

## Effect of acupuncture at Foot-Yangming Meridian on gastric mucosal blood flow, gastric motility and brain-gut peptide

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### Abstract

**AIM:** To observe the effect of acupuncture at Foot-Yangming Meridian on gastric mucosal blood flow (GMBF), gastric motility and brain-gut peptide.

**METHODS:** Sixty SD rats were randomly divided into 6 groups: normal control group, model group (group with gastric mucosal damage, GMD), Sibai group (with acupuncture at Sibai point + GMD), Tianshu group (with acupuncture at Tianshu point + GMD), Zusanli group (with acupuncture at Zusanli point + GMD) and non-acupoint group (with acupuncture at non-acupoint + GMD). The GMD model group was induced by infusing pure alcohol into gastric cavity. H<sub>2</sub> Gas Clearance Test (HGCT) was used to measure GMBF, the frequency and amplitude of gastric motility were measured by the method of aerocyst, the content of brain-gut peptide in sinus ventriculi and bulbus medullae were detected by radioimmunoassay.

**RESULTS:** Inhibitory effect of the frequency and amplitude of gastric motility were shown in model group, and the rates of frequency and amplitude changes were remarkably different from the normal control group (-19.41 ± 17.21 vs -4.71 ± 10.32,  $P < 0.05$ ; -51.61 ± 29.02 vs 1.81 ± 14.12,  $P < 0.01$ ). In comparison with control group, the GMBF was 0.52 ± 0.161 mL vs 1.03 ± 0.255 mL per 100g tissue/min,  $P < 0.01$ , the content of motilin in sinus ventriculi and bulbus medullae was 63.04 ± 7.77 pg/mL vs 72.91 ± 8.42 pg/mL,  $P < 0.05$  and 50.96 ± 8.77 pg/mL vs 60.76 ± 8.05 pg/mL,  $P < 0.05$ , but the content of somatostatin in sinus ventriculi and bulbus medullae was 179.85 ± 43.13 ng/g vs 90.54 ± 40.42 ng/g,  $P < 0.01$  and 532.86 ± 122.58 ng/g vs 370.91 ± 76.29 ng/g,  $P < 0.05$ , respectively. In comparison with model group, the amplitude of gastric motility was 1.52 ± 20.13, -6.52 ± 23.31, 6.92 ± 25.21 vs

-51.61 ± 29.02,  $P < 0.01$  and GMBF was 0.694 ± 0.160 mL vs 0.893 ± 0.210 mL, 1.038 ± 0.301 mL vs 0.52 ± 0.161 mL per 100g tissue/min,  $P < 0.01$ , respectively in Tianshu, Sibai and Zusanli groups, the content of motilin in sinus ventriculi and bulbus medullae was 71.64 ± 9.35 pg/mL vs 63.04 ± 7.77 pg/mL,  $P < 0.05$ ; 58.96 ± 6.69 pg/mL vs 50.96 ± 8.77 pg/mL,  $P < 0.05$  in Zusanli group and 72.84 ± 8.62 pg/mL vs 63.04 ± 7.77 pg/mL,  $P < 0.05$  in Sibai group, while the content of somatostatin in bulbus medullae in Tianshu, Sibai and Zusanli groups was 480.62 ± 151.69 ng/g, 388.53 ± 155.04 ng/g, 365.76 ± 143.93 ng/g vs 532.86 ± 122.58 ng/g,  $P < 0.05$ , respectively.

**CONCLUSION:** Electro-acupuncture at acupoints of Foot-Yangming Meridian could enhance the gastric motility, improve GMBF, and regulate the content of motilin and somatostatin in sinus ventriculi and bulbus. The effects of acupuncture on GMBF and gastric motility may be related to the content of brain-gut peptide.

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**Key words:** Electro-acupuncture; Foot-Yangming Meridian; Gastric motility; Motilin; Somatostatin

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### INTRODUCTION

The relationship between Foot-Yangming Meridian and stomach is an important issue in the field of meridian study. Our previous study had proved that acupuncture at Foot-Yangming Meridian could regulate gastric motility, gastric myoelectricity, tension of musculus sphincter pyloricus, gastric emptying and gastric pressure wave to protect gastric mucosa, and brain-gut peptide is one of the substantial factors in the relationship between Foot-Yangming Meridian and stomach. The present study examined the effect of acupuncture at points of Foot-Yangming Meridian on gastric motility, the content of motilin and somatostatin in GMD rats to explore the connection channel and substantial basis between Foot-Yangming Meridian and stomach.

## MATERIALS AND METHODS

### Materials

Electric acupuncture apparatus (SDE-II, Huatuo, Suzhou Medical Supplies Co.), desk-top high speed freezing centrifuge (TGL 16 M, by Kewei Industry Co.), FJ2008 radio-immune  $\gamma$  counter (262 Factory of Xi'an), and RM-46 electrophysiograph (Photoelectricity Factory, Japan) were used. Somatostatin and motilin radioimmunoassay reagent package were purchased from Huaying Biotechnology Institute of Beijing. SP radioimmunoassay reagent package was obtained from Navy RI Technique Center of Beijing. Other chemicals used were of analytical grade.

### Animal experiment

Sixty healthy SD rats of either sex weighing 200-300 g were obtained from Animal Technology College of Hunan University of Agriculture. They were randomly divided into 6 groups: normal control group, model group (group with gastric mucosal damage, GMD), Sibai group (with acupuncture at Sibai point + GMD), Tianshu group (with acupuncture at Tianshu point + GMD), Zusanli group (with acupuncture at Zusanli point + GMD) and non-acupoint group (with acupuncture at non-acupoint + GMD). The bilateral Sibai, Zusanli and Tianshu acupoints were chosen. The Sibai point was at the middle of infraorbital border. The Zusanli point was 5 mm under capitulum fibulae. The Tianshu point was 0.5 cm beside bellybutton. And the non-acupoint was 2 cm under anterior superior iliac spine.

### Experimental procedure

In normal control group, the gastric motility wave was recorded for 150 min. In model group, the gastric motility wave was recorded for 45 min, and the rats were infused with pure alcohol into gastric cavity to induce gastric mucosal damage. After that, the gastric motility wave was recorded for 60 min. In Tianshu, Sibai and Zusanli groups, the gastric motility wave was recorded for 45 min. Then the rats were treated with electro-acupuncture for 20 min, and were infused with pure alcohol into the gastric cavity to induce gastric mucosal damage and the gastric motility wave recorded for 60 min. At last, GMBF and ulcer index (UI) were observed and the sinus ventriculi and bulbus medullae were collected for motilin and somatostatin detection for each group.

### Acupuncture method

Stimulus parameters: condensation and rarefaction wave with the rarefaction wave of 4 Hz and condensation wave of 20 Hz, pulse wide of 0.5 ms; output voltage of 2-4 V, output current of 4-6 mA. The negative electrode was connected with the acupoint on the right side, while the positive electrode with the tail of a rat.

### Measurement

Frequency and amplitude of gastric motility wave were measured. The rats were fasted for 12 h and anesthetized with an intraperitoneal injection of 10% urethane (1 mL/100 g). Through a midline laparotomy, an aerocyst was implanted into sinus ventriculi and infused with gas to keep its water pressure at 2.5 kPa-4 kPa. The

Table 1 Effect of acupuncture on UI and GMBF (mean  $\pm$  SD,  $n = 10$ )

Groups	UI (mm <sup>2</sup> )	GMBF (mL/100g tissue/min)
Normal control	2.28 $\pm$ 1.56	1.03 $\pm$ 0.255
Model	4.46 $\pm$ 1.77 <sup>a</sup>	0.52 $\pm$ 0.161 <sup>b</sup>
Tianshu	3.09 $\pm$ 1.62	0.694 $\pm$ 0.160 <sup>d</sup>
Sibai	3.05 $\pm$ 1.38	0.893 $\pm$ 0.210 <sup>c,d</sup>
Zusanli	2.44 $\pm$ 1.68	1.038 $\pm$ 0.301 <sup>d,f,h</sup>
Non-acupoint	4.12 $\pm$ 1.82	0.671 $\pm$ 0.282 <sup>b,d</sup>

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs normal control group; <sup>d</sup> $P < 0.01$  vs model group; <sup>c</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$  vs Tianshu group; <sup>h</sup> $P < 0.01$  vs non-acupoint group.

aerocyst was connected with pressure transducer and electrophysiograph through a tube. The parameters were set as follows: Channel pattern: gastrointestinal motility scan; scan speed: 4 s/div; frequency filtering: 30 Hz; and sensitivity: 1 mm Hg. The frequency and amplitude of 10 waves were measured in every 10 min. The data measured before model preparation was regarded as the normal value while the data measured after that was the effective value. Rate of change = (Effective value - Normal value)/Normal value  $\times$  100%. According to the above formula, the average rate of frequency and amplitude change in 60 min was calculated as the final result. GMBF was evaluated by H<sub>2</sub> Gas Clearance Test (HGCT). The sinus ventriculi and bulbus medullae were collected and put into centrifuge tubes with about 2-3 mL boiled normal saline pro rata, and were kept on boiling for 3 min in thermostatic water bath at 100°C. After combination with 0.5 mL glacial acetic acid (1 mol/L), the tissues were centrifuged at 19000 r/min for 5 s three times at an interval of 1 min. The clear supernatant liquid was separated by centrifugation at 3000 r/min at 4°C for 30 min and kept in EP tube at -20°C for motilin and somatostatin assay according to the description in radioimmunoassay kit.

### Statistical analysis

All results were expressed as mean  $\pm$  SD. Statistical significance of differences was performed by employing the ANOVA with SPSS software (version 10.0). Differences were considered to be significant when  $P$  value was less than 0.05.

## RESULTS

### UI and GMBF

UI in model group was higher than that in normal control group which suggested successful model preparation. GMBF in model group was significantly lower than that in normal control group; GMBF in each group treated with electro-acupuncture was significantly higher than that in model group. It suggested that electro-acupuncture could improve GMBF (Table 1).

### Gastric motility

The frequency and amplitude of gastric motility in model group were significantly lower than that in the normal control group. The average descending level of frequency and amplitude of gastric motility in Sibai, Tianshu, Zusanli group was lower than that in model group. The average

**Table 2** Effect of acupuncture on average rates of frequency (%) and amplitude changes (%) (mean  $\pm$  SD,  $n = 10$ )

Groups	Frequency change (%)	Amplitude change (%)
Normal control	-4.71 $\pm$ 10.32	1.81 $\pm$ 14.12
Model	-19.41 $\pm$ 17.21 <sup>a</sup>	-51.61 $\pm$ 29.02 <sup>b</sup>
Tianshu	4.52 $\pm$ 20.62 <sup>c</sup>	1.52 $\pm$ 20.13 <sup>dh</sup>
Sibai	-3.03 $\pm$ 18.12	-6.52 $\pm$ 23.31 <sup>df</sup>
Zusanli	-1.62 $\pm$ 14.21	6.92 $\pm$ 25.21 <sup>df</sup>
Non-acupoint	-21.62 $\pm$ 16.42	-38.12 $\pm$ 26.42 <sup>b</sup>

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs normal control group; <sup>c</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  vs model group; <sup>e</sup> $P < 0.01$  vs non-acupoint group.

**Table 3** Effect of acupuncture on content of motilin in sinus ventriculi and bulbus medullae (mean  $\pm$  SD,  $n = 10$ )

Groups	Sinus ventriculi (pg/mL)	Bulbus medullae (pg/mL)
Normal control	72.91 $\pm$ 8.42	60.76 $\pm$ 8.05
Model	63.04 $\pm$ 7.77 <sup>a</sup>	50.96 $\pm$ 8.77 <sup>a</sup>
Tianshu	66.06 $\pm$ 10.43	51.27 $\pm$ 7.77 <sup>a</sup>
Sibai	72.84 $\pm$ 8.62 <sup>c</sup>	56.61 $\pm$ 8.29
Zusanli	71.64 $\pm$ 9.35 <sup>c</sup>	58.96 $\pm$ 6.69 <sup>ce,g</sup>
Non-acupoint	65.29 $\pm$ 8.31	50.66 $\pm$ 9.46 <sup>b</sup>

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs normal control group; <sup>c</sup> $P < 0.05$  vs model group; <sup>e</sup> $P < 0.05$  vs Tianshu group; <sup>g</sup> $P < 0.05$  vs non-acupoint group.

rates of amplitude change in Sibai, Tianshu and Zusanli were higher than that in model group. The average rate of frequency change in Tianshu group was significantly different from the model group (Table 2).

### Motilin

The content of motilin in sinus ventriculi and bulbus medullae of model group was lower than that of normal control group. The content of motilin in sinus ventriculi of Sibai and Zusanli was higher than that of model group. And the content of motilin in bulbus medullae of Zusanli group was higher than that of model, Tianshu and non-acupoints groups. The results suggested that electro-acupuncture at Sibai points could increase the content of motilin in sinus ventriculi and electro-acupuncture at Zusanli could increase the content of motilin in both sinus ventriculi and bulbus medullae (Table 3).

### Somatostatin

The content of somatostatin in sinus ventriculi and bulbus medullae of model group was higher than that of normal control group. The content of somatostatin in sinus ventriculi decreased in each acupuncture group. The content of somatostatin in sinus ventriculi of Sibai group was lower than Tianshu and non-acupoints groups. The content of somatostatin in sinus ventriculi of Zusanli group was lower than that of model, Tianshu and non-acupoints. The results suggested that electro-acupuncture at Sibai and Zusanli could regulate the content of somatostatin in sinus ventriculi. The content of somatostatin in bulbus medullae decreased after acupuncture at Sibai. Tianshu and Zusanli compared with model group (Table 4).

**Table 4** Effect of acupuncture on content of somatostatin in sinus ventriculi and bulbus medullae (mean  $\pm$  SD)

Groups	Sinus ventriculi (ng/g)	Bulbus medullae (ng/g)
Normal control	90.54 $\pm$ 40.42	370.91 $\pm$ 76.29
Model	179.85 $\pm$ 43.13 <sup>b</sup>	532.86 $\pm$ 122.58 <sup>a</sup>
Tianshu	177.58 $\pm$ 42.42	480.62 $\pm$ 151.69 <sup>c</sup>
Sibai	130.26 $\pm$ 69.23 <sup>ef</sup>	388.53 $\pm$ 155.04 <sup>cd,g</sup>
Zusanli	131.72 $\pm$ 38.27 <sup>cd,ef</sup>	365.76 $\pm$ 143.93 <sup>ce</sup>
Non-acupoint	165.26 $\pm$ 56.19	476.22 $\pm$ 131.17

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs normal control group; <sup>c</sup> $P < 0.05$  vs model group; <sup>e</sup> $P < 0.05$ , <sup>d</sup> $P < 0.01$  vs Tianshu group; <sup>g</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$  vs non-acupoint group.

## DISCUSSION

The study of interconnections between viscera and meridians reveals the principle of the connection between the upper and lower, exterior and interior of the human body. The principle has been proved in clinical and experimental studies, but the connected channel and mechanisms are not yet clear. Foot-Yangming meridian belongs to stomach and connected with spleen. The acupoints of Foot-Yangming Meridian are always used to treat gastrointestinal disease. In recent years, the connected channel and mechanism of "Interconnections between Foot-Yangming Meridian and stomach" have been studied. Tianshu point is the acupoint of Foot-Yangming Meridian and also the "Mu Point" of large intestinal meridian. Zusanli is the "He Point" of Foot-Yangming Meridian and always used to treat gastrointestinal disease in clinical practice. Our previous studies proved that acupuncture at Sibai could regulate sinus ventriculi area and gastric motility<sup>[1-3]</sup>, and the regulation effect was even better than that of acupuncture at "Zusanli". In this study, the effect of acupuncture at Tianshu, Sibai and Zusanli on GMBF, gastric motility and brain-gut peptide was observed.

Sufficient gastric mucosal blood flow is important to maintain normal secretion, digestion and motility of stomach and plays an important role in development of ulcers. Ischemic gastric mucosa is the main pathogenesis of stress ulcers<sup>[4]</sup>. Results of this study showed that gastric mucosal blood flow is decreased by gastric mucosal damage. After acupuncture at Sibai and Zusanli points, gastric mucosal blood flow increased and gastric mucosal damage was relieved. It suggested that acupuncture could protect gastric mucosa by increasing gastric mucosal blood flow.

Regulating function of electro-acupuncture in gastric motility has been proved<sup>[5-7]</sup>. Our previous study found acupuncture at acupoints of Foot-Yangming Meridian could increase amplitude of gastric motility wave, indicating a strong relevance between Foot-Yangming Meridian and stomach<sup>[8]</sup>. The present study showed that amplitude and frequency of gastric motility wave were suppressed after gastric mucosa was damaged. Acupuncture at Sibai, Tianshu and Zusanli could promote the gastric motility, while the effect of acupuncture at non-acupoints was not obvious. It suggested acupuncture at acupoints of Foot-Yang, Meridian could regulate gastric

motility and the relevance between Foot-Yang Meridian and stomach was proved again.

Gastrointestinal peptide can regulate gastrointestinal motility through nervous system and body fluid. In the nervous system, gastrointestinal peptides, such as motilin, can regulate gastrointestinal spontaneous rhythmic contraction induced by ICC cells in the gastrointestinal wall<sup>[9]</sup> and autokinetic movement of intestinal smooth muscle mediated by enteric nervous system (ENS)<sup>[10]</sup>. Some kinds of gastrointestinal peptide, for example, vasoactive intestinal peptide (VIP), can also regulate gastrointestinal motility as neurotransmitter of peptidergic nerve fiber. In addition, gastrointestinal peptide such as substance P (SP) and somatostatin can regulate gastrointestinal motility in the central nervous system<sup>[11]</sup>. On the other hand, gastrointestinal peptide can influence gastrointestinal motility in endocrine mode. The gastrointestinal tract is a huge endocrine organ. Some kinds of gastrointestinal peptide have been found in endocrine cells, some in gastrointestinal nerve system, and some in both. They regulate gastrointestinal functions in paracrine secretion and neurocrine mode. At present, motilin and SP are regarded as peptides which can stimulate gastrointestinal smooth muscle, while somatostatin and VIP as peptide exerts a suppressive effect.

Motilin is a kind of polypeptide composed of 22 amino acids<sup>[12]</sup>. It has been found in central, peripheral and nervous system of gastrointestinal wall. The main physiological functions of motilin are regulating gastrointestinal motility, inducing smooth muscle contraction of sinus ventriculi and segmentation movement of intestine, promoting movement of colon and gall bladder<sup>[13-15]</sup>. Yang *et al*<sup>[16]</sup> found that gastric emptying was closely related to content of motilin in plasma. Motilin could promote gastric motility and gastric electrical activity persistently<sup>[17]</sup>. Acupuncture has bidirectional effects on motilin. In other words, acupuncture could decrease exorbitant content of motilin as well as increase abnormally low content of motilin<sup>[18,19]</sup>. We found that electro-acupuncture could promote motility of stomach and Oddi sphincter muscle, which might be related to the regulating effect of acupuncture on release of motilin and CCK in the periphery<sup>[20]</sup>. The present study demonstrated that the content of motilin in sinus ventriculi and bulbus medullae decreased significantly accompanied with the suppression of gastric motility after the gastric mucosa was damaged, while acupuncture at Sibai could increase the content of motilin in sinus ventriculi and acupuncture at Zusanli could increase it both in sinus ventriculi and bulbus medullae. It suggested that acupuncture might regulate content of motilin to influence gastric motility.

Somatostatin can inhibit almost all the internal and external secretion and regulate other neuropeptides and neurotransmitters<sup>[21]</sup>. It can also inhibit release of gut hormones such as motilin. Somatostatin has been found in peripheral nerve, gastrointestinal mucosa and other parts of the brain besides the hypothalamus<sup>[22]</sup>. The chief biological effect of somatostatin on digestive system is inhibition. It can inhibit motility of stomach intestine and gall bladder, proliferation of mucosal cells; secretion of digestive juice, for example, gastric acid,

and gut hormones such as motilin, CCK, VIP, pancreatic polypeptide (PP) and gastric inhibitory peptide. It can also suppress absorption of nutrient substances such as electrolytes by small intestine, secretion of glicentin and release of growth hormone<sup>[23]</sup>. The reports of somatostatin in ulcer are not accordant. Sun *et al*<sup>[24]</sup> found content of somatostatin in serum and sinus ventriculi of gastric ulcer model group was lower than that of normal group. Increased content of somatostatin in gastric mucosa of ulcerated rats and decreased content of somatostatin in the rats after acupuncture have also been reported<sup>[25]</sup>. Xu *et al*<sup>[26]</sup> found content of serum gastrin increased and somatostatin decreased during spontaneous healing of stress ulcer in rats. The results of our study showed the content of somatostatin in sinus ventriculi and bulbus medullae increased after the gastric mucosa was damaged but increased after acupuncture. On the one hand, a compensative feedback effect diminished because acupuncture relieved the damage of gastric mucosa. On the other hand, somatostatin could inhibit synthesis of DNA and have a down-regulating effect on cell proliferation. The results suggested acupuncture at acupoints of Foot-Yangming Meridian could regulate the content of somatostatin to promote healing of gastric mucosal damage.

To sum up the above arguments, electro-acupuncture at acupoints of Foot-Yangming Meridian could regulate content of motilin and somatostatin in sinus ventriculi and bulbus medullae, improve gastric motility and gastric mucosal blood flow. But acupuncture at different acupoints had different effects on gastrointestinal peptide. Acupuncture at Sibai points regulate content of motilin and somatostatin mainly in sinus ventriculi, while acupuncture at Zusanli points regulate content of somatostatin in sinus ventriculi but motilin in bulbus medullae. It suggested that effects of acupuncture at different acupoints on gastric motility were related to different brain-gut peptides.

Effect of acupuncture at acupoints of Foot-Yangming Meridian on gastrointestinal functions was considered to be related to neural and humoral factors<sup>[27]</sup>. Our previous study demonstrated acupuncture at acupoints of Foot-Yangming Meridian could change the release of peripheral brain-gut peptide to protect gastric mucosa<sup>[28-30]</sup>. It could be deduced that Foot-Yangming Meridian was correlated with peptidergic nerve of stomach. The results of this study showed acupuncture at acupoints of Foot-Yangming Meridian could regulate content of motilin and somatostatin in sinus ventriculi and bulbus medullae, which suggested electro-acupuncture regulated gastric motility through regulating release of central and peripheral neuropeptides. The effect of acupuncture on viscera was slow and persistent which was similar to the active mode of neuropeptides as neurotransmitters. It further proved the correlation between Foot-Yangming Meridian and peptidergic nerves.

## COMMENTS

### Background

In recent years, there have been more studies on effect of acupuncture on brain-gut peptide. Whether acupuncture could regulate digestive tract motility

by influencing content of brain-gut peptide remains a problem to address. Acupuncture at Foot-Yangming Meridian could regulate gastric myoelectricity and increase content of motilin and gastrin in plasma and sinus ventriculi. The effects of acupuncture on gastrointestinal tract were related to brain-gut peptide.

### Research frontiers

Foot-Yangming Meridian is an important meridian of human body. The authors investigated whether acupuncture at Foot-Yangming Meridian could regulate gastrointestinal motility and content of brain-gut peptide in sinus ventriculi and bulbus medullae.

### Innovations and breakthroughs

The effect of acupuncture at acupoints of Foot-Yangming Meridian on gastric motility and content of brain-gut peptide was observed. It provided scientific references for correlation between Foot-Yangming Meridian and brain-gut peptide.

### Applications

Acupuncture could improve gastric motility and gastric mucosal blood flow, and regulate content of motilin and somatostatin in sinus ventriculi and bulbus medullae. Acupuncture at acupoints of Foot-Yangming Meridian could protect gastric mucosa by regulating content of brain-gut peptide.

### Terminology

Brain-gut peptide: Brain-gut peptide as a kind of neurotransmitter exists in brain and gastrointestinal tract.

### Peer review

This paper tries to explore the mechanism of this connection using electric-acupuncture to stimulate Foot-Yangming Meridian and investigate various responses of the stomach model. The idea of this study is novel and innovative.

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