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THE PATHOGENESIS AND CONTROL OF EXPERIMENTAL GASTRIC ULCERATION IN THE RAT

by

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THIS MANUSCRIPT IS DEDICATED

TO

DR. ANIS A. MIKHAIL

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ABSTRACT

It has been claimed that stomach ulcers could be induced by stressful psychological procedures which involve the use of electric shock. The literature also suggests that the variable of shock per se is the essential agent responsible for the formation of experimental ulcers. With these conflicting views in mind, Experiment 1 indicated that ulcer incidence and severity covaried with shock intensity. When the psychological stress of unpredictability and uncontrollability was held constant, ulcers did not develop when shock intensity was low. Only the high level of shock intensity was ulcerogenic. Experiment 2 tested the interactive effects of shock, restraint, food deprivation, and strain differences during a period of 96 hours. Most glandular ulceration occurred in restraint stress and in Sprague-Dawley rats. It was suggested that ulcer susceptibility in different strains of rats may be a treatment-specific phenomenon. In Experiment 3, gastric acidity increases during a period of food deprivation or during a period of restraint were selectively neutralized by the administration of an antacid drug (aluminum hydroxide) during either or both of these periods. The results clearly indicated that when hyperacidity due to food deprivation was blocked, restraint-induced

ulceration decreased markedly. The prophylactic effects of ascorbic acid on food deprivation ulcers were examined in Experiment 4. Contrary to previous research, no glandular ulceration was observed. Indeed, the administration of vitamin C during food deprivation was associated with an increase in the incidence and severity of rumenal ulcers. It was suggested that procedural differences may account for the inconsistent findings.

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CHAPTER 1

INTRODUCTION

Numerous procedures have been used to induce gastric ulcers in animals. These include physical treatments such as drug administration, surgery, or restraint and "psychological" treatments such as conflict or conditioned fear. Several decades of research have yielded little progress in the etiology of ulceration, particularly with respect to the pyschological aspects of ulcer pathogenesis. That physical procedures reliably induce ulceration is well documented with respect to restraint (Brodie, 1968; Levine and Senay, 1970), pylorous ligation (Sun and Chen, 1963; Dai and Ogle, 1973a; 1973b) and drugs (Luparello, 1969; Nixon, 1974). Considerable controversy has arisen, however, regarding psychologically-induced ulcers. Early studies using conflict and conditioned fear reported that psychological factors (e.g. anxiety, approach-avoidance conflict) had definite etiological significance in gastric ulcerogenesis (Sawrey and Weisz, 1956; Sawrey, Conger and Turrell, 1956; Conger, Sawrey, and Turrell, 1958). Mikhail (1966; 1969; 1971), however, suggested that the ulcers appearing in these studies were largely due to depriving the animals of food. Subsequent studies emphasized the

ulcerogenic properties of food deprivation per se (Mikhail and Hirschberg, 1972; Glavin and Mikhail, 1975). Recently, Weiss (1970; 1971a; 1971b; 1971c) suggested that the unpredictable and uncontrollable shock procedure used in his studies eliminated the confounding effects of physical variables and resulted in psychogenic ulcers. The shock intensity used in these studies, however, was very high and may have contributed to the results. This suggestion will be considered in the present work.

It is evident that the relative contributions of physical and psychological factors to the formation of experimental ulcers are matters of considerable debate. The following section provides a review of both physical and psychological variables involved in ulcer production with an emphasis on food deprivation and shock as confounding experimental factors.

Physical Variables

1. Restraint

Selye (1936) first reported using restraint with rats. His method involved either tying the forelimbs and hindlimbs together or wrapping the animal in a towel for several hours. The classic general adaptation syndrome resulted, including thymic involution and adrenal hypertrophy. Ulceration data was not reported in this study.

More recently, Rossi et al. (1956) and Bonfils and Lambling (1963) have used the same restraint procedure to produce rapid and uniform gastric ulcers. They called their procedure "psychogenic" in origin, but they noted that the "psychological factor is always accompanied by one or more physical actions (p. 155)", making it difficult to isolate specific etiological factors. Brodie and Hanson (1960) restrained rats by wrapping them in a wire screen for 24 hours. Without prior food deprivation they reported an ulcer incidence of 86% with this technique. They suggested that an increase in gastrointestinal motility might have accounted for these results since restrained animals had significantly less food in their stomachs at autopsy than did controls. Brodie and Hanson also noted that recovery from restraint-induced ulcers could be delayed by food deprivation. They concluded that "fasting was found to be a particularly effective means of increasing the effect of any stressor agent (p. 358)".

Acidity changes during restraint were examined by the chronic gastric fistula procedure (Brodie, Marshall and Moreno, 1962). It was found that restraint decreased the volume of secretion but increased the concentration. They reported some pilot data indicating that drugs which prevented the rise in acid concentration (e.g., atropine) reduced the incidence of restraint ulcers.

Guth and Mednick (1964) used the same restraint procedure as Brodie and his co-workers, in order to examine the effects of repeated periods of restraint on ulceration in rats. The procedure involved 18 hours of food deprivation followed by restraint for 4 hours per day. Two hours of ad lib food were given between each successive restraint period. Consequently, few ulcers were reported, yet Guth and Mednick concluded that adaptation to restraint had occurred.

Ader (1964) restrained rats at either the peak or the trough of their activity cycles. Some ulceration occurred in those rats restrained at the height of their activity, despite the availability of food. Barboriak, Wilson, Schulte and Knoblock (1972), however, noted that active rats in activity wheels will "voluntarily" decrease their food intake, often leading to a "self-starvation". Rats restrained at the low point in their activity cycle did not develop ulcers and Ader noted that some animals still had food in their stomachs at autopsy. He concluded that the presence of food in the stomach offered protection against restraint-induced lesions. Sines (1966) accounted for Ader's results by hypothesizing that rats which, for no apparent reason, are more ulcer-susceptible than others, have a higher activation level than non-susceptible rats. He suggested that ulceration was a function of (1) genetic (physiological) predisposition,

(2) activation level and (3) the type of stressor used, and that extreme values of any two factors interact to produce ulcers.

Mikhail and Holland (1966) used a plaster-of-Paris corselet wrapped tightly around the rat's body as a restraint technique. Twelve hours of food deprivation preceded restraint. After 12 hours of restraint, ulceration did not develop, "probably due to the protective effects of food still remaining in the stomach (p. 346)". The authors recommended as standard procedure the use of 20 hours of food deprivation followed by 24 hours of restraint, to produce a reliably high glandular ulcer incidence. Within an activation level framework, the authors suggested that the presence of the corselet around the rat's abdomen contributed to abnormally high sensory input to the animal (higher than its normal level of activation). Neural and hormonal changes thus occurred, resulting in the formation of gastric ulcers.

Pfeiffer (1967) studied the effects of chronic restraint in the rat. His results showed that when rats were fed ad libitum but restrained for up to five weeks, no ulceration developed. Starvation for five days prior to restraint, however, resulted in lesions confined to the rumen of the stomach of adult rats, and to the glandular

portion of the stomach in weanling rats.

Senay and Levine (1967) combined cold exposure (4° - 7°C) and restraint for two hours to produce ulcers. Rats were deprived of food for 24 hours prior to the experiment. The combined treatments produced significantly more glandular ulceration than either treatment alone. The authors used commercial plexiglass restraint cages in their experiment and recommended their use in restraint studies since the plaster-of-Paris corselet procedure tends to produce hyperthermia in rats. In addition, Senay and Levine noted that with the commercial restraint cages, volume could be maintained at a relatively uniform level across studies.

Hornbuckle and Isaac (1969) attempted to provide further support for the activation level hypothesis and gastric ulceration. Rats were restrained under low levels of illumination with their frontal cortex removed. These conditions had been shown to result in maximum motor activity in the rat. Rats in this group showed a high "erosion" incidence, however, the definition of erosion is unclear from this study. No specific mention of food deprivation was made. The authors reported, however, that the lesioned subjects lost an average of 51 grams of body weight while sham controls lost an average of 20 grams of body weight. This data indicates that the ulcerogenic effect of

food deprivation was involved in this study and, given the weight loss figures, probably contributed to the observed "erosions".

Perchach and Barry (1970) compared the pathological effects whole body restraint and neck restraint only, hypothesizing that the greater amount of struggling afforded the rat by neck restraint only, would increase stress responses. Rats were allowed free access to food throughout the restraint periods which were either 1, 4, or 24 hours in duration. Results indicated that both types of restraint produced significant increases in plasma corticosterone, with neck restraint producing a higher level. Although ulceration was not measured in this study, it is worthy of note that both kinds of restraint significantly decreased food intake and resulted in a significant body weight loss. These results provide further evidence that the variable of food deprivation cannot be ignored even in studies using physical stressors.

Levine and Senay (1970) used restraint (in commercial plexiglass cages) and cold (4^{o} - 7^{o}C) for two hours, preceded by 24 hours of food deprivation, to produce glandular ulcers in rats. The results showed that intragastric pH correlated significantly with these ulcers. In addition, it was found that pretreatment of rats with Basaljel (a

gastric antacid) was associated with a significant reduction in ulcer incidence. In some rats, the antacid pretreatment was found to be ineffective. Autopsies revealed that the antacid had been cleared from the stomach, thereby negating its effects. It appears that restraint promotes gastric clearance, leaving an empty stomach prone to the corrosive effects of gastric acid.

Caul and Buchanan (1971), Herner and Caul (1972), and Buchanan and Caul (1974) developed a standardized restrainer for rats which permits recording of electrophysiological data, food and water intake, shock administration and monitoring of excreta. Their procedure consistently employs a period of 24 hours of food deprivation prior to a subsequent manipulation, thereby acknowledging the contribution of food deprivation to experimental ulcers. In fact, Buchanan and Caul (1974) concluded "The influence of food deprivation upon ulceration has been well documented and must be considered in determining the effect of psychogenic variables upon ulceration (p. 587)".

Recently, Tran (1974) suggested that hypothermia may play a role in restraint induced ulcers. He noted that glandular ulceration was associated with hypothermia in rats restrained for 30 hours. This is not surprising given that the restrained rat cannot move about very much and given that food intake decreases during restraint.

2. Pharmacological Manipulation

Many substances have been used to induce gastric ulcers including caffeine (Pfeiffer and Gass, 1962; Roth and Valdes-Dapena, 1963a), reserpine (Hartry, 1962; Lambert, 1963), histamine (Watt, 1963), acetylsalicylic acid (Roth and Valdes-Dapena, 1963b), cincophen phenyl quinoline carboxylic acid (Varro and Csernay, 1963a), phenylbutazone (Vano and Csernay, 1963b), pilocarpine (Mallik and Gupta, 1963), gold thioglucose (Luparello, 1969) and acetic acid (Nixon, 1974). Again, food deprivation proved important in these studies, especially with pilocarpine. Mallik and Gupta (1963) noted that "fasting is essential for development of gastric lesions in rats treated with pilocarpine (p. 305)". One commonality in the studies mentioned above is that most of the authors concluded that the drugs used rendered the gastric mucosa more susceptible to the actions of pepsin and gastric acid, probably by alterations in the mucous lining. The same situation occurs in the empty stomach in a state of food deprivation (Berg, 1942; Wlodek, 1968).

3. Surgical Manipulation (Pylorous Ligation)

Shay et al. (1945) introduced the technique of pylorous ligation for the production of rumenal ulcers in the rat. The pylorous was tied off in a brief operation and the rats were sacrificed 17 to 19 hours later. This

method produced a very high incidence of severe rumenal ulcers. The authors noted that a period of food deprivation was essential prior to this procedure since "the absence of ulceration...could be related to the considerable food rests still present when the pyloric ligation was done (p. 47)". They noted that 48 hours of food deprivation was necessary with lighter rats ($<$ 180 gr) while 72 hours of starvation was required with heavier animals ($>$ 180 gr). Ulceration was attributed to the continual secretion and accumulation of gastric acid (pH $<$ 1.6), since the gastric contents could not be passed through the stomach due to the pyloric obstruction. Sun and Chen (1963) examined the influence of time after pylorus ligation on ulceration. Their results showed that after 16 hours, 100% of the rats developed severe, often perforating rumenal ulcers. They concluded that procedural standardization was necessary in order to reduce the variability of results obtained with the pylorous ligation technique, particularly with respect to the preoperative food deprivation period. They noted that the older and heavier the rat, the longer the duration of preoperative starvation was required for ulcer development.

Recently, Dai and Ogle (1973a; 1973b; 1974) refined the Shay et al. (1945) procedure in an attempt to eliminate the possible ulcerogenic effects of surgery and anaesthesia.

Dai and Ogle loosely looped a stainless steel wire around the pylorous 7 to 10 days prior to their experiments, during which time the rats recovered from surgery. On the day of the experiment, the two ends of the wire were gently pulled and the pylorous thereby occluded. Rats were deprived of food for 48 hours prior to pyloric ligation and were sacrificed 2 to 5 hours later. A second group of rats was prepared according to the Shay et al. (1945) method. Results showed that the wire implant procedure produced significantly more ulcers after 4 and 5 hours of ligation than did the Shay method. In addition, both the rate of gastric secretion and the total acid output of the Shay rats were less than the wire implant rats. The authors suggested that their method was superior to Shay's since it was less unphysiological than Shay's and did not interfere with gastric secretion thereby enabling this data to be collected. They concluded that since the rumen is the non-acid secreting portion of the rat stomach, it was not resistant to the action of gastric juice. Since gastric juice increasingly accumulates in the stomach during pylorous ligation, the incidence of rumenal ulcers was proportional to the time elapsed since ligation. Further observations indicated that ether anaesthesia supressed gastric secretion for 2 hours, while surgery inhibited secretion for 5 hours. Dai and Ogle

concluded that their wire implant procedure eliminated these confounding effects.

Mikhail, Gabriel, and Glavin (1975) recently examined the role of solid substances in the stomach of pylorous ligated rats. Rats were either food deprived or allowed access to solid non-nutritive bulk (silica and methyl-cellulose) for 48 hours prior to pylorous ligation by the method of Shay et al. (1945). Results showed that rats given non-nutritive solid bulk prior to surgery developed significantly fewer and significantly less severe rumenal ulcers than did the food deprived group. It was concluded that the empty stomach is more ulcer-susceptible and that even solid non-nutritive bulk is effective in reducing the severe ulceration which normally results from pylorous ligation.

Psychological treatments used to produce experimental ulcers in rats have generally used shock. They will be discussed under the headings of conflict, conditioned fear, predictability and controllability. As with the physical treatments, the role of food deprivation and shock in these procedures will be emphasized.

Psychological Variables

1. Conflict

The use of conflict procedures to produce experimental ulcers arose out of the need for an animal analogue

of the human "approach-avoidance" conflict. Sawrey and Weisz (1956) attempted to "demonstrate that gastric ulcers can be produced psychologically; that is, without some direct physiological manipulation (p. 269)". Rats were placed into the center compartment of a box, at the ends of which were food and water respectively. Between the rat and each end of the box was an electrically charged grid. Thus the animals had to endure shock in order to obtain food or water. The authors described this procedure as "strong and chronic approach-avoidance conflict (p. 269)". Six of nine experimental animals developed glandular ulcers and the authors concluded that psychological conflict was responsible. Several subsequent reports from Sawrey and his associates indicated (a) that weight loss and shock were important "only in interaction" with conflict (Sawrey, Conger and Turrell, 1956), (b) that animals placed into conflict alone and animals reared alone were more susceptible to ulcers than animals tested together (Conger, Sawrey and Turrell, 1958), (c) that females were more resistant to conflict ulcers than males and that Wistar rats were more resistant than Sprague-Dawley and Long-Evans (Sawrey and Long, 1962), and (d) that reserpine potentiated conflict-induced ulcers (Sawrey and Sawrey, 1964). In 1962, however, Pare showed that a weight loss of up to 40% of free feeding

weight occurred in rats exposed to the chronic conflict procedure. The group which received shock only (no conflict procedure) developed significantly more lesions than any group. Pare concluded that shock stress played a primary role and conflict a secondary role in gastric ulcerogenesis. He noted that "ulcers previously reported as having been developed by psychological stress may be attributed, to a greater degree, to the physical stress of electric shock (p. 225)". The most recent report from Sawrey indicated that Pare's suggestions had been incorporated. As a result of their conflict procedure, Sawrey and Sawrey (1966) suggested that age, weight, and large differences in food consumption were "possible" contributing factors in the etiology of "psychologically-induced" conflict ulcers.

Paré and Livingston (1970) examined the gastrointestinal consequences of a different conflict procedure. Rats were randomly shocked on 50% of the occasions when they emitted a food collecting response following completion of a VR-10 food reinforced schedule. This conflict procedure was in effect 22 hr. per day for three days. A control group which received only food deprivation was also included in this experiment. The results showed that food deprivation alone produced some ulceration, implying that "the ulcers observed in the experimental animals....may

have been partially influenced by the reduction in food consumption (p. 217)". These results clearly indicate that a physical factor (food deprivation) was contributing to so-called "psychological" ulcers, especially since the ulcers observed in this study were found in the rumen of the stomach. It is well known that the rumen is the primary site of food deprivation ulcers (Mikhail, 1966; Mikhail and Hirschberg, 1972; Paré and Temple, 1973).

In a subsequent study, Paré (1972a) showed that the temporal relationship between food reward and shock delivery in a conflict situation is a critical variable. In general, the results demonstrated that the closer the shock was to the consummatory response, the higher the ulcer incidence. Pare stressed the need for the control of food deprivation especially in studies using shock, since many results indicate that shock decreases food intake (Sterritt, 1962; Sterritt and Shemberg, 1963).

Paré (1972b) examined the role of the food deprivation schedule in chronic conflict studies. Animals given access to food every 24th hour developed significantly fewer ulcers than animals given food only every 48th hour. He noted that "the longer deprivation period is a necessary prerequisite for the occurrence of conflict-induced ulcers (p. 167)" thereby highlighting the role of physical variables in "psychological" ulcers. Again in this study, most

of the ulcers were found in the rumen of the stomach, providing further support for the role of food deprivation in conflict ulcerogenesis. Paré concluded "any conflict paradigm which opposes an approach to food with an avoidance to shock, should carefully consider the direct gastrointestinal effects of inanition and its relative contribution to ulcer development, especially if these ulcers are to be considered psychogenic ulcers (p. 171)".

Recently, the conflict controversy was revived by Wald, MacKinnon and Desiderato (1973) who reported a high incidence of glandular ulcers following only 6 hours of conflict. Rats were placed into a box with a continuously charged grid floor. The animals could escape the shock by jumping onto a small platform in the centre of the cage, however here, shock was delivered via a tail electrode on a VI 60 sec. schedule. The authors noted that up to 60 hours of food deprivation preceded the conflict treatment, yet in neither the experimental subjects, nor in the food deprivation only control group were rumenal ulcers observed. In order to eliminate the ulcerogenic effects of punishing shock per se, a yoked control group was included. These subjects received the same frequency, duration and temporal pattern of shock as did the experimental rats, but were never exposed to the conflict situation. Few of these

subjects developed ulcers. It may be, however, that the two hour rest period following stress termination was responsible for the ulceration observed in this study since Desiderato, MacKinnon and Hissom (1974) observed that "Significant ulcer production was not found unless animals experienced a minimum of 2 hour poststress rest prior to sacrifice (p. 208)". It should be noted that Brady (1958) also showed that gastric acidity showed a "rebound" increase during the periods immediately following termination of shock avoidance. In addition, the lengthy period of food deprivation used in this study might have contributed to the ulcer results. It thus appears that a true conflict ("psychogenic") ulcer has yet to be demonstrated.

2. Conditioned Fear

Mahl first examined the possible ulcerogenic effects of conditioned fear in dogs (1949). His procedure involved pairing a buzzer CS with a shock US of undetermined intensity, in a "non-predictable manner (p. 33)". Dogs were conditioned in this manner for 12 hours during which time they received 74 "trials", only 18 of which were reinforced with shock. The animals were then allowed to recover for an unspecified time on an ad lib diet. Finally, the "fear" period was instituted, wherein the CS was periodically presented but never reinforced with shock. The results

indicated that (a) behavioral manifestation of fear correlated highly with increased gastric acidity (both free and total acid), (b) those animals showing increased Hcl secretion also showed increased gastric emptying time, (c) removal of the subjects from the conditioning situation resulted in a return of acid levels to basal control valves and (d) no ulcers were observed in any subject at any time. Mahl concluded that although acid secretion may have been involved in fear-induced ulcers, the general notion of increased sympathetic arousal in states of fear was not supported. Given the feeding schedule used in these studies, however, it is not surprising that ulceration was not observed.

Sawrey (1961) attempted to separate the ulcerogenic effects of a psychological variable (fear) from the effects of physical variables (shock, food deprivation). Rats were given fear conditioning in two ways. One group received shock every time a light CS occurred, while for a second group, the CS was followed only half the time by shock in a random fashion. In order to equate the number of shocks in both groups, the second group received the other 50% of the shocks on a random basis in no relation to the CS. Thus both groups received the same amount of stimulation but in different temporal patterns of "predictability" and

"unpredictability". Animals were allowed access to food for 4 hours per day for the 14 day conditioning period. The results indicated that the unpredictable shock group developed significantly more ulcers than the predictable shock group. Ulceration was observed in both portions of the stomach but Sawrey noted that most ulcers were in the body of the stomach. It is difficult to see how shock and food deprivation were separated in this study, since both groups were exposed to both of these physical parameters. It remains questionable as to whether "conditioned fear" was the only ulcerogenic factor in this study.

In a subsequent study, Sawrey and Sawrey (1963) found that the duration of fear conditioning was positively related to increasing resistance to ulceration. With the durations used in this study, food deprivation must be considered an important variable and the weight loss data supports this interpretation.

Restraint was added to conditioned fear by Sawrey and Sawrey in their next study (1964). Rats were given various amounts of fear conditioning with 0, 10 or 40 shocks presented on an unpredictable schedule. Restraint for 48 hours followed, during which the CS was presented every four minutes. Shock was never administered during restraint. Ulcers were counted "without regard to location

in the stomach (p. 151)". Results indicated a significant difference between the 0, 10 and 40 shock groups, however, whether these ulcers were due to food deprivation, restraint or a combination of these factors cannot be ascertained from this study. The conclusion that ulceration rate may be a measure of the strength of fear appears unwarranted.

Bell, Hendry and Miller (1967) provided an interesting result which suggests a possible confounding factor in the studies using prenatal conditioned fear and subsequent ulcer-proneness in the rat. They found that rats given prenatal conditioned fear coincident with the development of the fetal gut developed ulceration as adults when exposed to restraint. No effect was observed if conditioned fear was given after the fetal gut had developed.

Sawrey and Sawrey (1968) followed up their 1964 study, varying shock intensity and duration. Results indicated that ulceration rate increased as a function of shock intensity but not duration. As in the previous study, however, it is difficult to isolate an etiological variable since the rats were restrained, however, the data do implicate, at least in part, the role of shock in ulcerogenesis.

Mikhail (1969) examined the effects of conditioned anxiety on gastric ulceration and acidity in rats. No differences were found between experimental and control

groups on either measure. It was concluded that the inhibitory effects of sympathetic arousal usually associated with conditioned fear did not support a relationship between fear, acidity and gastric ulcers.

Support for Mikhail's position arose from a study by Gliner and Shemberg (1971) who examined the effects of applying conditioned fear in a continuing cycle of stress and rest periods. No ulceration resulted from the conditioned fear treatment. The authors concluded that "conditioned fear is not an effective dimension relative to increases or decreases in gastric pathology (p. 22)".

Mikhail (1971) found that neither acute nor chronic exposure to conditioned fear increased gastric acidity in pylorous-ligated rats. In addition, some experimental subjects had significantly lower gastric acidity levels than controls. Mikhail concluded that chronic fear-provoking situations may suppress rather than increase gastric acidity, and as such, conditioned fear could not be responsible for ulcer formation. The suggestion implied by Mikhail's (1969, 1971) research was that conditioned fear might retard ulcer development since this treatment tends to suppress gastric acid. Mikhail (1972) induced ulcers by restraint and then administered conditioned fear during recovery on either a continuous or intermittent schedule. Delayed recovery of the restraint-induced glandular ulcers was not observed as

a result of conditioned fear, however, it was noticed that rumenal ulcers developed. Further experiments indicated that the rumenal ulceration observed was related to food deprivation since shock was shown to have a marked inhibitory effect on food intake (Sterritt, 1962; Sterritt and Shemberg, 1963). Mikhail concluded that "it appears that the procedures of conflict and conditioned anxiety exert their ulcerogenic effects partly through reduction of the animal's food intake and partly through direct physical damage by shock (p. 120)".

3. Stress Predictability

Seligman, Maier and Solomon (1971) defined predictability as a situation in which the occurrence of a CS did not change the probability of a US occurring; that is, a CS stood in a predictive relationship to a US if and only if the probability of a US given that a CS had occurred or p (US/CS) was not equal to the probability of a US given that a CS had not occurred or p (US/$\overline{CS}$). If these conditional probabilities were equal, so that whether a CS occurred had no effect on the occurrence of a US, then the situation was defined as unpredictable. When measured behaviorally, it appears that unpredictable aversive events are more stressful than predictable ones. "Relative aversiveness" studies and "preference" studies support this

conclusion (Lockard, 1963; Perkins, Levis and Seymann, 1963; Pervin, 1963; Lockard, 1965; Badia, Suter and Lewis, 1967; Glass, Singer and Friedman, 1969; Glass, Riem and Singer, 1971). Studies measuring physiological responses to unpredictable aversive stimulation also indicate that this procedure is more stressful, however, the conclusion that the critical variable involved is a psychological one remains premature.

Seligman (1968) exposed rats to a chronic unpredictable shock situation for two and one-half months. Relative to rats in the predictable shock group, the unpredictably shocked rats developed significantly more ulcers. As with previous studies, food deprivation appeared to be involved in Seligman's experiment. He noted that unpredictable shock eliminated instrumental responding for food, while no such behavioral suppression occurred in the predictable shock group. Given this data, it is not surprising to find the ulceration confined to the unpredictable shock group.

Weiss (1968b) examined ulceration in rats as a function of unpredictable shock delivered via fixed tail electrodes. This procedure eliminated the possibility of the rats' escaping grid shock by jumping or reducing the shock effectiveness by lying on their backs. Rats were

food deprived for 24 hours, given predictable or unpredictable shock for 19 hours, and then rested without food for a further 6 hour period before being sacrificed. As with the previous studies, Weiss found that rats in the unpredictable shock condition developed significantly more ulcers than either the predictable or non-shocked groups. Several physical factors, however, may have contributed to these results. First, Weiss used a very high shock intensity (3.5 ma for 2.0 sec duration) which may have produced electrical injuries rather than "psychological ulcers". This is evident by noting that 67% of the rats in the predictable shock condition developed ulcers despite the fact that the "psychological trauma" to these animals was supposedly much less than that for the unpredictable rats. Secondly, six hours elapsed between the end of the conditioning period and sacrifice. As previously noted, Desiderato, MacKinnon and Hissom (1974) found that ulceration increased in the post-stress rest period, probably due to the "rebound" gastric acid secretion first noted by Brady (1958). Finally, a total of 49 hours of food deprivation was involved in Weiss' study, despite the fact that only 24 hours of deprivation preceded the start of the experiment. Thus, Weiss' conclusion that "such (psychological) variables can be even more important than the

presence or absence of the physical stressor (p. 264)" appears premature.

In a later series of studies, Weiss (1970) found that rats given unpredictable shock lost significantly more body weight than predictably shocked or non-shocked rats. In addition, he noted that food intake was significantly lower in the unpredictable shock group. Water intake differences occurred, but only after the first day of conditioning. These results provide additional support for this writer's interpretation of Weiss' (1968b) previous study.

Seligman and Meyer (1970) exposed rats to high intensity (1.4 ma) or low intensity (.6 ma) predictable or unpredictable shock for 81 days. The results indicated that unpredictability shocked rats showed profound suppression of lever pressing for food, and consequently developed the most ulcers. Predictably shocked rats did not show as severe a degree of response suppression and as such obtained more food and developed less ulcers. It was also found that the high shock intensity suppressed lever pressing significantly more than the low shock and that no recovery occurred in the high shock group while complete recovery was seen in the low shock group. The correlation between response suppression (i.e., food deprivation) and ulceration

was .74. It is interesting to note that the extent of ulceration in Seligman and Meyer's unpredictable high shock (1.4 ma) group was far less than that seen in Weiss' (1968b, 1970) unpredictable shock groups (3.5 ma), indicating that shock intensity *per se* may have ulcerogenic consequences.

Price (1972) attempted to replicate Weiss' (1968b) study in the unrestrained rat. Rats were given unpredictable shock (3.0 ma for 2.0 sec) on a 6 hour "on" - 6 hour "off" schedule (Rice, 1963) for 12 out of 24 hours. No ulceration was observed in any subject, however, "pinprick-sized blood clots (p. 422)" were observed in some rats. Analysis of these areas did not differentiate between the unpredictable, predictable or control groups. Price concluded "That no ulcers were found was attributed to the absence of restraint (p. 423)". In a second experiment, rats were restrained and given the same shock conditions as in the first experiment. The ulcer results showed that 5 of 10 unpredictably shocked subjects developed ulcers, while 3 of 10 predictably shocked rats had ulcers. One non-shocked rat developed ulceration. Price concluded that although restraint was important, unpredictability was still a potent psychological stressor.

Caul, Buchanan and Hays (1972) examined ulceration and heart-rate in the restrained rat given unpredictable

shock. In presenting their results, the authors noted that "the gastric erosions found were identical to those described in the literature as resulting from immobilization (p. 670)". In addition, the ulcerogenic effects of shock per se may have contributed to the ulcers observed in this study, since the authors noted that "The trend was for more animals to ulcerate in the groups which received twice the number of shocks (p. 672)". These results, in addition to the lack of an unrestrained control group do not support the conclusion that unpredictability is a potent ulcerogenic variable.

Paré and Livingston (1973) measured gastric acid secretion in the chronic gastric fistula rat as a function of shock predictability. Unpredictable grid shock resulted in a significant decrease in gastric secretion and total acid output as compared to the predictable shock and no shock conditions. When restraint was added to the shock conditions, both unpredictable and predictable shock decreased stomach acidity as compared to restrained animals who were not shocked. Paré and Livingston concluded that gastric hypersecretion was not involved in ulceration induced by unpredictable shock, and that other gastrointestinal response systems were probably involved.

Paré and Isom (1975) found that both predictable

and unpredictable shock increased gastric acid secretion as a function of chronic (8 day) stress compared to acute stress (12 hours). The increased acidity values under chronic stress, however, were not significantly greater than pre-stress baseline values, and the authors concluded that initial inhibition of acid secretion was followed by "rebound" hypersecretion but only to baseline values. Again, it appeared that acid hypersecretion was not the primary factor in "psychological" ulcer etiology.

4. Stress Contollability

Uncontrollable aversive stimulation occurs when an organism either (a) has no mechanism available whereby a response can be produced to ameliorate the stimulation or (b) where such a mechanism is available but the probability of the stimulation occurring following an instrumental response is equal to the probability of the stimulation occurring in the absence of such a response (Seligman, Maier and Solomon, 1971). Like unpredictable aversive stimulation, the uncontrollable aspect has been claimed to be a powerful psychological factor in gastric ulcerogenesis.

Mowrer and Viek (1948) examined the behavioral consequences of "helplessness" in rats. Two groups of rats were given shock. One group could terminate shock by jumping vertically so that all four feet were off the ground while

the second group could not terminate shock; that is, had no "control" over the shock. The results showed that animals which could escape shock by jumping (i.e., had "control" over the shock) exhibited fewer behavioral manifestations of fear (inhibition of eating in the shock situation) than those animals that could not terminate shock. Physiological changes were not measured in this study.

Brady, Porter, Conrad and Mason (1958) examined the ulcerogenic effects of uncontrollable shock in their well-known "executive" monkey study. In this study, pairs of monkeys were restrained and shocked on a Sidman avoidance schedule. The "executive" monkey's lever was functional and at least one lever press every 20 sec postponed shock for a further 20 sec. The other monkey in the pair was a yoked control and as such, received the same number of shocks in the same temporal pattern as the "executive" monkey. The lever of the yoked control monkey was disconnected so that it had no control over the shock. Thus, only the "executive" monkey had control in this study. The results showed that the "executive" monkeys developed severe ulcers and often died, while controls showed no gastric pathology. Weiss (1971c) however, pointed out a subtle factor which may have operated in Brady's et al. study to confound the results. Subjects were not randomly assigned to groups; that is, to

the executive or control group. They were assigned on the basis of a pre-test for the rapidity of acquisition of the avoidance task. Those monkeys which acquired the task rapidly were assigned to the "executive" group while slower monkeys were designated as controls. Sines, Clelland and Adkins (1963) and Lepanto, Moroney and Zenhausern (1965) have shown that rats which are genetically ulcer susceptible acquire an avoidance response more rapidly than non-susceptible subjects. Therefore, it may be that the "executive" monkeys in Brady's et al. study were constitutionally more emotional and prone to ulcer development than were the yoked control monkeys.

Foltz and Millett (1964) replicated that "executive" monkey study with a larger number of subjects and without the pre-test for avoidance which might have biased Brady's results. No ulceration in the "executives" was observed in this study. One control monkey died and at autopsy, it was found to have three gastric ulcers close to perforation. The authors noted that in one situation, both an "executive" monkey and its yoked control died during an equipment failure in which they received "an inordinate number of shocks (p. 449)". Since the shock intensity used in this study varied from 5 ma to 20 ma for 0.5 to 1.0 sec duration, it is conceivable that ulceration or even more severe

pathology could be induced without any "psychological" stress added to the situation.

Weiss (1968a) examined the effects of coping responses on stress ulcers. Rats were tested in triplets, with one rat able to avoid shock by touching a plate with its nose, a second rat as a yoked control to the avoidance subject and a third rat serving as a non-shocked control. Shock started at .4 ma and increased to a maximum of 1.6 ma during the 21 hr conditioning sessions. All rats were restrained during this period. Subjects were removed from the apparatus, placed in their home cages and allowed free access to water for 12 hours before being sacrificed and examined for ulceration. The results showed that ulcer incidence did not differentiate between avoidance and yoked subjects, however, yoked animals had more severe stomach pathology. These results, however, may have been influenced by (a) the 12 hour rest period which occurred between the end of the shock session and sacrifice or (b) the restraint employed during shock. The latter factor may have been particularly important since Weiss noted that the ulcers observed were in the glandular portion of the stomach and looked similar to those ulcers seen in restraint research. Again, it seems that psychological factors alone are not sufficient to produce ulceration.

Moot, Cebulla and Crabtree (1970) assessed the severity of physiological responses to shock as a function of the degree of instrumental control over the noxious stimulation provided to the subjects. One group of rats received shock whenever a lever press response for food was emitted (VI - 5 min schedule). Shock could be terminated by emitting a wheel turning response. A second group received shocks each time a lever press response for food occurred, however, shock duration was independent of instrumental responses in this group. Shock duration was fixed with the restriction that the total duration per session was equal to that in the first group. A third group of rats was yoked to the second group such that whenever an animal in the second group received either food or shock, an animal in the third group received food or shock simultaneously and independently of instrumental responding. Finally, a food deprivation only control group was included. All rats were deprived of food and water for 24 hours prior to the experiment and food was available throughout the study in amounts sufficient to maintain the subjects at 80% of their free-feeding weight. Three 20 hour testing sessions constituted the experiment. The results indicated that the two groups with minimal control over the shock developed more ulcers than the group which could respond to terminate the shock.

The authors noted, however, that approximately 150 shocks were given to the subjects in each 20 hour testing period and that "slightly positive" correlations were found between shock duration and ulceration, although no data was given. It may be that shock duration and the concomitant decrease in food intake interacted to produce the ulceration observed. This factor is especailly important in the study of Moot et al. since it appeared that the rats were shocked when they emitted a lever press response for food. Paré (1972) showed that the temporal relationship of shock to the consummatory response is a critical variable in gastric ulcerogenesis. He found that when shock was delivered after food-reinforced lever presses, significant body weight loss and ulceration. Again, it seems unlikely that "control" or a lack thereof, is an ulcerogenic variable per se, but rather exerts its ulcerogenic influence only in interaction with physical factors such as shock, food deprivation, etc.

Weiss (1971a; 1971b; 1971c) proposed a theory to explain how psychological factors determine the amount of ulceration produced by predictable-unpredictable and controllable-uncontrollable stress situations. As in his previous studies, Weiss (1971a) found that yoked animals developed more ulcers than did rats which had control over

the shock. The addition of a warning signal reduced ulceration in all subjects. Weiss theorized that ulceration is a function of (a) an increasing number of coping responses and (b) a decreasing amount of relevant feedback from these responses. In a second study, Weiss (1971b) attempted to decrease the relevant feedback from coping responses to an absolute minimum - even below zero or negative. He accomplished this by punishing coping responses with shock. Rats which could avoid or escape shock by emitting a wheel turning response were shocked every time such a response occurred. According to Weiss' theory, this situation should have produced even more ulceration than that in the yoked control subjects. The obtained results agreed with this prediction. Weiss concluded that this experiment was essentially a "conflict" study and that conflict could now be subsumed by his theory by noting that conflict simply decreases relevant feedback below zero. Conflict was viewed as a special case of an otherwise "ordinary" avoidance-escape situation wherein animals have at least a minimal amount of feedback (but greater than zero). Finally, Weiss (1971c) increased the amount of feedback generated from coping responses by providing subjects with a tone immediately following avoidance-escape responses. These subjects developed significantly less ulceration than subjects which could avoid or escape

shock but which received no feedback signal following their coping responses. There is considerable variability in Weiss' results, particularly when controllability is held constant and predictability is varied (Table 1.1). When shock was unsignalled (i.e., unpredictable) but controllable (i.e., rats could avoid or escape shock) ulceration varied considerably between studies, however, when shock was both predictable and controllable, consistent ulcer results were obtained. It may be, as in previous studies, that ulceration varies with the amount of shock received including frequency (shock density), which is not reported in Weiss' studies. Finally, all three of Weiss' recent studies involved a total of 72 hours of food deprivation, but no control groups for this variable were included. It again seems reasonable to conclude that a necessary (and probably sufficient) condition for the production of psychologically induced pathology is an empty stomach, which, by virtue of its emptiness, then becomes a prime target for the action of stress-induced acid and hormonal changes leading to gastric ulceration.

Food Deprivation and Ulceration

From the preceding review of the literature, it can be concluded that food deprivation is critically involved, both directly and indirectly in the production of

TABLE 1.1

SUMMARY OF WEISS' ULCER RESULTS IN TERMS OF PREDICTABILITY

Shock	Shock Conditions	Mean Ulcer Length (mm)	Mean Number of Ulcers	Shock Parameters
Weiss, 1968b	unsignalled-controllable	8.9	6.6	3.5 ma for 2.0 sec
Weiss, 1971a	unsignalled-controllable	2.0	3.5	variable 1.6 ma increasing by .6 ma/12 hr to maximum 3.4 ma for .2 sec
Weiss, 1971c	unsignalled-controllable	3.0	7.0	variable 1.6 ma increasing by .6 ma/12 hr to maximum 3.4 ma for .2 sec
Weiss, 1968a	signalled-controllable	1.5	1.2	3.5 ma for 2.0 sec
Weiss, 1970	signalled-controllable	1.6	1.0	variable with maximum 3.0 for 5 sec
Weiss, 1971a	discrete signal-controllable	1.0	2.0	variable 1.6 ma increasing by .6 ma/12 hr to maximum for 3.4 ma for .2 sec
Weiss, 1971a	progressive signal-controllable	1.0	2.0	variable 1.6 ma increasing by .6 ma/12 hr to maximum of 3.4 ma for .2 sec

experimental ulcers. Evidence for direct involvement arises from studies which show that unless a period of food deprivation precedes a stress treatment, ulceration does not result. For example, Weinstein and Driscoll (1972) found that in wild rats (rattus norvegicus), restraint ulcers could be produced in 12 to 24 hours "given that pre-immobilization food deprivation was sufficiently long (p. 39)". Essman and his co-workers noted that in some of their subjects, food was still present in the stomachs at autopsy. In particular, Frisone and Essman (1965) noted that "the severity of the gastric pathology in those animals with undigested food was found to be consistently low (p. 944)". The authors concluded that the presence of food may have absorbed gastric acid and thereby reduced its corrosive action. Finally, Frisone and Essman suggested that restraint might have increased gastric emptying time since no animals in this condition were found to have retained undigested food, while many subjects in the food deprivation only condition did retain food. As a result of subsequent studies on gastric food retention, Essman (1966a; 1966b) concluded "gastric food retention serves a protective function insofar as ulcerogenesis and ulcer severity are concerned (Essman, 1966b, p. 252)". Finally, Essman, Essman and Golod (1971) suggested that stress conditions such as restraint, tend to minimize gastric retention and as such constitute an

initial and essential step in ulcerogenesis. The integrity of the stomach is then further weakened by the biochemical and hormonal changes induced by the stressor, which persists after the stomach has emptied.

Additional research indicated that not only food, but even non-nutritive bulk provides protection to the stomach. Taylor and Bruning (1967) suggested that "a rat, when hungry, will accept non-nutritive bulk to fill its stomach rather than remain completely deprived (p. 355)". Mikhail and Hirschberg (1972) examined this phenomenon and found that starvation-induced rumenal ulcers could be significantly reduced by feeding rats a non-nutritive but bulky solid diet. Subsequent studies confirmed the protective effect of non-nutritive bulk in shock situations (Glavin and Mikhail, 1976) and with the pylorous-ligation treatment (Mikhail, Gabriel and Glavin, 1975). It appears that the development of ulceration is dependent upon the absence of bulk (either nutritive or non-nutritive) in the stomach.

Indirect evidence which supports the role of food deprivation arises mainly from studies using shock as a stressor. Sterritt (1962) and Sterritt and Shemberg (1963) found that eating was inhibited by shock even during the intervals when the shock was turned off. The final result was that shocked subjects ate significantly less overall

than non-shocked subjects, regardless of whether they were starved or sated prior to the experiment.

Paré (1964, 1965) subjected rats to chronic (22 days) predictable shock and found that food intake was significantly reduced in these subjects as compared to non-shocked rats. Paré (1964) concluded that "in an experimental situation where subjects have continuous access to food and water, shock trauma produces a reduction in consummatory behavior (p. 149)". Paré also noted that the ad lib feeding condition was sufficient to prevent ulceration from developing.

Mikhail (1966), however, suggested that fear provoking procedures were not effective in inducing corpus ulcers and argued that rumenal ulcers which appeared in his work and earlier research, were a manifestation of depriving the animals of food. An alternate formulation of the mechanism whereby psychological stress produces gastric ulcers was suggested by Mikhail (1973): conflict, conditioned anxiety, or shock ⟶ starvation ⟶ rumenal ulceration. Glandular ulceration formation appeared to be somewhat different, resulting primarily from restraint or extremely high shock intensities. Mikhail (1973) suggested that (a) ulcer etiology in the two portions of the rat stomach were different, (b) food deprivation is the primary pathogenic

agent in rumenal ulceration, (c) without food deprivation, rumenal ulcers do not develop, and (d) when rumenal ulceration is used or a dependent variable, it reflects dietary deficiency and not the influence of psychological stress. He concluded that "a convincing experimental demonstration of the relationship of conflict or anxiety to gastric ulceration is lacking at present (p. 641)".

Paré and Temple (1973) attempted to quantify the severity and location of ulceration resulting from either starvation alone or a combination of starvation and shock stress. After five days of starvation, all rats developed rumenal ulceration, while some developed ulcers after only two days. Small glandular ulcers were observed in two rats after four days of starvation. The addition of shock stress to the starvation condition did not significantly increase ulcer incidence. It is interesting to note that Paré and Temple used a low shock intensity (.5 ma for .5 sec) and found no glandular ulcers even after five days of starvation and shock. This suggested that the glandular ulceration observed in some studies may reflect electrical injuries as a function of elevated shock intensities, rather than psychological stress. The authors concluded that rumenal ulcers reflect food deprivation rather than the manipulation of psychological variables, while "studies reporting glandular ulcers are probably on firmer ground in postulating an

etiology of a psychogenic nature (p. 374)".

Is there a psychologically-induced ulcer? Conversely, is there a physiologically-induced ulcer? It is apparent that a complex interaction of both physical and psychological factors produces gastric ulceration. This is not inconsistent with the current "pluricausal" view of illness in which disease is seen as resulting not from one specific pathogen but rather from several contributory factors (Selye, 1971; Mason, 1974). With respect to the disease in question - ulceration - the task of the researcher appears to be that of determining the relative contributions of both physical and psychological factors to its etiology, rather than trying to demonstrate the existence of a "purely" psychological or "purely" physical ulcer. The present experiments were designed with this task in mind.

CHAPTER II

PHYSICAL VERSUS PSYCHOLOGICAL EFFECTS OF SHOCK ON GASTRIC ULCEROGENESIS

Weiss (1968b; 1970) attempted to demonstrate the production of ulcers through psychological procedures by varying "predictability" and "controllability" of shock. It was noted in the preceding discussion, however, that the shock intensity used in Weiss' work was quite high (3.5 ma). Therefore, the present study (Experiment 1) examined the effects of shock intensity in a close replication of Weiss' (1968b; 1970) experiments. One group of rats received unpredictable and uncontrollable shock with the same duration and intensity as used by Weiss (3.5 ma for 2.0 sec). A second group received the same treatment, but at a reduced shock intensity (1.2 ma for 2.0 sec). A third group received very low shock intensity (.5 ma for 2.0 sec). If the direct and physically damaging effect of shock rather than a psychological effect was the main contributing variable in this paradigm, then the group receiving the lowest shock intensity should develop the least stomach pathology.

Method

Subjects. Subjects were 40 male Sprague-Dawley rats

approximately 100 days of age at the start of the experiment.[1] The mean body weight was 340 gr (S.D. = 7 gr). All rats were randomly assigned to treatment conditions.

Apparatus. Conditioning cages of three sides of stainless steel and a plexiglass front were used. Each cage measured 22.5 x 15.5 x 22.5 cm. The floor of each cage consisted of a grid of metal bars .25 cm in diameter and placed 1.5 cm apart. Each cage was covered with a sheet of milkglass through which a light stimulus was presented. The volume of each cage was reduced to that used in Weiss' work, by fastening wood around the inside of each cage. Tail electrodes as described by Weiss (1967) were used to administer shock. A rat fitted with a tail electrode and placed in a cage is shown in Figure 2.1. An electronic programmer was used to present the lights and shocks on a VI 60 sec schedule. All rats were killed with ether overdose (Fisher). Acidity measurements were obtained with a flat surface penicillin selective enzyme electrode[2] described by Cullen, Rusling,

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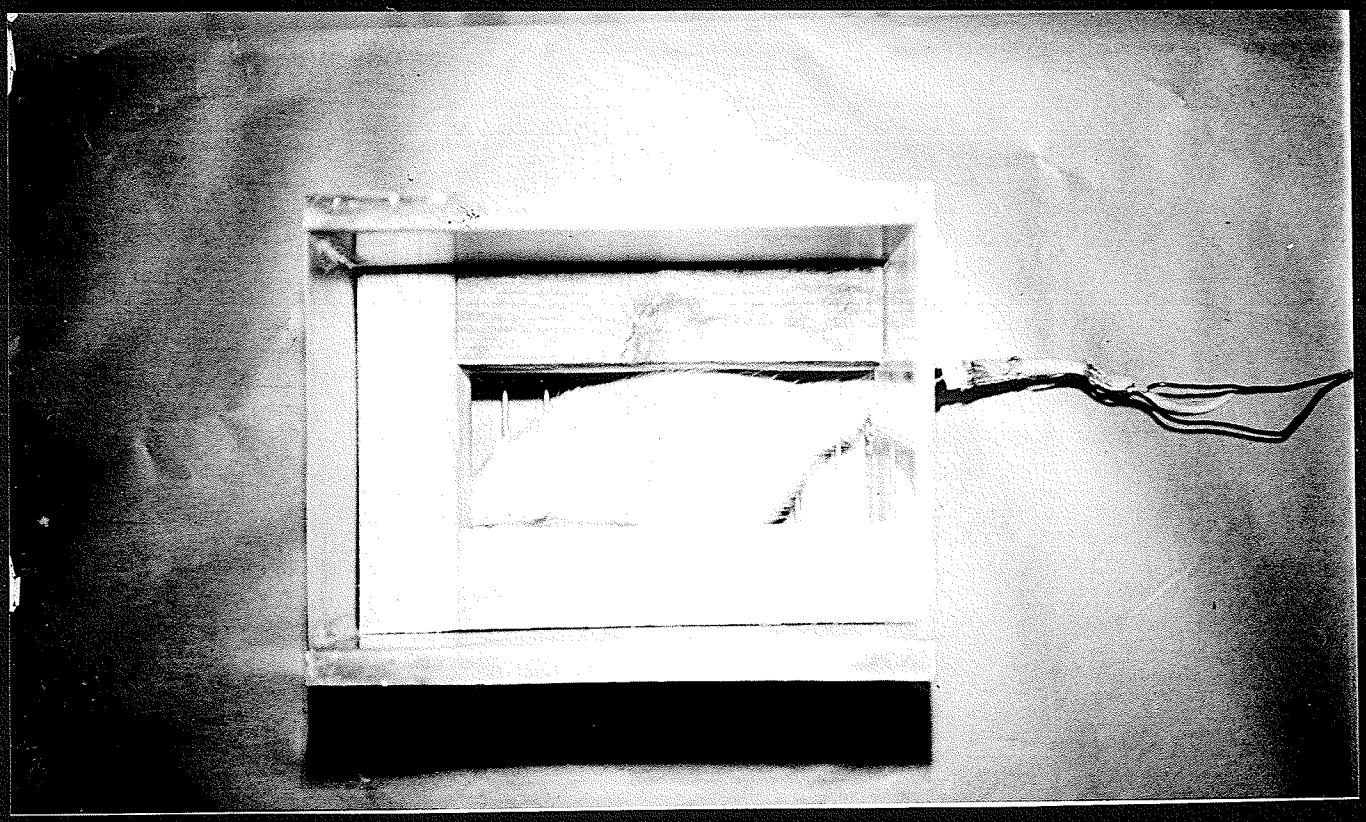
[1]Obtained from Hormone Assay Laboratories in Chicago, Illinois (as did Weiss, 1970).

[2]Markson Science Inc. Cat. No. 1207.

Figure 2.1

Figure 2.1 A rat fitted with tail electrodes and placed in a conditioning cage as used in Experiment 1.



Schleifer and Papariello (1974) connected to a Beckman Zeromatic S-33 pH meter[1] with full pH scale (0-14) and temperature range 0°C to 100°C.

Procedure. Weiss' (1968b; 1970) procedure was followed, with slight modifications. All rats were food deprived for 24 hours prior to the experiment. After being placed in the cages and their tails secured as in Figure 2.1, the electrodes were attached in the following manner. The hair and outer scales of skin on the tail were scraped away with a scalpel on the two areas where the electrodes were to be attached. It should be noted that this procedure caused neither visible discomfort to the animal nor blood to be drawn. These areas were then thoroughly rubbed with a solution of 75% saline and 25% glycerine. Finally, standard electrode paste was applied to the surfaces. The two electrodes, constructed of ½-inch pieces of braided wire, were then placed over the two prepared areas. A short length of polyethylene tubing was placed over each piece of braided wire to secure the electrodes in place. These procedures reduced the resistance of the rat's tail to 9 K ± 500 ohms. Without this preparation, the resistance of the rat's tail in

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[1]Cat. No. 9609, Model No. 96.

such a circuit varies from 50 to 500 K ohms.

After 1 hour of habituation, the program of lights and shocks began and continued for 19 hours. Group 1 (n = 10) received lights and shocks of high intensity (3.5 ma for 2.0 sec) programmed separately (i.e., unpredictable shock). Group 2 (n = 10) received lights and moderate intensity shock (1.2 ma for 2.0 sec) programmed in an unpredictable manner. Group 3 (n = 10) received lights and low intensity shock (.5 ma for 2.0 sec) programmed in an unpredictable manner. After the experimental period, all rats were returned to their home cages and allowed water but not food for six hours. Following this period, all rats were killed with ether overdose and their stomachs excised and examined for acidity and ulceration. An untreated control group of 10 rats was also examined in the same manner at this time. The acidity of both the rumen and the corpus was assessed by touching the flat surface electrode to the surface of either portion of the stomach and determining the pH from the meter. All stomachs were then washed and illuminated above a sheet of milkglass for ulcer counting, rating and photographing according to the method of Mikhail and Holland (1966) and Lambert (1968). A second observer who was naive with respect to which group the subject belonged also examined and rated the stomachs. In cases of disagreement, the naive observer's judgment was used.

Results

In the present and remaining series of experiments, several dependent measures were used to assess the effects of the treatments employed. To analyze data derived from several criterion measures, multivariate analysis of variance is an appropriate procedure (Gabriel and Hopkins, 1974). This method is an extension of univariate analysis of variance procedures with the advantage that the overall experimentwise type I error probability remains at α regardless of the pattern of intercorrelations among the set of dependent measures which are considered simultaneously. In addition, interpretation of the analysis proceeds exactly as does that of a univariate analysis. Gabriel and Hopkins (1974) noted that "The p value given by the multivariate test may be viewed as a 'protection level' for stating conclusions based on the univariate ANOVA's (p. 387)". In all experiments, analysis of variance was performed using version IV of Finn's (1968) MULTIVARIANCE program.

The results of this experiment indicated that increasing shock intensity increased rumenal acidity[1] and glandular ulcer severity (Table 2.1). All ulcer data of the

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[1]Note that increased acidity corresponds to a decrease in pH value.

TABLE 2.1

MEAN GLANDULAR pH, GLANDULAR ULCER INCIDENCE AND GLANDULAR ULCER SEVERITY IN THE EXPERIMENTAL AND CONTROL GROUPS

Group	Shock level	ulcer incidence	ulcer severity	glandular pH
Exp. 1	3.5 ma	4/10	0.7	2.5
Exp. 2	1.2 ma	2/10	0.2	2.8
Exp. 3	0.5 ma	2/10	0.2	2.7
Control	0.0 ma	0/10	0.0	3.8

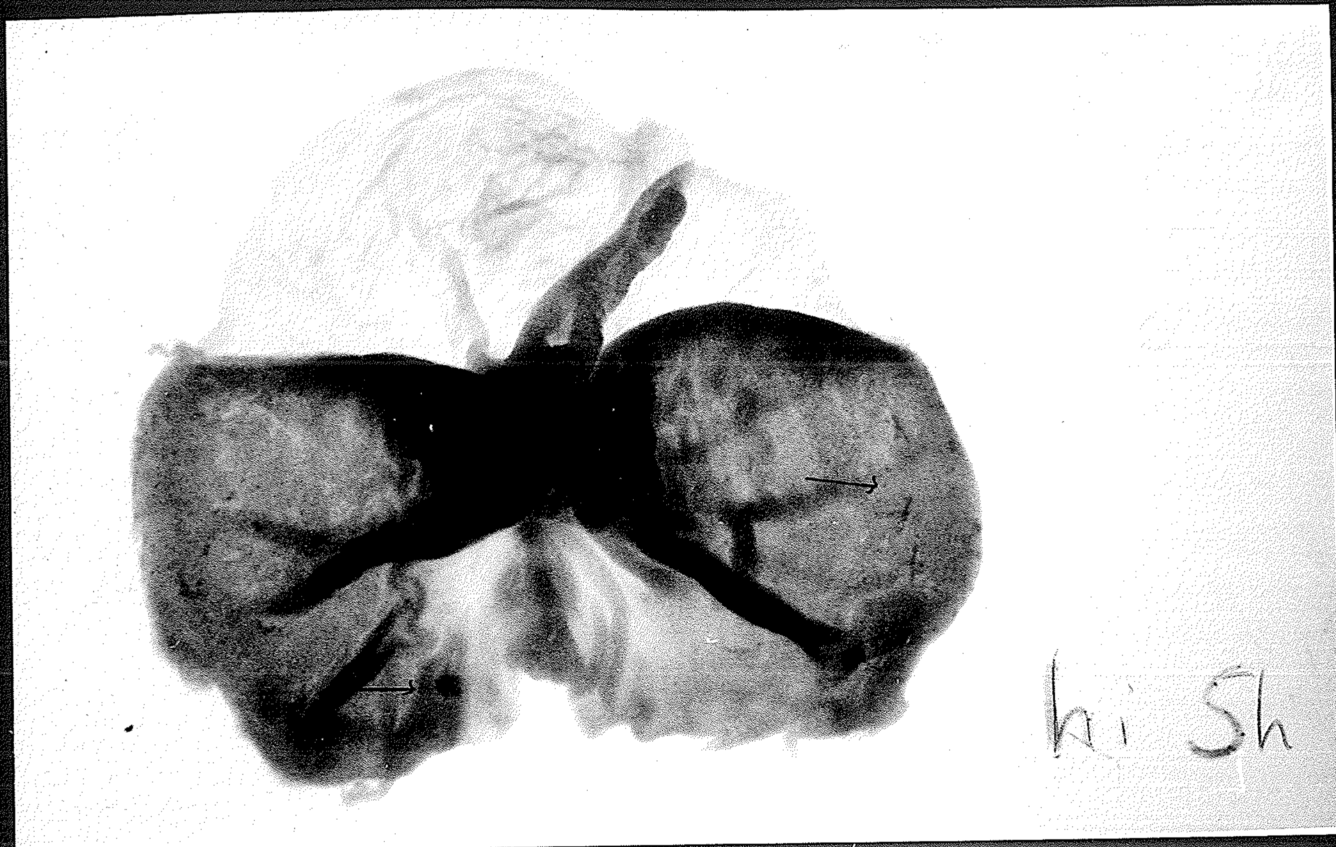
present study refers to glandular ulcers since no instance of rumenal ulceration was observed. Note the presence of several small glandular ulcers in the stomach of a rat from the high shock group (Figure 2.2), while ulcer incidence declined as shock intensity decreased (Figures 2.3, 2.4 and 2.5).

The overall multivariate analysis of variance indicated that the experimental and control groups differed significantly (Table 2.2). The variables contributing to the overall difference were located by univariate analysis of variance and found to be rumenal acidity ($p < .07$) and glandular ulcer severity ($p < .05$), both of which decreased with decreasing shock intensity. Tukey's HSD procedure confirmed that the high shock group developed significantly more severe glandular ulceration than either the moderate shock group ($p < .05$) or the low shock group ($p < .05$).

Figures 2.2 and 2.3

Figure 2.2 The stomach of a rat from the high shock (3.5 ma) group. Note the presence of several glandular ulcers.

Figure 2.3 The stomach of a rat from the medium shock (1.2 ma) group. Note the single ulcer in the body of the stomach.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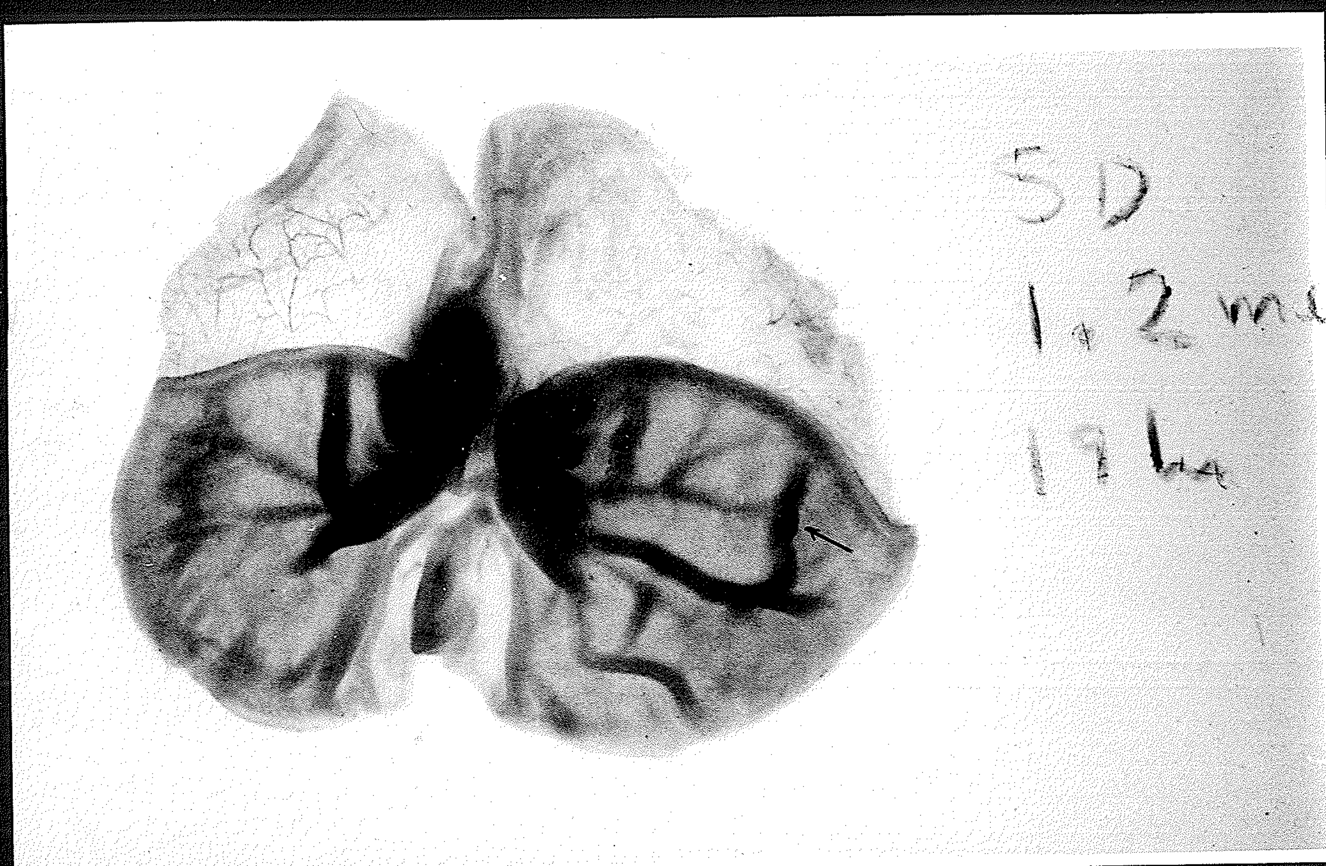
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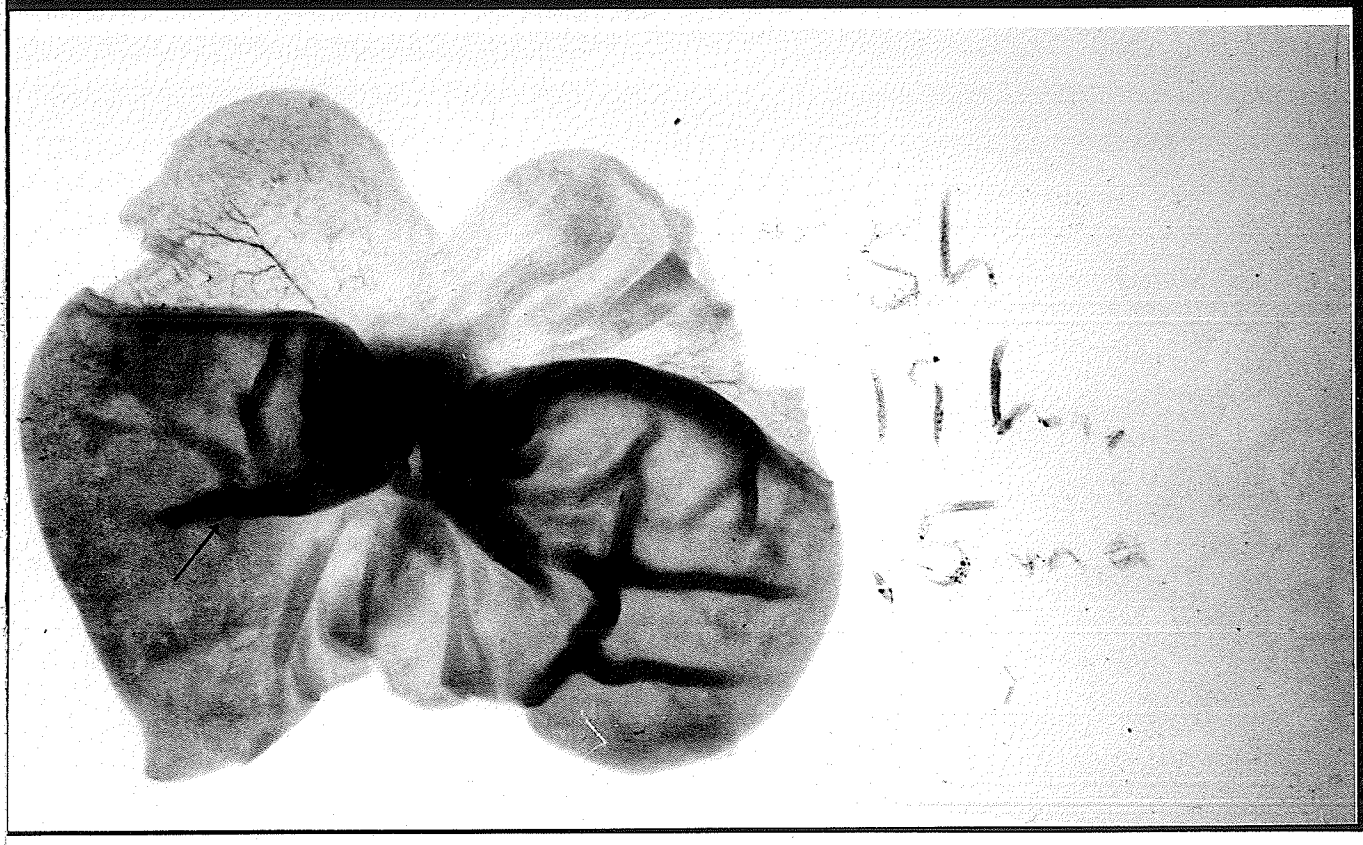
Figures 2.4 and 2.5

Figure 2.4 The stomach of a rat from the low shock (.5 ma) group. Note the one small glandular ulcer.

Figure 2.5 A normal stomach from a control rat which received no shock. Note the absence of pathology in this stomach.



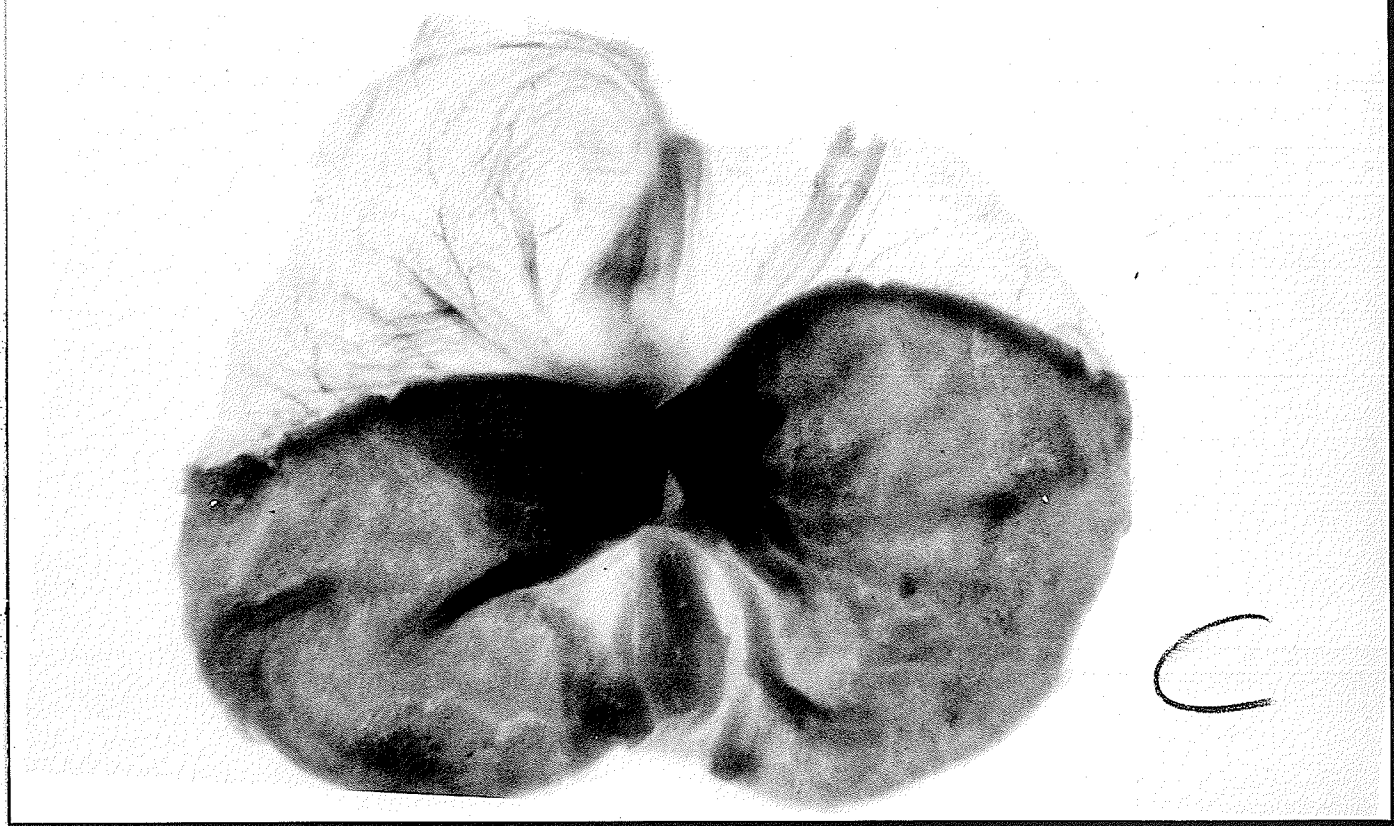


TABLE 2.2

ANALYSIS OF VARIANCE[1] OF SHOCK INTENSITY

Variable	MS	F[2]	p
rumenal pH	3.11	2.60	.0673
glandular ulcer severity	0.89	2.84	.0515
glandular pH	0.09	0.12	.9488

[1] multivariate $F_{9,82.9} = 2.10$; $p < .0385$

[2] all univariate F values were tested at 3 and 36 df.

Discussion

Weiss (1968; 1970) produced ulcers by using a paradigm in which physical conditions (e.g., shock) were constant, and psychological conditions (e.g., predictability and controllability of aversive stimulation) varied. A critical ulcerogenic variable in Weiss' work is shock intensity. Although shock conditions were constant, they were maintained at a very high level (3.5 ma for 2.0 sec). In the present experiment, psychological conditions were held constant while physical conditions (shock) were varied. If the ulceration in Weiss' research was due to high shock intensity, then reducing shock intensity but keeping the psychological conditions of predictability and controllability constant should result in decreasing ulceration. The results of experiment 1 show clearly that decreasing shock intensity decreased both ulcer incidence and severity despite the fact that psychological parameters associated with its delivery were constant. When Weiss' overall procedure is considered, the data do not provide support for its effectiveness in producing experimental ulceration in rats. Even in the maximally ulcerogenic (according to Weiss) condition of this experiment, that is, the highest shock intensity which was both unpredictable and uncontrollable, only four of ten rats developed ulcers, the severity of which was quite low

relative to that observed by Weiss.

The present study is not unique in that Weiss' findings were not replicated. Many earlier studies which employed shock in conflict and conditioned fear paradigms were generally ineffective in inducing experimental ulceration. Any ulcers occurring in such experiments were largely due to the effects of shock and/or starvation (Paré, 1962; Paré and Livingston, 1970, 1972a; Paré, 1972b; Mikhail, 1969; Mikhail, 1972). Even in a replication of Weiss' work, Price (1972) could not find stomach ulcers. When he restrained rats and then gave them unpredictable and uncontrollable shock via tail electrodes, ulcers developed. Price concluded that the absence of restraint was the agent responsible for the lack of ulceration in his first study. Paré (1975) also found a low incidence of ulceration when using Weiss' procedure. Given the efficacy of restraint in producing ulcers (as will be seen from the results of experiments 2 and 3), the conclusion is warranted that restraint played an essential role in Weiss' research.

The cage volume in Weiss' apparatus is comparable to that of a restraint cage. Thus, in his experiments, both restraint and shock intensity must be considered as important agents contributing to the ulceration which resulted from his procedure. In the present study, both restraint

and high shock intensity were not employed, leaving only the psychological factors of unpredictability and uncontrollability. In this situation, very little ulceration resulted. It is now abundantly clear that when restraint and shock were excluded, the psychological variables employed in Weiss' paradigm had little, if any, ulcerogenic potential. Ulceration appeared only when shock of a high intensity was made unpredictable and uncontrollable.

To conclude, it appears that the ulcers which had been formed by Weiss' procedure were induced partly by shock and partly by restraint, while the psychological factors of uncontrollability and unpredictability produced negligible pathological effects when the shock was of low intensity.

CHAPTER III

EFFECTS OF STRESS DURATION ON ULCER DEVELOPMENT AND GASTRIC EMPTYING TIME IN THREE STRAINS OF RATS

As previously noted in Chapter I, considerable disagreement exists with respect to the optimal duration of pre-stress food deprivation (Weinstein and Driscoll, 1972). In many cases, food was found in the stomachs of subjects at autopsy, rendering these animals useless in terms of obtaining reliable ulceration data (Frisone and Essman, 1965; Essman, 1966a; 1966b). In general, it can be concluded that if either food (Essman, 1966a; 1966b) or even non-nutritive bulk (Mikhail and Hirschberg, 1972; Glavin and Mikhail, 1976) remain in the stomach, ulceration will either be eliminated or significantly reduced. A demonstration of gastric emptying time as a function of commonly used stress treatments should provide a useful referent of the appropriate duration of food deprivation required prior to the exposure to stress. The confounding (antiulcerogenic) effects of food remaining in the stomach can thereby be eliminated. Experiment 2 examined the role of food deprivation in ulcer production by the commonly used treatments of unpredictable-uncontrollable shock and restraint in the most commonly used strains of rats including Sprague-Dawley, Wistar and Long-Evans. A further untreated, but food

deprived group of rats was also included.

Method

Subjects. Subjects were 80 male rats of each of the Sprague-Dawley, Wistar and Long-Evans strains (a total of 240 rats). All rats were approximately 100 days of age at the start of the experiment and were randomly assigned to treatment conditions as shown in Table 3.1. The mean body weights for the Sprague-Dawley, Wistar and Long-Evans rats were 318 gr (S.D. = 10 gr), 310 gr (S.D. = 8 gr), and 300 gr (S.D. = 9 gr), respectively.

Apparatus. For the shock condition, the same cages, programmer and tail electrodes as described in experiment 1 were used. Only the high shock intensity (3.5 ma for 2.0 sec) was used in this study. For the restraint condition, plexiglass restraint cages (Fisher Scientific Co.) as shown in Figure 3.1 were used. All animals were killed with ether overdose (Fisher). Stomach pH measurements were obtained with a flat surface pH electrode as in experiment 1.

Procedure. On the first day of the experiment (8:00 A.M.) subjects in the shock condition were placed into the cages, their tails were rubbed with electrode paste at the contact points and the tail electrodes were attached. At this time, food but not water was removed from all subjects.

TABLE 3.1

NUMBER OF SUBJECTS PER CELL IN EXPERIMENTAL AND CONTROL CONDITIONS

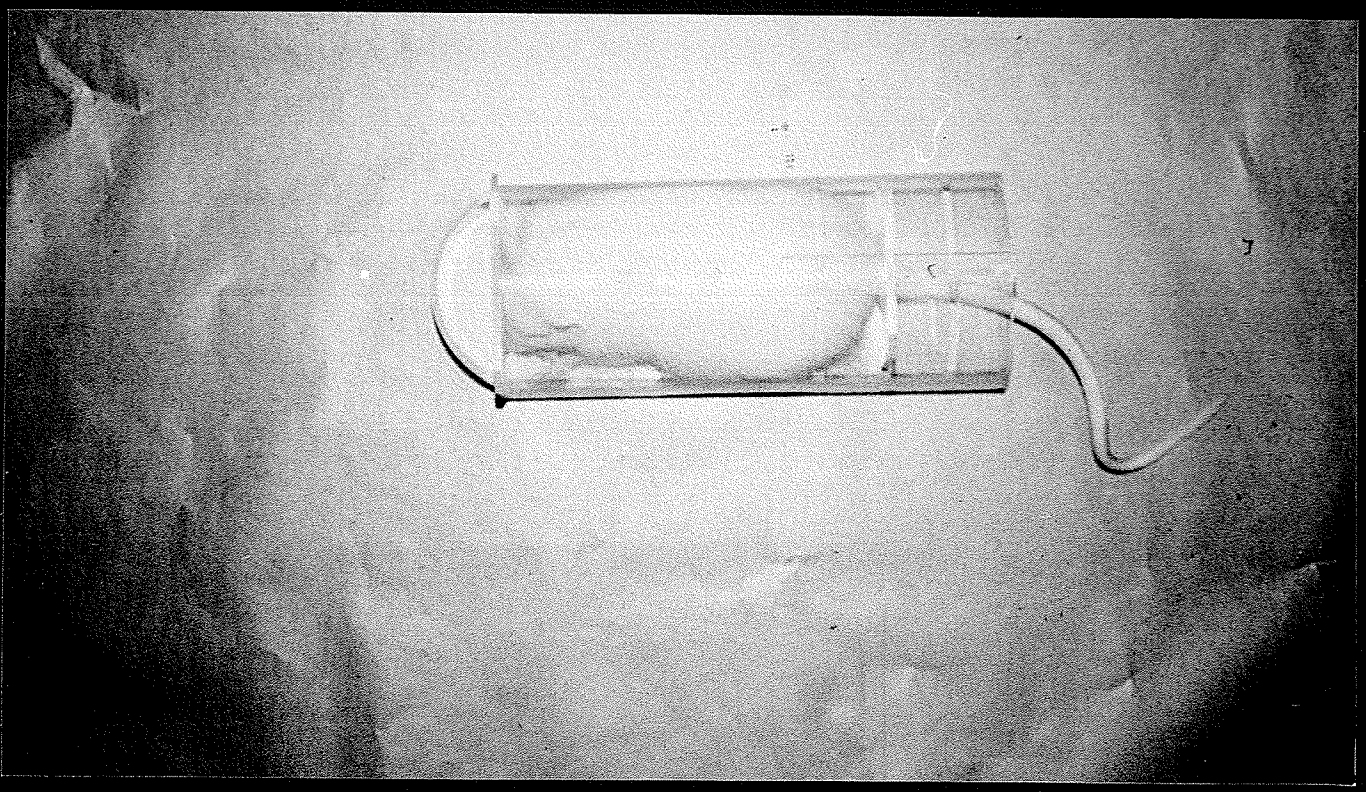
Treatment	Duration (hr)			
	24	48	72	96
Unpredictable-uncontrollable shock (3.5 ma - 2.0 sec.)	18[a]	18	18	18
Restraint	18	18	18	18
Food deprivation only	18	18	18	18
Control	6[b]	6	6	6

[a]Each cell of n=18 was comprised of 6 Sprague-Dawley, 6 Wistar, and 6 Long-Evans rats.

[b]Since ulcer incidence in untreated rats is between 0.0% and 0.2% only 8 rats of each strain served as home cage controls.

Figure 3.1

Figure 3.1 A rat placed in a commercial plexiglass restraint cage as used in the present experiment and in Experiment 3.



Following 24, 48, 72 or 96 hours of shock, six subjects of each strain (18 rats) were killed and the amount of food remaining in the stomach was weighed in the following manner. The stomach and contents were first weighed on a Sartorius[1] balance (±.001 gr). The stomach was then washed of its contents, dried and then weighed again within a standard period of time (30 sec). The difference between these two weights was used as the measure of the stomach contents. The stomachs were then examined for pH and ulceration and photographed as in Experiment I.

Subjects in the restraint condition were placed into the plexiglass restraint cages at 8:00 A.M. on the first day and deprived of food but not water. Following 24, 48, 72 or 96 hours of restraint, six subjects of each strain (18 rats) were killed and the amount of food remaining in the stomach was weighed. Acidity and ulceration data were obtained in the same manner as with the shock subjects.

Subjects in the food deprivation only group remained in their home cages throughout the study. They were deprived of food not water for 24, 48, 72 or 96 hours. Following a

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[1]Sartorius-Werke AG, Model No. 2604

given time period, six subjects of each strain (18 rats) were killed and the amount of food remaining in the stomach was weighed. Acidity and ulceration measures were obtained in the same manner as the other groups.

The untreated control rats remained in their home cages with food and water available ad libitum. Randomly selected rats (8 of each strain) were killed and examined at 24 hour intervals for four consecutive days (i.e., 24, 48, 72, and 96 hours) to verify the presence of food and the absence of ulcers in the stomachs of untreated rats.

Results

The overall pattern of results in the present experiment (Table 3.2) indicated a general increase in rumenal ulceration and a general decrease in glandular ulceration over time. It appears that Sprague-Dawley rats were most ulcer-susceptible and that restraint was the most ulcerogenic stressor. Figures 3.2 through 3.13 illustrate the pattern of both rumenal and glandular ulceration over time in restraint. This pattern is illustrated first for the Sprague-Dawley strain, and then for the Long-Evans and Wistar rats. Figures 3.14 to 3.17 show the strain x treatment interaction in that some strains were most ulcer-susceptible under one stressor and most ulcer-resistant under another. This treatment-specific nature of the pathological responses of different strains to different treatments is evident in the above photographs. In the case of food deprivation, Wistar rats developed more severe pathology than Long-Evans rats, while the opposite occurred in the shock condition. Sprague-Dawley rats were not seriously affected by either of these treatments, but responded maximally to restraint.

The multivariate analysis of variance for the treatment main effect indicated significant differences among the

TABLE 3.2

ULCER INCIDENCE, MEAN RUMENAL AND GLANDULAR ULCER SEVERITY, MEAN RUMENAL AND GLANDULAR pH AND MEAN AMOUNT OF FOOD REMAINING IN THE STOMACH IN ALL EXPERIMENTAL AND CONTROL GROUPS

Variable	Condition	Rumenal ulcer Incidence	Glandular ulcer Incidence	Mean Rumenal pH	Mean Glandular pH
Stress Treatment	Shock	20/72	4/72	2.68	3.09
	Restraint	21/72	8/72	2.75	2.83
	Food Deprivation	13/72	0/72	2.61	2.91
	Control	0/24	0/24	3.69	3.04
Strain	Sprague-Dawley	19/80	9/80	2.94	2.95
	Long-Evans	17/80	2/80	2.92	3.11
	Wistar	19/80	1/80	2.94	3.07
Time	24	0/60	8/60	2.83	2.97
	48	3/60	2/60	2.84	2.84
	72	11/60	1/60	2.93	3.19
	96	40/60	1/60	3.15	3.17

TABLE 3.2 (CONTINUED)

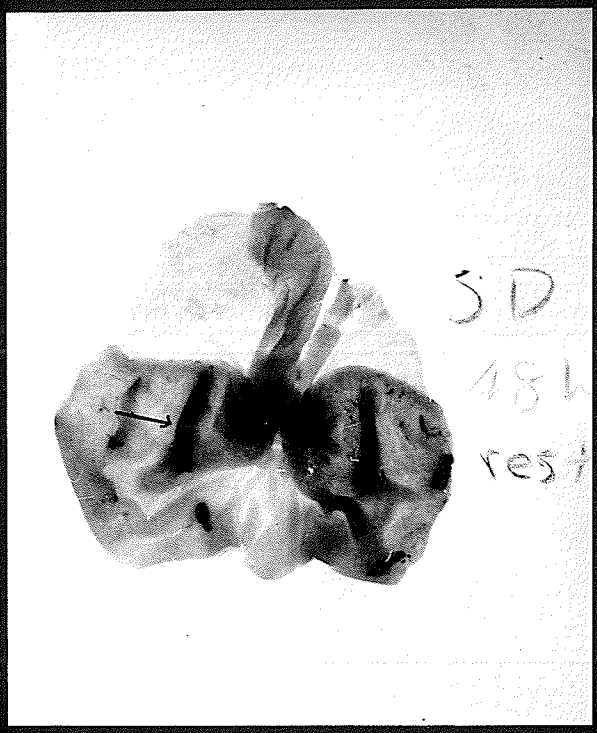
ULCER INCIDENCE, MEAN RUMENAL AND GLANDULAR ULCER SEVERITY, MEAN RUMENAL AND GLANDULAR pH AND MEAN AMOUNT OF FOOD REMAINING IN THE STOMACH IN ALL EXPERIMENTAL AND CONTROL GROUPS

Variable	Condition	Mean Rumenal Ulcer Severity	Mean Glandular Ulcer Severity	Mean amount of food remaining in the stomach
Stress Treatment	Shock	.514	.055	.400
	Restraint	.583	.236	.383
	Food deprivation	.306	.000	.356
	Control	.000	.000	3.511
Strain	Sprague-Dawley	.385	.188	1.22
	Long-Evans	.364	.021	.935
	Wistar	.302	.010	1.33
Time	24	.000	.194	1.19
	48	.070	.070	1.00
	72	.236	.028	1.30
	96	1.10	.014	1.16

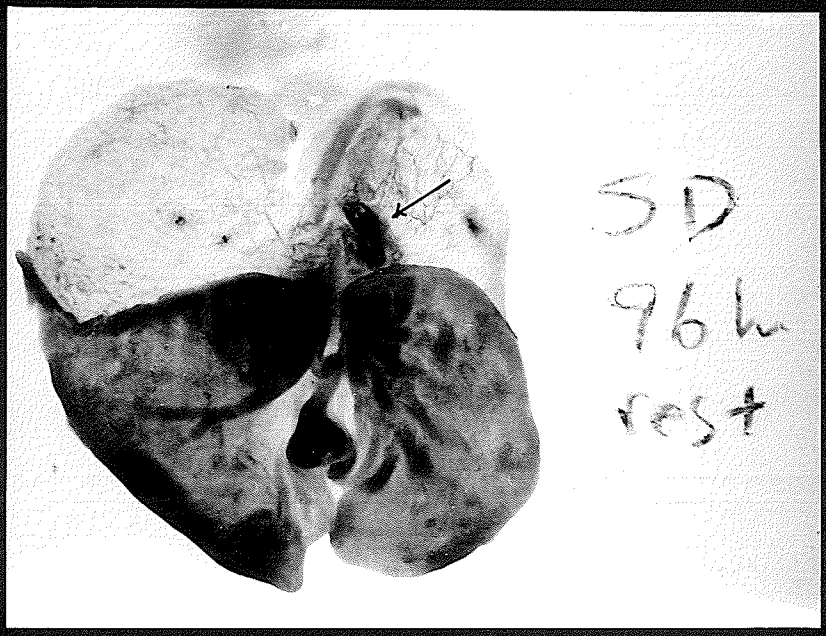
Figures 3.2, 3.3, 3.4, and 3.5

Figures 3.2, 3.3, 3.4, and 3.5 The stomachs of Sprague-Dawley rats after 24, 48, 72, and 96 hours of restraint. Note the glandular ulcers were more severe at shorter stress durations and then decreased in frequency, while rumenal ulceration increased over time.


SD
24 h
rest


SD
rest

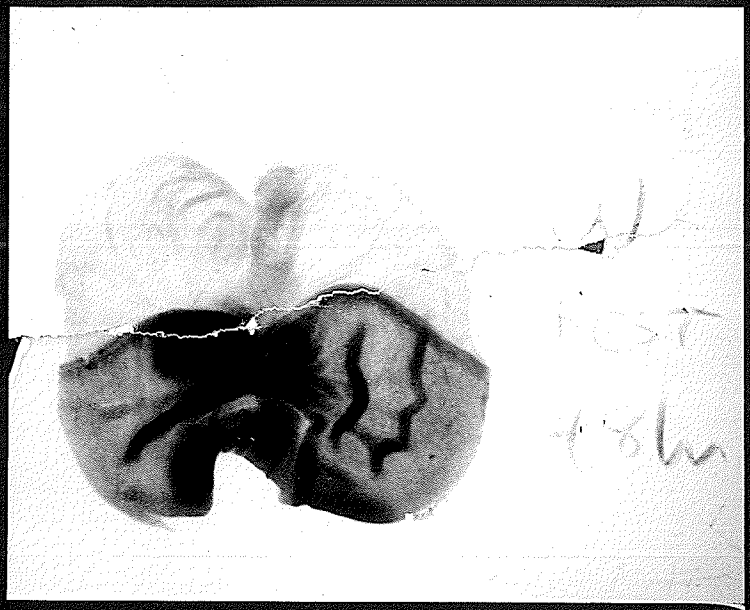

SD
72 h
rest


SD
96 h
rest

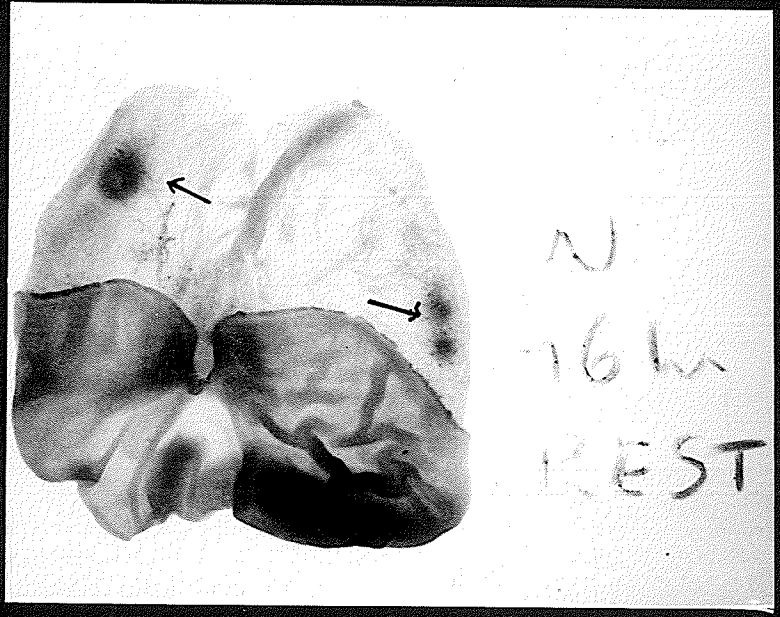
Figures 3.6, 3.7, 3.8, and 3.9

Figures 3.6, 3.7, 3.8, and 3.9 The stomachs of Long-Evans rats after 24, 48, 72, and 96 hours of restraint. Again, glandular ulceration was more severe at shorter stress durations and decreased over time, while rumenal ulcers generally increased over time.


W
24hr
REST


REST
+16hr

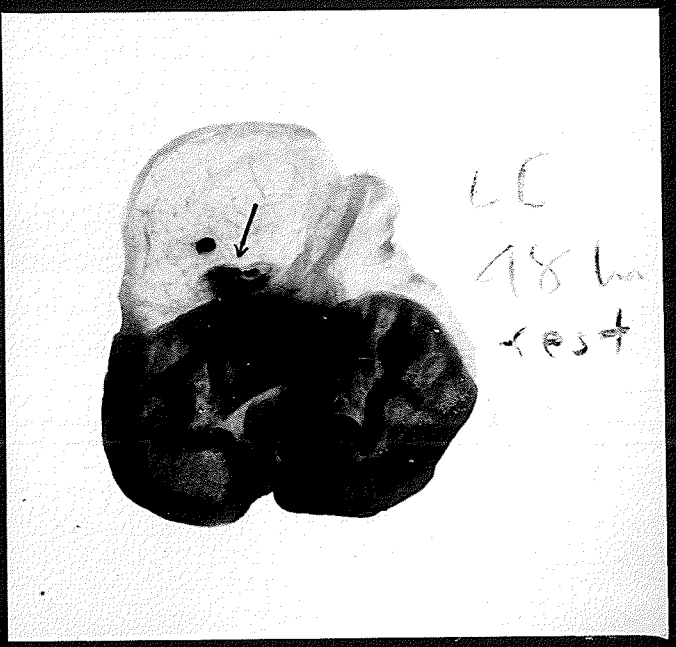

W
12hr
REST


W
16hr
REST

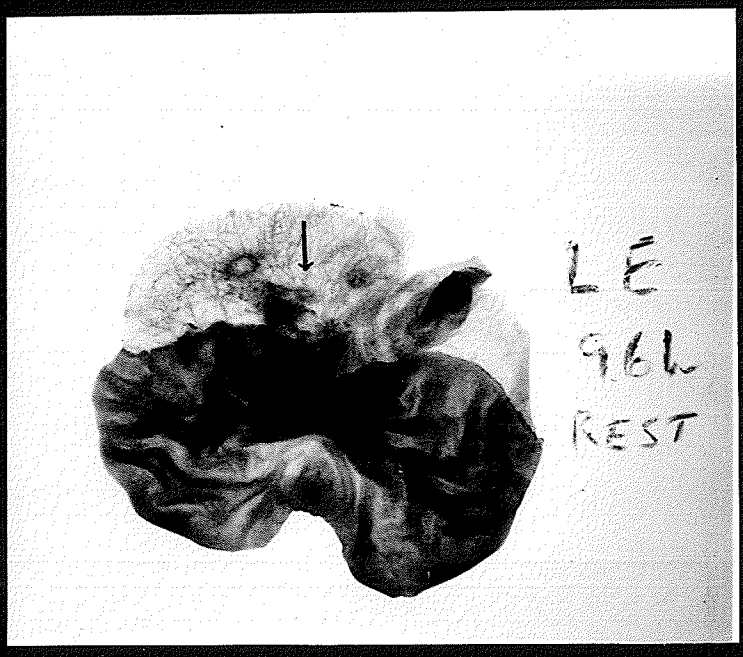
Figures 3.10, 3.11, 3.12, and 3.13

Figures 3.10, 3.11, 3.12, and 3.13 The stomachs of Wistar rats after 24, 48, 72, and 96 hours of restraint. No ulceration was seen until 96 hours. Rumenal ulceration finally developed at this duration, probably reflecting the effects of starvation rather than restraint.


LE
24 hr
REST


LE
48 hr
rest


LE
72 hr
rest


LE
96 hr
REST

Figures 3.14 and 3.15

Figure 3.14 The stomach of a Long-Evans rat after 96 hr of food deprivation, showing moderately severe rumenal ulceration.

Figure 3.15 The stomach of a Wistar rat after 96 hr of food deprivation. Note how the rumenal ulcers in the Wistar rat were more severe than those in the Long-Evans rat.

LE
96hr
FD

W
FD
96hr

Figures 3.16 and 3.17

Figure 3.16 The stomach of a Long-Evans rat after 96 hr of shock showing severe rumenal ulceration.

Figure 3.17 The stomach of a Wistar rat after 96 hr of shock. Note that the rumenal ulceration in this condition was less severe in the Wistar rat than in the Long-Evans rat.

LE
96h
5h

WT
5h
96h

stressors used (Table 3.3). These differences were particularly evident in the amount of food remaining in the stomach ($p < .0001$), rumenal ulcer severity ($p < .0013$), rumenal pH ($p < .0001$) and glandular pH ($p < .0005$). Restraint stress produced more frequent ulceration than the other treatments (Table 3.2), and produced significantly more glandular ulceration than food deprivation ($p < .001$; Scheffe's S method). Treatments were not significantly different with respect to rumenal ulceration. The amount of food remaining in the stomach did not differentiate among stress treatments. However, less food remained in the stomachs of all rats in all experimental conditions than in the stomachs of control rats.

The strain main effect indicated multivariate significance (Table 3.4), particularly with respect to glandular ulceration ($p < .0003$). Reference to Table 3.2 shows that Sprague-Dawley rats developed the most pathology in the body of the stomach. Scheffe's S method confirmed that more glandular ulcers occurred in Sprague-Dawley rats than in the Long-Evans ($p < .05$) or Wistar ($p < .003$) strains.

Glandular pathology was not uniform over time (Table 3.5), but decreased as stress duration increased ($p < .003$). Rumenal ulceration increased over time ($p < .0001$) and the rumen became less acidic across testing periods ($p < .08$).

TABLE 3.3

SUMMARY OF ANALYSIS OF VARIANCE[1] OF THE TREATMENT MAIN EFFECT

Variable	MS	F[2]	P
Amount of food left in stomach	70.61	395.16	.0001
Rumenal ulcer severity	2.58	5.45	.0013
Rumenal pH	7.59	8.02	.0001
Glandular ulcer severity	0.80	6.18	.0005
Glandular pH	0.68	1.75	.1592

[1]Multivariate $F_{15,519.38} = 41.04$; $p < .0001$.

[2]All univariate F values were tested at 3 and 192 df.

TABLE 3.4

SUMMARY OF ANALYSIS OF VARIANCE[1] OF THE STRAIN MAIN EFFECT

Variable	MS	F[2]	P
Amount of food left in stomach	0.33	1.83	.1633
Rumenal ulcer severity	0.22	0.46	.6333
Rumenal pH	0.17	0.18	.8372
Glandular ulcer severity	1.14	8.79	.0003
Glandular pH	0.63	1.61	.2017

[1]Multivariate $F_{10,376} = 2.42$; $p < .0084$

[2]All univariate F values were tested at 2 and 192df.

TABLE 3.5

SUMMARY OF ANALYSIS OF VARIANCE[1] OF THE TIME MAIN EFFECT

Variable	MS	F[2]	P
Amount of food left in stomach	0.39	2.21	.0888
Rumenal ulcer severity	22.25	47.03	.0001
Rumenal pH	2.13	2.25	.0837
Glandular ulcer severity	0.63	4.82	.0029
Glandular pH	1.21	3.10	.0279

[1]Multivariate $F_{15,519.38} = 9.89$; $p < .0001$.

[2]All univariate F values were tested at 3 and 192 df.

Glandular acidity first increased and then decreased over time ($p < .03$), paralelling the decline in ulceration in this portion of the stomach. Trend analysis of the time main effect indicated that the increase in rumenal ulceration and the decrease in rumenal acidity were both linear ($p < .001$ and $p < .02$, respectively). Glandular ulceration and acidity both decreased in a linear fashion also, ($p < .0007$ and $p < .05$, respectively). A significant quadratic trend was found for the amount of food remaining in the stomach ($p < .05$).

Differences among stress treatments were not uniform across the three strains of rats used (Table 3.6), particularly with respect to the amount of food remaining in the stomach ($p < .0001$) and glandular ulcer severity ($p < .0004$). Figures 3.18 and 3.19 illustrate the treatment x strain interaction for amount of food left and for glandular ulceration, respectively. Note that restraint produced maximal ulceration in the Sprague-Dawley strain, while the three stressors produced few differences in glandular ulcers between Long-Evans and Wistar rats.

Treatment conditions produced non-homogeneous effects across the four stress durations used in this study (Table 3.7). Rumenal ulceration produced by restraint, shock, and food deprivation increased over time ($p < .005$) as shown in Figure 3.20, while restraint-induced glandular ulceration

TABLE 3.6

SUMMARY OF ANALYSIS OF VARIANCE[1] OF THE TREATMENT X STRAIN INTERACTION

Variable	MS	F[2]	P
Amount of food left in stomach	2.21	12.35	.0001
Rumenal ulcer severity	0.69	1.45	.1967
Rumenal pH	0.73	0.77	.5909
Glandular ulcer severity	0.56	4.33	.0004
Glandular pH	0.42	1.08	.3737

[1]Multivariate $F_{30,754} = 3.78$; $p < .0001$

[2]All univariate F values were tested at 6 and 192 df.

Figure 3.18

Figure 3.18 Mean amount of food remaining in the stomach in the Sprague-Dawley, Long-Evans, and Wistar strains following three stress treatments: restraint, food deprivation and shock.

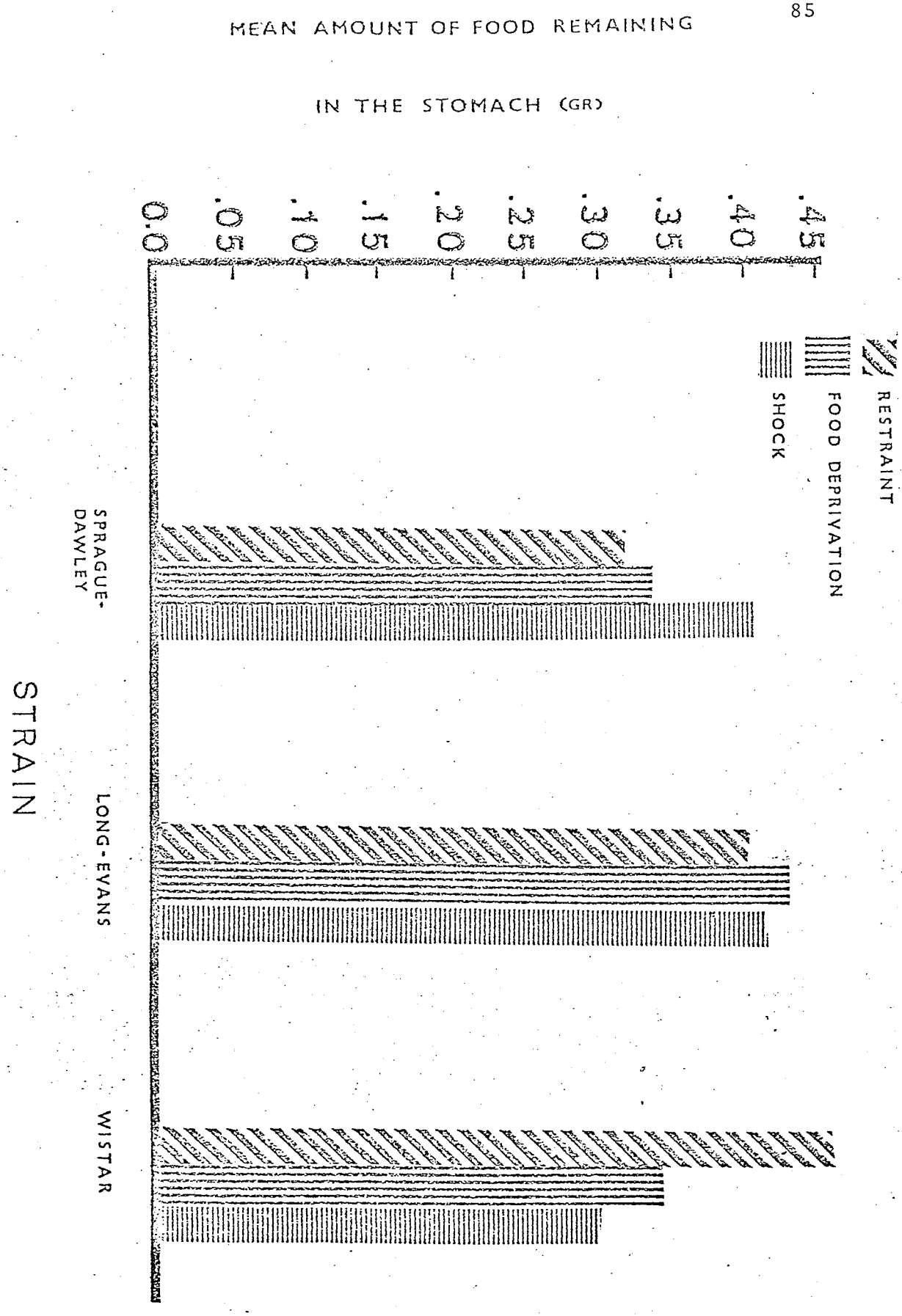

MEAN AMOUNT OF FOOD REMAINING
IN THE STOMACH (GR)
0.0
.05
.10
.15
.20
.25
.30
.35
.40
.45
RESTRAINT
FOOD DEPRIVATION
SHOCK
SPRAGUE-DAWLEY
LONG-EVANS
WISTAR
STRAIN

Figure 3.19

Figure 3.19 Mean glandular ulcer severity in the Sprague-Dawley, Long-Evans, and Wistar strains following restraint, shock, or food deprivation. Note the extreme susceptibility of Sprague-Dawley rats to restraint stress.

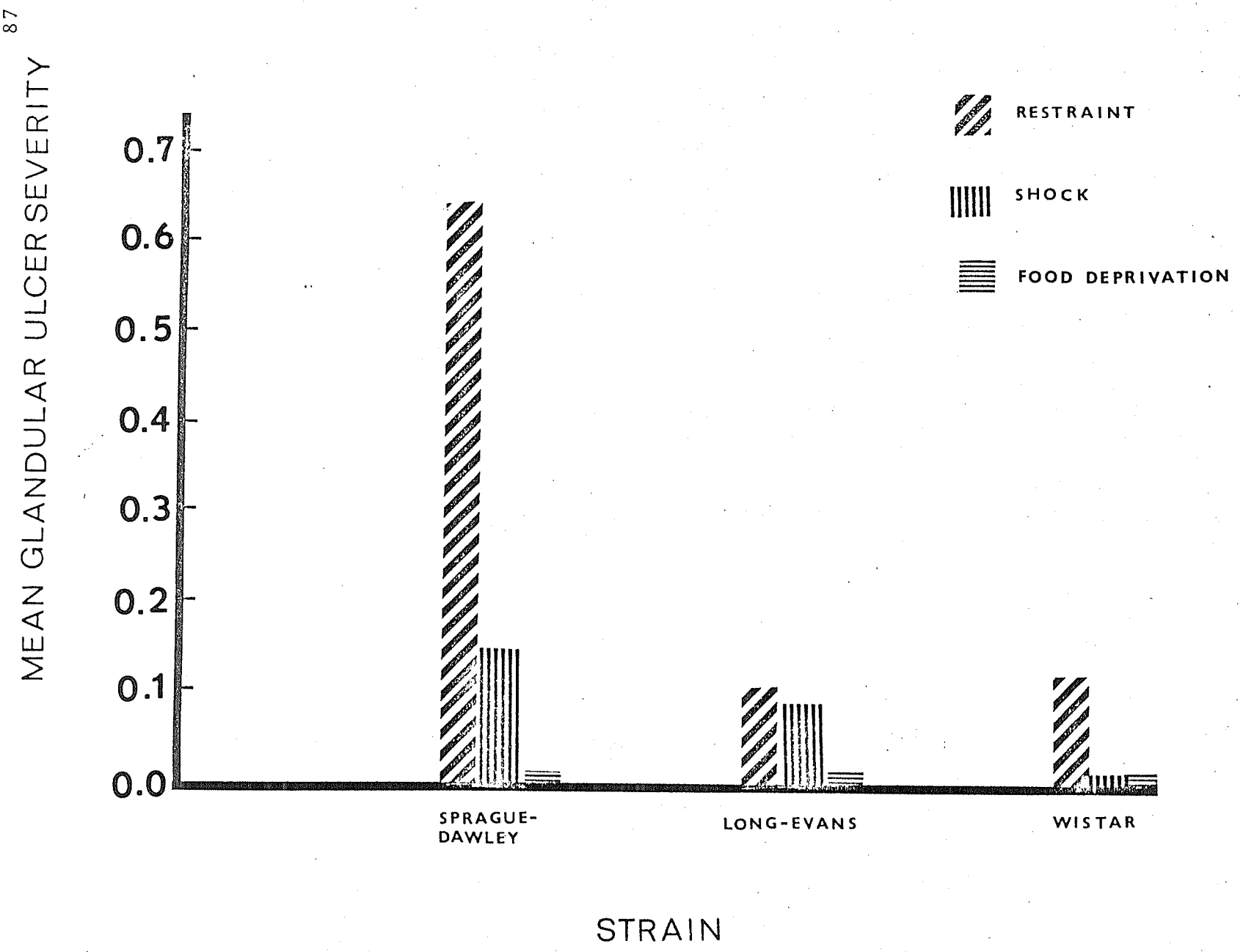

MEAN GLANDULAR ULCER SEVERITY
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
RESTRAINT
SHOCK
FOOD DEPRIVATION
SPRAGUE-DAWLEY
LONG-EVANS
WISTAR
STRAIN

TABLE 3.7

SUMMARY OF ANALYSIS OF VARIANCE[1] OF THE TREATMENT X TIME INTERACTION

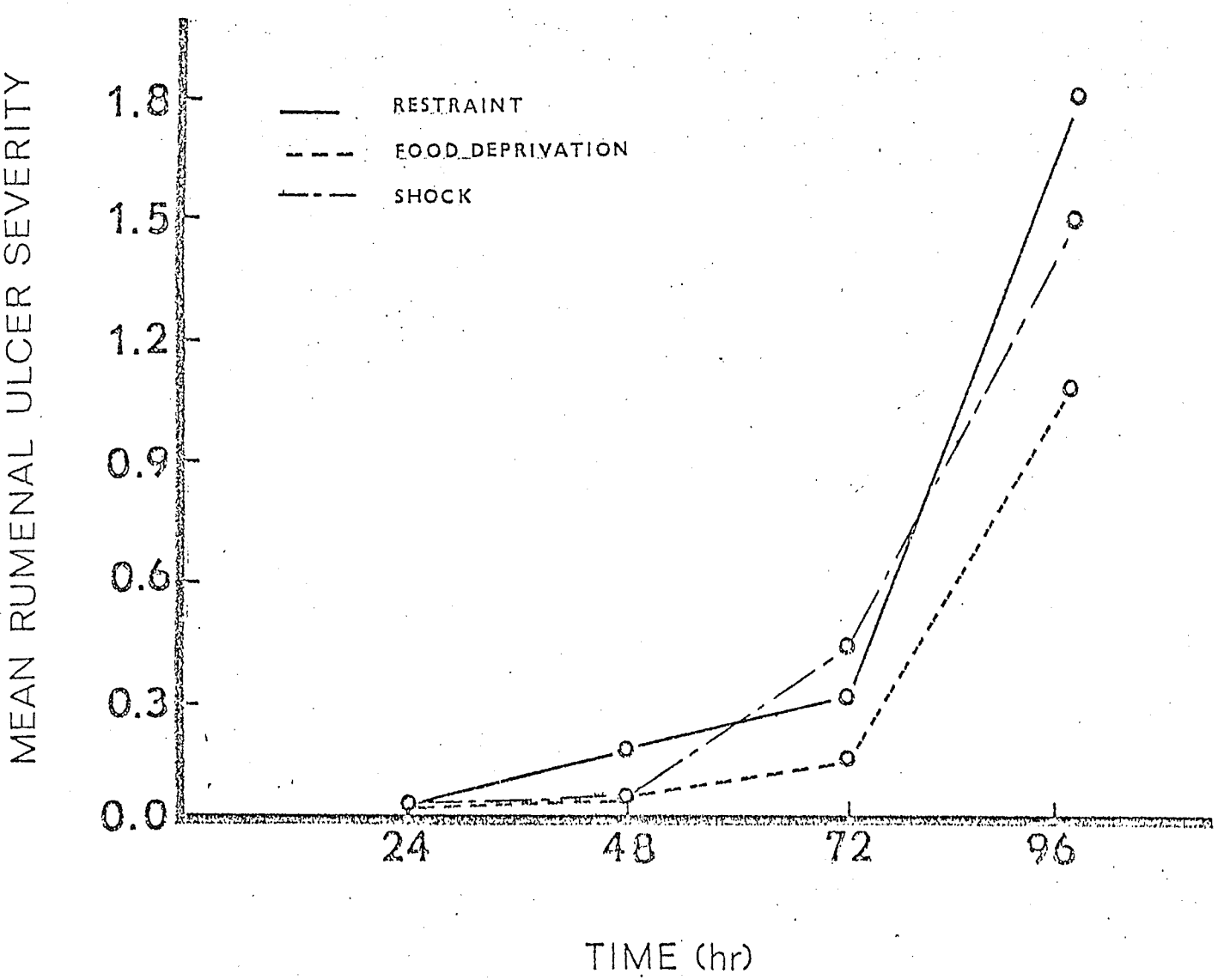
Variable	MS	F[2]	P
Amount of food left in stomach	0.54	3.03	.0021
Rumenal ulcer severity	1.30	2.76	.0048
Rumenal pH	0.63	0.66	.7420
Glandular ulcer severity	0.29	2.24	.0212
Glandular pH	0.48	1.23	.2804

[1]Multivariate $F_{45,844.07} = 2.04$; $p < .0001$

[2]All univariate F values were tested at 9 and 192df.

Figure 3.20

Figure 3.20 Mean rumenal ulcer severity over time in the restraint, shock, and food deprivation treatments. Ulceration increased over time in all treatments.


MEAN RUMENAL ULCER SEVERITY
1.8
1.5
1.2
0.9
0.6
0.3
0.0
RESTRAINT
FOOD_DEPRIVATION
SHOCK
24
48
72
96
TIME (hr)

decreased over time ($p < .02$) as shown in Figure 3.21.

Rats of different strains ulcerated differentially across the four stress durations of the present study (Table 3.8). This was particularly evident with respect to glandular ulceration ($p < .0004$) in Sprague-Dawley rats (Figure 3.22). Note the large discrepancy in glandular ulceration among strains at 24 hr which gradually decreased until 72 hr of treatment.

Analysis of the treatment x strain x time interaction (Table 3.9) confirmed that Sprague-Dawley rats were the most ulcer susceptible strain (Figure 3.23) and that restraint was the most ulcerogenic stressor,particularly with respect to glandular ulceration ($p < .0004$).

Figure 3.21

Figure 3.21 Mean glandular ulceration over time in the restraint, shock, and food deprivation treatments. Note that restraint produced the most pathology in the body of the stomach.

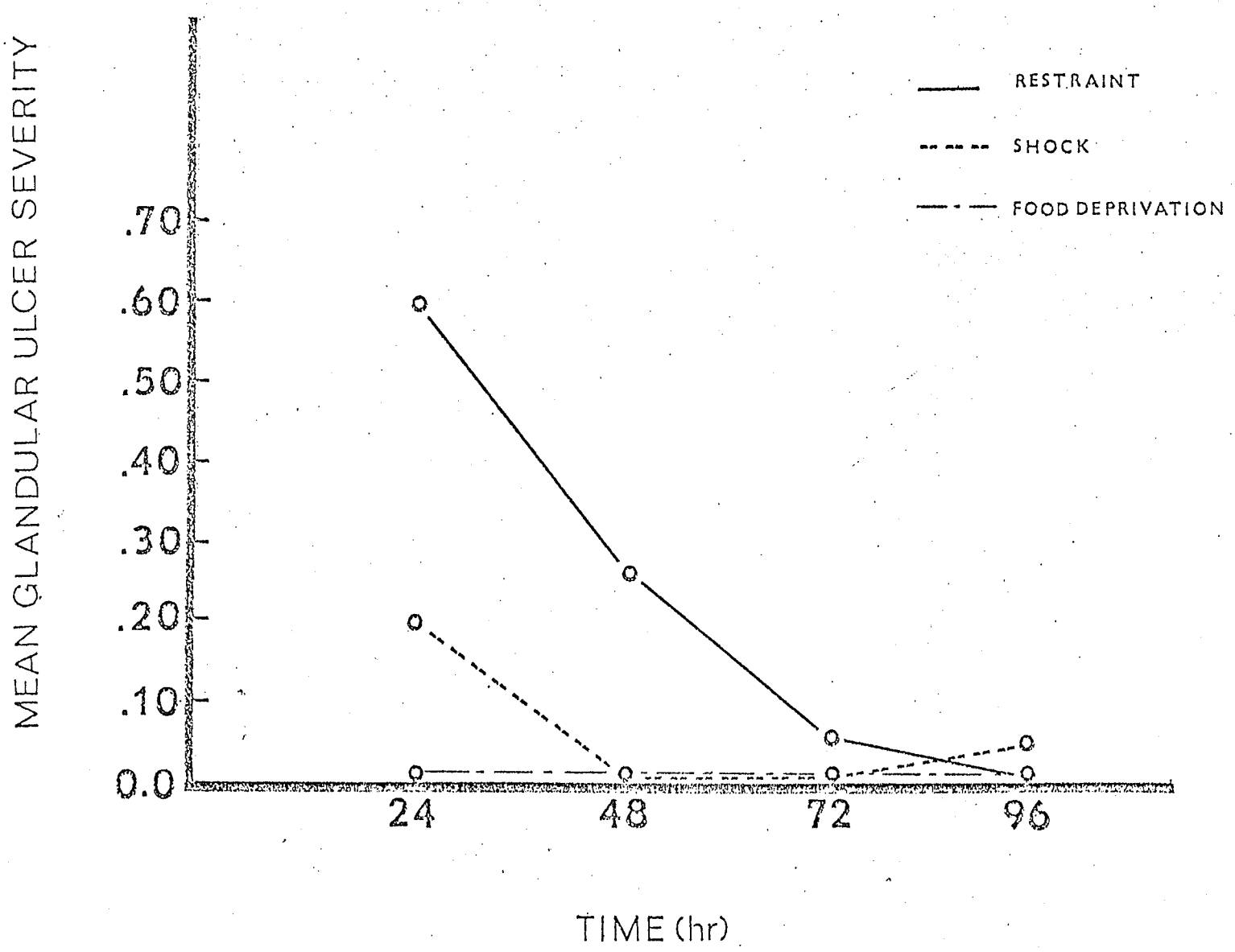

MEAN GLANDULAR ULCER SEVERITY
.70
.60
.50
.40
.30
.20
.10
0.0
24
48
72
96
TIME (hr)
RESTRAINT
SHOCK
FOOD DEPRIVATION

TABLE 3.8

SUMMARY OF ANALYSIS OF VARIANCE[1] OF THE STRAIN X TIME INTERACTION

Variable	MS	F[2]	p
Amount of food left in stomach	0.41	2.29	.0369
Rumenal ulcer severity	0.18	0.38	.8938
Rumenal pH	1.73	1.82	.0962
Glandular ulcer severity	0.58	4.46	.0004
Glandular pH	0.59	1.50	.1811

[1]Multivariate $F_{30,754} = 2.02$; $p < .0011$

[2]All univariate F values were tested at 6 and 192 df

Figure 3.22

Figure 3.22 Mean glandular ulceration over time in Sprague-Dawley, Long-Evans, and Wistar rats. Sprague-Dawley rats were generally most ulcerogenic, particularly at the earlier stress durations.

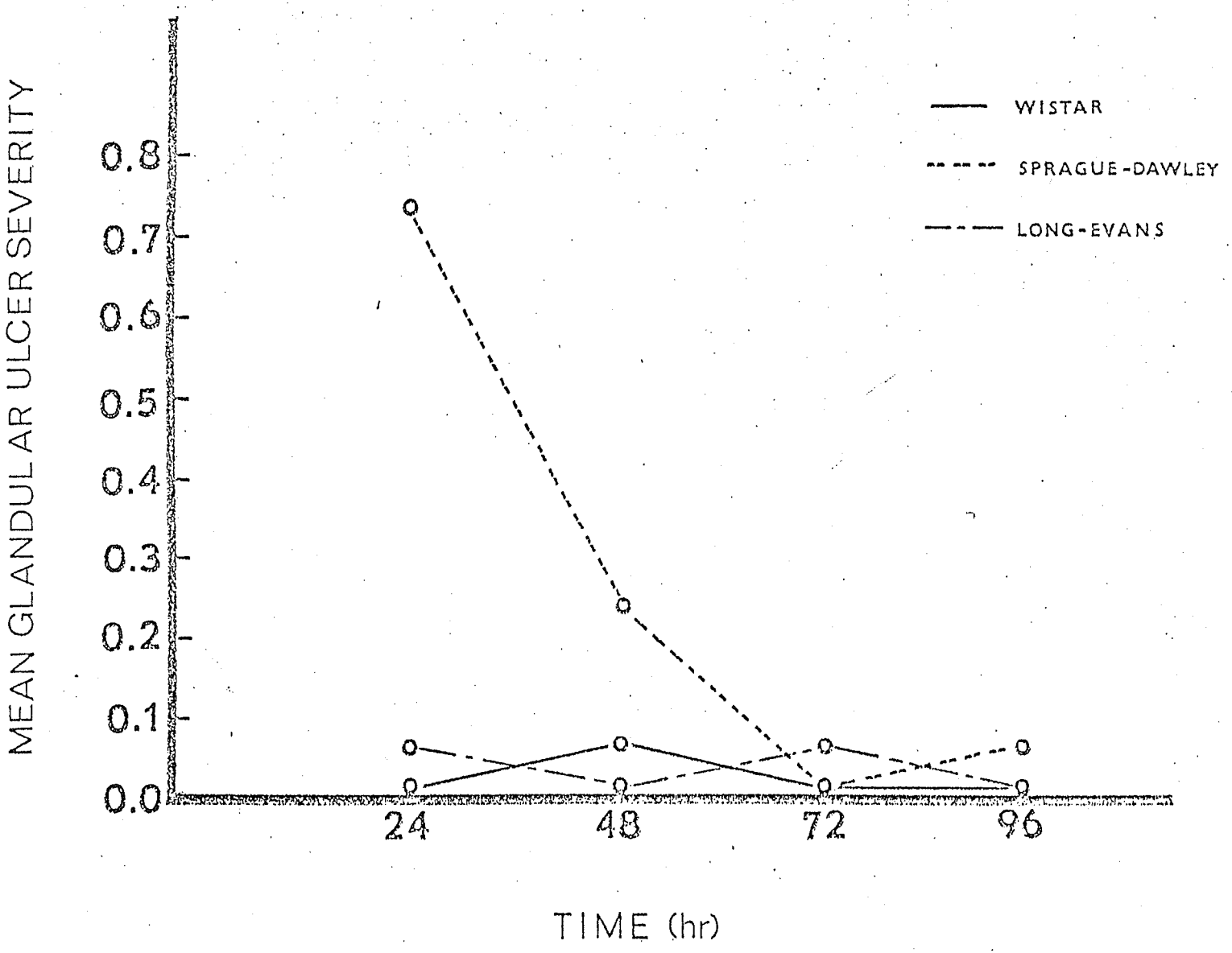

MEAN GLANDULAR ULCER SEVERITY
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
24
48
72
96
TIME (hr)
WISTAR
SPRAGUE-DAWLEY
LONG-EVANS

TABLE 3.9

SUMMARY OF ANALYSIS OF VARIANCE[1] OF THE TREATMENT X STRAIN X TIME INTERACTION

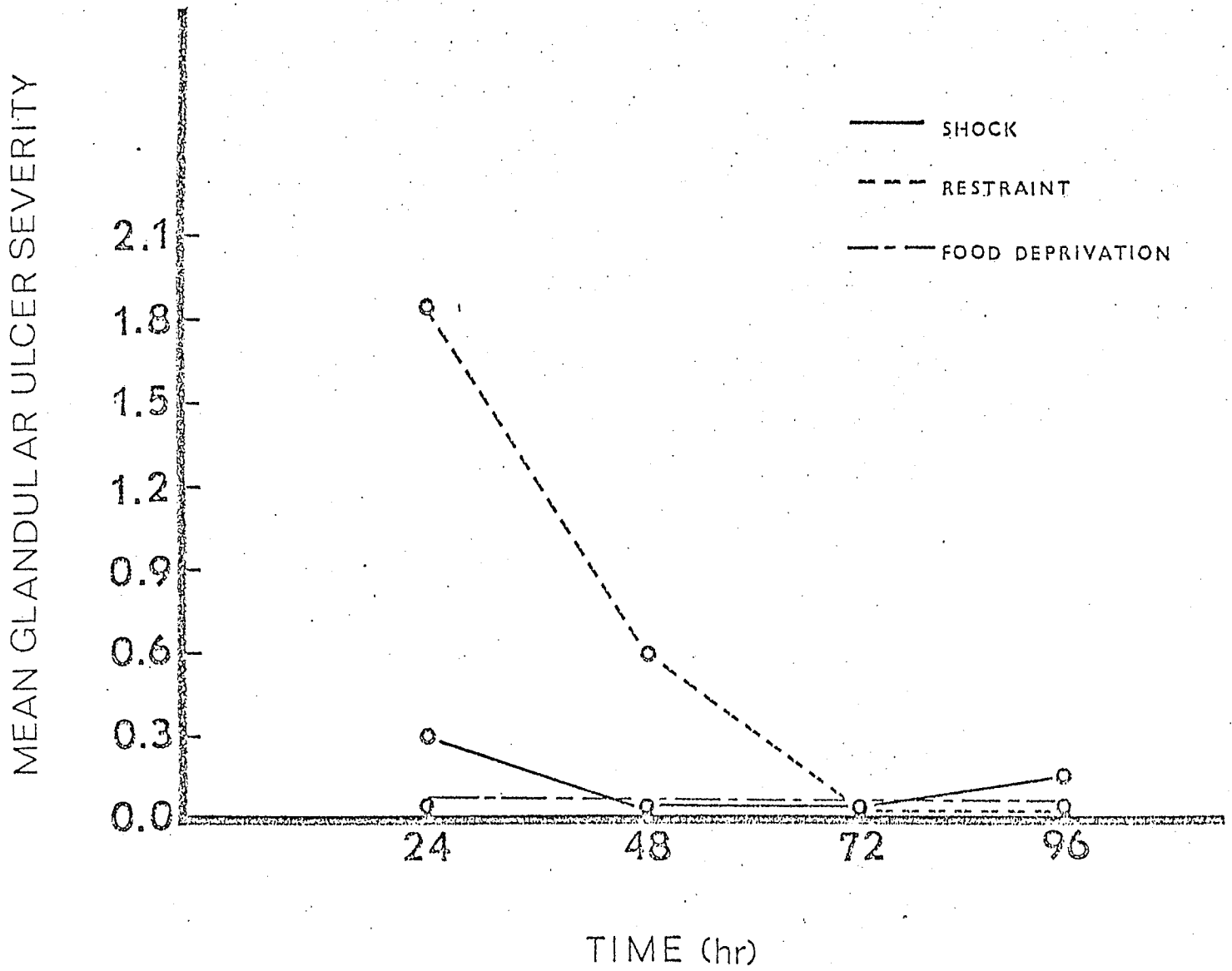
Variable	MS	F[2]	P
Amount of food left in stomach	0.30	1.68	.0466
Rumenal ulcer severity	0.55	1.15	.3042
Rumenal pH	1.11	1.17	.2870
Glandular ulcer severity	0.35	2.73	.0004
Glandular pH	0.48	1.22	.2464

[1]Multivariate $F_{90,916.55} = 1.59$; $p < .0007$

[2]All univariate F values were tested at 18 and 192 df

Figure 3.23

Figure 3.23 Mean glandular ulcer severity in Sprague-Dawley rats over time in restraint, shock, and food deprivation treatments. Note how Sprague-Dawley rats were most susceptible to restraint stress.


MEAN GLANDULAR ULCER SEVERITY
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0.0
24
48
72
96
TIME (hr)
SHOCK
RESTRAINT
FOOD DEPRIVATION

Discussion

1. With respect to the treatment effect, it is clear from Table 3.2 that restraint was the most ulcerogenic procedure. As in Experiment 1, the shock procedure produced a low incidence and severity of glandular ulceration. The rumenal ulcers observed in this condition and in the others, can be attributed to the prolonged starvation common to all conditions. It is interesting that food deprivation alone resulted in fewer rumenal ulcers than did either of the two stressors combined with food deprivation. It may be that when animals are subjected to food deprivation and another stressor, the stomach empties faster than under the stress of food deprivation alone, leaving more time for the gastric secretions to exert their corrosive action on the empty, unprotected stomach.

2. Gastric pathology increased over time. This was true for acidity in both portions of the stomach and especially for rumenal ulceration. In terms of the trends of these increases, both rumenal and glandular acidity increased in a linear fashion over time (that is, a decrease in pH value), rumenal ulceration increased linearly, while amount of food remaining in the stomach displayed a quadratic trend over time. The nature of the trend for gastric emptying deserves consideration. Across both strains and treatments,

the stomach contents decreased until approximately 48 hr and then increased until 96 hr. At first, this result seems inconceivable since all rats were food deprived throughout the entire experiment. This inconsistent result may be due to either an experimental artifact in the measurement of the stomach contents or to physiological changes occurring during the more chronic stress durations. The amount of food remaining in the stomach was measured by weighing the freshly excised stomach, then washing it of its entire contents, drying it, and then weighing it again. The difference between the two weights was used as a measure of food remaining in the stomach. Until 48 hr of treatment, this measurement was probably accurate, however, after this time, it appears that gastric secretion increased markedly, such that by 72 hr and 96 hr of treatment, the measure of food remaining in the stomach was being artificially increased by the increased volume of gastric secretion. Therefore, any remaining food may long since have been cleared from the stomach, but the volume of secretion had increased, thereby increasing the weight of the gastric contents. A more refined measure of food remaining in the rat stomach is clearly required.

3. It is evident from Table 3.2 that Sprague-Dawley rats were the most ulcer-susceptible strain used in

this experiment; particularly with respect to glandular ulcers. Long-Evans and Wistar rats were equally but less susceptible to ulceration. This data is not consistent with the findings of previous research examining strain differences in ulcer susceptibility. Ader, Beels, and Tatum (1960) used restraint and found that Sprague-Dawley rats were most ulcer-resistant, while Wistar rats developed the most ulceration. Opposite findings were reported by Sawrey and Long (1962) using conflict. In this study, both males and females of the Wistar strain developed less ulceration than Long-Evans rats of both sexes. Using a more chronic conflict situation, Paré (1972) found that Long-Evans rats were most ulcer-susceptible and Wistar rats most resistant to conflict-induced stomach pathology. Paré suggested that experimental procedural differences (e.g., age of rats, type of stressor used, length of food deprivation) could account for the inconsistent strain finding across studies. For conflict situations, (Sawrey and Long, 1962; Paré, 1972) it seems that Long-Evans rats are most susceptible to ulcers. When different stressors are used, different strain effects might reasonably be expected as in the present experiment. Within a particular stressor, consistent strain differences should occur (assuming procedural similarities), however, across stressors, few consistent findings are seen. It

appears that ulcer susceptibility in different strains of rats is a treatment (stressor) - specific phenomenon.

4. The treatment x time interaction suggested that treatments exerted differential effects at different time periods (Figures 3.20 and 3.21), with respect to both rumenal and glandular ulceration. In general, glandular ulcers were most severe at 24 hr and decreased over time, while rumenal ulcers generally increased over time, particularly for the restraint condition. That glandular ulcers occurred with maximum severity at 24 hr can be attributed to the strain x time interaction (Figure 3.22) and the treatment x strain interaction (Figure 3.19). It is clear from these figures that Sprague-Dawley rats were maximally sensitive to restraint at 24 hr and 48 hr of treatment, while few differences across time or among treatments were observed for other strains, in terms of glandular ulceration. The results of this study suggest that for a given treatment, results could be biased either for or against a hypothesis depending on whether an unusually susceptible or unusually resistant strain of rat was chosen. Reference to strain differences should be made within the bounds of research using a particular stressor rather than generalizing between stressors, since it appears that strain differences in ulceration are stressor-specific.

The findings of the present study are consistent with those of previous reports (Mikhail, 1966; Paré and Temple, 1973) in that rumenal ulcers occurred during prolonged food deprivation, while glandular ulcers occur in response to other forms of stress.

CHAPTER IV

THE RELATIVE CONTRIBUTIONS OF FOOD DEPRIVATION AND STRESS TO EXPERIMENTAL GASTRIC ULCERS

The purpose of Experiment III was to selectively block the action of gastric acidity in the following conditions: (a) the food deprivation treatment prior to stress, (b) the stress treatment alone, (c) both the food deprivation and stress treatment or (d) neither treatment. Considerable evidence has accumulated which indicates that gastric acid is critically involved in ulcer formation. One mechanism for the development of stress ulcer in the rat has been postulated as follows (Levine and Senay, 1970): stress ——→ increased histidine decarboxylase ——→ increased histamine ——→ (increased gastric acidity?) ——→ increased incidence and severity of ulcers.

The "missing link" in this sequence is that of gastric acid. It is known that histamine mediates gastric acid secretion in the rat (Code, 1965; Levine, 1965; Levine and Senay, 1968), however, Levine and Senay (1970) noted that "whether or not stress is associated with increased gastric acid secretion in the rat is not generally agreed upon (p. 61)". These authors examined the pH of the stomachs of restrained rats and found a strong correlation between intragastric acidity and glandular ulcer development. Glavin and Mikhail

(1975) obtained a similar correlation in the rumenal portion of the stomach and concluded that gastric acid was a significant pathogenic agent in ulcer formation.

Additional support for the role of acid in the development of ulcers arises from a study by Levine and Senay (1970). Rats were food deprived for 24 hours and then restrained and placed in a cold environment (4^{o} - 7^{o}C) for two hours. Prior to restraint, one group of animals was given a non-absorbable antacid (Basaljel). The results showed that pretreatment with antacid was associated with a significant reduction in ulcer incidence. Despite antacid pretreatment, animals which had low intragastric pH had a higher incidence of ulceration. When the pH of the stomach rose, ulcer incidence declined. The authors concluded that the protective effect of the antacid was due to the neutralization of gastric acidity.

The choice of an appropriate antacid, however, is not an arbitrary one. Harvey (1970) defined a gastric antacid as a compound which neutralized or removed acid from the gastric contents. Clark (1965) suggested that an "ideal" antacid would raise the pH (in man) from the normal range of 1 to 2 up to 3 to 4. An increase in alkalinity of this extent corresponds to the disappearance of free acid from the gastric contents. The corrosive effects of both the

gastric juice (HCl) and pepsin are thereby reduced. Clark also noted that both systemic (some absorption of the antacid ions by plasma) and non-systemic (non-absorbable) antacids neutralize gastric acid, however, Goth (1970) suggested that systemic antacids should be avoided due to the potential development of systemic alkalosis (increased CO_2 content and increased plasma pH leading to renal insufficiency and death). Harvey (1970) suggested aluminum hydroxide as a suitable antacid since it is known to raise stomach pH to 4, it is non-systemic, and it neutralizes gastric acid for a relatively long time period. Two commonly used antacids (Calcium carbonate and sodium bicarbonate) were not used in this study since Harvey (1970) suggested that the liberation of carbon dioxide by these compounds may cause stomach distention which is particularly dangerous if a gastric ulcer near perforation is present in the stomach.

If ulceration produced by unpredictable-uncontrollable shock or by restraint is primarily due to the food deprivation pretreatment, then the administration of a gastric antacid only during the food deprivation period should attenuate ulcer formation. If the stress procedure is the primary pathogen, then administering an antacid only during the stress phase should retard ulcer formation. Experiment III examined these predictions.

Method

Subjects. Subjects were 50 male rats of the Sprague-Dawley strain. All rats were approximately 100 days of age at the start of the experiment and were randomly assigned to treatment conditions. The mean body weight was 300 gr. (S.D. = 10 gr).

Apparatus. Restraint (72 hr) was the stress treatment used in this experiment. For this procedure, the same apparatus as described in Experiment II (Figure 3.1) was used. Amphojel[1] (Aluminum hydroxide gel, U.S.P.) was used as a gastric antacid. All rats were killed with ether overdose (Fisher). Stomach pH measurements were determined with a flat surface pH electrode as in the first two experiments.

Procedure. Four groups of rats were used. Rats in Group 1 (n = 10) were deprived of food for 72 hours and were then restrained for 72 hours. Group 2 (n = 10) received the same treatment as Group 1, but was given aluminum hydroxide gel (300 mg in 5 mL) by force feeding, once every 24 hours during both the deprivation and restraint periods. Group 3 (n = 10) was given the same treatment as Groups 1 and 2 but was given aluminum hydroxide only during the

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[1]Wyeth Laboratories Inc.

deprivation period. Group 4 (n = 10) received the same treatment as Group 1, 2, and 3 but received aluminum hydroxide only during the restraint period. Water was available ad libitum to all groups during the experiment. All rats were killed with ether overdose and their stomachs examined for ulceration and pH as in the first two experiments. An additional untreated control group of ten rats was maintained in the home cage colony room. Rats in this group were killed at the same time as the experimental subjects and their stomachs were examined in the same manner.

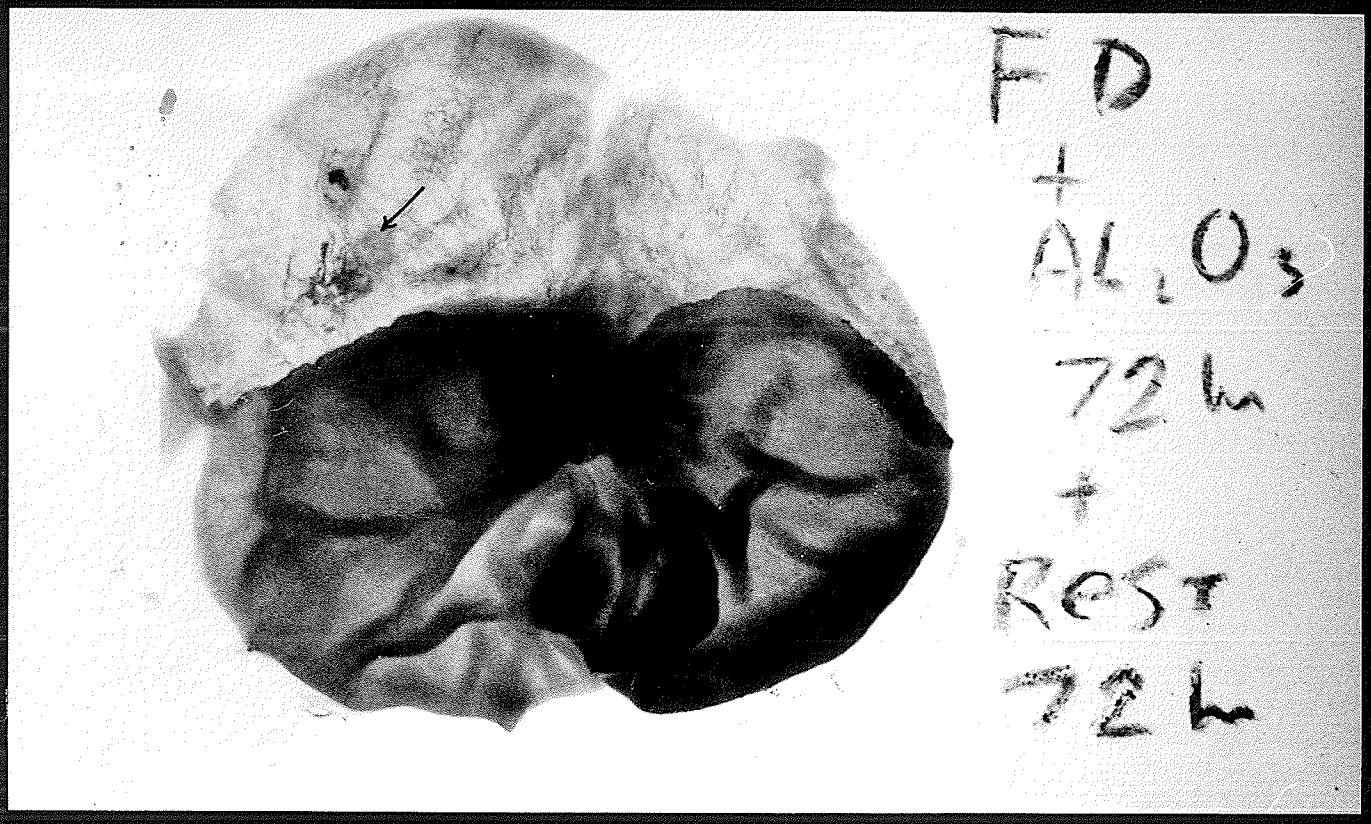
Results

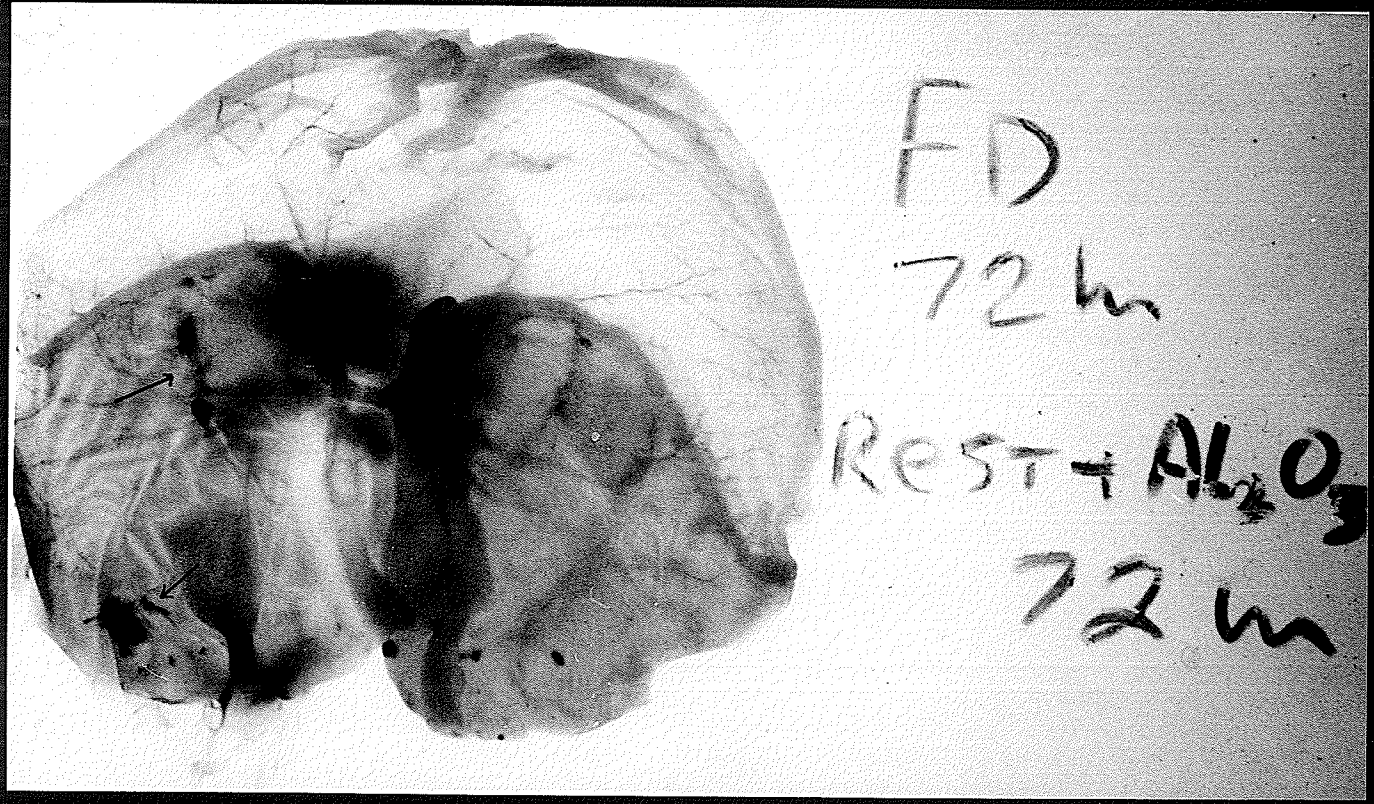
A comparison of Figures 4.1 and 4.3 with Figures 4.2 and 4.4 clearly indicates that when aluminum hydroxide was administered during the food deprivation period regardless of subsequent drug treatment, ulceration was markedly reduced. Scheffe's S method confirmed this result for rumenal ulceration ($p < .001$) and for glandular ulceration ($p < .001$), when the groups were combined according to drug administration during food deprivation or during restraint. It is interesting to note that all indices of gastric pathology were less severe in rats given aluminum hydroxide during the food deprivation period (Table 4.1). Table 4.1 also indicates that animals which did not receive the drug during the food deprivation period, had significantly more severe rumenal and glandular ulcers and significantly higher acidity (lower pH) in both portions of the stomach. Drug administration produced significant differences in rumenal ulcer severity ($p < .0001$), rumenal pH ($p < .0001$), glandular ulcer severity ($p < .0001$) and glandular pH ($p < .04$) as shown in Table 4.2, confirming the main finding that blocking acidity changes during food deprivation, significantly reduced stress-induced stomach pathology.

Figures 4.1 and 4.2

Figure 4.1 The stomach of a rat given aluminum hydroxide during the food deprivation period only. Note the mild rumenal ulcers and the absence of glandular pathology.

Figure 4.2 The stomach of a rat given aluminum hydroxide only during the restraint period. Note the severe glandular ulceration.


FD
+
Al_2O_3
72 h
+
REST
72 h

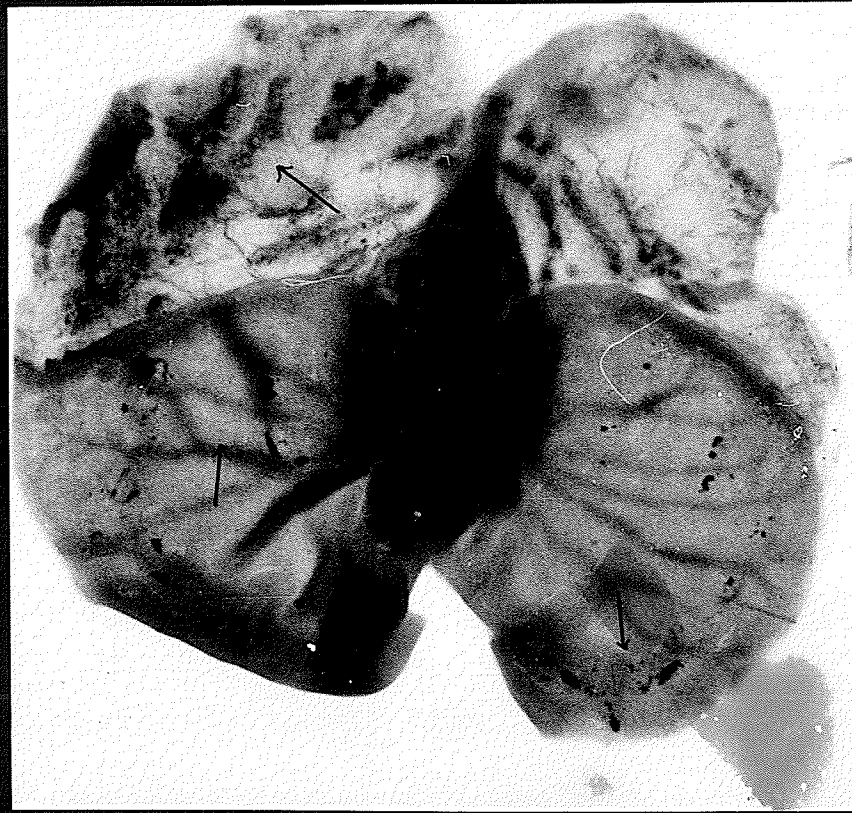

FD
72 h
REST + Al_2O_3
72 h

Figures 4.3 and 4.4

Figure 4.3 The stomach of a rat given aluminum hydroxide during both the food deprivation and restraint periods. Note the presence of only a small number of mildly severe glandular ulcers.

Figure 4.4 The stomach of a rat subjected to both food deprivation and restraint without aluminum hydroxide administration at any time. Note the severe ulceration in both portions of the stomach.

FD +
Al_2O_3
72 hr
Rest +
Al_2O_3
72 hr



FD
72 hr
+
REST
72 hr

TABLE 4.1

MEAN pH, RUMENAL AND GLANDULAR ULCER SEVERITY AND ULCER INCIDENCE IN THE EXPERIMENTAL AND CONTROL GROUPS

Group	Drug Administration Period	Rumenal ulcer incidence	Glandular ulcer incidence
Experimental 1	During food administration	2/10	3/10
Experimental 2	During restraint	9/10	10/10
Experimental 3	During both food deprivation and restraint	1/10	6/10
Experimental 4	During neither food deprivation nor restraint	10/10	9/10
Control	No stress or drug treatment	0/10	0/10

TABLE 4.1 (CONTINUED)

MEAN pH, RUMENAL AND GLANDULAR ULCER SEVERITY AND ULCER INCIDENCE IN THE EXPERIMENTAL AND CONTROL GROUPS

Group	Mean Rumenal ulcer severity	Mean Glandular ulcer severity	Mean rumenal pH	Mean glandular pH
Experimental 1	0.5	0.3	2.7	3.1
Experimental 2	1.1	2.0	2.7	3.9
Experimental 3	0.1	1.1	3.2	3.3
Experimental 4	2.8	1.8	2.3	3.0
Control	0.0	0.0	4.1	2.5

TABLE 4.2

SUMMARY OF ANALYSIS OF VARIANCE[1] OF DRUG ADMINISTRATION PERIOD

Variable	MS	F[2]	P
Rumenal ulcer severity	13.15	22.85	.0001
Rumenal pH	4.69	8.29	.0001
Glandular ulcer severity	7.83	10.18	.0001
Glandular pH	2.72	2.83	.0354

[1]Multivariate $F_{16,128.9} = 9.79$; $p < .0001$.

[2]All univariate F values were tested at 4 and 45 df.

Discussion

The above mentioned results clearly implicate the food deprivation pre-treatment as a primary contributor to stress ulceration. Groups 2 and 4 which received aluminum hydroxide during food deprivation regardless of subsequent treatment, developed significantly less frequent and significantly less severe ulceration in both portions of the stomach. Groups 3 and 5 which did not receive the drug during food deprivation, developed frequent and severe rumenal and glandular ulcers. Thus, preventing or reducing the corrosive action of gastric acid during the food deprivation period prior to the application of a stressor, markedly reduces ulcer frequency and severity. That this preventative effect is not due to the administration of the drug during restraint, is evident in Table 4.1. If the groups are recombined according to administration of the drug during restraint, widely discrepant results occur. Groups 3 and 4 both received the drug during restraint, while groups 2 and 5 did not. The discrepant ulcer results observed in this combination can be attributed to whether or not the drug was administered during the food deprivation period. Evidence that the antacid drug exerted its effects on stomach acidity can be obtained from the glandular pH data. Note that the pH of the body of the stomach in groups

2, 3, and 4 which received the drug at some time during the experiment was higher (that is, more basic) than that in groups 1 and 5 which did not receive the drug at any time. It appears that food deprivation is most effective at the initial 24 - 48 hr periods than at later times in bringing about pathophysiological changes (hyperacidity) which are a pre-requisite for the induction of ulcers by some experimental ulcer procedures. The data from the present study suggest that food deprivation produces increases in stomach acidity which, if prevented, reduce ulceration ultimately resulting from a subsequent stressor. If such acidity changes are unaltered, stress-induced gastric pathology is accentuated.

CHAPTER V

VITAMIN C AND THE PREVENTION OF STARVATION INDUCED GASTRIC ULCERS

L-ascorbic acid (vitamin C) is a water soluble vitamin involved in protein and amino acid metabolism and has also been prescribed to detoxify poisons and to combat infectious diseases (Rosenberg, 1945). Vitamin C is also essential for the formation of normal connective tissue (collagen). Clinical manifestations of ascorbic acid deficiency include failure to form new collagen, weakening of capillaries, reduced resistance to infection and defective wound healing (Scrimshaw, 1971). Johnson (1949) noted that human patients with ulcers metabolized 20% more ascorbic acid than normal subjects. Schlegal, Pipkin, Nishimura and Shultz (1970) found that bladder carcinoma could be prevented by l-ascorbic acid. Recently, Raineri and Weisburger (1974) reported that ascorbic acid significantly reduced the incidence of gastric cancer in rats. Given that ascorbic acid is essential in maintaining tissue integrity and given that gastric ulcers are characterized by tissue degeneration in the stomach mucosa, it is possible that large doses of vitamin C could retard or prevent the formation of ulceration. Cheney and Rudrud (1974) examined the effects of vitamin C on starvation-induced gastric ulcers

in rats. Animals were deprived of food for 47 hours per day for three days, being allowed access to food for one hour per day throughout the experiment. The results indicated that rats which received l-ascorbic acid in their drinking water prior to and during starvation developed significantly less severe glandular ulceration than rats given either deactivated or no ascorbic acid. The food deprivation schedule used, however, suggests that these results are questionable. Rats in the Cheney at al. study had access to food for one hour per day or were given sufficient food to maintain them at 70% of their free-feeding weight. Consistent results from this laboratory have shown that total food deprivation for three days is the minimum treatment required to produce rumenal ulcers. Glandular ulceration as a function of food deprivation in the adult rat has rarely been observed (Paré and Temple, 1973; Glavin and Mikhail, 1975; 1976). The report of glandular ulcers in the Cheney at al. study is also unclear due to the inclusion of the vague term "an area of hemorrhage (p. 1)" as ulceration. Therefore, the purpose of experiment 4 was to examine the role of ascorbic acid in the attenuation of rumenal (and glandular) ulceration in the totally food deprived rat.

Method

Subjects. Subjects were 30 male rats of the

Sprague-Dawley strain. All rats were approximately 100 days of age at the start of the experiment and were randomly assigned to treatment conditions. The mean body weight was 300 gr (S.D. + 10 gr).

Apparatus. Subjects were maintained in standard Wahmann home cages throughout the experiment. Ascorbic acid (Fisher[1]) was used as an anti-ulcer agent. All rats were killed with ether overdose (Fisher) and stomach acidity was determined with a flat surface pH electrode as in the first three experiments.

Procedure. Two groups of rats were used. Group 1 (n=10) was totally deprived of food but not water for 96 hours. Group 2 (n=10) was given the same treatment as group 1 but was administered ascorbic acid orally (6 gr/200 ml water) during the deprivation period. Ascorbic acid intake was recorded daily at the same time (8:00 A.M.). All rats were killed with ether overdose and their stomachs examined for ulceration and pH as in the first two experiments. An additional untreated control group of 10 rats was maintained under home cage conditions and killed and examined at the same time and in the same manner as the experimental subjects.

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[1]Fisher Scientific Co., Cat. No. A-61 (Lot No. 733061).

Results

The administration of ascorbic acid to food deprived rats increased both rumenal ulcer severity and acidity (Table 5.1). Note the relatively mild ulceration in the stomach of a food deprived rat (Figure 5.1) compared to that of a rat given ascorbic acid during food deprivation (Figure 5.2). No glandular ulcers were observed in any subject, nor was glandular pH significantly different among the three groups. Multivariate analysis of variance of ascorbic acid administration indicated an overall difference among the groups (Table 5.2). Univariate analyses of variance confirmed that rumenal ulcer severity ($p < .01$) and rumenal pH ($p < .0001$) were significantly different among the three groups, although rumenal ulcer severity was not significantly higher in the vitamin C group than in the non-drug group (Tukey's HSD procedure).

TABLE 5.1

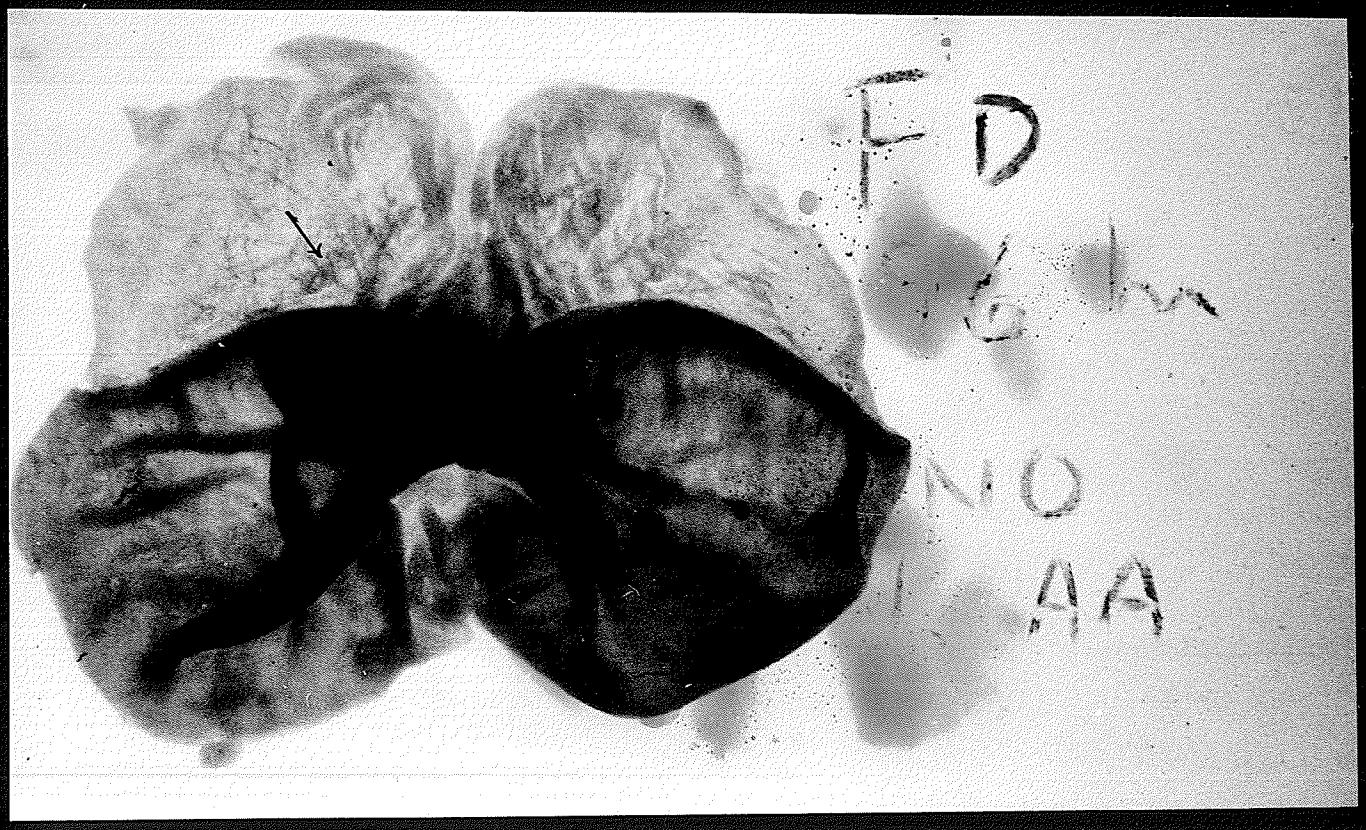
ULCER INCIDENCE MEAN RUMENAL ULCER SEVERITY AND MEAN RUMENAL AND GLANDULAR pH IN THE EXPERIMENTAL AND CONTROL GROUPS

Group	Rumenal ulcer incidence	Mean rumenal ulcer severity	Mean rumenal pH	Mean glandular pH
Food Deprivation	5/10	0.9	2.83	2.75
Food Deprivation + L-AA	7/10	1.3	2.49	2.89
Control	0/10	0.0	4.40	3.13

Figures 5.1 and 5.2

Figure 5.1 The stomach of a rat after 96 hr of food deprivation. No ascorbic acid was given during this time. Note the mild rumenal ulceration.

Figure 5.2 The stomach of a rat after 96 hr of food deprivation during which time ascorbic acid was administered. Note the increased severity of rumenal ulceration compared to the rat which did not receive ascorbic acid.


FD
NO
AA

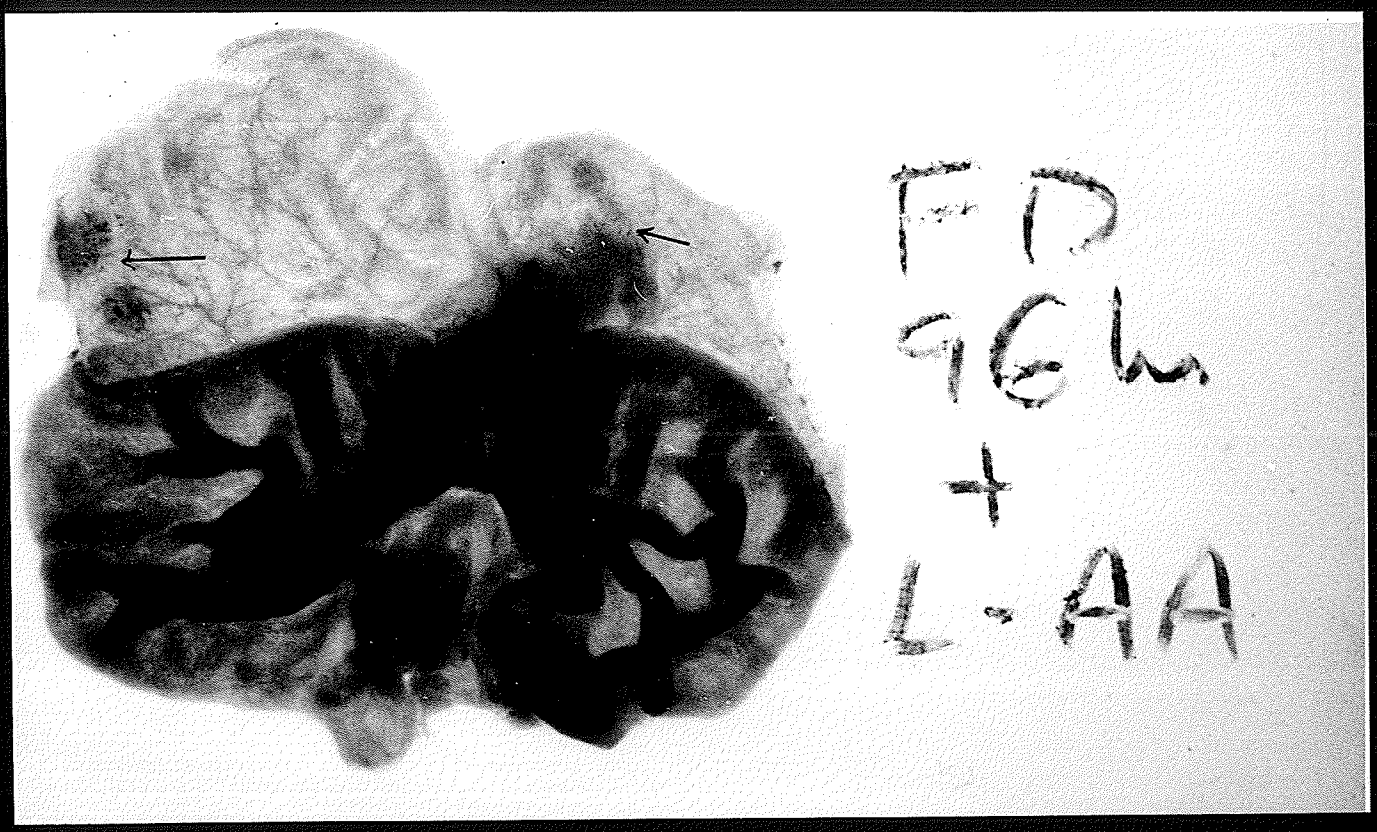

FD
96hr
+
L-AA

TABLE 5.2

SUMMARY OF ANALYSIS OF VARIANCE[1] OF ASCORBIC ACID ADMINISTRATION

Variable	MS	F[2]	P
Rumenal ulcer severity	4.43	5.20	.0123
Rumenal pH	10.38	14.61	.0001
Glandular pH	0.37	1.83	.1791

[1]Multivariate $F_{6,50} = 2.31$; $p < .0478$

[2]All univariate F values were tested at 2 and 27 df.

Discussion

As mentioned earlier, the purpose of this experiment was to replicate Cheney and Rudrud's (1974) finding that l-ascorbic acid (vitamin C) reduced starvation-induced glandular ulceration in rats, because glandular ulceration as a result of food deprivation has not been observed in our laboratory and only rarely in others (Paré and Temple, 1973), and because Cheney and Rudrud used a subtotal food deprivation regimen (47 hr or maintenance at 70% of their free-feeding weight).

The results of this study indicated that treatment with l-AA during starvation increased rumenal ulcer incidence and severity relative to rats which received no drug during food deprivation. Procedural differences between the present study and that of Cheney and Rudrud, however, do exist. Cheney at al. administered l-AA for eight days prior to the starvation period. This was not done in the present study, since the purpose of the experiment was to examine the effects of l-AA during the starvation period only.

In both studies, the l-AA was administered to food-deprived rats in a liquid form. Mikhail and Hirschberg (1972) have shown that liquid diets in the absence of bulky substances, can be ulcerogenic. It therefore seems that the absence of bulk in the food deprived rats may have

contributed to ulcer formation. This hypothesis is supported by the fact that only rumenal ulcers were observed in this study, and that the severity of ulcers in the 1-AA group was more severe than in the starved group. Ulcers in starved rats given liquid sucrose during food deprivation are also more severe than those seen in rats which are not given diets or drugs during the starvation period (Mikhail and Hirschberg, 1972; Glavin and Mikhail, 1975; Glavin and Mikhail, 1976).

The present writer has examined the stomachs of rats which were maintained at 70% of their free-feeding weight for up to eight months and found no sign of gastric pathology (Glavin and Hemingway, 1973). Only rarely have other researchers observed glandular ulcers in response to food deprivation and even in these exceptional cases, only mild ulceration was seen (Paré and Temple, 1973). Cheney's et al. production of severe glandular ulceration using a sub-total food deprivation regimen is clearly at variance with the bulk of the experimental ulcer literature and it is apparent that some unspecified variable (stressor) was operating in their study.

CHAPTER 6

SUMMARY AND CONCLUSIONS

What is mentioned below summarizes the conclusions of the following experiments.

1. Experiment 1. In this experiment, shock intensity was manipulated within a constant unpredictable-uncontrollable shock paradigm. The results suggested (a) that the physical effects of shock exert an ulcerogenic effect which is independent of the psychological factors associated with the delivery of such aversive stimulation; (b) that the ulcers which developed by Weiss' procedure are more likely attributable to the high shock intensity rather than the psychological variables of stress predictability and controllability; and (c) that the shock variable at a high intensity or even in the form of psychologically stressful procedures did not produce a reliably high incidence of ulceration.

2. Experiment 2. The effects of restraint, shock, and food deprivation on gastric emptying time and the development of gastric pathology in Sprague-Dawley, Long-Evans, and Wistar rats were examined in this study. On the whole, gastric pathology increased over time. This increase was particularly noticeable in the Sprague-Dawley rats and in the restraint stress condition with respect to glandular

ulceration. The pattern of interactions between strains and treatments suggests that strain differences in ulcer susceptibility were a treatment-specific phenomenon. The overall pattern of ulceration in Experiment 2 suggests that rumenal ulcers developed independently of psychological stress and primarily reflect the effects of starvation. Similar conclusions were reached by Mikhail (1966) and by Paré and Temple (1973). It was observed in Experiment 2 that when restraint or shock stress was added to food deprivation, the stomach emptied faster than under conditions of starvation alone. Rumenal ulcers were generally more severe in the latter case than in the former.

3. Experiment 3. Gastric acidity effects were selectively blocked during periods of food deprivation alone, restraint alone, or during both periods together, in order to assess the relative contributions of these treatments to ulcer formation. The results showed clearly that when the antacid drug (aluminum hydroxide) was administered during food deprivation, both rumenal and glandular ulcer incidence and severity were markedly reduced regardless of whether or not the drug was given further during restraint.

4. Experiment 4. The final experiment examined the role of l-ascorbic acid (vitamin C) in the prevention of starvation-induced ulceration. In a previous study (Cheney and Rudrud,

1974) it was found that glandular ulcers occurred in response to food deprivation and that the administration of vitamin C decreased the incidence and severity of such pathology. The results of Experiment 4 were not consistent with the above report. It has seldom been observed that glandular ulcers occur in response to food deprivation (Mikhail, 1966; Paré and Temple, 1973; Glavin and Mikhail, 1975). Since starvation produces ulcers confined almost exclusively to the rumen, it was not surprising to find that only rumenal pathology was observed in this experiment. Treatment with ascorbic acid increased the incidence and severity of rumenal ulceration relative to non-treated and control rats, but not significantly. It was suggested that procedural differences were responsible for these discrepant results.

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