THE PRODUCTION OF GASTRIC ULCERATION IN RABBITS BY PERIVAGAL INJECTIONS OF TETANUS TOXIN

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Gastric erosions have been produced in rabbits by subperitoneal injections of tetanus toxin along the vagus nerves in relation to the oesophagus or anterior wall of the stomach, and into the central end of the cut cervical vagus on one side.

Ten rabbits injected in this way with 0.3–1 mg of toxin all showed acute erosions, often haemorrhagic, after periods of 12–146 hr.

(a) Four control rabbits treated identically with boiled toxin did not exhibit ulceration. (b) Of 19 normal rabbit stomachs, examined at random, only one had a small erosion. (c) The intravenous injection of toxin was without ulcerative effect.

In 9 rabbits the vagi were both cut subdiaphragmatically. Four were killed either 6 or 42 days later, and had no erosion. The other 5 were killed (or died) at comparable times but had been injected in the usual way with toxin 28–48 hr. before death; all showed erosions.

Acute gastric ulceration can be produced in laboratory animals by a variety of experimental procedures, which have been reviewed in a book by Ivy, Grossman and Bachrach (9). Despite certain contradictions, it is clear, that those metods whereby overactivity of the parasympathetic nerve supply to the stomach has been induced, have most consistently yielded ulcers. On the one hand gastric erosions have been obtained as a result of irritative lesions in the brain, and by electrical stimulation of certain hypothalamic centres (4). On the other hand, the administration by various routes, of parasympathomimetic drugs such as pilocarpine, and of pituitrin (6,7), has also resulted in peptic ulcerations, although usually these were small and inconstant acute erosions.

It has not hitherto been satisfactorily shown, that any of these procedures involving either prolonged vagal stimulation or prolonged administration of parasympathomimetic drugs, result in chronic peptic ulcer. Most of the methods employed fail to satisfy the requirement

that the stimulus must be continuous and maintained over a sufficiently long period of time. An illustration of this point is given by Ivy et al. (9) who gave 2 mg. of histamine to dogs every two hours night and day for two months. They supposed the drug action to be continuous; even so ulcers were not produced. When however, histamine was given in beeswax in the same daily dosage, ulcers developed.

The same considerations apply to artifical stimulation of the vagus, either pharmacologically or electrically. For instance, the technical difficulties of maintaining continuous electrical stimulation of the vagus in a survival experiment are considerable. So far it has been achieved for short periods of time only, which probably accounts for the equivocal nature of some of the results.

In this paper the problem is approached from another angle. It is known that tetanus toxin exerts both central and peripheral actions. Thus it is fairly certain, that it may exert a quite localized action upon a spinal or medullary nerve centre if injected into the peripheral »field« of this centre (1, 2). It is also clear from the work of Harvey (8) that the toxin may produce »local tetanus« at the periphery, in a muscle whose nervous connection with the central nervous system has just been severed. This was supposed by Harvey to be due to an action on the cholinergic nerve-endings resulting in continuous activity localized in them.

We have reported elsewhere (2) some experiments in which local tetanus of the vagus produced by perivagal and intravagal injections of toxin, led to marked slowing and irregularity of the heart in rabbits. In the course of this investigation, it was noticed that the injections were followed by appearance of acute erosions in the stomach. As these are of rare occurrence in normal rabbits the experiments were repeated with suitable controls and it has been found that the results could be reproduced with a 100% incidence of ulceration.

METHODS

A powdered preparation of toxin precipitated with amonium sulphate from the highly toxigenic CN 655 strain of Cl. Tetani was kindly supplied to us by Prof. G. Payling Wright. This was dissolved in sterile 0.9% NaCl immediately before injection, and 0.5–1 c. c. of this solution containing 0.3 to 1.0 mg. of toxin was injected into the tissues surrounding the abdominal portion of the vagi in rabbits as follows.

Rabbits of mixed stock were used and were anaesthetised with intravenous »veterinary« Sodium pentobarbital (26 mg./kg). The abdomen was opened by a mid-line incision through the linea alba from the xiphisternum to the umbilicus. The stomach was delivered through this wound, both its anterior and posterior surfaces being carefully examined for any visible signs of internal ulceration. The left vagus was then

identified on the anterior wall of the oesophagus and stomach. Toxin was injected to one side of the nerve, subperitoneally, with great care to avoid any contact between the syringe-needle and the nerve itself. The precaution was also taken to avoid undue trauma to the exposed parts of the stomach, by handling it gently.
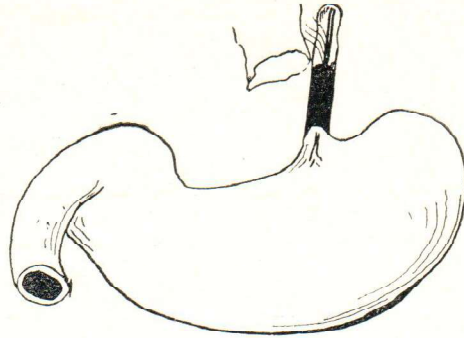

Fig. 1 a. Site of injection of toxin upwards
along the oesophagus (Type a).

In the first few experiments, sterile Indian ink particles were injected together with the toxin, in order to observe its distribution at the time of injection. Two types of paravagal injection were used; firstly up-
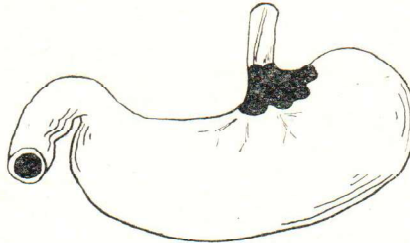

Fig. 1 b. Injection over the lesser
curvature (Type b).

wards and along the oesophagus (type a) in 13 experiments (see fig. 1 a.), secondly along the lesser curvature at 3 or 4 loci, and between the branches of the left vagus (type b) in 11 experiments (see fig. 1 b.)

Control animals were injected in precisely the same manner using the toxin solution, with or without Indian ink, boiled for 2 min.

Nine animals of mixed stock were vagotomised subdiaphragmatically. The two vagi were exposed below the caudate lobe of the liver and were divided carefully at a point just above the cardiac sphincter.

Four of the animals were killed after an interval of 6 days (1 animal) and 42 days (3 animals) (Table 2 B). Of the remaining 5 animals (see

Table 1
Production of gastric erosions by injections of tetanus toxin. (a) paravagally and (b) onto the anterior wall of the stomach

A. Active Toxin

Rabbit No.	Weight (Kg.)	Dose of toxin (mg.)	Time (hr.) after injection when the animal was killed (or died)	Results
1	1.4	1* (—ink)	48	1 large ulcer (lesser curvature); confirmed histologically.
2	0.9	0.55*	56	1 large ulcer (lesser curvature).
3	—	0.5+	Died between 4 and 18	Mucosa shows 8 black spots (early ulcers) and haemorrhagic points.
4	—	0.5++ (+ink)	50	Several small ulcers on the lesser and greater curvature. Confirmed histologically.
5	1.4	0.5++ (+ink)	72	1 large ulcer on the greater curvature and an area of multiple minor ulceration.
6	1.2	0.5*	42	Several large punched out ulcers on lesser curvature. Small haemorrhagic erosions on anterior wall. Confirmed histologically.
7	1.5	0.5*	48	Clear cut punched out ulcers with haemorrhagic bases. Confirmed histologically.
8	3	0.5++	146	Gross ulceration of stomach.
9	1.8	0.45*	76	1 haemorrhagic ulcer; small haemorrhages in the mucosa.
10	0.9	0.3+	46	Multiple small punctate erosions with black necrotic surfaces. Also haemorrhages. Confirmed histologically.

B. Controls. Boiled toxin

Rabbit No.	Weight (Kg.)	Dose of toxin (mg.)	Time (hr.) after injection when the animal was killed (or died)	Results
1	1.5	1+ (+ink)	64	No ulceration. One hyperaemic patch on the greater curvature.
2	1.1	0.5+ (+ink)	144	No ulceration. Stomach perfectly clean.
3	—	0.5++	48	No ulceration. Stomach normal.
4	—	0.45+ (+ink)	47	No ulceration. Mucosa absolutely clean.

+ = Type a injection (along the vagi, in the space between the peritoneum and the abdominal portion of the oesophagus.)

++ = Type b injection (between the branches of the vagus nerves, along the lesser curvature and anterior wall of the stomach, just under the peritoneum).

* = Combined type a and type b.

In rabbit No 8 the vagi were cut in the neck under local anaesthesia, 2 days before death.

Table 2 A) two were injected with toxin (0.5–0.75 mg.) 4 days later, and other three, 40 days later; all 5 were killed or died within 28–48 hr. of the injection.

Seven rabbits were injected intravenously with lethal doses of tetanus toxin (0.25–1.5 mg.) and after death (in from 12 to 78 hr.) the stomachs were removed and examined as usual.

RESULTS

A. Normal animals injected with toxin

The animals survived from 4 to 146 hr. when they either died as a result of cardiac failure, or were killed after experiments which are reported elsewhere (Ambache and Lippold, 1949). Of 10 rabbits injected with toxin all showed acute ulceration of the gastric mucosa, often haemorrhagic (see Table 1). The gastric mucous membrane was examined fresh, immediately after death, except where there is a statement to the contrary. In many instances these ulcers were visible through the wall of the unopened stomach in situ, whereas such gross lesions were definitely not present at the time of injection. The lesser curvature and fundus yielded most ulcers; only one was found on the anterior wall. Ulceration was often multiple.

Macroscopic appearance. The mucous membrane of the ulcerbearing area presented a diffuse mottled reddening, being studded with small petechial haemorrhages. Larger haemorrhagic lesions often had a darkly pigmented floor, (fig. 2) probably consisting of altered blood. Large punched out ulcers, up to 0.5 cm. in diameter, sharply demarcated and with an oedematous margin, extending into the muscle coat of the stomach, occurred in four animals.

Microscopic appearance. Four of these ulcers were examined histologically, and photomicrographs are shown in Fig. 3. These sections show the extensive necrosis and mucosal deficiency associated with typical acute peptic ulceration. Ulcers extend into the submucous coat but not deeper. There were haemorrhagic and inflammatory changes in the floor of the ulcers, which contained pigment granules (probably of acid hematin) and a covering of fibrinous exudate.

B. Control experiments (boiled toxin)

Control experiments were performed on four rabbits (Table 1 B) using sterile boiled toxin in equivalent doses, with or without Indian ink. None of these animals showed any sign of ulceration when killed after a comparable interval (46–144 hr.)

In addition, 19 normal rabbit stomachs taken at random were examined immediately after death; of these only one had a small single

erosion of a non-haemorrhagic type situated on the lesser curvature near the pylorus.

C. Animals injected with toxin after subdiaphragmatic double vagotomy

All of 5 animals which were injected with toxin (0.5–0.75 mg.) after both vagi had been cut previously and allowed time to degenerate, developed gastric erosions within 28–48 hr. In 2 of these, the injection was performed 4 days after vagotomy and was of type b); in the other 3, the injection was made 40 days after vagotomy and was of type a). The results are shown in Table 2 A.

Table 2

Production of gastric erosions by tetanus toxin in rabbits after subdiaphragmatic double vagotomy

A. Active toxin after vagotomy

Rabbit No.	Weight (Kg.)	Dose of toxin (mg.)	Time of injection after vagotomy (in days)	Time of killing		Results
				after vagotomy (days)	after injection (in hr.)	
1		0.5+	40	42	48	Small scattered punctate haemorrhages.
2		0.5+	40	42	48	Multiple scarred ulcers especially on the lesser curvature. Haemorrhagic erosions diffusely scattered.
3		0.5+	40	42	48	Single large haemorrhagic erosion 6 mm. in diameter surrounded by circumscribed area of petechial haemorrhages.
4	1.1	0.5++ (+ink)	4	6	48	1 or 2 small star-shaped ulcers.
5	1.1	0.75++ (+ink)	4	5	28	Ulcers present.

B. Controls. Vagotomy alone

Rabbit No.	Weight (Kg.)	Dose of toxin (mg.)	Time of injection after vagotomy (in days)	after vagotomy (days)	after injection (in hr.)	Results
1	1.4			42		No ulcerations or petechiae.
2	1.4			42		No ulcerations or petechiae.
3	1.4			42		No ulcerations or petechiae.
4	1.4			6		No ulcerations or petechiae.

+ and ++ as in Table 1.

D. Control animals; vagotomy alone

Four control animals, in which the vagi were cut in exactly the same way, were killed – one after 6 days and the rest after 42 days. No ulcers were found in this control group (Table 2 B), showing that erosions found in the above group of 5 animals (Table 2 A) were not due to the vagotomy, but, as before, to the toxin.

E. Intraneural injections of toxin into the cervical vagus

Autopsies were performed on 5 animals which had been used in the course of an investigation, which is reported elsewhere (Ambache and Lippold, 1948), on the occurrence of bradycardia of central origin after intraneural injections of toxin into the vagus nerve in the neck. The details of these are given in Table 3 below. It will be seen 'hat the injection of toxin into the central end of one vagus nerve produced gastric erosions in 2 rabbits (Nos. 2 & 3) but not in No. 1 which died too soon (after 24 hr.) The same amounts of toxin injected into the peripheral end of one vagus, had no ulcerative effect in rabbits Nos. 4 and 5.

Table 3

Production of gastric erosions by intraneural injections of tetanus toxin into one cervical vagus

Rabbit No.	Procedure	Dose of toxin (mg.)	Time of death or killing after injection (hr.)	Results
1	Lt. X cut; ganglion nodosum injected	0.5	24	Small petechiae over lesser curvature. No large ulcers.
2	Lt. X cut; central end injected	0.7	Died between 25–41 hr.	Numerous blackish early erosions.
3	Rt. X cut; central end injected	0.75	53	Multiple haemorrhagic ulcers.
4	Lt. X cut; peripheral end injected	0.5	Died between 48–96 hr.	Stomach shows 2 areas of diffuse red-staining (? postmortem) but no break in the mucosa.
5	Rt. X cut; peripheral end injected	0.75	102	No ulceration.

F. Intravenous injections of toxin

The stomachs of the seven animals given intravenous toxin in various doses (lethal and supralethal) showed no ulcerative changes, although the animals all succumbed eventually, with the typical changes of general tetanus. The heart rate however, was increased in the terminal stages, indicating that the specific effect that we have described upon the vagal innervation did not occur in these animals. In one of these animals a few petechiae were observed in the mucous membrane.

DISCUSSION

The results show (a) that spontaneous gastric erosions are of rare occurrence in rabbits of mixed stock and (b) that the ulcers produced by our experimental procedure were caused neither by the surgical operation nor by a mechanical irritation of the vagus nerve by the injection, since identical amounts of boiled toxin, injected in exactly the same way, were ineffective. The constancy with which gastric erosions were obtained when active toxin was injected, appears to indicate that this was a genuine effect of tetanus toxin.

The precise way in which the toxin produces these erosions, i. e., whether by inducing hypersecretion or a change in gastric motility, has not been elucidated as yet. Also, there is no means at present of distinguishing whether the toxin is here acting upon the nervous apparatus of the stomach or possibly more peripherally, i. e. directly upon the effector cells in the smooth muscle or the glands of the stomach. However, it has been shown previously, in the rabbit's eye, that tetanus toxin does not appear to exert any direct action on cholinergically innervated smooth muscle (3). Indeed, the present belief is that tetanus toxin is specifically neurotropic, and does not, so far as we know, induce hyperactivity in any tissue other than nerve. And there is some evidence that it is mainly the cholinergic nerves or rather their endings which are primarily involved; for instance the adrenergic and the sensory nerve fibres within the eye appear to be unaffected by the toxin (3). The ulcerative action of the toxin is therefore, in our opinion, most easily explained by assuming an effect upon the cholinergic innervation of the stomach. We have applied toxin in the immediate vicinity of the vagus nerve, and have shown elsewhere (2) that such a procedure will produce, regularly, a bradycardia of central origin. These experiments, and those of Acheson, Ratnoff and Schoenbach (1), show quite clearly that the injection of toxin into the peripheral »field« of
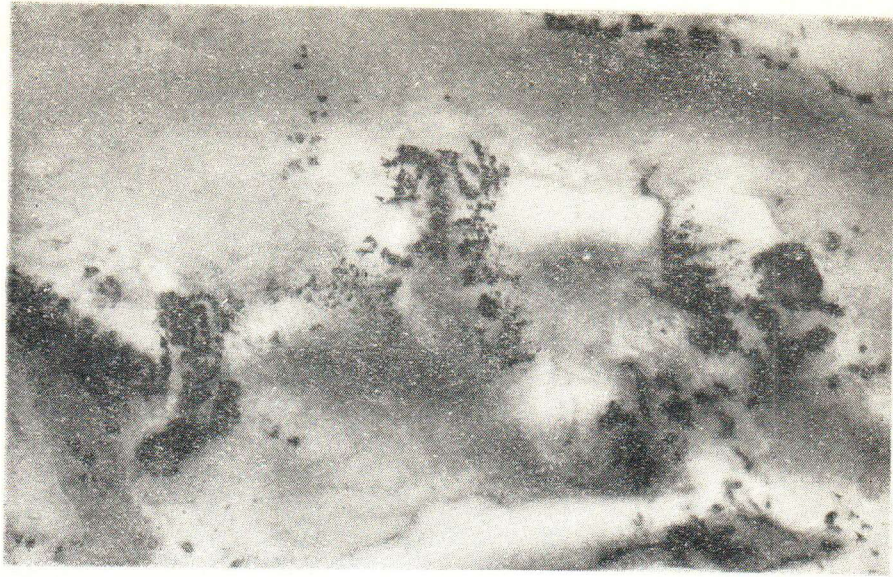
Fig. 2. *The macroscopic appearance of early ulcers produced by the injection of tetanus toxin into the vagus (Type a). Photograph from rabbit No 3 table 1A. Mag. 5.5 times.*
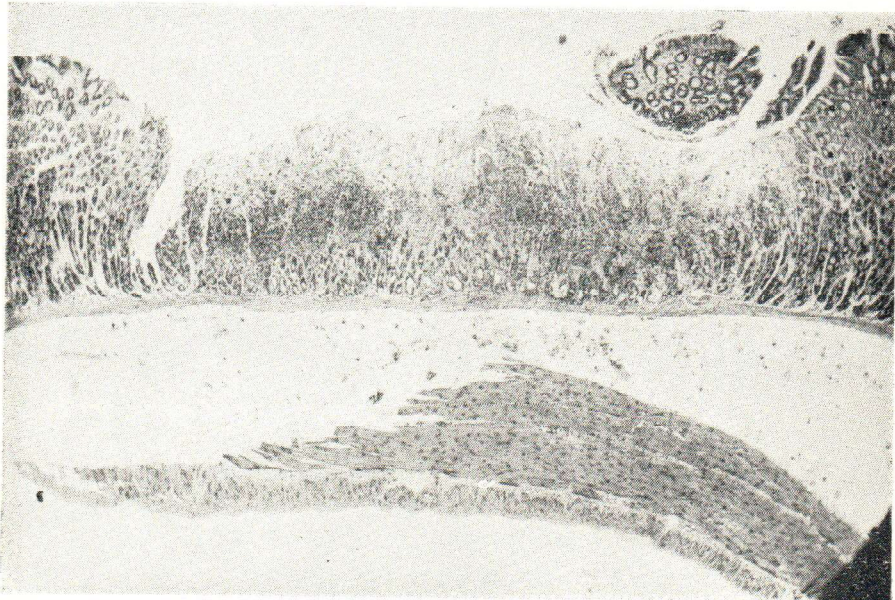

Fig. 3. *Photomicrographs of typical ulcers produced by injections of tetanus toxin into the cervical vagus.*
Section (a) from rabbit No 1 table 3
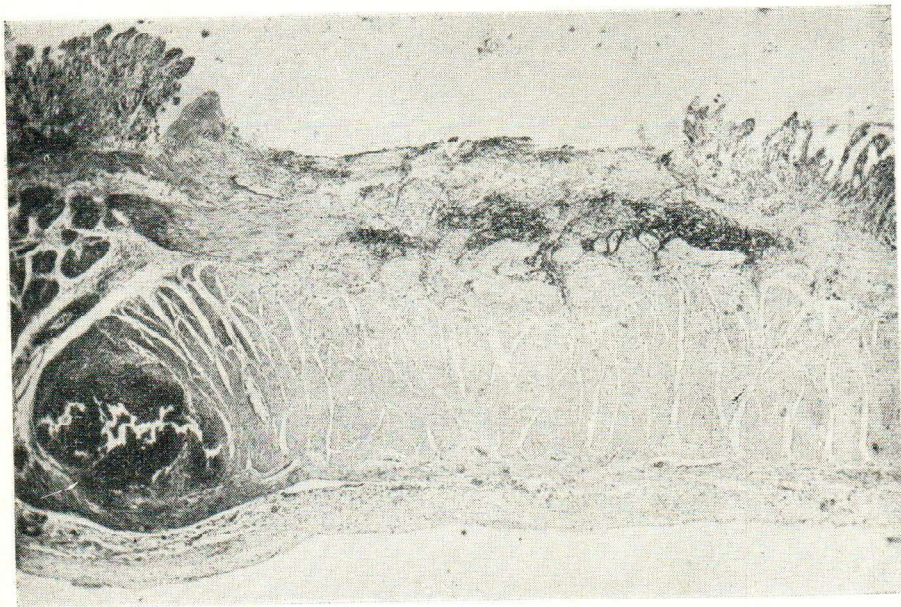*Sections stained with Hematoxylin & eosin. X 42.*

Fig. 3. *Photomicrographs of typical ulcers produced by injections of tetanus toxin into the cervical vagus.*
Section (b) from rabbit No 2 table 3
(c) from rabbit No 3 table 3
Sections stained with Hematoxylin & eosin. X 42.

a spinal or medullary nerve results in tetanic hyperactivity of its centre, which can be best explained on the basis of a transport of toxin along the nerve (or around it) to its centre. The injection of the central end of the cut cervical vagus reproduces this condition of the centre and has led to ulceration (Table 3); it is also possible that when the vagi were intact, part of the effect of the abdominal injections of toxin were due to its central migration.

Effect after vagotomy. The production of ulcers by the toxin in doubly vagotomised animals appears at first difficult to explain on such a theory. However, subdiaphragmatic vagotomy will result only in the degeneration of the preganglionic fibers of the vagus. Although it severs the connection between the stomach and the vagus centre, it will not in fact result in complete cholinergic denervation of the stomach. It will leave the synapses of the enteric plexuses proper, of Auerbach and Meissner, and these are known to act as local nerve-centres mediating peripheral visceral reflexes. It is possible that, even after vagotomy, the toxin is capable of exerting its usual tetanic effect upon these nerve-centres, to which it diffuses, with, at least at first, a phase of hyperactivity resulting in ulcer formation.

The following possibility has come to our notice since the completion of the experiments (10). It appears that section of the two main trunks of the vagus below the diaphragm does not result in complete preganglionic denervation of the stomach. It is claimed that this is due to aberrant vagal fibers embedded in the oesophageal wall and also to fibers travelling to the stomach in the connective tissue at a considerable distance from the oesophagus. Since no secretion or motility tests were made in our experiments, it is quite possible that the vagal resection was incomplete.

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Sadržaj

IZAZIVANJE ŽELUČANIH ULCERACIJA
PERIVAGALNOM INJEKCIJOM TETANUS TOKSINA

Želučani ulkusi bili su izazvani kod kunića subperitonealnim injekcijama tetanus toksina uzduž vagusa na ezofagusu i prednjoj stijenci želuca, i injiciranjem centralnog kraja prerezanog cervikalnog vagusa s jedne strane.

10 kunića bilo je injicirano na taj način dozama od 0,3–1 mg toksina i svi su pokazali akutne erozije, često hemoragične, nakon perioda od 12–146 sati.

4 kontrolna kunića, koji su bili tretirani na isti način kuhanim toksinom, nisu pokazali znakove ulceracija. Izvršeni su pregledi želudaca 19 normalnih kunića, te je samo kod jednoga nađena mala erozija. Intravenozna injekcija toksina nije izazvala ulceracioni efekt.

Kod 9 kunića bila su oba vagusa prerezana subdijafragmalno, 4 su bila ubijena nakon 6–42 dana i nisu pokazivali nikakvih erozija. Ostalih 5 je ubijeno (ili je uginulo)) u istim vremenskim razmacima. Njima se na uobičajeni način injicirao toksin 28–48 sati prije smrti, i svi su pokazivali erozije.

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