The activity of the upper gastrointestinal tract is periodic. It concerns the gastrointestinal and gallbladder motility, gastrointestinal blood flow, gastric, intestinal, pancreatic and biliary secretions, rate of nutrient absorption, and many other physiological events. Nowadays, the periodic activity of the gastrointestinal tract is considered as a basic physiological pattern in conscious animals and humans. Unfortunately, there are considerable species- and age-related as well as individual differences, therefore experimental protocols should consider first describing the individual periodic pattern in the examined animals. A lot of confusion may appear with data interpretation if the periodic activity is neglected, in particular when low physiological-like doses of test substances are used. For instance, the effect of CCK or VIP administration on the exocrine pancreas may differ from negligible effect to strong one depending of the phase of pancreatic secretion. The action of secretagogues on the gastrointestinal tract will also be discussed in terms of the ultradian and circadian cycles.

**Key words:** migrating motor complex, pancreatic secretion, gastric secretion, gut regulatory peptides, chronic experiment

**INTRODUCTION**

One who ever studied function of the gastrointestinal (GI) tract in a chronic experiment often might observe a phenomenon presented in *Fig.1*. The variability of the data in the control conditions was so high and did not allow to get any statistical significance between control and experimented factors. This could be observed in experiments when stimulating and inhibiting factors were
studied, in particular when the examined factors were given in doses close to a physiological range. Increasing number of observations or number of animals (usually, a number of 6 animals is required in chronic gastrointestinal studies) did not reduce a high standard deviation in the control. This problem may happen while studying many of gastrointestinal functions like, GI motility and digesta flow, gastric and pancreatic secretions, nutrient absorption, GI blood flow as well as plasma concentrations of gut regulatory peptides like, pancreatic polypeptide (PP) and motilin. The reason for this high variability in control conditions is related mostly to the periodic activity of the GI tract. This pattern when neglected during designing the study protocol may cause considerable problems with data interpretation. Another confusing situation is shown in Fig. 2, when the effect of examined factors produces variability which does not allow to get the statistical significance, even when the statistical tests considering repeated measurements are employed for analysis. Again, there is a problem with data interpretation: is the treatment of no physiological relevance or maybe some existing physiological phenomena that interfere the data have been overlooked? This paper will briefly discuss some of these reefs which exist in chronic animal studies. The navigation
map presented here may be of help in understanding the importance of periodic activity of the GI tract and designing study protocols and interpreting the data accordingly.

**Brief history of studies on the periodic activity of the gastrointestinal tract**

Bush (1) when published in 1858 his observations on fasting contractions in a woman with an abdominal fistula was probably the first to introduce the concept of periodic activity in the gastrointestinal tract. The history was continued by Boldyreff (2) who in 1911 published his studies on conscious dogs with pancreatic, gastric and Thiry-Vella fistulae. Boldyreff clearly demonstrated that in the preprandial or fasting condition the exocrine pancreas secretes juice in a cyclic pattern which is synchronised with other changes in gastrointestinal activity - gastric and intestinal luminal pressures and gastric and bile secretions. The secretion of pancreatic juice showed clear peaks and nadirs which coincided with the peaks and nadirs of bile secretion, respectively, as well as with the periods of alternating gastric contraction and rest. This pattern repeated itself periodically and the duration of the work-and-rest cycle in dogs was between 90 and 110 min (Fig. 3). Feeding, and to a certain degree the sight and smell of the food, disrupted the periodic pattern and enhanced the secretions. Boldyreff’s observations of periodic changes in secretion were soon confirmed by Babkin and Ishikawa (3)

![Fig. 3. Boldyreff’s original drawing demonstrating the periodic activity of the exocrine pancreas (I), bile secretion (II) and gastric pressure (III) in a fasted conscious dog. Arabian numbers in the bottom indicate hours of observation. Each rectangle represents horizontally a 15 min sampling period, and vertically reading of juice flow (0.2 cm³), bile flow (0.1 cm³) and gastric contractions recorded by water manometry (2 cm H₂O). (Reprinted from ref. 2).](image)
who also were the first to demonstrate that atropine abolishes pancreatic and intestinal periodic activities and keeps pancreatic secretion at nadir.

The periodic activity pattern was forgotten (with isolated exceptions: 4) for many years. Further impact to the understanding of GI motility was given by Szurszewski in 1969 (5) who have shown in conscious dogs with implanted electrodes in the stomach and small intestine that in the fasting conditions the electromyography signal migrates along the small intestine in a strictly organized pattern called the migrating myoelectric/motor complex (MMC). Code and Marlett (6) gave detailed description of the four-phased MMC pattern in conscious dogs: phase I of electrical quiescence with no spiking activity (only slow waves are recorded), phase II of irregular spiking activity, phase III of regular intense spiking activity, and transient phase IV when the spiking activity is reduced and replaced with quiescence (phase I) again. One sequence of four phases took about 120 min, and was disrupted by feeding for more than 12 hours. Renaissance of the studies on the periodic GI activity begun following the articles by DiMagno et al. (7) and Vantrappen et al. (8) who published in 1979 their observations in adult dogs and men confirming findings by Boldyreff. In the following years, several research groups worked on understanding of the nature of this basic physiological phenomenon. Extensive reviews concerning gastrointestinal periodic activity have been published by Wingate (9), Sarna (10), Davenport (11), Szurszewski (12), and Zabielski and Naruse (13).

**Periodic activity in the gastrointestinal tract**

The periodic activity of the GI tract seem to exist in all mammals, including carnivorous, omnivorous and herbivorous species though there are some differences between the species. This activity was studied also in humans (8), and also in birds and probably is present in other vertebrates though there were far less studies done.

In dogs (7), pigs (14, 15), rats (16), cattle (17, 18), and sheep (19) the stomach, small intestine, pancreas and gall bladder manifest periodic oscillations of their function. Among them, the MMC pattern of the small intestine is the most clear-cut and frequently studied. The MMC is also used to help in distinguishing the other periodic GI functions, involving secretion, absorption and blood flow. In humans and dogs, one small intestinal MMC cycle takes on the average 120 min, in the pig - 80 min, in cattle - 60 min, and in rats - 15 min. In young animals it is of shorter duration than in adults, namely in human and cattle neonates it takes 20 to 30 min (13). The duration of the cycle influences the duration of data recording in a chronic study, minimum one to two complete control cycles should be recorded before each treatment.

The electromyography pattern of the small intestine well reflects the intestinal motor function and the flow of intestinal content (20, 21, 22). In carnivorous animals the MMC is considered a “house keeper” that cleans the gut out of the
digesta remnants, bacteria and desquamated epithelia and prepares it for the next prandial pattern (5). In the herbivorous and omnivorous animals, however, the MMC is an important mean of digesta transport. In these animals, during phase I of MMC in the examined segment of the intestine there are no contractions and no flow of the intestinal content. During phase II, the backward and forward contractions increase in amplitude to mix the intestinal content, and the number of propulsive contractions increases during late phase II. During phase III the intestinal contractions are maximal and propulsive thereby allowing transport of all the content along the small intestine caudally. Besides dogs, in the other species the phase IV is transient (about 1% of the MMC duration) and usually skipped.

Physiological oscillations in the secretion of digestive juices, gastric, intestinal, pancreatic and bile coincide with the small intestinal, in particular duodenal MMC. The secretions are low during phase I of the MMC (often called as a nadir secretion or asecretory phase) and increase even several-fold during phase II of the MMC (a peak secretion or secretory phase) in regard to the juice volume and its composition (Fig. 4). Enhanced mixing contractions during phase II of MMC help in mixing the digestive juices with the luminal content thereby improving digestion of food. For instance, in calves positive

![Fig. 4. A representative recording of a three-phased MMC in the duodenum and a secretion of pancreatic juice in a 6 week-old calf. E1, E2, and E3 on-line frequency analysis of myoelectrical activity from the proximal and mid-duodenum (SPM, spikes per minute). The interdigestive pancreatic secretion recorded by a photoelectric drop counter fluctuates in phase with the duodenal MMC. The lowest secretion is observed during phase I of MMC and the highest during late phase II of MMC. Feeding with 3 l milk replacer (Milk) disturbed the MMC and pancreatic periodic pattern, and transiently increased the pancreatic secretion. The pancreatic periodic secretion reappeared together with the duodenal MMC (adapted from ref. 51).](image-url)
correlations were found between the intensity of spikes in the duodenum and secretion of protein in pancreatic juice (Leśniewska and Zabielski - unpublished data). In dogs, the secretion of pepsin is strongly associated with gastric and duodenal motility but independent of changes in the secretion of gastric acid (23). Konturek and Thor (24) reported that the duodenal alkaline secretion fluctuates cyclically in phase with canine duodenal motility, being maximal during phase II of the duodenal MMC. The peak in volume of pancreas juice in dogs, is only 7% of the maximal response to exogenous secretin but the protein peak is no less than 60% of the maximal response to cholecystokinin (23). In calves, relevant quantitative and qualitative differences in the composition of pancreatic juice enzyme proteins between the nadir and peak secretion were found (17, 25). The resolution of SDS-PAGE electrophoresis of pancreatic juice proteins collected strictly in phase of duodenal MMC led to a conclusion that there are much less animal-to-animal variations than the phase-to-phase variations in the protein composition of pancreatic juice. This corroborates with previous studies by Padfield and Case (26) who showed that stimulation with pharmacological doses of secretin and cholecystokinin (thus apparently removing physiological cycles) results in a pattern of pancreatic juice enzymes in guinea pigs which is more uniform and easier to compare between the animals. The secretion of bile into the duodenum is periodic, the peak of bile secretion in fasting dogs precedes the duodenal motility and pancreatic secretion peaks by 30 to 40 min, and about 80% of interdigestive bile is secreted during the secretion peak (27).

The endocrine activity of the small intestine is periodic in regard to some gut regulatory peptides, and is manifested by fluctuations in the release of PP, motilin, somatostatin (28, 29, 30, 31, 32) and secretin (33) into the circulating blood. The perfection in the timing of PP and motilin peaks during MMC cycles raised the question of their role in the origin and regulation of intestinal and pancreatic periodic activity. However, in studies on dogs with an autotransplanted pancreas, the coexistence of the interdigestive MMC and motilin cycles but not PP cycles was found (34) suggesting that fluctuation of gut regulatory peptides is rather a consequence not the cause. Changes in the peripheral blood plasma concentrations of PP and motilin associated with the MMC pattern were also observed in pigs (35), sheep (36), and calves (37). The relevance of motilin in regulation of MMC in sheep has been questioned by Plaza et al. (36).

An association between gastrointestinal motility and blood flow has been shown in conscious dogs, both in the stomach and in the small intestinal circulation. During the quiescent period in the stomach of the fasting dog, the blood flow in the left gastric artery was stable (38). During the contraction period each peristaltic contraction was coupled with a rapid fall and subsequent rise in blood flow but there was a sustained elevation in blood flow coinciding with the contraction phase of the stomach. Small intestinal blood flow measured by an electromagnetic flow probe implanted on the mesenteric artery showed
clear MMC-related variations in fasted dogs (39). These relations (high blood flow during phase II and low blood flow during phase I of the jejunal MMC), however, disappeared when the intestinal content was drained out. Feeding induced a rapid but transient increase in gastric blood flow, and periodic increases in blood flow did not appear until the next gastroduodenal MMC started (40). The pancreatic blood flow was also found to change in phase with MMC in conscious dogs (41).

Depending on the nature of food and the level of caloric load per unit of time, the periodic pattern is differently disturbed or masked. However, in omnivorous and herbivorous animals the periodic pattern is little affected by food in comparison with carnivores in which it can be seen only when the upper GI tract is empty. In sheep, Fioramonti et al. (42) have shown that the absorption of sugars is not constant and fluctuates in phase with the MMC in the jejunum.

**Animal health, well-being and the gastrointestinal periodic activity**

Code and Marlett reported low variability in the duration of the MMC cycles and high day-to-day punctuality in the appearance of the MMC phases in their “healthy, happy, conscious, well adjusted dogs” (6). Yao et al. (43) have observed similar regularity, in particular at night, in the appearance of duodenal MMC phases in pigs fed twice a day in fixed hours. In the later study the mioelectric activity was recorded using telemetry system, thus minimally affecting animal behaviour. The periodic GI activity reacts to external stimuli, for instance food expectation as well as fear and stress influence the regularity, duration and amplitude of the cycles. Experimented animals, e.g., calves and pigs, randomly removed from their own cages and placed for recordings in a different room secrete less pancreatic juice and do not manifest a regular periodic pattern. Usually this problem disappears after a few days of training, though I have experienced the occasional piglets which did not show the pancreatic cycles at all. These animals, though clinically healthy, did not become accustomed to experimental conditions (cage, connection to the extension tubing and cables, etc) regardless of training, and noisily manifested their discomfort immediately after the recording session was started.

Diarrhoea induced by overfeeding disrupted pancreatic cycles in suckling calves (44). Depending on the severity of the diarrhoea, the pancreatic cycles were either irregular and poorly synchronised with the duodenal MMC, or indistinguishable. The duodenal MMC was also affected by the diarrhoea, seen as a marked shortening of the duration of phase II of MMC. These cycle disturbances in pancreatic secretion and intestinal MMC were registered several hours before any clinical symptoms appear. In similar manner, exogenous enterotoxin given into the duodenum disrupted the synchronicity between the pancreatic secretion and duodenal MMC in calves (44). The periodic activity of the GI tract seems therefore to be a sensitive indicator of animal health and well-
being. Clinical studies demonstrated lack of synchronicity between pancreatic juice cycles, duodenal motility and plasma PP in chronic pancreatitis human patients (45).

Problems with recording of periodic activity

The phenomenon of periodic activity produces a number of problems when it is neglected as well as when it is regarded. In the past, often the physiological cycles seen during recording were considered an accidental problem, for instance peaks of pancreatic juice flow in the collecting system, and the results likely to be discarded, a known, circulating anecdote but hardly to be found in scientific publications. Any alteration in the control (figure 1) is unwanted because the responses to examined stimuli are then less clear and provide problems with statistical evaluation. However, the periodic activity is a difficult pattern to demonstrate, e.g., the secretory cycles (gastric, pancreatic and biliary) should be confirmed by parallel recording of motor or myoelectric activity of the stomach or upper small intestine. This complicates the animal model - extra electrodes or strain gauges and recording systems are necessary. PP and motilin analyses would be of help to discriminate the phases of the GI periodic activity but the RIA data are usually available long after the experiment is completed. The frequency and amplitude of physiological fluctuations may differ from animal to animal (17, 27, 46), thus it is important to know the individual characteristic in one-two separate control experiments that cover several cycles before any treatment begin. It is also necessary to mention on the differences related to the animal model applied, for instance due to method of cannulation. Lee et al. (32) demonstrated that the mean length of pancreatic secretory and duodenal motility cycles in dogs with Herrera cannulas was significantly shorter than that of dogs with Thomas cannulas. The motility cycles in the latter were of normal duration, similar to those of non-cannulated dogs. Moreover, the secretion of pancreatic juice (peak secretion and secretion per cycle) was higher in dogs with Herrera cannulas than in dogs fitted with Thomas cannulas, while the opposite was true for bicarbonate output. Time limitations for the duration of recording during one day could also be important - the dog, even if well trained, could only be restrained for a relatively short period of time. To register the periodic activity in his dogs, Boldyreff conducted recording for 6 to 8 hours without interruption (2). In the studies by Magee and Naruse, on each experimental day control recordings were usually made on the dog for 2 to 4 hours before any treatment was begun (27, 46). To describe a wave form, no less than 7 consecutive samplings per cycle should be made; thus in experiments on dogs manifesting a periodic activity of about 2 hours it is necessary to collect the digestive juices and withdrawn the blood at 10-min intervals. Reducing number of samplings would affect the amplitude of cycles. Home-made tools, like drop counters and flow meters are helpful for recording secretion on-line, increase sensitivity of measurement, and provide with new
information on the kinetic of flow. For instance, a flow-meter recording of the circadian pattern of pancreatic juice secretion in preruminant calves revealed the presence of “a night peak” of pancreatic secretion that appeared in most of examined preruminant calves between the midnight and 3 a.m. (Guilloteau and Zabielski - unpublished data). From the above, it seems that successful recording of periodic activity depends more on a great deal of labour than on sophisticated laboratory equipment.

From a literature review from last one to two decades, it seems that even currently the GI periodic activity is often neglected thereby chronic studies are performed strictly according to the time of the day. For instance, the animals or human volunteers are restrained from eating overnight, and in the materials and methods section we read that the time schedule of the experiment is rigorously kept: an experiment starts, let say, at 8 a.m. with the control collection which is short (lasting usually for one hour) that is followed by treatment e.g., an infusion of examined substance. Such standard protocol apparently does not take into account the periodic activity of the GI tract meaning that on each experimental day the test substance may be administered at an unspecified and different phase point of the GI periodic activity, although at the strict time according to the researcher’s watch. Is this methodologically correct? It could be, if the examined subjects are well synchronised in phase or if the response, inhibition or stimulation, is strong and obviously exceeds over the control, e.g. when pharmacological doses of secretagogues are applied. However, it is doubtful when lower doses, within a physiological range are used and the response is subtle like in Fig. 1. The changes may be then easily overlooked if the periodic activity is not monitored. In an étude study in calves fitted with pancreatic catheters and duodenal electrodes we checked how much the time point of secretagogue administration can influence the pancreatic response. Instead of a continuous infusion which might elicit secondary mechanisms affecting the periodic pattern, we used brief 5 min iv infusions of CCK-8 and VIP (100 pmol/kg of body weight). On each experimental day either VIP or CCK-8 was given during well-identified phase III of the duodenal MMC. After recording 2 to 3 MMC cycles the same dose was repeated during late phase I of MMC (47). The results showed that both VIP and CCK-8 evoked a surprisingly different pancreatic responses depending on the moment of administration (Fig. 5). The response to the infusion during phase III of MMC was weaker than that during late phase I. This observation helps to understand the situation shown in Fig. 2. There is also another supporting evidence coming from human studies with “sham” and real meal stimulation showing how much the timing of treatment is important. The existence of a cephalic phase of gastropancreatic secretion following “sham” feeding depends on the moment at which the treatment is performed (48). Subjects “sham” fed at the beginning of phase II of the small intestinal MMC showed a distinct elevation in pancreatic enzyme output lasting for 30 min. When the stimulation was made at other phase of the cycle the results were unclear. Stimulation with a meal during late phase III
resulted in a 10-20 min delay in the pancreatic response, whereas the same meal eaten during late phase I or early phase II resulted in an immediate stimulation (49). Considering that the responsiveness of the GI organs to exogenous stimuli vary in phase, the phases of physiological cycle seem to be a more appropriate timekeeper for the experimental protocol than the arbitrarily set hours and minutes. The story is further complicated, however, by the circadian cycles in the gastrointestinal tract (43, 48, 50).

Finally, the question arises how to compare the results on the periodic activity obtained from different animals. Usually, the descriptions in the published papers are extremely brief. In most cases, like in the pioneer article by Boldyreff (2) a representative trace from single experiment is shown (Fig. 3 and 4). To analyse the periodic activity of digestive juices secretion, the samples are usually renumbered by choosing either peaks or nadirs as origins, and the means and their standard errors are calculated for each of the newly numbered samples (27, 30, 46). A cycle amplitude is analysed following extracting the peak and nadir values (32). Plasma concentrations of gut regulatory peptides, to obtain mean values, are usually grouped according to the phase of the duodenal MMC (32, 34). The cycle length is determined by calculating the interval between two consecutive peaks. Due to the differences in the cycle duration, problems with the presentation of amplitude appear when mean data are shown (Fig. 6). These types of presentations which are used, e.g., following normalization of the cycles by recalculating them to the mean duration or after elimination of the data from the animals that are far from the mean, are criticized but there is no better solution so far.

In conclusion, the periodic activity nowadays is not terra incognita in the gastrointestinal tract physiology but a quite well recognized pattern, however, it still gives a chance for fascinating scientific adventures. In particular in regard to recent progress in chronopharmacology and chronotherapy.
Acknowledgments

Supported by grant from the Ministry of Science nr PBZ-KBN-093/P06/2003.

REFERENCES


Fig. 6. The result of calculating the mean of two cyclic patterns that differ in cycle duration. The amplitude of the averaged second and third cycle is incorrectly reduced.


**Received:** 27 March 2004  
**Accepted:** 28 May 2004

Authors address: Prof. Romuald Zabielski, PhD, Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, 02-766 Warsaw, Poland  
E-mail: rzabielski@plusnet.pl