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The Production and Prevention of Stomach Ulcers in Rodents

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THE PRODUCTION AND PREVENTION OF STOMACH ULCERS IN RODENTS

by

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Eric H. Rudrud

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ABSTRACT

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Rats given L-Ascorbic Acid in their drinking water prior to and during starvation did not develop severe ulceration in the glandular portion of their stomachs. Control rats which were either nontreated or given deactivated L-Ascorbic Acid developed severe stomach pathology on the starvation regimen.

The present study was based on the finding that food deprivation results in severe rumenal ulceration in rats. Given that L-AA is essential in maintaining tissue integrity and that ulcers are examples of tissue degeneration, the L-AA in large amounts could retard, or prevent, the formation of starvation induced stomach ulcers in rats.

The results show that large amounts of active L-AA were beneficial in maintaining the integrity of rat gastrointestinal tissue exposed to starvation conditions which, in the vitaimin's absence, induces deterioration.

(32 pages)

INTRODUCTION

Origin and nature of the problem

Ulcers are thought to originate from stress. Stress causes an increase in tonus of the vagus nerve and stomach muscles along with decreased blood circulation to the stomach mucosa. These factors and others cause ulceration to occur in man and other species. Basic research in the field of induced stomach ulceration has primarily been concerned with finding environmental and psychological factors that lead to the development of ulcers in animals. Ulceration has been shown to develop from conditioned "fear", Gliner and Shemberg (1971), Mikhail (1969), Sawrey (1961), Sawrey and Conger (1956), Sawrey and Sawrey (1964), and Seligman and Meyer (1970); approach-avoidance conflicts, Pare and Livingston (1970), and Sawrey and Weisz (1956); CS and UCS factors, Brady, Thorton, and Fischer (1962), Friedman and Ader (1965), Lovibond (1969), Pare (1971), and Seligman (1970); escape-avoidance responses, Brady (1958), and Pare (1971); restraint, Ader (1967), Brodie, Marshall, and Moreno (1962), Brodie (1971), Doteuchi (1971), Essman and Essman (1971), Frisone and Essman (1965), Levine (1971), Levine and Senay (1968), Levine and Senay (1970), Lovibond (1969), Singh (1971), and Weiss (1968); and starvation, Mikhail (1972,1973), and Ogawa, Chiles, and Necheles (1960).

Current research has primarily limited itself to identifying causal factors because it has been thought that the environmental causal factors can be understood and thence manipulated to prevent ulceration. In order to investigate potential programs for the

prevention of ulcers, one must first be able to reliably produce ulcers so as to provide an adequate baseline for testing treatment variables. Problems associated with many of the ulcer production methods listed above include the time and materials needed to produce ulcers, and the ultimate unreliability of ulcer production. For example, restraint requires the animal be placed in a body cast for 48 hours and the incidence of ulceration varies between 40 to 70 percent.

Mikhail (1972) subjected rats to a starvation regimen consisting of three 47 hour periods of total food deprivation, with each period followed by a 5 gram pellet of rat chow, with water ad lib. At the end of this starvation diet, seven out of eight animals had severe stomach ulcerations. Almost all other induction methods include the use of food deprivation, i.e. with restraint the animals are deprived 24 to 48 hours during the experiment. Therefore, these methods may not be valid methods of ulcer production since they are contaminated with the food deprivation variable.

Since many methods are used to produce ulcers, it is had to say what exactly causes ulceration. Perhaps almost anything! However, ulcers appear to develop in weak mucosal or submucosal tissue of the stomach. The gastric juices erode through the mucosa and submucosa causing bleeding and in severe cases perforation of the stomach wall. Medical management of ulcers includes diet, rest, antacids, drugs, and surgery. Diets and antacids are prescribed to neutralize the acidity of the gastric juices. Rest and drugs are used to reduce the amount of acid secreted into the stomach by reducing the stimulation of the vagus nerves. Surgery is employed to reduce the amount of acid secreted

into the stomach by severing the vagus nerves and/or remove the ulcerated tissue.

Medical management of the ulcer is concerned with reducing the amount of acid secreted on weakened tissue or removing the weakened mucosal tissues.

Vitamin C (L-Ascorbic Acid, L-AA) has important biological and metabolic functions. L-AA plays an essential part in the biosynthesis of collagen, intercellular ground substance, and connective tissue formation (Scrimshaw, 1971). Stomach ulcers are characterized by tissue breakdown in the stomach mucosa and this may be prevented or reduced by increased amounts of collagen. The healing of an ulcer requires ground tissue to be formed over the erosion (McMinn, 1969). From these facts the working hypothesis of this thesis may be stated: Given that L-AA is essential in maintaining tissue integrity, and further that starvation induced ulcers are in fact tissue degeneration, it may be the case that L-AA in large amounts will at least retard, or at best prevent, the formation of starvation-induced ulcers.

This hypothesis may possibly suggest a radical approach to the treatment of ulcers. Instead of treating the ulcer by dealing with gastric acidity and the factors that are thought to elicit it, this research would suggest one strengthen the stomach tissue and thus prevent gastric erosion or enhance healing where erosion has already occurred.

Objectives

1. To verify starvation as a reliable method of ulcer production in rats.

2. To investigate the role, if any, of L-Ascorbic Acid in the prevention of starvation-induced ulceration in rats.

REVIEW OF LITERATURE

General statement of why a model is justified

Research in the area of stomach ulceration has involved many species; for example rats, mice, monkeys, dogs, and guinea pigs (Pfeiffer, 1971). Since stomach ulceration does not occur spontaneously in subhuman species, a method of inducing ulcers has been developed. This "model" has been used to study the induction, etiology, pathology, and treatment of ulcers in the laboratory. The criterion for ulcers include the loss of tissue involving the mucosa, submucosa, and muscular layer of the stomach; changes in stomach vascularity; and perforated hemorrhaging (Kirsner, 1971). The prevention of ulcers in subhuman species would possibly allow generalization of our findings to the human ulcer, since both ulcers exhibit the same criterion. Various methods of ulcer production were investigated and final selection was restricted to the rat species.

Stomach ulcers

Three main types of gastric lesions have been defined: peptic gastric, and stress ulcer.

Peptic ulcer. Peptic ulcer is characterized by a sharply circumscribed loss of tissue, involving the mucosa, submucosa, and muscular layer, occurring in areas of the digestive tract exposed to acid-pepsin gastric juice. Peptic ulcers are often multiple, vary in diameter from millimeters to one or two centimeters, and usually occur along the lesser curvature of the stomach in the first 3 or 4 cm. of the duodenum (Kirsner, 1971). Peptic ulcer generally results from the inability of

localized areas of the gastroduodenal mucosa to withstand the digestive action of the acid-pepsin gastric content and develops only in areas of the digestive tract exposed to acid gastric juice and only in persons secreting hydrochloric acid (Kirsner, 1971). In individuals suffering from peptic ulcer, the amount of HCL secreted is above normal.

Gastric ulcer. Gastric ulcer resembles peptic ulcer in its clinical manifestations, but differs in that the secretion of hydrochloric acid is normal or less than normal. The muscular layer is interrupted completely by the ulceration. Activity and healing coexist in the same lesion. There may be inflammation and degeneration of nerve fibers and disappearance of ganglion cells.

Stress ulcer. Stress ulcers are multiple, superficial erosions (involving the mucosa and submucosa) which occur mainly in the fundus of the stomach, usually superimposed on a background of erosive gastritis (Silen, 1973). They develop after shock, trauma, and sepsis. Silen suggests that gastric ischemia is the basic pathogenic mechanism resulting in diminished mucosal blood flow. In addition, the presence of acid within the lumen of the stomach is necessary for the ischemic insult to cause erosive gastritis and ulcerations.

Although much is known about its development, the cause of the ulcer remains obscure (Kirsner, 1971). An ulcer results from an imbalance between aggressive gastric factors and resistance factors. These resistance factors include the rate of mucosal regeneration, the quantity and quality of mucous production, the vascular conditions of the mucosa, etc. (Pfeiffer and Sethbhakdi, 1971).

Spiro (1970), stated that duodenal ulcers appear to develop from

deficient buffering of the gastric ac id by the mucous. Kirsner (1971), attributed normal tissue resistance to five components: (1) the integrity of the mucosal cells, (2) the rapid, continuous regeneration of epithelium, (3) the mucous barrier, (4) the abundant vascular supply and (5) the permeability of the gastroduodenal mucous membrane. He explained that unidentified protective factors within the gastroduodenal wall and secretory inhibitors probably contribute to this tissue defense.

Healing of an ulcer occurs from below (inside) upwards, with the growth of granulation tissue and young fibroblasts (Kirsner, 1971). McMinn and Pritchard (1969), demonstrated that during the first days of the healing process, epithelial cells migrate from the sides of the erosion over a floor of developing granulation tissue. The granulation tissue that develops in the floor of the lesion does not appear to differ from that found in other wound sites.

Historic stress models

Ulceration, supposedly due to environmental stress, has been demonstrated in mice, rats, guinea pigs, dogs, cats, and monkeys. Mahl (1949) reported that chronic "fear" in dogs caused a rise in rate of HCL secretion and a rise in acidity of gastric contents. Ader (1965) found more ulcers in group housed rats as compared to individually housed animals, and that pre-weaned handled animals were less susceptible to erosions than other handled or non-handled animals. Brady (1958), with a six hour stress/six hour rest paradigm, demonstrated ulcers in monkeys. Two monkeys were restrained side by side in a shock delivering apparatus. The "executive" monkey could press a

lever when a light came on and prevent shock. The other monkey was restrained and received shock, along with the executive, if the executive failed to press the lever. As the experiment progressed only the executive died from stomach ulceration.

Conditioned fear. Gliner and Shemberg (1971), Mikhail (1969, 1973), Sawrey (1961), and Sawrey and Conger (1956) produced ulceration in rats using conditioned fear. Seligman and Meyer (1970) paired shock with food reinforcement contingent upon bar pressing in rats. Fear was measured by suppression in bar pressing and was correlated with ulceration. Sawrey (1961) and Sawrey and Sawrey (1964) found a high positive correlation with number of fear conditioning trials with incidence and severity of ulceration.

Approach-avoidance conflicts. Pare and Livingston (1970) and Sawrey and Weiz (1956) paired shock with the delivery of food, and observed ulceration in rats. Sawrey, Conger, and Turrell (1956) produced ulcers in rats by using approach-avoidance conflict with food and water deprivation. The results indicated that hunger and shock interaction caused ulceration but water deprivation did not contribute significantly in the production of ulcers.

CS and UCS factors. Brady, Thorton, and Fischer (1962), using rats, separated the effects elicited by a pre-aversive stimulus and the effects of the aversive stimulus. Using three groups, group 1 had shock preceded by a buzzer, group 2 had shock with no buzzer, and group 3 served as a control for the buzzer. The mortality and morbidity were greatest for group 1 and least for group 3. Friedman and Ader (1965), using mice, found that those subjected to periodic shock preceded by a stimulus light lost a greater amount of weight than

other groups. Lovibond (1969) compared aversive and appetitive CS's of the incidence of gastric lesions. Low ulceration occurred in a group with appetitive CS, which suggests that a stimulus previously associated with safety from shock and presentation of food reduces the effect of stress.

Predictability of shock. Seligman and Meyer (1970) and Friedman and Ader (1965) found that unpredictability of shock caused an increase in ulceration. Pare's (1971) results, though not significant, showed that contingent shock produced greater ulceration than random shock. Using rats on a 6 hour stress and 6 hour rest schedule, for 21 days, Pare found more severe ulceration in the avoidance group as compared to the escape group.

Restraint. Ulceration due to restraint has been demonstrated by Ader (1967), Brodie, Marshall, and Moreno (1962), Essman, Essman, and Golod (1971), Frisone and Essman (1965), Lambert, Truchot, Andre, and Chayvialle (1971), Levine (1971), Levine and Senay (1968,1970), Lovibond (1969), Singh (1971), Weiss (1965), and Wilson (1971). Restraining devices have included three-fourths length plaster casts; wire, plastic and sheet metal body length cages; and other methods such as adhesive tape and clamps. A common factor in the use of restraint is food deprivation for 24-48 hours preceeding restraint, and continuing another 24-72 hours while in restraint, and also employing light animals generally weighing about 160-180 grams.

Ader (1967) found that rats immobilized for six hours at the peak of the 24 hour activity cycle were more susceptable to ulcers than those immobilized at other times of the activity cycle. Levine (1972) and Levine and Senay (1968,1970) produced ulceration in rats by a

twenty four hour food deprivation schedule, ad lib water, and then placed in a cold environment, 4-7 degrees for 4 hours.

The typical lesion that develops from restraint, is characterized by erosions of the gastric mucosa that extend either to or through the submucosa, a breakdown of connective tissue and capillaries, with hemorrhaging (Brodie, 1971). This is a stress ulcer.

Drugs. Ulcers have also been shown to develop from drugs such as reserpine, acetic acid, histamine, and aspirin. Okabe (1971), using rats, injected various dosages of acetic acid into the submucosal layer of the anterior wall of the glandular stomach. The larger the injection, the more severe and persistant was the ulceration. Eagleton and Watt (1971), using guinea pigs deprived of food for 12-16 hours, injected 5 mg HAP/kg (histamine) and observed ulceration within 1-6 hours following the injection. They also stated that the method of injection was a critical factor in determining the type of ulceration to occur. IM injections produced duodenal ulcers and IP injections produced gastric lesions (stress ulcers). Hay, Varco, and Code (1942) used an IM injection of histamine in beeswax and mineral oil to produce ulceration. Watt (1959), Eagleton and Watt (1967), and Lillehei and Wangensteen (1948) produced ulceration in the guinea pig using histamine injections. Doteuchi (1971), using rats, combined electrical shock with reserpine injections to produce gastrointestinal ulceration.

Starvation. Ogawa, Chiles, and Necheles (1960) deprived mice of food for three days and observed ulceration. Morris (1941) found that the frequency of intermittent feeding to be more effective than the quantity of food in prolonging the life of fasting rats. Morris showed that fasting and partial inanition were found to be more important in

ulceration than individual deficiencies of protein, carbohydrates, or fats. He concluded that food in the stomach acted as a protective mechanism. Essman, Essman, and Golod (1971) combined immobilization with varying intervals of food deprivation, with mice, and found that deprivation for 36 and 48 hours caused ulceration in non-immobilized animals. Essman also stated that retention of food served as a protective mechanism in stomach ulceration.

Mikhail (1972), using rats weighing approximately 280 grams, put each animal of a food deprivation schedule consisting of three 47 hour deprivation periods, with water ad lib, followed by one five gram rat chow pellet. Severe ulceration occurred in seven out of eight animals. Mikhail (1973) replicated food deprivation as a reliable method of ulceration.

Ulceration by the various methods has been observed in different strains of rats (Sprague Dawley, Long Evans, and other inbred strains) and in both sexes.

Pilot studies. Using food deprivation alā Mikhail (1972), ulceration has been reliably produced in our lab in both sexes of Long-Evans rat. The studies have found that the weight of the animal influences the degree of ulceration and we have now identified optimum weight levels. The heavier the animal, the longer the animal can stay on the starvation diet: for instance, a rat weighing 160 grams will have ulcers in three days, whereas a rat weighing in excess of 350 grams will go seven days on this diet before ulceration occurs. The critical factor is the loss of 30 percent free feeding weight after which ulceration is observed.

Medical management of ulcers

Drug therapy. Medical management of ulcers typically seeks to protect the gastrointestinal mucosa from the digestive action of hydrochloric acid and pepsin. "This goal might be achieved by increasing tissue resistance, but no method is currently available for directly improving the tissue defense in peptic ulcer," (Kirsner, 1971, p. 1273). Peptic ulcers heal in the presence of hydrochloric acid, but the healing process is facilitated and recurrences are diminished, when the acidity in the stomach is decreased or neutralized. Antacids are prescribed to neutralize acid, but none are ideal (Kirsner, 1971) and many lead to other gastrointestinal complications (Jeffries, 1973). These complications may be a lack of response to oral iron therapy, diarrhea, or alkalosis, which is a biochemical distrubance involving an increase in serum ph.

Gastric acidity may be reduced by the use of antisecretory drugs. When administered, these agents suppress the vagal and antral mechanism of gastric secretion. Jeffries (1973) states that complications may arise such as blocking of the esophageal peristalsis or inhibition of "resting tone" of the lower esophageal sphincter. This may allow reflux of gastric acid through a lower esophageal sphincter and may lead to peptic esophagitis. Other complications, due to the effects of drugs on the mucosa, include disruption of the gastric mucosal barrier to acid back-diffusion (aspirin, alcohol), inhibition of cell renewal (corticosteroids, phenylbutazone), stimulation of acid secretion (caffeine, reserpine), and depression of mucosal blood flow (vasopressin).

Surgery. Gastric secretion may be reduced by a vagotomy. This surgical procedure severes the vagal neural connections to the stomach an eliminates the neurogenic phases of gastric secretion.

Gastroenterostomy connects the upper intestine to the stomach and relieves obstruction at the pylorus or duodenum. This encourages healing by diverting acid from the ulcer and by facilitating neutralization through the alkaline intestinal secretion.

Gastrectomy removes the affected area of the stomach and sutures the tissue together. Complications with gastroenterostomy and gastrectomy are that the gastric secretory potential is unchanged, and these procedures are usually accompanied by a vagotomy.

Diet. The dietary treatment of an ulcer is characterized by being bland with no acids. Kirsner (1971), however, has stated that there was no conclusive evidence that coarse or highly seasoned foods retard healing or that a soft diet enhanced the healing process. Johnson (1949) noted that citrus fruits usually caused irritation of the ulcer and were excluded from many diets. This caused a deprivation of L-AA and symptoms of scurvy were not infrequent. Attention to nutrient intake, including L-AA is an essential part of the medical treatment of many illnesses. For example, "the diet prescribed for peptic ulcer may almost free of ascorbic acid, a situation which favors bleeding rather than healing." (Scrimshaw, 1971, p. 1447). Spiro (1970) felt that if the diet was to be restricted for no more than three to six weeks, then no vitamin supplementation was necessary. If it was to be carried out for longer periods of time, then it was to be supplemented with half strength orange juice or 50 mg of L-AA daily.

Of the diets reviewed (Chaney and Ross, 1971 and Mayo Clinic

Therapeutic Diets, 1971) most seemed to be lacking in L-AA. Few suggested citrus fruits and those that did were of minimal levels. None mentioned the possibility of L-AA administered IV.

Rest. Different psychological factors are presumed to predispose a person to peptic ulceration by increasing gastric secretion via the vagal mechanism and by decreasing tissue resistance. "The tensions, strains, and competitive efforts of modern life apparently increase the tonus of the vagus nerves involving both motor and secretory fibers." (Kirsner, 1971, p. 1262). Duodenal ulcers are more frequent among people whose occupations involve administrative and professional responsibility and competitive effort. Emotional disturbances are common in patients with peptic ulcers, and the recurrences and complications are often associated with periods of stress, frustration, and anxiety.

Brodie (1971) stated that stress sensitizes the stomach by changing the vascularity so that it is less resistant to the digestive enzymes, and thus bleeding occurs. Kirsner (1971) felt that emotional stress may increase the secretion of HCL and the susceptibility of the gastroduodenal mucosa to ulceration, and that rest and relief of tension are important. "The precipitating factors recognized and emphasized most often are physical fatigue, nervous tension, insomnia, excessive smoking, dietary indiscretions, irritating drugs and intercurrent illness." (Kirsner, 1971, p. 1276).

Rest is prescribed for the prevention of recurrance by dealing with the problems of physical fatigue, anxiety, frustration, nervous tension, and insomnia. Emotional arousal increases the tonus of the vagus nerves involving both motor and secretory fibers, causing an

increase in stomach acidity. Reducing the emotional arousal, by rest, would maintain secretion of hydrochloric acid in the stomach at a minimum, improve the vascularity of the stomach and mucosa, and also restrict the body's requirements for vitamin C.

Ascorbic acid

L-Ascorbic acid. L-Ascorbic Acid (vitamin C) is a water soluble vitamin, that oxidizes readily, and when in the body has a reversible oxidation reduction capacity, (Rosenberg,1945). It reaches its maximum plasma concentration in 1.5 hours with excess amounts secreted in the urine. Normal plasma concentration is 1.2 mg ascorbic acid/100 cc plasma. Rosenberg and others report no ill effects due to hypervitaminosis of ascorbic acid. Ascorbic acid (L-AA) is involved in protein and amino acid metabolism and has been prescribed to detoxify poisons and combat infectious diseases (Rosenberg, 1945).

L-AA is needed for the formation of normal connective tissue (collagen and ground substance). Scurvy is caused by a prolonged deficiency of L-AA. The clinical manifestations of L-AA deficiency are the formation of defective ground substance, failure to form new collagen, and weakening of the capillaries, reduced resistance to infection and microbial diseases, and defective healing of wounds. L-AA is transported to wounds soon after injury, with its concentration rising there as the plasma concentration falls, Scrimshaw (1971). Patients with thermal burns may develop L-AA deficiency and large dosages of L-AA are necessary to maintain normal plasma concentrations. Silen (1973) and others report that ulcers often develop in burn patients.

The intake requirements to maintain tissue saturation of L-AA increases in patients with diseases such as tuberculosis, chronic infections, burns, peptic ulcers, alcoholism, and other infectious diseases. Johnson (1949) stated that ulcer cases utilize 20 percent more ascorbic acid than normals. O'Keane (1972) showed that as a group alcoholics are subclinically scorbutic and that this was associated with an insufficient dietary intake of L-AA.

Schlegel, Pipkin, Nishimura, and Shultz (1970) reported that L-AA prevented bladder tumors in mice by oxidizing an implanted carcinogenic pellet. Hodges and Hotston (1971) reported that the L-AA concentrations in the guinea pigs adrenals were depleted during stress.

D-Ascorbic acid. D-Ascorbic Acid is one of two types of deactivated L-AA. D-Ascorbic Acid is chemically the mirror image of L-AA. It does not have any of the antiscorbutic properties of L-AA (Rosenberg, 1945). No known beneficial effects of D-AA have been found.

The other method used in deactivating L-AA is to allow L-AA to oxidize in the atmosphere. When this occurs it is not reversible and it loses its antiscorbutic properties. Rosenberg (1945) stated that the deactivation reaction is increased in the presence of heat and Fe, Mg, and Ca ions. L-AA also becomes deactivated when exposed to sunlight and water.

Ascorbic acid (L-AA) is involved with amino acid and protein metabolism, maintains tissue integrity, is capable of reversible oxidation and reduction processes, is necessary for the formation of ground substance and maintaining capillary integrity. L-AA is transported to the site of tissue injury, is necessary for the formation of

scar tissue, and the intake requirements are increased due to tissue insult and stress.

An ulcer results from an imbalance between aggressive gastric factors and resistance factors. These resistance factors include the rate of mucosal regeneneration, the quantity and quality of mucus production, the vascular conditions of the mucosa, etc. (Pfeiffer and Sethbhakdi, 1971).

METHOD

Subjects

Eighty female Long-Evans rats weighing 230 (± 15) grams were used in the study.

Apparatus

The Ss were housed in their individual cages throughout the experiment. L-Ascorbic Acid was obtained from the Sigma Chemical Company (St. Louis, Mo.).

Procedure

Twenty rats designated C 1 received no dietary L-AA treatment or stress but were simply maintained in their home cages with ad lib water and Purina Rat chow. Twenty were starved but not L-AA treated, group E 1. Twenty others, group E 2, were treated with deactivated L-AA and also starved. The final 20 rats, group E 3, were treated with active L-AA and also starved. Starvation consisted of three consecutive 47 hour periods of total food deprivation with water ad lib. At the end of each of the first two 47 hour periods, all subjects were given one Purina Chow pellet (approximately 5 grams).

The L-AA treatment consisted of supplying 6 grams of L-AA in 200 ml of cold tap water in the rats normal drinking bottle. The drinking bottle was opaque plastic to retard deactivation of the ascorbic acid by light. The L-AA was deactivated by mixing it with 200 ml of water

and then heating and stirring it for one hour at 95 degrees. It was then stored at room temperature in an opaque bottle. The vitamin dose was provided for 8 days prior to the starvation regimen as well as throughout the 6 starvation days. Fresh water and vitamins were prepared and supplied every 48 hours.

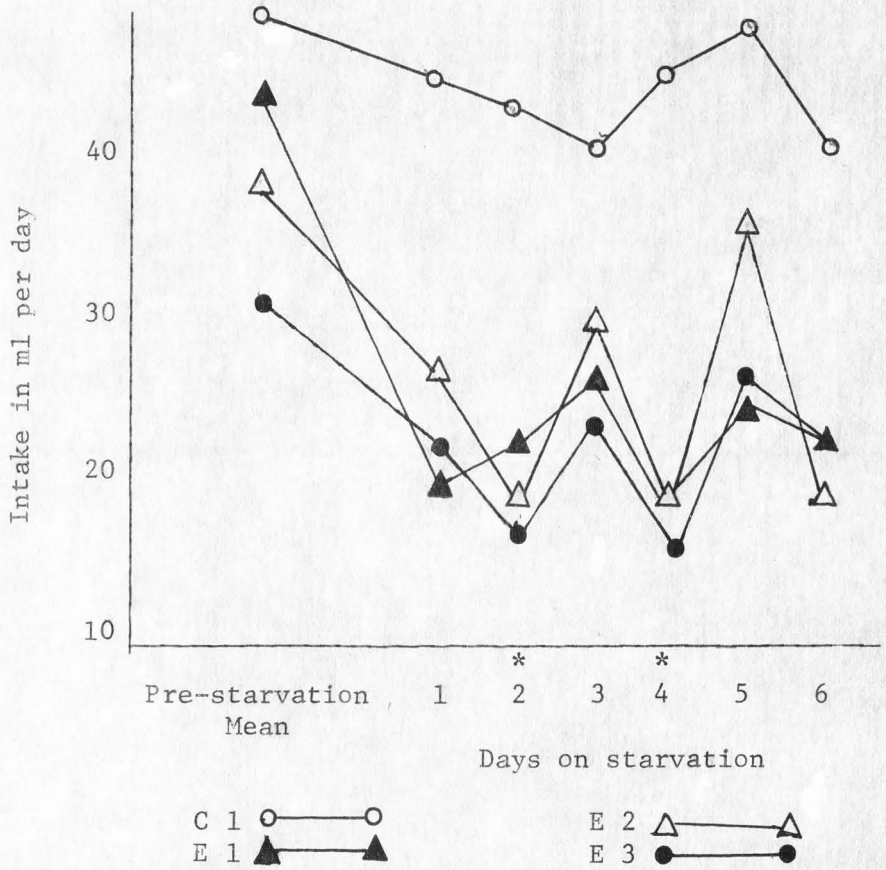
All Ss were decapitated at the end of the third 47 hour period or when they reached the criterion of 30 percent loss of freefeeding weight, which ever came first. The stomachs were rapidly removed and opened by cutting, with scissors, along the greater curvature. The mucosal surface was then rinsed with cool tap water and examined under a 4X power dissecting glass. The individual examining the tissue was unaware of the group from which the specimen came. The stomachs were then classified according to severity of ulceration, "None" meaning no lesions, "Mild" having 1 to 3 lesions, "Moderate" having 4 to 7 lesions, and "Severe" having more than 8 ulcers. An ulcer was defined as a well defined, bleeding lesion in the gastric mucosa, and/or a perforation of the stomach tissues themselves.

Each Ss weight was recorded daily through out the experiment. Baseline free feeding weight was the mean of the last three days during the pre-starvation interval.

Each Ss water intake in mililiters, was recorded each day throughout the experiment.

RESULTS

The mean water intake in mililiters per day for each group during starvation is shown in Figure 1.



Overall Mean

	Pre-starvation	Starvation
C 1	39	40
E 1	50	22
E 2	30	21
E 3	36	24

Figure 1. Mean water intake in ml per group per day throughout the experiment.

* indicates delivery of 5 grams of food.

The mean intake per day during the pre-starvation period for the deactivated L-AA and the L-AA groups was less than the groups receiving water ad lib. During the starvation period the mean water intake per day for these groups was similar.

Peaks in water intake per day during starvation were observed, see Figure 1. During starvation the water intake per day was reduced by approximately 10 ml per day for the deactivated L-AA and L-AA groups, and approximately 20 ml per day for the water group. This is due to the fact that water intake is regulated by the amount of food ingested. The peaks in water intake during starvation were the result of the Ss receiving a pellet of chow at the end of the 47 hour period of food deprivation. This increase in ingested food caused an increase in the amount of water consumed.

The incidence and severity of ulceration are shown in Table 1.

Table 1. The incidence and severity of ulceration

Group	None	Mild	Moderate	Severe	Total
C 1 (N=20)	20	0	0	0	0
E 1 (N=20)	0	2	2	16	20
E 2 (N=20)	3	5	6	6	17
E 3 (N=20)	16	3	1	0	4

C 1 was the control group receiving no starvation with water ad lib. E 1 received starvation with water ad lib. E 2 received starvation with deactivated L-AA. E 3 received starvation with L-AA ad lib.

Figure 2 shows stomachs that were removed and classified by visual inspection as "Mild", "Moderate", and "Severe" ulceration.

Figure 2. Classification of stomach ulceration, "Mild", "Moderate", and "Severe".

The results speak for themselves. The results show that L-AA was significant in reducing the incidence of ulceration, E 3 and E1 Chi Square of 23.437 with 1 df which is significant at the .001 level; E 3 and E 2 Chi Square of 14.436 with 1 df which is significant at the .001 level. L-AA treatment was not significantly different from the control group, Chi Square of 2.5 with i df. The deactivated group was not significantly different from the non-treated group, E 1 and E 2, Chi Square of 1.441 with 1 df.

L-AA was also significant in reducing the severity of ulceration, E 3 and E 1, Chi Square of 28.125 with 3 df which is significant at the .001 level; E 3 and E 2, Chi Square of 14.156 with 3 df which is significant at the .01 level. L-AA treatment was not significantly different from the control group, E 3 and C 1, Chi Square of 1.583

with 3 df. The deactivated group was not significantly different from the nontreated group, E 1 and E 2, Chi Square of 6.711 with 3 df.

The average percent of free feeding weight loss per day for the groups on starvation are shown in Figure 3.

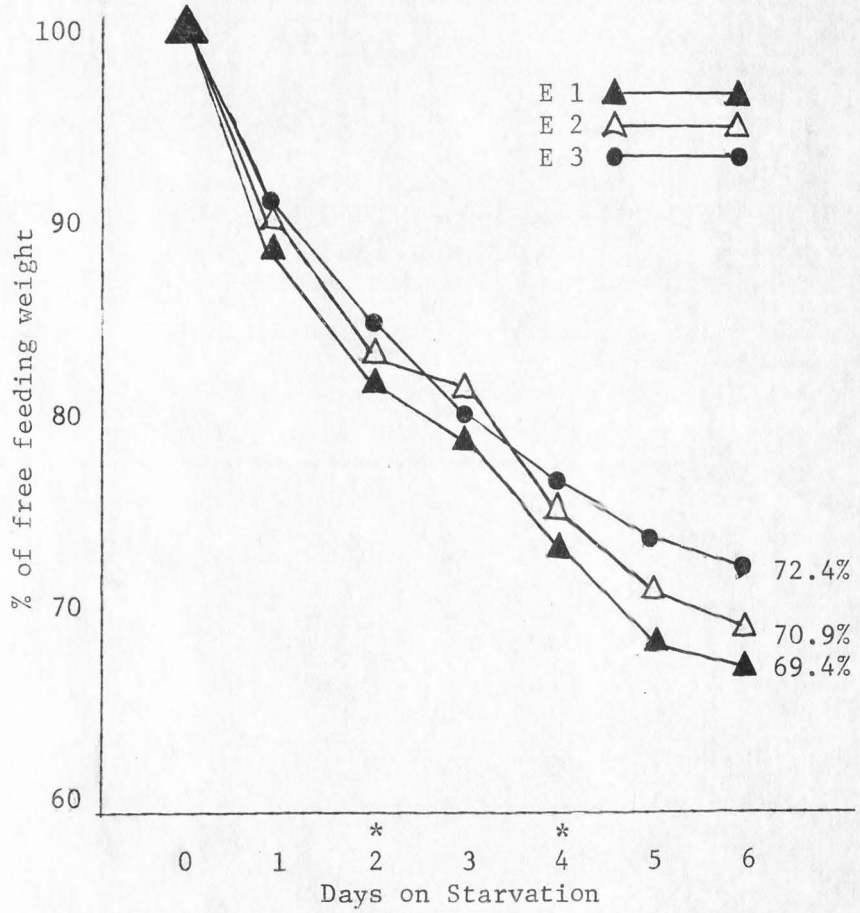


Figure 3. Mean percentage of free feeding weight lost per day.
* indicates delivery of 5 grams of food.

The weight losses for the groups are similar, except at 74-75% free feeding weight, when groups E 1 and E 2 show sharp drops in body weight. It is hypothesized that at this point active hemmorrhaging of the lesions occurs and the blood is digested and secreted. This would account for the sharp drop in body weight.

The L-AA treated group did not show a similar drop in weight. This is consistent with the above hypothesis, since hemmorrhaging did not occur blood was neither digested nor secreted.

Parameters affecting the incidence and severity of ulceration are the weight of the animal and the percentage of free feeding weight lost on the starvation regimen. The percentage of free feeding weight lost is the main parameter in ulceration by the starvation regimen. All animals will have ulcers at 70-72 percent of their body weight. The number of days required to reach this criterion is influenced by the weight of the animal. The percentage of weight lost is very critical, in that animals at 73 percent body weight will not exhibit ulceration.

The weight of the animal determines the amount of time the animal is on the starvation regimen before ulceration occurs. Animals weighing 160-170 grams show ulceration in three to four days, whereas heavier animals, 250-260 grams, require 5 to 6 days on the starvation regimen before ulceration occurs. This is probably due to the lack of fat deposits in the lighter animals that would supply "food" to the organism while on the starvation regimen. The grams of weight lost per day in the lighter animals influences the percentage of weight lost more than the same amount of weight lost in heavier animals.

Varying concentrations of L-AA were used during pilot studies. Five grams per 200 ml of water and 7.5 grams per 200 ml of water. The results obtained using 5 grams showed ulceration in 30 percent of the animals. The concentration using 7.5 grams prevented ulceration in all animals. However, this concentration caused a decrease in water consumption during pre-starvation to 20 ml per day.

Sections of the ulcerated stomachs were veiwed by a pathologist (courtesy of Dr. John W. LaBree St. Mary's Hospital Minneapolis, Minnesota). The report stated that the sections were not "classical ulcers" but did show a breakdown of the mucosa and capillaries. This is consistent with Silen's (1973) definition of the stress ulcer. The sections viewed were from stomachs removed at 72 percent body weight. Earlier studies showed deeper submucosal erosion at 69 percent body weight.

CONCLUSIONS

The results of the study verify starvation as a reliable method of ulcer production in rats. Group E 1 with 144 hours of nearly total deprivation showed 100% ulceration. It was found that the main parameter in ulceration by this method was the reduction to 71 percent of the Ss free feeding weight. The Ss initial weight determines, some what, how long the animal must be deprived before it reaches this criterion. Light animals require three to four days on the starvation regimen while heavier animals require 6 to 7 days.

L-AA was significant in preventing starvation induced ulceration in rats and in reducing the severity of ulceration. L-AA was also beneficial in maintaining stomach tissue integrity in the rat and prevented starvation induced tissue degeneration.

L-AA helped maintain the integrity of the mucosal cells by forming ground substance and collagen, providing amino acid and protein synthesis for the regeneration of the epithelium tissues, and also played a significant role in maintaining an abundant vascular supply to the stomach by strengthening the capillary walls.

This research has contributed to the study of ulcers by providing a reliable baseline to investigate other possible treatments for ulcers. It has established this baseline without interfering variables such as chronic stress, restraint, or fear. It was demonstrated that ulcers were tissue breakdown and has provided the opportunity to investigate a method of strengthening the tissues themselves with large dosages of L-AA.

These data suggest a critical role for L-AA in maintaining tissue integrity and, further, that the study of peptic ulcer may benefit from a closer analysis of the diet and eating habits of the organism.

The food pellet delivered every 47 hours elicited drinking and therefore the L-AA intake was increased.

Since the rats were deprived of food for 6 days, it is possible that they were showing signs of scurvy. This is plausible; however, rats weighing 25 grams more will go one day longer on this diet before ulceration occurs. It is questionable that this extra weight would prevent scurvy in these animals. Further research suggested would be to put the animals on a starvation diet and administer sucrose IP. This would maintain nutrients in the animals while keeping their stomachs empty.

Other research suggested by this data is the investigation of the role of L-AA in the prevention of histamine induced ulceration and the role of L-AA in the prevention of ulceration due to ischemic factors. Other parameters to be investigated in starvation-induced ulceration are the effects of gradual reduction to 70 percent body weight and the effects of frequent feeding of smaller amounts of food vs. single feeding of 5 grams every 47 hours. Other studies to be done with L-AA are its effects on rate of healing of starvation-induced ulceration in the rat.

LITERATURE CITED

Ader, R. Effects of Early Experience and Differential Housing on Behavior and Susceptibility to Gastric Erosions in the Rat. Journal of Comparative and Physiological Psychology, 1965, 60:233-238.

Ader, R. Behavioral and Physiological Rhythms and the Development of Bastric Erosions in the Rat. Psychosomatic Medicine, 1967, 29:345-353.

Brady, J. P., Ulcers in "Executive" Monkeys. Scientific American, 1958, 199:95-103.

Brady, J. P., Thornton, D. R., Fisher, D. E. Deleterious Effects of Anxiety Elicited by Conditioned Pre-Aversive Stimuli in the Rat. Psychosomatic Medicine, 1962, 24:590-595.

Brodie, D. A. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

Brodie, D. A., Marshall, R. W., Moreno, O. M. Effect of Restraint of Gastric Acidity in the Rat. American Journal of Physiology, 1962, 202:812-814.

Chaney, M. S., Ross, M. L. Nutrition, Boston, Mass.: Houghton Mifflin Co. 1971.

Doteuchi, M. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

Eagleton, G. B., Watt, J. Paradoxical Effect of Anti-histamines on Histamine-Induced Bastric and Duodenal Ulceration in the Guinea Pig. Federal Procurement, 1967, 26:541.

Eagleton, G. B., Watt, J. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

Essman, W. B., Essman, S. G., Golod, M. I. Metabolic Contributions to Gastric Ulcergenesis in Mice. Physiology and Behavior, 1971, 7"509-516.

Friedman, S. B., Ader, R. Parameters Relevant to the Experimental Production of "Stress" in the Mouse. Psychosomatic Medicine, 1965, 27:27-30.

Frisone, J. D., Essman, W. B. Stress-induced Gastric Lesions in Mice. Psychological Reports, 1965, 16:941-946.

Gliner, J. A., Shemberg, K. M. Conditioned Fear and Gastric Pathology in a Continuing Stress-Rest Paradigm in Rats. Journal of Comparative and Physiological Psychology, 1971, 74:20-22.

Hay, L. J., Varco, R. L., Code, C. F. The Experimental Production of Gastric and Duodenal Ulcers in Laboratory Animals by the Intra-Muscular Injection of Histamine in Beeswax. Surgery, Gynecology, and Obstetrics, 1942, 75:170-182.

Hodges, J. R., Hotston, R. T. Suppression of Adrenocorticotrophic Activity in the Ascorbic Acid Deficient Guinea Pig. British Journal of Pharmacology, 1971, 42:595-602.

Jeffries, G. H. Gastrointestinal Complications of Drug Therapy. G. I. Tract, 1973, 3(3):14-19.

Kirsner, J. B. In: Cecil-Loeb Textbook of Medicine, 13th edition, by Beeson, P. B., McDermott, W. (eds.) Philadelphia, Pennsylvania: W. B. Saunders Co. p. 1259-1284. 1971.

Lambert, R. J., Truchot, R., Andre, C., Chayvialle, J. A. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

Levine, R. J. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

Levine, R. J., Senay, E. C. Histamine in the Pathogenesis of Stress Ulcers in the Rat. American Journal of Physiology, 1968, 214:892-896.

Levine, R. J., Senay, E. C. Studies on the Role of Acid in the Pathogenisis of Experimental Stress Ulcers. Psychosomatic Medicine, 1970, 30:61-65.

Lillehei, C. W., Wangensteen, O. H. Effects of Muscular Fatigue on Histamine Provoked Ulcer with Observations on Gastric Secretion. Proceedings of the Society of Experimental Biology (N.Y.), 1948, 67:49-52.

Lovibond, S. H. Effect of Patterns of Aversive and Appetitive Conditioned Stimuli on the Incidence of Gastric Lesions in the Immobilized Rat. Journal of Comparative and Physiological Psychology, 1969, 69:636-639.

Mahl, G. F. Effect of Chronic Fear on the Gastric Secretion of HCL in Dogs. Psychosomatic Medicine, 1949, 11:30-44.

Mayo Clinic Diet Manual 4th ed. Philadelphia: Saunders Co. 1971.

McMinn, R. H. M., Pritchard, J. J. Tissue Repair, London: Acedemic Press. 1969.

Mikhail, A. A. Stress and Ulceration in the Glandualr and Non Glandular Portions of the Rat's Stomach. Journal of Comparative and Physiological Psychology, 1973, 85:636-642.

Mikhail, A. A. The Effects of Conditioned Anxiety on the Recovery From Experimental Ulceration. Journal of Psychosomatic Research, 1972, 16:115-122.

Mikhail, A. A. Relationship of Conditioned Anxiety to Stomach Ulceration and Acidity in Rats. Journal of Comparative and Physiological Psychology, 1969, 68:623-626.

Morris, H. P. Production of Gastric Lesions in Rats by Fasting, Partial Inanition, and Deficiency of Certain Dietary Constituents. Journal of the National Cancer Institute, 1941, 2:459-477.

Ogawa, T., Chiles, T., Necheles, H. Starvation Ulcer in the Mouse. American Journal of Physiology, 1960, 198: 619-620.

Okabe, S., Pfeiffer, C. J. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

O'Keane, M., Russel, R. Il, Goldberg, A. Ascorbic Acid Status of Alcoholics. Journal of Alcoholism, 1972, 2:6-11.

Pare, W. P. Six Hour Escape-Avoidance Work Shift and Production of Stomach Ulcers. Journal of Comparative and Physiological Psychology, 1971, 74:459-466.

Pare, W. P., Livingston, A. Grain Norepinephrine and Stomach Ulcers in Rats Exposed to Chronic Conflict. Physiology and Behavior, 1970, 5:215-220.

Pfeiffer, C. J. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

Pfeiffer, C. J., Sethbhakdi, S. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co. 1971.

Rosenberg, H. R. Chemistry and Physilogy of the Vitamins, New York: Inner Science Publications Inc. 1945, pp. 289-338.

Sawrey, W. L. Conditioned Responses of Fear in Relationship to Ulceration. Journal of Comparative and Physiological Psychology, 1961, 54:347-348.

Sawrey, W. L., Conger, J. H., Turrell, E. S. An Experimental Investigation of the Role of Psychological Factors in the Production of Gastric Ulcers in Rats. Journal of Comparative and Physiological Psychology, 1956, 49:457-461.

Sawrey, W. L., Sawrey, J. M. Conditioned Fear and Restraint in Ulceration. Journal of Comparative and Physiological Psychology, 1964, 57:150-151.

Sawrey, W. L., Weisz, J. D. An Experimental Method of Producing Gastric Ulcers. Journal of Comparative and Physiological Psychology, 1956, 49:269-270.

Schlegel, J. V., Pipkin, G. E., Nishimura, R., Shultx, G. N. The Role of Ascorbic Acid in the Prevention of Bladder Tumor Formation. The Journal of Urology, 1970, 103:155-159.

Scrimshaw, N. W. In: Cecil-Loeb Textbook of Medicine, 13th ed., by Beeson, P. B., McDermott, W. (eds.) Philadelphia, Pennsylvania: Saunders Co., 1971, pp. 1445-1447.

Seligman, M. E., Meyer, B. Chronic Fear and Ulcers in Rats as a Function of the Unpredictability of Safety. Journal of Comparative and Physiological Psychology, 1970, 73:202-207.

Silen, W. Stress Ulcers. G. I. Tract, 1973, 3(3):14-19.

Singh, G. B. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co., 1971.

Spiro, H. M. Clinical Gastroenterology. New York: MacMillan Co., 1970, p. 214-321.

Watt, J. The Mechanism of Histamine Ulceration in the Guinea Pig. Gastroenterology, 1959, 37:741-759.

Weiss, J. M. Effects of Coping Responses on Stress. Journal of Comparative and Physiological Psychology, 1965, 65:251-260.

Willson, J. In: Peptic Ulcer, by C. Pfeiffer (ed.) Philadelphia, Pennsylvania: J. B. Lippincott Co., 1971.

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