STUDIES ON THE MECHANISM OF VOMITING PRODUCED BY STAPHYLOCOCCUS ENTEROTOXIN

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Among the toxins produced by certain strains of staphylococci is one which is capable of causing food poisoning symptoms in man. Although a number of workers including Denyes (1), Owen (2), and Barber (3) had called attention to the etiological rôle of staphylococci in certain outbreaks of acute illness characterized by nausea, vomiting, and diarrhea, it was not until the work of Dack (4) and Jordan (5) and their coworkers that interest was stimulated in staphylococcus food poisoning. These later investigators demonstrated that the symptoms were due to a filterable toxin which was thermostable. This toxin was found to be quite distinct from the hemolytic, lethal, and dermo-necrotic toxins which are relatively thermolabile. Since its main effect seemed to be on the gastro-intestinal tract, it was termed enterotoxin.

The clinical symptoms in man usually begin 2 to 4 hours after ingestion of the contaminated food, and include dizziness, nausea, vomiting, diarrhea, and weakness. Recovery is rapid, and the individual feels practically well 24 hours later. Fatalities are rare, and are probably caused by an actual tissue invasion by the staphylococcus rather than by the enterotoxin alone. In the fatal case reported by Blackman (6), the illness began with the above symptoms, but at necropsy a staphylococcal infection of the jejunum and ileum was found.

The investigation of enterotoxin-producing organisms has been retarded by the fact that, until recently, the only known susceptible subjects were man and the monkey, and these exhibited considerable variation in the response to enterotoxin. In 1936, Dolman, Wilson, and Cockcroft (7) demonstrated that kittens injected intraperitoneally with formalinized or

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boiled filtrates from certain strains of staphylococci developed marked lassitude, weakness, vomiting, and diarrhea. Numerous workers (8) have verified the validity of this test for the presence of enterotoxin.

Since vomiting is one of the most conspicuous signs of the effect of enterotoxin on man and lower animals, experiments have been designed to determine the mechanism of this activity. In the present paper are presented the results of an investigation of the physiology of emesis in the cat produced by the parenteral introduction of staphylococcus enterotoxin.

Preparation of Enterotoxin

Staphylococcus enterotoxin was prepared according to the method of Dolman and Wilson (9) using a strain of food poisoning staphylococcus U.D. received from Dolman. The organism was grown in semisolid agar medium for 40 hours at 37°C. The toxin was freed of agar by passing through filter paper. After Seitz filtration to remove the bacteria, the filtrate, which contained the enterotoxin, was held at 0–5°C. until needed. Just before use the preparation was placed in a boiling water bath for 20 minutes to destroy the lethal toxin. The material was then tested for potency by intraperitoneal injection into cats in a dose of 1.0 cc. per kilo. Any preparation which did not produce vomiting was discarded.

Enterotoxin Syndrome in Cats

Following the intraperitoneal or intravenous administration of 1.0 cc. per kilo of boiled enterotoxin, there is a period of 5 to 100 minutes (usually 15 to 30 minutes) in which the cat appears and acts normal. This is followed by a period frequently ushered in by urination and defecation, during which the animal shows symptoms of apparent unsteadiness, weakness, and drowsiness, with loss of appetite. This period, possibly corresponding to the subjective symptom of nausea in human beings, may be of variable duration, and when the dosage is small or the enterotoxin of low potency, no further symptoms may develop. Shortly before emesis occurs, the animal usually exhibits marked restlessness. It begins to lick its lips and show symptoms of salivation. The act of vomiting is ordinarily preceded by a few contractions of the abdominal musculature. Intermittent vomiting usually begins 20 to 60 minutes following the injection. It has been observed as early as 5 minutes, and as late as 2 hours after intraperitoneal administration. There may be from one to ten spells at intervals of 2 to 30 minutes. Only rarely does vomiting persist for more than an hour, although one cat was noted to vomit as late as 24 hours after a single injection. Following emesis, the cat usually shows evidence of marked lassitude, weakness, and diarrhea, and fails to eat for a period of 2 to 24 hours. Occasionally an excessive dose may produce progressive

symptoms ending in death. Recovery, however, commonly takes place rapidly, and in most cases the animal appears normal in all respects the following day. When the dosage was regulated according to body weight, essentially similar results were obtained in cats varying from 350 to 4500 gm. in weight.

Postmortem examination of an animal dying from toxin or operative procedures following toxin injection has shown an excessive amount of mucus in the entire gastro-intestinal tract. The urinary bladder was always contracted, and the gall bladder usually distended. Other than

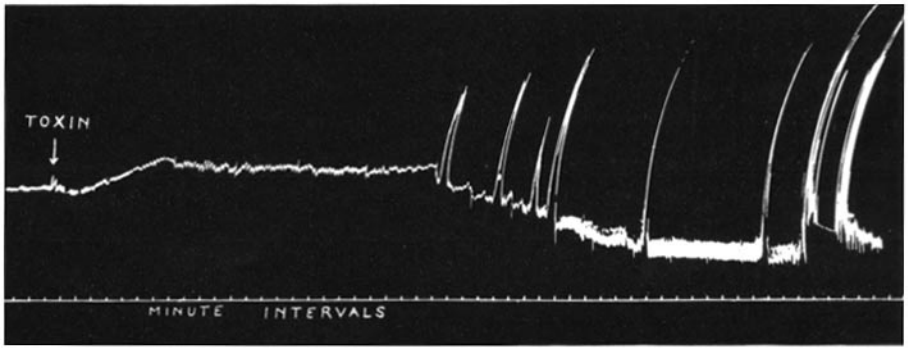

FIG. 1. Intragastric pressure changes following intraperitoneal administration of staphylococcus enterotoxin. The enterotoxin was administered at point labelled "toxin." This was followed by an increased intragastric pressure. After an interval of 25 minutes, slight retching movements began with a gradual decrease of stomach tone. 50 minutes following the toxin injection, frank emesis occurred as indicated by the large excursions at intervals of a few seconds.

this, the gross pathology has not been constant. The necropsy findings confirm those of Dolman, Wilson, and Cockcroft (7).

In order to obtain a better understanding of the rôle of the stomach and the somatic musculature in the emetic act, kymograph records were made of intragastric pressure following enterotoxin injection. In a group of five cats, a stomach tube with a balloon was inserted by way of the mouth. The balloon was filled with air, and then connected with a recording tambour marking on a smoked drum. The animals were kept under deep pentobarbital anesthesia, since in light anesthesia the control animals showed vomiting signs when the stomach tube was inserted. Enterotoxin was given intraperitoneally in doses of 2 cc. per kilo. A typical kymograph record of the emetic activity is shown in Fig. 1.

As indicated by this record, a few minutes following the intraperitoneal injection of enterotoxin, there is a gradually increasing tonicity of the

stomach musculature. This reaches its maximum level in about 5 minutes' time, and is then maintained at this level for a 10 to 30 minute period. Eventually retching movements begin, as indicated by the sudden upward excursion of the kymograph marker. It should be noted, however, that in the intervals between the retching movements a gradual decrease in gastric tonus takes place. This decrease in tone is probably due to the relaxation of the cardiac portion of the stomach. These observations are consistent with those of Cannon (10) who has shown by means of the x-ray that the rôle of the stomach in emesis is mainly passive, consisting of relaxation of the cardia, while the expulsive force is produced by contractions of the diaphragm and muscles of the body wall. The retching move-

TABLE I

Occurrence of Emesis in the Cat According to the Route of Administration of Staphylococcus Enterotoxin

Route	Amount per kilo	Number of animals	Result
	cc.		
Oral	30	8	Negative
Subcutaneous	8	6	"
Intramuscular	8	6	"
Intravenous	1–2	8	Emesis
Intracardial	1	8	"
Intraperitoneal	1	>100	"

ments occur at intervals and with increasing severity, finally resulting in emesis when the contractions are powerful and spaced at short intervals.

Effect of the Route of Administration of Enterotoxin

Dack (4), Jordan (5), and Dolman (11) have produced vomiting in human beings by oral administration of as little as 2 cc. of a potent filtrate. Dolman *et al.* (7) as well as Minett (12) have also produced emesis by feeding large amounts (50 cc.) of filtrate to cats. However, only a small proportion of the animals vomited even when fed these large amounts. Davison, Dack, and Cary (13) produced vomiting by intracardial as well as intraperitoneal administration.

In this study potent enterotoxin was administered to kittens orally, subcutaneously, intramuscularly, intracardially, intravenously, and intraperitoneally. In order to inject the kittens intravenously or intracardially, it was necessary to anesthetize them with sodium pentobarbital or ether. The effect of the various routes of administration is shown in Table I. The dosage listed refers to the smallest amount of enterotoxin that regularly

produced vomiting, or the largest amount that failed to produce it in those cases in which emesis did not occur.

The results show that cats are susceptible to enterotoxin when it is introduced into the blood stream or into the peritoneal cavity, and relatively insusceptible when it is given orally, subcutaneously, or intramuscularly. It is probable that these latter routes of administration do not give sufficiently rapid absorption to affect the vomiting mechanism in the cat in the dosages which were employed. Introducing the enterotoxin intravenously appeared to require slightly more enterotoxin, and to produce less severe vomiting signs than intraperitoneal introduction. Approximately the same period of time elapsed between administration of the toxin and the onset of emesis in both intravenous and intraperitoneal methods.

Action on Isolated Intestinal Strips

Small strips of intestine from freshly killed cats and rabbits were placed in aerated Ringer's solution maintained at 37°C. Recordings of the movements of the strips were made on a kymograph record. To the Ringer's solution was added 0.1 per cent to 1.0 per cent amounts of potent enterotoxin. As a control, staphylococcus toxin formed by a non-enterotoxin producer (Wood strain) was used. Both untreated and boiled toxins were tested.

No consistent differences were noted between the intestinal strips treated with enterotoxin and the control strips. Apparently the enterotoxin has no direct action upon smooth muscle.

Influence of Various Drugs on Emesis Produced by Enterotoxin

Experiments were made to determine the effect of a number of drugs on the emetic act in the cat following administration of staphylococcus enterotoxin. Sodium pentobarbital was tested because this drug was used as an anesthetic in some of the experiments reported in this paper. The effect of morphine was tested because this drug is frequently used in the treatment of staphylococcus food poisoning in man, and it is desirable to have some clue regarding its mode of action. Ergotoxine and atropine were tested because these drugs are supposed to paralyze the sympathetic and parasympathetic nervous systems, and because their effect on the emetic action of various chemicals has been extensively studied by Hatcher and Weiss (14). The results of our experiments are summarized in Table II.

Sodium Pentobarbital.—When administered to cats in doses of 25 to 30 mg. per kilo body weight, this drug produced excellent anesthesia and relaxation. The intraperitoneal administration of 2 cc. of enterotoxin per kilo body weight caused vomiting in the animals anesthetized with sodium pentobarbital as regularly and in approximately the

same length of time as in unanesthetized animals. Observations were made on more than 100 cats under pentobarbital anesthesia, and on a greater number without anesthesia.

No attempt was made in these experiments to determine slight differences in the minimal emetic dose, although Phatak and Pentler (15) have recently reported that slightly larger doses of enterotoxin are necessary to produce vomiting when the kitten is anesthetized.

Morphine.—Morphine sulfate was administered to five cats subcutaneously in a dosage of 5 to 10 mg. per kilo body weight. After an interval of 15 to 30 minutes, several times the amount of enterotoxin necessary to produce emesis was administered. Morphine also prevented vomiting when the cat was under sodium pentobarbital anesthesia. In one case the animal vomited once 5 minutes after administration of the morphine. None of the five cats vomited following administration of the enterotoxin.

TABLE II

Effect of Drugs on the Emetic Action of Staphylococcus Enterotoxin

Drug	Dosage of drug per kilo	Number of animals	Result
	mg.		
Pentobarbital	25–50	>100	Emesis
Atropine	1–2	6	"
Ergotoxine	1–2.5	5	Delays or inhibits
Morphine	5–10	5	Inhibits

From these experiments it is evident that morphine greatly inhibits or abolishes the emetic action of enterotoxin.

Ergotoxine.—Five cats were given ergotoxine ethanesulfonate subcutaneously in amounts varying from 1 to 2.5 mg. per kilo. 30 to 90 minutes later, when the ergotoxine was manifesting its effects of marked irritability, two to four times the emetic dose of enterotoxin was injected intraperitoneally. In two animals no vomiting occurred. In the other two, vomiting did take place, but not until 3 to 5 hours after the enterotoxin was given. In one animal, the ergotoxine produced emesis 10 minutes after injection, but no further vomiting occurred after administration of the enterotoxin.

The experiments of Hatcher and Weiss (14) indicated that the main effect of ergotoxin was an inhibition of afferent emetic impulses from any part of the body from which they might arise. There was no evidence that the drug acted directly on the vomiting center. Interpreting the present experiments on this basis, it appears that the action of staphylococcus enterotoxin is principally on the peripheral afferent nervous system.

Atropine.—Six cats were given 1 to 2 mg. of atropine sulfate intramuscularly. 15 to 30 minutes later, following dilation of the pupil with failure to react to light, enterotoxin was injected. All the cats vomited from 15 to 60 minutes following its injection. The symptoms in no way varied from unatropinized cats given enterotoxin.

It is evident that the vomiting reflex in these cats was unaffected by atropine.

Effect of Removal of the Gastro-Intestinal Tract and the Celiac Ganglion

In order to study the rôle of the gastro-intestinal tract in vomiting due to staphylococcus enterotoxin, various portions were removed, and the effect noted. In addition, the sympathetic nerve supply to the abdominal viscera by way of the celiac ganglion was also eliminated.

These experiments were divided into four groups according to the structures removed: (*a*) Removal of the celiac ganglion in two cats following which emesis was produced in the usual manner by enterotoxin. (*b*) In three animals the stomach was removed, with the result that emesis always occurred subsequent to intraperitoneal enterotoxin injection. Although some retching and vomiting of mucus did occur following the gastrectomy, but prior to the injection of the enterotoxin, there was a marked increase in the severity and frequency of these symptoms following the administration of the enterotoxin. (*c*) In two animals the large and small intestine from pylorus to rectum was removed leaving the stomach, the spleen, and part of the pancreas intact. Following intraperitoneal enterotoxin injection, no symptoms were observed in one animal while the other exhibited only slight retching movements. (*d*) Seven cats under pentobarbital or ether anesthesia were eviscerated from the cardia to the lower one-third of the rectum. The spleen and pancreas were likewise removed. Injection of the enterotoxin was either into the inferior vena cava or into the tightly sutured peritoneal cavity. Control animals were injected with an equal quantity of boiled non-enterotoxic toxin. The animals were watched for vomiting signs for 2 or 3 hours. In four animals slight contractions of the abdominal muscles were observed which might be interpreted as mild retching, while three animals exhibited no abnormal movements. Except for the median incision, the abdominal musculature in these animals was intact, as was also the diaphragm and the intercostal muscles. Since it is probable that true emetic movements of these muscles could occur, the absence of such movements must be interpreted as due to lack of adequate stimulation.

Removal of the stomach did not interfere with vomiting produced by enterotoxin. This demonstrates that sensory and motor fibers to and from that organ are not necessary to the emetic act. Moreover, the extensive sympathetic nerve supply to the stomach and intestines by way of the celiac ganglion is likewise not essential since emesis may be induced by enterotoxin in the absence of this ganglion. The removal of the large and small intestine along with the stomach, however, reduces the sensory

impulses to such an extent that marked retching movements are no longer elicited.

Destruction of Vomiting Center

Thumas (16) has described a "vomiting center" located in the floor of the fourth ventricle approximately in the region of the ala cinerea. The observations of Hatcher and Weiss (14) confirm those of Thumas. Attempts were made to induce emesis with enterotoxin following bilateral destruction of this area. The operative approach was made aseptically, under pentobarbital anesthesia. The lesion as produced occupied the region of the floor of the fourth ventricle bilaterally anterior to the obex and involved the ala cinerea, the hypoglossal trigone, and the nucleus solitarius, which contains sensory components of the cranial nerves VII, IX, and X. Anatomists widely agree that extensive visceral afferent terminations of the vagus are also located in the posterior portion of this nucleus. Care was taken to avoid direct injury to the surrounding brain stem and cerebellar structures. Following recovery from the period of narcosis, 2 cc. per kilo of the enterotoxin were injected intraperitoneally. Injections were made as late as 3 days postoperatively. In no experiment did vomiting follow, regardless of the mode or amount of injection, which indicates that this area in the floor of the fourth ventricle is a necessary part of the vomiting reflex.

Injection over the Vomiting Center

In order to test the direct effects of the enterotoxin on the cells of the vomiting center, the following procedure was performed:—

Five cats were placed under sodium pentobarbital anesthesia. With aseptic technique, the muscles covering the occipital bone were dissected apart, and the bone trephined. It was necessary to enlarge the exposure ventrally by rongeuring away the bone down to the foramen magnum. An injection needle was then inserted through the posterior medullary velum, and cerebrospinal fluid withdrawn from the fourth ventricle. 0.2 cc. of toxin was injected into the ventricle immediately over the vomiting center. When no signs of vomiting appeared in 30 minutes, an additional 0.4 cc. was injected. This was repeated at 10 to 30 minute intervals until 2 to 3 cc. had been injected. Care was taken to withdraw cerebrospinal fluid before injecting the toxin, since a rise in intracranial pressure will of its own accord produce vomiting. The muscle and skin were then sutured, and the animal allowed to recover from the anesthetic. The following day the injection schedule tabulated above was repeated, care being taken to avoid continued increased intracranial pressure. In two cats the pial membrane overlying the vomiting center was stripped off prior to the injection of the enterotoxin.

Since vomiting never resulted in any of these animals, they were then checked for possible immunity factors by intraperitoneal injection of 2 cc.

per kilo enterotoxin. This induced vomiting in every case. Autopsies were then done to check for brain injuries, especially for lesions of the floor of the fourth ventricle.

From the experience of others, notably Hatcher and Weiss (14), it is known that emesis can be produced by applying certain drugs to the region designated as the vomiting center. The failure to produce emesis by the application of enterotoxin to this area indicates that either the toxin does not act directly on the center, or else it was prevented from gaining access to the nerve cells. Howe and Peele (17) have shown that the pia mater membrane serves as a barrier to poliomyelitis virus, and the possibility existed that this membrane likewise holds back staphylococcus enterotoxin. That this was not an important factor is indicated by our experiments in which no vomiting occurred even when this membrane was stripped off before the enterotoxin was placed in the fourth ventricle. The slight injury occasioned by removal of the membrane was believed insufficient in itself to damage materially the vomiting center.

Injection of the Enterotoxin Following Lesions of the Spinal Cord

Sympathetic outflow to the abdominal viscera takes place below cervical segments 7 and 8. The question arises as to what spinal cord levels are necessary for the vomiting act to attain completion. Transections of the cord were made at C_7, T_2, and T_5. In these transections all afferent and efferent impulses below the level of the lesion were, of course, eliminated.

Lesions were made with aseptic precautions in nine animals under sodium pentobarbital anesthesia. 2 cc. or more of enterotoxin per kilo of body weight were given intraperitoneally immediately after recovery from the anesthesia, and again 18 to 24 hours later. The animals were carefully watched for evidence of emesis. Table III includes the results of these experiments. Mild retching movements occurred subsequent to total spinal cord transections at C_7, while lesions at T_2 and T_5 allowed emesis to take place. At the conclusion of the experiments the animals were killed and the stomach and the esophagus examined.

The parasympathetic nerve supply and the innervation of the diaphragm is intact with lesions at C_7. However, with a lesion at this level, central control of the sympathetic nervous system, as well as the influence of the central nervous system on the abdominal wall musculature is impaired. With lesions at T_2 or caudad to this, the sympathetic nervous system plays an increasing rôle as far as innervation of the viscera is concerned. According to present anatomical concepts, a lesion at T_2 allows a sympathetic outflow to the stellate ganglia, as well as to the three pairs of cervical ganglia through which fibers pass to visceral structures in the thorax. It is interest-

ing to note that the main sympathetic outflow to the abdominal viscera is by way of greater and lesser splanchnic nerves which arise below the level of a lesion at T_2, hence the sympathetic supply is without central control below this level. The production of emesis by enterotoxin following the above transections of the spinal cord indicates that: (*a*) The abdominal and intercostal musculature which receive innervation from below T_2 are not essential for severe retching movements. (*b*) Sympathetic, parasympathetic, and visceral afferent nerve fibers arising below this level are also not essential for severe retching movements. Since it is the dia-

TABLE III

Effect of Operative Procedures upon Emesis Due to Enterotoxin

Operative procedure	Number of animals	Result
Gastrectomy	3	Emesis
Removal of celiac ganglion	2	"
Removal of small and large intestine	2	Nothing or slight retching
Removal of gastro-intestinal tract	7	" " " "
Ablation of vomiting center	4	No emesis or retching
Application over vomiting center	5	" " " "
Spinal cord transection C_7	3	Mild retching
Spinal cord transection T_2	3	Retching or emesis
Spinal cord transection T_5	3	" " "
Single vagal section	3	Emesis
Double vagal section	8	Usually slight retching
Central nervous system transection between pons and hypothalamus	7	No emesis or retching

phragm without the coordinated abdominal and intercostal musculature which is producing these movements, actual emesis may not occur.

Vagus Nerve Studies

The vagus nerve carries visceral afferent fibers from and parasympathetic fibers to the heart, lungs, esophagus, stomach, parts of the small intestine, and certain other structures. Parasympathetic fibers to the organs in the lumbar and sacral regions come from the sacral segments of the spinal cord. Relaxation of the cardia of the stomach is part of the vomiting mechanism (10) and since the vagus innervates that part of the stomach, experiments were made following destruction of this nerve.

These studies can be grouped into two divisions: (*a*) In three animals one vagus nerve was sectioned. Following recovery from the anesthetic, enterotoxin was injected.

Vomiting with the usual vigor and in the usual length of time occurred in every case. (*b*) In eight animals both vagi were sectioned. In order to maintain the animals alive, it was necessary to introduce intratracheal cannulas before sectioning the vagi, because the recurrent laryngeal nerves were cut at the same time. Upon recovery from the anesthetic, enterotoxin was administered. A second injection was usually given on the following day. All operative procedures were performed under pentobarbital anesthesia. In contrast to the results following sections of one vagus, when both vagi were sectioned, emesis rarely occurred. Several animals exhibited only mild retching movements, while some showed no evidence at all of retching or emesis. In only one animal of eight on which double vagotomy was performed was frank emesis produced.

These experiments indicate that fibers traversing the vagus are an important component of the reflex arc. Included in the vagus are afferent fibers running from the heart, esophagus, stomach, and intestine, and parasympathetic nerves which supply the same organs. The principal parasympathetic fibers directly concerned in the emetic act are those supplying the cardia. Since the animals with bilateral vagotomy did not show signs of vomiting against an obstruction such as an unrelaxed cardia, it is concluded that the visceral afferent fibers in the vagus form one of the important pathways of the emetic impulse.

Complete Transverse Section Anterior to the Pons and Posterior to the Hypothalamus

In order to eliminate the influence of higher centers on the so called vomiting center located in the medulla, complete transverse sections were made anterior to the pons. Only three of seven cats showed decerebrate rigidity. 2 cc. per kilo of enterotoxin were injected both before and after recovery from the anesthesia. No animal was maintained alive for longer than 24 hours postoperatively. Immediately after the animal was killed, the level and extent of the lesion was checked, and the stomach and the esophagus were examined for the presence of food and bile. None of the animals made visible retching or vomiting movements. In one instance vomitus was found in the esophagus postmortem. In one animal, transection was made in the posterior hypothalamic area during emetic activity, and this activity was immediately suspended.

In these lesions the cerebrum and thalamus, including both geniculates, were completely separated from the rest of the central nervous system. Thus any possible cerebral cortical control of vomiting was eliminated as was any influence which the hypothalamus has on the sympathetic and parasympathetic nervous systems. The action of the diaphragm in respiration would indicate that the nervous and muscular systems below the level of the lesion are capable of functioning. It is evident therefore that brain centers anterior to the pons also influence the vomiting reflex.

DISCUSSION

Vomiting is a reflex act which involves the coordinated actions of the muscles of the throat, esophagus, diaphragm, abdominal wall, and stomach. In the experiments reported in this paper, an effort has been made to determine the mode of action of staphylococcus enterotoxin on this reflex, and to define the pathways of the reflex arc.

In considering the initial site of action, the studies on smooth muscle indicate that the enterotoxin does not act directly on the gastro-intestinal musculature itself. This is not surprising in view of the fact that the active elements in vomiting are not movements of smooth muscle, but of the striated musculature. In view of this, the action of enterotoxin initially on some type of afferent nerve ending must be considered.

These afferent nerve endings may be found in various organs. It is well known that mechanical stimulation of the pharynx in man and certain animals will produce gagging and vomiting. Goldberg (18) found that reflex vomiting could be induced by mechanical stimulation of an isolated pyloric pouch. Certain drugs such as digitalis are supposed to act on the heart to produce emesis. In these studies removal of the stomach did not affect the ability of the enterotoxin to cause marked retching movements. When the gastro-intestinal tract from the lower end of the esophagus to the lower one-third of the rectum was removed, no retching or emesis occurred. Occasionally very slight twitchings of the abdominal wall which might be interpreted as feeble attempts at vomiting were noted. A similar finding was observed when the stomach and small intestine were removed, leaving the large intestine intact. On the basis of these observations, it can be concluded that the gastro-intestinal tract, especially the small intestine, contains the main portion of the necessary afferent nerve endings.

At the same time it should be recalled that section of the spinal cord at the level of C_7 permitted only mild retching movements after administration of enterotoxin, while a similar section at the level of T_5 did not materially affect the vomiting act. It is not known whether this result was due to interruption of afferent pathways from some of the organs in the thorax, through the stellate ganglion, which enter the cord between C_7 and T_5, or whether it was due to interference with the innervation of some of the somatic muscles involved in the vomiting act. While organs other than the intestine must be considered as possible loci of the necessary afferent nerve endings upon which enterotoxin acts, nevertheless it seems clear that most of these endings are located in the gastro-intestinal tract.

Hatcher and Weiss (14), from their studies on the emetic action of vari-

ous drugs, concluded that, depending on the drug and the organ upon which the drug was acting, the afferent emetic impulses might pass through the vagus, the sympathetics, or both. Goldberg (18) found that in emesis produced by mechanical stimulation of an isolated pyloric pouch, the afferent side of the reflex arc was entirely through the vagus nerves. Walton, Moore, and Graham (19) have presented evidence which shows that vomiting as a result of peritonitis induced by *B. coli* results from afferent nervous impulses traversing both vagal and sympathetic paths. Staphylococcus enterotoxin apparently sets up afferent impulses which pass mainly through the vagi since cutting of these nerves usually abolished emesis, although allowing mild retching movements frequently to occur. That the vagi are not the only pathway is indicated by actual emesis being produced in one animal following bilateral vagotomy. Also the diminution in the severity of the retching movements following spinal cord transections at C_7 indicates that afferent impulses may possibly arise to some extent in the heart, and pass over the sympathetic pathways in the region of the stellate ganglion. Removal of the celiac ganglion had apparently no influence on emesis produced by enterotoxin, indicating that fibers belonging to this ganglion are of little importance in transmitting impulses concerned with vomiting.

The muscular mechanisms of emesis are governed by a so called vomiting center in the medulla which receives impulses arising in various viscera and discharges impulses along numerous efferent nerves to bring about the coordinated movements of vomiting. This vomiting center of Thumas (16) comprises an area in the posterior region of the floor of the fourth ventricle and includes the dorsal motor nucleus of the vagus nerve, the motor nucleus of the hypoglossal nerve, and the nucleus solitarius. Ablation of this area abolishes emesis due to enterotoxin, as well as that due to various other emetic substances as shown by the studies of Hatcher and Weiss (14). In such a lesion not only are the nerve cells destroyed, but also nerve fibers passing through this area. Since the motor and sensory nuclei of the vagus are located in this area, it is to be expected that this injury would interrupt the reflex arc.

In the experiments of Hatcher and Weiss, it was found that the amount of certain emetic substances such as apomorphine and picrotoxin necessary to produce vomiting when the substance was applied directly to the vomiting area was much less than when the drug was injected intravenously. Other emetics such as ouabain and tartar emetic were completely inactive when applied directly to the vomiting center. In the experiments reported here, no evidence of emesis or retching appeared following application of

enterotoxin to the area of Thumas, even though the ependymal lining was first removed. It appears, therefore, that staphylococcus enterotoxin does not act directly on the vomiting center.

A point hitherto inadequately investigated is the ability to produce emesis following lesions of the brain anterior to the pons. In man it is recognized that psychic influences can produce emesis. Miller and Sherrington (20) have reported retching and deglutition in the cat following buccopharyngeal stimulation with obnoxious substances subsequent to decerebration. In the present experiments with total transecting lesions between the anterior border of the pons and posterior portion of the hypothalamus, neither retching nor emesis occurred following enterotoxin injection. Although it might be argued that the failure to produce emesis was due to the physical shock of the operation, yet the ability of the diaphragm to function in respiration, would indicate that at least the diaphragm was capable of taking part in the vomiting act. Since there is an extensive area of the brain separated from the lower brain stem structures by such a lesion, it is not possible to say what localized areas influence the medullary vomiting center. One of the higher centers that possibly affects the vomiting act, the hypothalamus, was eliminated by this transection. This region of the diencephalon has considerable influence over the sympathetic and parasympathetic nervous system. The rôle of the cerebral cortex and other higher centers in the emetic act cannot be assayed on the basis of the present experimental evidence.

The efferent pathways from the vomiting center are obviously the nerves innervating the muscles involved in the emetic act. The diaphragm is supplied by the phrenic, while the intercostals and abdominal musculature are supplied by their somatic nerves from the spinal cord. It should be pointed out that in the transections of the spinal cord at T_2, although marked retching occurred, emesis frequently did not, due to the lack of coordinated contractions of the diaphragm. Although the rôle of the stomach is relatively a passive one (10), the impulses bringing about relaxation of the cardia, pass through the vagus. In double vagal section not only are the cranial visceral afferents eliminated, but also the cranial visceral efferents. It might be thought that it is the elimination of the latter which is the more important, since it might be expected to maintain constriction of the cardia and not allow vomitus to reach the mouth. However, this does not appear tenable since retching movements were absent or mild except in one case, and never appeared to be acting against an obstruction.

Observations of the effect of operative procedures on emesis produced by enterotoxin have been supplemented by a study of the influence of

various drugs. Hatcher and Weiss (14) found evidence that ergotoxine inhibited normal afferent emetic impulses through fibers passing along with either the sympathetic or parasympathetic nerves. Since this drug markedly delayed or inhibited the action of enterotoxin, it appears that the action of the latter is on the peripheral nervous system rather than directly on the vomiting center. One of the main actions of atropine, however, is to paralyze the myoneural junction of the parasympathetic nerves (21). The occurrence of emesis following enterotoxin in atropinized cats shows that efferent impulses traversing the parasympathetics are not essential to emesis. This again indicates the greater importance of the visceral afferents passing through the vagus as compared with the visceral efferents (parasympathetics). Morphine acts mainly on the central nervous system. It depresses the brain, especially the higher functions. The medullary centers are first stimulated and then depressed. It will produce vomiting when applied directly to the vomiting center, and also occasionally by other routes of administration (14). This narcotic is widely used in the form of camphorated tincture of opium in the control of food poisoning symptoms. It is probable that it prevents emesis by depressing the vomiting center and probably other higher centers connected with the vomiting act. Barbiturates such as pentobarbital depress the cerebral cortex, and also the hypothalamic portion of the diencephalon and the subcortical ganglia, while the bulbo-spinal reflexes are very little affected (22, 23). The failure to affect emesis indicates that possibly the above mentioned areas are not a necessary portion of the vomiting reflex.

On the basis of the experiments reported in this and other papers, it is concluded that staphylococcus enterotoxin probably produces emesis by acting on peripheral sensory structures of the viscera, especially the small intestine. The impulses pass to the vomiting center mainly through the vagus nerves. Brain centers cephalad to the medulla also affect the vomiting mechanism. From the vomiting center located in the region of the dorsal motor nucleus of the vagus and the nucleus solitarius, motor impulses pass to the diaphragm by way of the phrenic; to the trunk musculature through the somatic nerves; and to the enteron by way of the vagus and the sympathetic nervous system.

SUMMARY

The emetic action of staphylococcus enterotoxin was tested on young and adult cats under various experimental conditions with the following results:

1. No direct action on isolated intestinal strips was observed.
2. Emesis resulted following intravenous, intracardial, and intraperi-

toneal injections, but failed to appear subsequent to oral, subcutaneous, or intramuscular administration.

3. Emesis occurred following (*a*) celiac ganglionectomy, (*b*) gastrectomy, (*c*) spinal cord transection at T_2 or caudad, and (*d*) unilateral vagotomy.

4. Mild retching movements and rarely emesis resulted subsequent to enterotoxin injection following (*a*) double vagotomy, (*b*) abdominal evisceration, and (*c*) spinal cord transection at C_7.

5. Emesis never occurred following (*a*) destruction of the vomiting center, (*b*) injection of enterotoxin into the fourth ventricle over the vomiting center, and (*c*) transection of the central nervous system between the anterior border of the pons and the posterior border of the hypothalamus.

6. Morphine inhibited, ergotoxine inhibited or delayed, while atropine and pentobarbital had little or no effect on emesis due to enterotoxin injection.

These experiments indicate that the action of staphylococcus enterotoxin on peripheral sensory structures is of greater importance in the initiation of emesis than direct action of the enterotoxin on the vomiting center. The principal pathways of the afferent and efferent impulses are described.

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