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Experimental Gastric and Duodenal Ulcer.

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(Introduced by B. P. Babkin.)
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From a review of the literature concerning peptic ulcer one receives the impression that great caution should be exercised in drawing conclusions from the results of experiments in which ulcers have been produced. Although ulcers can be produced readily in certain animals, the conditions necessary to do this are artificial and alter seriously the normal anatomical and physiological relationships of the organs involved. Usually these alterations are far different from any that could possibly occur in human subjects with peptic ulcer. Species differences, too, constitute a hazard in comparing experimental ulcer in animals with spontaneous ulcer in man.

The evidence tends to show that the two outstanding factors in the formation of experimental gastric and duodenal ulcers are the digestive action of acid gastric secretion, and the lowering of mucosal resistance due to local vascular abnormality. The most successful experimental methods for the production of ulcers utilise one or both of these factors.

It is evident that subacute or chronic ulcers resembling those in man are very difficult to produce. While chronic ulcers appear after the establishment of surgical duodenal drainage,[1] it should be realized that this operation has other profound effects, due to the short-circuiting of bile, pancreatic and duodenal secretion past the greater part of the small intestine.

Chronic combined histamine and nitroglycerine stimulation[2,3] is one of the few experimental methods for the production of gastric and duodenal ulcers utilizing the factors both of acid-peptic digestion and of local tissue ischemia in the intact animal. The reported high incidence of lesions in rabbits caused by this technic suggested it to be dependable in an animal that is refractory to other methods.

Code and Varco introduced the method of chronic histamine stimulation. It consists in embedding histamine in a mixture of beeswax and mineral oil before intramuscular injection. This mixture prolongs the action of the contained histamine by delaying its absorption. In this way a large dose of histamine may be injected intramuscularly, with a resultant chronic effect lasting 24 hours or longer. One intramuscular injection daily is, therefore, sufficient to maintain continuous stimulation of gastric secretion.

It was desired, in our laboratory, to use an efficient method for the production of gastric and duodenal ulcers in rabbits. Reports from Wangensteen's laboratory indicated that it was difficult or impossible to provoke ulcers or erosions in rabbits by the chronic action of histamine alone.[3] However, Baronofsky and Wangensteen[4] were successful in producing ulcers in rabbits by means of chronic combined histamine and nitroglycerine stimulation. They reported erosions or ulcers in 9 of 12 rabbits within an experimental period of 6 days. They explained the occurrence of these lesions on the basis of local tissue ischemia (due to the chronic action of nitroglycerine) which rendered the mucosa more susceptible to the digestive action of a highly acid gastric secretion (caused by the chronic action of histamine). In our experiments a longer period was used, with the hope that a higher incidence of ulcers would occur.

[1] Mann, F. C., and Williamson, C. S., *Ann. Surg.*, 1923, **77**, 409.

[2] Code, C. F., and Varco, R. L., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 475.

[3] Hay, L. J., Varco, R. L., Code, C. F., and Wangensteen, O. H., *Surg. Gynec. and Obstet.*, 1942, **75**, 170.

[4] Baronofsky, I. D., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 127.

There are conflicting reports[5-7] in the literature concerning the development of gastric and duodenal ulceration following bilateral vagotomy in experimental animals, particularly rabbits. Some investigators have observed the development of ulcerative lesions in more than 30% of vagotomised rabbits, the incidence increasing progressively with the interval of time after vagotomy. Our results in experiments of a similar nature are reported herein. In addition, we investigated the type and incidence of ulcers of the stomach and duodenum of vagotomised rabbits, provoked by the chronic combined sitmulation of histamine and nitroglycerine.

Materials and methods. In order to demonstrate that our beeswax and mineral oil mixture (prepared according to the method of Code) effectively prolonged the action of histamine, experiments were carried out on 2 dogs, one with a Spivack gastrostomy and the other with a metal gastric fistula. The animals were conditioned to the laboratory so that their fasting secretion was minimal. Control specimens were taken, and 0.5 mg histamine injected subcutaneously. The secretion was collected every 15 minutes and volume and pH determined. Subsequently, in each animal, 30 mg of histamine embedded in beeswax and mineral oil was injected intramuscularly. Specimens were collected every 30 minutes for as long as 5¼ hours and the volume and pH of each determined.

A control series of experiments, using 6 rabbits, was performed to determined whether or not daily intramuscular injections of nitroglycerine, embedded in beeswax and mineral oil, produce gastric or duodenal lesions. These rabbits received the stock diet of Purina rabbit pellets, carrots and water. Daily intramuscular injections of 1 mg of nitroglycerine in beeswax and mineral oil were given. Injections were continued for 14 days in survivors and these rabbits were sacrificed on the 15th day for gross and microscopic examination of the involved organs.

It was felt that Code and Varco had demonstrated amply that injections of beeswax and mineral oil alone do not stimulate gastric secretion or produce ill effects in animals. Therefore, no control series of experiments was carried out to confirm this point.

Series I included 32 adult rabbits maintained on the stock laboratory diet. In this series were investigated the type and incidence of lesions of the stomach and duodenum of rabbits produced by the chronic combined action of histamine and nitroglycerine. Daily administration to each rabbit of 30 mg of histamine and 1 mg of nitroglycerine was carried on until death of the animal or until 14 daily injections had been given. Each drug was embedded in beeswax and mineral oil mixture and injected intramuscularly early in the afternoon. Those animals which died during the experimental period were examined as soon as possible, while all survivors were examined on the 15th day.

Series II included 12 adult rabbits in which investigation was made of the incidence of lesions of the stomach and duodenum following bilateral subdiaphragmatic vagotomy. In performing the vagotomy at least 2.0 cm of esophagus was completely cleared of surrounding tissues down to its muscular layer to ensure division of all fibers of both vagus nerves. As a rule these rabbits completely recovered from the effects of the anesthetic within a few hours and were soon lively and eating. Those animals which died were examined as soon as possible after death. The remaining rabbits were sacrificed at different intervals up to 47 days from the time of operation. Survivors were killed by a blow on the neck and autopsies were performed at once.

In Series III 19 rabbits were vagotomised. After a few days they were given daily intramuscular injections of histamine and nitroglycerine, each embedded in beeswax and mineral oil mixture. Injections were carried on for 15 days in survivors, which were sacrificed on the 16th day.

Results and discussion. Fig. 1 shows the results of the experiments performed using Dog No. 1. The results of both parts of the

5 Beazell, J., and Ivy, A. C., *Arch. Path.*, 1936, **22**, 213.

6 Ophuls, W., *J. Exp. Med.*, 1906, **8**, 181.

7 Alvarez, W. C., Hosoi, K., Overgard, A., and Ascanio, H., *Am. J. Physiol.*, 1929, **90**, 631.

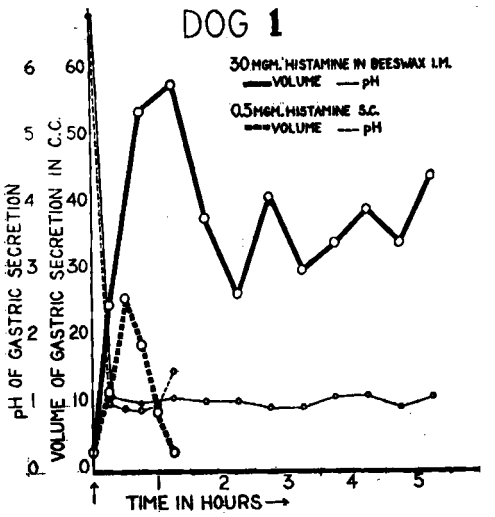

Fig. 1.

Gastric secretory response of dog No. 1. The arrow indicates the time of injection of histamine. Certain volumes are recorded as 15-minute volumes and others as 30-minute volumes.

experiment are plotted on the same graph to emphasize the contrast between the gastric secretory response to a subcutaneous injection of 0.5 mg of histamine in aqueous solution

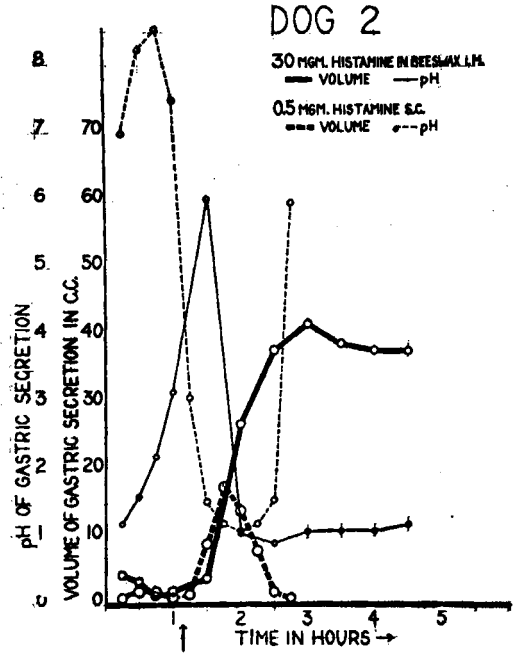

Fig. 2.

Gastric secretory response of dog No. 2. The arrow indicates the time of injection of histamine. Certain volumes are recorded as 15-minute volumes and others as 30-minute volumes.

and the prolonged response to an intramuscular injection of 30 mg of histamine embedded in beeswax and mineral oil mixture. Fig. 2 shows the results of similar experiments, using Dog No. 2.

Following the intramuscular injection of 30 mg of histamine in beeswax and mineral oil our animals showed no evidence of toxic effects of the drug. This was taken as evidence that the large dose of histamine was not absorbed rapidly. In both experiments (Dog No. 1 and Dog No. 2) the rate and acidity of gastric secretion continued at high levels, showing little change in the first few hours. In Dog No. 1 the specimen of gastric secretion obtained 24 hours after the injection still showed a large volume and high acidity of gastric secretion.

These results were taken as proof of the prolonged effects of intramuscular injection of histamine in beeswax and mineral oil. The nitroglycerine control series (6 rabbits) yielded the following results. Two rabbits died of pneumonia during the injection period, one on the 8th and one on the 10th day. Four survivors were sacrificed on the 15th day. Autopsy revealed no gastric or duodenal lesions. This finding was taken as evidence that the chronic action of nitroglycerine alone is incapable of producing ulcerations within a period of 15 days.

Results summarizing the findings in Series I are shown in Table I. In this series, involving 32 rabbits, 12 died during the experimental period. Of these, 9 had perforation of the fundus of the stomach, one had perforation of the first part of the duodenum, one had hemorrhage into the gastrointestinal tract, and one died without a demonstrable lesion to account for its death. Of the 20 rabbits that survived until their sacrifice at the end of the experimental period, 19 had no demonstrable lesion and one had a recent small perforation of the first part of the duodenum with associated localised peritonitis. All the rabbits in this series lost weight during the experiment, so that some were emaciated at the time of death or sacrifice, although food was found in all their stomachs at autopsy.

All perforations of the fundus of the stomach occurred within the first 9 days of the

TABLE I—Series I.

Rabbit	No. daily inj. of histamine and nitroglycerine	Died	Sacrificed	Gross autopsy findings
1	3	X		Perforation, fundus of stomach
2	11		X	No lesion
3	11		X	" "
4	11		X	" "
5	6	X		Perforation of fundus and blood in bowel
6	7	X		Perforation of fundus
7	8	X		Perforation of fundus and blood in bowel
8	14		X	No lesion
9	14		X	" "
10	14		X	" "
11	14		X	" "
12	14		X	" "
13	14		X	" "
14	14		X	" "
15	14		X	" "
16	14		X	" "
17	14		X	" "
18	1	X		Perforation of fundus of stomach
19	2	X		Perforation of fundus
20	3	X		" " "
21	3	X		" " "
22	5	X		" " "
23	11	X		No lesion
24	12	X		Intestinal hemorrhage, no lesion found
25	13	X		Perforation of duodenum
26	14		X	No lesion
27	14		X	" "
28	14		X	" "
29	14		X	" "
30	14		X	" "
31	14		X	Perforation of duodenum
32	14		X	No lesion

experiment; namely, after 1, 2, 3, 3, 3, 5, 7 and 8 injections, respectively. The short time required for the production of these lesions, as well as their microscopic appearances indicate that they were in every sense acute. The perforations of the duodenum, one fatal after 13 daily injections and the other discovered after 14 daily injections, required a somewhat longer time to develop. They were much smaller in size than the gastric perforations.

Generally speaking, the gastric wall near the site of perforation showed very little inflammatory cell infiltration and only moderate edema. All layers of the gastric wall appeared relatively normal to within a short distance of the edge of the perforation, except for scattered superficial mucosal erosions in several instances. The muscularis, at the site of perforation, came to an abrupt end with a small zone of necrosis. The mucosa was absent over a narrow zone about the perforation, leaving the submucosa exposed. This may have been due to retraction of the mucosa after perforation occurred. In some instances minimal leucocytic infiltration was noted, chiefly in the submucosa. Frequently great dilation of blood vessels in the submucosa or subserosal zone was seen.

The preceding brief description of the appearance of sections of gastric wall adjacent to a perforation may be considered representative of the findings in all nine instances of gastric perforation. The gross and microscopic appearances (Table I, Fig. 3, 4) together with the rapid development of these lesions, suggest an acute necrosis of all layers of the gastric wall with digestion of the necrotic tissue resulting in the large, ragged type of perforation. Acute peritonitis, due to the escape of gastric contents into the peritoneal cavity, with or without hemorrhage amply explains the fatal issue in these rabbits.

The two perforations of the first part of the duodenum, one discovered after death of

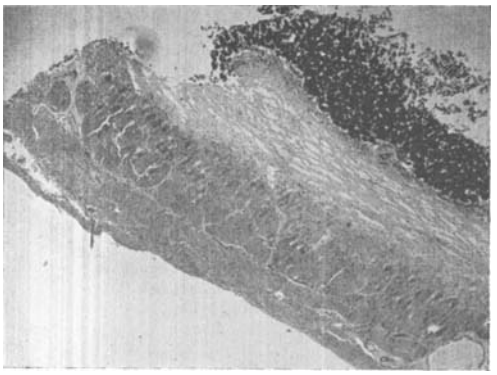

Fig. 3.

Photomicrograph: Rabbit 20, Series I. This shows a section of gastric wall at the edge of the perforation of the fundus. The mucosa shows superficial necrosis, with numerous shallow erosions. The muscularis mucosae and the submucosa are both edematous, so that there is great thickening of this zone of the wall. The submucosa is exposed for a short distance adjacent to the perforation. The muscularis is somewhat edematous and shows a narrow zone of necrosis at the edge of the perforation. The subserosal tissue is edematous and the serosa is necrotic and detached for a short distance. The blood vessels in the submucosa and muscularis are markedly engorged with blood, and the veins, particularly are greatly dilated. There is absence of inflammatory cell infiltration and there is no sign of a repair process.

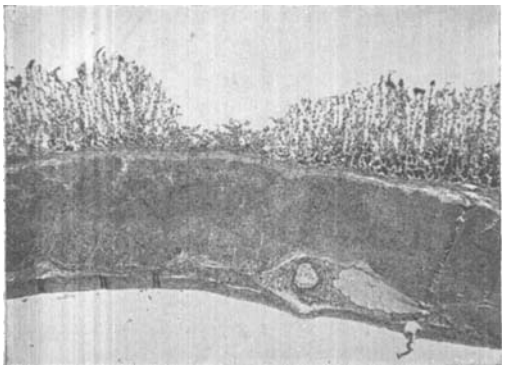

Fig. 4.

Photomicrograph: Rabbit 21, Series I. This shows a section of a gastric mucosal erosion near a perforation of the fundus. There are scattered superficial erosions of necrotic areas of mucosa. The submucosa, muscularis and serosa appear relatively normal apart from marked engorgement and dilatation of the subserosal blood vessels. No apparent inflammatory cell infiltration or evidence of repair is present about the bases of the erosions.

the animal, the other at the time of sacrifice (Fig. 5), were quite similar to one another in microscopic appearance. Sections were cut so as to pass through the involved duodenal wall immediately adjacent to the perforation. There was a moderate degree of acute inflammatory cell infiltration (polymorphonuclear leucocytes and lymphocytes) in the wall of the duodenum surrounding the perforated ulcer. In places there were focal collections of lymphocytes, particularly in the mucosa and submucosa, which were considered to be lymphoid follicles and not part of the inflammatory reaction. The wall of the ulcer contained necrotic tissue with a somewhat homogeneous, eosinophilic appearance. In one instance a thrombosed submucosal artery was seen in the wall of the perforated duodenal ulcer. The ulcer crater was cone-shaped, wider in diameter at the mucosal side of the wall. The duodenal wall at a distance from the perforated ulcer appeared essentially

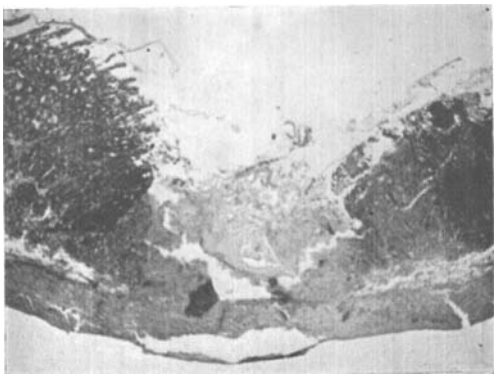

Fig. 5.

Photomicrograph: Rabbit 25, Series I. This is a tangential section through the wall of a perforated duodenal ulcer showing the tissues immediately adjacent to the actual perforation. On each side of the perforation, a short distance away, the wall of the duodenum appears essentially normal. Occasional collections of lymphocytes are present in the mucosa and submucosa representing naturally-occurring follicles and not part of an inflammatory process. The tissue immediately adjacent to the perforation is necrotic, the zone being wider towards the mucosal side and narrower towards the serosal surface. A mass of poorly-stained necrotic debris is lying semi-detached at the level of the mucosa and submucosa. The continuity of the muscularis is seen in this section near the perforation but it is necrotic and no cellular detail is visible. The serosa is necrotic and partially detached. There is a zone of edema of all layers near the necrotic area and a very few polymorphonuclear leucocytes are to be seen in the submucosa at this point. No definite evidence of repair can be detected.

TABLE II—Series II.

Rabbit	Survival time, days after operation	Died	Sacrificed	Autopsy findings
1	47		X	No lesion
2	47		X	" "
3	32	X		Pneumonia
4	20	X		Ear infection
5	43		X	No lesion
6	18	X		Infected wound
7	5	X		Pneumonia
8	38		X	No lesion
9	31	X		Pneumonia
10	31		X	No lesion
11	25	X		Pneumonia
12	4	X		"

normal. No definite evidence of repair was detected in the sections so that these lesions were considered to be acute.

Gross and microscopic examination of the small perforations of the first part of the duodenum suggested the process to have been due to infarction of an area of the duodenal wall followed by the development of an acute perforating ulcer. Although acute in appearance the lesion had developed slowly enough to permit inflammatory cell infiltration about it. This may be contrasted with the large, rapid perforations of the fundus of the stomach which developed in other animals.

In the 21 animals showing no gross lesions of the stomach or duodenum at autopsy, the microscopic appearance of sections of the gastric and duodenal wall was essentially normal.

Table II summarizes the findings in the 12 experiments in Series II. None of the rabbits in this series developed lesions of the stomach or duodenum within periods varying from 4 to 47 days following vagotomy. Seven of the 12 animals died after vagotomy, on the 4th, 5th, 18th, 20th, 25th, 31st and 32nd day, respectively. Of these, 5 died of pneumonia, one of a wound infection and one of an ear infection. The remaining 5 were sacrificed on the 31st, 38th, 43rd, 47th, and 47th days, respectively, after vagotomy.

At autopsy the stomach of each of these animals was found stuffed with food and appeared definitely larger with greater intragastric pressure than was noted during autopsies upon non-vagotomised rabbits. The esophagus was empty in each instance, while the duodenum was either empty or contained a small quantity of bile-stained fluid. No gross lesions were found in other organs apart from those mentioned previously as a cause of death. In each case, the microscopic appearance of the stomach and duodenum was essentially normal. The incidence of pneumonia in this series was notably greater than in animals not subjected to vagotomy. Vagotomy may permit aspiration pneumonia from regurgitation of gastric contents.

It would appear, from the results of this series of experiments, involving 12 vagotomised rabbits, that gastric or duodenal ulcers are not likely to develop following this operation within periods of up to 47 days.

Results summarising the findings in Series III are shown in Table III. Although 24 rabbits were vagotomised in this series, 5 died before the injections of histamine and nitroglycerine were begun. Two rabbits died of pneumonia; one did not recover consciousness following the operation; and the causes of death of the other two are unknown as no autopsies were performed upon them. The three rabbits examined did not have lesions of the stomach or duodenum at the time of death.

Since 5 rabbits died before the injections were begun, it was necessary to exclude them; so that this series actually consisted of nineteen vagotomised rabbits injected daily as described. Of these, nine died before the 16th day of the experiment, at which time the survivors were sacrificed for examination. Of the 9 deaths, 4 were caused by pneumonia, one by infection of an ear, while the other

TABLE III—Series III.

Vagotomised rabbit	No. daily injections	Died	Sacrificed	Autopsy findings
1	15		X	No lesion
2	15		X	" "
3	0	X		Pneumonia
4	15		X	No lesion
5	15		X	" "
6	15		X	" "
7	15		X	" "
8	15		X	" "
9	0	X		Pneumonia
10	15		X	No lesion
11	15		X	" "
12	15		X	" "
13	0	X		(Anesthetic death)
14	0	X		(No autopsy performed)
15	0	X		" " "
16	2	X		Infection—ear
17	5	X		No lesion (emaciation)
18	5	X		Pneumonia
19	6	X		No lesion (emaciation)
20	6	X		Pneumonia
21	9	X		"
22	9	X		No lesion (emaciation)
23	12	X		Pneumonia
24	14	X		No lesion (emaciation)

4 deaths could not be accounted for by any demonstrable lesion apart from marked emaciation. Autopsies revealed no gastric or duodenal ulcerative lesions in any animal in this series of experiments. Microscopic examination of the stomach and duodenum of the animals in this series revealed no abnormalities.

Conclusions. 1. Daily intramuscular injections of 1 mg of nitroglycerine embedded in a mixture of beeswax and mineral oil does not provoke gastric or duodenal ulceration in rabbits within a period of 15 days.

2. Bilateral subdiaphragmatic vagotomy does not produce gastric or duodenal ulceration within periods as long as 47 days in rabbits maintained on a diet of Purina rabbit pellets, carrots and water.

3. Daily intramuscular injections of histamine (30 mg) and nitroglycerine (1 mg) embedded in a mixture of beeswax and mineral oil produce acute, necrotic, perforating lesions of the stomach or duodenum of approximately 30% of rabbits. These lesions are fundamentally different from typical human peptic ulcers.

4. Bilateral subdiaphragmatic vagotomy apparently exerts a measure of protection against the development of acute ulcerative lesions of the stomach and duodenum of rabbits produced by the chronic action of histamine and nitroglycerine.

5. Gastric and duodenal ulcers produced in rabbits by chronic combined histamine and nitroglycerine stimulation are not considered to be suitable lesions for the experimental assessment of ulcer prevention measures.