Modulation of the Migrating Myoelectric Complexes by Cholecystokinin and Gastrin in the Gastrointestinal Tract of Chickens

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ABSTRACT Several mammalian avian species, including the chicken, show migrating myoelectric complexes (MMC) both in unfed and fed states. In these species, postprandial hormones seem to modulate but not to disrupt the MMC. To gain more information in this modulatory role, we evaluated the role of cholecystokinin (CCK) vs gastrin on the regulation of intestinal motility in chickens. Birds were implanted with eight electrodes for electromyography in the stomach, duodenum, jejunum, and ileum. In feed-deprived animals, CCK infusion (10^{-12} mol/kg per min × 3 h) did not disrupt the MMC but induced changes in the MMC pattern similar to those induced by a meal. Infusion of CCK in fed animals induced dose-dependent effects: CCK infused at 10^{-11} and 3×10^{-11} mol/kg per min × 2 h, progressively elongated the MMC and slowed the speed of propagation of Phase 3. Furthermore, CCK infused at 10^{-10} mol/kg per min × 2 h disrupted the MMC but a Phase 3 appeared just after the end of the infusion. By contrast, chicken gastrin (10^{-10} mol/kg per min × 2 h) did not modify the MMC pattern. In conclusion, CCK influence on the intestinal motility of chickens ranges from the modulation of the MMC to total disruption, depending on the dose. Moreover, this study suggests that the mechanism of action of CCK could be similar in both mammalian and avian small intestines. (*Key words*: gastrointestinal motility, gastrin-cholecystokinin peptides, migrating myoelectric complex, chicken, feed deprivation)

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INTRODUCTION

In most mammals, (e.g., dogs, rats, and humans), migrating myoelectric complexes (MMC) have been described as an "all or nothing" phenomenon that disappears during the postprandial period (Ruckebush and Fioramonti, 1975; Konturek *et al.*, 1987). These species have only sporadic meals, the MMC pattern disappears for several hours during the postprandial state, and a fed-motility pattern appears during this period (Weisbrodt, 1987). However the MMC pattern is present both in fed and unfed states in those species, such as ruminants or pigs, that have frequent and small meals (Gregory *et al.*, 1986; Rayner and Wenham, 1986; Ruckebusch, 1989). Consequently, in these species, the MMC has been described as an ultradian rhythm (Ruckebusch and Bueno, 1977).

Postprandial hormones, such as cholecystokinin (CCK) or gastrin, both exogenously and endogenously, disrupt the MMC pattern, turning it into a fed-like motor activity (Mukhopadhyay *et al.*, 1977; Konturek *et al.*, 1987). Moreover, the exogenous administration of CCK in dogs does not modify the MMC until a threshold dose is reached, which totally disrupts the MMC (Wingate *et al.*, 1978a,b). Similar actions on the MMC pattern have also been described for gastrin (Weidsbrodt *et al.*, 1974; Marik *et al.*, 1975; Wingate *et al.*, 1978a; Thor *et al.*, 1988). The fact that the postprandial state disrupts the MMC pat-

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tern in some species but does not in others suggests that the regulatory mechanisms of gastrointestinal motility might be different between species.

Less is known about the biology of the gastrin-CCK family and the control of gastrointestinal motility in avian species. Chicken gastrin (cG) has recently been isolated from chicken antrum as a molecule showing a CCK-like structure but a gastrin-like biological activity (Dimaline *et al.*, 1986; Dimaline and Lee, 1990). Although CCK has not been isolated from the chicken gut, there is good chromatographical (Dockray, 1979), immunological (Martínez *et al.*, 1993b), and functional (Martínez *et al.*, 1993a) evidence supporting its presence in the chicken intestine. A previous study also demonstrated different actions for CCK and cG in the control of gastroduodenal motility in chickens, supporting the presence of a functional form of CCK in the avian gut (Martínez *et al.*, 1993a).

Migrating myoelectric complexes have been described in avian species as intestinal motor patterns present both in the fed and the feed-deprived states. As in mammals, they are characterized by a sequence of three phases: Phase 1, a basic pattern of quiescence; Phase 2, irregular spiking activity; and Phase 3, intense regular activity, which migrates along the intestine. In chickens, the MMC cycle length is very constant (about 60 min) and the propagation speed of Phase 3 is about .6 cm/min (Clench *et al.*, 1989; Mueller *et al.*, 1990; Jiménez *et al.*, 1994). Major differences between postprandial and unfed MMC are an elongation of Phase 2, a jejunal origin of the MMC, and a reduction of the speed of propagation of Phase 3 in fed state (Jiménez *et al.*, 1994).

In order to extend our knowledge of the control of gastrointestinal motility from a comparative point of view, the objectives of this study were to study the actions of CCK and cG on intestinal electrical activity in chickens.

MATERIALS AND METHODS

Animal Preparation

Ten male White Leghorn chickens (*Gallus gallus*) 7 to 10 wk of age that consumed a standard diet *ad libitum* were used in this study. Birds were surgically prepared for electromyographic studies, under general anesthesia with sodium pentobarbital (20 mg/kg i.v.) and preanesthetic medication with atropine (.1 mg/kg i.m.) and diazepam (15 mg/kg i.m.). Eight groups of electrodes[2] (120 μm diameter; Ni/Cr, 80:20), were implanted in each animal, as previously described (Jiménez *et al.*, 1992), in the proventriculus, the cranial thin and caudal thick muscles of the gizzard, the proximal (7 cm from the pylorus) and distal duodenum (19 cm from the pylorus), the jejunum (60 cm from the pylorus), the ileum (80 cm from the pylorus), and one about 11 cm aborad to the ileo-ceco-colic junction (104 cm from the pylorus). Electrode location was determined at necropsy.

Electromyographic Recordings

Experiments were conducted 3 wk after surgery, at which time the birds were totally recovered and showed a clear MMC pattern all along the small intestine. Electrodes were connected to high gain amplifiers.[3] Low (50 Hz, –6 dB) and high (.03 s, –6 dB) pass filters were used in order to select spike-bursts. Electromyographic (EMG) signals were registered on a polygraph and simultaneously digitized using an Analog/Digital converter (8 bits resolution) with a sampling frequency of 50 Hz. Digitized data were stored on hard disk for later analysis (Jiménez *et al.*, 1992).

Substances

Substances used in this study were: synthetic CCK-octapeptide[4] (sulfated form) (CCK8) and cG.[5] The CCK8 was dissolved in 1% aqueous sodium bicarbonate and cG in .9% NaCl aqueous solution to 10^{-5} mol. Further dilutions of these peptides were made in saline solution.

[2]Microfil Industries, Renens, Lausanne, Switzerland.

[3]Lectromed MT8P, Lectromed Limited, Jersey Channel Islands, UK.

[4]Peptide Institute Inc., 4-1-2 Ina, Minoh-Shi, Osaka 562, Japan.

[5]Peninsula Laboratories, Inc., Belmont, CA 94002.

Experimental Procedures

The CCK8 and cG were perfused i.v. (2 h infusion, 2 mL final volume) by mean of a catheter placed in the wing vein. The CCK8 was infused at doses of 10^{-11} (n = 6), 3×10^{-11} (n = 5), and 10^{-10} (n = 5) mol/kg per min and cG was infused at a dose of 10^{-10} mol/kg per min (n = 5). Infusions were made with a syringe infusion pump located outside the cage. Chickens were allowed to move freely throughout the experiment. At least one complete MMC was recorded before the beginning of the infusion period. The infusion was initiated 10 min after a clear Phase 3 recorded at the electrode located at the jejunum. After finishing the infusion, the recording lasted for 2 h more. A control recording, lasting at least 4 h, was taken from all animals. All the treatments were performed in a randomized manner.

Six chickens were used to study the effects of CCK8 on gastrointestinal motility in the unfed state. These animals were not fed for 18 h prior to the experiments. After 3 h of control recording, and 10 min after a clear Phase 3 was recorded at the electrode located at the jejunum, the infusion of CCK8 (10^{-12} mol/kg per min) was started. The CCK8 infusion lasted for 3 h.

Data Processing and Statistical Analysis

Electromyographical recordings were integrated at time intervals of 1 min. From the integrated recording, the following characteristics of the MMC were evaluated: 1) total duration; 2) duration of Phases 1, 2, and 3; 3) speed of propagation of the Phase 3 (between consecutive electrodes and all along the intestine); and 4) anatomical site of origin of the Phases 3. Data were statistically compared by means of ANOVA followed by an appropriate post-hoc test (Bonferroni or Dunnett). The site of origin of the MMC was compared using a chi-square test. Data were considered significantly different when $P < .05$.

RESULTS

Myoelectrical Activity in Fed and Unfed States

Both in fed and unfed animals, electromyographical recordings showed a pattern of MMC present all along the small intestine (Figure 1). Gastroduodenal electri-

TABLE 1. Migrating myoelectric complex (MMC) duration (mean ± SEM) in fed and unfed states and during the infusion of cholecystokinin

Organ	State	Unfed state (6)[1]	Unfed + CCK8[2] (6)	Fed state (10)
		(min)		
Jejunum	Total duration	75.2 ± 7.6	69.6 ± 7.4	59.6 ± 3.8
	Phase 1	46.3 ± 7.2[a]	38.1 ± 6.7[ab]	21.4 ± 1.5[c]
	Phase 2	22.1 ± 4.2	25.4 ± 3.1	33.9 ± 3.5
	Phase 3	6.8 ± .3[a] (15)[3]	6.2 ± .4[a] (13)	4.4 ± .2[b] (29)
Proximal ileum	Total duration	89.3 ± 6.5[a]	80.3 ± 8.6[a]	56.6 ± 3.3[b]
	Phase 1	59.1 ± 6.0[a]	38.4 ± 6.1[b]	22.2 ± 1.7[c]
	Phase 2	24.3 ± 3.0	36.2 ± 4.5	30.3 ± 2.4
	Phase 3	6.0 ± .5[a] (12)[3]	5.6 ± .6[ab] (11)	4.1 ± .3[b] (26)
Distal ileum	Total duration	85.0 ± 9.8	65.1 ± 7.6	63.1 ± 4.1
	Phase 1	56.3 ± 11.1[a]	32.4 ± 6.4[ab]	20.3 ± 1.3[b]
	Phase 2	23.1 ± 2.8[b]	29.6 ± 4.2[ab]	38.8 ± 3.5[a]
	Phase 3	5.6 ± .5[a] (7)[3]	6.0 ± .5[a] (9)	4.0 ± .3[b] (9)

[a-c]Means in row with no common superscript differ significantly ($P < .05$) according to Bonferroni test.

[1]Number of chickens.

[2]Dose of CCK8: 10^{-12} mol/kg per min × 3 h.

[3]Number of MMC recorded.

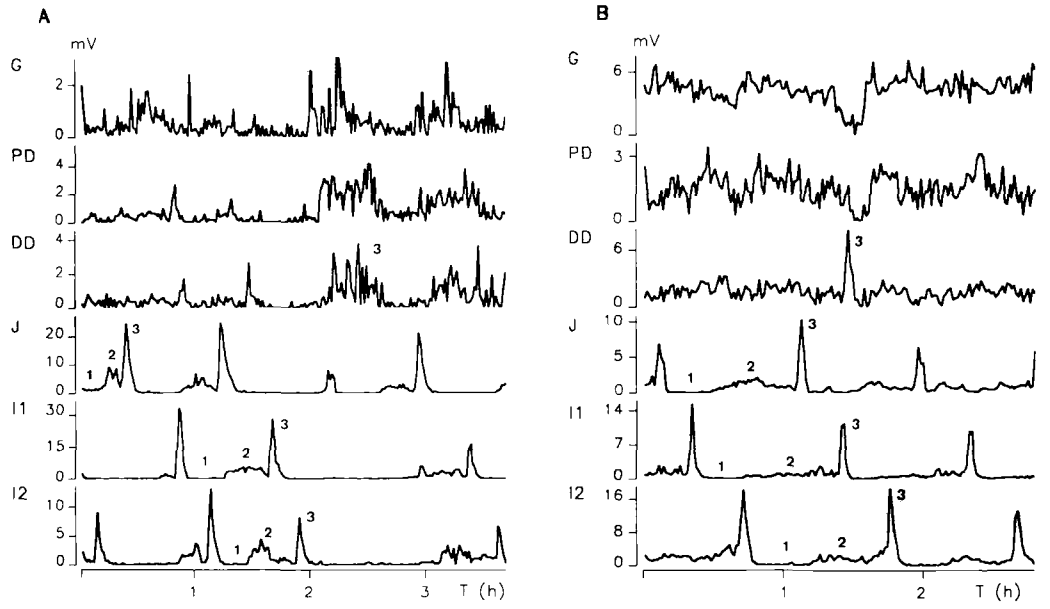

FIGURE 1. Integrated recordings (time set interval 1 min) of the electrical activity of the gastrointestinal tract of a unfed (A) and a fed (B) chicken showing the intestinal migrating myoelectric complex pattern. G, craniodorsal thin gastric muscle of gizzard; PD and DD, proximal and distal duodenum, respectively; J, jejunum, I1 and I2, *proximal and distal ileum, respectively.*

cal activity was highly coordinated, each gastric contraction was followed by one or more duodenal spikes propagated from the proximal to the distal duodenum. These basic motility patterns exhibited some differences between fed and unfed states.

Migrating myoelectric complexes recorded in unfed chickens were slightly elongated compared with the postprandial state (Table 1). Significant differences were found in the relative duration of the phases of the MMC. Phases 1 and 3 were of longer duration in unfed animals, whereas Phase 2 was slightly shorter (Table 1). In all cases the speed of propagation of Phase 3 decreased along the small intestine and was always faster in unfed than in fed animals (Figure 2).

Both in unfed and fed states, MMC recorded in the small intestine were preceded by an increase of electrical activity in the duodenum, a reduction of the frequency of gastric spikes, and a suppression of gastroduodenal coordination. We previously proposed (Jiménez *et al.*, 1994) that these duodenal hyperactivities can be

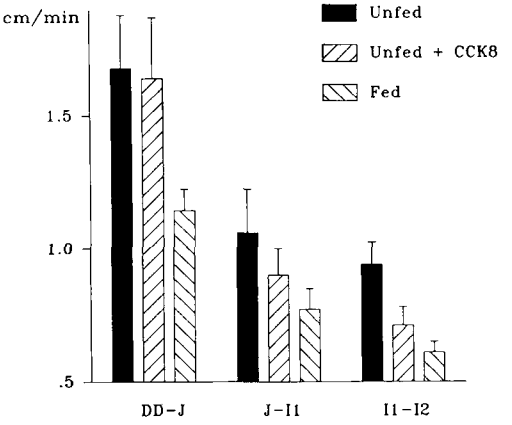

FIGURE 2. Speed of propagation of the Phase 3 of the MMC between two consecutive electrodes: DD-J, from the distal duodenum to the jejunum; J-I1, from the jejunum to the proximal ileum; and I1-I2, from the proximal to the distal ileum. Speed of propagation was faster ($P < .05$) in unfed than in fed animals in all the intestinal segments. CCK8 (10^{-12} mol/kg per min × 3 h) partially mimicked the fed state.

TABLE 2. Frequency distribution of the number of Phases 3 originated at each gastrointestinal area[1]

Organ	Unfed	Fed	Unfed + CCK8	n
	(%)			
Stomach	88.2	3.3	5.9	17
Duodenum	5.9	56.7	64.7	29
Jejunum	5.9	40.0	29.4	18
n	17 (100%)	30 (100%)	17 (100%)	64

[1]Chi-square analysis: $P < .001$. Notice the similar distribution found in fed and unfed animals infused with CCK8 (10^{-12} mol/kg per min × 3 h).

considered as duodenal Phase 3 and, accordingly, we will use the term duodenal Phase 3 in this study.

The fed state displaced aborally the site of origin of the Phases 3 compared with the unfed conditions. In fed animals, about 57% of the Phases 3 were generated at the duodenum and 40% at the jejunum (Table 2). In unfed animals, about 88% of the Phase 3 originated at the stomach. In these cases, the stomach showed an increase of its electrical activity prior and during the appearance of a duodenal Phase 3 (Figure 1).

These results show that although the MMC pattern is not disrupted in chickens during the postprandial state, there are clear differences between the "fed MMC pattern" and the "unfed MMC pattern" according to the duration, speed of propagation, and origin of the MMC.

Effects of Cholecystokinin on Unfed Intestinal Motility

The CCK8 infusion (10^{-12} mol/kg per min × 3 h) in unfed animals transformed the "unfed MMC pattern" into a "fed MMC-like pattern" (Figure 3). Table 1 shows the duration of the MMC phases in each treatment. The MMC phases during CCK8 infusion in unfed animals moved from values observed in unfed birds to intermediate values closer to those found in fed animals (Table 1). The speed of propagation of Phase 3 was also modified by CCK8 infusion in unfed animals (Figure 2). Values were never different from the postprandial state. The CCK8 reduced the speed of propagation of Phase 3. This effect was significant at the distal ileum (Figure 2). Similarly, CCK8 infusion during feed deprivation mimicked the effect of feeding in relation to the site of origin of the Phases 3 (Table 2 and Figure 3). In these conditions, about 65% of the Phase 3 had a duodenal origin and 29% were jejunal, whereas only 6% originated at the stomach. No differences were found between the fed pattern and that observed during CCK8 infusion in unfed animals.

Effects of Cholecystokinin on Fed Intestinal Motility

The CCK8 effects on MMC during the postprandial state were dose-dependent. Lower doses of CCK8 (between 10^{-11} and 3×10^{-11} mol/kg per min × 2 h) produced a progressive elongation of the MMC (Table 3) with a reduction of the speed of propagation of the Phase 3 (Table 4), whereas doses greater than 10^{-10} mol/kg per min totally disrupted the MMC pattern (Figure 4).

The CCK8 infusion at a dose of 10^{-11} mol/kg per min × 2 h did not modify the MMC parameters at the proximal areas of the small intestine (Figure 4B). Phase 3 was not modified at any intestinal area, whereas Phase 2 was significantly elongated and Phase 1 significantly shortened both at the proximal and distal ileum (Table 3). The speed of propagation of the Phase 3 was not modified at this dose (Table 4). Similarly, gastroduodenal coordination was not modified by this dose of CCK8.

Infusion of CCK8 at a dose of 3×10^{-11} mol/kg per min × 2 h induced significant changes in the MMC pattern in all the studied intestinal areas (Figure 4C). The MMC was elongated at the jejunum and proximal ileum, showing a duration of between 80 and 90 min. In two out of five chickens, no complete MMC was recorded at the distal ileum. In the other three chickens MMC were significantly elongated. Moreover, all the intestinal areas

TABLE 3. Migrating myoelectric complex (MMC) duration (mean ± SEM) in fed chickens under several experimental conditions

Organ	State	Control (10)[1]	CCK8 (10^{-11} mol/kg per min) (4)	CCK8 (3×10^{-11} mol/kg per min) (5)	CCK8 (10^{-10} mol/kg per min) (5)
		(min)			
Jejunum	Total duration	59.6 ± 3.8	65.4 ± 1.8	89.5 ± 14.0**	Disruption
	Phase 1	21.4 ± 1.5	26.2 ± 3.7	11.8 ± 4.6*	
	Phase 2	33.9 ± 3.5	34.3 ± 4.4	73.5 ± 16.3**	
	Phase 3	4.4 ± .2	4.6 ± .7	4.2 ± .7	
		(29)[2]	(5)	(6)	
Proximal ileum	Total duration	56.6 ± 3.3	63.7 ± 6.2	83.9 ± 13.0**	Disruption
	Phase 1	22.2 ± 1.7	10.7 ± 4.6*	5.3 ± 2.6**	
	Phase 2	30.3 ± 2.4	48.5 ± 9.0	74.9 ± 13.1**	
	Phase 3	4.1 ± .3	4.5 ± .6	3.7 ± .7	
		(26)[3]	(4)	(7)	
Distal ileum	Total duration	63.1 ± 4.1	80.5 ± 12.8	115.6 ± 8.7**	Disruption
	Phase 1	20.3 ± 1.3	11.5 ± 4.9	11.0 ± 5.8	
	Phase 2	38.8 ± 3.5	64.8 ± 13.3*	99.0 ± 8.9**	
	Phase 3	4.0 ± .3	4.2 ± .7	5.6 ± .3	
		(29)[3]	(4)	(3)	

[1]Number of different chickens.

[2]N = number of MMC (the number of MMC at 3×10^{-11} is smaller because the peptide induced disruption in two out of five chickens).

*$P < .05$ in relation to the control according to Dunnett test.

**$P < .01$ in relation to the control according to Dunnett test.

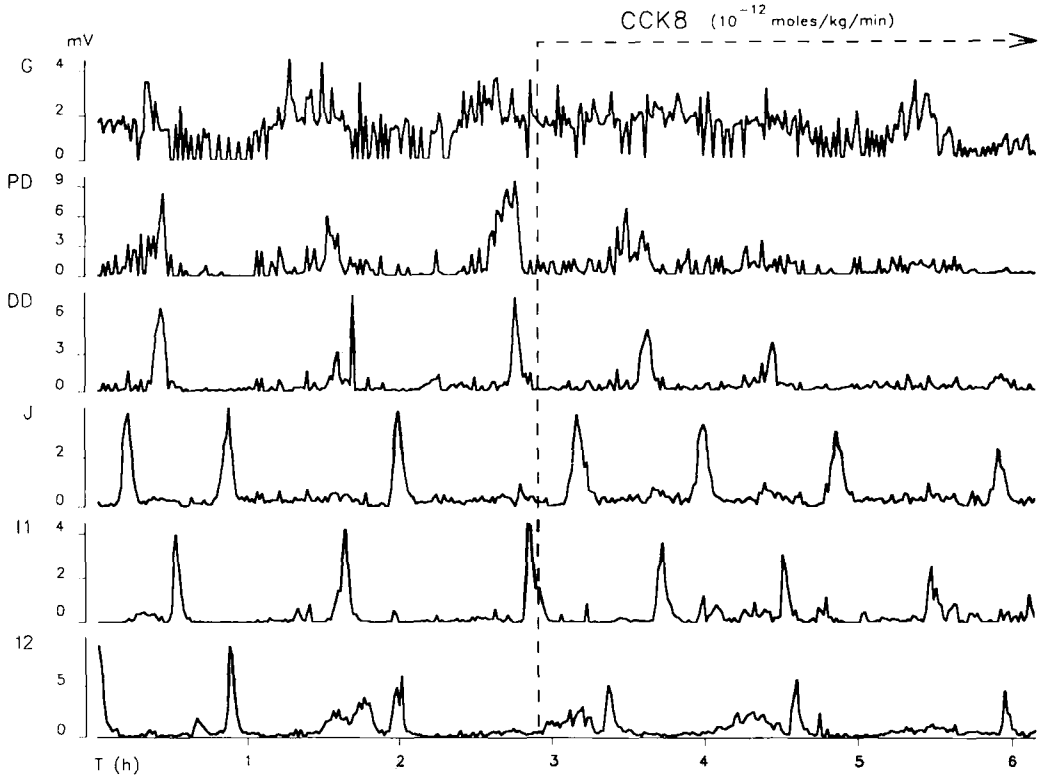

FIGURE 3. Integrated recording (time set interval 1 min) of the electrical activity of the gastrointestinal tract of a fasting chicken showing the effects of an i.v. infusion of CCK8. Similar responses were observed in each animal (n = 6). G, gastric cranial thin muscle; PD and DD, proximal and distal duodenum, respectively; J, jejunum; I1 and I2, proximal and distal ileum, respectively.

showed a shortness of the Phase 1 (duration between 5 and 12 min) and an elongation of the Phase 2 (duration between 74 and 99 min) (Table 3). Speed of propagation of the Phase 3 was significantly reduced at the distal small intestine but not at the proximal small intestine (Table 4). During CCK infusion, gastroduodenal coordination was modified. Gastric activity was reduced (in some cases suppressed), whereas duodenal activity was either not modified or slightly increased.

TABLE 4. Speed of propagation (mean ± SEM) of the Phase 3 in fed animals during infusion of cholecystokinin

Treatment	DD-J[1]	J-I1[1]	I1-I2[1]
	(cm/min)		
Control	.59 ± .03	.71 ± .06	.66 ± .04
CCK8 (10^{-11} mol/kg per min)	.62 ± .08	.81 ± .14	.58 ± .07
CCK8 (3×10^{-11} mol/kg per min)	.43 ± .08*	.45 ± .14	.43 ± .09*
CCK8 (10^{-10} mol/kg per min)	Disruption	Disruption	Disruption

[1]Speed of propagation between distal duodenum and jejunum (DD-J), jejunum and proximal ileum (J-I1), and proximal and distal ileum (I1-I2).

*$P < .05$, in relation to the control according to Dunnett test.

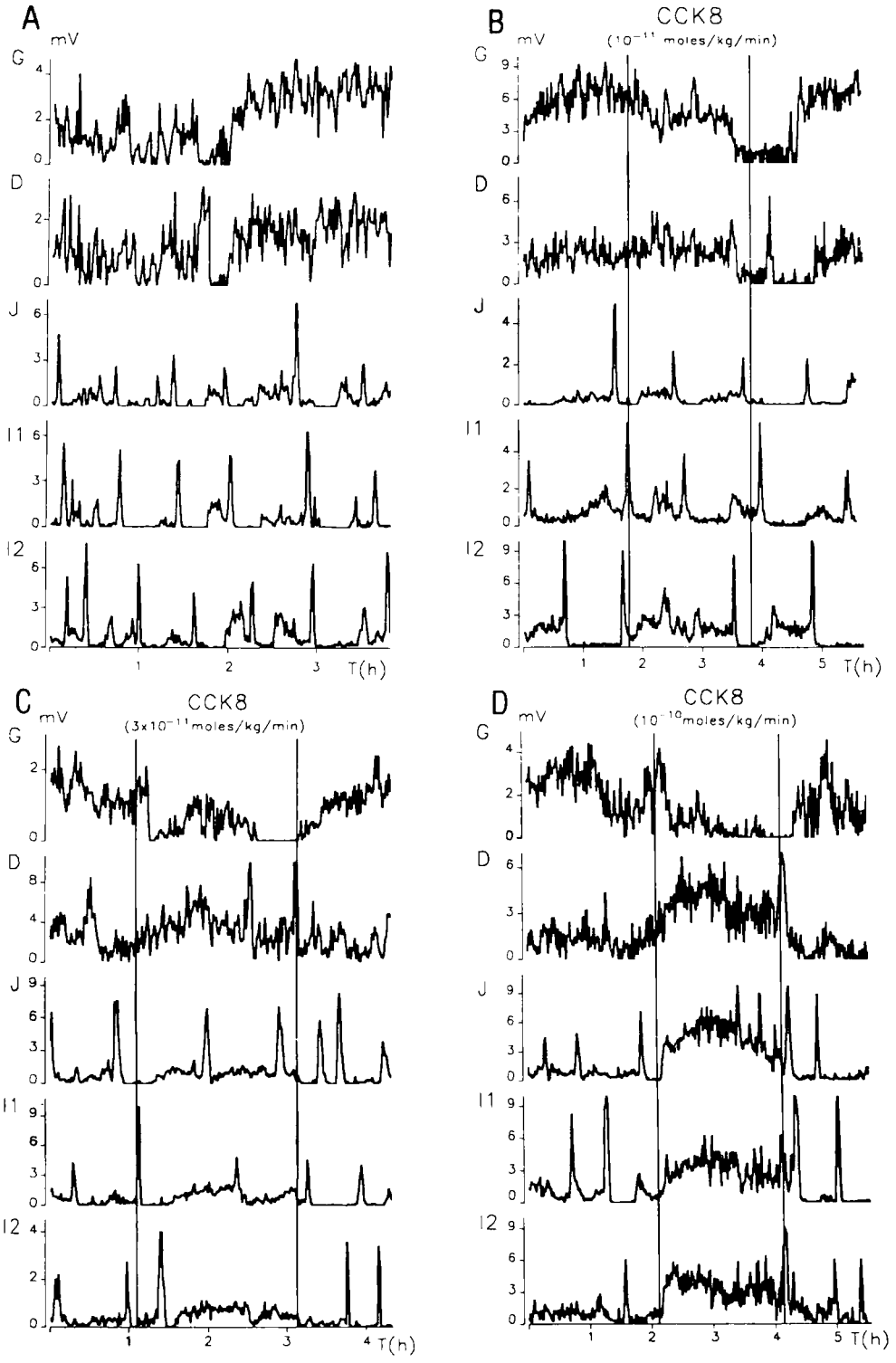

FIGURE 4. Integrated recordings (time set interval 1 min) of the electrical activity of the gastrointestinal tract of a chicken that ate *ad libitum*, in several experimental situations: A) control; B) infusion of CCK8 (10^{-11} mol/kg per min × 2 h); C) infusion of CCK8 (3×10^{-11} mol/kg per min × 2 h); and D) infusion of CCK8 (10^{-10} mol/kg per min × 2 h). Similar responses were observed in all the animals. G, gastric cranial thin muscle; D, distal duodenum; J, jejunum; I1 and I2, proximal and distal ileum, respectively.

Infusion of CCK8 at a dose of 10^{-10} mol/kg per min × 2 h produced a total disruption of the MMC pattern, which lasted during the whole infusion time (Figure 4D). During this period the intestine showed an irregular and disorganized motility pattern, characterized by the presence of continuous spike-bursts of high amplitude appearing almost simultaneously in all the intestinal areas. Neither orad or aborad propagation of these spike-bursts could be established. A careful visualization of the recordings during CCK8 infusion allowed us to establish clearly that the Phase 3 electrical pattern was absent during the infusion (Figure 5). However, when CCK infusion finished, a migrating Phase 3 appeared almost immediately at the jejunum. In fact, a significant correlation (ANOVA test) between the dose of CCK infused and the time of appearance of the first Phase 3 after infusion was observed (23.7 ± 8.2, 12.5 ± 3.8; and 7.0 ± 2.1 min after infusion with CCK8 at 10^{-11}, 3×10^{-11}, and 10^{-10} mol/kg per min × 2 h, respectively).

Simultaneously, during 10^{-10} mol/kg per min × 2 h CCK8 infusion, gastric electrical activity was significantly reduced or totally suppressed, whereas duodenal activity was increased, showing a continuous motility pattern, similar to that observed at the small intestine (Figure 5).

Effects of Chicken Gastrin on Fed Intestinal Motility

The infusion of cG (10^{-10} mol/kg per min × 2 h) did not modify any of the MMC parameters studied. However, during the infusion of cG, gastric activity was signifi-

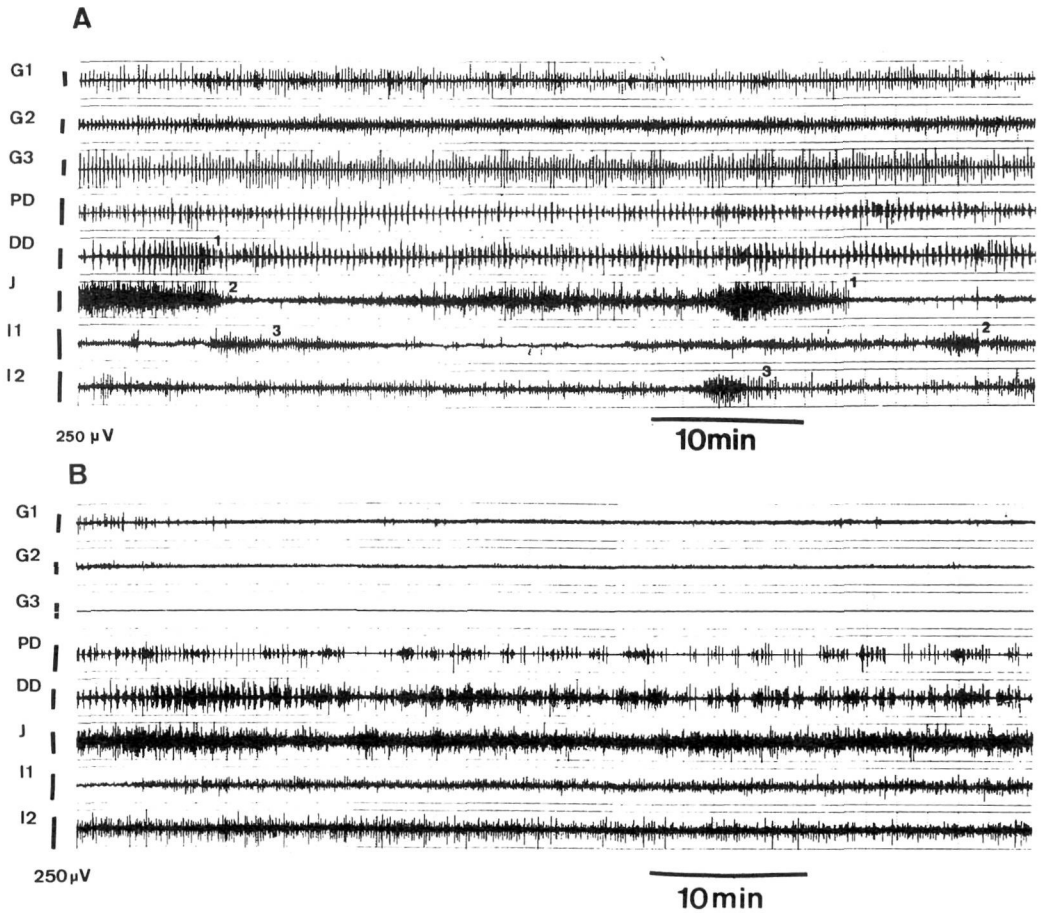

FIGURE 5. Electromyographical recordings of the intestinal activity of gastrointestinal tract of a fed chicken during the control period (A) (numbers —1, 2, 3—indicate three different MMC and their migration) and during CCK8 (10^{-10} mol/kg per min) infusion (B) C1, proventriculus; G2 and G3, gastric cranial thin and caudal thick muscles, respectively; PD and DD proximal and distal duodenum, respectively; J, jejunum; and I1 and I2, proximal and distal ileum.

cantly reduced and in most of the cases totally suppressed, whereas the duodenum showed phases of inhibition alternated with phases of increased activity (Figure 6).

DISCUSSION

Chickens, like other avian species and some mammals, show MMC both in fed and unfed states. The results of this study corroborate that consuming a meal modulates the MMC pattern without disrupting it. This modulation consists in: 1) elongation of the Phase 2 simultaneous with a reduction of Phase 1; 2) reduction of the speed of propagation of the Phase 3; and 3) aboral displacement of the site of origin of the Phase 3. In those species that show differentiated patterns of intestinal motility during unfed (MMC) and fed states (disorganized pattern), both CCK and gastrin participate in the conversion of an unfed motility into a fed motility pattern (Weidsbrodt *et al.*, 1974; Marik *et al.*, 1975; Mukhopadhyay *et al.*, 1977; Wingate *et al.*, 1978a,b; Konturek *et al.*, 1987; Thor *et al.*, 1988). Similarly, in unfed chickens, the exogenous administration of CCK mimicked the fed-MMC pattern, suggesting that the role of CCK in regulating intestinal motility may be similar in both groups of animals. In fact, it has recently been suggested that the motility pattern induced by consuming a meal might not completely disrupt the MMC, because a cyclical activity, similar to the motor complex, has been described in the rat (Zenilman *et al.*, 1992). Moreover, the presence of several Phases 3 has also been described in dogs during the intraluminal infusion of nutrients (Schmid *et al.*, 1992). These results suggest that an MMC-like pattern may also be present during the postprandial state even in those species in which feeding apparently disrupts the MMC pattern.

Our results show that there is an inverse correlation between Phases 1 and 2 and the feeding state. Whereas the duration of Phase 1 increases during feed deprivation, the duration of Phase 2 is significantly longer postprandially. According to Schmid *et al.* (1992), Phase 2 of the MMC and irregular spiking activity recorded postprandially cannot be distinguished. In our study, a correlation between the duration of Phase 2 and the dose of CCK was established. Accordingly, a dose-related decrease of the duration of Phase 1 was measured. These results suggest that the higher the postprandial stimuli the longer the Phase 2 duration, until there is a total disruption of the MMC. The unfed state also produces an increase in the speed of propagation of Phase 3, as has been observed previously (Jiménez *et al.*, 1994), suggesting that the intraluminal content could be an important stimulus in reducing the speed of propagation of Phase 3. Reductions in the speed of propagation might also contribute to the final disruption of the MMC at higher doses.

The nutritional state also seems to participate in regulating the anatomical site of origin of the MMC. During the unfed state, Phase 3 originated mainly at the stomach, whereas the fed state dis-

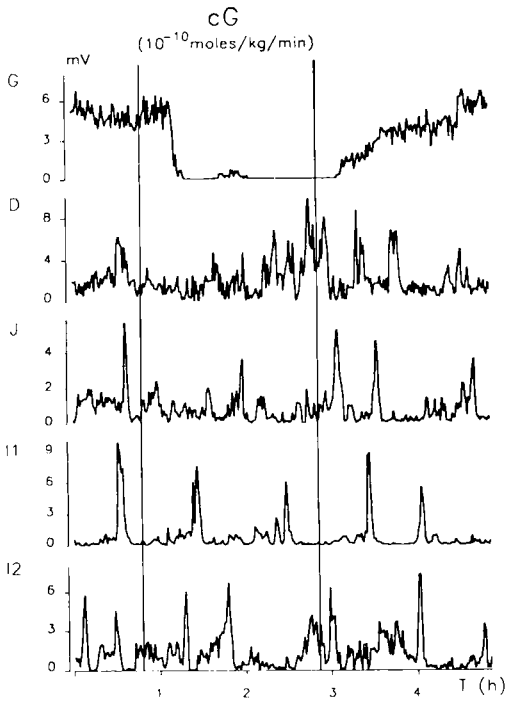

FIGURE 6. Integrated recording of the electrical activity from the same gastroduodenal areas than in Figure 5, showing the effect of an infusion of chicken gastrin (10^{-10} mol/kg per min × 2 h) in a fed chicken. Similar responses were observed in each animal (n = 5).

placed caudally this site of origin to the duodenum and jejunum. Until now it was thought that in dogs and humans, for instance, only two differentiated states were possible: either the MMC pattern originated at the stomach during feed deprivation or there was a total disruption of the MMC after meals. However, as previously mentioned (Schmid *et al.*, 1992; Zenilman *et al.*, 1992), in dogs and rats, but also in humans, it is possible to observe the MMC pattern during some postprandial states. An intestinal site of origin of these MMC was reported in these cases (Wilmer *et al.*, 1993).

Although the stomach of the chicken and that of mammals are very different anatomically, they show strong similarities in their functions. In mammals, the mechanical grinding of the food and the gastric emptying mainly depend on the antrum motor activity (Malagelada and Azpiroz, 1989). In chickens, this function depends on the motor activity of the gizzard (Vergara *et al.*, 1989), so that mammalian antrum and the gizzard of chickens may be considered to be functionally equivalent. Therefore we can consider that in feed deprivation MMC have the same origin both in mammalian and avian species. However, there is some controversy concerning the existence of the MMC pattern in the duodenum of avian species (Clench *et al.*, 1989; Mueller *et al.*, 1990), although "repetitive spike bursts" migrating distally have already been described in earlier studies (Roche and Ruckebush, 1978). We recently proposed that migrating repetitive spike bursts have enough similarities to be considered as a duodenal Phase 3. However, the strong gastroduodenal coordination in avian species does not allow a clear distinction between Phases 1 and 2 at the duodenum (Jiménez *et al.*, 1994). Another explanation for this discrepancy might be that most of the studies were done in postprandial states and, as we clearly demonstrate here, in such conditions MMC originate mostly at the intestine.

The administration of CCK in unfed chickens reproduces the gastrointestinal motility pattern present during the fed state: the MMC characteristics, duration, and speed of propagation of the Phase 3 show a tendency to be displaced to the fed state values, and the site of origin of the MMC was displaced caudally. These results suggest that in chickens, similar to observations in other species, CCK participates in the conversion of an unfed pattern into a fed one. In this case, CCK modulates the characteristics of the MMC, whereas in those species that do not show MMC during the fed state, it disrupts the MMC, generating a fed motility pattern (Wingate *et al.*, 1978a,b). The fact that CCK does not reproduce exactly the fed characteristics of the MMC indicates that this peptide is not the only neuroendocrine modulator released after the meal that regulates gastrointestinal motility. Presumably, other regulatory substances, released by both chemical and mechanical stimuli, participate in this process.

In studies with dogs, the MMC has been described as an "all or nothing" phenomenon, with a threshold dose of CCK that disrupts this pattern (Wingate *et al.*, 1978a,b). In chickens, CCK8 has a clear dose-dependent effect on the MMC. Doses of CCK8 of 10^{-10} mol/kg per min or greater produce an irregular motility pattern all along the intestine with a disappearance of the fed-MMC activity. Lower doses of CCK8 produce a modulatory effect, consisting of a dose-dependent elongation of the MMC with a progressive reduction of the speed of propagation of Phase 3. In fed animals the modulatory actions of CCK8 are especially evident at the most distal areas of the small intestine, suggesting that the disruption of the MMC starts distally. However, lower doses of CCK only displace the site of origin of the MMC caudally. Collectively, these results suggest that the sensitivity of the gastrointestinal tract of chickens to CCK depends on two different factors: the nutritional state (unfed vs fed) and the anatomical area. Controversially, other authors have described the distal canine small intestine as an especially resistant area to CCK actions (Wingate *et al.*, 1978a,b; Thor *et al.*, 1988).

In dogs, infusion of the specific CCK antagonist L364,718 did not prevent the conversion of the MMC pattern into the postprandial pattern, suggesting a weak role of CCK on the control of unfed

motility in those species with different motility patterns in fed and unfed states (Konturek *et al.*, 1987; Thor *et al.*, 1988). Unfortunately, this correlation cannot be studied in avian species because the CCK antagonists L-364,718 and L-365,260 are not active *in vivo*, suggesting some differences between avian CCK receptors and their mammalian counterparts (Campbell *et al.*, 1991; Martínez *et al.*, 1993a).

Cholecystokinin has not been isolated from the chicken gut and despite the efforts we made to measure CCK in plasma, using specific antibodies for a series of several fragments from mammalian CCK, results were not reproducible and were inconstant. Thus, we do not have valid data for CCK concentration in chicken plasma. Therefore, circulating CCK concentrations are unknown for avian species and no relation between them and changes in the intestinal motility can be made. In mammals, CCK is released endogenously by chemical stimuli derived from the meal (Rehfeld, 1989). Conceivably, chickens, which are continuous eaters, might have a more or less constant concentration of circulating CCK at any time. These circulating concentrations should be enough to modulate the site of origin of the MMC, its duration, and the speed of propagation of Phase 3. Posterior changes in the MMC pattern might be due to a supraphysiological release of CCK that would appear only in cases of saturation of the digestive process and by a direct stimulation of those areas of the intestine richest in CCK, such as the proximal ileum (Martínez *et al.*, 1993b). In other species, such as humans or dogs, in which the stomach can be totally emptied between meals, basal CCK concentration might be lower and the increase due to meal sharper, thus explaining a more dramatic effect of CCK on the motility patterns. However, this hypothesis cannot be demonstrated without a reliable test to measure chicken plasma CCK.

A recent study, already mentioned in this discussion (Zenilman *et al.*, 1992), suggests that even when an apparent disruption of the MMC is present, a cyclic activity with the same frequency as that of the MMC can still be distinguished. We have carefully analyzed our tracings from the higher doses of CCK, where an apparent disruption of the MMC is clear, and, although we did not use any frequency spectra analysis, we could not find any activity similar to that observed during Phase 3. Moreover, immediately at the end of CCK infusion we observed the appearance of a Phase 3, indicating that CCK was blocking the Phase 3 induction mechanism and that a resetting of the mechanism occurred afterwards. We do not suggest this is a general mechanism for all the species, but similarities observed in the MMC induction mechanism make this hypothesis plausible. In our opinion, rats do not eat as large a meal as dogs do, in spite of what has been thought until now. They must be in an intermediate point between those species showing a total disruption of MMC by meals and those in which the pattern is only slightly modified.

It should be pointed out that cG, the only peptide of the gastrin-CCK family isolated from chicken gut (Dimaline *et al.*, 1986), at the high dose tested (10^{-10} mol/kg per min × 2 h), did not modify the MMC pattern. This result supports the idea that cG has a weak role in the control of the MMC pattern, although it might participate in the regulation of gastroduodenal motility (Martínez *et al.*, 1993a). In contrast to plasma RIA for CCK, immunohistochemical differentiation of two populations of cells, one containing cG and the other containing a CCK-like peptide, was clearly demonstrated in a previous study (Martínez *et al.*, 1993b). The CCK-like containing cells had an intestinal location, in contrast to gastrin-containing cells, which were located at the antrum. Results of the present study lend functional support to the idea of the existence of CCK in chicken gut as a peptide clearly differentiated from cG.

In conclusion, these results suggest that in avian species the MMC should be described not as an all-or-nothing phenomenon but as a continuous event in the intestine and that the effect of CCK on the MMC should be described as a modulator but not as disruptive.

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REFERENCES

Campbell, B. J., A. Garner, R. Dimaline, and G. J. Dockray, 1991. Hormonal control of avian pancreas by gastrin-releasing peptide from the proventriculus. Am. J. Physiol. 261:G16–G21.

Clench, M. H., V. M. Piñeiro-Carrero, and J. R. Mathias, 1989. Migrating myoelectric complex demonstrated in four avian species. Am. J. Physiol. 256:G598–G603.

Dimaline, R., and C. M. Lee, 1990. Chicken gastrin: a member of the gastrin/CCK family with novel structure-activity relationships. Am. J. Physiol. 259:G882–G888.

Dimaline, R., J. Young, and H. Gregory, 1986. Isolation from chicken antrum, and primari aminoacid sequence of a novel 36-residue peptide of the gastrin/CCK family. FEBS Lett. 205:318–322.

Dockray, G. J., 1979. Cholecystokinin-like peptides in avian brain and gut. Experientia 35:628–630.

Gregory, P. C., V. Rayner, and G. Wenham, 1986. The influence of intestinal infusion of fats on small intestinal motility and digesta transit in pigs. J. Physiol. 379:27–37.

Jiménez, M. V., V. Martínez, A. Rodriguez-Membrilla, A Rodriguez-Sinovas, E. Goñalons, and P. Vergara, 1994. Rythmic oscillating complex: characterization, induction and relationship to MMC in chickens. Am. J. Physiol. 266: G585–G595.

Jiménez, M., V. Martínez, A. Rodriguez-Membrilla, A. Rodriguez-Sinovas, E. Goñalons, 1992. A method of analysis of the electrical activity of the proximal gastrointestinal tract of the chicken. Poultry Sci. 71:1531–1539.

Konturek, S. J., J. Konturek, and P. Thor, 1987. Physiological role of CCK in pancreatic secretion and intestinal motility. Pages 183–193 *in*: Gastrin and cholecystokinin. Chemistry, Physiology and Pharmacology. J. P. Bali and J. Martínez, ed. Elsevier Science Publishers B. V., Amsterdam, The Netherlands.

Malagelada, J.-R., and F. Azpiroz, 1989. Determinants of gastric emptying and transit in the small intestine. Pages 909–938 *in*: Handbook of Physiology. The Gastrointestinal System. Vol. 1. S. G. Schultz, J. D. Wood, and B. B. Rauner, ed. American Physiological Society, Bethesda, MD.

Marik, F., F. Charles, and M. D. Code, 1975. Control of the interdigestive myoelectric activity in dogs by the vagus nerves and pentagastrin. Gastroenterology 69:387–395.

Martínez, V., M. Jiménez, E. Goñalons, and P. Vergara, 1993a. Effects of cholecystokinin and gastrin on gastroduodenal motility and coordination in chickens. Life Sci. 52:191–198. [Erratum: Life Sci. (1993) 52:1667].

Martínez, V., A. Rodríguez-Membrilla, M. Jiménez, E. Goñalons, and P. Vergara, 1993b. Immunochemical differentiation of gastrin and cholecystokinin in gastrointestinal tract of chickens. Poultry Sci. 72:2328–2336.

Mueller, L. R., G. E. Duke, and O. A. Evanson, 1990. Investigations of the migrating motor complex in domestic turkeys. Am. J. Physiol. 259:G329–G333.

Mukhopadhyay, A. K., P. J. Thor, E. M. Copeland, L. R. Johnson, and N. W. Weisbrodt, 1977. Effect of cholecystokinin on myoelectric activity of small bowel of the dog. Am. J. Physiol. 232:E44–E47.

Rayner, V., and G. Wenham, 1986. Small intestinal motility and transit by electromyography and radiology in the fasted and fed pig. J. Physiol. (Lond.) 379:245–255.

Rehfeld, J. F., 1989. Cholecystokinin. Pages 337–358 *in*: Handbook of Physiology. The Gastrointestinal System. Vol. 2. S. G. Schultz, G. M. Makhlouf, and B. B. Rauner, ed. American Physiological Society, Bethesda, MD.

Roche, M., and Y. Ruckebusch, 1978. A basic relationship between gastric and duodenal motilities in chicken. Am. J. Physiol. 235:E670–E677.

Ruckebusch, Y., 1989. Gastrointestinal motor functions in ruminants. Pages 1225–1282 *in*: Handbook of Physiology. The Gastrointestinal System. Vol. 1. S. G. Schultz, J. D. Wood, and B. B. Rauner, ed. American Physiological Society, Bethesda, MD.

Ruckebusch, Y., and L. Bueno, 1977. Electrical spiking activity of the small intestine as an ultradian rhythm. Page 789 *in*: Abstract of Round Tables and Lectures. XXVII Congress, Vol. XII. Paris, France.(Abstr.)

Ruckebusch, M., and J. Fioramonti, 1975. Electrical spiking activity and propulsion in small intestine in fed and fasted rats. Gastroenterology 68: 1500–1508.

Schmid, H. R., H. J. Ehrlein, and C. Feinle, 1992. Effects of enteral infusion of nutrients on canine intestinal motor-patterns. J. Gastrointest. Motil. 4:279–292.

Thor, P., J. Laskiewicz, P. Konturek, and S. J. Konturek, 1988. Cholecystokinin in the regulation of intestinal motility and pancreatic secretion in dogs. Am. J. Physiol. 255:G498–G504.

Vergara, P., C. Ferrando, M. Jiménez, E. Fernández, and E. Goñalons, 1989. Factors determining gastrointestinal transit of several markers in the domestic fowl. Q. J. Exp. Physiol. 74:867–874.

Weisbrodt, N. W., 1987. Motility of the small intestine. Pages 631–663 *in*: Physiology of the Gastrointestinal Tract. Vol. 1. 2nd ed. L. R. Johnson, ed. Raven Press, New York, NY.

Weidsbrodt, N. W., E. M. Copeland, R. W. Kearley, E. P. Moore, and L. R. Johnson, 1974. Effects of pentagastrin on electrical activity of small intestine of the dog. Am. J. Physiol. 227:425–429.

Wilmer, A., G. Coremans, T. Andrioll, J. Janssens, and G. Vantrappen, 1993. Do the characteristics of phase 3 motor activity after the end of the fed pattern differ from those after a prolonged fasting period? Gastroenterology 104:A603. (Abstr.)

Wingate, D. L., E. A. Pearce, M. Hutton, A. Dand, H. H. Thompson, and E. Wünsch, 1978a. Quantitative comparison of the effects of cholecystoki-

nin, secretin and pentagastrin on gastrointestinal myoelectric activity in the conscious dog. Gut 19:593–601.

Wingate, D. L., H. H. Thompson, E. A. Pearce, and A. Dand, 1978b. The effects of exogenous cholecystokinin and pentagastrin on myoelectrical activity in the small intestine of the conscious fasted dog. Pages 47–58 *in*: Gastrointestinal Motility in Health and Disease. H. L. Duthie, ed. MTP Press Limited, Lancaster, England.

Zenilman, M. E., J. E. Parodi, and J. M. Becker, 1992. Preservation and propagation of cyclic myoelectric activity after feeding in rat small intestine. Am. J. Physiol. 263:G248–G253.