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Effect of D-amphetamine, Guanethidine, Disulfiram, and Stress on Gastric Ulceration in the Rat

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EFFECT OF D-AMPHETAMINE, GUANETHIDINE, DISULFIRAM, AND STRESS

ON GASTRIC ULCERATION IN THE RAT

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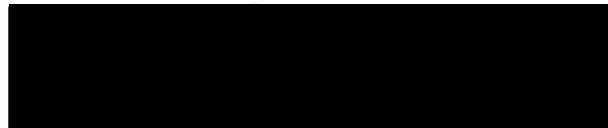Members   Michael W. Etkin, Ph.D.

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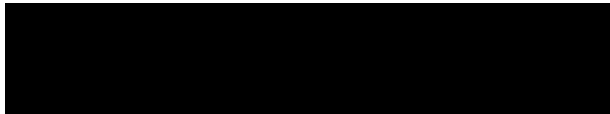

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
ANS	autonomic nervous system
C	centigrade
CA	catecholamine(s)
CNS	central nervous system
DA	dopamine
E	epinephrine
GAS	general adaption syndrome
IP	intraperitoneal
NE	norepinephrine
PSN	parasympathetic nervous system
SN	sympathetic nervous system

ABSTRACT

Albino rats were injected with various doses of d-amphetamine (.02 mg/kg-
9 mg/kg) and subjected to 4 hours restraint in a cold (+5°C) environment. Differ-
ential effects on ulceration were observed as a function of the d-amphetamine
dose level. Pretreatment with a .50 mg/kg injection of d-amphetamine signific-
antly inhibited ulceration over that of saline injected, control animals, while
a 9 mg/kg dose injection of the drug significantly facilitated it. Such results
were explained in terms of a model interaction between sympathetic and parasym-
pathetic nervous system activity, and the effect that such activity has on
gastric conditions conducive to ulceration.

A second experiment was conducted to further delineate the properties of
the proprosed theoretical model using drugs which were known to deplete norepi-
nephrine. Differential effects of disulfiram and guanethidine on ulceration
were observed and these results were discussed in reference to the theoretical
model. Alternative explanations for these results were also presented.

Experiment I

The use of the restraint technique has proven to be a consistently reliable method of producing gastric ulcers in the rat (Ader, 1967; Brodie & Hanson, 1960; Boles, 1970). It has been combined with various other factors for the purpose of investigating their effects on gastric ulceration (Levine & Senay, 1970; Bonfils and Lambling, 1963; Selye, Pierre & Cantin, 1962). One such factor under investigation has been the effect of adrenergic substances, especially amphetamine, on the development of ulcers. D-amphetamine is a potent sympathomimetic drug which has been reported to increase gross locomotor activity (Cole, 1967) and lower the behavioral and EEG thresholds for arousal in some species (Bradley & Elkes, 1957). Sines (1966) has postulated that such increased motor activity and lowered arousal thresholds may represent an increase in neurological activity and a higher level of activation as measured by response rate. He further suggested that an interaction existed between activation level, type of stress and a physiological predisposition to ulceration. Any extreme value or increase in these factors could effect stress or restraint induced ulcers in the rat.

If Sine's proposed relationship does exist, then it could be hypothesized that a combination of stress, or stressors, along with an injection of amphetamine which would serve to increase the animal's activation level (Boyd, 1969), would produce an increase in gastric ulceration. This has been borne out to some extent by Zabrodin (1967) who found an increase in ulceration in rats for a combination of 4.0 mg/kg dose of d-amphetamine and three hour restraint plus shock. However, Boyd (1969), employing

relatively low doses of d-amphetamine, reported a decrease in ulceration for 0.2 mg/kg and 0.025 mg/kg doses of d-amphetamine after 24 hours of restraint. Bruckel and Gallaire (1967) observed a decrease in ulceration in rats subjected to two hours of restraint plus cold with an injection of 2.5 mg/kg of d-amphetamine. In a pilot study by the author, it was shown that with three hours of restraint plus cold, ulceration rate was signi- ficantly inhibited by a 0.4 mg/kg does of d-amphetamine when compared to saline injected controls. In terms of Sine's hypothesis, such conflicting results could possibly be accounted for by the disparity between drug dosage levels, types of stress, duration of stress and strain of rats.

The purpose of the present experiment was to investigate the effect of d-amphetamine on gastric ulceration employing various dosage levels and a combined stressor of 4 hours restraint in cold. This stressor was employed in an attempt to avoid the lethal effects reported by Body (1969) for a 0.40 mg/kg or 0.80 mg/kg dose of d-amphetamine and twelve hours of restraint. Based on previous results, it was proposed that at low dose levels of d-amphetamine an inhibition of ulceration would be observed while at higher doses, a facilitatory effect would be noted.

<div align="center">Method</div>

Subjects:

The Ss were 48 Wistar-Lewis male albino rats 60 to 120 days old, weighing between 160 to 350 grams. Ss received handling only in transport and were kept in the colony a minimum of two days on ad libitum feeding prior to use in any experimental condition. Ss were food and water deprived 24 hours prior to being placed in stress conditions.

Apparatus:

The restraint apparatus was a partially flattened cylinder of hardware

cloth with a base of two inches and a height of approximately one and one-half inches. Volumetric restriction was approximately 380 ml. (Bonfils & Lambling, 1963). This flattened cylinder was fastened to a 12 x 14 inch wooden base. The rat's horizontal movement was restricted by inserting a metal bar through the cylinder underneath the animal's tail. The other end of the cylinder was sealed by a large, removable wooden block attached to the base. Cold was supplied by an International Harvester commercial refrigerator with interior dimensions of 19 x 15 x 27 inches at 5 ± 1 C. The refrigerator interior was partitioned into four compartments so that Ss could not observe each other and darkness was maintained under all experimental conditions.

Ss were randomly assigned to eight groups consisting of six animals each. Each group received a different logarithmic dose level of d-amphetamine. These were: 0 mg/kg (saline control), -1.7 mg/kg, -1.2 mg/kg, -0.7 mg/kg, -0.3 mg/kg, 0.2 mg/kg, 0.7 mg/kg, and 0.9 mg/kg. Injections were given intraperitoneally to all Ss immediately preceding their placement into the restraint plus cold condition for four hours. The cold plus retraint stressor has been shown to have a facilitative effect upon the production of gastric ulcers in the rat in a relatively short period of time (Levine & Senay, 1967; Martin, Martin, Andre & Lambert, 1969; Brodie, Lotti & Bauer, 1970; Bruckel et al., 1967). Normal saline was the control injection given to all Ss in the first group and the volume of all injections was proportional to the weight of the animal. All injections were administered between 8:30 and 9:00 a.m. The order of administration of injections was randomly determined. At the end of the restraint-cold period, the Ss were removed and immediately

sacrificed by injecting 1 cc. of Nembutal intraperitoneally. Their
stomachs were then removed, cut open along the lesser curvature, and
washed with water. Examination of the stomach was done by the experi-
menter, and the number of lesions, defined as an area of mucosal erosion
of the fundus and body with or without hemorrhage, was counted. A second
count was taken by another examiner who had no knowledge of the content
and/or dose level of the injection received by the animal. The correlation
between the two counts was +0.94 (Pearson Product Moment). The experimenter's
ulcer count was employed in the statistical analysis. All ulceration was
observed to have occurred in the glandular portion of the stomach.

Results

A log (X + 1) transformation of raw scores, as suggested by Winer (1962)
to stabilize variances was computed. A one way analysis of variance across
dose levels revealed a significant overall F value of 6.25 (df = 7/40) at the
.01 level. The linear and quadratic analyses of the treatment effect were
also significant at the .01 level with F's of 10.50 and 27.75 respectively
(df = 1/40).

Subsequent comparative analyses of different dose level groups with
the control group using Dunnett's t statistic revealed only two dose level
means to be significantly different at the .05 level from that of the control
group mean. These were the -0.3 (.50 mg/kg) logarithmic dose of d-amphetamine
group (t = -2.63, df = 8/40), and the +0.9 (9.00 mg/kg) logarithmic dose of
d-amphetamine group (t = +3.00, df = 8/40).

Figure I shows the mean ulceration for each group computed from the
transformed raw data.

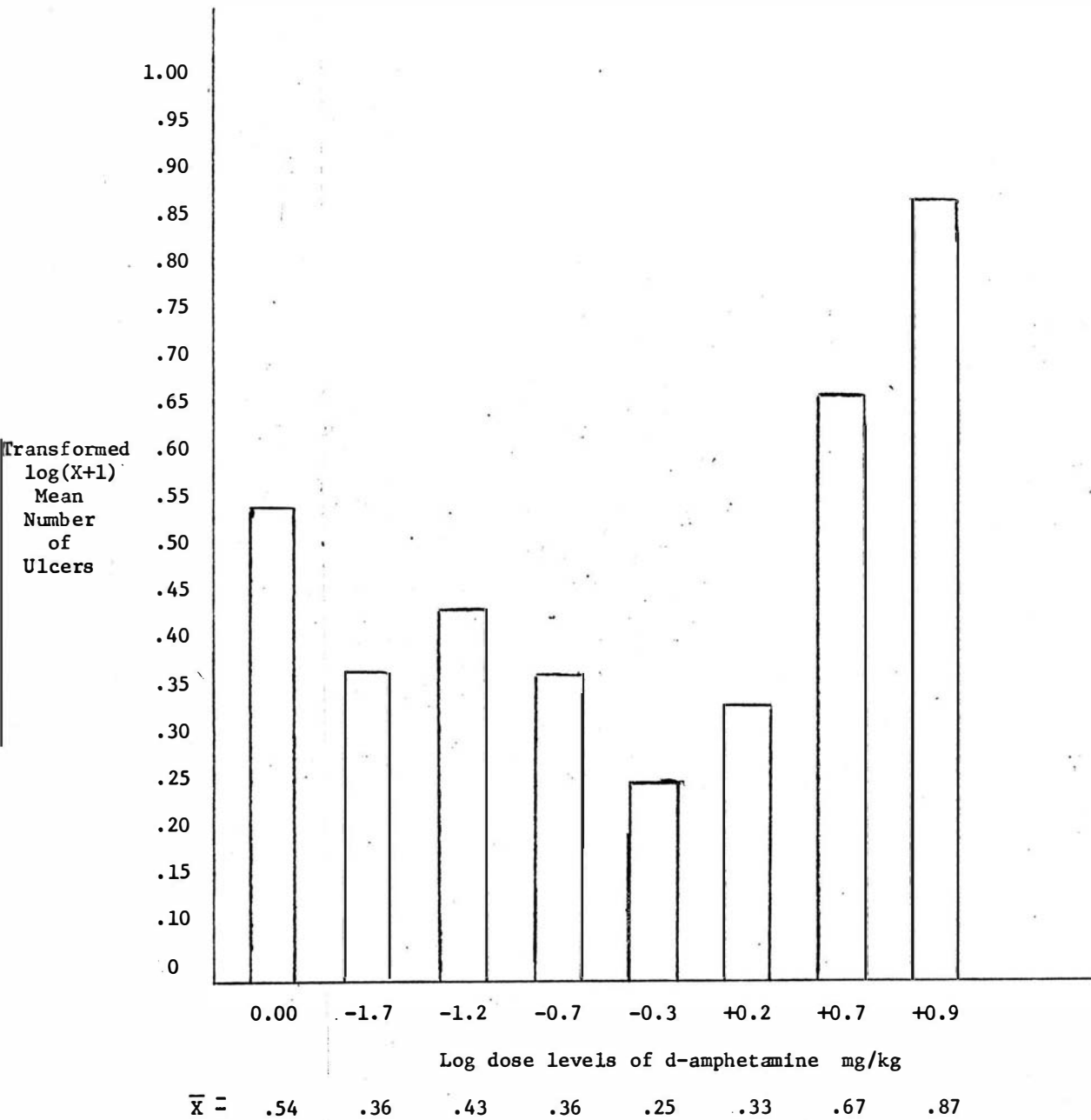It appears that at lower dose levels of d-amphetamine (.02 - 1.6 mg/kg)

Figure I. Mean ulcer production under different dose levels of d-amphetamine

there is less incidence of ulceration while at higher doses (4.8 - 9.0 mg/kg) there is more when compared to a saline injected control group.

Discussion

Results of this study would seem to indicate that with 4-hour restraint-cold stressor, low doses of d-amphetamine have an inhibitory effect on ulceration while higher dose levels facilitate it. With such a paradoxical effect of d-amphetamine, the question of why it occurs is a puzzling one. The observed inhibitory effect at low doses cannot be dismissed on the basis that such small amounts are physiologically inactive since dose levels between .01 and 0.50 mg/kg IP are sufficient to cause behavioral activation in rats as indicated by home cage activity and performance in a Sidman avoidance (Fuxe & Ungerstedt, 1970; Maickel, R., Cox, R., Miller, D., Segal, D. & Russell, R, 1969). The difficulty of explaining this paradoxical effect of d-amphetamine is further compounded by the fact that the literature on gastric ulceration in the rat has presented no empirically established etiology for the restraint stress ulcer itself. Thus it becomes exceedingly difficult to speculate on the reasons for the differential effect of d-amphetamine on restraint plus cold ulcers when the reasons for the restraint plus cold ulcers themselves are not clearly uncerstood. However, a tentative stress ulcer etiology can be proposed and developed in conjunction with a discussion of the differential effect of d-amphetamine on the restraint plus cold ulcer.

REVIEW OF GASTRIC CONDITIONS IN ULCERATION

Attempts to explain the stress ulcer in terms of a single physio-
logical factor have proved inconclusive. At present, the literature
suggests that at least three factors seem to be involved in the production
of the stressed induced gastric ulcer. It appears quite probable that the
stress ulcer is produced by an interaction between 1) gastric secretion and/
or acidity, 2) gastric motility and 3) vascular changes in the mucosa
of the stomach.

Support for this view came indirectly from Shay (1954) who proposed
an increase in gastric secretion to be an important factor in the production
of gastric ulcers and presented a schema, involving the vagus nerve and
the pituitary-adrenal axis, to explain the mechanisms through which stress
may act to stimulate gastric secretion. Later, Hartry (1962) implicated
the importance of stomach motility along with gastric acid secretion in
the pathogeneis of the stress induced ulcer. Brodie (1962) concurred but
added that, "...vascular changes may be as important in the etiology of
the restraint ulcer as the changes in gastric secretion and motility (108)."
Frankel and Kark (1965) in studying ulceration in man described the pre-
dominant features of the disease as: 1) low or normal acid, 2) hypomotility
of the stomach and 3) atrophy of the gastric mucosa surrounding the ulcer.
It should be noted, however, that this was a post hoc observation.

It would seem then, that explanations employing these three proposed
physiological factors in dynamic interaction with each other, while consid-
erably more complex, may prove more fruitful in studying the gastric
mechanisms involved in restraint-induced ulceration in the rat. Examination
of these three gastric factors separately to discover how their changing

parameters correlate with the incidence of ulceration in the restraint,
or restraint plus cold, stressed rat may offer some insight into the
etiological mechanisms of the stress induced gastric ulcer.

Gastric Acidity:

Menguy (1960) employing a 20-hrs. restraint stressor on pylorus
ligated rats, animals whose stomachs are tied off from the intestines at
the duodenum, reported a 94% inhibition of gastric secretory activity in
comparison with non stressed controls. Similar results were obtained by
Eagleton and Sines (1962) using rats that were not pylorus ligated. Brodie,
Marshall and Moreno (1962) showed that, with 24-hrs. restraint, chronic
fistula rats produced a significant decrease in gastric secretory volume
and a significant increase in free and total acid concentration. This
concentration was double that of control animals. They suggested that
acid concentration was an important variable and that restraint may alter
gastric secretion by causing a decrease in secretion of the non-acid
components of gastric juice, thus allowing a more concentrated gastric acid
juice to come in contact with the gastric mucosa. Hanson (1963) added
that "...it is probably the concentration rather than the amount of acid
secreted that is important in the restraint induced gastric pathology in
the rat (395)."

Ader (1963) observed that blood plasma pepsinogen levels were higher
in rats showing stress induced ulceration. Pepsinogen is the inactive
precursor of pepsin, a powerful digestive enzyme. Guth (1969) reported
that neutralization of gastric acid in rats subjected to 4-hrs. restraint
offered partial protection against stress ulceration. This finding was
replicated by Levine and Senay (1970) using 2-hrs. restraint plus cold

stressor. They concluded that there was a strong correlation between intragastric acidity and the development of the stress ulcer.

In summary, it would seem that a decrease in gastric secretory volume and/or a probable increase in acid/pepsin concentration are important in the development of gastric stress ulcers in the rat.

Stomach Motility:

Brodie and Hanson (1960) were the first to observe that motility in the restraint stressed rat increases. Its plausibility as an important factor in the pathology of the stress ulcer came with the finding of Eagleton and Sines that a breed of ulcer susceptible rats showed significantly more gastric motility than control groups. Hartry (1962) cited a gastric acidity-motility interaction in an explanation of her results on the effects of reserpine on gastric ulceration. She suggested that increases in gastric motility may be correlated with increased ulceration. Hartry noted that, "Gastric motility was increased in the ulcerated animal, draining out all excessive gastric secretion and contents (721)." The import of this statement on the pathogeneis of gastric ulceration is indicated by George's (1968) statement that, "A rapidly emptying stomach which only secretes a small amount of acid may achieve a higher concentration of acid than a stomach secreting more acid but emptying more slowly (376)." That this is an over-simplification of events is indicated by his rejoinder that, "...the longer the stomach takes to empty, the longer the acid, pepsin or any other gastric irritant will have to act on the gastric mucosa (376)." A close reading will reveal that these two statements are far from paradoxical and suggest at least two possible modes of action for the development of the stress ulcer just employing the two factors of motility and acidity. Increased acid concentration with high motility may facilitate ulceration while high

motility and low acidity would not. In either case, simple restraint can be shown to increase gastric motility, decrease gastric volume and possibly increase acid/pepsin concentration.

Vascular Changes:

Brodie (1962), observing that artificial derangement of the gastric blood supply produced massive hemorrhage, cited the importance of vascular changes in the pathology of stress ulceration. Additional support for this view was given by Bonfils and Lambling's (1963) observations that acute vascular lesions of the gastric mucosa, observed soon after the first half an hour restraint, were of primary importance in the occurrence of gastric lesions. Bonfils, Richir, Potet, Liefooghe, and Lambling (1959) also reported that besides finding gastric erosions in the restraint stressed rats, they also observed numerous capillary pits or areas of intense vasodilation in the gastric mucosa. Indirect evidence of vascular importance has come from Wolff's (1950) observations of a man with a large gastric fistula whose stomach, when he became angry, engorged with blood and increased in motility to the extent that strong peristaltic action was capable of producing small lesions in the mucosa. Hartry (1962) theorized that once such a lesion was begun, HCl and pepsin might then serve to further irritate the lesion. Guth and Hall (1966) have presented more concrete evidence along these lines. They reported "...a marked increase of blood in the mucosa immediately below the surface epithelium of the glandular portion of the rat stomach in response to restraint stress. This vascular change occurred with one half an hour of restraint and prior to the development of mucosal ulceration (564)." Animals restrained only one half an hour showed this vascular change, but only a couple had very small mucosal lesions. They further reported that, "...when

ulceration did occur, the area of erosion involved this hyperemic region
of the mucosa adjacent to the lumen (570)." Guth suggested that vascular
engorgement may lead to ulceration by decreasing mucosal resistance to the
normal acid content in the restrained rat's stomach.

Summary:

It was previously stated that the pathology of the stress induced
gastric ulcer was a dynamic interaction of at least three main factors:
motility, acidity and vascular change. It can be reasonable conjectured
that a stomach subjected to high motility, increased acidity (either by
volume or concentration) or pepsin, and a marked vascular engorgement, would
develop ulcers. By the same reasoning, a stomach with low motility, de-
creased acidity or pepsin and no vascular engorgement, would not. It is
in the realm of other possible combinations of these three factors that
the data is less supportive. It is not entirely implausible to suggest that
high motility and acute vascular engorgement may produce ulceration in the
presence of normal or low gastric acid concentrations. The same may be said
for acute vascular engorgement and high acidity coupled with low motility,
or high acidity and motility with little vascular engorgement. The important
considerations to be derived are: 1) that all three factors must be considered
in any attempt to define the pathological mechanisms of the stress induced
ulcer, 2) that it is possible that the presence of two of the three factors,
in sufficient amounts, is capable of producing gastric ulceration and 3) that
the action of these three factors on ulceration are empirically testable.

EFFECT OF RESTRAINT PLUS COLD STRESSOR AND D-AMPHETAMINE ON GASTRIC CONDITIONS

Restraint plus Cold Stressor:

Four hours of simple restraint has been shown to increase motility (Brodie, et al., 1960), decrease gastric secretion and acidity, though possibly increasing acid concentration (Brodie, et al., 1962) and promote vascular engorgement (Guth et al., 1966). These are conditions which appear to be excellent predisposing factors for ulceration.

The effects of cold on gastric motility, acidity and vascularization has not been as well researched. Witty and Fong (1970) have reported an increase in acid and pepsin output for pylorus ligated rats exposed to three hours cold. Brodie et al. (1963) stated that vascular factors appear to be involved in the cold plus restraint gastric hemorrhage though little direct information has been obtained bearing on gastrovascular changes in rats subjected to cold. No direct evidence has been found in the literature on the rat's gastric motility in the cold; however, Perkins, Nicholas, Lassen and Gertler (1950) reported that slow cooling of smooth muscles in vitro causes contraction of the muscle. It is quite plausible that, as long as the animal maintains homiothermic temperature, motility is increased in the cold since increases in heat production can be brought about by muscular activity and contraction.

In summary the restraint plus cold stressor apparently produces vascular engorgement, increases gastric motility and possibly increases gastric acid/pepsin concentrations (or at least maintains normal secretory conditions in the stomach). These are gastric conditions which would seem to facilitate the pathogeneis of the stress induced gastric ulcer.

Amphetamine:

A 2 mg/kg dose of d-amphetamine has been shown to decrease the motility of the gastrointestinal tract in rats over that of controls in a forty minute period after injection (Van Liere, Strickney, Northrup & Bell, 1951) while Vane (1960) has reported that in vitro d-amphetamine produces relaxation of the rat stomach strip. Nitescu, Groza, Dumitrescu and Sanduliscu (1958) observed that an intramuscular injection of d-amphetamine (0.1 mg/kg) inhibited the reflex secretion following distension of the stomach or sham feeding in the dog. The vasoconstrictive action of d-amphetamine is well documented (Goodman & Gilman, 1965). With this data in mind the proposed stress ulcer etiology, it is possible that a .50 mg/kg dose of d-amphetamine attenuates the production of the restraint plus cold ulcer by lessening the increased motility, acidity and vascular engorgement. The problem then arises as to why higher doses of d-amphetamine facilitate ulceration and how a low dose of d-amphetamine produces those gastric conditions inhibitory to ulceration. It would appear that additional factors, through their effect on those gastric conditions conducive to ulceration, play an important role in the proposed stress ulcer etiology. Specifically, these factors may be the autonomic nervous system and the adrenal glands.

ROLE OF THE AUTONOMIC NERVOUS SYSTEM AND ADRENAL GLAND IN STRESS ULCER ETIOLOGY

Autonomic Nervous System:

The vagus nerve is the main pathway for the parasympathetic nervous system's (PSN) inervation of the stomach. Vagotomies performed on rats prior to restraint, or restraint plus cold stress reduced the incidence of ulceration anywhere from 42% to 88% that of similarly stressed controls (Hanson, 1963; Bonfils et al., 1963; Menguy, 1960; Brodie et al., 1970). It has been also shown to decrease gastric motility (Davenport, 1965), gastric secretion and acidity (Brodie et al., 1970) and vascular engorgement (Guth & Kozbur, 1968), conditions which under many circumstances should alleviate ulceration. This data along with additional observations that anticholinergic drugs decrease ulcer incidence (Kramer, 1960), led Hanson and Brodie (1960) to speculate that, "...an increase in PSN activity produced by the stress situation, acting directly on the midbrain rather than indirectly through an increase in cortical function, is the central factor in the production of gastric elcers (293)." To this Sines (1963) added that, "These ulcer susceptible animals might be functionally sympathectomized or PSN dominant (397)." Support for this view was presented by Francois and Sines (1961) with rats whose sympathetic ganglia cells had been markedly reduced through injection of a nerve-growth-protein-antiserum. There was a significantly higher incidence and severity of ulceration in the antiserum restraint stressed rats than control restraint stressed rats. Indirectly, Richter (1957) wrote that wild rats who die suddenly on being subjected to a forced swimming stress, while other rats swim on for hours, "... died a so-called vagus death which is the result of overstimulation of the PSN rather than the sympathicoadrenal system (196)."

However, the assumption that gastric ulceration is solely mediated by the PSN system is as gross an oversimplification as that of one factor being predominantly ulcerogenic in the rat stomach. It must be recalled that a vagotomy is not entirely successful; it does not eliminate all ulcers in all rats. Thus there would appear to be at least one other condition of importance in the pathogeneis of the stress ulcer, quite possibly the adrenals or pituitary-adrenal axis (Menguy, 1960).

Adrenal Glands:

Adrenalectomy has been shown to have no effect on gastric ulceration (Bonfils, Lieffoogh, Rossi & Lambling, 1957) or to increase it (Hanson, 1963). Such disparate results may be due to the number of hours of restraint employed; the former being seven hours and the latter being twenty four. To further compound such conflicting results, it has been demonstrated that the administration of adrenal corticosteroids, and ACTH, can aggravate gastric ulceration in the restraint stressed rat (Brodie et al., 1960; Selye, 1956; Bonta, 1961), and plasma steroid levels have been shown to be positively correlated with stress ulceration (Weiss, 1971 a & 1971 b). A plausible mechanism through which adrenal steroids have their ulcerogenic effect has been aptly demonstrated by Selye (1956) and concerns the effect that these corticosteroids have on the gastric wall, making it more sensitive to degradation by gastric secretion. It is this process which may explain the remaining ulceration that is sometimes seen to occur following a vagotomy. The question which then arises is how can the removal of the adrenals, whose corticosteroids are clearly implicated in promoting the stress ulcer, worsen ulceration when logically it would be assumed that the removal of the source of corticosteroids would lessen ulceration. The answer to this disparity

may hold the key to the conflicting results of d-amphetamine on ulceration.

The adrenals, besides secreting cortical hormonal substances, also secrete medullary epinephrine (E) and norepinephrine (NE); the latter being an adrenergic transmitter substance crucial for sympathetic nervous system (SN) activity. Rats subjected to restraint plus cold show an increased output of both corticosteroids and catecholamines (CA) (Leduc, 1961; Smith & Dugal, 1964; Knigge, Perrod & Schindler, 1959; Gordon, Spector, Sjoerdsma & Udenfriend, 1966; Perhuch & Barry, 1970). It might be plausible to assume the adrenal's increased secretion of E and NE has an inhibitory effect on ulceration. Such an assumption seems even more plausible when combined with the view that such increases in CA output represent an increased SN system activity which may promote a concomitant decrease in PSN system activity (Bovard, 1961). In earlier discussion it was shown that PSN activity was integrally related to ulcer production, so perhaps by increasing SN activity, those conditions which favor ulceration in the rat's stomach might be post-poned. SN stimulation of the stomach has been shown to inhibit gastric secretion and motility and cause vasoconstriction of intestinal blood vessels (Grossman, 1967), gastric conditions which do not appear to be favorable to stress ulceration.

Brodie et al. (1963) showed that as injection of .48 mg/kg IP of E had an inhibitory effect on stress hemorrhage in rats subjected to one hour restraint plus cold, while Linich (1969) reported that a 2 mg/kg IP dose of NE aggravated ulceration in rats subjected to 3-hrs. restraint plus shock. Based on these results, a tentative observation might be that low doses of CA inhibits ulceration while higher doses facilitate it, like d-amphetamine.

It would seem logical to conjecture that increasing the amount of CA in an organism at a time when the body itself is producing and utilizing

more would facilitate the effects of the SN system; one of which would be the lessening of stress ulceration. However, evidence that higher doses of NE does not do this, but actually aggravates stress ulceration, indicates some type of ceiling effect which refutes this simplified view. As with conditions conducive to stress ulceration, a more complex interaction appears to be the case.

MODEL OF AUTONOMIC NERVOUS SYSTEM ACTIVITY UNDER STRESS

Since the organism's initial response to a stressor would appear
to be the SN-mediated "fight or flight" reaction, and the etiology of
the stress induced gastric ulcer has been shown to be predominantly PSN-
mediated, it may be concluded that it is the interaction between these two
components which is of importance in determining the occurrence of the
stress induced gastric ulcer. A schematicized conceptual model developed
by the author may elucidate the nature of this interaction. The following
figure can be used to represent a theoretically typical initial ANS response
in a rat subjected to stress:
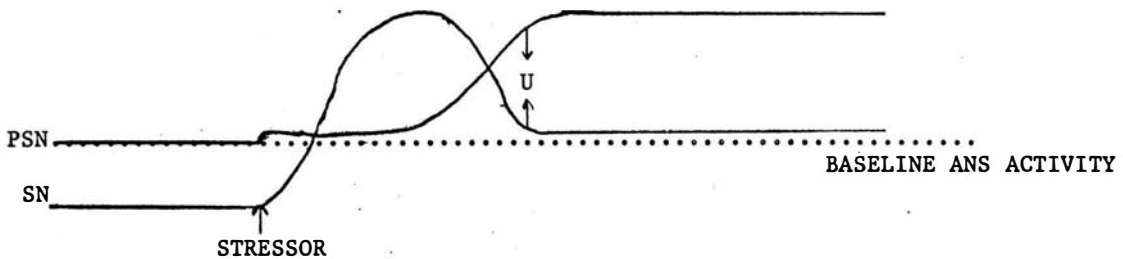
Initially, baseline or ongoing activity is represented by the PSN system
being slightly dominant, or controlling a majority of the animal's biologic
functions. At the inception of stress, i.e., restraint plus cold, the SN
"fight or flight" system takes over and neuronal activity is greatly increased.
PSN activity is initially greatly attenuated in relation to SN activity, but
due to the imposed stressor, both systems are still elevated above baseline

activity. As the stress continues, and the animal's "fight or flight" response has proven futile, the SN system activity recedes either through exhaustion of CA stores and/or organismal readjustment to the stress situation. The net result is that PSN activity becomes ·dominant. It is at this point that conditions ideal for stress ulceration and mortality begin. The distance "U" between the activity levels of the two systems at this point can be taken as an indication of the severity of stress ulceration; the greater the distance, the more severe the ulceration.

Some important aspects of this model are: 1) that whichever system is dominant after the inception of stress, it is at a considerably higher level than its resting state, 2) that these two systems are reciprocally inhibitory and 3) that increased SN activity will lead to increased PSN activity in the final stages of the acute reaction. This means that, due to the temporal nature of the stress situation, the amount of initial SN activity has an effect on subsequent PSN activity and thus on ulceration.

Keeping in mind the assumption that each type of stressor elicits different amounts of SN activity, it can be predicted that a low dose of CA would slightly increase and/or prolong the SN "fight or flight" response without unduly increasing the subsequent ulcer producing PSN activity. At much higher dose injections of CA, an overblown SN reaction, for that particular stressor, would occur and subsequent PSN activity would also be elevated, thus causing increased ulceration.

Indirect support for such a conceptual schematicization comes from Bovard (1962), who states in a discussion about positive and negative brain system activity that there are two reciprocally inhibitory systems. A positive system, which is inhibitory with respect to the neuroendocrine

response to stress and mediates parasympathetic function; and a negative system which is excitatory with respect to the neuroendocrine response to stress and mediates sympathetic autonomic effects. The former system is considered to be a cholinergic or serotogenic system and the latter to be adrenergic. Bovard suggests that, "...under emotional stress there is a built in tendency for the positive-negative system complex, taken as a whole, to drift into a state of extreme negative system dominance (123)." He adds that:

> ...under extreme stress, inhibition of the positive system is only a first phase that is followed by increased activity of the psoitive system to counterbalance the protein catabolic and other consequences of extreme negative system activity. That is to say, under long-continued stress, we may consider the possibility that some normal balance between, for example sympathetic and parasympathetic output to the viscera has to be maintained by counteractivity of the positive system even in the absence of external reinforcing stimulation. Such a balance between positive and negative systems, where both are hyperactive, must be considered highly unstable compared to the normal resting balance (124).

Assuming that CA levels are indicative of SN activity, additional support for the proposed theoretical model can be found in studying the effects of stress on CA. In the brain, these effects are biphasic. The initial tendency at the inception of stress is for the amine to be elevated; but if the stress is sufficiently intense or prolonged, it may be lowered (Welch et al., 1970). Four to eight hours restraint has been shown to lower brain NE in rats (Corrodi, Fuxe & Hokfet, 1968; Moore & Larivere, 1964), while severe cold stressors have been shown to increase their excretion of E and NE initially and then cause depletion (Leduc, 1961). It would appear that the effects of stress on CA closely parallels SN activity under stress in the proposed model.

The effect of stress plus d-amphetamine of CA has been observed to be

dose related. Welch et al. (1970) reported that mice subjected to high

doses of d-amphetamines and stress showed lowered brain NE levels while

Moore and Larivere (1963) observed that in rats subjected to restraint

for four hours, the NE levels of animals who had received a 3 mg/kg dose

of d-amphetamine were higher than those animals that had been just restraint

stressed although the overall brain NE levels of both groups was lower

than a group of unstressed controls. This difference was not seen at 10

or 30 mg/kg of d-amphetamine and 4-hrs. restraint. Welch et al. (1970)
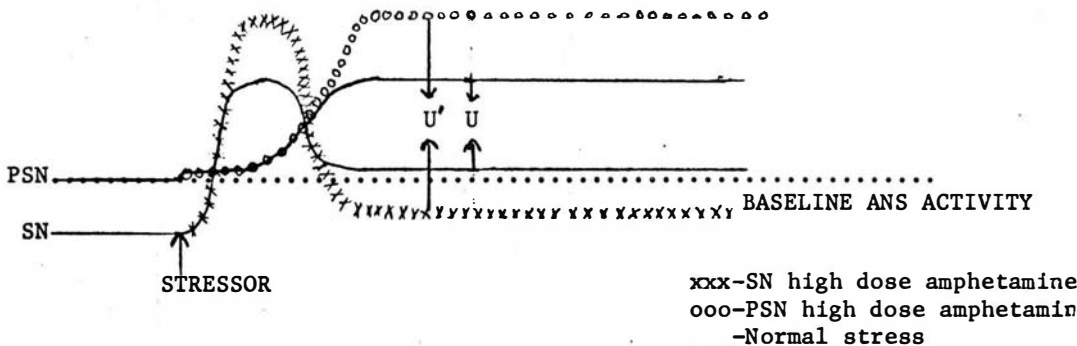
felt that:

> Rapid and substantial increases in the concentration of nor-
> epinephrine, dopamine, and serotonin occur in the brain at
> the inception of natural stress and also within minutes after
> the administration of low doses of d-amphetamine. This has
> obvious functional significance in that increased amounts of
> neurotransmitter amines are made available at the very times
> when more are needed to meet the increased requirements that
> are imposed by an accelerated rate of neurotransmission (439).

The data available seems to indicate that d-amphetamine in low doses is

capable of releasing central and peripheral NE and facilitating the release

which is induced by nerve stimulation (Carlson, 1970). This release appears

to occur preferentially from extragranular stores in the cytoplasm of NE

carrying nerve fibers. In larger doses, however, d-amphetamine also

partially blocks the NE reuptake mechanism and apparently acts on the

granules themselves causing overt CA depletion (Axelrod, 1966; Fuxe &

Ungerstedt, 1970; Welch et al., 1970). Specifically, it has been shown

that low doses (1 mg/kg) of d-amphetamine increases NE while doses greater

than 5 mg/kg cause a decrease of NE in the rat (Leonard & Shallice, 1971).

Although most of the data showing these effects deal with central NE levels,

Carlsson (1970) states, "There is no reason to believe that the action of

amphetamines on the catecholamines should be fundamentally different centrally

or peripherally (298)."

By combining the data on d-amphetamine's effects on CA levels and the effects of restraint plus cold on CA levels and presuming that CA levels are indicative of SN activity, it is possible to explain the differential effect of d-amphetamines on ulceration using the theoretical schematicization of the ANS stress reaction.

Typically, then, a rat subjected to a 4-hr. restraint plus cold stressor responds initially with an increase in SN activity or CA synthesis and release. As readjustment and/or exhaustion occurs, the PSN becomes hyperactive and pathogenic ulceration begins. A low dose injection of d-amphetamine, much like a low dose injection of CA, increases the NE available to the organism for its initial reaction thus prolonging, and perhaps accentuating it, without significantly increasing the PSN rebound which is to follow. A high dose of d-amphetamine, similar in some respects to a high dose of NE, greatly increases the NE released, but it also blocks its reuptake, thus in effect, causing a maximal SN reaction which leads to rapid SN exhaustion and a significnatly heightened PNS reaction. This effect can be schematicized:

PSN .... BASELINE ANS ACTIVITY

SN _____

STRESSOR

xxx-SN high dose amphetamine
ooo-PSN high dose amphetamin
____-Normal stress

For a given stress, or stressors, the animal responds with a
certain level of SN activation dependent upon many variables. Among
them are: sex, strain, deprivation, time of day, past experience, and
even the nature of the stressor itself. For some stressors, like restraint,
no, or little, SN activity (and therefore no PNS reaction) would be ideal
for the animal to avoid ulceration. Other stressors, like cold, due to their
physiological aversiveness, require some degree of increased SN activity.
With the addition of d-amphetamine at a given dose level, it becomes possible
to promote an overreaction to some stressors while improving underreaction
to others. Zabrodin (1967) reported that a 4 mg/kg dose of d-amphetamine
facilitated ulceration in rats who were 3-hrs. restrained plus shocked.
Shock has been shown to lower NE in rats (Bliss & Zwanziger, 1968) and
combined with d-amphetamine, it may even prove lethal. Weiss (1961) reported
that the L D_{50} for shocked rats was 2.9 mg/kg of d-amphetamine while for
non-shocked animals, it was 49.5 mg/kg. In the present study, no mortalities
were reported, and it appears that, for 4-hrs. restraint plus cold, a .5
mg/kg dose of d-amphetamine boosted the animal's SN response to a more
optimal level. However, before any truly meaningful interpretations can
be derived from any pharmacological data on stress ulceration, dose response
curves, standardized stressors, and homogenour animal strains must be employed.

Although many possible studies suggest themselves in order to empi-
rically test this proposed model of stress ulceration, it would be of
initial additional value to know if this d-amphetamine release of NE, which
has been proposed to play a crucial role in its effect on gastric ulceration,
is important peripherally, or centrally and peripherally. This is the purpose
of Experiment II.

Experiment II

It has been proposed that the differential effect of low and high doses of d-amphetamines on gastric ulceration in the 4-hrs. restraint plus cold stressed rat is due to the differential effect that these dose levels have on CA levels. It was assumed that CA levels in the animal reflect on SN activity and that the interaction of SN and PSN activity is an important factor in the production of the stress induced gastric ulcer. It has been specifically suggested that the low dose of d-amphetamine (.5 mg/kg) releases additional NE which the organism is able to use in maintaining a SN state of arousal, while the high dose (9 mg/kg) results in acute depletion of the adrenergic system. The question was raised of whether this low dose release effect acted on peripheral or central and peripheral stores of CA.

Disulfiram and guanethidine are two drugs which have been shown to cause depletion of NE with the important difference that while the former acts both centrally and peripherally, the latter appears to act only peripherally. I would be expected, in terms of the theoretical model, that a rat given an injection of either disulfiram or guanethidine at an equivalent dosage prior to an injection of d-amphetamine, (.5 mg/kg) would develop ulceration equal to or worse than a disulfiram or guanethidine pretreated only control group since the low dose of d-amphetamine would only accentuate the response of a SN system that already has lowered NE levels. By the use of these two drugs, it may be possible to determine whether NE depletion, either peripherally or centrally and peripherally, is more important in the etiology of the stress ulcer. Significant differences between the two control groups would give some indication of where d-amphetamine's anti-

ulcerogenic effects are most pronounced. If there is no difference between the disulfiram injected only control and the guanethidine injected only control group, then a more important peripheral depletion would seem to be indicated. If, however, the disulfiram control group's ulceration was worse, this would argue more for additional central factors. Although the mode of action of disulfiram and guanethidine has been shown to different (Musacchio, Kopin & Snyder, 1964; Goldstein & Nakajima, 1967; Kuntzman, Costa, Gessa & Brodie, 1962; Cass & Spriggs, 1961; Chang, Costa & Brodie, 1962; Sheppard & Zimmerman, 1960), it is hoped that the activation of CA in response to the stressor will be widespread enough that pharmaceutical depletion, by whatever mechanism of action, will facilitate ulceration.

Equivalent dose levels for these two drugs in the present experiment consisted of those doses which caused an equal amount of NE depletion from some peripheral organ. In this manner, any additional ulceration seen with disulfiram might be attributed to its central effect. Musacchio et al. (1964) reported that two injections of 400 mg/kg of disulfiram seventeen hours apart decreased the endogenous NE content of the rat heart by 50% two hours after the final injection. Kuntzman et al. (1962) observed the same effect in rat heart NE two hours after rats were given a single injection of 25 mg/kg of gaunethidine. Thus it appears that two hours after the final injection of 400 mg/kg of disulfiram, or the single dose injection of 25 mg/kg of guanethidine, heart stores of NE are depleted by 50%.

Using Musacchio et al.'s and Kuntzman et al.'s dose levels of disulfiram and guanethidine respectively, rats were subjected to 4-hrs. restraint plus cold stressor at a time which corresponded to the equivalent depletion of heart NE by both drugs. In addition, an injection of d-amphetamine (.5 mg/kg)

.was given to some animals immediately prior to stressing them. In brief four groups were used: 1) guanethidine injected (25 mg/kg) two hours before being restraint-cold stressed for 4 hours, 2) guanethidine injected (25 mg/kg) two hours before receiving a .5 mg/kg dose of d-amphetamine and being restraint-cold stressed, 3) disulfiram injected (400 mg/kg) 19 and 2 hours before being subjected to restraint-cold stressor and 4) disulfiram injected (400 mg/kg) 19 and 2 hours before receiving a .5 mg/kg dose of d-amphetamine and being subjected to restraint-cold stress.

It was predicted that all groups would show increased ulceration to that of saline injected, restraint-cold stressed controls of experiment I, and that group 4 would show the most ulceration since the NE depletion should be the greatest in this group. Progressively less ulceration should be seen with groups 3, 2, and 1 since NE depletion should also be progressively reduced in these groups. These predictions were based on the assumption that central factors are implicated in the stress response (Bovard, 1962).

<div align="center">Method</div>

Subjects:

20 rats of the same strain, age and weight as those used in experiment I.

Apparatus:

Same as experiment I.

Procedure:

Ss were randomly assigned to one of four groups, each group having five animals in it. Groups 1 and 2 received an intraperitoneal injection of 25 mg/kg of guanethidine between 6:30 and 7:00 a.m. on the day in which the animal would be stressed. They also received a saline control injection

between 2:00 and 2:30 p.m. in the afternoon of the day prior to the one

on which the animals would be stressed. Rats in group 1 also received a

saline control injection immediately prior to undergoing the 4-hrs. restraint

plus cold stressor, while animals in group 2 received a .5 mg/kg dose of

d-amphetamine. Groups 3 and 4 received an IP injection of 400 mg/kg of

disulfiram between 2:00 and 2:30 p.m. in the afternoon of the day prior to

undergoing the restraint-cold stressor, and at 6:30 and 7:00 a.m. on the

day in which they would be stressed. Animals in group 3 were also given

a saline control injection immediately prior to the imposition of the

restraint-cold stressor while the animals in group 4 were given a .5 mg/kg

dose of d-amphetamine. All animals were subjected to the 4-hrs. restraint

plus cold stressor between 8:30 and 9:00 a.m., and all animals had three

pre-stress injections of drugs and/or saline. The volume of all injections

was proportional to the weight of the animal. The order of administration

of injections was randomly determined. Procedure at the end of the 4-hrs.

restraint-cold stressor was the same as in experiment 1. Correlation be-

tween rater 1 and rater 2's ulcer counts was +0.91 (Pearson Product Moment).

Results

Using rater 1's data, an analysis of variance (Winer, 1962) computed

across the four drug conditions revealed no overall statistically signifi-

cant effect. Specific comparisons, as indicated in the introduction,

were also computed with no statistically significant results, although a

certain notable trend was observed in the comparison of the guanethidine

only group (1) with the guanethidine plus d-amphetamine group (2).

Figure II represents the mean number of ulcers for each group.

A t test between group 2, which had the least ulceration of the four

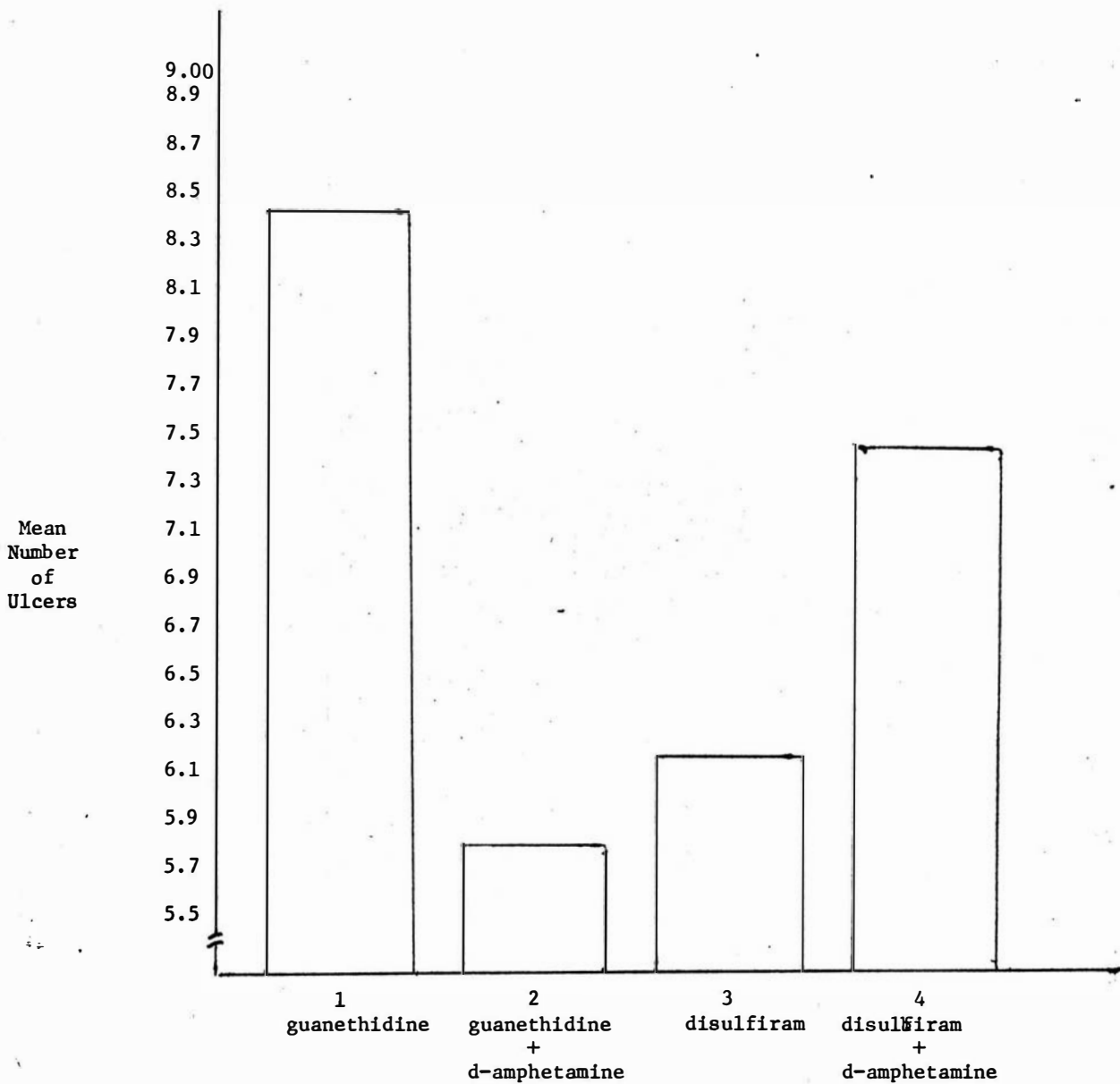groups, and the saline control group of experiment I yielded a significant

Figure II. Mean ulceration with disulfiram (400 mg/kg), guanethidine (25 mg/kg), and d-amphetamine (.50 mg/kg)

t of 2.47 (df = 9) at the .05 level. Thus ulceration in all four groups was worse than that of experiment I's saline control group.

Discussion

Although the results of this study were not statistically significant, the data for the different drug groups was not in accordance with some of the hypotheses developed in the introduction. It was initially predicted that the disulfiram only group (3) would have more ulceration than the guanethidine only group (1). Instead, an opposite effect was observed. It was also predicted that the addition of a low dose of d-amphetamine (.5 mg/kg) would either have no effect, or worsen ulceration. This was found to be the case in comparing the disulfiram only group (3) to the disulfiram plus d-amphetamine group (4). However, in the comparison of the guanethidine only group (1) with the guanethidine plus d-amphetamine group (2), an inhibition of ulceration was seen in group 2, not unlike that observed with a low dose of d-amphetamine by itself in experiment I. The general prediction that ulceration in those four groups would be worse than that of the saline control group of experiment I was supported.

In terms of the proposed theory, the assumed peripheral depletion of NE by the guanethidine injection would seem to be the more potent or important locus of action for the induction of stress ulceration. However, a new implication appears to be indicated by the data of groups 2, 3, and 4. It would seem that a release of or a slight heightening of brain NE from its normal levels antagonizes the effect of the peripheral NE depletion and/or parasympathetic rebound. Since the low dose of d-amphetamine has NE releasing properties both centrally and peripherally, it would seem that peripheral stores of NE are going to be lowered further, thus producing even more ulceration. Since this is empirically not the case for

group 2, it would seem necessary to look at d-amphetamine's central
action as a possible mechanism for its observed antagonism to stress
ulceration. This central effect has been reported to be a release, and
sometimes heightening of brain NE (Welch, et al., 1970).

The mechanisms through which this slight increase and/or release
of brain NE might antagonize the peripheral PSN system has been suggested
by Bovard (1961, 1962) and Carlton (1963). In essence, it may be that
this slight increase and/or release of NE antagonizes a second cholin-
ergically-mediated system in the brain which serves as an important source
of inervation for the peripheral PSN system. By antagonizing this system,
PSN activity is reduced.

Other explanations for the results of experiment II are possible without
reference to the proposed theoretical model. It is possible that the
different modes of action of these two drugs accounted for the difference.
Disulfiram is an effective inhibitor of dopamine-B-hydroxylase. The
inhibition of dopamine-b-hydroxylase blocks NE brosynthesis at its terminal
stage. Thus central and peripheral depletion is seen as due to the fact
that no new NE is being systhesized; however, dopamine concentrations have
been reported to increase above normal levels (Musacchio, et al.; 1964).
Guanethidine has been shown to deplete NE stores in tissues, interfere wtih
sympathetic neuronal function and act as a false transmitter, being released
by sympathetic nerve stimulation (Blazkowski, 1968). It can be conjectured
that the multiple effects of guanethidine may account for its increased
ulceration rate and that the low dose of d-amphetamine conteracts these
effects. It might also be proposed that dopaminergic systems can antagonize

stress ulceration and the slight increase in DA seen in animals injected
with disulfiram was instrumental in reducing ulceration. Necina and
Kregci (1961) demonstrated that the gastric ulceration produced by reserpine
and cold stress could be inhibited by the administration of the NE pre-
cursor, dopa. Although initially presented as evidence supporting the
role of NE in stress ulceration, it might also be indicative of dopa-
minergic involvement in the stress ulcer etiology.

The dose levels of the drugs employed is another important consideration.
Although the drug dose levels were made equivalent for a given organ, the
heart, at a set period in time, the effects of these two drugs before,
and particularly after this period were unknown. It is entirely possible
that one drug continued to have a more potent NE depleting effect than the
other as the experiment continued. Thus group 1's greater ulceration
could be explained by the fact that, in reality, the dose of guanethidine
used had a stronger peripheral effect than disulfiram. Obviously, addi-
tional studies employing other experimental designs are needed to determine
if the alternative explanations are correct or have played an important
role in the present results.

In terms of the theoretical stress ulcer model, it might be tentatively
assumed that those drugs and stress conditions which lead to a lowering of
NE both centrally and especially peripherally, will facilitate stress
ulceration, while those drugs, conditions, surgical interventions, etc.,
that decrease PSN activity or boost SN activity a certain "optimal" amount
under stress will inhibit the resulting gastric ulceration. The proposed
model, is of course, grossly oversimplified. Other important factors

which may interact with, or even override, these proposed mechanisms –
and thus should be included in any final analysis of stress ulcer etiology –
are: amount and type of adrenal corticosteroid output, secretion of
various digestive enzymes and histamines, mast cell degranulation, genetic
predispositions and instinctual responding to a given stressor. Nevertheless,
as Ordy, Samorajski and Schroeder (1966) point out, "NE depletion in
response to anesthetics, tranquilizers, and behavioral stress provides
some compelling evidence for assigning an important role to NE as a possible
neurotransmitter substance in the integration of stress reactivity by
central and peripheral adrenergic mechanisms of the autonomic system (457)."

Summary

The effect of various doses of d-amphetamine (.02 - 9 mg/kg) on ulcer production in the 4-hrs. restraint plus cold rat was studied. It was found that low dosesoof d-amphetamine (.5 mg/kg) inhibited gastric ulceration while high doses (9 mg/kg) facilitated it. It was proposed that gastric ulceration is produced by various interactions of three factors: gastric motility, gastric acid and/or pepsin secretion, and gastric vascularity. It was suggested that increases in all three factors would lead to ulceration; it was further felt that an increase in two of these factors would also be sufficient for gastric ulceration. Evidence was presented that PSN inervation of the stomach played an important role in the production of stress ulcers or erosions, and that CA depletion was associated with increased ulceration. From this data, a model for stress ulceration was developed which posited an initial increased SN system activity followed by an increased PSN system activity. It was postulated that the stressors produced ulceration by depleting endogenous NE, thus lowering SN system activity and increasing PSN-ulcer conducive activity.

Data was then presented which indicated that low doses of d-amphetamine may slightly increase central NE without causing a subsequent, severe depletion later. This was not true of high doses. This information, combined with the proposed stress ulceration model, indicated that the low dose of d-amphetamine increased NE perhaps prolonging SN system activity and delaying the onset of PSN-ulcer producing activity. At higher doses, the NE depletion was augmented leading to a shortened period of SN activity and a heightened and prolonged PSN activity.

\ A second study was conducted to confirm this NE depletion hypothesis and to determine if peripheral, or central and peripheral, depletion was more facilitative of ulceration. Using two drugs, guanethidine (25 mg/kg) which has a peripheral depleting effect only and disulfiram (400 mg/kg) which has a central and peripheral depleting effect, animals were subjected to the same stressor as those in the first experiment. It was predicted that ulceration in these drug injected groups would be worse than the saline injected control animals of experiment I. This was supported by the results. It was further predicted that the addition of a low dose of d-amphetamine (.5 mg/kg), previously shown to have an inhibitory effect on ulceration, would not be effective, and that the disulfiram injected animals, because of the drug's additional central depleting effects, would cause the most ulceration.

Results, although not statistically significant, showed that animals treated with guanethidine alone had greater ulceration than animals pretreated with disulfiram only. This increased ulceration in the guanethidine pretreated group was inhibited by giving the animals a low dose of d-amphetamine (.5 mg/kg) prior to imposing the restraint plus cold stressor. This effect was not observed in the disulfiram pretreated animals.

These results were discussed in terms of the proposed model, and it was concluded that a slight or mild increase and/or release of NE centrally over previous levels, would antagonize the ulcerative-producing effect of peripheral NE depletion. Acute central NE depletion would have no effect or worsen ulceration.

Alternative explanations of the results of experiment II could be atrributed to: the mode of action of the two drugs used, their interaction

with d-amphetamine and the failure to use truly equivalent dose levels.
It was concluded that NE depletion is an important factor in the production
of stress ulcers through its indirect effect on SN and PSN inervation of
the stomach.

APPENDIX A

Analysis of Variance for Experiment I

Transformed scores: $\log(X+1)$

Component	SS	df	V	F	Fcrit
Treatment	1.75	7	.25	6.25*	3.12
Linear	.42	1	.42	10.50*	7.31
Quadratic	1.11	1	1.11	27.75*	"
Remainder	.22	5	.04	1.00	3.51
Error	1.60	40	.04		
Total	3.35	47			

* significant at .01 level

! approximately equal intervals obtained for linear and
quadratic analysis by transformation $\log(\text{dose level} + 1 \times 100)$

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