Vomiting is a complicated act which involves the coordinated activities of many motor systems of the body including both the somatic and autonomic nervous outflows. The orderly sequential action of the different muscle groups used in emesis has been clearly demonstrated by the now classical work of Cannon (1). Equally complex is the question of the sensory pathways of vomiting. The roles of the numerous afferents investigated by various workers on this problem are not in agreement. Our investigation concerns the essential sensory pathways for emesis arising from the gastrointestinal tract.

Miller (2) studied the afferent nerves in the emetic action of mustard, and concluded that the vagi alone were responsible for this function, since immediately following vagotomy emesis was no longer elicitable; this was not the case after section of the splanchnic nerves. The importance of afferents in the vagus was further stressed by Bayliss (3) who produced vomiting by intraperitoneal injection of staphylococcus enterotoxin and found that emesis rarely occurred following division of the vagi in the acute preparation. Goldberg (4) induced vomiting by distending an isolated pyloric pouch and found that this reflex also disappeared after vagotomy. On the other hand, many workers (5) observed that vagotomy did not interfere with vomiting in man. In addition, Walton, Moore and Graham (6) reported that in order to prevent the vomiting of experimental peritonitis, they had to section both the vagi and the splanchnic nerves.

The present report represents the results of a corollary study made during the course of experimental work on the vomiting center (7), in which a considerable quantity of data were amassed on the action of copper sulphate in the elicitation of emesis. This information affords the opportunity for evaluating the relative importance of the various visceral afferent components in vomiting arising from the gastrointestinal tract, and in addition, has uncovered other emetic properties of copper sulphate.

**METHODS**

Experiments were carried out on 11 mongrel dogs weighing from 6 to 10 kg. They were tested for the emetic response to intragastric copper sulphate in order to ascertain the threshold dose. A measured quantity of copper sulphate crystals...
(CuSO$_4$$\cdot$5H$_2$O) was dissolved in 50 cc. of distilled water and given by tube to an empty stomach. For normal dogs, 40 mg. of copper sulphate (calculated without including the weight of water) was given as a first trial. If the animal responded, the period of latency was noted and a second test with 20 mg. was tried on a different day. If, on the other hand, the animal failed to respond to the initial 40 mg., the test was usually repeated. If it failed again, twice the dosage (80 mg.) was given at a later date. None of the normal fasted dogs tested at this last dosage level failed to respond.

After the emetic threshold of copper sulphate was obtained, all dogs (one dog already had a bilateral medullary lesion in the dorsal region of the ala cinerea) were subjected to lower thoracic and abdominal sympathectomy or trans-thoracic vagotomy. When the animal had fully recovered from the acute operative effects, the threshold to copper sulphate was re-evaluated. Then denervation of the gastrointestinal tract was completed either by vagotomy or sympathectomy as was required in each case. For reasons to be discussed, some of these animals were subjected to another operative procedure later either for an end-to-end anastomosis of the divided esophagus or transection of the cauda equina.

Copper sulphate in amounts above 80 mg. when repeatedly administered by mouth and retained by the operated animal was prone to cause anemia (8) and bloody diarrhea. For this reason, the threshold determination during the postoperative period had to be executed judiciously by selecting the appropriate dosages for trial and also by spacing the tests at long intervals.

Sympathectomy was carried out in two stages according to the method of Cannon et al. (9), from T$_8$ to the last sacral ganglion inclusive bilaterally. Trans-thoracic vagotomy was performed through an incision in the sixth intercostal space on the left side; under artificial respiration, the thoracic cavity was opened and the esophagus elevated to the body surface. Three large vagal trunks (at this level, one anterior and two posterior branches) were easily identified and a portion of about one cm. in length was resected from each. The esophagus was then released and the thorax closed in the usual way. As concerns the operation for making the superficial lesion in the medulla oblongata, the method has been described fully in an early communication (7).

In all cases, apomorphine threshold was also determined during the pre- and postoperative periods. Apomorphine hydrochloride was administered via the intravenous route. Details on the testing procedure, dosage levels and response have been reported elsewhere (7).

After completing the individual experiments, each animal was sacrificed and subjected to autopsy in order to ascertain the completeness of the surgical treatment and to check for possible nerve regeneration. Brain lesions were examined histologically.

**Results**

The postoperative course of the 11 dogs used in this series was uneventful with the exception of one dog, which subsequent to vagotomy developed a bleeding ulcer of the right cornea and had to be sacrificed. Several of the dogs following the operation for bilateral vagotomy showed spontaneous vomiting, sometimes persistent for
a number of days; but eventually all overcame this emetic episode. Polyphagia of variable degree was evident in most of the vagotomized dogs. The stool was bulky and did not differ greatly in appearance from that of the ingested food. In order to insure complete gastric evacuation before the copper sulphate tests were to be made on these animals, it was advisable to withhold food for more than 24 hours.

Typical experimental data on individual animals are illustrated with two protocols which follow. A survey of the results for the entire series is presented in table I.

**Dog 2.** Female, weight 6.0 kg. Copper sulphate tests:

Preoperative: Vomited to 40 mg., latency 7 minutes. Did not vomit to 20 mg.

Postoperative:

- **Sympathectomy:** Day 36: Vomited to 40 mg., latency 10 minutes; day 43: Did not vomit to 20 mg.
- **Vagotomy** (day 51): Day 70: Did not vomit to 160 mg.; day 76: Vomited to 320 mg., latency 2 hours; day 115: Vomited to 320 mg., latency 2.5 hours.
- **Transection of cauda equina** (day 117): Day 122: Vomited to 320 mg., latency 2.5 hours.

Apomorphine hydrochloride threshold remained at 0.1 mg. throughout.

The dog was sacrificed on the day 122 and autopsy showed no regeneration of the sectioned nerves.

**Dog 10.** Male, weight 8.0 kg. Copper sulphate tests:

Preoperative: Vomited to 40 mg., latency 9 minutes, and also to 20 mg., latency 20 minutes.

Postoperative:

- **Vagotomy:** Day 13: Vomited to 80 mg., latency 27 minutes; days 14 and 23: Did not vomit to 40 mg.
- **Sympathectomy** (day 44): Day 55: Did not vomit to 160 mg.; day 63: Vomited to 320 mg., latency 3.2 hours.
- **Ablation of the emetic “trigger” zone in the ala cinerea** (day 94): Day 117: Did not vomit to 480 mg.

Apomorphine hydrochloride threshold remained at 0.05 mg. until lesions in the medulla were accomplished. Then, no vomiting was obtained following 5.0 mg., the largest dose tried on this animal.

The dog was sacrificed on the day 117 and autopsy showed no regeneration of the sectioned nerve. The lesions in the medulla are similar to the superficial lesions described in a previous communication (7).

It can be seen from table I that elimination of the lower thoracic and lumbar chains did not influence the threshold emetic dose of the intragastric copper or the latency of the response (see protocol of dog 2). Bilateral vagotomy alone, on the other hand, increased both the threshold and latency of emesis (see protocol of dog 10). In fact, it was observed in each of the 6 vagotomized dogs that the threshold was about doubled. All of the 9 animals following both operations, i.e., vagotomy and sympathectomy, showed a marked increase of threshold and extremely prolonged latency (see protocols). Vomiting, however, always occurred with remarkable reproducibility when 320 mg. of copper sulphate was delivered into the empty stomach. Neither esophageal anastomosis nor transection of cauda equina in addition to the above operative procedures modified this response. However, vomiting was not at all elicitable in the two dogs in which ablation of a small area in the dorsal region of the ala cinerea was also accomplished, even though a dose of 480 mg. of copper sulphate was administered orally. It is important to note that none of the procedures for peripheral denervation did alter the vomiting response of these animals to intravenously administered apomorphine.
According to Alvarez (5), much of the research work done on pathways in the vomiting mechanism is of doubtful value because of the great difficulties of interpretation. Operations on the brain, spinal cord and nerves are accompanied by a state of depression which, for some time, can so diminish the activity of structures near the operative site that erroneous conclusions are likely to be drawn. Another obvious difficulty with past investigations is that the techniques used did not permit the quantitative study of changes in threshold following an operative procedure. Indeed, for producing emesis by means of experimental peritonitis (6), it had not been possible even to repeat the observations on the same animal.

Using copper sulphate, Opackowski (quoted by Hatcher, 10) reported failure to induce emesis after vagotomy in the dog. However, Hatcher and Weiss (11) elicited

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>NO. OF DOGS</th>
<th>THRESHOLD</th>
<th>LATENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11</td>
<td>40 mg.</td>
<td>20-80</td>
</tr>
<tr>
<td>Sympathectomy</td>
<td>4</td>
<td>30 mg.</td>
<td>20-40</td>
</tr>
<tr>
<td>Vagotomy</td>
<td>6</td>
<td>80 mg.</td>
<td>40-160</td>
</tr>
<tr>
<td>Vagotomy and sympathectomy</td>
<td>9</td>
<td>320 mg.</td>
<td>320</td>
</tr>
<tr>
<td>Vagotomy, sympathectomy and esophageal anastomosis</td>
<td>3</td>
<td>320 mg.</td>
<td>320</td>
</tr>
<tr>
<td>Vagotomy, sympathectomy and transection of cauda equina</td>
<td>4</td>
<td>360 mg.</td>
<td>320-480</td>
</tr>
<tr>
<td>Vagotomy, sympathectomy and brain lesion</td>
<td>2</td>
<td>&gt;480 mg.</td>
<td>Nu emesis</td>
</tr>
</tbody>
</table>

vomiting with large doses of copper sulphate in 4 acutely vagotomized cats. They attributed this difference in results to the fact that they had not sought to determine the effect of vagal interruption with minimal doses of this substance.

Since publication of a previous article (7), we have extended the data on copper sulphate threshold and latency of emesis to 107 normal dogs, weighing from 6 to 10 kg. Ninety-one of them vomited to 40 mg. of copper sulphate in 50 cc. of distilled water given via stomach tube, with an average latency and standard deviation of 16 ± 9 minutes, and of these only 20 responded also to 20 mg. with a mean latency of 19 ± 11 minutes. On the other hand, all of the 16 dogs which did not respond to 40 mg. of copper sulphate vomited after the administration of 80 mg. with a latency of 19 ± 7 minutes. The large individual variation in latent period with low copper sulphate levels is another indication of the threshold nature of the dosage used, for with large doses, as with 160 mg. or more, the range of latency is narrow (4 to 10 minutes). Thus, 40 mg. of copper sulphate is an effective dose for emesis in 85 per cent of normal dogs, although 20 mg. is sufficient to produce vomiting in about one-fourth of these animals;
the latency has never exceeded 45 minutes. It is true that copper sulphate is relatively toxic, but if the test is used carefully it may be repeated almost indefinitely. These data serve as a sound basis for studying quantitatively the effect on emesis of any subsequent operative procedure in chronic animals.

For the 11 dogs used in this series, individual control copper sulphate tests yielded an average threshold value of 40 mg. with a latency of 12 minutes. In 6 dogs in which copper sulphate tests were repeated a number of days after vagotomy there was observed an increase both in threshold and in latency to roughly twice that of the preoperative values. On the other hand, for emesis due to intravenously administered apomorphine neither the threshold nor the latency was changed by this operative procedure. Thus, the failure of the vagotomized dogs to vomit following the administration of the preoperative effective dose of copper is not to be explained by the possible cardiospasm resulting from bilateral section of the efferent vagal fibers. Indeed, Hwang, Essex and Mann (12) maintained that the response to appropriate doses of apomorphine was exaggerated by vagotomy. Thus, the increase in minimal effective dosage of copper sulphate for emesis required by these operated animals must be due to the elimination of an important afferent component in the reflex mechanism for emesis.

Although lower thoracic and abdominal sympathectomy by itself produced no discernible change in the response to intragastric copper, elimination of these nerve pathways in addition to vagotomy caused a sharply pronounced increase in emetic threshold as well as an extreme lengthening of the latent period of response. In all 9 animals with completely denervated gastrointestinal tracts, emesis was obtained with unusual regularity but only following the oral administration of 320 mg. or eight times the normal average threshold of copper sulphate. Again, in no case was the apomorphine threshold altered.

The very long latency before vomiting by these gut-denervated animals suggested certain possibilities for additional routes of action by the copper. Since it has been reported that there may be intrinsic vagal fibers in the esophageal wall (12, 13), the esophagus in 3 of the gut-denervated animals was divided and its continuity resumed by an end-to-end anastomosis to interrupt unquestionably all central connections of the vagus nerve. This procedure did not eliminate the copper sulphate response nor did it influence apomorphine emesis. It also seemed possible that when the copper sulphate reached the lower part of the large bowel, deep vomiting might occur via stimulation of the sacral afferent system. But this is not the case, since subsequent section of the cauda equina in 4 of the gut-denervated dogs did not abolish the delayed response to intragastric copper. Another possibility was that the copper might have been absorbed into the systemic circulation during the long period of

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3 This was not supported by our experience with intravenously administered apomorphine. We found that the threshold remained unchanged after vagotomy (average, 0.1 mg., range, 0.05 to 0.2 mg. for dogs weighing from 6 to 10 kg. (7)). Hwang, et al. (12) gave apomorphine by the subcutaneous route with a dosage about two to three times greater than the threshold value which we used via the intravenous route. Nevertheless, a careful examination of their data does not substantiate the impression given by these workers that the sensitivity of the vagotomized animals to apomorphine is exaggerated since neither the threshold nor the latency of response was significantly changed.
latency and caused direct excitation of the central emetic mechanism. In support of this view is the fact that when copper is given orally it reaches its peak level in the blood of normal dogs (14) about the same time (1 1/2 to 2 1/2 hours) as the occurrence of the vomiting response in the gut-denervated dogs. Furthermore, we have found that copper is also a potent 'central' emetic since vomiting invariably occurred following the intravenous injection of 32 mg. of copper sulphate in normal as well as in the gut-denervated animals (15). We have demonstrated that i.v. injected copper acts on a chemoreceptor 'trigger' zone for vomiting located in the dorsal region of the ala cinerea; ablation of this specialized group of cells results in a preparation which is permanently refractory to both apomorphine and copper given i.v. (15). Final proof that central action of the absorbed copper is responsible for the delayed emesis in the gut denervated dogs is supplied by two experiments (see table 1, last condition) in which dogs with lesions of the dorsal region of the ala cinerea in addition to sympathectomy and vagotomy are shown to be completely refractory to as much as 450 mg. copper sulphate (or 750 mg. of CuSO₄ · 5H₂O) given to the empty stomach. Only by such means is it possible to eliminate totally the vomiting response to orally administered copper sulphate. It is important to stress here that ablation of the central emetic 'trigger' zone alone does not in any way alter the normal vomiting response of copper given by mouth (7).

Inasmuch as the emetic mechanism of copper is complex and the latency for emesis is relatively long, especially with the threshold dose (up to 45 minutes in some normal animals), it is difficult to assess the importance of the rise in serum copper level during this interval in facilitating the normal oral copper emetic response. According to Sachs et al. (14) the rise in serum copper in the first half hour is small. It is also known that the serum copper level in pregnancy and disease is raised (16, 17); the significance of this fact in explaining the emetic tendencies of patients in such conditions is not clear. All of these phenomena require further investigation.

**Summary**

The action of copper sulphate in causing emesis is twofold, that is it has a central as well as peripheral effect. In acting peripherally, it has been shown that afferents in both the vagus and splanchnic nerves are of importance. Interruption of the vagi had a more profound effect on the threshold and latency of vomiting than did sympathectomy which caused no discernible change. In animals with both abdominal sympathectomy and vagotomy, not only the threshold to copper sulphate was markedly increased (8-fold) but also its latency was greatly prolonged (2 hours or longer). There is no evidence of interference by the cardiac sphincter in causing this phenomenon, since the emetic threshold of apomorphine given i.v. remains unchanged.

Attempts to eliminate entirely the residual delayed response to intragastric copper by sectioning the intrinsic vagal fibers in the esophageal wall or interrupting the sacral innervation to the large bowel were of no avail. This leaves the one remaining possibility that copper is slowly absorbed into the systemic circulation and exerts an action as a central emetic. Evidence has been presented to show that this is the most probable explanation for the delayed emesis. Most convincing is the fact that
ablation of a central emetic 'trigger' zone in addition to gut-denervation totally eliminates the emetic response to lethal dose of intragastric copper sulphate.

REFERENCES