Endogenous ghrelin and 5-HT regulate interdigestive gastrointestinal contractions in conscious rats

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The characteristic feature of gastric MMC is different among species. In humans and dogs, MMC is usually observed every 90–120 min the interdigestive state. In contrast, MMC cycle is short (less than 20 min) in rats and not so regular as that of humans and dogs (2, 18, 53). Since it is rather difficult to distinguish three phases in rats, these phases are called as phase I-like contractions and phase III-like contractions in rats (3, 18, 53).

Ghrelin, a 28-amino acid peptide, was discovered as the endogenous ligand for growth hormone secretagogue receptor (GHS-R) from the rat stomach (37). Because of a structural resemblance to motilin, ghrelin is known as a motilin-related peptide (4, 54). Although motilin administration does not affect gastric emptying and gastrointestinal (GI) transit in rats (13), ghrelin stimulates GI motility in rats. Thus it is well established that motilin regulates gastric phase III contractions in dogs, whereas ghrelin regulates gastric phase III-like contractions in rats.

Ghrelin administration increases gastric motility in a dose-dependent manner in urethane-anesthetized rats (42). Ghrelin administration also causes phase III-like contractions in the antrum and duodenum in conscious rats (18, 58). We have previously showed that plasma ghrelin levels were highly associated with the occurrence of phase III-like contractions of the rat stomach (3). We also showed that spontaneous phase III-like contractions of the antrum were abolished by a GHS-R antagonist. These suggest that the spontaneous phase III-like contractions of the antrum are mediated via endogenously released ghrelin in rats (3). However, it still remains unknown whether endogenous ghrelin regulates spontaneous phase III-like contractions of the small intestine in rats.

The gastric and intestinal phase III contractions are controlled by different mechanisms in dogs. Plasma motilin level is highly associated with the appearance of gastric phase III in dogs (34). In contrast, phase III contractions in the small intestine sometimes occur without a concomitant increase in plasma motilin concentration (47).

Motilin antiserum inhibits the occurrence of phase III contractions only in the stomach, not in the intestine (38). After duodenectomy, no obvious phase III contractions were seen in the antrum, but migrating phase III contractions were seen in the upper jejunum in dogs (50). Gastric phase III is vagal dependent, whereas intestinal phase III is vagal independent in dogs (33).

The role of serotonin (5-HT) in regulating GI motility has been well documented for over 30 years (46). It has been shown that intestinal migrating myoelectrical activity are reg-
ulated via an endogenous 5-HT in rats (5, 40, 48). 5-HT stimulates phase II-like contractions when administered during phase I of the small intestine in dogs (45). In humans, 5-HT reuptake inhibitor (paroxetine) shortened the MMC cycle and increased the propagation velocity of intestinal phase III (25). In addition to motilin, 5-HT initiates gastric phase III contractions via 5-HT3 receptors in dogs (32). However, it still remains unclear whether 5-HT is involved in mediating gastric phase III-like contractions in rats.

Intestinal migrating myoelectrical activity was reduced by a 5-HT3 antagonist, but not by a 5-HT4 antagonist, in rats (40). In contrast, others showed that intestinal migrating myoelectrical activity was reduced by a 5-HT4 antagonist in rats (5). Thus it remains unclear which 5-HT receptor subtypes are involved in mediating intestinal MMC in rats.

Although 5-HT acts as a neurotransmitter of the enteric nervous system (23), the majority of 5-HT is stored in enterochromaffin (EC) cells of epithelial cells. EC cells have been considered to release 5-HT mainly into the blood vessels and/or intrinsic nerve terminal via a basolateral border (21). In contrast, others showed that 5-HT is also released into the intestinal lumen (1, 15, 28, 35). We have recently demonstrated that 5-HT is released into the lumen, but not into the portal circulation in response to luminal pressure increase of the rat proximal colon (55).

Immunoelectron microscopic study showed the anatomical evidence that 5-HT is released from EC cells in response to increase of luminal pressure of the rat duodenum. 5-HT is stored in the secretary granules of EC cells and released into the cytoplasmic matrix. Then 5-HT particles diffuse or are transported into the intestinal lumen in response to intraluminal pressure increase (17). However, it still remains unclear whether luminally released 5-HT regulates intestinal MMC in rats.

In the present study, we investigated whether ghrelin and/or 5-HT receptors are involved in mediating spontaneous phase III-like contractions of the duodenum in conscious rats.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats weighing 280–340 g were kept in group cages under conditions of controlled temperature (22–24°C), humidity, and light (12-h light cycle starting at 7:00 AM) with free access to laboratory chow and water. Protocols describing the use of rats were approved by the Institutional Animal Care and Use Committee of Duke University (Durham) and Zablocki Veterans Affairs Medical Center (Milwaukee) and carried out in accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals.” All efforts were made to minimize animal suffering and to reduce the number of animal in experiments.

**Animal preparation for GI motility recording.** After overnight fasting, the rats were anesthetized with intraperitoneal injection of pentobarbital sodium (45 mg/kg). Through the midline laparotomy, strain gauge transducers were implanted on the serosal surface of the antrum, duodenum, and jejunum. Duodenal transducers were implanted at 5 cm distal from the pylorus. Jejunal transducers were implanted at 15 cm (the proximal jejunum: J-1) and 25 cm (the distal jejunum: J-2) distal from the pylorus, respectively. The wires from the transducer were exteriorized through the abdominal wall and run under the skin toward the back. The intravenous catheter was inserted into the right jugular vein and similarly exteriorized to the back, as previously reported (2, 3). The catheter was filled with heparinized saline (100 unit/ml) to prevent coagulation. Wires and a catheter were protected by a protective jacket (Star Medical, Tokyo, Japan). After the surgery, the rats were housed individual and were allowed to recover for 1 wk before the experiments.

We have previously studied the effect of fixed feeding on plasma ghrelin levels and interdigestive gastric contractions in rats. Rats were trained to the assigned meal-feeding regimen once daily at 12:00–4:00 PM. We demonstrated that fixed feeding augments interdigestive gastric contractions (2). We also showed that the plasma ghrelin levels of fasted state are higher than that of fed state in rats (3). To obtain regular phase III-like contractions of GI tracts, we utilized the fixed-fed regimen, as previously reported (2).

After the implantation of transducers, rats were given food once daily at 12:00–4:00 PM. Experiments of GI motility recording were initiated 24 h after the feeding schedule. Feeding was initiated 30 min after the onset of each recording period. The feeding pattern is shown in Fig. 1.
started at 9:00 AM every day. The wires from the transducer were connected to the recording system (Power-Lab model 8SP, ADI instruments, Colorado Springs, CO). GI contractions were measured with free access to water in freely moving conscious rats. Spontaneous phase III-like contractions were observed for 2–3 h. Phase III-like contractions were defined as clustered contractions with amplitude of more than 4 g, followed by a silent phase (phase I-like contractions), as previously reported (53).

Effect of various antagonist on spontaneous phase III-like contractions in the interdigestive state. Two hours after recording the spontaneous contractions, various antagonists were administered. To study the involvement of ghrelin and 5-HT in mediating spontaneous phase III-like contractions, rats received truncal vagotomy, as previously reported (53).

We have previously showed that (D-lys3)GHRP-6 (1.0 μmol/kg) almost completely abolished phase III-like contractions induced by exogenously applied ghrelin (3). We selected doses of ondansetron (0.5 mg/kg) (40) and GR125,487 (5-HT4 receptor antagonist; 1.0 mg/kg) were administered intravenously (bolus).

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Five animals were randomly treated with each antagonist in cross-over design. Each study was performed at least 3 days apart. Motility index (MI), peak amplitude, and frequency of phase III-like contractions of GI tract were compared 30 min before and after the administration of (D-lys3)GHRP-6, ondansetron, and GR125,487. Saline injected rats served as controls.

Table 1. MI and peak amplitude of the GI tract between the fasted state and fed state

<table>
<thead>
<tr>
<th></th>
<th>Fasted State</th>
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<tbody>
<tr>
<td></td>
<td>MI, g·s</td>
<td>Peak Amplitude, g</td>
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<tr>
<td>Antrum</td>
<td>2267.8 ± 153.9</td>
<td>11.2 ± 0.8</td>
<td>3839.1 ± 283.0</td>
<td>15.8 ± 1.2</td>
</tr>
<tr>
<td>Duodenum</td>
<td>1920.3 ± 143.2</td>
<td>10.0 ± 0.7</td>
<td>2259.1 ± 255.2</td>
<td>10.8 ± 1.0</td>
</tr>
<tr>
<td>Jejunum-1</td>
<td>2352.6 ± 203.8</td>
<td>9.9 ± 0.7</td>
<td>2491.7 ± 283.2</td>
<td>7.5 ± 0.9</td>
</tr>
<tr>
<td>Jejunum-2</td>
<td>2059.1 ± 109.2</td>
<td>11.9 ± 1.0</td>
<td>2720.1 ± 261.4</td>
<td>9.0 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 rats. MI, motility index; GI, gastrointestinal.

Luminal concentration of 5-HT of the duodenum in the interdigestive state. To evaluate the relationship between the intraluminal concentration of 5-HT and phase III-like contraction of the duodenum, an intraduodenal catheter was inserted into the lumen of the duodenum. A transducer was also implanted on the duodenum. At 1 week after the surgery, duodenal juice (20 μl) was collected during the interdigestive state in a conscious state. 5-HT content of the duodenal juice was measured by HPLC, as previously reported (55).

Effects of vagotomy on phase III-like contractions in the interdigestive state. To investigate whether vagal pathways are involved in mediating phase III-like contractions, rats received truncal vagotomy, at the time of transducer implantation. As previously reported (44), the lower part of the esophagus was exposed and anterior and posterior branches of the vagal nerves were incised above the hepatic and celiac branches. In sham-operated rats, the vagal trunks were similarly exposed but not cut. One week after the surgery, interdigestive GI motility was recorded.

We have previously showed that continuous infusion of acyl ghrelin (active form; 0.8 μg·kg⁻¹·min⁻¹) augmented phase III-like contraction in the rat stomach (3). In our present study, acyl ghrelin (0.8 μg·kg⁻¹·min⁻¹) was continuously infused for 30 min in sham-operated rats and vagotomized rats.

Chemicals. Acyl ghrelin (Tocris Cookson, Ellisville, MO) and (D-lys3)GHRP-6 (Bachem, King of Prussia, PA) were kept in powder form at −70°C. Peptides were dissolved in saline immediately before use.

Statistical analysis. Results were shown as means ± SE. Statistical analysis of the data were performed by Student’s t-test to assess the difference between groups. One-way ANOVA followed by Dunnett’s post hoc test was also used to test the significance of differences among groups. A P value <0.05 was considered to be statistically significant.

RESULTS

Gastrointestinal contractions in the interdigestive state and postprandial state in conscious rats. It has been shown that spontaneous phase III-like contractions are observed at 12- to 15-min intervals of the stomach (3, 18, 53) in conscious rats.

In our present study, cyclic changes of contractions were detected in the antrum, duodenum, J-1, and J-2, including a

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In our present study, cyclic changes of contractions were detected in the antrum, duodenum, J-1, and J-2, including a
quiescence period (phase I-like contractions) followed by a
grouping of strong contractions (phase III-like contractions).
Spontaneous phase III-like contractions were observed every
14.8 ± 2.0 min at the antrum, 14.1 ± 2.0 min at the duodenum,
14.6 ± 1.3 min at the J-1, and 14.3 ± 1.5 min at the J-2 (n = 12).

The fasted motor patterns in the antrum, duodenum, J-1, and
J-2 were disrupted immediately after food intake and were
replaced by the fed motor pattern. There were no silent phases
(phase I-like contractions) in the antrum, duodenum, and jeju-
num during the fed state (Fig. 1 and Table 1).

**Effect of various antagonists on phase III-like contractions.**
After the administration of a GHS-R antagonist, spontaneous
phase III-like contractions of the antrum were significantly
attenuated for 30 min (Fig. 2). MI, peak amplitude, and
frequency of phase III-like contractions were significantly
reduced by a GHS-R antagonist in the antrum (Table 2,
Dunnett’s test, P < 0.05).

In contrast, MI, peak amplitude, and frequency of phase
III-like contraction at the duodenum, J-1, and J-2 were not
significantly affected by a GHS-R antagonist (Fig. 2 and Table 2).

**Table 2. Effect of (D-lys3)GHRP-6, ondansetron,
and GR125487 on % changes of MI, peak amplitude,
and frequency of phase III-like contractions of GI tract**

<table>
<thead>
<tr>
<th></th>
<th>Antrum</th>
<th>Duodenum</th>
<th>Jejunum-1</th>
<th>Jejunum-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% MI changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>99.2±2.0</td>
<td>101.2±0.6</td>
<td>97.9±2.0</td>
<td>100.3±3.1</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>126.0±4.2</td>
<td>127.1±1.7</td>
<td>125.8±4.3</td>
<td>120.0±4.5†</td>
</tr>
<tr>
<td>D-lys3-GHRP6</td>
<td>53.7±7.1</td>
<td>103.0±8.6</td>
<td>110.2±5.4</td>
<td>102.3±6.7</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>101.3±6.7</td>
<td>103.3±8.8</td>
<td>101.9±5.5</td>
<td>102.2±4.9</td>
</tr>
<tr>
<td>GR125,487</td>
<td>100.7±5.0†</td>
<td>109.6±5.9</td>
<td>49.1±4.3*</td>
<td>45.1±5.8*</td>
</tr>
<tr>
<td>Ghrelin+GR125,487</td>
<td>139.5±13.8*</td>
<td>132.9±9.6</td>
<td>63.7±13.2*</td>
<td>66.6±9.6*</td>
</tr>
</tbody>
</table>

| Peak amplitude, % |        |          |           |           |
| Saline   | 105.0±3.0 | 102.3±4.1 | 99.2±1.1  | 103.7±1.4 |
| Ghrelin  | 136.5±9.0* | 125.5±4.1 | 112.1±11.8 | 151.5±18.3† |
| D-lys3-GHRP6 | 66.3±6.4* | 100.4±5.3 | 98.3±3.4  | 100.6±3.9 |
| Ondansetron | 100.4±6.4 | 97.2±3.1  | 98.1±3.1  | 107.1±3.8 |
| GR125,487 | 97.9±4.0† | 90.3±7.7  | 65.2±7.2* | 47.5±11.3* |
| Ghrelin+GR125,487 | 143.0±12.6* | 125.7±3.9 | 68.3±4.8* | 62.1±9.2* |

**Effects of vagotomy on phase III-like contractions in the
interdigestive state.** In sham-operated rats, spontaneous phase
III-like contractions were occurred every 15 min in the antrum
and jejunum. In contrast, phase III-like contractions in the
antrum were no more observed in vagotomized rats. In the
jejunum, however, phase III-like contractions were still
observed in vagotomized rats (Fig. 6).

In sham-operated rats, phase III-like contractions were aug-
mented in response to ghrelin infusion at the antrum. In
contrast, ghrelin-induced contractions were significantly attenu-
ated in vagotomized rats. Peak amplitude of contractions at
the antrum in vagotomized rats (6.4 ± 1.1 g) was significantly
lower than that of sham-operated rats (12.2 ± 1.7 g, n = 5,
Student’s t-test, P < 0.05).

**DISCUSSION**

We have previously showed that GHS-R antagonists signif-
icantly inhibited spontaneous phase III-like contractions in
conscious rats (3), suggesting that endogenously released gh-
relin regulates spontaneous phase III-like contractions of the
rat stomach. We also showed the correlation between the
plasma ghrelin levels and occurrence of gastric phase I- and
III-like contractions of the antrum (3).
It is not clear whether intestinal phase III-like contractions are regulated by endogenously released ghrelin. Our present study showed that GHS-R antagonists inhibited phase III-like contractions in the antrum, but not the duodenum and the jejunum. This suggests that endogenously released ghrelin plays a major role to regulate gastric, but not intestinal, spontaneous phase III-like contractions in rats. Previous studies also showed that GHS-R antagonists did not affect phase III-like contractions in the duodenum (58).

It is well known that exogenously administered ghrelin augments phase-III like contractions of the antrum (3, 18). Our present study showed that exogenously administered ghrelin augmented phase-III like contractions in the jejunum as well as the antrum. In vitro study showed that ghrelin stimulates intestinal motility and that GHS-R is present in the enteric neurons of the rat jejunum (14). It is conceivable that exogenously administered ghrelin (pharmacological dose of ghrelin) causes phase-III like contractions at the jejunum, in addition to the stomach.

Ghrelin receptors are identified on the vagal sensory fibers of the gastric wall in rats (12). Ghrelin receptors are also localized to the nodose ganglia that project to the stomach in rats (49). It is highly likely that released ghrelin from the gastric mucosa stimulates primarily vagal afferents.

It still remains controversial whether the stimulatory effect of ghrelin on gastric motor function is mediated via intrinsic neurons or extrinsic neurons. In vitro muscle strip study showed that ghrelin itself (up to $10^{-5}$ M) failed to cause any
motor activity of the spontaneous contraction in rats (11, 13, 19) and mice (36), whereas others showed that ghrelin itself (10^{-7} to 10^{-6} M) stimulates contractions in a dose-dependent manner in rats (39).

Ghrelin enhances (10^{-9} M to 10^{-5} M) electrical field stimulation-evoked contractions of the gastric muscle strips in rats (11, 13, 19). Although these studies suggest the direct action of ghrelin on the gastric myenteric plexus, it still remains unclear whether the pharmacological doses of ghrelin (10^{-9} to 10^{-5} M) used for in vitro study are compatible to the doses used for in vivo study.

Our present study showed that spontaneous phase III-like contractions of the antrum completely disappeared in vagotomized rats. This suggests that spontaneous phase-III like contractions, which are regulated by endogenously released ghrelin, are mediated by the vagal pathway. We also showed that ghrelin infusion-induced gastric contractions were significantly attenuated in vagotomized rats. Others also showed that ghrelin-induced acid secretion is abolished by vagotomy in rats (61). Thus the direct action of ghrelin on the gastric wall (myenteric plexus) should be minor in physiological conditions.
It has been shown that ghrelin secretion is reduced after vagotomy (31, 59). Therefore, we cannot exclude the possibility that impaired spontaneous phase III-like contractions after vagotomy is due to decreased ghrelin secretion.

Previous reports suggest that gastric and intestinal phase III contractions are regulated by different mechanisms in dogs. Phase III contractions in the small intestine are independent of plasma motilin concentration (47). Motilin antiserum inhibits the occurrence of phase III contractions only in the stomach (38, 50). Acute vagal nerve blockade abolished gastric phase III without affecting intestinal phase III in dogs (29).

Our present study suggests that endogenously released ghrelin may regulate spontaneous phase III-like contractions of the stomach, but not the small intestine, in conscious rats. If so, what is the mediator regulating intestinal MMC in rats?

It has been demonstrated that 5-HT is involved in mediating interdigestive contractions of the small intestine in rats (46). Subcutaneous or intravenous administration of 5-HT can induce intestinal migrating myoelectrical activity in a dose-dependent manner in rats (40, 48).

Intestinal migrating myoelectrical activity was reduced by a 5-HT3 antagonist, but not by a 5-HT4 antagonist in rats (40). However, the same group, several years later, showed that intestinal migrating myoelectrical activity was reduced by a 5-HT4 antagonist as well as a 5-HT3 antagonist in rats (5).

Our present study showed that phase III-like contractions in the jejunum, not the antrum and duodenum, were significantly attenuated by 5-HT3 antagonists. In contrast, 5-HT3 antagonists did not affect phase III-like contractions in all of the upper GI tract. These suggest that spontaneous phase III-like contractions in the jejunum are mediated via 5-HT4 receptors, but not 5-HT3 receptors.

We also showed that phase III-like contractions were still observed in response to ghrelin infusion in the presence of 5-HT4 antagonists in the antrum and duodenum. In contrast, ghrelin failed to induce any phase III-like contractions at the jejunal mucosa in the presence of 5-HT4 antagonists. This further confirms that phase III-like contractions of the antrum are mediated via GHS-R and that phase III-like contractions of the small intestine are mediated via 5-HT4 receptors.

Although 5-HT acts as a neurotransmitter of the enteric nervous system (23), the majority of 5-HT is stored in EC cells of epithelial cells of the GI tract. EC cells release 5-HT into the blood vessels and intrinsic nerve terminal via a basolateral border (21). 5-HT is also released into the intestinal lumen from EC cells (1, 15, 28, 35).

Luminal pressure increase stimulates 5-HT release into the lumen, but not into the portal circulation, of the rat proximal colon (55). Immunelectron microscopic study also showed that 5-HT is released intraluminally from EC cells in response to luminal pressure increase of the rat duodenum (17).

Luminal applied 5-HT can move by passive diffusion across the intestinal wall of the guinea pig ileum (10). Recent study showed that 5-HT can cross the intestinal wall from the mucosa to the serosa (apical-to-basolateral direction) (41). Thus 5-HT into the intestinal lumen could reach the synaptic circuitry, resulting in stimulation of 5-HT receptors located on the lamina propria.

5-HT activates enteric afferent neurons to stimulate intestinal motor function (7, 16, 30). Intraduodenal administration of 5-HT also causes intestinal myoelectrical activity in rats (57).

Luminal administration of 5-HT into the proximal colon increased the fecal pellet output (20) and accelerated colonic transit (55) in rats.

5-HT3 receptors (43) and 5-HT4 receptors (52) are located on the cholinergic neurons of the myenteric plexus as well as sensory neurons of the intestinal mucosa (22). Nerve endings of sensory neurons may well be the targets for the 5-HT released from EC cells (6). It is generally accepted that 5-HT stimulates intrinsic nerve fibers via 5-HT4 receptors (16), whereas 5-HT stimulates extrinsic nerve fibers via 5-HT3 receptors (8, 22) in rats. 5-HT3 receptors are located on the nerve terminal of vagal afferent of the duodenal mucosa in rats (24). In contrast, there is no evidence of 5-HT4 receptors located on the vagal afferent or nodose ganglia.

5-HT released by mucosal stimuli initiates peristalsis by activating 5-HT4 receptors on sensory CGRP neurons of the rat colon in vitro (27). Ascending contractions and descending relaxations were inhibited by selective 5-HT4 blockers, but not by selective 5-HT3 antagonists in human jejunum in vitro (26). These suggest that 5-HT4 receptors play a major role in mediating an intrinsic neural reflex.

Because phase III-like contractions of duodenum and jejunum were not attenuated by a GHS-R antagonist, we hypothesized that ghrelin-independent intestinal phase III-like contractions may originate at the duodenum. We further hypothesized that 5-HT released from EC cells of the duodenum mucosa mediates intestinal phase III-like contractions.

To prove the hypothesis, we measured luminal concentration of 5-HT of the duodenum during the period of phase I-like contractions and phase III-like contractions. We showed that luminal concentration of 5-HT at the time of phase III-like contractions was significantly higher than that of phase I-like contractions. These results suggest that 5-HT released from EC cells of the duodenal mucosa initiates intestinal phase
III-like contractions in rats. Luminally released 5-HT from duodenal EC cells stimulates duodenal phase III-like contractions via 5-HT₄ receptors located on intrinsic primary afferent neurons. Our present study showed that phase III-like contractions of the jejunum were not affected by vagotomy. This indicates that intestinal phase III-like contractions occur independently of vagal pathway.

Our rat study demonstrated that spontaneous phase III-like contractions are mainly regulated by ghrelin and its own receptors in the antrum, whereas spontaneous phase III-like contractions are regulated by 5-HT and 5-HTᵣ receptors in the jejunum. Released ghrelin from the gastric mucosa initiates gastric phase III-like contractions. 5-HT is released from EC cells of the duodenal mucosa. Released ghrelin from the gastric mucosa initiates intestinal phase III-like contractions via 5-HT₄ receptors in rats (Fig. 7).

However, the stimulatory mechanism of ghrelin release from the stomach and 5-HT release from the duodenum remains to be investigated.

REFERENCES


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