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New Method for Evaluating Topical Action of Substances on Gastric Mucosa

R. S. ALPHIN and D. A. DROPPLEMAN

Abstract □ A new method is described to evaluate the topical action of drugs on the gastric mucosa of laboratory animals. Of distinct advantage is the ability to assess the effects of more than one drug simultaneously on adjacent mucosal areas. The method also has proven to be of value in studying the effects of such variables as duration of exposure, pH, and pepsin.

Keyphrases □ Gastric mucosa injury—multidrug simultaneous evaluation, animals □ Aspirin—gastrotoxic effects, cats □ Phenylbutazone—gastrotoxic effects, cats

An important and well-known facet of the pharmacology of various substances, particularly anti-inflammatory drugs, is their injurious topical action upon the GI tract. Most methods used to determine such action have involved assessing the gastric effects following the oral administration of drugs to guinea pigs (1–3), rats (4), dogs (5, 6), rabbits (3), and man (7). Some investigators introduced the substances under investigation directly into the stomach of several species of animals (3, 8, 9). Direct application of anti-inflammatory drugs on the buccal mucosa of man (9) and the explanted gastric mucosa of dogs (10) also were studied. Few, if any, of these methods provide for the simultaneous assessment of the damage produced by two or more agents on adjacent areas of the gastric mucosa. The apparatus and procedure described in this report make such an evaluation possible and, in addition, allow the experimenter to observe the progression of some events as they occur.

EXPERIMENTAL

Apparatus—A 10.2-cm. round bar of clear Plexiglas was bored to 6.5-cm. i.d. and cut into pieces 5.6 and 2.4 cm. in length for the two upper pieces of the apparatus. Two pieces of Plexiglas tubing (0.5 × 2.5 cm.) were glued into holes bored into opposite sides of the upper portion using Plexiglas solvent MC-25. Three holes were drilled down through the top ring and into the bottom ring; the bottom ring was threaded to receive 10–32 SS machine screws. Three machine screws were ground to a point for easier penetration of the mucosa.

A continuous groove, 0.3-cm. deep and 0.4-cm. wide, was cut in the center of the bottom surface of the upper ring where it contacts

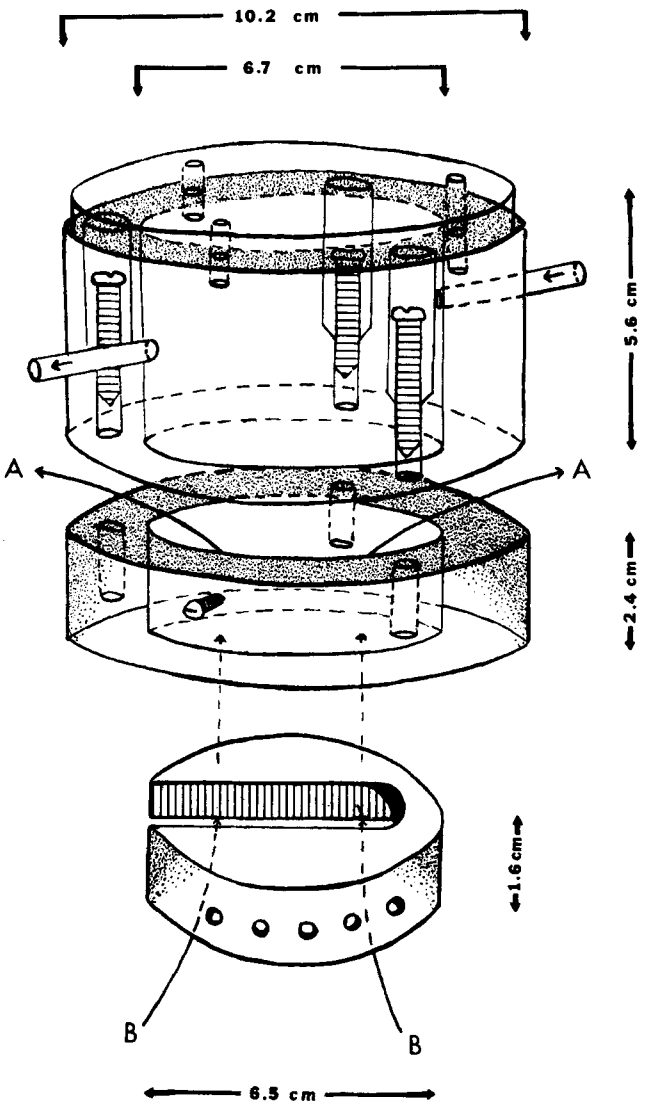

Figure 1—*Diagram of the Plexiglas chamber used for assessment of topical effects of substances on the gastric mucosa. Key: A, position of the stomach wall and mucosa; B, direction of major blood vessels. The lower Plexiglas disk, when placed into position, provides a relatively flat mucosal surface.*

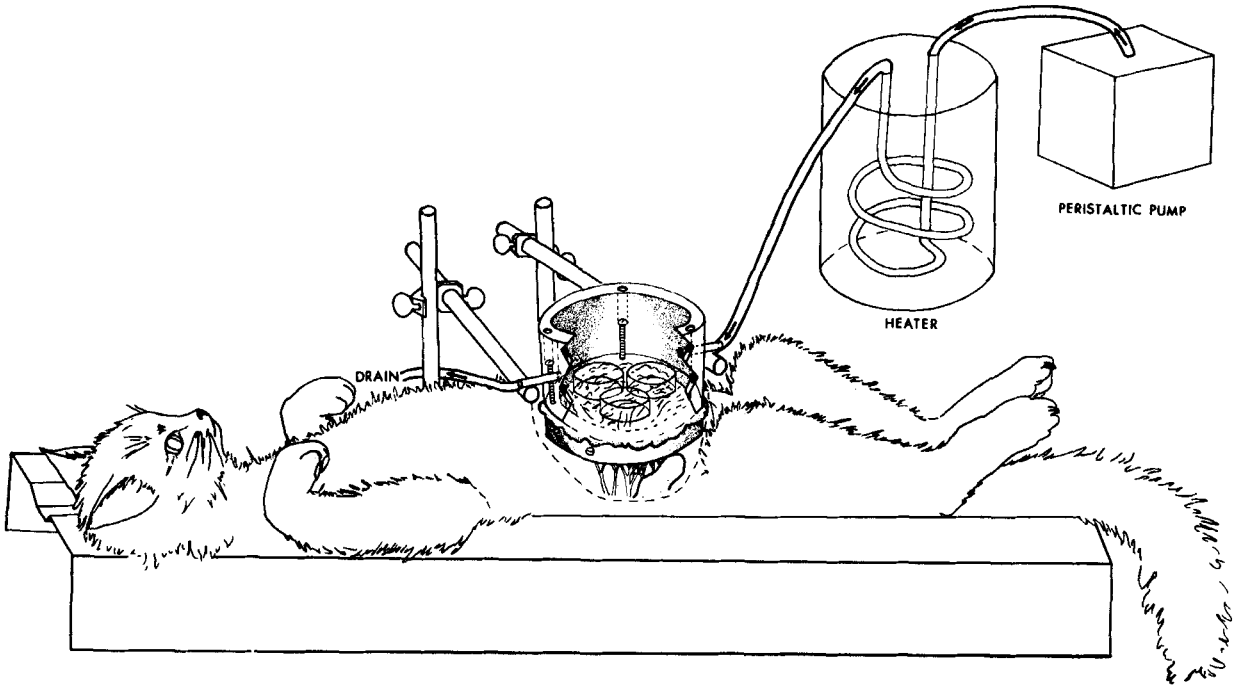

Figure 2—*Schematic drawing of the apparatus in position in the cat for assessing the topical action of substances on the gastric mucosa. Cutaway view of the chamber shows the position of the glass containers into which the test materials are placed.*

the mucosa. Likewise, a continuous tongue was cut in the top surface of the lower ring to fit into the groove. Gaskets of 0.2-cm. gum rubber were glued over the tongued surface and the grooved surface of the rings. A Plexiglas disk, 1.6-cm. thick, was made to fit inside the lower ring. A slot, 2-cm. wide, was cut into the disk to accommodate the vessels of the mucosa. The disk was held in place by a set screw. Holes were drilled into the sides of the disk so that the set screw could hold the disk more firmly. A diagram of the apparatus is shown in Fig. 1.

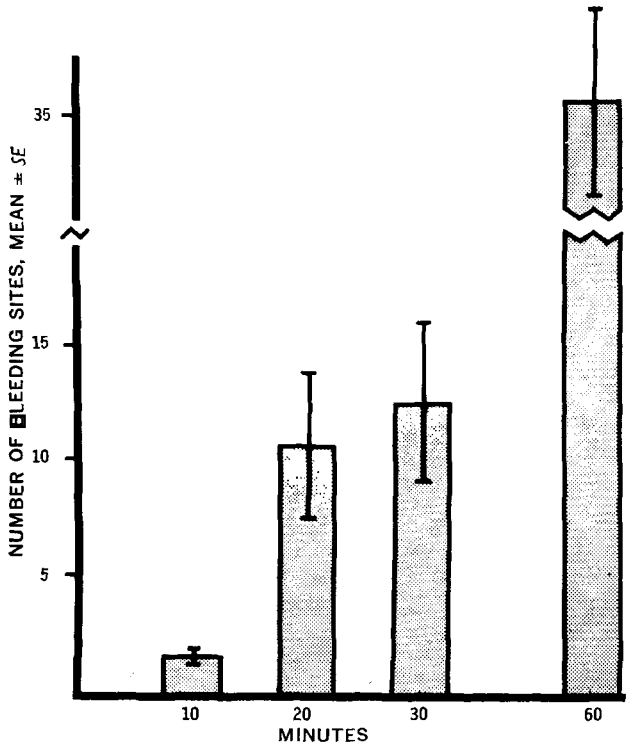

Figure 3—*Time–bleeding response pattern of aspirin (10 mg.) applied to the gastric mucosa of cats. The pH of the artificial gastric juice was 1.3. Results shown were obtained from 66 exposures in 11 cats.*

Procedure—Fasted cats of either sex, 2.5–5.0 kg., were anesthetized with phenobarbital sodium (130 mg./kg. i.p.). The stomach was exposed through a midline abdominal incision. The right gastric artery and vein were included with the ligature placed around the pyloric–duodenal juncture (excluding the right gastroepiploic artery and vein). Additional ligatures were placed around the esophagus, the left gastric artery and vein, and the larger vessels adjoining the lesser curvature. The stomach was further isolated by cutting through the esophagus and the pyloric ring distal and proximal to the respective ligatures. Eversion of the mucosa was made through an incision along the entire length of the lesser curvature.

Eight Allis clamps were attached to the stomach wall in a circular fashion and brought up through the Plexiglas ring (A in Fig. 1). The top part of the Plexiglas chamber was placed on the mucosa directly over the bottom ring and secured with screws. Blood vessels along the greater curvature were placed through the slot of the Plexiglas disk designed to provide a reasonably flat mucosal surface. Tissue blood supply was thus maintained in a similar fashion to that described by Rehm (11). In most of the experiments, physiological saline was allowed to remain on the mucosa for 1–2 hr. prior to perfusion of the chamber with artificial gastric juice USP.

The temperature within the chamber was maintained at approximately 37° by preheating the gastric juice prior to its entry into the chamber. This was accomplished by means of a Harvard peristaltic pump (model 1202) and a water bath (Blue M, model 120). The Tygon tubing leading from the water bath to the chamber was carefully insulated with asbestos tape to avoid heat loss. During the perfusion of artificial gastric juice, test substances were applied directly on the gastric mucosa with a tuberculin syringe (0.1 ml.) and confined to relatively small areas by means of glass rings (2.5 × 1.2 cm.). A schematic drawing of the apparatus in position in the cat is shown in Fig. 2. During the exposure to various substances, any visible bleeding sites on the mucosa were counted as they appeared.

Following termination of the experiment, the gastric mucosa was examined macro- and microscopically for injury, *i.e.*, erosions and ulceration[1].

[1] When it was desirable to record the progression of events, an EXA 35-mm. single-reflex camera (Exakta Camera Co., New York, N. Y.) was mounted directly above the chamber; Ektachrome EHB-135 film was used along with a 3200°K Photo-Flood lamp. A Polaroid Land camera (model Ed-10, film 108), mounted on a Bausch and Lomb Stereozoom 7 microscope, was also used successfully.

Table I—Comparative Topical Effects of Aspirin and Phenylbutazone on the Gastric Mucosa of Cats[a,b]

Drug	Exposure Time, min. 10	20	30	60
Aspirin	1.8 ± 1.8	14.3 ± 4.6	25.2 ± 4.9	44.9 ± 2.7
Phenylbutazone	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.3
p-Value	>0.05	<0.01	<0.001	<0.001

[a] Results expressed as the mean number ± *SE* of bleeding sites following the exposure of both phenylbutazone and aspirin to a total of 17 areas each in six cats. [b] Equimolar amounts of aspirin (18 mg.) and phenylbutazone (30.8 mg.) suspended in 20% arabic gum were applied to gastric mucosa perfused with artificial gastric juice (pH 1.3).

To illustrate the utility of the method, the gastric effects of the topical application of suspensions of aspirin and phenylbutazone are presented.

RESULTS AND DISCUSSION

The results of the application of equimolar amounts of aspirin and phenylbutazone on a total of 34 sites of the gastric mucosa in six cats are depicted in Table I. Both drugs were suspended in 20% arabic gum (USP powder), with the initial pH of the aspirin suspension being 3.3 and of the phenylbutazone being 4.4 prior to application on the gastric mucosa. Of 17 sites exposed to aspirin (18.0 mg.) for 60 min., 16 showed gastric bleeding and lesions; in the phenylbutazone (30.8 mg.)-treated areas, only slight bleeding and no lesions were observed. Four of the sites exposed to aspirin showed frank ulceration. The findings that phenylbutazone produced significantly less acute local gastric injury than aspirin are in agreement with those of Hitchens *et al.* (12); they found that upon oral administration to rats, phenylbutazone was less ulcerogenic than aspirin. Comparative clinical studies by Wood (13) indicated that, following oral administration of aspirin, 70% of the subjects studied showed occult bleeding whereas no reproducible bleeding occurred following phenylbutazone.

Quantitative assessment of the bleeding produced by 10 mg. of aspirin (0.1 ml. of a suspension with purified starch, pH 2.7) applied topically to the mucosa is shown in Fig. 3. The pH of the artificial gastric juice was 1.3 in all of these experiments. It is evident that the number of bleeding sites increased with time.

Another application of the method was shown by determining the effect of the pH of the artificial gastric juice on the bleeding produced by aspirin. The results obtained in 27 cats (Fig. 4) show that when the pH of the juice was maintained above 2.0, little or no bleeding resulted from the topical application of 10 mg. of aspirin. In contrast, as the pH decreased from 2.0, there was a marked increase in gastric bleeding.

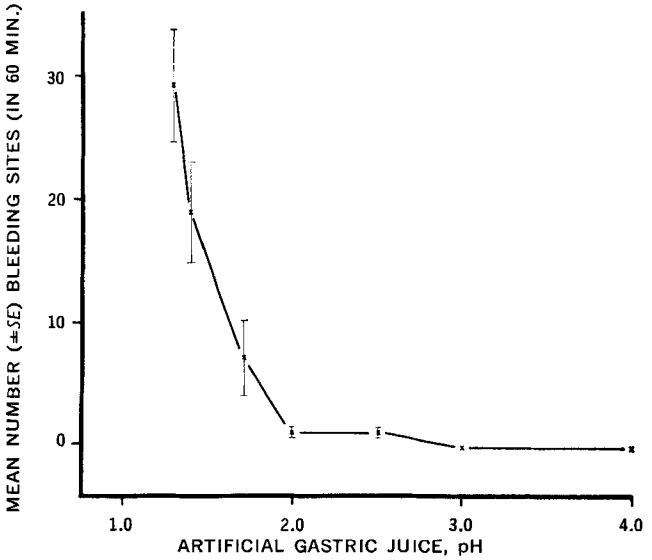

Figure 4—*Effect of pH of artificial gastric juice on bleeding produced by aspirin. The results were obtained from an average of 17 exposure sites to aspirin (initial pH of 2.7 in a suspension of 20% purified starch) with a minimum of three cats at each pH, with the exception of pH 4.0 where two cats were used.*

It appeared to be of interest at this point to determine whether or not, and if so to what extent, exogenous pepsin contributes to the bleeding response observed with aspirin. Following 1 hr. of exposure to aspirin (artificial gastric juice USP, pH 1.3), 18 areas in six cats showed a mean number of bleeding sites of 28.2 ± 4.5 *SE*. When under similar conditions 18 sites in five cats were exposed to aspirin with deletion of pepsin from the artificial gastric juice, the mean number of bleeding sites was 12.3 ± 3.7 *SE*. The difference between the number of bleeding sites with and without pepsin was significant ($p < 0.01$). Under the conditions used, pepsin appeared to contribute to the bleeding observed with aspirin.

The method as described has been used routinely in this laboratory for the assessment of the topical action of drugs on the gastric mucosa of laboratory animals. In one series of experiments, 10 mg. of aspirin (applied to 350 different areas of the gastric mucosa in 154 cats) produced 15.82 ± 1.00 *SE* bleeding sites within 30 min. When the time was extended to 60 min., the number of sites increased to 38.02 ± 1.11 *SE* (266 exposures in 134 cats).

Experience with the method has demonstrated that it offers the following advantages: (*a*) one or more substances may be evaluated simultaneously on adjacent mucosal areas; (*b*) the quantity of material applied and exposure time may be easily varied; (*c*) gastrotoxic effects can be observed as they occur; and (*d*) the apparatus may be adapted for use in large or small animals.

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