SITE OF EMETIC ACTION OF ORAL COPPER SULFATE IN DOGS (I) THRESHOLDS OF VARIOUS PORTIONS OF GASTROINTESTINAL TRACT TO LOCALLY APPLIED COPPER SULFATE

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Abstract—Emetic thresholds to copper sulfate administered into the Pavlov pouch, Forrest pouch, Thiry fistulas of the jejunum and ileum, and duodenal, jejunal and ileal catheters were measured in dogs to conjecture the site of emetic action of copper sulfate. The oral emetic threshold had been measured preoperatively. In the stomach, the pyloric antrum had a high sensitivity, while the corpus had a low sensitivity to the topically applied copper sulfate. In the intestine, the sensitivity was high in the duodenum, whereas a low sensitivity was noted in the jejunum. Almost no sensitivity was observed in the ileum. Thus it would appear that the site of the emetic action of copper sulfate was the pyloric antrum and/or duodenum.

Copper sulfate has long served as a prototype of the so-called peripherally acting emetics. According to Wang and Borison (1), a low oral dose stimulates the gastrointestinal tract to elicit vomiting as the gut-denervated dog fails to respond, whereas a dose of over 320 mg/head also affects central emetic mechanisms. The site on which copper sulfate acts in the gastrointestinal tract has not been elucidated.

Emetic thresholds of the various portions of the gastrointestinal tract to the locally applied copper sulfate are described herein and a comparison is made of the related sensitivities.

MATERIALS AND METHODS

Animals

Mongrel dogs, weighing 8 to 14 kg, of either sex, 0.5 to 1.5 years old and free from abnormal clinical signs, were obtained from a pound and bathed and quarantined for the close examination. In addition to the clinical observations, debris of feces and blood smears were routinely checked regarding eggs, oocysts and microfilariae. Ascaris or whipworm infection was treated with an adequate anthelminthic. Dogs which passed the quarantine were caged separately in a room, fed on commercial dog biscuits once daily and

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water was provided *ad libitum*. The vomiting experiment was carried out once a week after a one day fast.

**Determination of oral threshold**

Table 1 shows the method determining the threshold of oral copper sulfate emesis (2). A measured quantity of copper sulfate crystals was dissolved in 40 ml of distilled water and given by gastric tube into the empty stomach. Dogs were observed for 90 min, and retching, vomiting and latency were noted. Animals with a threshold of 160 mg/head or more were excluded from the experiments. Oral thresholds of copper sulfate emesis in dogs from the Tokyo district were usually high, 40 mg/head in about 40%, 80 mg/head in about 40% of the dogs (2), as compared with those in documentations from the U.S.A. (3-4).

In a group of dogs, the determined threshold dose was dissolved in 10 ml of water and given via gastric tube. If the dog vomited, 1/2 of threshold was tested the following week. Dogs in this group were kept in a constant condition (22±2°C, 55±5% humidity, 10 air changes/hr) during the quarantine and experiments.

**Operations**

Dogs of the first group underwent surgical procedures as illustrated in Fig. 1. Under Pentobarbital anesthesia, the Pavlov pouch, an innervated fundic pouch, and Forrest pouch, an innervated antral pouch were constructed using a spool-shaped hard PVC cannula. The main stomach was separated from the pouches by the mucosal bridges and the continuity was reestablished by the Billroth II operation, a gastrojejunostomy. A PVC tubing was set at the pyloric end of the duodenum and brought out of the body at the shoulder through

| TABLE 1. Determination of threshold for oral copper sulfate emesis in dogs |
| 1st week | 2nd week | threshold |
| 40 mg { vomited | 20 mg { vomited | 20 mg/head |
| 40 mg not vomited | 80 mg { not vomited | 40 mg/head |
| 80 mg { vomited | 80 mg/head | excluded |

**Fig. 1.** Schematic presentation of the operation in the text.

1. Pavlov pouch. 2. Forrest pouch. 3. duodenal catheter. 4. mucosal bridge.
the subcutaneous space and served as a duodenum indwelling catheter. All these animals were given benzylpenicillin benzathine 300,000 units routinely, were deprived of food and water for 2 days and given skim milk for the next 2 days. Fluid balance was maintained by the subcutaneous route for 6 days.

In the second group of dogs, a 2 step operation was carried out. In the first operation, a duodenal catheter at the pyloric end, jejunal catheter at about 5 cm below the duodenojejunal flexure and ileal catheter at the middle of the jejunum-ileum were inserted. After the determination of emetic thresholds to the intracatheter instillation of copper sulfate, a jejunal and/or ileal Thiry fistula was constructed. For the fistula, about 15 cm of the intestine was isolated from the middle of the jejunum or ileum, and the oral end was closed by a purse-string suture, the anal end was connected to a PVC cannula, the other end of which was protruded out of body through a cutaneous stab wound. The continuity of the intestine was restored by end-to-end anastomosis.

The postoperative recovery was assured by absence of infection and return of the normal appetite, and the animals were allowed one week of rest. Main postoperative occurrences were falling off of the cannula and rupture of the mucosal bridge. In such dogs, enucleation of the pouch was carried out after which the animal was excluded from the experiment.

Measurement of threshold by topical application

Copper sulfate dissolved in 10 ml of water was administered. For the application to a pouch or fistula, a cylindrical stopper with a PVC tubing in the center was used to plug the cannula, and all administered solution entered the pouch. The initial doses were 1/2 of the oral threshold for the antrum and duodenum, the oral threshold for the fundus and twice the threshold for the jejunum and ileum. If the dog vomited, 1/2 of the initial dose was given the following week. Unresponsive dogs were challenged with double the initial dose. A dose over 160 mg/head was never given since the central emetic mechanism may be effected.

Anatomical examination

After the experiments, 3 dogs of the first group were sacrificed with an overdose of Pentobarbital, the whole body was immersed in formalin solution for one month and then the tissues were dissected out in such a way so as to preserve the main branches of the vagus nerves to the pouches.

Drugs

Reagent grade copper sulfate was used and is referred to as anhydrate salt. The penicillin used was from a commercial vial.

RESULTS

Stomach

Results obtained are summarized in Table 2. In the Pavlov pouch, the oral thresholds were not effective in eliciting vomiting, but double the dose were usually effective. Threshold
doses in the Forrest pouch were the same as or 1/2 of the oral thresholds, with short latencies.

**Intestine**

In dogs with gastric pouches, the duodenum was separated from the stomach and copper sulfate was injected into the stump through the cannula. As is shown in Table 2, 40 mg/head, oral threshold or half, were effective, and extremely shorter latencies were noted. Results obtained from the dogs with the intestinal catheters and fistulas are summarized in Table 3. The threshold dose for the intraduodenal injection was also 40 mg/head, with a shorter latency. When copper sulfate was administered into the upper jejunum, only 1 out of 6 dogs responded when 4 times the oral threshold was given. A large dose given into the jejunal fistula caused vomiting in 2 out of 3 dogs. Neither intralveal administration nor application in the ileal fistula of a large dose evoked vomiting.

**Volume of administration on the oral threshold**

As shown in Table 4, the oral threshold doses of copper sulfate in 40 ml and 10 ml of solution were the same, but the latency with the smaller volume was significantly shorter than that with the usual volume of 40 ml (P<0.05, Student's t-test). No dog responded to

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**Table 2.** Emetic threshold doses of copper sulfate administered into gastric pouches and duodenal cannula

| dog number | oral (mg/head) | latency (min) | fundus (mg/head) | latency (min) | pylorus (mg/head) | latency (min) | duodenum (mg/head) | latency (min) |
|------------|----------------|---------------|------------------|---------------|------------------|---------------|-------------------|---------------|
| 1          | 40             | 8             | 80               | 10            | 40               | 4             | 40                | 4             |
| 2          | 80             | 13            | 160              | 9             | 40               | 7             | 40                | 3             |
| 3          | 80             | 19            | 160              | 17            | 40               | 12            | 40                | 4             |
| 4          | 40             | 12            | 80               | 10            | 40               | 7             | 40                | 4             |
| 5          | 80             | 21            | 40               | 21            | 40               | 9             | 40                | 3             |
| 6          | 80             | 11            | 40               | 9             | 40                | 6             | 40                | 3             |
| 7          | 40             | 22            | 40               | 17            | 20               | 16            | 40                | 3             |
| 8          | 40             | 17            | 40               | 8             |                   |               |                   |               |

**Table 4.** Effect of administration volume on the emetic threshold of oral copper sulfate

| dog number | 40 ml (mg/head) | latency (min) | 10 ml (mg/head) | latency (min) |
|------------|-----------------|---------------|-----------------|---------------|
| 15         | 20              | 24            | 20              | 12            |
| 16         | 40              | 12            | 40              | 6             |
| 17         | 40              | 9             | 40              | 6             |
| 18         | 40              | 24            | 40              | 6             |
| 19         | 80              | 12            | 80              | 9             |
| 20         | 80              | 11            | 80              | 6             |

| mean±S.D. | 15.3±6.2        | 7.5±2.3       |

Copper sulfate was dissolved in 40 ml or 10 ml of water.
Table 3. Emetic threshold doses of copper sulfate administered into intestinal catheters and fistulas

| dog number | oral   | duodenum | intracatheter | ileum | intrafistula |
|------------|--------|----------|---------------|-------|--------------|
|            | dose (mg) | latency (min) | dose (mg) | latency (min) | dose (mg) | latency (min) | dose (mg) | latency (min) | dose (mg) | latency (min) |
| 9          | 20     | 21       | 80           | nv    | 80           | nv          |
| 10         | 80     | 22       | 160          | nv    | 160          | nv          | 160       | nv          |
| 11         | 80     | 14       | 160          | nv    | 160          | nv          | 160       | nv          |
| 12         | 40     | 21       | 40           | 8     | 160          | 17          | 160       | 42          |
| 13         | 80     | 17       | 40           | 4     | 160          | nv          | 160       | nv          | 160       | nv          |
| 14         | 40     | 15       | 40           | 6     | 160          | nv          | 160       | 80          | 60        | 160         |

mean±S.D. 18.3±3.4  6.0±2.0  17  51.0±12.7

nv: not vomited
1/2 of the threshold.

DISCUSSION

The purpose of this study was to determine the site of emetic action of copper sulfate from various portions of the gastrointestinal tract. Limitations as the result of experimental conditions have, however, to be given consideration.

1. Although the Pavlov pouch, Forrest pouch and Thiry fistula are innervated, there are possible minor abscessions of the nerve supplies to the tract. Since vagotomy at the heart level plus total sympathectomy abolishes the emetic reflex by oral copper sulfate in dogs (1), all the afferent nerves related to the reflex are considered to be included in both nerves. According to the Mizeres' detailed description (5), the main branches of the both nerves were preserved in our animals. The accessory branches, however, seemed to be cut.

2. Dogs are probably in a state of stress after such major surgery and the gastrointestinal tract is probably more irritable than usual.

3. The intraluminal environment of the pouch or fistula is quite different from the normal lumen. For example, the mucosal surface of the normal pyloric antrum is exposed to a profuse amount of food, saliva and fundic secretion, while that of the antral pouch is exposed only to its own poor alkali secretion. Indeed, there is a possibility that the responsiveness of a portion may be determined by an intraluminal environment, not by the true tissue sensitivity.

Therefore, the exact inference of the site of action from a simple comparison of the thresholds of various portions on the strength of the oral threshold is not justified.

In the stomach, the fundus showed a low sensitivity. Since the fundus is capable of profuse acid secretion, dilution of the administered solution may to some extent be responsible for the low sensitivity. The high sensitivity of the pyloric antrum may be due to the poor alkali secretion. Despite these limitations, we concluded that the pyloric antrum might have a higher sensitivity than the fundus.

While the jejunum or ileum showed a very low or practically no sensitivity, an extremely high sensitivity was found in the duodenum. Lately we repeated the experiment under well controlled and more physiological conditions, and found that the oral thresholds instilled into the duodenum were always effective in eliciting vomiting with short latencies, and 1/2 of the thresholds were not effective in the most cases (6). Therefore, an increased irritability of the intestine after major surgery may explain such a finding.

The fistula construction from the duodenum is an extremely difficult operation in which the duodenum has to be freed from its tight fixation and the bile and pancreatic ducts are then connected to the jejunum.

In spite of the limitations, there was a sufficient difference of sensitivity between the parts of the small intestine. Other workers have also reported irritating the duodenum to evoke vomiting (7-9).

The reason we used 10 ml instead of 40 for local applications was the high emetic sensitivity of the pyloric pouch to distension. Therefore, the effectiveness of oral admin-
istration of 10 ml was compared with that of the routinely used 40 ml solution. A smaller volume i.e. a higher concentration was equally effective, but with a significantly shorter latency. Usually, we can expect a latency of more than 10 min after administration of 40 ml of intragastric copper sulfate and can discard data with a latency of less than 5 min, as such may be a response of pharyngolaryngeal origin (2). Thus, use of 40 ml for oral administration seems reasonable.

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