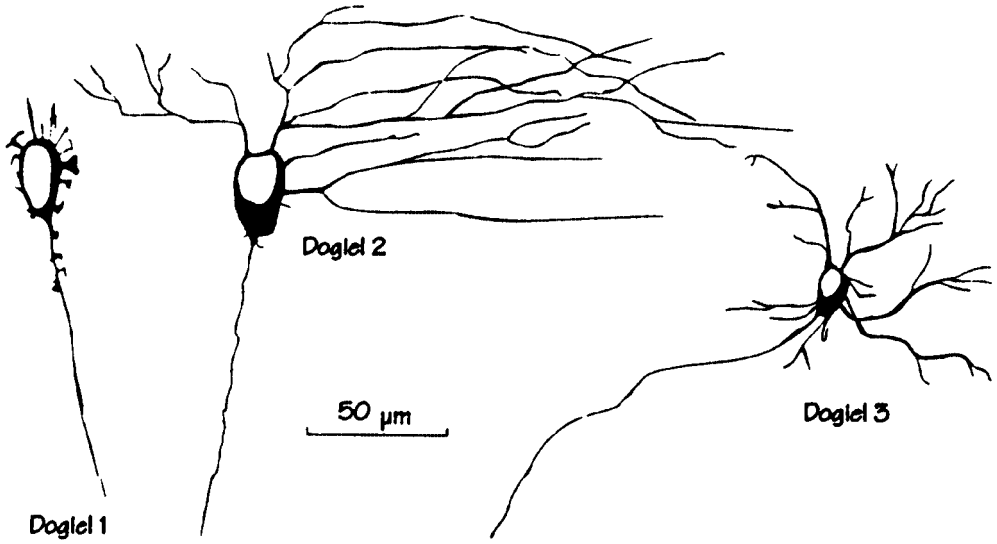

Figure 6: Diagrammatic representation of the three morphological categories of enteric neurons according to *Dogiel* (1899).

via the vagal nerve and the splanchnic and pelvic nerves.

However, the enteric nervous system has a remarkable independence from the central nervous system. It is the only division of the peripheral nervous system that is capable of mediating reflex activity in the absence of input from the brain and/or the spinal cord. It is now well established that the vast majority of the enteric neurons do not receive input from the central nervous system (there are only 2000 efferent fibres in the subdiaphragmatic vagus, versus 100 to 1000 million enteric neurons). Current concepts consider the enteric nervous system to be an independent integrative system that contains sensory neurons, interneurons and motor neurons which are all involved in the production of motility patterns (*Wood*, 1994).

On a morphological basis *Dogiel* (1899) identified 3 types of enteric neurons: type 1, 2 and 3 (Figure 6).

On the basis of their electrophysiological characteristics four types of enteric neurons have been identified (S/Type 1; AH/Type 2; types 3 and 4 (Table 1). S/Type 1 neurons of the small intestine discharge several action potentials, whereas AH/Type 2 neurons discharge only one action potential fol-

Table 1: Electrophysiological classification of enteric neurons

Properties	S/type 1	AH/type 2	Type 3	Type 4
Resting potentials	low	intermediate	high	intermediate
Input resistance	high	intermediate	low	intermediate
Spike discharge	repetitive	1 or more	none	one
Anodal-break discharge	yes	no	no	no
TTX-sensitive spikes	yes	no	-	yes
After-hyperpolarisation	no	yes	-	no

Table 2: Established and putative neurotransmitters present within the enteric nervous system

Amines:	acetylcholine norepinephrine histamine 5-hydroxytryptamine dopamine
Amino acids:	gamma-aminobutyric acid
Purines:	adenosine 5'-triphosphate
Non-related compounds:	nitric oxide?
Neuropeptides:	vasoactive intestinal polypeptide substance P enkephalins dynorphin calcitonin gene-related peptide somatostatin bombesin neurotensin neurokinin A cholecystokinin neuropeptide Y galanin gastrin-releasing peptide angiotensin adrenocorticotrophic hormone

lowed by a long after-hyperpolarisation. Studies of enteric neurons in different divisions of the digestive tract have revealed distinctive, region-specific electrical and synaptic properties (for example, AH/Type 2 neurons are absent from the oesophagus, the corpus of the stomach and the gallbladder). This variability probably reflects the adaptation of the microcircuits that control the specialized functions of different regions of the gastro-intestinal tract.

Over the past several years important information has been obtained regarding neurotransmitters and receptors for different neurotransmitters in the enteric nervous system.

Histochemical methods have allowed to visualize a large number of potential neurotransmitter substances or transmitter related enzymes in enteric neurons (*Costa* et al, 1987). These include amines (such as acetylcholine, 5HT and dopamine), amino acids (GABA), purines, nitric oxide.

There are also a large number of neuropeptides including VIP, substance P, enkephaline, etc. (Table 2).

Electrophysiological studies of enteric neurons and *in vitro* studies of enteric reflex activity have demonstrated a diversity of receptors for different neurotransmitters and messenger substances on enteric neurons. Some are ENS specific, some are confined to subclasses of neurons and there are regional differences in the presence of receptors.

The data, presented thus far only constitute a small fraction of the huge amount of data accumulated in recent years on the physiology of the enteric nervous system. It will be a major challenge for the future to put these data

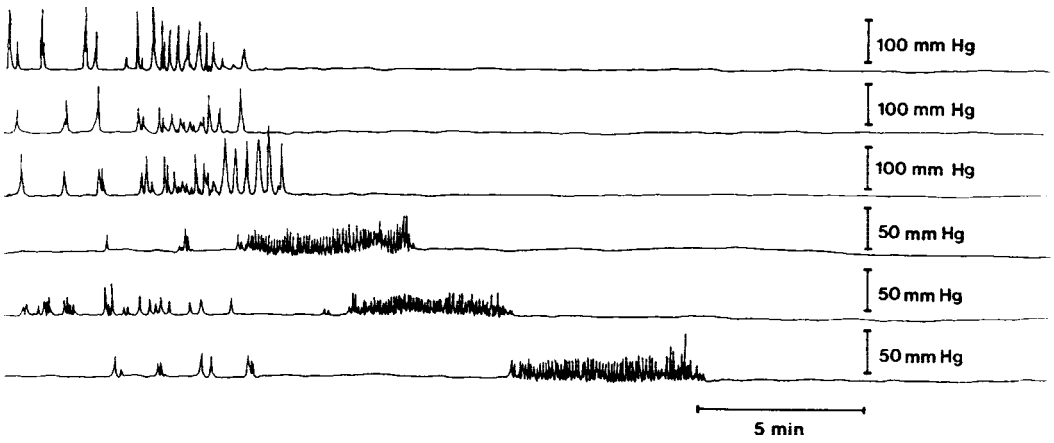

Figure 7: Manometric recording of the migrating motor complex (MMC) in man. The upper three traces were recorded in the antrum and the antropyloric region at 3 cm intervals; the lower three traces were recorded in the duodenum and upper jejunum (at 25 cm intervals).

all together in order to understand how the enteric nervous system really functions, and to determine the role of the enteric nervous system in the organisation of myogenically controlled contractions into specific motility patterns.

NORMAL AND ABNORMAL GASTRO-INTESTINAL MOTILITY PATTERNS

Recently it has become clear that well organized motility patterns occur in the small intestine in both the digestive and the interdigestive phases (*Code* and *Marlett*, 1975).

The best known interdigestive motility pattern is the migrating motor complex (MMC; Figure 7) and particularly phase 3 of this complex (the activity front). The burst of rhythmic contractions of phase 3 starts in the stomach. This burst of peristaltic contractions slowly migrates down the small intestine and dies out somewhere in the ileum. A new phase 3 then starts in the stomach. The duration of the MMC cycle is quite variable, but usually lasts between 90 and 120 min. This cyclic activity goes on and on until it is interrupted by food, which induces the so-called fed pattern. This fed pattern lasts for a variable period of time, depending upon the caloric load and composition of the meal.

At each level of stomach and small intestine the MMC comprises three phases. Phase 1 is a period of quiescence, with no or only very few contractions. During phase 2 the intensity of the motor activity gradually increases and contractions appear more and more frequently, until the burst of rhythmic, forceful contractions of phase 3 develops.

Distinct motility patterns have also been identified during phase 2 of the MMC i.e.: 1) single propagated (or peristaltic) contractions, 2) burst activity or clustered contractions (which may or may not be propagated); and 3) ultrarapid contractions of ultrarapid rushes (*Coremans*, 1987; *Vantrappen* et al., 1986).

Figure 8 is an example of an EMG

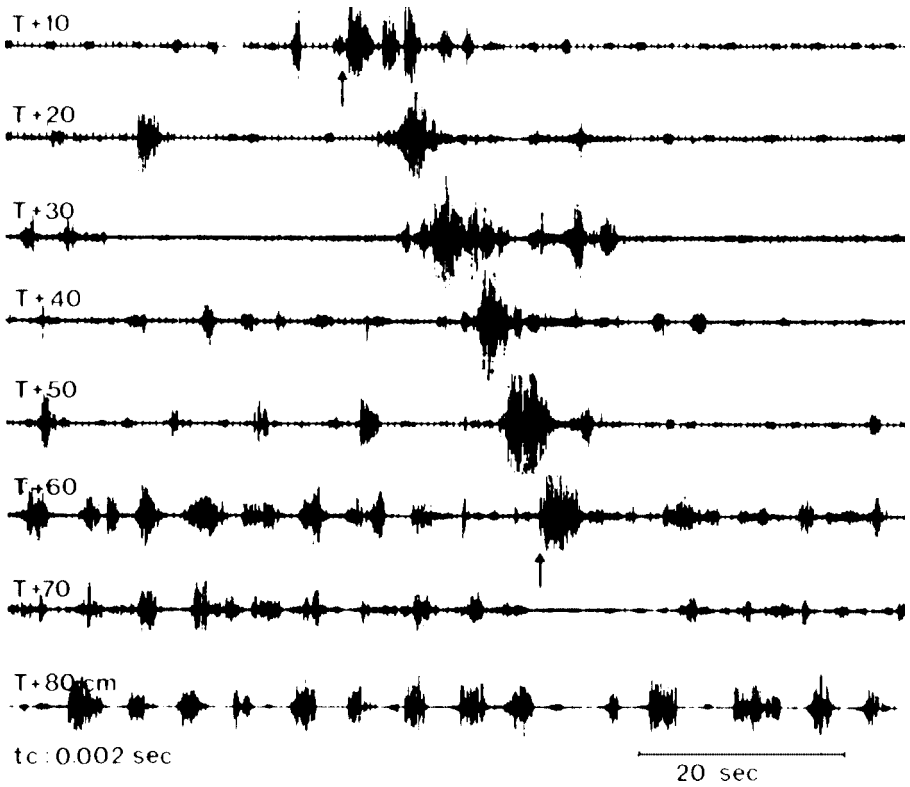

Figure 8: Electromyographic recording of a single propagated contraction i.e. a peristaltic contraction progressing from 10 cm below Treitz (T+10) to 60 cm below Treitz (T+60). The trace was recorded in a normal subject. (t.c.: time constant of the electrical recording).

recording of a single propagated contraction progressing from 10 cm below Treitz to 60 cm below Treitz.

On manometric examination a single pressure wave is seen to sweep down the intestine in a peristaltic way at a speed of 0.5 to 2.0 cm per second in the jejunum, but it is markedly slower in the ileum (0.2-1.8 cm/min). Such a lumen obliterating contraction results in propulsion of intestinal contents.

A second pattern observed during phase 2 of the MMC may be termed "clustered contractions". This pattern is characterized by the occurrence of a series of contractions which follow each other in close sequence and are separated from other contractions at that level by a period of quiescence before and after the cluster (Figure 9).

Clustered contractions may or may not be propagated. Propagated clusters are highly propulsive.

Both propagated single contractions and propagated clustered contractions occur rather infrequently in normal subjects. Their incidence is markedly increased in pathological conditions (*Coremans*, 1987). Stimulation of the small intestine by exposure of the mucosa to non-invasive bacteria results in an increased incidence of propagated single contractions, whereas exposure of the small intestine mucosa to invasive bacteria or other agents that cause mucosal damage induces propagated clustered contractions (*Mathias* et al., 1976; *Burns* et al., 1978).

An other type of motility pattern is: the retrograde giant contraction, which

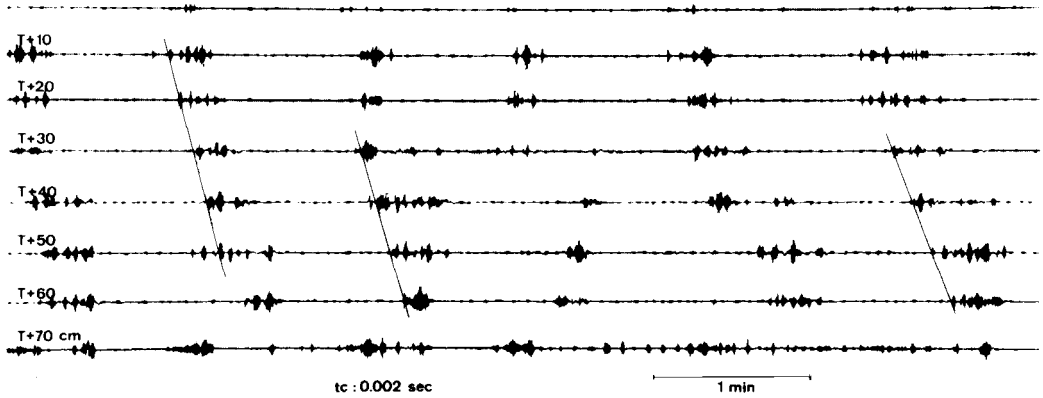

Figure 9: Electromyographic recording of clustered migrating contractions in the upper jejunum of a patient with diarrhoea (T+10: 10 cm below Treitz). Three clusters of propagated spike bursts are indicated by the oblique lines.

is one of the motor correlates of vomiting (*Lang* et al., 1986). It is a large-amplitude, long duration contraction that begins in the middle small bowel and rapidly migrates to the antrum. This retropulsive contraction precedes vomiting. Just prior to the occurrence of a giant retrograde contraction slow waves

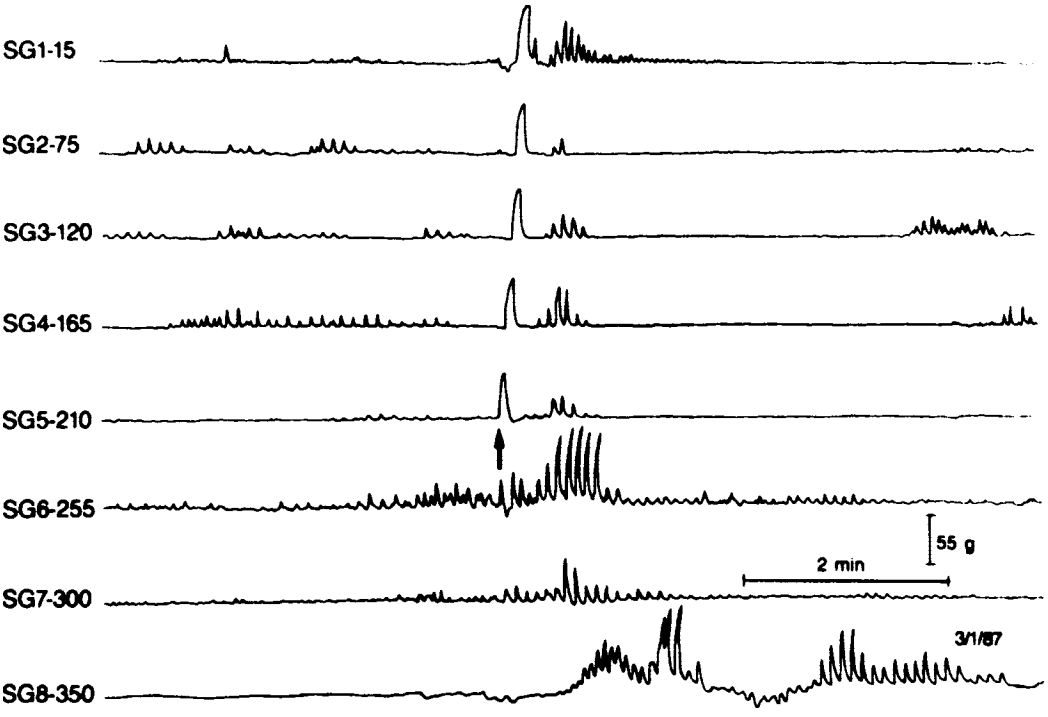

Figure 10: Retrograde giant contractions recorded from the canine small intestine. The arrow indicates the start of this contraction which migrated uninterruptedly from the mid small intestine to the proximal duodenum. SG1-SG8 are strain gauge transducers and the numbers indicate their distances from the pylorus. (From: *Sarna* and *Otterson*, 1989).

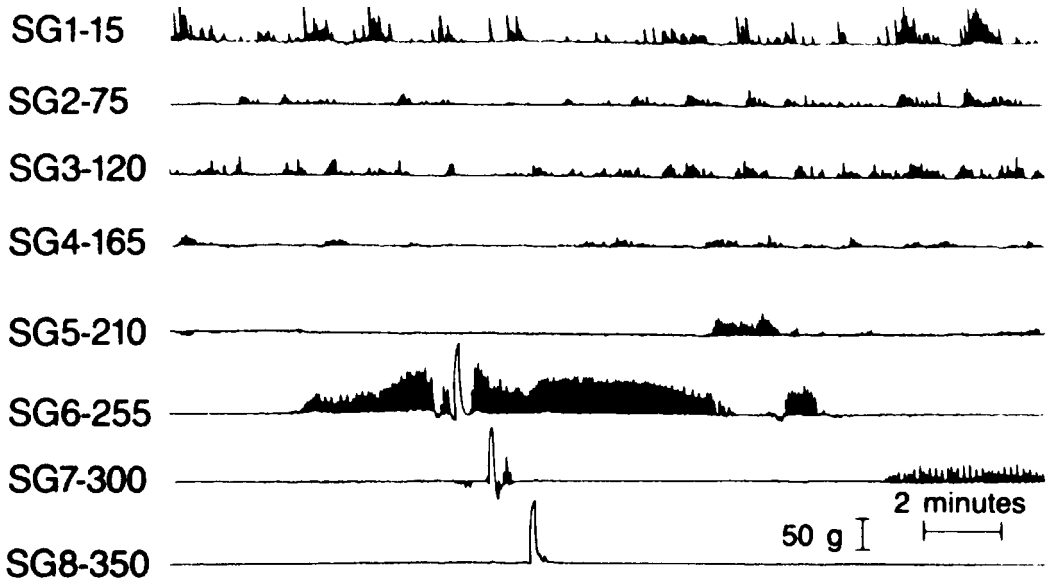

Figure 11: Giant migrating contractions recorded from the canine small intestine. The contraction started 255 cm distal to the pylorus and rapidly migrated to the terminal ileum. SG11-SG8 are strain gauge transducers and the numbers indicate their distances from the pylorus. (From: *Sarna* and *Otterson*, 1989).

disappear and phasic activity stops, apparently in order not to hamper the retropulsion of the intestinal contents into the stomach (Figure 10).

The giant migrating contractions (also called power contractions) also constitute a special motility pattern (*Sarna* and *Otterson*; 1989). These high amplitude, long duration contractions occur irregularly in the distal small intestine and migrate from their point of origin to the ileo-colonic junction and often into the colon (Figure 11).
These contractions occur only in the fasted state and can be initiated by a variety of stimuli including morphine, loperamide, erythromycin and intraluminal administration of short chain fatty acids. The function of the giant migrating contractions is probably to clean the distal ileum from food residues or refluxed colonic contents.

Abnormal pacemaker activity

If an intestinal distal pacemaker beats at an abnormally fast frequency i.e. at a frequency that is higher than the frequency of a more proximal pacemaker, there is an inversion of the slow wave frequency gradient. This then leads to a situation where contractions progress orally i.e. as antiperistaltic contractions which may cause chronic pseudo-obstruction (*Waterfall* et al., 1981).

A comparable situation may occur in patients with a Roux-en-Y type gastrectomy (*Vantrappen* et al., 1991). In studies on patients with a Roux-en-Y procedure we showed that 80% of the patients complaining of symptoms such as nausea and vomiting had a slow wave frequency in the distal part of the Roux limb that was higher than that of the more proximal parts. In other words they had an inversion of the slow wave frequency gradient in the Roux limb, the distal part of the Roux limb acting as a pacemaker for the more proximal parts.

Such a situation leads to the occur-

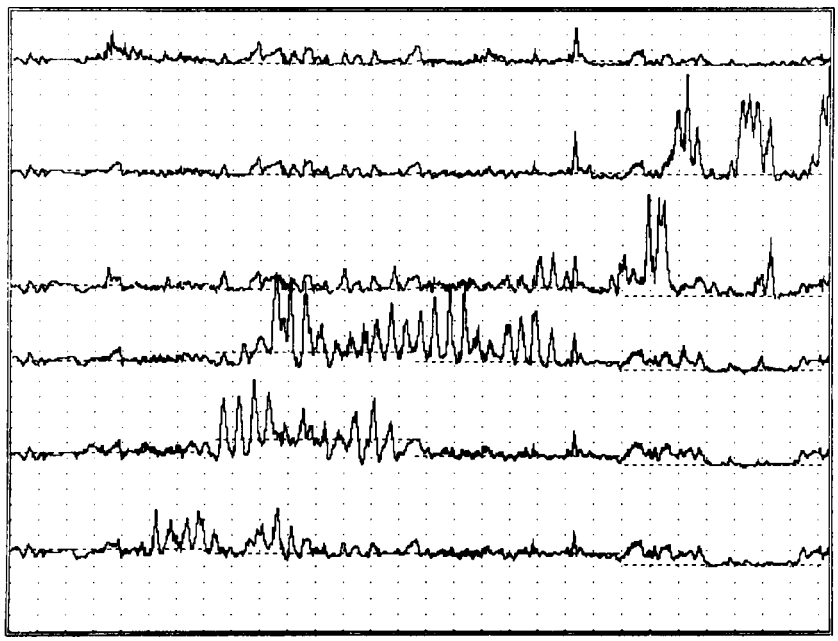

Figure 12: Retrograde phase 3-like contraction burst in a Roux-en-Y limb of a patient presenting with symptoms of nausea and vomiting after a Roux-en-Y procedure. Manometric recordings were made at 5 cm intervals in the Rouxlimb.

rence of antiperistaltic contractions and probably retropulsion. Even phase 3 of the MMC may move in an oral direction (Figure 12). This retrograde activity probably has a role in delaying gastric emptying and in the production of symptoms.

INTERACTION BETWEEN SMALL INTESTINAL MOTILITY AND SMALL INTESTINAL BACTERIA

There is a close interplay between the small intestinal microbial flora and small intestinal motility. Motility disorders may lead to bacterial overgrowth; conversely, exposure of the small intestinal mucosa to certain bacteria may lead to motility disorders.

Motility disorders may lead to bacterial overgrowth of the upper small intestine

Simultaneous manometric and radiocinematographic studies in dogs by *Code* and *Schlegel* (1974) and in man by our group had shown that the burst of peristaltic contraction waves of phase 3 are highly propulsive. They clear the bowel of all injected contrast material completely and quickly. *Code* and *Schlegel* (1974), therefore, proposed the concept that phase 3 of the MMC is the housekeeper of the small intestine and serves to keep the bowel clean. We speculated that, if the function of the MMC is to keep the bowel clean, absence of MMC's might result in stasis of food remnants, secretions and desquamated cells, thus creating an ideal culture medium for the development of small bowel bacterial overgrowth.

To test this hypothesis we studied

Table 3: Relation between presence or absence of phase 3 activity and bacterial overgrowth as demonstrated by bile acid studies

	Number	Phase 3 normal	Phase 3 absent or grossly abnormal
Normal subjects	18	18	0
Patients with negative bile acid breath test	9	9	0
Patients with positive breath test due to ileal malabsorption	6	6	0
Patients with positive breath test due to bacterial overgrowth	12	7	5

the interdigestive motor activity of the small bowel in 18 normal subjects, in 9 patients with various gastro-intestinal diseases but a normal 14CO2 bile acid breath test and in 18 patients with various gastro-intestinal diseases but a positive bile acid breath test (Table 3). In 6 of these 18 patients repeated bile acid breath tests after antibiotic treatment and radio-activity measurement in 24 h stool collections showed the positive bile acid breath test to be due to ileal bile acid malabsorption. In the remaining 12 patients the positive bile acid breath test seemed to be due to small intestinal bacterial overgrowth. In 7 of these 12 patients with bacterial overgrowth phase 3 of the MMC was normal; in 5 patients phase 3 was completely absent or grossly abnormal during recording periods of 6-8 hours. In other words, whenever phase 3 was absent or grossly abnormal there appeared to be bacterial overgrowth of the small intestine. Follow-up studies in these and in numerous other normal subjects and patients showed that: 1) absence of phase was always accompanied by a positive bile acid breath test that normalized after a course of antibiotics and was not accompanied by abnormal bile acid loss in the stool, and 2) when phase 3 had reappeared on later controls the evidence of bacterial overgrowth had disappeared.

That absence of phase 3 of the MMC may induce small bowel bacterial overgrowth was later proved experimentally by *Scott* and *Cahall* (1982). These authors treated rats with morphine or phenylephrine and cultured segments of the small bowel and its contents for aerobic and anaerobic bacteria. Opposite to its effect on the small bowel of man, morphine eliminated the activity front in rats and phenylephrine in the doses administered had the same effect. If the activity front was absent for more than 6 to 15 hours, the rats developed small intestinal bacterial overgrowth. If the rats were killed after the drugs had been stopped and the activity front had been allowed to reoccur, bacterial overgrowth was no longer present.

Bacteria in contact with the small intestinal mucosa may induce small intestinal motility disorders

Matthias et al. (1976) studied the myoelectrical pattern of the rabbit ileum in response to non-invasive diarrhoeagenic bacteria and their toxins. They noted in rabbit ileal loops exposed to live *Vibrio cholerae* or to cholera enterotoxin the occurrence of an abnormal electrical pattern they termed the "migrating action potential complex" (MAPC). It is a burst of spiking activity

of 2-5 seconds or longer which migrates down the ileal loop and propels the intraluminal contents in an aboral direction. This MAPC results in a single propagated (peristaltic) contraction. That this MAPC pattern was not merely a consequence of the increased intraluminal fluid secretion was suggested by the fact that the MAPC activity began 4 hours after loop inoculation, a time well beyond the well-known intestinal secretory effect of cholera enterotoxin.

These propagated single contractions have been described in the literature under various terms: MAPC, peristaltic rush, propagated contractions of the jejunum, prolonged propagated contractions of the ileum, type IV contractions, giant migrating contractions and power contractions, depending upon the animal species examined and the method of investigation used. The main characteristic of these propagated single contractions is their propagation velocity which is similar or approaches the propagation velocity of the slow waves (± 2 cm/sec in jejunum). As they are produced by a single spike burst associated with a single slow wave, or by a single spike burst spanning several slow wave cycles, their duration varies from a few seconds to approximately half a minute, and their amplitude is also quite variable.

Single propagated contractions of this type occur rather rarely in the normal jejunum. They can be elicited in experimental animals by a variety of stimuli. Non-invasive micro-organisms or their toxins may elicit this motility pattern. This has been demonstrated for *Vibrio cholerae* and its enterotoxin, for various strains of *Salmonella typhimurium*, for live toxigenic *E. coli* and its heat-labile enterotoxin, and there may be several others. Chemical stimuli which do not cause damage to the mucosa such as ricinoleic acid and prostaglandins also may cause this motility pattern. In patients with secretory diarrhoea the incidence of propagated single contractions is markedly increased.

Burns et al. (1978) studied the effect of invasive strains of *E. coli* on the myoelectrical activity of the small intestine of New Zealand white rabbits. They found two distinct complex patterns: repetitive bursts of action potentials (RBAPs) occurring predominantly in the infected ligated ileal loop and MAPC activity occurring predominantly in the uninfected small bowel oral to the ligated ileal loop. The authors concluded that MAPC activity was characteristic of "non-invasion" and that RBAPs correlated with enterocyte injury. RBAPs en MAPC activity have also been described after *Shigella dysenteriae* I enterotoxin, *Clostridium perfringens* A enterotoxin and *Clostridium difficile* enterotoxin.

These studies indicate that, at least in animals, specific motor patterns can be induced by diarrhoeagenic bacteria and that their toxins may contribute to the diarrhoea in these diseases. Analogous studies have not been performed in man.

These repetitive bursts of action potentials have been described in the literature under various names: clustered contractions, type II peristalsis, ricinoleic acid pattern of dogs, and in case of prolonged sequences of clustered contractions, it has been termed "minute rhythm".

The incidence of clustered contractions is increased in a variety of pathological conditions. We observed it in patients with infectious diarrhoea. The outbreak of chronic diarrhoea in raw milk drinkers in Brainerd, Minnesota, also resulted in clustered contractions. We observed it also in the uninvolved jejunum of patients with inactive Crohn's disease. Interestingly, the jejunal motility pattern of these Crohn's disease patients was characterized by decreased phase III activity and an in-

crease in both propagated single and propagated clustered contractions. Whether this is due to the presence of an abnormal bacterial flora of to other causes remains unknown.

The interaction between small intestinal motility and small intestinal bacteria was recently further investigated in a series of elegant studies by E. Husebye (*Husebye*, 1995; *Husebye* and *Engedal*, 1992; *Husebye* et al., 1992, 1994). Studies in healthy subjects and patients with late radiation enteropathy showed that intestinal motility is the main line of defence against colonisation with Gram-negative bacilli in the upper gut. The role of gastric acid in host defence is confined to restriction of microbial growth and metabolism in the stomach. Husebye's studies further showed that proximal intestinal dysfunction, as indicated by abnormal motility patterns and colonisation with Gram-negative bacilli, plays a key role in the pathogenesis of late radiation enteropathy.

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THE MYENTERIC PLEXUS IN THE CONTROL AND PHARMACOTHERAPY OF GASTRO-INTESTINAL MOTILITY

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INTRODUCTION

The innervation of the gastro-intestinal tract

Gastro-intestinal functions are controlled by a hierarchy of humoral, neural and myogenic mechanisms. The neural control of the gastro-intestinal tract is provided by three divisions of the autonomic nervous system: the parasympathetic, the sympathetic and enteric divisions. The subdivision is based on the location of the ganglia and the connections with the central nervous system. The parasympathetic and sympathetic nerves supply the extrinsic innervation of the gut. The enteric nervous system (ENS) comprises the intrinsic innervation of the gut.

The structure and function of the enteric nervous system

The ENS is made up of ganglia and interconnected nerve fibres, ranging from the oesophageal body to the internal anal sphincter (*Furness* and *Costa*, 1987). The ganglionated plexuses within the ENS are the myenteric plexus, found between the circular and longitudinal muscle layers, and the submucous plexus in the submucosal connective tissue layer. It is generally accepted that the myenteric plexus is mainly involved in the control of gastro-intestinal motility, while the submucous plexus is mainly involved in the control of secretion, absorption and bloodflow (*Furness* and *Costa*, 1987).

During health, two quite distinct functional states of the upper gastro-intestinal tract can be recognized; the interdigestive state is observed following a period of fasting, the fed state following a meal. The ENS is involved in the control of both these functional states.

The motor activity of the upper gastro-intestinal tract in the interdigestive state

The migrating motor complex (MMC) is a cyclical motor pattern of the stomach and the small intestine in the fasting state in most mammalian species, including man (*Szurszewski*, 1969; *Vantrappen* et al., 1977). The MMC consists of four phases of which phase 3, also called the activity front, is the most characteristic one. Phase 1 is a quiescent phase. It is followed by phase 2, a period of persistent but random contractile activity. The contractile activity reaches a maximum in frequency and intensity during phase 3, which is also called the activity front. Phase 4 is a phase of rapidly subsiding contractile activity and merges into phase 1. The duration of one cycle in man is about 100 minutes (*Vantrappen* et al., 1977). Recording gastro-intestinal motility from several sites simultaneously revealed that the MMC sequence progresses aborally from the stomach or the proximal small intestine to the

terminal ileum (*Szurszewski*, 1969).

It is now evident that the enteric nervous system is the medium through which the MMC is propagated (Sarna et al., 1981). The control of the initiation of the MMC is incompletely understood. In dog and in man, plasma levels of the hormone motilin are maximal just before and during phase 3 in the distal stomach (*Itoh* et al., 1975; *Vantrappen* et al., 1979; *Sarna* et al., 1983; *Bormans* et al., 1987), and administration of exogenous motilin induces a premature phase 3 which starts from the stomach (*Vantrappen* et al., 1979). These observations suggest that motilin has a physiological role in the induction of phase 3 of the MMC. The target for motilin in the gastro-intestinal tract is unknown.

The motor activity of the upper gastro-intestinal tract in the fed state

In the fed state, the principal motor tasks of the stomach are to break up the meal and to deliver it into the intestine at a rate that matches the processing capability of the intestine. The corpus and fundic regions of the stomach act as a reservoir for food storage. The antrum serves as a muscular pump that breaks up food, mixes it with gastric secretions and drives the semisolid chyme in a controlled way through the pyloric sphincter, into the duodenum.

HYPOTHESIS: THE MYENTERIC PLEXUS OF THE STOMACH PLAYS A KEY ROLE IN THE CONTROL OF GASTRIC MOTILITY

The stomach displays specific motor patterns in both of the interdigestive and the fed state. We hypothesized that the myenteric plexus of the stomach is providing the basic neural circuitry that governs these different patterns of contractile activity. In support of this hypothesis, we tried to demonstrate the following steps:

1. The presence of a diversity of myenteric neurones in the stomach becomes apparent using neurochemical electrophysiological morphological and pharmacological studies.
2. In support of a physiological role of the gastric myenteric plexus in the control of the fed state, endogenous substances that are involved in the control of gastric emptying, can be shown to act on myenteric neurones in the stomach.
3. In support of a role of the gastric myenteric plexus in the control of the interdigestive state, an interaction of motilin with myenteric neurones in the stomach can be demonstrated.
4. Pharmacological agents, used in the therapy of upper gastric motility disorders, act on myenteric neurones in the stomach.
5. Pharmacological studies of myenteric neurones in the stomach can identify new agents that will modify gastro-intestinal motility.

PHENOTYPIC DIVERSITY OF MYENTERIC NEURONES IN THE STOMACH

In order to control the various motor patterns exhibited by the stomach, several functional types of neurones must be present in the myenteric plexus of the

stomach. The presence of a diversity of myenteric neurones in the stomach becomes apparent using neurochemical electrophysiological, morphological and pharmacological studies.

Neurochemical studies of the myenteric plexus of the stomach

Using immunohistochemical techniques, we demonstrated the presence of several neurotransmitters in neurones of the myenteric plexus of the guinea-pig gastric antrum. Immunoreactivity for choline acetyltransferase was present in 62% of all neurones, indicating that the vast majority of neurones in the stomach are cholinergic. This is in agreement with the predominance of cholinergic mechanisms involved in the control of gastric motility. Immunoreactivity for nitric oxide synthase was present in 34% of the neurones, indicating a substantial nitrergic population. This is in agreement with several observations that demonstrate a role for nitric oxide in inhibitory neurotransmission in the stomach. Substance P-immunoreactive neurones (41%) and neuropeptide Y-immunoreactive neurones (33%) formed two distinct subpopulations. A subpopulation of neuropeptide Y-immunoreactive neurones was immunoreactive for nitric oxide synthase. The majority of neurones immunoreactive for substance P and for vasoactive intestinal polypeptide (22%) were separate populations. A subpopulation of nitric oxide synthase-immunoreactive neurones was immunoreactive for vasoactive intestinal polypeptide, but a distinct subpopulation of nitric oxide synthase-immunoreactive neurones was vasoactive intestinal polypeptide-negative. These data demonstrate that several neurotransmitters are present in gastric neurones, displaying specific co-localisation patterns. This supports the notion of functional diversity of gastric neurones.

Electrophysiological studies of the myenteric plexus of the stomach

We used intracellular recording methods to provide the first electrophysiological studies of antral myenteric neurones. On the basis of their electrophysiological characteristics, antral neurones were classified into four subtypes (*Tack* and *Wood*, 1992a). Gastric I neurones were characterized by repetitive spike discharge during intraneuronal injection of depolarising current pulses. Gastric II neurones discharged only one or two spikes at the onset of depolarising current pulses. In both cell types, the action potential was suppressed by TTX, suggesting exclusive involvement of inward Na^+ current in the depolarisation phase of the spike. Gastric III neurones never discharged spikes during depolarising current pulses. AH/Type 2 neurones were characterized by spikes which were not abolished by tetrodotoxin due to a calcium component of the inward current. Action potentials in these neurones were associated with a long-lasting hyperpolarising after-potential which began shortly after the positive after-potential of the spike and whose amplitude increased when an increasing number of spikes was fired.

Synaptic signals are the way in which enteric neurones communicate with each other. They consist of both excitatory and inhibitory potentials, and have a fast or slow time scale. We used intracellular recording methods and focal electrical stimulation of interganglionic fibre tracts to evoke synaptic potentials, which are likely to reflect the synaptic interactions in the neuronal networks of the gastric antrum (*Tack* and *Wood*, 1992b).

Nicotinic cholinergic fast excitatory postsynaptic potentials (EPSPs) could be evoked in every antral neurone. Most neurones received multiple inputs from

axons arriving in several different interganglionic fibre tracts and several neurones received input from multiple axons in individual fibre tracts. Application of acetylcholine (ACh) by microejection mimicked the fast EPSP in all neurones. In about one third of the neurones, this fast nicotinic response to ACh was followed by a long-lasting muscarinic depolarisation.

Slow EPSPs were evoked by repetitive stimulation of the interganglionic connectives. They consisted of a slowly activating depolarisation which persisted for several seconds after the termination of the stimulus. They were observed mainly in AH/Type 2 neurones. Atropine did not inhibit the slow EPSP. The neurotransmitter that mediates the slow EPSP is unknown, but substance P and 5-HT are likely candidates. Elevation of cyclic adenosine 3',5'-monophosphate by the application of forskolin mimicked the slow EPSP in antral AH/Type 2 neurones, suggesting cyclic AMP may function as an intraneuronal second messenger in the signal transduction process for slow synaptic excitation.

Slow inhibitory postsynaptic potentials (IPSPs) were hyperpolarising potentials evoked by repetitive stimulation of interganglionic fibre tracts. They were only rarely observed. The neurotransmitter mediating slow IPSPs and their functional role are unknown.

Morphological studies of the myenteric plexus of the stomach

Intracellular dye injection during electrophysiological studies of gastric neurones allows to study their morphological characteristics. The size of the neuronal cell bodies is variable. Most gastric neurones are unipolar, with one long axon. This axon can project in oral or anal directions. The dendrites can be short, broad and club-like (Dogiel I neurones), or fine, tapering and filamentous (Filamentous neurones). These data demonstrate that several morphological types of neurones are present in the stomach. This supports the notion of functional diversity of gastric neurones.

ROLE OF THE MYENTERIC PLEXUS IN THE STOMACH IN THE CONTROL OF GASTRIC EMPTYING

We hypothesized that the myenteric plexus in the gastric antrum plays a key role in the coordination of gastric emptying of solids. Therefore, we studied the interaction of endogenous and exogenous substances, that are known to modulate gastric emptying, with neurons in the gastric antrum.

Norepinephrine

Norepinephrine has a well established role as a neurotransmitter in the gastro-intestinal tract (*Furness* and *Costa*, 1987). Stimulation of adrenergic nerves inhibits gastric tone and antral motility (*Guimaraes*, 1969). We used electrophysiological methods to directly study the actions of norepinephrine on antral myenteric neurons, in order to elucidate the mechanism by which norepinephrine inhibits gastric motility. Our study revealed two different actions of norepinephrine on myenteric neurons (*Tack* and *Wood*, 1992c). Both mechanisms may contribute to the neurally mediated inhibitory action of norepinephrine on gastric contractility.

One action was presynaptic inhibition of fast and slow excitatory synaptic potentials: norepinephrine interacts with presynaptic a_2-receptors to inhibit the release of ACh and the (presently un-

known) non-cholinergic mediator for the slow EPSP. By this mechanism, norepinephrine may decrease the probability of suprathreshold responses in the excitatory motor neurons and may directly or indirectly decrease the amount of excitatory transmitters that reaches the gastric smooth muscle.

The second action of norepinephrine was a postsynaptic depolarisation with increased input resistance and enhanced excitability, which is mediated by an a1 adrenoceptor. In the stomach, these receptors are probably located on non-cholinergic inhibitory neurons (*Hillsley* et al., 1991) and by directly stimulating these inhibitory motor neurons, norepinephrine may increase the amount of inhibitory neurotransmitter that is released and may consequently also contribute to the neurally mediated inhibition of gastric motility by norepinephrine.

5-Hydroxytryptamine

A great deal of evidence has accumulated to support the hypothesis that serotonin, or 5-hydroxytryptamine (5-HT), is a neurotransmitter in the ENS (*Furness* and *Costa*, 1987). In the stomach in vitro, 5-HT causes a relaxation (*Van Nueten* and *Janssen*, 1980), but nerve-mediated excitatory actions of 5-HT in the stomach were also described (*Yamaguchi*, 1972). The purpose of our study was to directly study the actions of 5-hydroxytryptamine on the electrical and synaptic properties of antral myenteric neurons and to characterize the receptors involved. We found that 5-HT has multiple actions on myenteric neurons in the gastric antrum (*Tack* et al., 1992b). 5-HT evoked both fast and slow depolarising responses in antral myenteric neurons. Furthermore, 5-HT inhibited the stimulus-evoked release of acetylcholine and non-cholinergic neurotransmitters. Finally, 5-HT caused a slow hyperpolarising response in a small subgroup of antral myenteric neurons. Specific 5-HT receptor subtypes mediate the different responses to 5-HT. The fast depolarising response is mediated by a 5-HT_3 receptor. The slow depolarising response is mediated by a 5-HT_{1P} receptor. The hyperpolarising response and the presynaptic inhibition of neurotransmitter release are both mediated by a 5-HT_{1A} receptor.

It has been suggested that the 5-HT_{1P} receptor plays a role in the control of gastric emptying (*Mawe* et al., 1989). Recent studies by our group have shown that 5-HT_3 receptors are involved in the control of gastric activity fronts (*Wilmer* et al., 1993). Enteric 5-HT1A receptors may be involved in 5-HT-mediated gastric relaxation (*Meulemans* et al., 1991).

Cholecystokinin

Cholecystokinin (CCK) stimulates antral contractile activity in the guinea-pig stomach *in vitro* by acting on intrinsic cholinergic neurons (*Gerner* and *Haffner*, 1977). We used electrophysiological recordings to directly study the actions of CCK on antral myenteric neurons. Antral myenteric neurons responded to CCK in one or more of the following ways: a brief depolarisation, a prolonged hyperpolarising response or a prolonged depolarisation (*Tack* et al., 1992c). All responses seemed to be mediated by CCK-A receptors.

Prokinetic substituted benzamides

Substituted benzamides, such as cisapride, have clinical application in the treatment of gastroparesis. It is generally accepted that they act on myenteric neurons to stimulate gastric motility, possibly by acting on 5-HT_4 receptors. We conducted the first study of the effects of cisapride at its target: the myenteric neurons in the gastric antrum. We found that cisapride inhibits both 5-

HT_3 and 5-HT_{1P} receptor-mediated responses to 5-HT. However, cisapride also directly depolarized a subpopulation of antral neurons, through a non-5-HT mechanism (*Tack* et al., 1992d). We found no evidence for an interaction with 5-HT_4 receptors. Through a similar mechanism of action, cisapride also enhances cholinergic neurotransmission between antral myenteric neurons (*Tack* et al., 1992d). Our hypothesis that this non-5-HT-related action of cisapride is responsible for its gastrokinetic actions is supported by recent *in vivo* studies (*de Ridder* and *Schuurkes*, 1993).

Sumatriptan

Recently, we demonstrated that sumatriptan, a 5-HT_{1D} agonist at cerebral arteries, clinically used in the treatment of migraine, is an agonist at the 5-HT_{1P} receptor (*Vanden Berghe* et al., 1995). Thus, sumatriptan provides us with a tool to study the effect of 5-HT_{1P} receptor activation on gastro-intestinal functions *in vivo*. In the interdigestive state in man, administration of sumatriptan induces a premature activity front with jejunal onset and suppresses phase 3 motor activity in the stomach (*Tack* et al., 1995a). The observed effects on interdigestive motility are not accompanied by changes in the cyclical pattern of motilin plasma levels. Furthermore, sumatriptan inhibits somatostatin release. Gastric phase 3 activity induced by the motilin agonist erythromycin is blocked by sumatriptan (*Coulie*, 1996).

Sumatriptan increases the amplitude and the duration of oesophageal contractions (*Houghton* et al., 1994). In addition, administration of sumatriptan in man results in an immediate and profound relaxation of the gastric fundus (*Tack* et al., 1995b). This relaxation allows larger volumes to be accommodated before the thresholds for perception and discomfort are reached during gastric distention (*Tack* et al., 1996). Studies in cats suggest that activation of a nitrergic pathway underlies the relaxatory effect of sumatriptan on the gastric fundus (*Coulie*, 1996).

In the postprandial state, sumatriptan causes a significant increase in the gastric half emptying of both solids and liquids, and it increases the lag phase for the gastric emptying of solids (*Coulie* et al., 1997). Unlike other agents that delay gastric emptying, sumatriptan induces a prolonged lag phase for the gastric emptying of liquids.

ROLE OF THE MYENTERIC PLEXUS IN THE CONTROL of INTERDIGESTIVE GASTRO-INTESTINAL MOTILITY

The target for motilin in the gastrointestinal tract is unknown. In the rabbit and in man, the existence of motilin receptors on smooth muscle cells has been demonstrated *in vitro* (*Louie* and *Owyang*, 1984). In the dog however, no smooth muscle receptors for motilin could be demonstrated, although motilin is able to induce a premature gastric phase 3 in this species (*Itoh* et al., 1975). Moreover, the complicated and highly organized motility pattern of the MMC seems to suggest an underlying neural control mechanism. Therefore, we hypothesized that, besides a direct effect on intestinal smooth muscle, motilin also has a direct effect on intrinsic neural elements.

We used intracellular recordings to study the actions of motilin on the electrical behaviour of myenteric neurons of the guinea-pig gastric antrum *in vitro*. In a subpopulation of antral neurons, motilin evoked a long-lasting depolari-

sation of the cell membrane associated with enhanced excitability, thus mimicking slow synaptic excitation (*Tack*, 1992). The effects of motilin appeared to be directly on the neurons from which the recordings were made because they still occurred during synaptic blockade with tetrodotoxin or removal of Ca^{2+}. Dose dependency of the membrane depolarising action of motilin suggests that it is receptor mediated.

Erythromycin.

The motilin agonist erythromycin has been shown to stimulate gastro-intestinal motility and to accelerate gastric emptying in man (*Peeters* et al., 1989; *Tack* et al., 1992a; *Janssens* et al., 1990). Indirect observations suggest that erythromycin exerts its prokinetic effect by acting on motilin receptors on presynaptic enteric neurons (*Sarna* et al., 1991). We demonstrated that erythromycin directly depolarizes a subpopulation of myenteric neurons in the gastric antrum. Moreover, the same subpopulation is depolarized by motilin and motilin and erythromycin cause mutual desensitisation of each others effect (*Tack* et al., 1991). We therefore conclude that erythromycin is an agonist for the motilin receptor on myenteric neurons, and that this mechanism is involved in its prokinetic actions.

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MOTILIN AND THE DISCOVERY AND DEVELOPMENT OF MOTILINOMIMETICS

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SUMMARY

Motilin is a peptide which stimulates gastro-intestinal motor activity. Motilin has been mostly studied in relation to the migrating motor complex, and is thought to be involved in the regulation of this pattern. Motilin also increases pressure in the lower oesophageal sphincter, accelerates gastric emptying, induces gallbladder contraction and increases colon motility. Smooth muscle motilin receptors have been characterized pharmacologically in several species and organ-specific subtypes may exist. The existence of neuronal motilin receptors can be deduced from *in vivo* data. They probably resemble the smooth muscle receptors, and may be even closer related to the recently discovered central motilin receptors.

Erythromycin has been shown to be a motilin agonist. The development of motilin antagonists has removed all doubt in this regard. The successful application of erythromycin in patients with gastroparesis has stimulated the development of motilinomimetics, a new class of prokinetic drugs. This class encompasses motilides, derived from macrolides, and motilin analogues. Several motilides have already been proposed as well as two motilin analogues, and affinity for the motilin receptor has been a useful screening tool in their development. At the same time these studies have led to an understanding of the structure-activity relation of motilin, and to the development of antagonists. The motilin receptor may also be used to develop antibiotics with reduced gastro-intestinal side-effects.

One may safely predict that soon motilinomimetics will be available for the treatment of hypomotility conditions. This will again increase our understanding of the physiological role of motilin and of the regulation of gastro-intestinal motility.

INTRODUCTION

Two decades have passed since the discovery of motilin (*Brown* et al., 1972), and until a few years ago this peptide was reduced to the status of an orphan peptide: abandoned by its discoverer, who saw more future in GIP, it attracted the attention of only a few "motilinophiles". The picture changed when the hypothesis that erythromycin was a motilin agonist (*Peeters* et al., 1989), found a successful application in patients with diabetic gastroparesis

	1	6	11	16	21
pig, man	FVPIF	TYGEL	QRMQE	KERNK	GQ
dog	FVPIF	THSEL	QKIRE	KERNK	GQ
cat	FVPIF	THSEL	QRIRE	KERNK	GQ
rabbit	FVPIF	TYSEL	QRMQE	RERNR	GQ
chicken	FVPFF	TQSDI	QKMQE	KERNK	GQ

Figure 1: Amino acid sequence of porcine motilin, and substitutions found in other species.

(*Janssens* et al., 1990). Presentation of these data at the meeting of the American Gastroenterological Association in Washington in 1989 proved to be so stimulating, that at the next meeting in San Antonio in 1990 a whole symposium could be devoted to erythromycin's prokinetic effects. Since then interest continues to grow, as the program of the present Symposium again demonstrates. This paper is not an extensive overview, but a brief and broad summary with a perspective to the past as well as to the future.

MOTILIN

Motilin is a 22-amino-acid polypeptide, first isolated from the duodenal mucosa of the pig by J.C.Brown in 1972 (*Brown* et al., 1972). It was named motilin because of its ability to induce motor activity in the gastro-intestinal tract. Motilin has also been isolated from a few other species. Known sequences are shown in Figure 1.

Motilin has especially been studied in relation to the migrating motor complex (MMC). This is a motility pattern which is observed in the fasted state in man and in several other species and which consists of three phases. In phase 1 motor activity is absent. It is followed by phase 2 when contractions occur irregularly. During phase 3 contractions occur at their maximum frequency. This phase lasts only for some minutes, ends abruptly and may be followed by a brief period of declining irregular activity, phase 4. The migrating motor complex progresses distally from the lower oesophageal sphincter to the terminal ileum. In man this takes about 90 min, and this is also the time interval separating the occurrence of the same phase of the MMC at one particular location. It has been proposed that the MMC functions as the "intestinal housekeeper" keeping the gastro-intestinal tract free of residual food, desquamated cells and intestinal secretions, which would otherwise accumulate during fasting. The MMC may also limit bacterial growth in the proximal small intestine. Indeed bacterial overgrowth is associated with the absence of MMC activity (*Vantrappen* et al., 1977).

Administration of motilin induces phase 3 of the MMC, but the peptide has also other effects. In pharmacological doses, motilin increases pressure in the lower oesophageal sphincter, accelerates gastric emptying, induces gallbladder contractions and increases colonic motility (*Vantrappen* and *Peeters*, 1989). The physiological role of motilin is still debated, but the peptide is considered to be involved in the regulation of the migrating motor complex. The main arguments for this hypothesis are 1) the temporal relationship

between the occurrence of motilin plasma peaks and the occurrence of phase 3 activity in the gastroduodenal area; 2) the induction of phase 3 activity by the administration of motilin in doses which increase plasma levels to the same extent as the endogenous rise; 3) the inhibition of phase 3 activity in the gastroduodenal area by immunoneutralisation of motilin.

Motilin's mechanism of action has not been fully elucidated. *In vitro* it induces contractions in preparations from man, rabbit, cat and chicken which are mediated via smooth muscle receptors (*Strunz* et al., 1976; *Adachi* et al., 1981; *Depoortere* et al., 1993; *de Clercq* et al., 1995). Just as motilins from different species show amino acid substitutions, the smooth muscle receptors of the different species are not identical. This can be deduced from the differences in potency and in binding affinity (*Peeters*, 1993a). Besides these species differences, evidence has also been presented for organ-specific receptor subtypes in the rabbit. However the putative p- and d-receptors of, respectively, the pyloric region and the duodenum, are both smooth muscle receptors (*Peeters* et al., 1994a).

In vitro preparations from dog, rat and guinea pig do not respond to motilin, although the dog has been the model of choice to study *in vivo* effects. *In vivo* however, motilin's effect is neurally mediated, even in species in which a direct smooth muscle effect has been shown *in vitro* (man, rabbit). Motilin appears to stimulate the release of excitatory neurotransmitters via cholinergic, opioid and serotonergic pathways, but may also inhibit the release of inhibitory substances such as VIP and NO. This will not be discussed in detail, but an important finding in this respect is the motilin-evoked release of acetylcholine (*Kitazawa* et al., 1993). The *in vivo* data therefore indicate that besides the smooth muscle motilin receptors mentioned above, and characterized fairly well *in vitro*, there also exist neuronal receptors. Nevertheless all these receptors seem to be closely related, as *in vitro* potencies (reflecting the muscular receptor) correlate well with *in vivo* potencies (reflecting the neuronal receptor) (*Peeters* and *Depoortere*, 1994) . It seems likely that the neural motilin receptors are present in low density in the gastro-intestinal tract, and difficult to demonstrate or characterize in binding studies. This would certainly be the case if these neuronal receptors have a much lower affinity as was suggested by *Kitazawa* et al. (1993). Recently motilin receptors have been discovered in the brain (*Depoortere* et al., 1995). Besides the fact that this opens a new area of investigations, and points to other as yet unknown functions of motilin, one may hope that these central motilin receptors have the same characteristics as the neuronal receptors of the gastro-intestinal tract. This would much facilitate their characterization.

The lack of motilin antagonists has seriously hampered the study of motilin's mechanism of action and physiological role. Recently the first, although weak, motilin antagonist, ANQ-11125 i.e. [Phe^{3}, Leu^{13}] porcine motilin (1-14), was announced (*Peeters* et al., 1994b). Soon thereafter the extended form of this compound OHM-11526 i.e. [Phe^{3}, Leu^{13}] porcine motilin (1-22), was shown to have a slightly higher affinity than motilin itself (*Peeters* et al., 1993), but unfortunately it behaved as a weak agonist *in vivo* (*Matsuo* et al., 1994). The antagonist properties of both these compounds are due to the substitution of residue 3, proline, by phenylalanine. Very recently, it has been claimed that GM-109, a short cyclic peptide with structure Phe-cyclo[LysTyr(3-tBu)-βAla], is

a motilin antagonist, devoid of agonist activity *in vivo* (*Takanashi* et al., 1995). It will be noted that GM-109 has a tyrosine in the position corresponding to the phenylalanine residue in OHM-11526. It may be hoped that these compounds, and derivatives that will certainly be derived from them, will soon help to unravel the physiology of motilin. As will be mentioned below, they have already been helpful to elucidate the mechanism of action of erythromycin derivatives.

DISCOVERY OF THE EFFECT OF ERYTHROMYCIN ON GASTRO-INTESTINAL MOTILITY

The gastro-intestinal side-effects of erythromycin have been known for long, but the mechanisms involved in the induction of nausea, vomiting, cramping, upper abdominal pain and diarrhoea, although rather common, have received little attention. They were vaguely ascribed to changes in intestinal flora, caused by the antibiotic properties of the compound.

In 1984 two groups described, independently and almost simultaneously, that erythromycin stimulated small intestinal contractile activity in the dog. *Zara* et al., (1985) suggested that "part of the macrolide structure might have a direct effect on smooth muscle". *Itoh* et al. (1984) noted that erythromycin mimicked the effect of motilin, and because erythromycin caused a motilin release, they proposed that it acted through motilin.

We explored the possibility that erythromycin acted directly upon a motilin receptor, and showed that erythromycin displaced motilin bound to its receptor, and also mimicked motilin's effect on the rabbit small intestine *in vitro*. We therefore proposed that erythromycin was a motilin agonist (*Peeters* et al., 1989).

Out of the list of effects induced by motilin, acceleration of gastric emptying appears to be the most interesting one from a therapeutic point of view. Our group therefore studied the effect of erythromycin on gastric emptying in patients with diabetic gastroparesis. The results were clear-cut: erythromycin normalized the prolonged gastric emptying times for both liquids and solids in these patients (*Janssens* et al., 1990). As was mentioned in the Introduction, these findings largely stimulated interest in this area of research. The development of more powerful erythromycin derivatives, which lack antibiotic properties, suggests that a new class of powerful prokinetics has been discovered (*Itoh* and *Omura*, 1987).

MACROLIDES, MOTILIDES AND MOTILINOMIMETICS

Erythromycin was discovered in 1953, and is one of a group of substances produced by various species of Streptomyces, microorganisms found in soil. Because these substances all contain a large lactone ring, they are called macrolides. They are divided into 14-membered and 16-membered macrolides depending upon the number of atoms present in the ring structure. Erythromycin, produced by *Streptomyces erythreus*, has a 14-membered ring, while spiramycin, produced by *Streptomyces ambofaciens* is an example of a substance with a 16-membered ring.

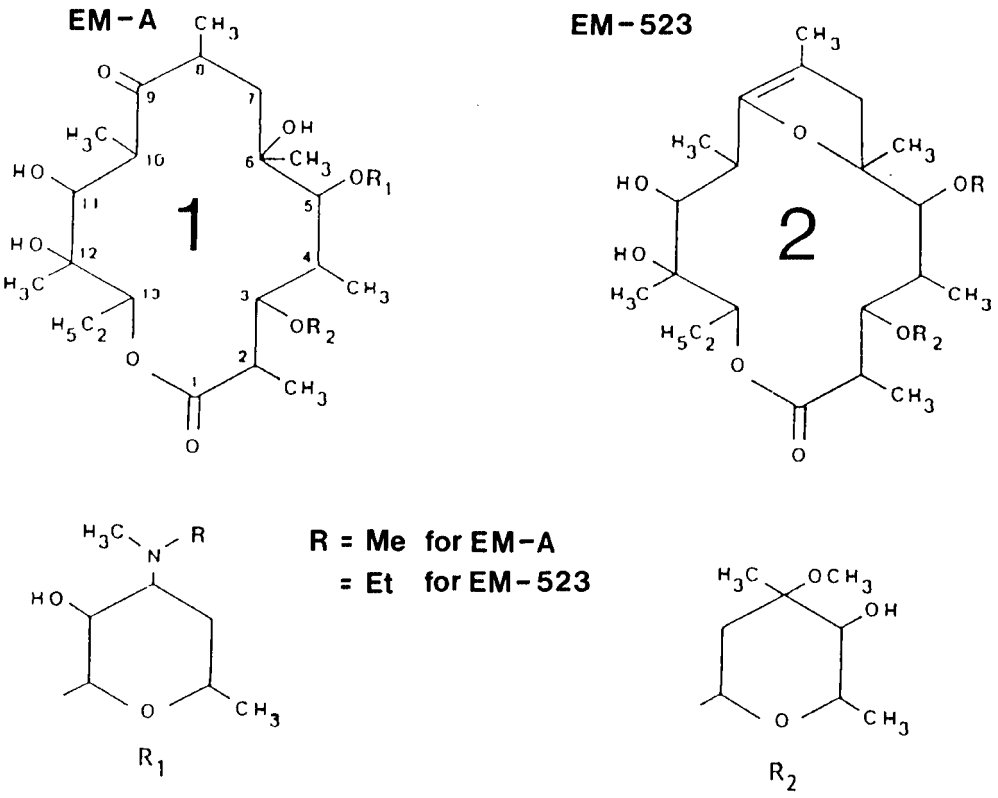

Figure 2: Structure of erythromycin A and of EM-523.

During fermentation, macrolides are produced as a mixture of closely related substances. Thus 3 erythromycin's, designated A, B and C, are produced with A being the major compound. The structure of erythromycin A is shown in Figure 2. As can be seen from this figure, sugars are attached to the ring. In erythromycin A, cladinose is attached in position 3, desosamine in position 5. Erythromycin B differs only from erythromycin A by the absence of the hydroxyl group in position 12, while erythromycin C has in addition a modification of the cladinose sugar.

Attempts have been made to improve the antibacterial properties and the stability of erythromycin (it is destroyed by the acidity of the gastric juice). In 1987 *Omura* et al. (1987) reported that a group of derivatives of erythromycin A lacked antibacterial properties, but had much improved potency in stimulating gastro-intestinal motor activity. *Itoh* and *Omura* (1987) proposed the name motilides for all macrolides with a) a direct contractile effect *in vitro* on rabbit duodenal segments; b) the capacity to induce *in vivo* phase 3 activity in dogs. Although the name motilides may suggest an interaction at the level of the motilin receptor, such an interaction was not being considered and the name mainly referred to the motility effects which did resemble those of motilin.

Our proposal that erythromycin was a motilin agonist (*Peeters* et al., 1989) was met with scepticism, mostly because structurally erythromycin and motilin seem to have little in common. Admittedly the first evidence was incomplete, but it has since then been

Table 1: Motilinomimetics in development.

Compound	Company	Structural features	pKd	Reference
EM-523	Takeda, Japan	14; enol	8.40	Depoortere et al., 1990
EM-574	Takeda, Japan	14; enol	7.94	Satoh et al., 1994
A-81229	Abbott, USA	14; enol	8.14	Nellans et al., 1994
GM-611	Chugai, Japan	14; enol	8.38	Takanashi et al., 1994
LY-274301	Lilly, USA	12; enol	ND	Greenwood et al., 1994
KC-11458	Solvay, Germany	12; enol	ND	Eeckhout et al., 1994
KW-5139	Kyowa, Japan	[Leu13]-po-motilin	9.18	Hanyu et al., 1993
OHM-11638	Ohmeda, USA	motilin fragment analogue	8.94	Macielag et al., 1995

strengthened by other findings. These arguments (for a review see *Peeters*, 1993b) remained indirect, but as mentioned above, motilin antagonists have been discovered recently and they allowed for the unequivocal demonstration that erythromycin is a motilin agonist (*Peeters* et al., 1994b).

Not only erythromycin is a motilin agonist. As expected, many derivatives have the same ability. The substances for which the name motilides was introduced, were mostly derived from 8,9-anhydroerythromycin A 6,9 hemiketal, which is obtained by mild acid treatment of erythromycin A. These conditions favour the addition of the hydroxyl in position 6 to the carbonyl in position 9, followed by dehydratation. The resulting double bond between carbons 8 and 9, and the oxygen bridging carbons 6 and 9, correspond to an enol ether. Introduction of this enol ether configuration in erythromycin's macrolide ring greatly enhances its motility effects. Derivative EM-523 [de(N-methyl)-N-ethyl-8,9anhydroerythromycin A 6,9-hemiketal] synthesized by Dr. Omura from the Kitasato Institute (Tokyo, Japan), is such a compound (Figure 2). We have shown that it is a motilin agonist and that *in vitro* it is about 1000 times more potent than EM-A (*Depoortere* et al., 1990). Other modifications of the ring structure tested so far reduce the potency. Also all macrolides with a 16-numbered ring do *not* interact with the motilin receptor. Structure-activity studies have further revealed that the sugars attached to the macrolide ring are also part of the pharmacophore. Especially modifications of the amino-N of the desosamine sugar may change the potency (*Depoortere* et al., 1989).

At this point we would like to introduce the term motilinomimetics for all compounds able to interact with the motilin receptor, because the term motilides may appear to be too narrow. Indeed, in developing such drugs there is an obvious alternative to the exploration of macrolide derivatives, namely motilin itself. Motilinomimetics therefore encompasses at least two types of drugs: motilin analogues and motilides.

l. Motilin analogues

In order to design a drug starting from motilin itself, a detailed knowledge of the structure-activity relationship is required. Exploration of the affinity for the motilin receptor and of the potency *in vitro* of N-and C-terminal fragments of motilin, and of analogues of the 1-14 fragment in which the residues 1 till 11 where systematically replaced by either alanine or their D-isomer (except residue 8, glycine, which was replaced by D-alanine), led to the conclusion that motilin's pharmacophore resides in the

N-terminal end and involves especially residues 1 (PHE), 4 (ILE) and 7 (TYR) (*Macielag* et al., 1992; *Peeters* et al., 1992).

Motilin has a short half-life, and shorter fragments will most likely also be rapidly metabolized. Very recently the first data on a stabilized motilin analogue, OHM-11638, have been presented (*Macielag* et al., 1995). This compound is an analogue of the (1-14) fragment of porcine motilin stabilized by methylation of the N-terminal amino group, by the introduction of D-Arg in position 12 and of leucine instead of methionine in position 13. Also, the last residue, 14, is lysine instead of glutamine. The affinity for the motilin receptor and the potency *in vitro* of OHM-11638 are comparable to motilin (Table 1). *In vivo* it induces phase 3 activity and accelerates gastric emptying in the conscious dog.

A method has also been developed to synthesize large amounts of porcine motilin by genetic engineering. Only methionine, residue 13, was replaced by leucine because this methionine is easily oxidized and the oxidation product seems to have a reduced biological activity. Encouraging reports on the use of this peptide, KW-5139, have begun to appear in the literature (*Hanyu* et al., 1993). Both OHM-11638 and KW-5139 may prove to be useful as prokinetic agents, but as they require intravenous administration, their application will be limited to acute care patients.

2. Motilides

The enol derivative EM-523 of erythromycin has already been mentioned, and this compound has been developed by Takeda Chemical Company. *Inatomi* et al. (1989) compared the effect of EM-523 on gastric motor activity in conscious dogs, with the effect of cisapride, trimebutine, metoclopramide and motilin. Although the threshold dose for EM-523 was 100 times higher than for motilin, it was 100 times lower than for cisapride which had the lowest threshold dose of the other prokinetics. These data may encourage work with motilin, but they also show that EM-523 is superior to existing prokinetics. Human studies with EM-523 reported up to this date show promising results, but data on a new derivative, EM-574, were recently made available (*Sakai* et al., 1993), suggesting that Takeda Chemical Company also considers other compounds. EM-574 is quite similar to EM-523, the only difference being the replacement of the ethyl group on the desosamine sugar with an isopropyl group.

Abbott Chemical Company has reported extremely high potencies for a compound with code name A-81229 which is closely related to EM-523. It has the enol ring-structure derived from erythromycin B (hydroxyl group missing in C12), the same modification of the desosamine sugar as in EM-523 and an additional modification in the cladinose (hydroxyl at C-4" removed) (*Lartey* et al., 1992). In several models it was shown to have superior prokinetic properties as cisapride (*Nellans* et al., 1994). Another enol-derivative is GM-611, de(N-methyl)-11-deoxy-12-O-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiketal from Chugai Pharmaceutical Company. The compound is acid-stable and has prokinetic properties when administered orally (*Takanashi* et al., 1994).

LY-267108 a ring-contracted derivative (12-membered), which incorporates the enol-structure and in which the two sugars still occupy analogous positions as in Erythromycin, has been developed by Eli Lilly. It increases LES pressure in cats (*Greenwood* et al., 1994). KC-11458, an erythromycin derivative produced by Kali-Chemie Pharma stimulates gallbladder emptying in dogs

(*Eeckhout* et al., 1994). Finally, FK-507, recently introduced as an immunosuppressant, seems to have motilide properties as well (*Ikoma* et al., 1993). Although a macrolide, its structure is quite different and it seems worthwhile to study the interaction of this compound with the motilin receptor.

As yet the motilin receptor has proved to be a useful tool in predicting the prokinetic potential of motilides. Indeed the potency to displace motilin bound to its receptor (pIC_{50}), correlates very well with the potency to induce contractions in segments of rabbit duodenum (pEC_{50}), and this correlates in turn with the potency *in vivo* in other animal models (*Peeters*, 1993a). With such a tool available, one may safely predict that still more potent compounds will be discovered, and that at least one of them will find clinical application.

DEVELOPMENT OF MACROLIDE ANTIBIOTICS

Antibiotic properties are undesirable in a motilinomimetic. Indeed, long term therapy with low doses of a substance with antibiotic properties involves the risk of disturbing the bacterial flora and of developing resistant bacterial strains. Although low doses of erythromycin have a clear prokinetic effect, physicians have been refrained for these reasons from using the drug to stimulate gut motility. It is an advantage of all the motilides that their increased prokinetic potency is accompanied by a strong reduction of their antibiotic properties.

It has been shown that at high doses, such as those used in antibiotic therapy, erythromycin induces abnormal motility patterns (*Sarna* et al., 1991) and it seems likely that these patterns are responsible for at least some of the adverse side-effects of erythromycin. Consequently, antibiotics with reduced affinity for the motilin receptor, may have less gastro-intestinal side effects, at least if they have comparable pharmacokinetical properties. It is interesting to note in this respect that oleandomycin, a 14-membered macrolide with a reduced affinity for the motilin receptor (*Depoortere* et al., 1989), is less potent in inducing motor activity (*Itoh* et al., 1985). Another erythromycin derivative, clarythromycin, is known to have less side effects, and its affinity for the motilin receptor is reduced (*Nellans* et al., 1991). Midecamycin, which like all 16-membered macrolides lacks affinity for the motilin receptor, does not induce gastro-intestinal motor activity and has no or only weak gastro-intestinal side-effects (*Sifrim* et al., 1992). Therefore determining the affinity for the motilin receptor may be useful to predict the likeness that an antibiotic may show gastro-intestinal side-effects.

CONCLUSION AND PERSPECTIVES

The discovery of the effects of erythromycin on gastro-intestinal motility has opened a new area of research and has led to a renewed interest into the peptide motilin. Although it is clear that erythromycin interacts with the smooth muscle motilin receptor *in vitro*, the *in vivo* effects of the motilides are neurally mediated (*Chaussade* et al., 1994). However as this is also the case for motilin, the neural motilin receptor needs characterisation. The discovery of a motilin receptor in the central nervous system (*Depoortere* et al., 1995) may be

very important in this respect.

Another promising development are the motilin antagonists. These compounds will also be helpful in determining the physiological role of motilin. If only recently almost everyone would agree that motilin was mainly related to events occurring in the fasted state, especially the MMC, then the present findings of the wide spectrum of action of motilin agonists, questions this concept.

Therefore, the research reviewed in this paper will not only lead to a new class of prokinetic drugs, and to an understanding of the interaction with the motilin receptor at the molecular level. At the same new insights into the physiological role of motilin, in the regulation of gastro-intestinal motility, and the relation between both will be gained.

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MACROLIDES AS PROKINETIC AGENTS

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INTRODUCTION

It is now firmly established that in the fasting state low doses of erythromycin induce phase 3 of the migrating motor complex, while higher doses only stimulate the stomach and may inhibit the small intestine (*Sarna* et al., 1991; *Tack* et al., 1992).

Derivatives of erythromycin were soon discovered with enhanced motility effects, and decreased antibiotic potency (*Omura* et al., 1987; *Itoh* and *Omura*, 1987). It was proposed to give the name motilides to all macrolides with a) a direct contractile effect *in vitro* on rabbit duodenal segments; b) the capacity to induce, *in vivo*, phase 3 activity in dogs. Besides these two effects, these compounds have many other properties in common with the peptide motilin.

GASTRIC EMPTYING AND GASTROPARESIS (Table 1)

Interest in the therapeutic potential of motilides has been largely stimulated by the original report by *Janssens* et al., (1990) that erythromycin markedly accelerates gastric emptying in patients with diabetic gastroparesis. This report has been confirmed by several groups (*Richards* et al., 1990; *Wadhwa* et al., 1991; *Boiron* et al., 1993) and erythromycin has also been successfully used in other conditions accompanied by gastroparesis such as postvagotomy (*Mozwecz* et al., 1990; *Hill* et al., 1993; *Hocking* et al., 1993), systemic sclerosis (*Dull* et al., 1990), cancer therapy (*Maliakkal* et al., 1991) and anorexia nervosa (*Stacher* et al., 1993).

The motor patterns responsible for the effect of erythromycin on gastric emptying have been studied in volunteers. Erythromycin induces powerful peristaltic contractions in the antrum (*Sarna* et al., 1991), improves antroduodenal coordination and induces phase 3-like patterns superimposed on the fed motility (*Annese* et al., 1992). Erythromycin also seems to affect the proximal stomach (*Edelbroek* et al., 1993). Erythromycin abolishes the lag phase (*Mantides* et al., 1993), and the emptying rate of solids and liquids are the same (*Janssens* et al., 1990). This may be due to the induction of phase 3 patterns, and suggests that erythromycin accelerates gastric emptying at the expense of grinding and sieving. Indeed in dogs erythromycin causes rapid gastric emptying of untriturated solids (*Lin* et al., 1994).

Table 1: Effects of motilides on gastric emptying in gastroparesis

Subjects	Dose[1]	Time[2]	Drug[3]	Effect	Reference
diabetics	200 mg i.v., 20 min	pp	EM	accelerates emptying of liquids and solids	Janssens, 1990
diabetics	250 mg p.o.	meal, -20 min,tid	EM	accelerates emptying of liquids and solids (less pronounced)	Janssens, 1990
diabetics,idiopathic	6 mg/kg i.v.	pp	EM	accelerates gastric emptying	Richards, 1990
diabetics	100 mg i.p.	meal, -30 min,tid	EM	normalizes gastric emptying, suppresses vomiting	Wadhwa, 1991
diabetics	100-500 mg i.v.	meal, +6 hrs	EM	emptying patterns improved	Boiron, 1993
diabetics	250, 1000 mg p.o.	meal, -30 min	EM	accelerates gastric emptying	Desautels, 1995
postvagotomy	250 mg p.o.	pp, tid	EM	normalizes gastric emptying	Mozwecz, 1990
postvagotomy	250 mg p.o.	tid	EM	improved rate of gastric emptying	Hill, 1993
postvagotomy	100 mg i.v.	qid	EM	tachygastria improved	Hocking, 1993
systemic sclerosis	250 mg p.o.	pp, tid	EM	improves gastric emptying	Dull, 1990
cancer therapy	250 mg i.v.	pp	EM	improves gastric emptying, symptoms	Maliakkal, 1991
anorexia nervosa	200 mg i.v.	pp	EM	accelerates gastric emptying	Stacher, 1993

[1]: all doses are given in mg, and were administrated over the time period indicated (i.v.: intravenously; p.o.: orally).
[2]: pp= postprandial; qid= four times daily; tid= three times daily.
[3]: EM= erythromycin

LES PRESSURE AND OESOPHAGEAL BODY MOTILITY; REFLUX DISEASE (Table 2)

Four groups reported that erythromycin increases LES pressure in man (*Chaussade* et al., 1994; *Janssens* et al., 1990; *Dalton* et al., 1990; *Pennathur* et al., 1993; *Pennathur* et al., 1994).

Erythromycin has less effect on the oesophageal body. In two studies with volunteers (*Chaussade* et al., 1994; *Pennathur* et al., 1993), and in three studies with patients with reflux disease (*Champion* et al., 1994; *Pennathur* et al., 1994; *Harrison* et al., 1991), only one found an effect on the amplitude of peristaltic contractions (*Harrison* et al., 1991). The duration of peristaltic contractions showed a tendency to increase in two of these studies (*Chaussade* et al., 1994; *Pennathur* et al., 1994), while a decrease in the propagation velocity was noted by *Champion* et al., (1994) and *Chaussade* et al., (1994).

These effects suggest that erythromycin could find application in the treatment of gastro-oesophageal reflux, but so far two studies did not find a favourable effect on reflux parameters (*Champion* et al., 1994; *Harrison* et al., 1991). However, in a small study with human volunteers the gastro-oesophageal reflux caused by white wine was reversed by infusing 3.5 mg/kg EM-A before ingestion (*Pfeiffer* et al., 1991), and the same group found that in patients with reflux disease erythromycin at 3 mg/kg given i.v. 10 min before lunch, shortened reflux duration (*Pehl* et al., 1994)

Although motilides appear to have properties useful in the treatment of reflux disease, it is obvious that more data are needed to evaluate their potential.

SMALL BOWEL MOTILITY AND SMALL INTESTINAL PSEUDO-OBSTRUCTION

In chronic idiopathic pseudo-obstruction erythromycin is able to induce phase 3 activity (*Miller* et al., 1990; Di *Lorenzo* et al., 1991). Improvement of clinical symptoms has also been reported (*Berger* et al., 1990; *Chami* et al., 1991).

In children with the presumptive diagnosis of chronic intestinal pseudo-obstruction, erythromycin facilitates the postpyloric passage of tubes during duodenal intubation for antroduodenal manometry. The authors assume that propagating antral contractions sweep the tube through the open pyloric channel into the duodenum (*Di Lorenzo* et al., 1990). Accordingly, erythromycin also facilitates the migration of an enteral feeding tube (*Keshavarzian* and *Isaac*, 1993).

Preliminary studies have also looked for an application of motilides in the treatment of postoperative ileus. In cholecystectomy patients erythromycin did not affect the appearance of flatus or bowel movements (*Bonacini* et al., 1993), but with EM-523 a dose-dependent decrease of the time until the start of bowel sounds was observed in a similar patient population (*Hanyu* et al., 1991)

Table 2: Effects of motilides on the human oesophagus

Subjects	Dose[1]	Time[2]	Drug[3]	Effect	Study[4]
volunteers	150 mg i.v., 20 min	fasting	EM	increases LES pressure and duration of peristaltic contractions	Chaussade et al., 1991
volunteers	3.5 mg/kg i.v.	phase 3, +5 min	EM	increases LES pressure	Janssens et al., 1990
volunteers	500 mg p.o.	pp	EM	increases LES pressure	Dalton et al., 1990
patients with reflux	250 mg p.o.	qid	EM	tendency to reduce long reflux episodes	Champion et al., 1991
patients with reflux	250 mg p.o.	tid	EM	no effect on reflux	Harrison et al., 1991
volunteers	3.5 mg/kg i.v.	pp	EM	suppresses reflux caused by white whine	Pfeiffer et al., 1991
volunteers	0.5-2.0 mg i.v., 15 min		EM-523	induces esophagal MMC	Itoh et al., 1991
patients with systemic sclerosis	150 mg i.v., 30 min		EM	increases LES pressure, and fundic contractions	Chaussade et al., 1991
volunteers	500 mg i.v., 30 min		EM	increases LES pressure	Pennathur et al., 1993
patients with reflux	500 mg i.v., 30 min		EM	increases LES pressure, prolongs duration of contractions in esophagus	Pennathur et al., 1994
patients with reflux	3 mg/kg i.v.	lunch, -10 min	EM	shortens reflux duration	Pehl et al., 1994

[1]: all doses are given in mg, and were administrated over the time period indicated (i.v.: intravenously; p.o.: orally).
[2]: pp= postprandial; qid= four times daily; tid= three times daily.
[3]: EM= erythromycin; EM-523= the erythromycin analogue developed by Takeda Chemical Company.
[4]: studies are given in chronological order.

IMPAIRED COLONIC MOTILITY (Table 3)

A few, mostly preliminary studies, have explored the effect of erythromycin on the human colon. The result is basically negative and has been interpreted as due to the absence or low density of motilin receptors.

In other species the situation may be different. In dogs intravenous erythromycin first induced a period of intense colonic motor activity which was followed by a period of inhibition (*Zara* et al., 1985). Erythromycin has also a marked effect on the colon of the rabbit, a species in which there is firm evidence for colonic motilin receptors (*Depoortere* et al., 1991).

Effects on colonic motility may warrant further studies because successful treatment of one patient with reflex ileus, and two with Ogilvie's syndrome has been reported (*Armstrong* et al., 1991; *Bonacini* et al., 1991).

GALLBLADDER

Erythromycin stimulates gallbladder contraction in normal volunteers, in post-cholecystolithotomy patients, and in diabetics with autonomic neuropathy (*Catnach* et al., 1992, 1993; *Fiorucci* et al., 1992). Low doses of EM-523 (0.5-2.0 mg i.v.) have been shown to be effective as well (*Itoh* et al., 1991). Motilides may therefore find application in patients with the risk of gallstone formation, such as those on parenteral nutrition, as a prophylactic to reduce recurrence, or to aid fragment clearance after extracorporeal shock-wave lithotrypsy (*Catnach* et al., 1992).

CONCLUSIONS

It is now well established that erythromycin has profound effects on gastro-intestinal motility. The most convincing data have been obtained in gastroparesis patients when erythromycin was administered intravenously. The efficacy of oral therapy may be less because of the conditions of such patients, and the timing of the administration may be crucial.

Motilides may also be useful in patients with a risk of gallstone formation, and given the limited number of therapeutic agents able to help patients with pseudo-obstruction, the potential of erythromycin in treating this condition should be further explored. The usefulness in gastro-oesophageal reflux should not be discounted too quickly. Trials in gastritis, small intestinal bacterial overgrowth, postoperative ileus and constipation should be considered.

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Table 3: Effects of motilides on the human Colon

Subjects	Dose[1]	Time[2]	Drug[3]	Effect	Reference
volunteers	200 mg i.v., 20 min	fasting	EM	induces contractions in the sigmoid region only	Bradette, 1991
patients, constipation	500 mg i.v., 1 hr	fasting	EM	no effect on colonic motility, nausea	Bassotti, 1991
patients, IBS	500 mg i.v., 30 min	fasting	EM	no effect on colonic motility, nausea	Delvaux, 1994
volunteers	6 mg/kg	fasting	EM	no effect on colonic motility, nausea	Yeaton, 1992
volunteers	500mg p.o., or 1.8mg/kg i.v.	fasting	EM	no effect on sigmoid motility or colonic transit	Jameson, 1992

[1]: all doses are given in mg, and were administrated over the time period indicated (i.v.: intravenously; p.o.: orally).
[2]: pp= postprandial; qid= four times daily; tid= three times daily.
[3]: EM= erythromycin

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OLD HERBORN UNIVERSITY SEMINAR ON GASTRO-INTESTINAL MOTILITY: REVIEW OF THE INTERNAL DISCUSSION

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INTRODUCTION

During the internal discussions which followed the oral presentations which were held on 26 June 1995, a selected group of scientists, enlisted above, exchanged ideas and recent findings in the field of gastro-intestinal motility. One of the main subjects concerned the current status of the methodological set.

The discussion on methodology followed the anatomy of the gastro-intestinal tract (GIT). Several methods concerning a range of both anatomical and functional tests were discussed in an organ-oriented fashion.

METHODOLOGY FOR MEASURING GASTRIC MOTILITY: THE STOMACH

Scintigraphy

Scintigraphy is an accepted method for monitoring of gastric emptying. It can be used for solid material: egg omelette or pancake, sometimes radiolabelled with Gallium or Technetium. (Note: Flower in pancake prevents binding of radiolabel).

Once the label is ingested, two camera's in sandwich position are used to measure the pattern of gastric emptying. The correlation between retardation gastric emptying and pathologic disorder is unfortunately still low. The advantage of the test is its convenience for both patient and doctor. A disadvantage concerns the use of radiolabels.

Paracetamol absorption

In this test the levels of paracetamol which appear in the blood stream after oral intake of a standard dose of paracetamol are measured. Paracetamol absorption is still an experimental method. Paracetamol is nearly completely absorbed in the duodenum. However, it is weakly acidic so that a small part of the dose is already absorbed in the stomach. In a healthy person, paracetamol can appear in the blood stream after 10 minutes. The period of measuring paracetamol levels in the blood after oral intake, should not exceed 90 minutes because after that time period paracetamol is being metabolized by the host.

(Note: A problem is that normal liquid emptying may be confused with pathological solid emptying. One has to make sure that no solid material is in the stomach when this test is performed).

H_2-breath test

In this method, after intake of an omelette labelled with C^{13}, the amount of exhaled H_2 is determined. This method is fast, cheap and the results correlate very well with the results of scintigraphy. Therefore this method is used both in research and in clinical settings.

Ultra sonography

Ultra sonography renders the possibility to image the boundaries of the empty compartment of the antrum pylori. In order to obtain sharp boundaries in the image, only liquid emptying is monitored. However, because this method has not been validated properly, it is only employed in research

Analysis of a "gastric emptying curve"

The half-life time of emptying is the time required to reduce gastric contents with 50%. To make the outcome reliable, the entire gastric emptying curve needs to be recorded. Furthermore, this parameter is not very indicative as the gastric emptying is not constant.

The slope of the curve at a defined point in time after the start of the measurement or at the linear part of the curve could also be used as a measure. This method, however, also depends strongly on the constancy of the gastric emptying rate. This type of evaluation has the advantage that only a portion of the curve needs to be recorded.

Antro-duodenal manometry

This method involves measurement of pressure differences in the antro-duodenal region. Especially the pressure induced by motor activity of the stomach during feeding may be of functional value. (Note: A problem with this method is that the catheter with its pressure-sensitive points may slide out of position into the stomach during motor activity. During feeding the stomach increases in size. The influence of this phenomenon on manometric patterns is not clear yet).

Using scintigraphy it can be seen that only 40% of the contractions is actually recorded by this method. This method only used in research centres to measure the postprandial motor patterns because of their clinical importance.

Electro-gastrography

This method of measuring myogastric activity is using electrodes placed on the outer side of the body. It is a convenient method for the patient. The method yields a cyclic signal (2.7 cycles/min). Interpretation of the data (expressed in mV/cm^2) is difficult

THE SMALL INTESTINE

Scintigraphy of the small intestine

Scintigraphy is probably a useful method. However, the anatomical overlap of the various parts of the small intestines is a problem; this may cause difficulties during image-interpretation. In addition, the method is not validated.

H2-breath test

With oral lactulose, this test gives a good indication of the absorption capabilities of the small intestine. (Note: A problem with this test concerns the fact that measurements are hampered by the fact that lactulose increases the duodenal transit time. The result of the method

need to be corrected for this phenomenon).

This method is only employed for research applications, e.g. for drug response testing and for manometry of the duodenum. It is the most frequently used test method at this moment; both in clinical practice and in research. The method can be applied both in a stationary and in a ambulatory setting; the latter yields a lot of noise in the data set.

The motor patterns obtained with this method allow differentiation between feeding and fasting patterns. An occasional pathological pattern has as yet no clinical meaning.

Statistical analysis of the manometry data of the duodenum is particularly useful. The data can be divided in three phases (phase I, II and III). All phases are probably related to a contraction stage. The use of combinations of one or more of these parameters provides combination parameters which may yield information that is easier to analyse with standard statistical methods. An example of the advantage of this method is the fact that "hidden phase III" activity (which is not visible in the raw data) may be detected.

Some manometrical observations important for pathology

PCC (propagated cluster contractions) are observed in the elderly, during irritable bowel syndrome (IBS) and during diarrhoea. Isolated bursts in upper small intestine are indicative for "bacterial overgrowth". Giant migrating bursts in upper small intestine and "bacterial overgrowth" are likely related to one or even several infecting agents. This concerns an influence of bacteria on motor patterns probably not caused by endotoxins.

THE COLON

Methods concerning the functioning of the colon can be divided in two functionally different groups:

a. Flow-measurements: scintigraphy and marker transit

b. Wall movements: radiology, manometry, barostatic measurement and EMG.

Scintigraphy and marker transit

This method (intake of 20 plastic pellets daily during 6 days, measurement of the location of the pellets with scintigraphy) can only be performed when the anatomy of the gastrointestinal tract is normal.

This method provides a quantitative measure for the colonic transit time. Obstipation can be quantified in this way. (Note: A problem is that method is nearly always employed in sick and/or old people. The obstipation measured may then be a secondary result of immobilization or drugs used for treatment).

During the discussion it was stated that the colonic flora itself has no influence on the flow-rate. Increased production of fatty acids by colonic flora will enhance the flow-rate (diarrhoea) but a possible (inhibitory) static effect could not be explained.

Manometry of the colon

Manometry of the colon is not used very often; no clear method for the analysis of the data is available today. Only rectal data are usable. (Note: A disadvantage is that the colonic lumen is in fact too wide; this gives poor data. The contraction rate may be higher during diverticulae. The colon has to be cleansed before manometry can be used).

Barometry of the colon

A barometer measures volume rather than pressure. The colon does not have to be empty. This technique is mostly employed to measure contractions in the rectal sigmoid area. Standardization of data is not yet possible.

Myoelectrogram

This technique concerns the measurement of the slow electrical waves which traverse the colon. There is as yet no clinical application. Data interpretation is very difficult.

MICROBIAL PRODUCTS AND MOTILITY

The kallikrein-kinnin system

The kallikrein-kinnin system, was originally identified by *Gordon* and colleagues as an utero-contractile and a villi contractile substance. Thus far only the influence of the system on colonic motility of the cat is studied. It appears that the system is present in the entire gastro-intestinal tract. *Bruckner* presented a scheme in which an influence on the a-pigment receptor is proposed.

Short chain fatty acids (SCFA)

Short chain fatty fcids (SCFA) are produced by the anaerobic microflora in the colon. Testing of several concentrations (after oral feeding) of SCFA in germfree and in conventional mice revealed a short-term increase of the motor complexes (MMC) along the intestinal tract. However, all animals showed quick adaptation to the elevated levels of SCFA and returned to normal MMC-values, even when intestinal SCFA-levels remained high. It is thought that SCFA's have a modulating effect on the enteric nervous system (receptors unknown). This may explain the rapid adaptation. Especially butyric acid seems to have an effect. (*Husebye* proposed implantation of butyric producing E. coli). Propionic acid would stimulate pancreas secretion.

Poly-unsaturated fatty acids (PUFA)

Germfree animals have higher levels of poly-unsaturated fatty acids (PUFA) in the enterocyte membrane. The function is unknown

Prostaglandins

No experience on the effect of prostaglandins was available. *Husebye* reported on an experiment in which the influence of NO, eicosanoids and prostaglandins on the immuno-histochemistry of the gut associated lymphoid tissue of germfree mice was tested. This appeared to have no microscopical effects.

Macrolides

Macrolides are antibiotic-like substances produced by the gut-flora. Most studied is motilin, which causes MMC increase in animals. Hypothesis: Motilin binds an muscular receptor or an neuronal receptor.

Tests with motilin antagonist ANQ-11168 are in progress; motilin is administered intra-nasally.

Serotinine

Serotinine has a strong gut motility normalizing effect. Serotonine is stored in the mucus but is metabolized very quickly. Serotonine can also be produced by the gut microflora. Which microorganism is responsible is not known. In humans, it is known that a substance (H-tryptophane, synthesized by the reduction of tryptophane) has a serotonine-like effect. 5H-tryptophane is produced *in vivo* by *Entamoeba histolytica*.

Biliary products

Experiments in rats (surgical: outlet of bile, pharmaceutical: stimulation by oral mannitol) show no influence of bile on the MMC's.

Endotoxins

The effect of endotoxin is difficult to measure because of the major side effects (multiple organ failure). There are no data available which make likely that there is an effect.

Nitric oxide (NO) and vaso-active intestinal peptide (VIP)

NO is produced in the muscular cells of the GIT. Arginin is converted to citrullin by NO-synthetase (the production is receptor mediated). NO is excreted and via a carrier molecule transferred to smooth muscle cells. In the smooth muscle cells, it induces muscle relaxation via the GMP second messenger system.

During the discussion a new hypothesis on the function of NO was proposed: NO is thought to give rise to the production of VIP (vaso-active intestinal peptide). Blocking of the VIP-receptor in nerve terminal blocks of the signal transduction, resulting in the absence of MMC's and thus dilatation of muscle cells (*Mutt* and *Said*, New Engl. J. Med., 1970) Nowadays VIP (28 amino acids long) is regarded a neurotransmitter.

Other vaso-active substances

Other vaso-active substances are:

- Calcitonin (not a CNS-peptide),
- Neurotensin,
- Oxytocin (stimulates muscle contraction),
- Opiates (blocks muscle contractions),
- Somatostatin (effect on GIT unknown),
- GABA (probably not effective),
- Diazepam (blocks stimulator GABA activity) has no effect.

Criteria for gut motility promoting mediators

To name a substance a gut motility promoting mediator (GMPM) it should meet several criteria. The following three criteria were proposed:

1. Substance should be present in the gut lumen,
2. Substance has to provoke a response upon luminal application,
3. substances effect should remain intact upon removal of the gut flora.

PROBLEMS

In addition to the substance being tested, a multitude of other substances may interfere with the test system applied. The gastro-intestinal tract responds probably to any substance as long as its concentration in high enough.

In the colon, nearly no slow contractions do exist (the outflow would be to high). Only giant migrating bursts are known. This poses a methodological problem

The size of the caecum. The influence of the caecum on MMC's in gut-motility experiments on germfree and specified pathogen free (SPF) animals is remarkable (it is thought that the large volume of the caecal contents in these animals has, because of its bulk, a large disturbing influence on the gut motility). Tests in which such animals are used, are strongly biased by this phenomenon.