ID_{50} method, and 1 out of 1-36 organisms (100-2.5%) by the LD_{50} method.

In contrast to these methods, the plaque assay is in effect a direct viable cell count (accuracy obtained with only a few plates is at least equal to that obtained by use of a large number of animals in calculating the LD_{50}). In addition, plaques can be detected in 4-5 days, regardless of size of inoculum, whereas mice inoculated with limiting dilutions die only after about 12 days. It has been demonstrated that each plaque is induced by a single parasite, and that large populations of toxoplasmata can be produced at will in which 1 of every 2 organisms is a plaque former.

One problem which has not yet been overcome is our inability to release more than an average of 2 parasites/cell. In view of the fact that chick embryo fibroblasts which line the plaques usually contain a large number of organisms, the low yield under conditions of saturating infections suggests the operation of some yield-limiting mechanism.

The clear definition of individual organisms as infectious units should facilitate further improvement of media with the ultimate aim of supplying the parasite with its needs for extracellular proliferation. Further work is planned leading to purification and biochemical studies of *Toxoplasma*.

Summary. The RH strain of *Toxoplasma gondii* induces formation of necrotic plaques on monolayers of chick embryo fibroblasts overlaid with nutrient agar. Correlation between number of organisms plated and number of plaques produced indicates that each plaque is induced by a single toxoplasma. Survival of toxoplasmata in various media has been tested, and it has been found that Hanks BSS containing chick embryo extract in concentrations of 5 to 50% preserves 80-100% of plaque forming units (pfu) for 3 to 4 hours, 50-60% for 6 hours. Adsorption of pfu on monolayers is maximal after 70 to 90 minutes (Kc = 0.024), can be retarded by increased viscosity of the medium, speeded by centrifugation. Artificial lysis of chick embryo fibroblasts infected at low multiplicity yields populations with a predictable efficiency of plating of about 50% of the counted organisms.

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Assessment of Antral Protective Mechanisms and Intestinal Susceptibility Gradient to Ulcer Formation. (25276)

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Development of peptic ulcer is dependent upon a shift in equilibrium between ulcerogenic factors (secretions of corrosive acid-peptic gastric juices in varying concentrations and volumes), the protective mechanisms (alkaline buffering secretions, *i.e.* bile, pancreatic juice and succus entericus), and bowel susceptibility to ulceration (quantity and character of mucus secretions, mucosal blood supply and regenerative potential, and intrinsic mucosal cellular resistance.) The antral phase of gastric secretion is largely an auto-regulatory system(1) when the antrum occupies its normal position. Potentiation of

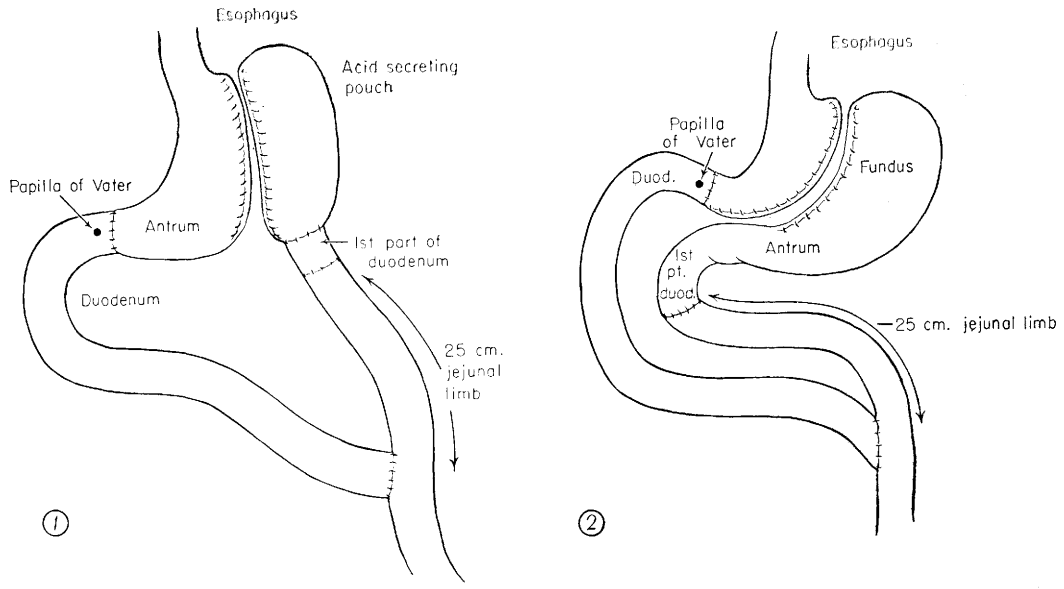

FIG. 1 and 2. Surgical preparation of Group I and Group II animals, respectively.

ulcer production by excluding the antrum from continuity with the proximal acid-peptic secretory area of the stomach is well known (2). A positive gradient of susceptibility to peptic ulceration of the bowel directly related to distance from pylorus was proposed by Mann and Williamson(3). This thesis has been both supported(4) and denied(5). The following preparation was designed for studying protective influences of the antrum and susceptibility gradient to peptic ulceration of the upper small bowel. The preparation also permits assessment of any protection accruing from antral exclusion when acid peptic juice continues to pour over the excluded antrum.

Methods. Two groups of mongrel dogs of both sexes were prepared as follows: (Fig. 1, 2). Procedures were carried out under intravenous nembutal anesthesia (30 mg/kg) and through a midline abdominal incision. In Group I a moderate sized (40%-50%) isolated (denervated) acid secreting gastric pouch was formed as illustrated in Fig. 1. The antrum was left in continuity with the remaining proximal gastric segment. The first part of the duodenum, about 3 cm long, between pylorus and papilla of Vater, was anastomosed isoperistaltically proximally to the isolated gastric segment and distally to a 25 cm Roux-en-Y upper jejunal limb. The distal duodenum containing the papilla of Vater was anastomosed to the antrum. In Group II, (Fig. 2) the stomach was transected obliquely across its longitudinal axis so that the antrum and an equal sized fundic pouch were separated from the proximal gastric segment remaining in continuity with the esophagus. The duodenum was transected about 1 cm proximal to the papilla of Vater. A 25 cm Roux-en-Y upper jejunal limb was anastomosed to the proximal duodenal segment. The distal duodenum containing papilla of Vater was anastomosed to the proximal gastric segment for reconstitution. Postoperatively, the animals were given regular kennel rations supplemented with canned cooked meat daily. Experiment was terminated and all animals sacrificed July 1, 1958. Autop-

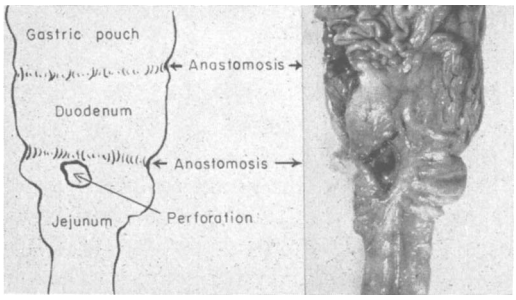

FIG. 3. Example of jejunal perforations occurring in 50% of Group I animals, showing intact proximal duodenal segment.

TABLE I.

	No. of dogs	Survival of sacrificed dogs (mo) Mean	Range	No. of dogs with ulcer	% incidence of ulcer	Site of ulcer	Time of ulcer appearance
Group I	6	8	4-10	3	50%	Jejunum	4, 9, 10 mo
" II	5	6	3- 9.5	0	0		

sies were performed and pertinent results are recorded in Table I.

Results. Of the 6 dogs in Group I, surviving 4-10 months to end of study or dying from peptic ulcer, 3 of them, 50%, developed perforating jejunal ulcers just beyond the site of duodenojejunal anastomosis. (Fig. 3). In none of the animals was the short proximal duodenal segment intervening between the fundic pouch and the jejunum ulcerated or eroded.

In the 5 animals of Group II surviving 3 to 9.5 months, to termination of experiment, there were no erosions or ulcerations in any portion of the bowel. Neither jejunal limb nor duodenal segment showed any evidence of peptic digestion. No evidence of esophagitis was noted in either group. One animal was sacrificed at 3 mo. because of suspected ulcer. No ulcer was found.

Discussion. It is significant that no ulcers were observed in preparation II, in the area of biliary papilla or between it and site of duodenogastric anastomosis (Fig. 2). Presence of antrum in continuity with the acid-secreting fundic pouch in Group II animals undoubtedly accounts for absence of stomal ulcer at this point. The acid secretion bathing the antral mucosa inhibits antral secretion, thereby decreasing secretions of the fundic pouch and the residual proximal gastric segment (auto-regulatory mechanism). Antral mucin and mucus undoubtedly furnish mechanical and chemical protection as well(6). The neutralizing influence of succus entericus of the upper duodenum was a factor common to both groups. Absence of neutralizing effect of bile and pancreatic juice at the neostoma of the isolated pouch was similarly common to both groups.

Upon examination of Group I results, it is evident that both the short upper duodenal segment and the contiguous jejunal Roux limb were exposed to the same acid peptic juice of the isolated pouch. The 50% incidence in this group of a jejunal perforation just distal to the duodenojejunostomy without duodenal involvement suggests definitely a greater susceptibility of the jejunal mucosa to peptic ulceration. The presence of Brunner's glands in this proximal duodenal segment in the dog may partially account for this difference in susceptibility.

This susceptibility gradient has been a controversial subject in the literature. Dillard and Merendino(5) interposed short segments of bowel from various levels between second and third parts of the duodenum to keep constant the alkaline buffering effect of the duodenal contents. Heidenhain or Pavlov pouches were anastomosed to the mid-points of the interposed segments. They failed to produce ulcers in any of these animals and concluded that a susceptibility gradient does not exist.

The phenomenon of decreasing buffering capacity and pH of intestinal contents at levels of small bowel progressively more distal from the papilla of Vater was amply demonstrated by Cross(7).

Summary and conclusions. 1) In Group II type of preparation described herein (antral exclusion with 50% of acid peptic secreting tissue attached to antrum) no abettment of ulcer diathesis was noted. Ulcer was not observed at either neostoma. 2) When, however, a similar fundic pouch was attached to the duodenum (Group I preparation) ulcer occurred at this neostoma in 50% of dogs studied and interestingly not in the short duodenal segment, but just over on the jejunal side, an occurrence which suggests an increased gradient of susceptibility to peptic ulceration.

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Epinephrine-Induced Alterations in Connective Tissue of Aortic Wall in Rabbits.* (25277)

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Alterations in rabbit aortae caused by epinephrine were first described by Josué(1). Later on several others have confirmed his results. Summaries have been given by Hueper (2) and Raab(3). Gross alterations have been observed as a response to epinephrine injected intravenously once daily through two weeks. The purpose of this experiment was to examine the influence of epinephrine on the connective-tissue ground substance in the aortic wall. The amorphous ground substance is characterized by its content of acid mucopolysaccharides, sulfated as well as non-sulfated, whereas to a considerable degree, the collagen is characterized by its content of hydroxyproline.

Materials and methods. Twenty white male rabbits were kept on a uniform, sufficient laboratory diet. The experimental animals had epinephrine† intravenously injected daily for 15 days in doses rising from 0.025 mg/kg once a day to 0.050 mg/kg twice a day during the last 10 days. On the 14th day experimental animals and controls were injected with ^{35}S sulfate, 1 mC/kg subcutaneously (carrier-free ^{35}S sulfate,‡ diluted to a suitable volume with a 0.005% solution of sodium sulfate in physiological saline). 48 hours later they were sacrificed with nembutal (ethyl-methylbutyl barbituric acid) injected intravenously. The aorta was removed in its whole length together with the heart. The gross alterations were roughly estimated. The thickness of the walls of the right and left heart ventricle was measured. Preparations for histologic examinations were removed from the thoracic and abdominal aorta. The tissues were fixed in a 4% aqueous solution of lead subacetate and a 4% aqueous solution of neutral formalin. The sections were stained with hematoxylin-eosin (Harris), orcein (Unna) and Sudan III. Further the sections were stained with a ½% aqueous solution of toluidine blue for metachromasia, and for calcium by the method of von Kossa. For the biochemical investigations the thoracic aorta from the aortic valve to the diaphragm, the site of the most pronounced gross alterations, was used. The aorta was cut longitudinally into 2 halves, and the intima plus media were peeled off. The water content was determined as the difference in weight before and after 48 hours drying in an oven at 37°C. The total hexosamine content was determined to estimate the content of acid mucopolysaccharides, by the method of Elson and Morgan(4) as modified by Boas(5). The analysis was carried out on half of the dried and minced tissue by hydrolysis with 2 N HCl as described by Kirk and Dyrbye(6). The sulfated mucopolysaccharides were estimated by the *in vivo* uptake of ^{35}S sulfate as devised by Moltke(7). After drying, the uptake was measured on the hydrolysate of the dried tissue, which was also used for the determination of the hexosamine. The activity was measured with a Geiger-Müller tube having a mica-end window weighing 2.7 mg/sq cm. Determination of the hydroxyproline

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† 0.1% solution of epinephrine hydrochloride.

‡ Obtained from the Radiochemical Centre, Amersham, England.