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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:

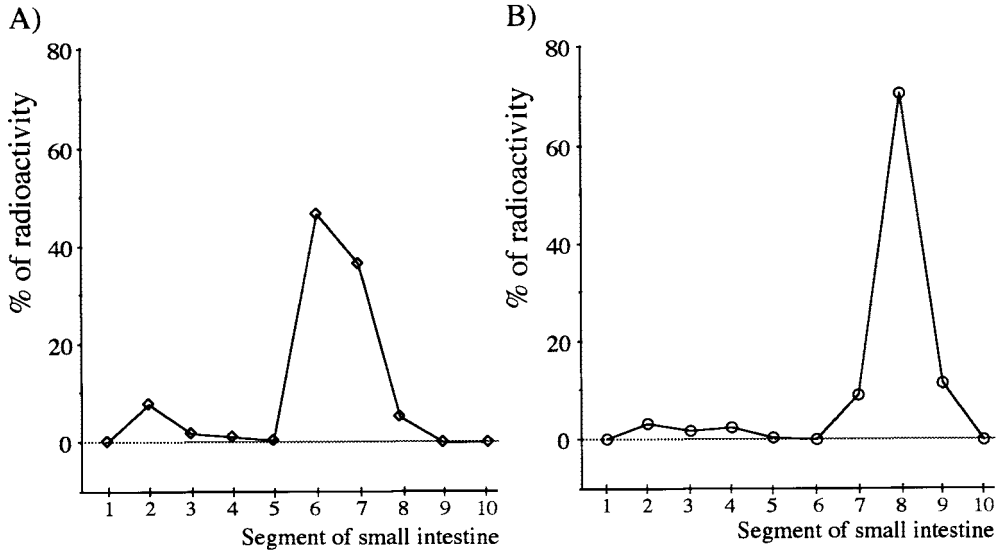

Figure 1: Linechart showing the distribution of radioactivity along the small intestine of A) a germfree and B) a conventional Sprague-Dawley rat 60 minutes after intraluminal instillation of marker ($Na_2{}^{51}CrO_4$) 15 cm distal to pylorus. The percent of total radioactivity in the small intestine 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

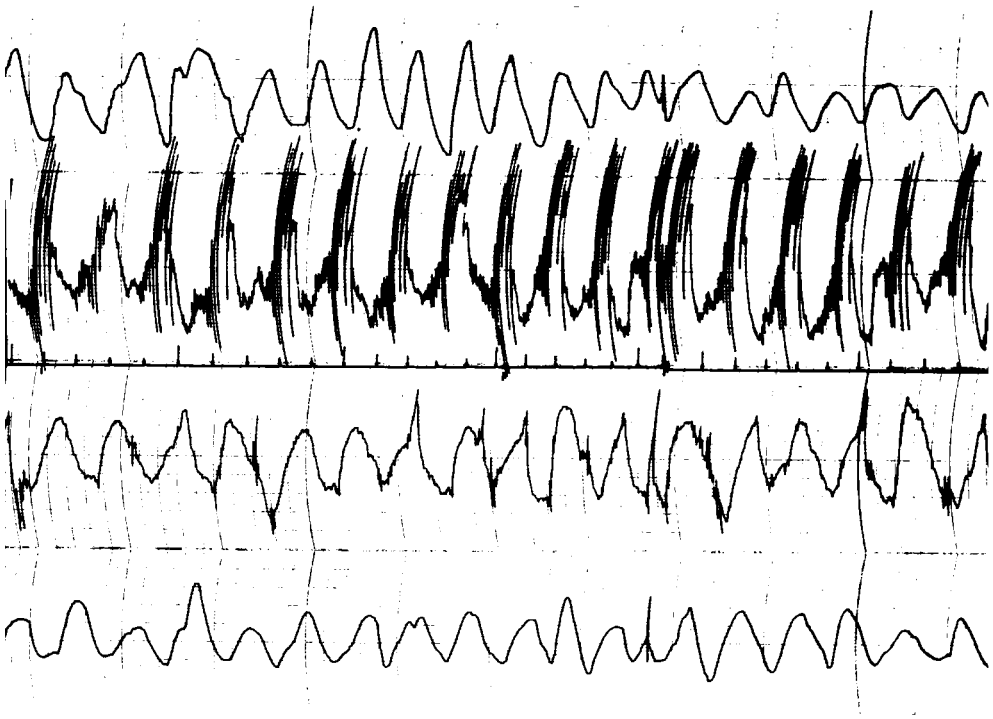

Figure 2: Fasting myoelectric activity of the small intestine of Sprague-Dawley rats recorded from jejunal electrodes 5 (first); 15; 25; 35 cm distal to the duodeno-jejunal junction. Time scale between second and third electrode with inter-marker distance of 1 sec. The first and the fourth electrode 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

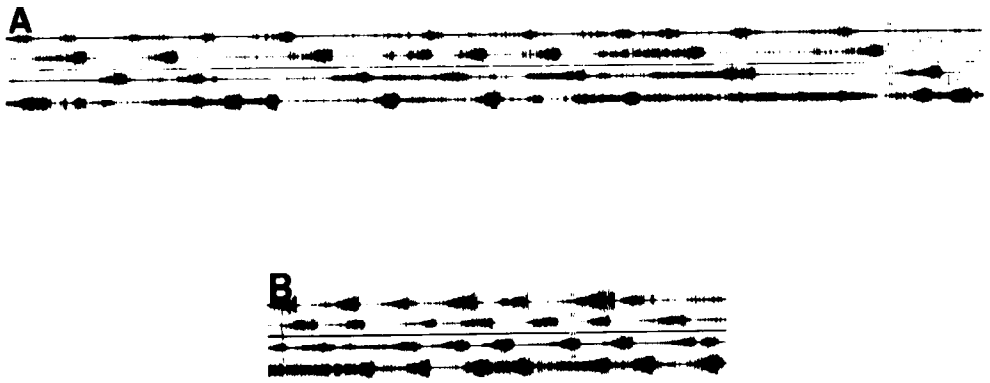

Figure 3: Fasting myoelectric recording in Sprague Dawley rat in A) the germfree state and B) one week after introduction of conventional intestinal flora. J1; J2; J3; J4 indicate jejunal electrodes 5; 15; 25; and 35 cm from the duodeno-jejunal junction. Activity fronts representing phase III of MMC 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

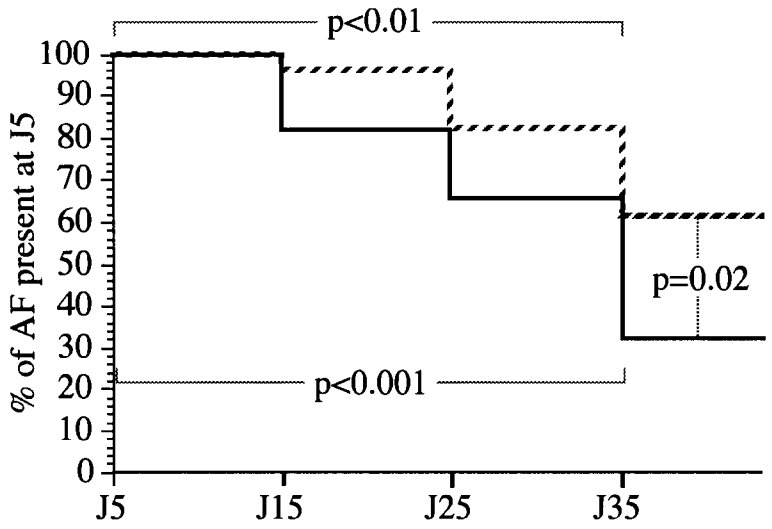

Figure 4: Plot showing how far activity fronts of MMC (AF) recorded five cm distal to the duodeno-jejunal junction (J5) propagated in the germfree state (solid line) and after conventionalisation (broken line), in six germfree Sprague-Dawley rats. Mean percentage of activity fronts that reached the respective 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.,

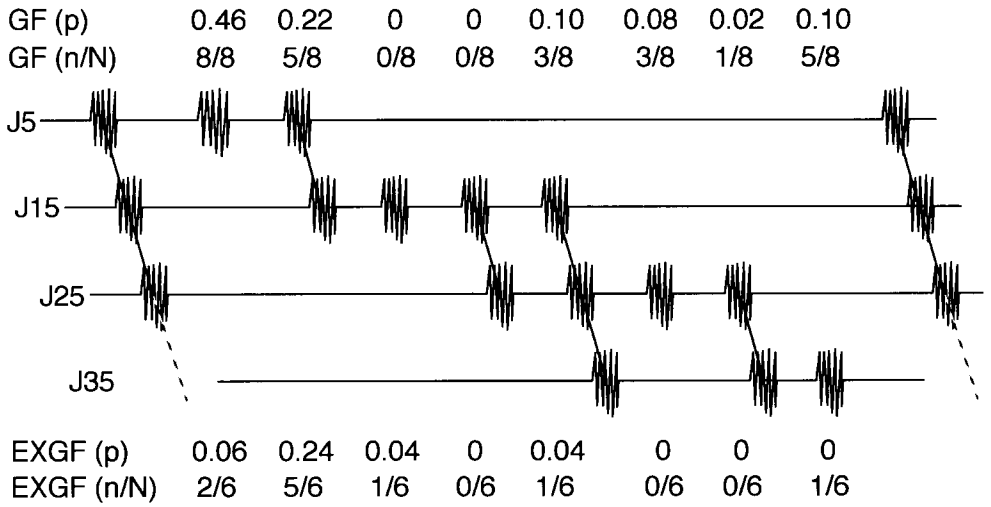

Figure 5: Schematic presentation of the possible patterns of propagation for activity fronts. GF and EXGF denotes germfree and ex-germfree state, respectively. (P) is the probability for a given pattern of propagation to occur during one complete MMC cycle, based on activity front propagating at least 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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