1951

Role of lysozyme in the pathogenesis of ulcerative alimentary disease

Keay Hachiya
University of Nebraska Medical Center

THE ROLE OF LYSOZYME IN THE PATHOGENESIS OF ULCERATIVE ALIMENTARY DISEASE

Keay Hachiya

Submitted in Partial Fulfillment for the Degree of Doctor of Medicine

College of Medicine, University of Nebraska

December 17, 1951

Omaha, Nebraska

INTRODUCTION

The investigations of Meyer and his associates (1 & 2) suggesting relationship between lysozyme and certain ulcerative diseases of the alimentary tract have aroused widespread interest. These workers reported that the concentration of lysozyme is increased above normal in the pyloric and duodenal mucosa as well as in the gastric juice of patients with peptic ulcer. Patients with ulcerative colitis were found to have extremely high concentrations of lysozyme in the feces. It was postulated by Meyer et al, that lysozyme removed the protective surface mucus from the colon by virtue of its mucolytic activity and consequently favored ulceration of the denuded mucosa by the proteolytic enzymes and indigenous bacterial flora.

It is the purpose of this paper to review the recent investigations to ascertain or disprove this claim.

Lysozyme is an enzyme characterized by its ability to effect the lysis of certain bacteria, notably Micrococcus lysodeikticus, which was isolated by Fleming (3). The lytic power of lysozyme is attributed to its ability to digest the mucoid capsule of the susceptible organism. In their attempt to purify the enzyme Meyer and his co-workers (4 & 5) found that the purest lysozyme preparations were basic in nature, being soluble only in acidified aqueous media and insoluble in pure organic solvents. They contained about 15 per cent nitrogen, a small amount of sulfur present as sulfhydryl, and a small amount of phosphorus. A Highly purified preparation had the following composition: C 48.65, H 6.44, N 15.33, ash 3.31, P .25, S .64. With phosphorus and sulfur as a basis, the minimum molecular weight is 25,000.

Lysozyme is apparently a polypeptide giving a number of protein reactions. From the presence of sulfhydryl, its inactivation by alkali, peroxide, iodine, and cuprous oxide, and its reactivation by hydrogen sulfide, sulfite and hydrogen cyanide, it was concluded that lysozyme acts only in the reduced state.

The lytic action of lysozyme on susceptible bacteria can not be explained on a physical basis; eg., lowering of surface tension. It has no protease, kinase, amylase,

lipase or phosphatase activity. The type of linkage attacked is not known.

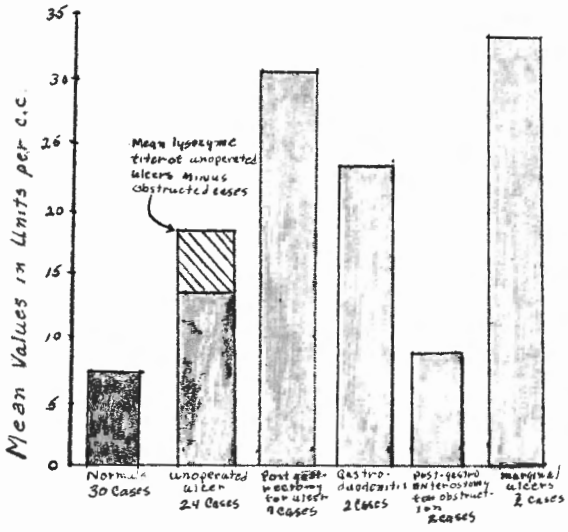
de Garille, et al., (6) established a complete amino acid formula of lysozyme. The molecular weight was found by them to be 14,400. They found it to contain no phosphorus, and it was possible, after hydrochloric acid hydrolysis of the protein, to account for all 191 atoms of nitrogen as constituents of the amino acids. Hydroxyproline and cysteine was found to be completely absent from the molecule of lysozyme thus invalidating the postulates of earlier authors that the enzymatic activity of lysozyme must be dependent on the presence of sulfhydryl group. The number of residues of all other 18 amino acids present in the molecule was determined. Lysozyme is widely distributed in nature, being found in plants as well as in animals. Early studies were confined mainly to the aforementioned lytic action.

In their original studies Meyer and his co-workers (1, 2, 7 & 8) measured lysozyme titers viscosimetrically. Analysis of the mucosa of the fundus, antrum, pylorus, and duodenum of six human stomachs resected for peptic ulcer showed a mean lysozyme concentration of 12, 120, 316, and 213 units per gram of wet weight respectively. The highest concentration of lysozyme occurred in the

duodenum and pyloric regions where peptic ulcers occur most frequently. Mean lysozyme titers of 7.69 and 14.3 units per cc respectively were found in assays of the gastric juice of 30 normal individuals and 29 unoperated ulcer cases. In the normal series there was only one titer above 25 units per cc, whereas there were 6 ulcer patients with titers exceeding 25 units per cc. While the difference in means was not great it was believed to be statistically significant. A mean lysozyme value of 1.7 units per cc was found in cases with obstruction. This low titer may have been due to greater dilution of the enzyme with gastric retention and or to the destruction of lysozyme by pepsin. The mean titer of the unoperated ulcer cases without the seven obstructed individuals was 18.3 units per cc. A graphic representation of the titers found in all other cases is given in Table I.

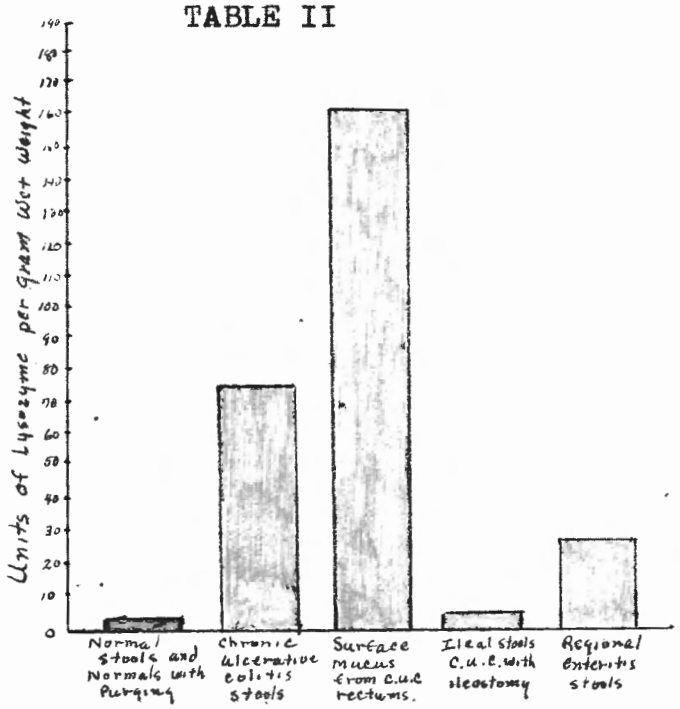
Experiments were conducted in which high concentrations of lysozyme was dissolved in pooled normal human gastric juice and instilled into a Pavlov pouch of an anesthetized dog over a period of four hours. All precautions possible were taken to prevent traumatization of the mucosa and to prevent any possible mechanical factor in producing loss of the mucosa of the pouch. At the conclusion of the experiment the animal was sacrificed and the stomach removed. Gross examination of the pouch

TABLE I



Graphic representation of mean lysozyme titers of all groups studied

TABLE II



A comparison of the mean lysozyme titer in 4 groups studied

From Meyer et al.
Am J Med. 5:482

showed an almost complete removal of the surface mucus and the mucosa gave a scrubbed appearance. Microscopic examination of representative histological sections showed that the protective mucous coat found regularly on the mucosa of the normal stomach was completely lacking. A small ulcerated lesion characterized by a destruction of the mucous cells within the gastric pits in the lamina propria resulted in the antral portion of the pouch.

A portion of the functioning stomach of the same dog was removed and treated with exactly the same technic for the purposes of control and comparison. Histological examination of this tissue disclosed a much greater amount of mucus on the epithelial lining of the stomach.

Instillation of egg-white lysozyme dissolved in saline through a Levine tube into the functioning stomach of a normal live dog resulted in almost a complete removal of the surface mucus. Microscopic studies of sections showed the deeper mucus apparently disappearing first leaving no support for the uppermost layer. Physiologic saline was used in this experiment in order to determine whether the dissolution of the mucus in the previous experiment was due to lysozyme or to the gastric juice.

In another experiment three normal dogs were

given crystalline egg-white lysozyme orally. The first dog was given 30,000 units of enteric coated lysozyme tablets twice a day for 38 days. The second dog was given 30,000 units in 20 cc cherry juice twice a day for 38 days. The third dog was given 270,000 units in gelatin capsules twice a day for 3 and 1/2 days. After the stated time the dogs were sacrificed and the alimentary tracts removed. The mucosa of the small intestine of the first dog appeared hyperemic and edematous throughout, whereas the stomach and colon appeared normal. There was an apparent lack of surface mucus in the antral and pyloric area. Five definite superficial ulcerations were found in the small intestine. The second dog revealed an essentially same general mucosal pattern and had three ulcerations. The third dog's stomach was grossly normal except for a superficial ulceration at the pylorus. The small intestine had numerous serpiginous ulcerations. These were distributed throughout the small intestines but were more numerous distally. The colon showed similar lesions with hyperemia and edema.

Histological sections showed fragmentation of the lamina propria of the valvulae conniventes, with edema and extreme hyperemia of the lamina propria. The lesions were completely lacking in superficial epithelium and superficial mucus.

Viscosimetric examination of lysozyme titers in stools of patients suffering from chronic ulcerative colitis indicated that lysozyme production is greatly increased in the diseased colon. It was believed that the colonic mucosa was the source of the enzyme because of the high titer found in the mucus. Ileal stools from three patients whose condition necessitated ileostomy had low titers, indicating that excessive lysozyme is produced in the colon rather than in the ileum in chronic ulcerative colitis. Table II graphically shows the assays of individual stools for various groups of patients. In contrast to the very high titers in active ulcerative colitis, stools of patients in remission showed low titers. Serial enzyme determinations showed high titers during exacerbation of the disease, and concomittant with clinical improvement there was a fall in the lysozyme titer in the stools.

Meyer's studies of lysozyme activity in chronic ulcerative colitis was corroborated by the findings of Gray and his co-workers (9). In their work Gray et al, determined the fecal lysozyme content in normal subjects, patients with chronic inactive ulcerative colitis, and patients in the acute phase of ulcerative colitis. Serial analysis of the fecal lysozyme content was made to follow the transition from the acute to the

chronic phase of the disease. The lysozyme titer in the stools of the 21 normal individuals showed a variation from 2.9 to 7.6 units per gram with a mean of 4.8 units. These subjects on a regular diet did not vary more than 1.0 unit per gram daily. Fourteen patients in the acute phase of ulcerative colitis demonstrated a marked increase in fecal lysozyme content. The mean level for this group was 109.4 units per gram as compared to the mean value of 4.8 units per gram as seen in the 21 normal subjects.

The lysozyme titer in the stools was found to parallel the course of the disease. With the subsidence of the acute phase of the disease, the lysozyme titer fell concomittantly with the remission. The remission of the disease was evidenced by a decrease in the daily number of stools, disappearance of blood from the stools, and a return to normal of the temperature, white blood cell count, and sedimentation rate and evidence of healing on proctoscopic examination. 11.9 units per gram was the mean lysozyme content of the stools in chronic inactive phase of the 19 patients studied.

The transition from the acute to the chronic phase of ulcerative colitis was followed by repeated fecal lysozyme determination over a period of 1 to 11 months. to observe the association between the activity of the

disease process and the lysozyme titer in the stools in 11 patients. The mean lysozyme titers was found to be greatly elevated in all cases during the acute phase of the disease. A high titer of 481 was reached in one case. During the remission period, titers decreased gradually as the disease passed into the chronic, inactive phase. Clinical improvement was seen uniformly with the fall in fecal lysozyme content.

Lysozyme inhibition studies in vitro were done by Gray and his co-workers using detergents and other enzyme inactivators. With oral administ ration of these inhibitors they produced a prompt fall in the fecal lysozyme titers within 48 to 72 hours to a normal level or to lysozyme titers characteristic of the remission or inactive phase. There was no clinical evidence that the administration of inhibitor and the consequent inhibition of fecal lysozyme altered the course of the disease process. There were no significant changes in the proctoscopic appearance of the mucosa in these patients, and the stool guaic, fever, white blood cell count or sedimentation rate did not improve more rapidly than would be anticipated from conservative medical management for an equal period of time .

The effect of lysozyme on the gastric mucosa of the rat in vivo was observed by Wang ahd his associates (10).

The experiments were carried out on rats which had been fasted for 24 hours. The abdomen w as opened in each case and the esophagus was ligated, excluding the blood vessels and vagus nerves. The solution of lysozyme was introduced into the stomach by way of a glass cannula tied in the duodenum 1 cm below the pylorus. At the conclusion of the experiment all gastric tissues were examined both grossly and microscopically.

Three series of experiments were conducted. In one series a solution of pure lysozyme (2mgm per cc) was introduced into the rat's stomach every half an hour over a period of 4 to 6 hours. The lysozyme was dissolved in Mc Ilvain's buffer solution. As a control the same buffer solution without lysozyme was used. In the second series, the same buffer solution and concentration of lysozyme was instilled into the stomach each half hour over a period of 3 hours. Then for an additional 3 hours a solution containing 0.2% pepsin in 0.15 normal hydrochloric acid containing 0.2 molar sodium chloride was introduced every half an hour. Two sets of control animals were used in this series. One group received the buffer solution alone as a substitute for the lysozyme solution. The second control group received saline solution in place of the acid-pepsin solution. The third series utilized a

solution containing 0.15 normal hydrochloric acid, 0.2% pepsin, 0.2 molar sodium chloride and 2 mgm of lysozyme per cc. Two sets of controls were again employed. One group received the lysozyme dissolved in buffer solution without acid-pepsin solution and the other received the acid-pepsin solution without lysozyme. All the solutions were changed every half an hour over a period of 4 to 6 hours.

Gross or microscopic damage was seen in 3 of the 6 rats in the first series. An ulcer penetrated the musculabis mucosa in one animal, and in two the damage consisted of erosions and hemorrhage. In a fourth animal congestion, hemorrhage and hemolysis were found in the submucosa beneath the normal mucosa. There were no gross or microscopic evidence of damage in the mucosa of the stomachs of the control animals treated with buffer solution without lysozyme. In the second series the stomachs of the animals which had been perfused with lysozyme in buffer solution and then with acid-pepsin solution revealed ulcers which occurred most frequently at the lesser curvature of the pyloric region. Hemorrhage was seen over the whole surface of the gastric mucosa in some cases; and in one portion of the gastric mucosa, edema was seen near the surface of the glands with edema and hemorrhage below. No

damage was found in the control animals whose stomachs were perfused with the solution without lysozyme. Erosions or hemorrhages were found in 4 of the 7 control animals treated with lysozyme in buffer solution followed by saline solution. In the third series damage was found in the stomachs of all animals treated with the lysozyme mixture. Slight damage was seen in both control groups. The effect found after the two enzymes had been perfused together was greater than after one alone. When one was perfused immediately after the other, the damage caused was usually greater than that caused by a mixture of the two in one solution.

In vitro experiments on canine gastric mucus were made to observe the action of lysozyme on mucus and cells. In periods of observation up to six hours in length, the surface cells suspended in the alkaline mucus showed no signs of disintegration in the presence of lysozyme in concentration of 2% in buffer at pH 5 to 6. Lysozyme in solution in acid gastric juice did not hasten the rate of disintegration. of cell bodies in the mucus above that of the disintegration caused by acid juice alone. These studies demonstrated that lysozyme is capable of damaging the mucous membranes, but it gave no evidence of the mechanism involved.

Nickel and his co-workers (11) and Grace and his associates (12) have also comfirmed the observations of Meyer. Nickel, in his experiment made direct daily observation of the gastric mucosa of experimental dogs. Isolated loops of colon were made with one end closed and the other patent and attached to the skin in the manner of a colostomy. Observation of the mucosa was made through this opening with an infant proctoscope. Ten per cent lysozyme was instilled into this loop daily for 2 weeks while daily observations were made. Varying degrees of hyperemia with occasional superficial erosions were seen. However, these lesions healed despite continued lysozyme instillations.

Grace and co-workers found that in normal subjects there may occur a rise in colonic lysozyme concentration in response to situational threats productive of anxiety and apprehension and during periods of anger, hostility, and resentment. The response was found to be transitory Daily observations were made on patients with ulcerative colitis. Lysozyme concentration was found to be low during remission which coincided with periods of relative self assurance and security. However, during exacerbations, usually marked by situations provocative of unexpressed anger, hostility and resentment, there occurred sharp rises in stool lysozyme titers. When such increases

in lysozyme concentrations were sustained for three to four days an episode of exacerbation of colitis with bloody stools and tenesmus ensued.

Observations on the treatment of human gastric mucus with lysozyme were made by Glass et al (13). Specimens of gastric mucus for the experiments were obtained from the following sources: 1) Visible gastric mucus taken directly from the exposed mucosa of "Tom", a fistulous subject. 2) Visible gastric mucus collected from alkaline secretions of stomachs during gastroscopy of two individuals who continually showed gastric anacidity. 3) Mucoprotein fraction of the dissolved gastric mucin from the pooled gastric juice of several individuals with duodenal ulcer. and 4) Mucoproteose fraction of the dissolved gastric mucin from the pooled gastric juice of several normal individuals. The above preparations and mucus specimens were exposed to lysozyme in various concentrations ranging from 50 to 3,000 units per cc of mixture of lysozyme and mucus substrate. Viscosimetric determinations, chemical analysis for the digestion products of mucus by determination of mucoproteose content after lysozyme action by the above mentioned method, and volumetric determination after centrifugation of the non-dissolved visible mucus before and after exposure to lysozyme were made on the samples. Control determinations

were made with distilled water, saline, Mc Ilvaine buffer solution crystallized Armour pepsin in solution, and native gastric juice containing lysozyme added to tested mucus or its preparation.

It was not possible to demonstrate any mucolytic action of lysozyme on gastric mucus or its constituents in any of the procedures outlined even under optimal conditions for lysozyme activity. There was no appreciable difference in action of the solutions containing lysozyme as compared to the controls.

It was believed by Reifenstein and his associates, (14) who were not able to corroborate Myer's findings, that the lysozyme content of the gastric juice was not determined under uniform standard conditions by Meyer; and hence, reevaluated the status of lysozyme in peptic ulcer by measuring its concentration in the gastric juice in fasting state and following maximum gastric stimulation.

In their experiments gastric juice was aspirated from 15 normal individuals, 16 patients with gastric ulcer, and 12 patients with active duodenal ulcer after a 12 to 15 hours period of fasting, and three consecutive 15 minute samples were collected with constant suction for 45 minutes. Histamine diphosphate or insulin administered and fractional samples were obtained at

15 minute intervals for the next 60 to 160 minutes. All the samples were stored in an ice bath to minimize the loss of enzymatic activity.

Analyses were made on the samples for lysozyme activity, pepsin, mucoproteose, and mucoprotein concentration; and the volume, pH and free acid and total acid were measured. Determinations for lysozyme and pepsin were made upon filtered gastric juice. The mucoproteose and mucoprotein fractions were measured in the supernatant fluid following centrifugation of the gastric juice.

The determination of lysozyme content of the gastric mucosa was made immediately after gastric resection. Representative tissue sections were taken from the gastric ulcer site and from the mucosa at the pylorus and fundus of the stomach and stripped away from the underlying tissue. Extraction was done by tituration of one gram of mucosa with ten times its weight of tenth normal hydrochloric acid, and the filtrate was used for lysozyme analysis.

In the normal subject the lysozyme content of the individual fasting samples of gastric juice varied considerably with a range of 7 to 32 units per cc. The mean lysozyme titer of the gastric juice prior to histamine administration was 17.2 units in the 15 normal

subjects. Although the consecutive 15 minute gastric juice samples varied in the same subject, depending somewhat upon the changes in acidity, a lysozyme titer was obtained in each individual which was reproducible on repeated analysis. The lysozyme content of the gastric juice fell sharply to levels of 0.2 to 2 units per cc with a mean of 0.9 units following histamine administration in the normal subjects.

The 16 patients with gastric ulcer did not differ from the normal in gastric juice lysozyme content. The mean titer was 16.8 with a range of 7.4 to 30.4 units per cc of gastric juice. Following the administration of histamine, there was a decrease in the lysozyme content of the gastric juice to levels of 0.4 to 2.2 units with a mean level of 1.0 unit per cc.

The gastric juice lysozyme concentration in patients with duodenal ulcer was significantly lower than that observed with gastric ulcer or normal subjects. There was a variation from 0.2 to 27 units per cc in the individual samples with a mean titer of 9.8 units for the group. The concentration dropped rapidly with the administration of histamine to low levels of 0.2 to 1.5 units per cc with a mean of 0.8 units.

The response to histamine was the same for patients with duodenal ulcer, gastric ulcer and in normal subjects.

There was, in each case, a rapid decrease in gastric juice lysozyme following histamine administration. Within 15 to 30 minutes after histamine administration, the lysozyme titer fell precipitously; and there was a simultaneous increase in gastric acidity. The concentration of lysozyme remained low for 30 to 45 minutes following the injection of histamine and gradually returned to the pre-histamine levels.

The effect of pepsin and acid on lysozyme content of the gastric juice was observed by this group. In vivo studies were made, and inhibition of lysozyme by acid was demonstrated. The lysozyme titer of the gastric juice was less than 3 units per cc whenever the pH fell to 1.5 or below. When the pH of the gastric juice exceeded 1.5, there appeared to be no relationship between pH and lysozyme concentration. There appeared to be no relationship between gastric juice lysozyme and pepsin content, either prior to or following histamine stimulation.

Tissue lysozyme determinations were made of the mucosa at the border of the ulcer in 14 patients with acute gastric ulcer and 9 patients with chronic ulcer. The mucosa at the edge of the ulcer was stripped away from the underlying tissue and analyzed for lysozyme. The mean lysozyme titer of the mucosa at the border of the ulcer in acute group was 52.8 units per gram,

which was significantly higher than the mean titer of 8.4 units per gram found in the 9 chronic ulcer patients. The titer of the tissue ranged from 20 to 175 units per gram in the acute ulcers and 2.8 to 15 units per gram in the chronic cases. The lysozyme content of the uninvolved mucosa at a distance from the ulcer was approximately the same as that of the mucosa at the border of the chronic ulcer. The lysozyme content of the mucosa of the fundus, antrum and pylorus was essentially the same in the patients with gastric ulcer as in the duodenal ulcer group.

Continuing Meyer's experiments Prudden et al (15) observed the effect of orally and intra-arterially administered lysozyme on the canine gastrointestinal mucosa. Crystalline egg-white lysozyme in gelatin capsules was administered orally to 4 dogs. At the completion of the experiment, the dogs were sacrificed, and the gastrointestinal tracts were immediately removed and examined grossly without washing. In this series no hyperemia or edema comparable to that seen in previous experiments were noted. All mucosal depressions and suspicious erosions were fixed in Bouin's solution and examined histologically. The gastrointestinal tracts of four dogs sacrificed for other experimental purposes were used as controls.

Mucosal defects occupied by masses of mucus densely packed with groups of desquamated mucosal cells retaining a viable appearance characterized the lesions produced in the lysozyme fed animals. The villi in, adjacent to, and often deep to the lesion were devoid of epithelium to a greater depth than seen in the control sections. No significant change in the surface mucus was noted histologically in three dogs; however, the fourth dog showed only fragmentary remnants of surface mucus in the majority of sections.

Lysozyme in concentration of 2 milligram per cc was injected into the superior mesenteric artery of four dogs over a 5 hour period to determine the effect of the uniform interstitial distribution of the enzyme in a segment of the gut. Five hours after the initial injection the dogs were sacrificed. Three control dogs received approximately the same injections of inactivated lysozyme.

The control dogs exhibited no gross lesions and no alteration in the normal amounts of surface mucus or in the vascularity of the mucosa. Two of the experimental dogs exhibited mucosal hemorrhages. In one it was of patchy character and in the other it was more diffuse. The other two showed no such areas as compared to the controls. The lack of surface mucus

in the distribution of the superior mesenteric artery was definite in two dogs, minimal in the third and not appreciable in the fourth. Microscopically, all lysozyme dogs showed wide capillary dilatation in the lamina propia. Extravasation of red blood cells could be seen in all lysozyme dogs.

The possibility that the increase in lysozyme titers in the stools of patients with ulcerative colitis is an accompaniment rather than an etiological factor was investigated by Marshall and co-workers.(16). To evaluate this possibility the lysozyme concentration was measured before and during experimentally induced injury to the bowel of dogs.

Six anesthetized dogs were subjected to electrocautery through a proctoscope. Subsequent washings from the cauterized rectum showed significantly high concentrations of lysozyme comparable to that seen in patients with ulcerative colitis.

Lysozyme concentration in the washings from cauterized and non-cauterized blind loops of colon in the same animal were measured at intervals of up to 24 hours. Lysozyme activity of the washings from the cauterized loop continued to rise and reached a peak in five and a half hours after surgery and cautery. In the non-cauterized loop the lysozyme remained at a low level for the duration of the experiment.

DISCUSSION

Lysozyme is regarded by most of the investigators as having a significant role in the pathogenesis of ulcerative alimentary disease. The distribution of lysozyme in the various histologic areas of the stomach is consistent with this supposition. The exceedingly high lysozyme titer found in stools of patients in the acute phase of ulcerative colitis suggest that lysozyme either plays an important role in the pathogenesis or reflects tissue reaction to an injury.

Marshall and his asociates observed marked rise in lysozyme titer within the gastrointestinal tract of dogs after injury by electrocautery and postulated, therefore, that the increase in lysozyme titer observed in the feces and bowel of patients with active ulcerative colitis may be an accompaniment rather than an etiologic factor in the disease.

As high lysozyme content is present in granulation tissue, the question of whether the high titer in colitis might be accounted for on this basis was raised. This may be explained on the grounds that high stool lysozyme titers are found in the absence of occult blood and with sigmoidoscopically non-ulcerated mucosa. This does not imply that a portion of the stool lysozyme content may not originate from granulating surface when ulceration

is present. Granulation tissue is a form of tissue response to injury. Production of bacteriolytic agents is another form of tissue response. Since lysozyme has bacteriolytic properties and ulcerative colitis is associated with granulation tissue formation, the elevated fecal lysozyme titer in this disease may be a reflection of the tissue reaction to an injury.

The hypothesis that lysozyme is causative rather than corollary in ulcerative alimentary disease is substantiated by the effects demonstrated experimentally on the gastrointestinal mucosa. Experimental animals and humans treated with varying concentrations of lysozyme showed removal of the surface mucus with subsequent loss of superficial epithelium, hyperemia, and edema of the gastrointestinal tract. Vacuolization of mucus and disorganization in the surface epithelial cells was also noted. However, it must be borne in mind that the concentrations of lysozyme used in the studies were considerably higher than those encountered clinically even under pathologic conditions.

The close association of high fecal lysozyme titer to acute exacerbation of the disease process and a concomittant decrease in the fecal lysozyme titer with clinical remission of the disease was noted, and it appeared to be a good measure of the activity of the disease.

Lysozyme inhibition studies were carried out to try to produce a remission of the disease by lowering the lysozyme titer. No clinical improvement followed prolonged inhibition of fecal lysozyme by detergents. This inhibition of lysozyme in the stools by detergents, however, does not necessarily reflect inactivation of the enzyme within the mucosal cells of the colon.

Meyer theorized the pathogenesis of ulcerative alimentary disease as occurring in two stages. First the removal of the surface mucus (by lysozyme) with dissolution of the mucus cells and second, necrosis of the denuded tissue by proteolytic enzymes. However, Glass and Gray and their co-workers were not able to demonstrate any mucolytic properties in lysozyme, hence lysozyme can not be considered a solvent agent for the mucus of the human colon. No substrate for lysozyme action has been isolated from gastric mucus or the gastric mucosa.

The role of pepsin and hydrochloric acid in the pathogenesis of peptic ulcer, according to Meyer, is secondary also to the removal of surface mucus by lysozyme. Peptic ulcer do not ordinarily occur in the area where pepsin and hydrochloric acid are produced, but the high concentration of lysozyme in the ulcer bearing area is in accord with this hypothesis.

This, however, does not explain the occurrence of single ulcers. It would have to be supposed that a highly localized overproduction of lysozyme occurs in the ulcerating area.

Reifenstein and his associated in well controlled experiments on gastric juice of patients with duodenal and gastric ulcers were unable to demonstrate any difference in lysozyme content of the gastric juice taken both in a fasting state and after histamine administration. from that of normal subjects. They found that gastric juice of high acidity had low lysozyme content which tend to negate the significance of lysozyme in the pathogenesis of duodenal ulcer. Analysis of ulcerated tissue revealed greatly increase lysozyme content with the highest mean titer occurring in the tissues taken from the ulcer margin including a portion of the ulcer base. The lysozyme content in the tissue of chronic ulcer cases did not differ significantly from the normal. Inflammation, cellular reaction and granulation observed in the acute cases were minimal in these cases and fibrosis was the predominant characteristic.

SUMMARY

1. Lysozyme content is increased significantly in the stools of patients with ulcerative colitis.
2. Stool lysozyme titer in these patients increase with acute exacerbations and decrease concomittantly with clinical remission of the disease.
3. An increase in tissue lysozyme was observed at the margins of active ulcers. The tissue lysozyme was normal at the border of inactive chronic ulcers.
4. Lysozyme content is greatly increased in granulation tissue suggesting that it may be a response to tissue injury rather than the cause of the injury.
5. Fecal lysozyme content was greatly increased in experimental dogs whose gastrointestinal mucosa was injured by surgery and electrocautery. The titer did not increase in control animals.
6. In man the mean lysozyme content of the gastric mucosa was found to be low in the fundus and found to increase to a maximum in the first portion of the duodenum.
7. The mean lysozyme titer in gastric juice was found by Meyer to be elevated in patients with peptic ulcer, however, another investigator found that the titers did not differ significantly from that of normal subjects in well controlled experiments.

8. Lysozyme content of gastric juice varied directly with mucoproteose content and inversely with the mucoprotein content which suggest that lysozyme has its origin within the surface epithelial cells of the stomach.

9. Histamine produced a rapid fall in lysozyme concentration in gastric juice of normal subjects and patients with peptic ulcer indicating that the gastric gland does not secrete lysozyme and that it is not under vagal control.

10. High gastric acidity was associated with inhibition of lysozyme activity.

11. Ulcerations were produced by lysozyme administration orally, intra-arterially and through perfusion in gastric and colonic mucosa in experimental animals. However, the concentrations of lysozyme used greatly exceeded that encountered clinically even under pathological conditions.

12. Lysozyme inhibition studies revealed no clinical remission of acute colitis after prolonged inhibition of stool lysozyme by detergents.

13. Other laboratories were not able to demonstrate mucolytic activity in lysozyme and that it acted as a solvent for gastric and colonic mucus as described originally by Meyer.

14. No substrate of lysozyme has been demonstrated in the alimentary tract.

CONCLUSION

Lysozyme in extremly high concentrations is capable of producing erosions and hemorrhages in the gastric and colonic mucosa. However, the mechanism by which this is accomplished is not explained. Meyer's contention that lysozyme removes the surface mucus from the mucosa has not been supported by most imvestigators.

Further investigations will be necessary to substantiate the claim that lysozyme has a significant role in the pathogenesis of ulcerative alimentary disease. With the information at hand, it is concluded by this author that the high lysozyme content seen in this disease process is a corollary rather than a cause of the disease.

BIBLIOGRAPHY

1. Meyer, Karl; Prudden, John; Lehman, William; and Steinberg, Anita: Lysozyme Content of the Stomach and Its Possible Relationship to Peptic Ulcer. Soc Exp Biol & Med. 65:220, 1947

2. Meyer, Karl; Gellhorn, Alfred; Prudden, John; Lehman, William; and Steinberg, Anita: Lysozyme in Chronic Ulcerative Colitis: Soc Exp Biol & Med. 65:221, 1947

3. Fleming, Alexander: On a Remarkable Bacteriolytic Element Found in Tissues and Secretions. Proc Roy Soc London Series B. 93:306, 1922

4. Meyer, Karl; Thompson, Richard; Palmer, John; and Kharazo, Devorah; The Purification and Properties of Lysozyme. J Biol Chem. 113:303, 1936

5. Meyer, Karl; Palmer, John; Thompson, Richard; & Kharazo, Devorah: On the Mechanism of Lysozyme Action. J Biol Chem. 113:479, 1937

6. de Garille, M.P.; & Fromageot, C.: Amino Acid Formula of an Enzyme: Lysozyme. Biochem & Biophys Acta 4:509, 1950

7. Meyer, Karl; Gellhorn, Alfred; Prudden, John; Lehman, William; and Steinberg, Anita: Lysozyme Activity in Chronic Ulcerative Colitis. Gastroenterology 16:476, 1949

8. Meyer, Karl; Prudden, John; Lehman, William: & Steinberg, Anita: Lysozyme Actvity in Ulcerative Alimentary Disease. I Lysozyme in Peptic Ulcer, II Lysozyme in Chronic Ulcerative Colitis. Am J Med. 5:482, 5:496, 1948

9. Gray, Seymour; Reifenstein, Robert; Connolly, Edward; Spiro, Howard; and Young, Gordon: Studies on Lysozyme in Ulcerative Colitis. Gastroenterology 16:687, 1950

10. Wang, K.J.; Grant, Rhoda; Janowitz, Henry; and Grossman, M.I.: Action of Lysozyme on Gastrointestinal Mucosa. Arch Path. 49:298, 1950

11. Nickel, William; Gordon, Glenn; & Andrus, William: Studies of Lysozyme as an Etiological Agent in Chronic Ulcerative Colitis. Gastroenterology 17:406, 1951

12. Grace, William; Seton, Paul; Wolf, Stewart; & Wolff, Harold: Studies of Human Colon: Variation in Concentration of Lysozyme with Life Situation and Emotional State. Am J Med Sci. 217:241, 1949

13. Glass, Jerzy; Pugh, Betty; Grace,William; & Wolf, Stewart: Observation on the Treatment of Human Gastric and Colonic Mucus with Lysozyme. J Clin Observ. 29:12, 1950

14. Reifenstein, Robert; Gray, Seymour; Spiro, Howard; Young, Gordon; and Connolly, Edward: The Relationship of Lysozyme to Other Components of Gastric Secretion in Peptic Ulcer. Gastroenterology 16:2 p387, 1950

15. Prudden, John; Lane, Nathan; & Meyer, Karl: The Effect of Orally and Inta-arterially Administered Lysozyme on the Canine Gastrointestinal Mucosa. Am J Med Sci. 219:291,1950

16. Marshall, Homer; Moeller, Hugo; & Kirsner, Joseph: Lysozyme Production in Response to Injury of the Gastrointestinal Tract in Dogs. J Lab & Clin Med. 36:6 p960, 1950