

THE EFFECT OF ACIDS AND OTHER SUBSTANCES IN THE PRODUCTION OF ACUTE GASTRIC ULCERS*

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PLATE 14

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Gastric ulcers have been produced by many experimental methods but in very few instances have they shown any tendency toward chronicity. Numerous investigators have attributed to hyperacidity the power of converting acute into chronic ulcers. There are certainly many factors involved but none have been so consistently present as hyperacidity. The fact that a moderate number of ulcers have been recorded in which hyperacidity was not present is evidence enough that other factors play important rôles in the production of the condition. Since the etiology of ulcer is still not well understood we have attempted to study the problem experimentally. It is a problem of the first importance, not alone because of the dangers and disabilities caused by ulcer, but because this so often leads to cancer of the stomach.

O'Shaughnessy (1) recently attempted to produce chronic ulcers in dogs by injecting histamine into the muscular layer of the stomach wall. In two instances of a large series he was able to produce ulcers which resembled the chronic forms seen in man. The same observation had been previously made by Bolton who used gastrotoxic serum and a similar technique. In the remaining experiments of his series, O'Shaughnessy was able only to produce acute ulcers and these healed rapidly. He suggests that histamine is more than likely an important factor in ulcer production. The fact is well recognized that histamine is a powerful stimulant to hydrochloric acid secretion and although subcutaneous injections of histamine do not in themselves produce chronic ulcers, the local injection into the stomach

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wall may, according to some observers, bring this about through an induced over-secretion of acid.

In the experience of Ivy (2) hyperacidity is not of great significance for the chronicity of ulcer. However, many investigators have shown that instillations of hydrochloric acid into the stomach and duodenum produce not only acute and chronic gastritis and duodenitis but also multiple erosions and acute ulcers. Gallagher found that when an element of trauma was added to the administration of acid by mouth, chronic ulcers could be produced.

More recently, the importance of acid in the production of ulcer has been discussed by Lindau and Wulff (3) who have been able to corroborate Buchner's finding that ulcers arise in the areas of activity of gastric juice, whereas the area of production possesses by nature a relatively high power of resistance. Ulcers rarely occur in the area of the fundus glands; they are usually found in the region of the pyloric glands. From their survey Lindau and Wulff conclude, that "in the biochemical theory, we have an exceedingly good explanation of the nature as well as the location of the peptic lesions with ulcer formation, confined to the esophagus, cardia, area of the pyloric glands, duodenum, jejunum (postoperatively) and in Meckel's diverticulum. Everywhere the lesion is to be found in the area of activity of hydrochloric acid."

Ivy and Shapiro (4) in 1925 were able to produce acute ulcers of the stomach in dogs and rabbits on the basis of local anaphylaxis to foreign proteins. In a later series of experiments (5) they describe in detail their method of producing ulcer. They employed egg albumin, beef protein, oat protein, horse serum, edestin, squash and other substances and found that when these substances were injected into the stomach wall without previous sensitization no ulceration resulted, but when previously sensitization had been carried out with the respective sensitizing proteins, ulcerations were found at the site of injection in the stomach. They also noted that the healing times of the ulcers were not affected by the gastric acid. On the other hand, Bolton (6) maintains that the acid of the gastric juice is the chief damaging factor to a devitalized portion of the stomach, and where motor insufficiency occurs, the effect of the acid is more marked and the healing of acute ulcerations is definitely delayed. Although Bolton emphasizes the fact that hyperacidity is concerned as a factor in the pathological process of an ulcer, yet he maintains that proof has never been afforded that an ulcer gives rise to hyperacidity. Dragstedt (7), after producing ulcers in Pavlov pouches in animals by the local injection into the stomach wall, of 4 per cent silver nitrate, concluded that acidity had no effect upon the healing time of acute ulcers.

Methods

In our experiments we tested the effect of injections of various substances into the muscular coat of the stomach and their relation to the production of hyperacidity and acute ulcers.

The following materials were injected: sterile water, normal salt solution, glu-

cose 50 per cent, skimmed milk, whole milk, lipo-protein (the commercial preparation, omnadin was used), lipiodol, bismuth subcarbonate, pituitrin S., 5 per cent casein (the commercial preparation, activin, was used), histamine, adrenalin, insulin, 0.3 per cent hydrochloric acid, 0.5 per cent hydrochloric acid, 1 per cent hydrochloric acid, 10 per cent hydrochloric acid.

It will be seen that substances representing fats, carbohydrates, proteins, metals and other chemicals were used in tests of their local effects. It was thought that perhaps chemicals of certain structure might act more specifically than others in producing gastric defects. As control, the effect of trauma alone was noted.

Determinations of the gastric acidities were made following histamine stimulation. It is important to call attention to the fact that the average acidities in most of our dogs following this stimulation varied but little. Even after the injection of substances into the stomach wall, no changes in acidity were noted. The resistance of the gastric tissues to the injected material varied considerably in different dogs. Where the dog is well nourished, its resistance is likely to be far better than in the underfed animal. Lowered resistance is recognized to be conducive to ulcer formation.

The dogs were operated upon under ether anesthesia. The stomach was exposed through a right rectus incision, care being taken to prevent unnecessary trauma. Injections of 0.5 cc. of various substances were made into the muscular coat of the lesser curvature using a hypodermic needle of very small gauge. In a few instances the greater curvature was selected for injection. The injections were made through the serosa, the stomachs not being opened. We made certain that the injected materials were placed in the muscular coat not only by the resistance offered to the needle but also by the bulge which appeared after each injection and in the case of lipiodol and bismuth by x-ray verification. The area injected was marked exactly by the placement of a colored bead. The beads were attached only to the serosa and care was always taken not to include any of the deeper structures. The lesser curvature was the site most frequently selected in these experiments since it is the favorite area of ulcer formation. In some instances, however, the greater curvature was selected and it is of interest to note (1) the difference in the resistance of the muscle layers to the hypodermic needle, and also (2) the difference in rapidity of diffusion of the injected substances in the two areas. In other words, it was found that the greater curvature offered less resistance and allowed the material to diffuse much more rapidly.

Utilizing the method of multiple injections for the detection of hypersensitivity to certain substances, just as in the case of the skin, we injected as many as four different materials into the stomach wall of the dog, placing them so that they did not interfere with one another. For example, two injections were made on the anterior wall and two on the posterior wall, each separated from the other by a considerable area. Colored beads were again used, each substance being represented by a distinct color. This made identification relatively simple.

The dogs were fed on the ordinary laboratory diet and there was no interference with their usual habits. When acid was administered, this was done through a

stomach tube. The animals were eventually killed with ether and autopsies were performed as routine. The stomach, after complete removal, was opened along the greater curvature and spread out. The mucosa was examined with meticulous care and the injected area corresponding to the bead, which was easily identified, was located and excised with a wide margin. Histological studies were then made.

EXPERIMENTAL

Under histamine stimulation numerous gastric analyses were performed in a large series of dogs and an average normal acidity established. Repeated examinations showed but slight variations from this average except in some instances in which excessive mucus was present. Instances in which this was the case were not included in obtaining the average normal.

After the injection of various substances into the stomach wall, gastric analyses were made at frequent intervals. We were unable to demonstrate any tendency toward hyperacidity, the average normal being about the same. Even after the formation of an acute ulcer, no increase in the acidity was observed.

Trauma.—As controls, beads were placed over areas traumatized by a threaded needle which was passed through the serosa. Upon subsequent examination the stomachs were found to be normal in every respect.

1. *Sterile Water.*—For this experiment four dogs were utilized. 0.5 cc. of sterile water was injected into the muscular coat of the lesser curvature of the stomach. After 17 days, two of the dogs were killed and on macroscopic examination the mucosa was found to be normal. This observation was verified by histological studies. The other two dogs were killed after 22 days, after having been fed 1100 cc. (60 to 120 cc. *q. d.*) of 0.3 per cent hydrochloric acid. This additional administration of acid did not produce any change in the end-result, since both microscopic and macroscopic examinations were negative.

2. *Normal Salt Solution.*—The experiments performed with sterile water were repeated, using normal (physiological) saline. The results obtained were also found to be normal.

3. *Glucose (50 Per Cent).*—With the use of this agent the findings were exactly similar to those obtained after the injection of sterile water and saline solution.

4. *Skimmed Milk*.—Five dogs were used in this experiment and after the 5th day three dogs were killed. These revealed slight submucosal edema and congestion, the mucosa remaining normal. The other two dogs having been fed with 800 cc. of 0.3 per cent hydrochloric acid were killed on the 15th day and no changes were detected.

5. *Whole Milk*.—Five dogs were utilized. Of three, which were killed after 5 days, there was found in two, a slight submucosal edema and congestion, while the remaining one revealed no abnormalities. The other two dogs had been fed with 15 cc. of 0.3 per cent hydrochloric acid twice daily for 15 days when they were killed. These showed no abnormal changes.

6. *Omnadin (a Combination of Proteins and Lipoids Used in Non-Specific Immunization Therapy)*.—Five dogs were used. In three, killed on the 5th day, the mucosa was normal; the submucosa showed in two instances a localized leucocytic infiltration with large phagocytic cells, while, in the other, no changes of this type were observed. The remaining two dogs were fed with 300 cc. of 0.3 per cent hydrochloric acid twice daily for 15 days and in these no changes were found.

7. *Lipiodol*.—Four dogs were utilized. In order to determine and verify the exact location of the injected material, the dogs were radiographed. It was found that the opaque substance was definitely located in the gastric wall. (Lipiodol was used to represent a foreign body, not easily absorbed and therefore possibly causing a prolonged irritative action by pressure.) Two dogs were killed on the 17th day. Macroscopically these showed large indurated nodules, 1 cm. in diameter, which represented the unabsorbed lipiodol. Further studies under the microscope gave no indication of tissue injury but showed large globules of unabsorbed lipiodol. The remaining two dogs had been fed daily with 30 cc. of 0.3 per cent hydrochloric acid for 21 days and despite the administration of this dilute acid the end-results were the same.

8. *Bismuth Subcarbonate*.—As another type of non-absorbable foreign body, bismuth was employed in a manner exactly identical with the lipiodol experiment. The final results were a repetition of those obtained with the lipiodol except that the bismuth microscopically revealed itself as a brownish black pigment.

9. *Pituitrin (S.)*.—Five dogs were utilized. One dog died after 4

days and revealed no gastric changes at autopsy. After 21 days, two dogs were killed and whereas macroscopic examination showed a slight redness and edema, the microscopic examination revealed a normal mucosa with only slight submucosal edema. After 25 days one dog was killed and similar findings were noted. The remaining dog was fed with hydrochloric acid, receiving 750 cc. of 0.3 per cent hydrochloric acid in small doses of 15 cc., and being killed after 15 days. Macroscopically, no abnormalities were observed but microscopically slight submucosal edema was found.

10. *Activin (5 Per Cent Casein)*.—Six dogs were used. One died after 4 days and revealed no gastric changes at autopsy. In two dogs killed after 21 days, the mucosa was found to be normal except for slight redness over the injected area; the submucosa showed slight edema; otherwise, no abnormalities were noted. In one dog killed after the 25th day, a slight mucosal erosion was noted on both macroscopic and microscopic examinations of the stomach. In addition, vascular engorgement and submucosal edema were observed. Two dogs were fed with acid; one received 765 cc. of 0.3 per cent hydrochloric acid in small doses, and when killed on the 15th day, presented no gastric changes. The other, killed on the 14th day after having been fed with 350 cc. of diluted hydrochloric acid, likewise showed no pathological changes.

11. *Histamine*.—Six dogs were used. One died after the 4th day and revealed no gastric changes at autopsy. Two dogs, killed after 21 days, presented a normal gastric mucosa and a pronounced submucosal edema. In another dog, killed after the 25th day, there was found a small healed ulcer which on microscopical study revealed a slight mucosal erosion with infiltration of polymorphonuclear leucocytes and mononuclear cells in the stroma between the glands, engorgement of the blood vessels and moderate submucosal edema. In two other dogs, fed respectively with 350 and 765 cc. of 0.3 per cent hydrochloric acid, there were no macroscopic nor microscopic changes after 14 days.

12. *Adrenalin*.—Three dogs were used. In one dog, killed after 25 days, there was found on macroscopic examination a small congested area and on microscopic examination an infiltration between the glands, moderate engorgement of blood vessels and submucosal edema.

In the other two dogs, killed after 21 days, the mucosa was found to be normal and the submucosa slightly edematous. In each instance macroscopic swelling of the tissue corresponding to the injected area was observed.

13. *Insulin*.—Three dogs were used. One dog died after 4 days, and showed no gastric changes at autopsy. Two dogs were fed with 0.3 per cent hydrochloric acid, 350 and 765 cc. respectively, and revealed no microscopic nor macroscopic changes.

14. *0.3 Per Cent Hydrochloric Acid*.—Nine dogs were utilized in this experiment. In two dogs 0.5 cc. of 0.3 per cent hydrochloric acid was injected into the muscular layer of the lesser curvature of the stomach. These were killed after 16 days and on macroscopic and microscopic examinations no abnormalities were noted. After the injection of 0.5 cc. of 0.3 per cent hydrochloric acid into the muscular layer of the stomachs of three other dogs, they were, in addition, fed with 30 cc. of 0.3 per cent hydrochloric acid twice daily for 15 days. In two of these, definite small mucosal ulcers were noted and in the other no macroscopic nor microscopic changes were observed. As a check on these findings two other dogs were injected and fed with 0.3 per cent hydrochloric acid. After 12 days of this treatment the dogs were killed. One showed a definite small ulcer filled with necrotic tissue and leucocytes. In the other, the mucosa was found to be normal and the submucosa showed a moderate edema. In two dogs, 0.5 cc. of 0.3 per cent hydrochloric acid was injected into the muscular coat of the anterior aspect of the *greater curvature* near the pyloric antrum. On the 16th day these dogs were sacrificed and examination disclosed no abnormal changes. These dogs were not fed with acid.

15. *0.5 Per Cent Hydrochloric Acid*.—Two dogs were utilized. These dogs were not fed with acid. On the 9th day they were killed. In one, a definite deep ulcer was noted surrounded by an indurated edge. Microscopically the ulcer was surrounded by leucocytes and fibrin. The base was found to be infiltrated down to the muscular coat. The other showed a definitely thickened area but no ulceration nor erosion.

16. *1 Per Cent Hydrochloric Acid*.—Eight dogs were utilized in this experiment. Two were killed after the 9th day. No acid was fed to these dogs. In one of them, the macroscopic examination revealed a perforated ulcer walled off by omentum. Microscopically, the per-

forated ulcer was found to be surrounded with inflammatory exudate. In the other there was found a definite ulcer of the penetrating type verified by both macroscopic and microscopic examinations. In two dogs the injection of 0.5 cc. of 1 per cent hydrochloric acid was made into the muscular coat, on the anterior aspect of the greater curvature near the pyloric antrum of the stomach. On the 12th day these dogs were killed and no abnormal changes were observed. In the four remaining dogs, the injections of 1 per cent hydrochloric acid were again made into the muscular coat of the *greater curvature*. They were killed on the 17th day. One of these revealed a perfectly normal mucosa, while in three, definite penetrating ulcers were observed. In this series of experiments the animals had not been fed with acid.

When higher concentrations of HCl are employed (*e.g.* 10 per cent), either by introduction into the viscus or injection into the muscular coat, destructive effects are displayed which lead to perforating ulcers. The lesions are of acute character and should not be confused with the chronic ulcerating processes in human cases.

The Effect of Histamine Given Subcutaneously.—Acute ulcers were produced in four dogs by the direct injection of 1 per cent hydrochloric acid into the muscular coat of the stomach. It was thought that histamine given subcutaneously might cause the acute ulcer to become chronic by maintaining a maximum secretion of acid or by the toxic effect of the excessive amounts of histamine administered. To that end the four dogs were given 0.5 mg. of histamine subcutaneously, one to three times daily. One dog died 23 days following the production of the ulcer after receiving 54 injections of histamine. Autopsy disclosed a large penetrating punched out ulcer of the acute type. In the three remaining dogs which were killed on the 95th day after receiving 146 injections, autopsy revealed no ulcer in two and in the other a very superficial erosion was noted. In a second control group of four dogs acute ulcers were produced in the same way but in these no histamine was administered and small healed ulcers were observed in each instance probably illustrating the fact that subcutaneous injection of histamine did not have a significant influence upon the production of chronic ulcers but on the contrary seemed to favor complete healing. Despite the small number of dogs the results obtained pointed clearly, it seemed to us, to the fact that histamine given as described had no

pronounced effect upon acute ulcers. It is interesting that even after the continuous use of histamine over a long period of time the acidities were not markedly affected and the relationship between ulcer, healing ulcer, scar and acidity was too variable to be of any significance.

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DISCUSSION AND SUMMARY

Although other investigators have attempted to demonstrate that histamine has the power of producing acute ulcers when injected locally into the stomach wall, our experiments show clearly that this is a very inconstant finding for, of six instances, we were only able to obtain a small healed mucosal ulcer in one. Even when dilute acid (0.3 per cent hydrochloric acid) was fed to these animals they did not develop ulcers or erosions. It has also been stated that histamine, given subcutaneously, may produce ulcers secondarily to oversecretion of acid, especially when at the same time histamine has been injected into the stomach wall. We have been unable to confirm this observation.

In our series of experiments on dogs which were observed over a varying period of time ranging from 4 to 95 days we utilized other substances in addition to histamine. After establishing controls, sterile water, normal saline, 50 per cent glucose, skimmed milk, whole milk, omnadin, lipiodol, bismuth subcarbonate, pituitrin (S.), activin, histamine, adrenalin and insulin were injected into the muscular coat of the stomach and it was found that such procedures rarely produced any definite changes in the stomach mucosa even when supplemented by the feeding of dilute hydrochloric acid (0.3 per cent hydrochloric acid). We were able to demonstrate conclusively that the trauma involved in our operative procedure could not in itself account for any of the pathological findings. We were also able to show that the pressure due to the injected materials did not produce erosions or ulcerations. The injections of acids produced varying results. Diluted acid (0.3 per cent) injected into the stomach wall gave almost uniformly negative results but when combined with the feeding of acid of equal

strength erosions were occasionally produced. Our results with 0.5 per cent hydrochloric acid were also inconstant. The 1 per cent hydrochloric acid produced, in many instances, definite ulcerations even when not accompanied by the feeding of acid.

CONCLUSIONS

1. The various substances utilized in these experiments when injected into the muscular coat of the stomach wall did not have any significant relationship to the production of ulcer.

2. Weak solutions of hydrochloric acid had no corrosive effect upon the gastric mucosa and rarely produced ulceration when injected into the muscular coat of the stomach. Stronger solutions produced definite ulcerations.

3. No relationship could be demonstrated between the injection of various substances into the stomach wall and the production of hyperacidity.

4. The prolonged use of histamine administered subcutaneously was not a factor in the production of chronic ulcers even after 1 per cent hydrochloric acid was injected into the muscular coat of the stomach.

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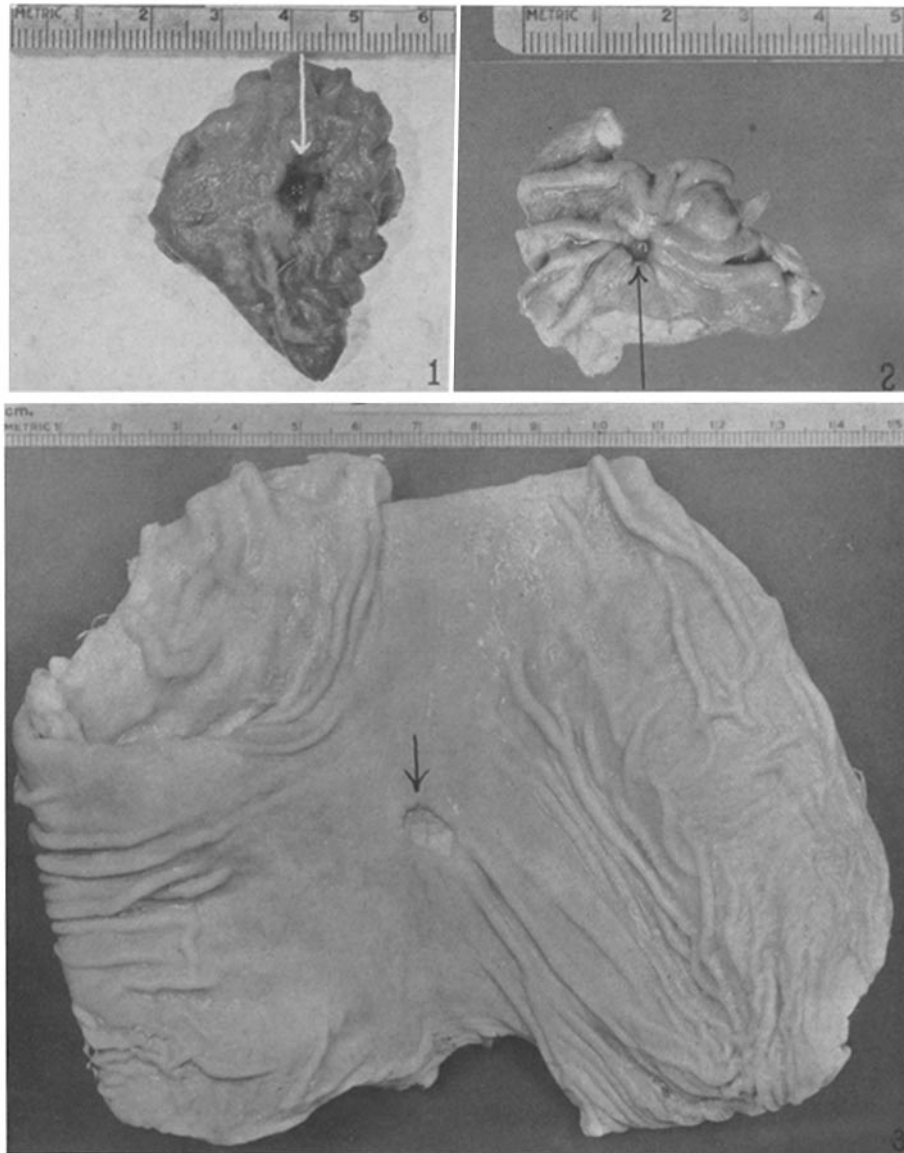
EXPLANATION OF PLATE 14

FIG. 1. A perforated ulcer, walled off by peritoneum, produced by the injection of 1 per cent hydrochloric acid. The dog was killed on the 8th day.

FIG. 2. A small penetrating ulcer, deep puckered type, with thickened elevated edge. Mucosal folds converge towards the ulcer. This ulcer was produced by

the injection of 1 per cent hydrochloric acid into the wall of the stomach. The dog was killed on the 19th day.

FIG. 3. Deep punched out ulcer of penetrating type, extending down into the muscularis produced after the injection of 1 per cent dilute hydrochloric acid into wall of stomach, following daily subcutaneous injections of histamine over a period of 23 days. This occurred in only one dog of the series. In another dog only a small mucosal erosion was encountered, while in two dogs a perfectly normal mucosa was found.



(Friedenwald *et al.*: Acids in production of acute gastric ulcers)