ACUTE AND CHRONIC PHYSIOLOGICAL EFFECTS OF PROLONGED STRESS

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ABSTRACT

The study was conducted to assess the effects of stress on certain physiological correlates of emotional behaviour, and to test for the perserverance of these effects beyond the stress period. In two experiments a total of sixty-eight albino rats were exposed to a prolonged period of unavoidable light-shock pairings. Gastric acid secretion was examined in pylorus ligated rats. It was observed that gastric acidity decreased during the period of exposure to stress and that ulceration developed in the rumen of the rat's stomach. The drop in gastric acidity was not detectable in rats which received a post-stress rest period prior to testing. While blood eosinophil count, body weight, and thymus weight decreased both during and following stress, the light-shock pairings had no effect on adrenal weight and blood sugar level. It was concluded that the autonomic aspects of emotion show a non-uniform pattern of activity not only during stress but also during the post-stress period. The influence of stress on water intake and the problem of generalizing rat ulceration to human ulcerogenesis was also considered.

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CHAPTER 1

INTRODUCTION

1.1 The Approach of the Study

The experimental production of lasting disturbances of emotional behaviour by prolonged stressful conditions is important - especially to the clinician trying to find ways to relieve patients of their disturbed behaviour. Although a large body of literature is devoted to the study of lasting emotional disorders as a result of stress, little recent work deals with the production and post-stress persistence of the disorders utilizing the allegedly sensitive correlates of emotional behaviour. The situation exists despite the psychophysiologist's emphasis on using the autonomic indicators of emotional behaviour in his research.

Recent work by Brady (1969) exemplifies this approach. He used as a stressor a conditioned avoidance situation in which shocks were presented to the feet of rhesus monkeys every 20 seconds unless the animal pressed a lever within that interval; each lever press postponed the shock for another 20 seconds. Brady found that exposure to a continuous 72-hour avoidance session produced an increase in plasma 17-hydroxycorticosteroid levels and a decrease in plasma pepsinogen levels during the avoidance period. The post-avoidance period was characterized by elevated 17-OH-CS levels for 48 hours, and increased levels of pepsinogen for several days (Figure 1). Animals were repeatedly exposed to such 72-hour continuous stress sessions on six separate occasions over a six month period with approximately 4 weeks rest between each exposure. The

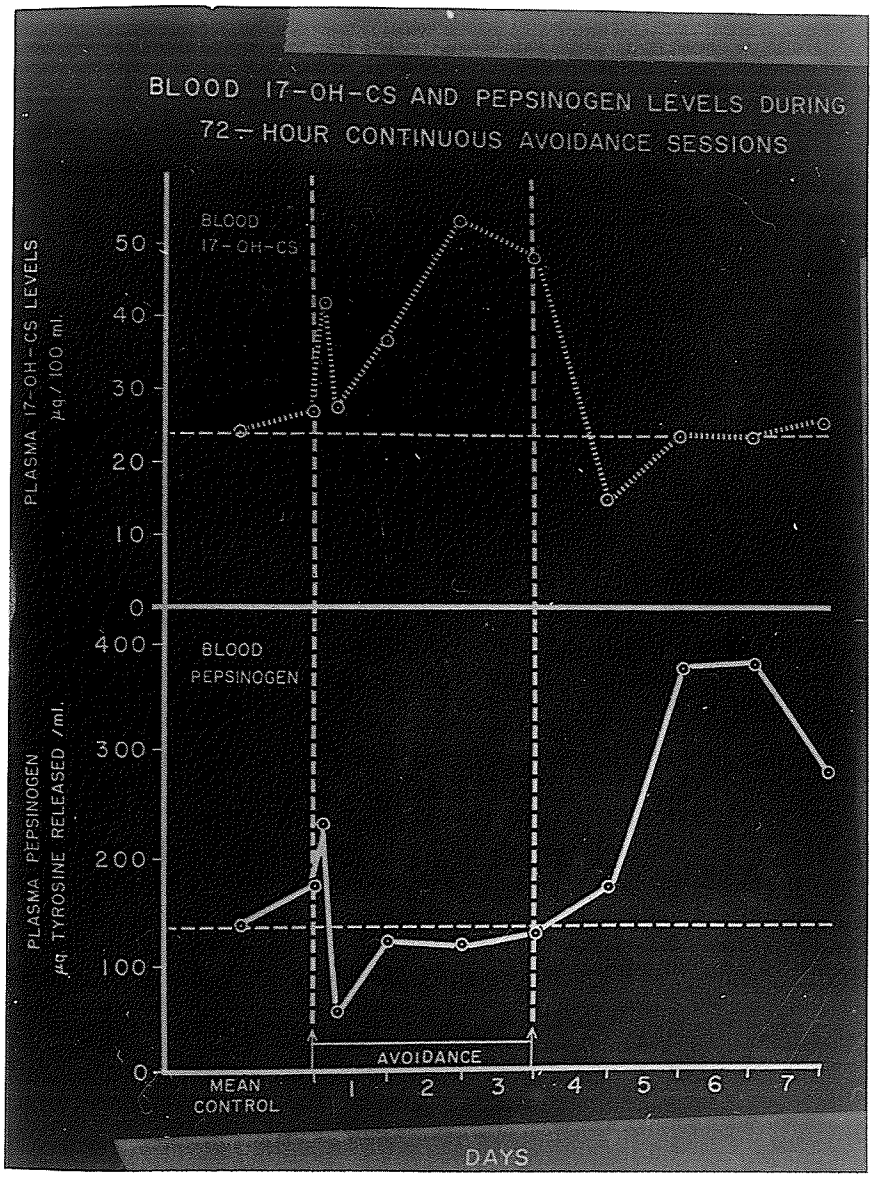


Figure 1. Result of the Brady Study: Mean levels 17-OH-CS and pepsinogen during 72-hour continuous avoidance session.

17-OH-CS changes related to the repeated 72-hour avoidance sessions showed consistent and replicable elevations of steroid levels during the avoidance session with recovery delayed for approximately 6 days after the avoidance session. Significant changes related to the extended avoidance performance were also observed in catecholamine, gonadal, and thyroid hormone levels, with recovery delayed in some instances (thyroid) for 3 weeks after the termination of avoidance.

Aside from Brady's experiment, little recent literature deals with the post-stress recovery period using autonomic measures. More research in line with Brady's approach, but using different autonomic measures, may be useful. This study will, therefore, use autonomic measures in determining the effects of stress.

1.2

Stress and Behaviour Disorders

Stressful conditions are often implicated in the development of lasting disturbances of behaviour. A recent symposium held at the University of Windsor opened with the statement:

> "...no dimension of behaviour (which) has been studied from more vantage points by psychologists than that of stress and anxiety (Rourke, 1969, p. viii)."

Similarly Spielberger (1966) said that anxiety is generally regarded as a product of stress.

Furthermore, the literature abounds with conclusions and theories that account for the initiation and persistence of disorders as due to stressful conditions maintained for some time. Two major approaches in the literature have been the physiological and psychological explanations

of disturbed behaviour.

(a) Physiological Explanations of Disturbed Behaviour

Pavlov (1927) described an experiment performed by his co-worker, Dr. Rickman (p. 302). The experimenter positively conditioned a dog's salivary secretion to an electric light, strong tone, weak tone, and buzzer. The animal was then taught an inhibitory alimentary conditioned reflex to a metronome rate of 60 beats per minute. After the reflex to the metronome had been well established (by a criterion of no salivary secretion to the 60 beat signal during 20 seconds of signal presentation), the negatively conditioned stimulus was then repeatedly and positively reinforced. The animal at first learned to secrete saliva to the previously negative stimulus. At this point, however, the dog also secreted equally to all stimuli whereas before the reinforcement to the metronome signal was changed from negative to positive, the animal had responded more (i.e., secreted more saliva) to certain stimuli (strong stimuli) than to others (weak stimuli). On succeeding trials an inhibitory effect developed and the animal refused to respond to the metronome signal. Moreover, upon presentation of the signal, the dog also failed to respond to all other stimuli on succeeding trials. The condition progressed to a point where not only the metronome signal, but also all stimuli (except very weak ones) completely disrupted the animal's responses. At this point the animal also showed signs of general excitement and either refused to eat or took the food reluctantly. The disturbance continued for an unspecified long period of time.

The experiment was repeated with cutaneous stimuli by Dr. Federov

(p. 311), another of Pavlov's co-workers. His dog developed symptoms similar to Dr. Rickman's animals, but the general disturbance of behaviour reached such an intensity that the experiment had to be discontinued. Furthermore, the animal's disordered behaviour continued beyond the stress period and was relieved only after "prolonged administration of bromides and by disuse of both positive and negative tactile stimuli (p. 311)."

In still further experiments, Pavlov showed that not only the conflict situation depicted above, but also very strong or unusual stimuli can result in similarly disturbed behaviour. He hypothesized that the disturbed behaviour developed as a result of conflict between the inhibitory and excitatory processes in the cortex. Having emphasized the permanent character of the disturbance in the activity of the cortex, he stated:

> "...the disturbance must be regarded as a result of a strictly localized functional interference ... a chronic functional lesion of some circumscribed part, the stimulation of which produced an immediate effect upon the function of the whole cortex, and finally leads to a protracted pathological state (p. 310)."

To summarize Pavlov's view, stress resulted in a disturbance of nervous activity, a change resulting in a chronic (non-reversible or slowly reversible) disturbance of behaviour.

The hypothesis that stress produces structural damage which leads to disturbed behaviour was expressed by Solomon and Wynne (1954). Their view developed from four consistent observations on several dogs in an experiment where the animals were required to jump back and forth over a barrier to avoid being shocked. The observations were: (a) that the

dogs performed the avoidance response for months without extinction after having received only a few intense shocks during the acquisition of the response; (b) that although overt signs of anxiety gradually disappeared, the dogs became more and more stereotyped in their jumping and their latencies to the CS progressively shortened, indicating a state of heightened anxiety; (c) that the animals, rather than extinguishing the anxiety response when prevented from jumping, showed increasing signs of overt anxiety; and (d) that if a dog had a long latency on a trial, he appeared upset after the response and jumped very quickly on the next few trials.

To account for their observations the authors reviewed the literature on learning. They found the two-process learning theory deficient as an explanation since the theory predicts that if the animal avoids, the CS is no longer followed by a UCS (shock) and the CS ceases to evoke an anxiety response. The CR should, therefore, extinguish since it no longer is reinforced by anxiety reduction. The latter prediction contradicts the first observation made by Solomon and Wynne: that the avoidance response failed to extinguish. The same criticism was made against the "anxiety conservation phase theory (Solomon and Wynne, 1954)." According to the theory, as the latency to a CS decreases, the animal performs the avoidance response too swiftly to experience the anxiety response. Since the non-reinforced exercise of a CS-CR relationship is the necessary condition for extinction, the anxiety response is not extinguished. The authors point out, however, that since the CR of avoiding is not reinforced by anxiety reduction, there is an increase in the latency to the CS until the CS again elicits the anxiety response.

The latency immediately decreases again, but unless the anxiety response is reinforced by a UCS, the CS-anxiety bond has been weakened somewhat. The repetition of this cycle of events eventually extinguishes the anxiety and in turn the avoidance response; a prediction contrary to Solomon and Wynne's observations (a) and (b).

Since their review of learning theory failed to account for their observations, Solomon and Wynne hypothesized "a partial irreversibility of classical conditioning as a result of traumatic stimuli (p. 361)" was the basis of their findings. The partial irreversibility was due to the fact that stressful stimuli result in a "permanent increase in the probability of occurrence of an anxiety reaction (p.361)." The permanent change was thought of "as a decreased threshold phenomenon or as a sensitization phenomenon which is relatively permanent (p. 361)." Wolpe (1958) pointed out that since the hypothesis of partial irreversibility excludes learning as a cause, a lesion of some kind is implied. Solomon and Wynne themselves propose some form of structural reorganization of neural circuits to explain the permanent decreased threshold or increased sensitization.

It should be noted, however, that, as shown in the section below on psychological explanations of disturbed behaviour, Solomon and Wynne did not exhaust the possible learning theory explanations for the persistence of the animal's behaviour.

The reorganization of neural circuits, according to Schaffer (1954) occurred in the relationship of the cortex to the lower cerebral centers. He recalled the similarity between animals whose behaviour was disturbed as a result of stressful conditions and those who have under-

gone surgical decortication. Both cases exhibit a heightened degree of emotional sensitivity to all stimuli (violent agitation, biting, trembling, struggling, vocalization, piloerection, mydriases, high pulse rate and blood pressure, irregular respiration, decreased control over micturation and defecation), and changes in both learning rates and learning ability. According to Schaffer, the relationship of the cortex to the lower cerebral centers in the normal animal is one of dominance of the cortex over the more primitive centers so that behaviour is more cortically determined. Under conditions calling forth emotional behaviour, the author proposed that subcortical mechanisms are activated and a "shift in emphasis occurs from the cortex to subcortical centers (p. 327)." The shift is brought about by inhibition of the physiological activity of the cortex by discharges from subcortical centers. The continued stressful conditions, therefore, result in a pathological state that may be regarded as a chronic disturbance in the relationship of the cerebral centers.

On the other hand, Liddell (1964) found that the disturbance associated with stressful conditions may lie in the balance between the sympathetic and parasympathetic divisions of the autonomic nervous system. Using a classical conditioning paradigm in a restraint situation (metronome signal followed by shock twenty times a day for a period lasting months), he produced disturbed emotional behaviour in sheep. The behaviour was characterized by rigidity and other manifestations of an overreaction of the parasympathetic system. Using a difficult differentiation paradigm, the author produced an emotional condition resembling an overreactivity of the sympathetic system. Either type of abnormal

behaviour was maintained for a long time (5 years in one case) and was manifested both in the experimental situation and in the barn and pasture. Thus a protracted stress situation led to a permanent disturbance of emotional reactions due to a chronic overreaction of a part of the autonomic nervous system, a view earlier expressed by Szasz (1950).

A similar conclusion was arrived at by Gellhorn (1963; 1969). He hypothesized that in the normal animal the autonomic divisions are reciprocally inhibited and that the immediate history of the animal predisposes increased activity of one division with increased reciprocal inhibition of the other (a process termed tuning). In a situation in which the reactivity of one system is quite high due to a strong tuning influence, continued stressful conditions may further facilitate the system to the point of massive discharge. In this circumstance, the overreactive system may 'spill over' into the antagonistic system, which instead of remaining suppressed, may now also discharge. For example, the author cited Richter's experiments on wild rats forced to swim in conditions in which the animals died within a brief period of time. The heart rate of the rats at first accelerated, but declined markedly later; at death the heart seized in diastole. Richter also found that cholinergic drugs aggravated and atropine retarded these effects. According to Gellhorn, the data suggested that intensive parasympathetic discharges were associated with states of strong, prolonged sympathetic excitation.

Further evidence that prolonged excitation of one autonomic division may 'spill over' into the antagonistic division was provided by experiments on dogs subjected repeatedly to inescapable shock. The

paradigm, which closely resembled Liddell's study quoted above, resulted in the same neurotic symptoms accompanied by "strong parasympathetic discharges (Gellhorn, 1963, p. 198)." Thus strong sympathetic discharges resulted in simultaneous, strong parasympathetic discharges. For the opposite situation, Gellhorn reported that in an inhibitory neurotic state, augmentation of the inhibitory (parasympathetic) discharge produced a simultaneous sympathetic discharge. For example, in human catatonia the prevalent parasympathetic inhibitory state is combined with an increased excretion of noradrenaline, indicating a concurrent sympathetic discharge. Similarly Pavlov's inhibitory dogs discussed above showed signs of general excitement during their periods of response inhibition.

Gellhorn summarized that under stressful conditions:

> "It seems that the more or less simultaneous activation of the two divisions of the hypothalamic system, which induces a prolonged or permanent shift in autonomic balance, is responsible for the production of experimental neurosis and that the type of imbalance determines its qualitative character (1963, p. 203)."

and:

> "Whereas reciprocal relations (of inhibition) exist between the ergotropic (sympathetic) and trophotropic (parasympathetic) systems in the normal organism, the reciprocity is absent in the neurotic state (1969, p. 293)."

A breakdown of inhibition as a product of stress was also considered by Malmo (1957), although he did not relate it to the autonomic balance. Malmo and Shagass (1952) found that neurotic patients exposed to pain-heat stimulation, a mirror drawing task, or a rapid discrimination task gave an exaggerated increase in blood pressure that did not level off as in the case of normal people. The authors suggested that

a regulatory mechanism was not functioning. Malmo (1957) stated that the regulatory process was an active inhibition of arousal measures mediated by a chemical transmitter in particular kinds of cells in the nervous system. According to the author, prolonged stress causes the chemical transmitter to lose effectiveness, resulting in a lasting state of overarousal characterized by a low threshold of arousal to all stimuli. Thus the emotional conditions resulting from prolonged stress could be considered a chronic disease of overarousal.

(b) Psychological Explanations of Disturbed Behaviour

Lang (1964) maintained that a condition of pathological anxiety may be produced by stress and that this condition may sustain itself beyond the stress situation by interoceptive stimuli conditioned for anxiety, such as phases of the digestive process (e.g., peristalsis). To substantiate his view he cited the experiments of Russian investigators who have used an externalized loop of intestine as the site of a conditioned stimulus.

According to Mednick (1958, 1959) not only conditioning, but also generalization is involved in the initiation and persistence of disturbed behaviour. He noted that schizophrenics show increased generalization and, in situations where irrelevant stimuli are controlled, they exhibit faster conditioning than normals. A Hullian concept was invoked to explain the observation: a stressful stimulus may induce a period of intense anxiety and this state contributes to drive strength. Heightened drive state increases the response strength of <u>any</u> habit tendencies that may be aroused in a given situation, which produces heightened generali-

zation responsiveness. The increased generalization gradient now provides many more stimuli that are anxiety producing. A vicious circle sets in, which results in a spiralling generalization gradient to both relevant and irrelevant stimuli. The result is a relatively permanent state of emotional hyperarousal. An escape hatch is provided when the patient withdraws from all anxiety producing stimuli, a move that reduces the spiralling anxiety. The withdrawal leads to a chronic state of hypoarousal (the 'flat effect' or emotionalessness of schizophrenia).

Similarly Wolpe (1958, 1966) also invoked primary and secondary generalization and conditioning. The more intense or poorly defined the stressful stimulus, the more stimulus aspects are likely to acquire some measure of anxiety conditioning. The gross generalization thus established as well as the failure of autonomic responses to generate reactive inhibition (Wolpe, 1966) would explain the extraordinary persistence of the disturbance.

Another possible explanation for the persistence of the disturbance comes from the work of Halasz (1961). A cat was taught to deflect a pendulum to forestall 80-volt shocks which began one minute after the onset of a buzzer CS. When the cat was performing the avoidance response immediately upon CS onset, the response was partially extinguished by terminating the CS after only 5 seconds after its onset - a period too short for the cat to complete the avoidance response. The cat was then given test sessions in which the CS was again left on for the animal to terminate or not at will, but no shock was delivered regardless of the cat's actions. On the first test trial the animal did not respond to the CS immediately, but finally did so as the CS duration approached one

minute. On succeeding trials the cat once again deflected the pendulum immediately upon CS onset. Halasz considered this reconditioning without UCS delivery as "second order reconditioning (p. 287)." His interpretation was that the 1-minute CS paired with the UCS also extended, by generalization, to briefer CS's. The brief, unreinforced CS presentations extinguished the CR to them without affecting the 1-minute CS. Finally, by induction, presentation of the still potent 1-minute CS resulted in reactivation of the previously extinguished brief CS's.

However, Gantt (1944, 1962) noted that a difficult auditory discrimination not only produced lasting marked abnormalities in dogs' emotional behaviour (high degree of excitation; refusal to eat), but that the condition increased overnight when the animal was supposedly resting. He accounted for the observation with the principles of schizokinesis and autokinesis. Schizokinesis referred to a discrepancy between general autonomic and specific or somatic components of an animal's adaptation to a stressful environment. Thus an animal may appear superficially undisturbed, but in the autonomic components of the response there may be violent agitation. Thus in normal dogs the heart rate conditional reflex was regularly found after only one reinforcement, while there may have been no accompanying motor reflex. Furthermore, the heart rate increase could not be extinguished. According to Gantt, the agitation in the autonomic components may maintain itself and increase through an internal development process, independent of stimulus control, which he termed autokinesis. The autokinesis accounted for the increase in the dogs' symptoms overnight and for the long duration of those symptoms.

Eysenck (1968) developed a theory of anxiety which appears to elaborate on Gantt's principle of autokinesis and to incorporate a spiralling generalization gradient notion similar to Mednick's and Wolpe's. Autokinesis (incubation in Eysenck's formulation) occurs as follows: all stimuli associated with an aversive stimulus situation become conditioned, producing conditioned responses of anxiety (e.g., autonomic responses such as heart rate change). These CR's, in turn, become stimuli that produce the anxiety reaction. A positive feedback loop is produced without the necessity of further CS-UCS combinations. The positive feedback loop is similar to the notion of a spiralling generalization gradient. The following case history served to clarify the theory:

> "Mr. X suffers from impotence on a particular occasion due to drink, fatigue, or illness; the CS's associated with the occasion produce fear/anxiety as a CR. On the next occasion these CR's follow upon $\overline{CS}$ (a CS not followed by the primary reinforcement) and cause reciprocal inhibition of sexual reflexes; this failure causes additional anxiety/fear CR's which produce an even stronger reaction the third time, thus setting in motion a positive feedback circle which continues without the necessity of a new UCS-CS combination (Eysenck, 1968, p. 313)."

One observation that led to the formulation of the theory was an experiment by Napalkov (1963). When a dog was presented various novice stimuli, the result was an increase in blood pressure of less than 50 mm. Hg., with complete adaptation after some 25 applications. A single conditioning trial, however, followed by repeated administration of the CS, but not the UCS, brought about final increases in blood pressure of 190-230 mm. Hg. The latter condition lasted up to a year.

From the above, one can delineate that a chronic disturbance of emotional behaviour can result from stressful conditions maintained for

a prolonged period of time.

As stated above, it may be fruitful to study the effects of stress using the correlates of emotion as did Brady (1969). The importance of demonstrating persistence of the effects of stress on autonomic measures even beyond termination of the stress period must be emphasized: both for the verification that stress is the determining factor, and for further research into the acquisition and elimination of the changes.

1.3

Choosing the Autonomic Indicators of Emotion for the Study

There are many alleged physiological indicators of emotion (Gellhorn, 1963, Lazarus, 1966; Sternbach, 1966). However, on the basis of the feasibility of their use with the rat (the experimental animal of this study) and for the reasons discussed below, the following measures were chosen for use in the study: (a) gastric acid secretion, (b) ulceration, (c) body weight, (d) thymus weight, (e) adrenal weight, (f) blood sugar level, and (g) circulating blood eosinophil level.

1.4

Gastric Acid Secretion and Ulceration

Ulceration of the upper digestive tract occurs among all peoples in all regions of the earth (Gallart-Mones, 1959; Kurokawa, 1959; Pulvertaft, 1958; Watkinson, 1958). Autopsy and X-ray studies have shown that 10% to 12% of all people at some time in their lives suffer from a gastric or duodenal ulcer (Eusterman, 1935), and the numbers are increasing (Gallart-Mones, 1959).

Yet less is known of the phenomenon of ulceration than almost any

other human disease (Robbins, 1967). Both the pathological development of ulcers and the relationship of psychological variables to gastric acid secretion and ulceration remain enigmas.

The current thinking on the problem is at the stage where conditions evoking emotional behaviour are associated with changes in gastric acid secretion and "all efforts are directed to the investigation of acid secretion alone (Wlodek, 1968a; p. 125)" in the study of ulceration.

However, as Dr, Wlodek pointed out:

> "Our knowledge of the physiology of gastric secretion has been increasing rapidly, but in spite of all the material published, the etiology and pathogenesis of peptic ulcer is a continuing mystery and the accepted principles of gastric secretion are still controversial and unresolved. It is as if an impasse had been reached (1968a; p. 125)."

Both a reexamination of previous findings and further experimentation are required to resolve the "impasse" that Wlodek spoke of. With this in mind experiment 1 was designed to use gastric acidity and ulceration in the rat as dependent measures; the effects of prolonged stress on these measures were examined, and the perserverance of any effects beyond the stress period was tested for.

Pathology: Acid-Ulcer Relationship

The importance of gastric acidity in the etiology of ulceration has long been recognized; in 1910 Schwarz enunciated the dictum "No acid - No ulcer." However, the specific relationship of the two variables has not been clear.

Puhl (1932) introduced 0.4%-0.6% HCl into the stomachs of dogs and produced degenerative and erosive changes in the stomach and duodenum. Mann and Bollman (1932) reported ulcers in dogs after about four weeks of

instilling 0.4% HCl into the stomach via gastric fistula. The acid was introduced for 8 hours daily at a rate of less than 1 cc. per minute.

On the other hand, Schmidt and Fogelson (1937) introduced 300 cc. of 0.36% HCl twice daily via esophageal fistual into the stomach of dogs which had been sham-fed for 50 days. After 10 days the acid concentration was increased to 0.5% and treatment was continued for 42 days. None of these dogs developed any lesions. Overgaard (1931) also reported no ulceration after giving dogs 350-400 cc. of 0.5% HCl once or twice daily for periods ranging from 5 weeks to 22 weeks.

The notion that acid causes ulcers has often led to the idea that ulcers in the upper gastrointestinal tract was due to hypersecretion of gastric acid (Wolf and Wolff, 1947; Brodie, Marshall, and Moreno, 1962). This conclusion is equivocal since the question of the relative roles of gastric secretion and various other potential ulcerogenic variables still require elucidation. Thus Stewart (1955) reported that of 116 human cases of gastric ulcer examined, 70% showed normo- or hypochlohydria. Levine et al. (1948, 1950, 1952) reported that although patients with duodenal ulcers were hypersecretors, those with gastric ulcers were hyposecretors. Similarly Wlodek (1968b) stated:

> "The association of duodenal ulcers with gastric hypersecretion and of gastric ulcers with hyposecretion is well established. Duodenal ulcer patients characteristically have greater acid output than normal persons. Individuals with gastric ulcers have an equivalent volume of gastric acid secretion, but the gastric acid concentration is greatly decreased (p. 483)."

It seems, therefore, that there are two distinct etiological factors involved in ulceration of the duodenum and gastric ulceration. The former may well be due to the action of high quantities of gastric acid; the latter, however, must involve other factors.

Use of the Rat in the Exploration of Ulcer Etiology

The rat is an animal commonly used in the attempt to experimentally reproduce human ulceration. The fact that the animals can be kept cheaply in limited space, and their high resistance to trauma and post-operative infections are the main arguments in favour of using rats.

However, as can be seen from the above discussion of acid-ulcer pathology, to properly evaluate the results of ulceration in rats in terms of the human disease, one must specify which analogue is being studied - human gastric ulceration or human duodenal ulceration. The ulcers produced in rats usually occur in the stomach and avoid the 'duodenum.' If the gastric ulceration in rats is to be studied as an analogue of human duodenal ulceration, a positive correlation between gastric acid secretion and ulceration extent must be demonstrated; while a negative correlation or no correlation would indicate that the rat ulcer may be similar to the human gastric ulcer, or unrelated to the human disease at all.

Experimental evidence in this area is vague. Levine and Senay (1970) reported a high positive correlation between increased acidity and ulceration in the rat. On the other hand, Boles and Russell (1970) found that although their experimental groups developed ulcers, acidity levels showed no concomitant changes.

Clarification of the conflict is essential. Experiment 1 was designed in part to test the acid-ulcer relationship in rats.

Extraneous Factors in the Study of Ulcerogenic Effects in the Rat

The following variables have been found to strongly influence

results in the study of gastric ulceration in rats:

(a) Rearing conditions (social effect): The results vary depending upon whether the animal has been raised individually or in a group. Rearing in a group sensitizes the animal to the ulcerogenesis as shown by Ader (1965), who obtained 82% of ulcers in rats reared in a group, and 66% in rats raised alone. Similar results were obtained by Stern et al., (1960).

The rats, therefore, should all be raised under strictly comparable conditions if comparable results are desired. Moreover, if greater generality of the results are desired, balanced groups involving rats with different rearing histories should be used;

(b) Genetic effect: a constitutional factor in susceptability to ulcer formation has been demonstrated by Sines (1959). He selected the sensitive animals of the Sprague Dawley race and submitted them to restraint stress. Results were as follows: unselected rats - 58% ulcers in the male and 68% ulcers in the female; selected group F1 - 79% in the male and 87% in the female; generation F2 - 92% in the male and 96% in the female; generation F4 - 100% ulceration.

Experimental groups should, therefore, be counterbalanced for the number of animals in each group from the same litter;

(c) Sex effect: as can be seen from Sines (1959) experiment cited above, there is a greater sensitivity to ulceration in the female rat. Thus one must assure that either only one sex is used in an experiment, or that proper counterbalancing is performed.

In order to increase the potential generalization of the results of the study, the groups were balanced for the extraneous variables

mentioned above, rather than restricting the animals to only one of each of the possible conditions mentioned.

Psychologic Effects on Acidity and Ulceration

Among the most frequent of the so-called "psychosomatic" disorders is the development of ulcerous pathology and physiological changes in gastric secretion in the gastrointestinal tract. It is alleged that these effects arise as a result of exposure to stress. However, the results of experimental investigation of the relationship of the physiological effects to psychological effects are highly equivocal; the literature contains many conflicting claims.

Wolf and Wolff (1947) with the aid of a subject who had a gastric fistula, carried out a pioneer investigation of the relationship between emotional stress and stomach secretions. They found that feelings of anxiety were associated with increases in gastric secretion and that fear associated with decreased acidity. In contrast, Coddington (1968), studying an infant with a fistual, found that gastric secretion rates decreased with all ingoing affects such as depression as well as fear.

Brodie, Marshall, and Moreno (1962) reported that chronic fistula rats restrained for 24 hours exhibited a significant increase in free and total acid concentrations, but no changes in free acid outputs. Yet Singh, Sharma, and Kar (1967), who reported ulceration in fasted guinea-pigs after being rocked to-and-fro at 200 movements per minute for only 1 hour, found that the volume of gastric juice secreted by these animals was high, but they did not differ in their free acid concentrations from controls; nor was any ulcer-acid correlation exhibited. Moreover, the

authors reported that in rats the gastric juice secretion was inversely proportional to the severity of the restraint.

Brady (1958) and Brady, Porter, Conrad, and Mason (1958) found that emotional stress produced in monkeys by a conditioned avoidance task on an intermittent schedule maintained for about three weeks led to both an increase in acid secretion and gastrointestinal lesions. They also noted that the increase in acidity occurred not during the periods of avoidance, but upon their termination. Norman (1969) also noted a trend towards suppressed acid secretion in humans during a 90-minute shock or shock-avoidance session, while the post-stress period was characterized by hypersecretion. On the other hand, attempts to duplicate the Brady (1958) study have been in vain (Foltz and Fay, 1964).

Several experimenters have reported the production of gastric ulcers in various animals in response to both chronic and acute stressful conditions: Weisz (1957) produced stomach lesions in rats subjected to intermittent shock, deprivation, and/or conflict for a period of 30 days; fasted golden hamsters developed gastric lesions after 4-7 hours of restraint (Arcari et al., 1968); Leveine and Senay (1970) produced stomach ulcers as well as increases in acid secretion by subjecting rats to restraint for 2 hours in a cold environment; and Fife (1970) reported lesions in the stomachs of fed rats subjected to a 48-hour period of restraint during which stimuli previously paired with shock were repeatedly presented. Contradicting these findings are: Pare (1962) - no ulcers found after conflict and/or shock conditions for 8 hours per day for four weeks (rats); Snapper, Shoenfeld, and Locke (1966) - no ulcers in rats after a conflict and food deprivation paradigm for 2 hours every

other day for 11 sessions; Pfieffer (1967) - light restraint of fed rats for periods up to 5 weeks failed to produce ulcers (rats fasted for 5 days straight did show some lesions); and Pare (1968) - no ulceration in rats after 24 days of unavoidable tone-shock presentations for 20 hours per day.

Recently Mikhail (1969) demonstrated that short exposure (18-24 hours) of rats to a conditioned fear stimulus in both a restraint and non-restraint situation produced neither gastric lesions nor changes in stomach acidity. This supported a much earlier finding by Mahl (1949) who reported no changes in secretion rates in dogs for the first five days of a conditioned fear paradigm. Beyond the 5-day period, the dogs showed marked hypersecretion of acid. On the other hand, Grecheshkina (1967) found that dogs exposed to noxious stimuli for 3-4 weeks show a marked decrease in gastric acidity. Furthermore, Mikhail (1970) has reported a similar decrease in acidity in rats in response to prolonged stress.

The above research gives little ground for optimism in the use of gastric acidity and ulceration as correlates of emotional behaviour. However, the stimulus-response specificity principles discussed below will prove useful in explaining the discrepancies.

1.5

Body Weight, Thymus Weight, Adrenal Weight, Blood Sugar Level, and Eosinophil Level

The situation of claim and counterclaim in the literature exists not only for the measures of acidity and ulceration, but also for the other dependent variables chosen for the study, as can be seen from the

brief outline given below.

Body Weight

A decrease in body weight as a result of shock-stress and conditioned aversive stimuli presentation was reported by Brady, Thorton, and DeFisher (1962). The decrease was noted after the first day of continuous presentation of the stressful conditions, and the weights continued to remain suppressed after a chronic period of the stress (12 days). Similarly, Hale (1964) found that rats subjected to electroconvulsive shock 8 times daily for 10-17 days weighed significantly less than controls that did not receive stress treatment.

Pare (1965) reported that rats subjected to tone-shock presentations for several days inhibit their water intake throughout the stress period. The results implied that loss of body weight in response to stress may be due to inhibition of consummatory behaviour. However, Ragusa, Shemberg, and Rasbury (1968) have shown that the decrease may be considered a physiological response to stress rather than purely consummatory. They found that of all rats equally deprived of food, those enduring shock-stress for 23½ hours lost significantly more body weight.

Contrary to the above, Weisz (1957) did not produce body weight loss in food deprived rats (partial deprivation) after 30 days of shock, conflict, and/or fear conditions. Nor did Boles and Russell (1970) find any weight loss with 36-hour restraint of rats.

Adrenal Weights

Selye (1950) reported that physical trauma and noxious stimuli resulted in an increase in adrenal weight. The increase was confirmed

in both acute stress situations: Jarratt and Nowell (1970) using cold stress for 24 hours; and chronic situations: Pare (1962) testing conflict and shock stress maintained for 8 hours per day for 4 weeks on rats; Nitschkoff, Krwizkaja, and Gnüchtel (1967) using noise stress for 5 minutes twice per day throughout 28 weeks (rats); Welch and Welch (1969) subjecting mice to 5-10 minute fighting periods for 14 days; and Jarratt and Nowell (1970) placing rats in a cold environment for 25-130 days.

However, Boutwell, Brush, and Rush (1948) found no adrenal weight changes associated with chronic deprivation of food. Snapper, Shoenfeld, and Locke (1966) produced a decrease in adrenal weight as a result of a conflict paradigm maintained for 2 hours every other day for 11 sessions: Pfieffer (1967) noted that young rats exhibited an increase in adrenal weight (calculated relative to body weight) after 5 days of light restraint, while no weight difference from controls existed after the 5 days; in mature rats no change in weight was observed at any time.

Thymus Weight

Selye (1950) reported a decrease in thymus weight in response to physical stressors. Support for this finding came from the Boutwell, Brush, and Rush (1948) and the Snapper, Shoenfeld, and Locke (1966) studies cited above. Both sets of experimenters noted that their respective stressful treatments resulted in decreased thymus weights in rats. Pfieffer (1967) contradicted the findings, reporting no difference in thymus weights for either young or mature rats after a chronic period of restraint greater than 5 days.

Blood Sugar Level

As far back as 1913, Loewy and Rosenberg had shown that an operation involving some pain increased blood sugar in dogs. Cannon (1920) reported experiments that confirmed the increase in blood sugar in response to pain, extensive handling, restraint, and fear. He stated:

> "... it is reasonable to conclude that just as in the cat, dog, and rabbit, so also in man, emotional excitement produces temporary increase in blood sugar (p. 75)."

These results and conclusions have been reaffirmed more recently by: Bodo and Benaglia (1938) - dogs subjected to chronic food deprivation; Gellhorn (1953, 1963) - rats exposed to sudden loud noise, and cats subjected briefly to barking dogs; and Telford (1968) - used lobsters removed for a brief period from water (a process that acts as a stressor just as an air breathing animal is under stress when placed under water).

However, Jarratt and Nowell (1970) noticed that acute (24 hours) periods of cold-stress produced hypoglycemia, which, upon continuation of the stress, converted to hyperglycemia for 25 days, and then returned to pre-stress levels regardless of how long beyond the 25 days the stress was maintained. Ter Haar, Van Riet, Thijssen, and Schwartz (1969) reported no changes in blood sugar levels in humans during or after acute work-stress. Pruett (1970) found that 6-hour periods of work-stress in humans led to decrease in blood sugar levels.

Eosinophil Levels

Selye (1950) proposed that eosinopenia (a decrease in circulating blood eosinophil count) is a highly sensitive and constant sign of the stress reaction. This was supported by Gabrilove (1950) - studying trauma;

Dreyfuss (1956) - with emotional stress; Kerr (1956) and McDonald and Yagi (1960) - mental stress; Gerber (1966) - sound stimulation; and Moyer and Bunnel (1960) and Gollender (1960, 1967) - shock stimulation.

On the other hand, Koltek (1970) found no difference in eosinophil levels in rats after a period of acute (3.5 hours) shock or light-shock presentations, while prolongation of the stressor to 24 hours duration resulted in eosinophillia (increased eosinophil level). Biro (1959, 1962, 1963) and McDonald and Yagi (1961) have verified the eosinophillic response with various stressors. Furthermore, Burton, Sluka, Besch, and Smith (1967) reported that chickens exposed to chronic (180 days) acceleration stress do not manifest any changes in circulating blood eosinophil levels.

As stated above, the principle of stimulus-response specificity may provide an explanation of the divergent results from the various researchers.

1.6

Stimulus-Response Specificity

The problem of the unidimensionality - or apparent lack of it - of the various physiological correlates of emotional behaviour in response to stress has plagued the psychophysiologist's measurement of the stress reaction.

The unidimensional approach states that stress will produce a generalized sympathetic response in all stressed animals; the correlates of emotion should all indicate activity in only one division of the autonomic nervous system. The unidimensional view was proposed originally

by Cannon (1920). He had shown that the same sympathetic nervous system activity occurred in three states - fear, anger, and responses to pain. He emphasized that only the sympathetic branch responded to stressful stimuli such as pain, extensive handling, restraint, and stimuli conditioned for fear; and that all sympathetic effectors showed equal maximum activity to meet emergency situations. More recently, Selye (1946, 1950) has supported Cannon's notion of a generalized response to stress, so much so that he termed the response a syndrome (i.e., an identically reproducable series of events in response to all kinds of stressors). Despite the varying kinds of stressors Selye employed (cold, exercise, physical trauma, injury, and injection of drugs), the same pattern of end-organ responses always occurred: discharge of adrenal cortical steroids, shrinking of the thymus gland, eosinopenia, and bleeding stomach ulcers. On the other hand, various other researchers began to differentiate patterns of responses in terms of the type of stressor used. Wenger and Cullen (1958) measured 9 autonomic variables in response to 14 different stimulus conditions (including electric shock and its anticipation, insertion of a needle, infusion of saline, adrenaline, and noradrenaline, exercise, hyperventilation, cold pressor, mental arithmetic, and carbon dioxide inhilation). The authors concluded that patterns of autonomic responses seem to vary with the kinds of stimuli which elicit them.

An extension of the Wenger and Cullen conclusion was the notion of "directional fractionation of response" named by Lacey (1959). The idea relates to instances in which the direction of change in one physiological variable is contrary to what one might expect under Cannon's

and Selye's unidimensional approach to the stress reaction. Thus Lacey noticed instances in which heart rate decelerated, but skin conductance increased. These findings were verified and extended by Lacey et al., (1963).

In the face of the low correlations between various end-organ responses, the concepts of stimulus and response specificity have been proposed (Lazarus, 1966; Sternbach, 1966). "These concepts imply that different autonomic reaction patterns are associated with specific threat or noxious stimulus (stimulus specificity) and, further, that different autonomic reaction patterns are consistently found in different individuals across different kinds of stress (individual response specificity), (Lazarus, 1966, p. 375)."

It would appear, then, that the contradictions in the literature on stress measurement arise in part from the stimulus-response specificity effects. The effects of individual response specificity that is constitutionally determined was studied by Lambert (1968). He studied the ulcerogenic effect of phenylbutazone in 210 rats of the Wistar race. All the rats were given an equal daily dose of phenylbutazone and were sacrificed on the tenth day. Although the animals were all of the same race, they came from several different sources. The results showed that the rats differed in their susceptibility to ulcerogenesis depending on which source they came from (Table 1). The experiment was repeated using restraint for 24 hours as a stressor on 690 rats of the Wistar race. Individual differences were again found (Table 2). In addition, stimulus specificity was demonstrated in the experiments. As can be seen in Tables 1 and 2, while only 6% of Lambert's own rats developed lesions

in response to injection, 43% did so with restraint.

The expectation that individual differences would manifest themselves even more across species was verified by Sines (1962). He observed formation of restraint ulcer in 20% of Sprague Dawley rats, 21% of August rats, 17% of Fisher's rats, and 64% of A.C. rats. Ader et al., (1960) also noted large differences in the ulcer score in Sprague Dawley, Long Evans, and Wistar rats.

Therefore, to make various results comparable, researchers must specify and attempt to equate as closely as possible both the stimulus and organism characteristics used in their respective experiments.

Corollaries from the above are as follows: (a) should the various measures used in this study indicate activity in alternate divisions of the autonomic nervous system, this would indicate a lack of support for the unidimensional view of the stress reaction; and (b) should the lack of unidimensionality be exhibited, one may also find similar incongruities in the persistence of the various changes resulting from the stress period (i.e., some measures recover upon removal of stress while others remain chronically changed).

1.7

Statement of the Purpose of the Study

The main purpose of this study was to ascertain the effects of prolonged exposure to stress upon certain alleged physiological concomitants of emotional behaviour, and to test for the perserverance of these effects beyond the stress period. Two experiments were designed to carry out the above, the first measured gastric acid secretion and ulceration,

Table 1

Phenylbutazone Ulcers

	# Treated	Developed Ulcers		Died	
	#	#	%	#	%
Own Rats	45	3	6	7	15
Source 1	45	21	46	27	60
Source 2	40	10	25	15	37
Source 3	80	17	21	39	48
Total	210	51	24	88	42

(From Lambert, 1968)

Table 2

Restraint Ulcers

	# Treated	Developed Ulcers	
	#	#	%
Own Rats	160	70	43
Source 1	100	40	40
Source 2	124	101	81
Source 3	79	60	76
Source 4	63	48	76
Source 5	106	64	60
Source 6	58	46	79
Total	690	429	62

(From Lambert, 1968)

and the second used body weight, adrenal weight, blood sugar level, and eosinophil level as the physiological measures.

In addition to the main purpose, the study attempted to deal with the following problems:

(a) To see whether the measures being used responded unidimensionally to stress or not;

(b) To determine whether prolonged stress leads to an increase or a decrease in gastric acidity and/or ulceration in the species of animal used, and to find out the relationship between gastric acidity and ulceration in the rat in order to assess whether it may be an analogue of human gastric ulcer or human duodenal ulcer; and

(c) To check the Pare (1965) finding that rats under stress inhibit water intake and that this consummatory behaviour may influence the physiological measures.

The experimental animals were exposed to prolonged stress, and physiological measures were taken at the end of the stress period and after a period of rest (14 or 20 hours) beyond the stress period. Measures that changed in response to stress, but recovered to control levels during the rest period were termed acute effects of stress, while those in which the changes persisted were termed chronic effects.

CHAPTER 2

EXPERIMENT 1

2.1

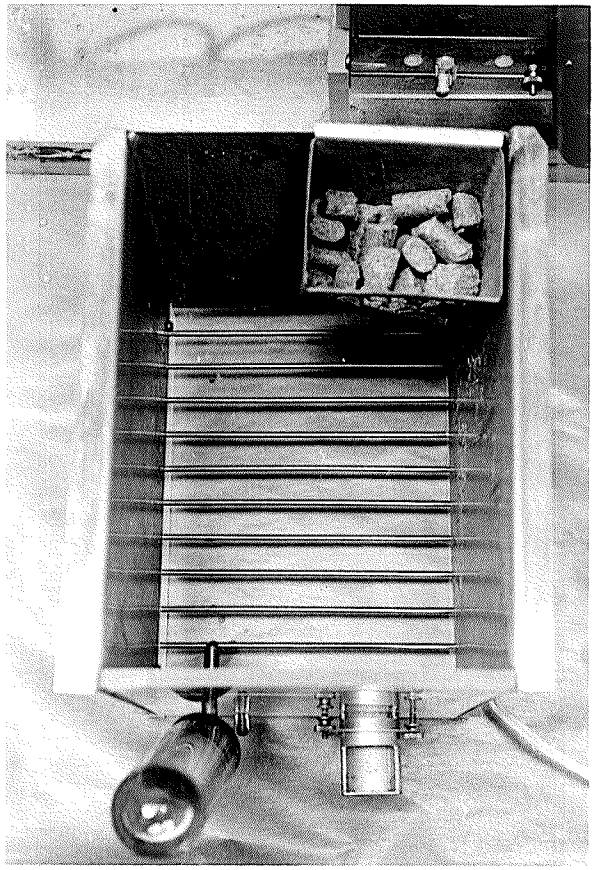
Subjects

Forty-four albino rats of the Sprague-Dawley strain (22 male, 22 female) age 90-100 days at the start of the experiment were used. All were bred and raised at the University of Manitoba: 12 males and 12 females were raised one to a cage; the rest were raised two of the same sex to a cage. Each rat was assigned to one of four groups of eleven subjects each. Each group was balanced with the others for weights of the animals, the number of animals from each litter (genetic effects), the number of animals of each sex, and the number of animals raised alone or in pairs (social effects).

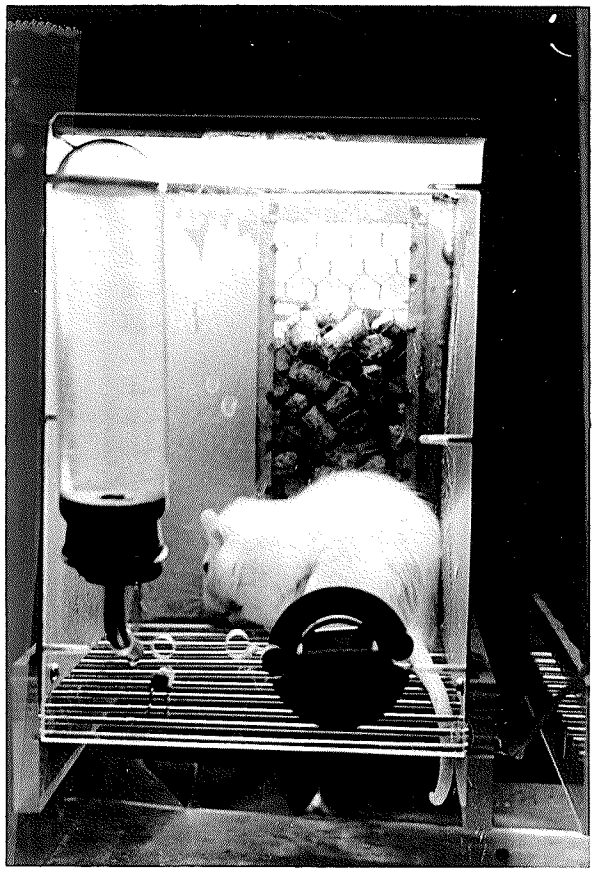
2.2

Apparatus

The conditioning cages had three sides of stainless steel and a plexiglass front. They measured 9 x 6 x 9 inches. The floor of each cage consisted of an electrified grid of metal bars 1/8 inch in diameter and 1/4 inch apart, through which shock was administred (Figures 2 and 3). Each cage was covered by a sheet of milk glass through which a 60 watt electric bulb homogeneously transmitted a light stimulus of 27 foot-candles measured from the reflectance of a Kodak standard 18% grey card held at 45° to the front of the cage at the level of the grid floor. An automatic programmer was used to maintain the animals on their respective schedules.



Conditioning Cage (Top View)



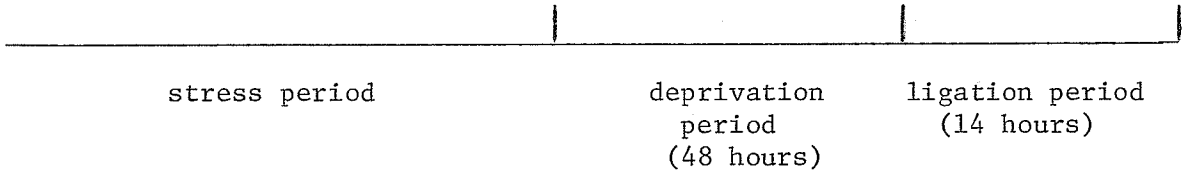
Conditioning Cage (Front View)

2.3

Procedure

During the course of the experiment Ss were subjected to their respective treatments in quartets, one from each group being studied simultaneously.

The temporal paradigm of the experiment may be diagrammed as follows:



The four groups of animals were classified as: Group I - continuously stressed group; Group II - recovery group A; Group III - recovery group B; and Group IV - control group. The continuously stressed group received stress treatment throughout the stress, deprivation, and ligation periods. The group served to determine if stress had any effect on the variables being studied. Recovery groups A and B were used to see whether the effects of stress were apparent even after a 14-20-hour recovery period. Therefore recovery group A was subject to stress treatment during the stress and deprivation periods, but not during ligation, and recovery group B received stress treatment during the stress period and for the first 42 hours of the deprivation period only. The control group received no stress treatment and served as a comparison to the other groups to see if the stress and/or recovery cycles resulted in changes from the measures obtained from animals that had not undergone experimental manipulation.

Starting from 7:00 P.M. on the day scheduled for the initiation of stress treatment, the room housing the conditioning cages was darkened. For five consecutive 24 hour periods, Groups I, II, and III received 1-minute presentations of light (CS) of which 50% were randomly followed by a 2.0 ma. shock of 0.25 second duration. The interval between each CS presentation was 5 minutes. Group IV animals received no treatment. All animals were fed and watered ad libitum during this period.

Beginning at 7:00 P.M. on the fifth day, all the rats were deprived of food (but not water) for 48 hours (deprivation period). CS-shock treatment was maintained throughout this period for the continuously stressed group and recovery group A; recovery group B Ss received the CS-shock presentations for the initial 42 hours of the deprivation period and were then returned to their home cages without food.

At the end of the deprivation period, the technique of pylorus ligation (Shay and Kamarov, 1945) was applied to all Ss. Under light ether anaesthesia, the stomach was reached by a 2 cm. ventral mid-line incision and a cotton thread ligature was tied around the pylorus. After wound closure with individual sutures placed 1/8 inch apart, the wound was covered with a colloid.

The Ss were deprived of both food and water for the 14 hours of pylorus ligation. During this period of ligation, Group I rats were maintained in the stress situation (CS-shock presentations); all other rats were returned to their home cages. At the end of this period all the animals were anaesthetized with ether, the esophageal entrance to the stomachs was ligated with cotton thread, and the animals were sacri-

ficed by ether overdose. This method of termination was used to prevent the regurgitation of stomach contents upon death. The gastric juice was collected in a graduated centrifuge tube and the stomachs were opened along the outer curvature and examined for ulceration.

The 14-hour gastric juice sample was centrifuged for 5 minutes, then filtered through No. 1 Whatman filter paper. Free and total acidity (normality) were determined by titrating a 1.0 ml. sample of the filtrate to a pH of 4.1 for free acidity and 8.5 for total acidity with 0.01 N NaOH, using a Coleman Metrion III pH meter. The 14-hour free and total acid outputs were calculated in milliequivalents by multiplying the total gastric juice volume (in ml.) by the free or total acid concentrations (acidity in milliequivalents per ml.).

Stomach ulceration was rated on a relative scale of 0-4 points, from least ulceration to worst respectively (Lambert, 1968). Ratings were performed by two observers, one of whom was naive as to which animal the stomach being rated belonged. In case of disagreement (one occurrence only) the rating of the naive observer was taken.

Description of an Ulcer

An ulcer is a circumscribed area of loss of tissue. The ulcer is a penetrating process beginning in the mucosa and invading the deeper layers of gastric or duodenal wall, perhaps perforating the wall completely. The lesion usually has a sharp, clean-cut border and is surrounded by normal or slightly inflamed mucosa. The border may be flat, but it is usually elevated and rounded owing to extensive edema of the submucosa. The floor of an ulcer is usually clean. It is covered by a

thin layer of fibrinopurulent exudate and the base consists of granulation and fibrous tissue.

Evidence of extension of the ulceration and of healing usually are evident at the same time. Ulceration may progress in one part of a lesion while healing is progressing in another. If ulceration is dominant, perforation ensues; if healing is dominant, scar tissue proliferates and normal mucosa returns.

2.4

Results

The mean volume of total gastric secretion for the 14-hour ligation period was calculated, as well as the mean values per group for free and total acidity and free and total acid outputs. The mean ulceration rating per group was also calculated. One animal died as a result of ether overdose and was not included in the results.

A Kruskal-Wallis one way analysis of variance was used to determine overall differences between groups on each of the dependent variables. Free acidity, free acid output, and ulceration were found to differ significantly across groups at the .05 level of probability. The results are summarized in Table 3.

For the variables demonstrating significant differences on the Kruskal-Wallis test, individual means of the continuously stressed group and recovery groups A and B were compared to the control group. The Group I - Group IV comparison showed whether the continuous stress had any effect on the variable being studied; while the Group II - Group IV and the Group III - Group IV comparisons showed whether the

measures had returned to pre-stress levels or if a difference still remained. The comparisons were made by the Mann-Whitney U-test, corrected for ties and adjusted for multiple comparisons by the Dunn method (Ferguson, 1959; Siegal, 1956; Kirk, 1968).

The results are summarized in Table 4. Free acidity and free acid output were significantly depressed as a result of stress while ulceration markedly increased. The acidity and acid outputs no longer differed from controls on the recovery group A - control group, and recovery group B - control group comparisons while ulceration ratings were the same for the experimental and control rats on the recovery group B - control group comparison.

A Spearman's coefficient of rank correlation was calculated to establish the relationships between total acidity and ulceration, total acid output and ulceration, free acidity and ulceration, free acid output and ulceration, and total gastric juice volume and ulceration. The student's t-test was used to test the hypothesis that each correlation coefficient equaled zero. As shown in Table 5, the free acidity and free acid output as well as the total acidity exhibited significant inverse relationships with ulceration.

The author also noted that in all cases of ulceration except one, the ulcers occurred only in the rat's forestomachs. The latter is simply a reservoir covered with an esophageal type mucosa and does not secrete any acid. In the one exception, major ulceration occurred in the forestomach, but there were several large lesions in the glandular region as well.

Table 3

Group Means for Gastric Secretion Measures and Ulceration in Rats After Exposure to Prolonged Stress

Group	Continuously Stressed	Recovery A	Recovery B	Control	H & p
Free Acidity (meq/l)	.05	.06	.08	.09	H = 9.0 p = .03
Total Acidity (meq/l)	.09	.10	.12	.11	H = 7.4 N.S.
Free Acid Output (meq)	.53	.73	1.00	1.26	H = 9.8 p = .02
Total Acid Output (meq)	.92	1.15	1.46	1.63	H = 7.2 N.S.
Volume of Gastric Secretion (ml)	10.03	11.75	12.64	13.89	H = 5.0 N.S.
Ulceration Rating	2.55	2.45	2.18	1.30	H = 8.2 p = .04

Table 4

Comparison of Individual Group Means for Variables Showing Significant Overall Differences with the Kruskal-Wallis Test

Variable	Comparison		
	Continuously Stressed - Control	Recovery A - Control	Recovery B - Control
Free Acidity	$p < .05$	N.S.	N.S.
Free Acid Output	$p < .05$	N.S.	N.S.
Ulceration	$p < .05$	$p < .01$	N.S.

Table 5

Spearman's Coefficient of Rank Correlation for Ulceration Versus (a) Volume Gastric Juice, (b) Free Acidity, (c) Free Acid Output, (d) Total Acid OUtput, and (e) Total Acidity

Ulceration versus	rho	t	p
(a) Volume Gastric Juice	-.02	- .14	N.S.
(b) Free Acidity	-.45	-3.23	<.01
(c) Free Acid Output	-.41	-2.99	<.01
(d) Total Acid Output	-.21	-1.37	N.S.
(e) Total Acidity	-.37	-2.56	<.05

CHAPTER 3

EXPERIMENT 2

3.1

Subjects

Twenty-four albino rats (12 male, 12 female) of the Sprague-Dawley strain aged 80-90 days at the start of the experiment were used. All were bred and raised at the University of Manitoba: six males and six females were raised one to a cage; the rest were raised two of the same sex to a cage. Each rat was assigned to one of three groups of eight Ss each. The groups were balanced for sex, mode of raising, number of animals from the same litter, and weight.

3.2

Apparatus

The conditioning boxes and the programmer were identical to those used in Experiment 1. To determine the number of circulating blood eosinophils, Certified Fisher white blood cell diluting pipets and the Fuchs-Rosenthal counting chamber were used. Blood glucose was determined in 25 ml. glass volumetric flasks and sugar levels were established on a Klett-Summerson photoelectric colorimeter using quartz cuvets.

3.3

Procedure

During the course of the experiment, Ss were subjected to their respective treatments in triads, one from each group being studied simultaneously.

Starting from 7:00 P.M. on the day scheduled for stress treatment, the room housing the conditioning cages was darkened. A blood sample was drawn from the tail of each animal at this time. For the next consecutive seven 24-hour periods, Group I (continuously stressed group) and Group II (recovery group) animals received 1-minute CS presentations of light of which 50% were randomly followed by a 2.0 ma. shock of 0.5 second duration. The interval between each CS presentation was 5 minutes. Group III animals received no stress treatment and served as controls. All animals were fed and watered ad libitum throughout the experiment, and 24-hour water intakes were recorded daily.

At 7:00 P.M. on the seventh day, the recovery group was removed from the stress situation for a 14-hour period of rest. Group I was maintained on stress treatment during this period. At the end of the 14 hours, a blood sample was drawn from each rat's tail and the animals were sacrificed by ether overdose.

Blood Sampling and Analysis

With the rat under light ether anaesthesia, the tail was washed in warm water to avoid sluggish flow or rapid clotting, then dried. The tip of the tail was cut and the blood allowed to drip freely. A drop of blood was drawn into the blood diluting pipette for the circulating eosinophil count. The sample was treated as outlined by Miale (1967) (see Appendix A). The counting procedure outlined by Wintrobe (1956) was followed, using the Fuchs-Rosenthal counting chamber.

A 0.5 ml. blood sample was also taken for blood sugar analysis. A 1:10 protein free filtrate was prepared by mixing the 0.5 ml. blood

sample with 0.5 ml. of 10% sodium tungstate, 3.5 ml. distilled water, and 0.5 ml. of 2/3 N H_2SO_4. After shaking well in a stoppered test tube, the solution was left standing for 10 minutes and was then filtered through a No. 1 Whatman filter paper. The determination of the blood glucose level was then performed according to the Folin-Wu (1920) method (see Appendix B).

Autopsies

After the animal had been sacrificed, a ventral mid-line incision was made extending from the level of the sternohyoideus muscle, through the sternum, to the sacrum. The abdominal viscera were drawn out of the way and the adrenals excised one at a time. Each adrenal was cleaned of fat and adhering tissue on wax paper, and weighed within 2-3 minutes after removal on an electronic scale to the nearest 0.01 mg.

The thymus was removed by blunt dissection, cleaned on wax paper of adhering fat and thyroid tissue, and weighed to the nearest 0.01 mg.

3.4

Results

The mean adrenal weight of each animal was determined and the mean weights of the adrenal glands and the thymus glands for each group were calculated. The mean weights per group of the glands relative to the animals' body weights (organ to body weight ratio) were also calculated.

The blood sugar levels at the time of death were recorded. In addition, the percentage changes of the eosinophil counts, blood sugar levels, and body weights from the beginning of the experiment to the time of death were determined.

A Kruskal—Wallis one way analysis of variance was used to ascertain overall differences on each of the above variables. Where overall significance was indicated, the means of the continuously stressed and recovery groups were individually compared to the control group using the Mann-Whitney U-test corrected for ties and multiple comparisons (see Results section in Experiment 1). The Group I - Group III comparison showed whether any change had occurred as a result of stress, and the recovery group - control group comparison showed whether stress and a period of rest resulted in measures equal to animals that had not undergone experimental manipulation, or whether a change persisted.

As can be seen from Tables 6 and 7, thymus weight (both relative and absolute), change in eosinophil count, and change in body weight were all suppressed by the stressful conditions. None of these measures returned to the level of the controls after a period of rest. On the other hand, adrenal weight and blood sugar measures were not significantly affected by the prolonged stress.

The mean 24-hour water readings per group are shown in Figure 4. A Kruskal-Wallis analysis of variance revealed that the difference in water intake during the first 24 hours of stress was significant at better than the .01 level of probability. Mann-Whitney U-tests indicated that the difference was between the experimental groups and the control group, while the two experimental groups did not differ from each other. There were no significant differences on any other day of the stress schedule. The latter is contrary to the findings of Pare (1965).

Mean Values for Adrenal Weight, Thymus Weight, Respective Organ/Body Weight Ratios, Percentage Change in Eosinophil Counts, Blood Sugar Level at Death and Percentage Change in Blood Sugar Level, and Percentage Change in Body Weight in Rats After Prolonged Stress

Also Results of the Kruskal-Wallis Tests

Group	Continuously Stressed	Recovery	Control	H & p
Thymus Weight (mg.)	359.48	322.76	490.97	H = 9.554 p <.01
Thymus/Body Weight Ratio	1.37	1.41	2.10	H = 10.16 p <.01
Adrenal Weight (mg.)	26.82	28.16	29.26	H = 0.06 N.S.
Adrenal/Body Weight Ratio	.11	.13	.13	H = 0.85 N.S.
% Change in Eosinophil Counts	24.10	3.15	146.80	H = 9.54 p <.01
Blood Sugar at Death (mg. %)	91.90	89.50	107.30	H = 2.3 N.S.
% Change in Blood Sugar	12.18	11.59	61.75	H = 4.2 p N.S.
% Change in Body Weight	-1.75*	-0.34*	7.80	H = 12.8 p <.01

*A negative value indicates a decrease in the value of the variable.

Table 7

Comparisons of Individual Means For Variables Showing Significance on the Kruskal-Wallis Test

Variable	Comparison	
	Continuously stressed - Control	Recovery - Control
Thymus Weight	$p < .05$	$p < .05$
Thymus/Body Weight Ratio	$p < .05$	$p < .05$
% Change in Eosinophil Counts	$p < .05$	$p < .05$
% Change in Body Weight	$p < .05$	$p < .05$

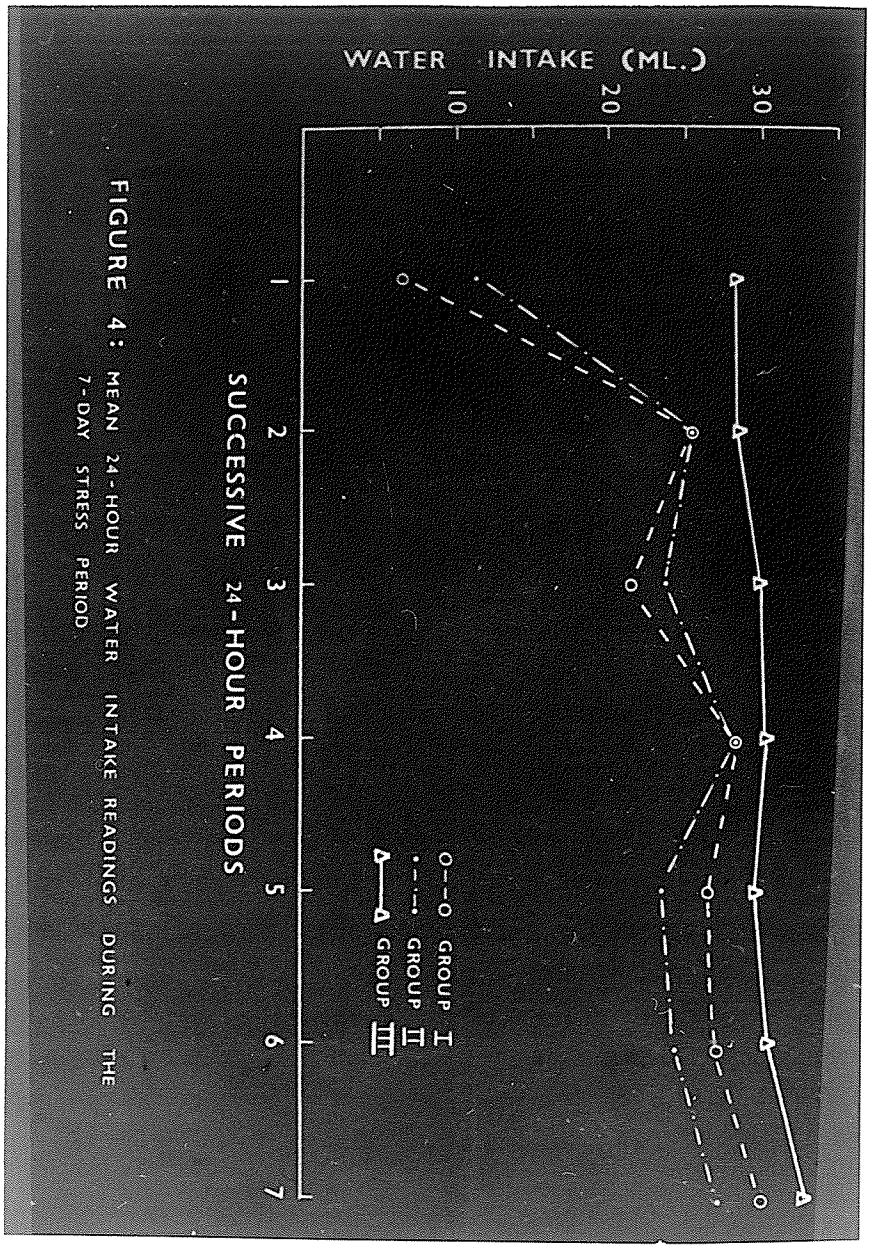


FIGURE 4: MEAN 24-HOUR WATER INTAKE READINGS DURING THE 7-DAY STRESS PERIOD

DISCUSSION AND CONCLUSIONS

The findings of this study can be summarized as follows:

(a) The results of Experiment 1 indicate that gastric acid secretion in the rat is suppressed during the stress period. Acid levels in animals that have received a rest period following stress, however, cannot be distinguished from those in control rats. The decrease in acid secretion during stress is consistent with the results of Greschiskina (1967) who used a noxious stimulus paradigm on dogs, and Mikhail (1970) who used a similar paradigm and rat strain as that used in this study. Furthermore, the return of the acidity level in the stressed rats to the level of the control rats during rest confirms the results of Mahl (1949).

(b) In Experiment 2 the prolonged stress resulted in decreased blood eosinophil counts, body weights, and thymus weights, while no effects were produced on the adrenal weights or blood sugar levels. Furthermore, the decreased eosinophil levels, body weights, and thymus weights were maintained beyond the stress period. Water consumption was depressed during the first 24-hour period of stress, and returned to the level of the control rats thereafter.

The lack of change in the adrenal weights agrees with Pfieffer's (1967) results, whereas the decrease in thymus weights is contrary to his findings. The thymus weight decrease agrees with the results of Selye (1950), Boutwell, Brush, and Rush (1948), and, more recently, Snapper, Shoenfeld, and Locke (1966). It appears that the light restraint paradigm used by Pfieffer produces effects that are distinct

from the effects resulting from unavoidable light-shock pairings, food deprivation, noxious stimuli, or conflict paradigms.

The lack of change in the blood sugar levels is contrary to the findings of Jarratt and Nowell (1970), who predicted an initial decrease followed by an extended period of hyperglycemia.

The persistent decrease in the blood eosinophil count is contrary to the results of Koltek (1970). The latter used the same strain of rats and a similar paradigm as that used in this study to produce eosinophillia at 24 hours of continuous stress. The results of this study indicate that the eosinophillic response noted by Koltex may occur only at the 24 hour point during a period of continuous stress, or it may represent some rhythm in the eosinophil response to stress. Another explanation may be that the eosinophillic response was the result of sensitization to the blood sampling technique which wore off after the seven day period of stress employed in this study.

Conclusions:

(1) The results of the Experiments 1 and 2 indicate that physiological measures do not react to stress unidimensionally. Whereas body weight, thymus weight, eosinophil levels, and gastric acidity levels decreased as expected with increased activity of the sympathetic division of the autonomic nervous system, the adrenal weights and blood sugar levels did not change. Furthermore, while some changes persisted during the recovery period (e.g., eosinophil level), others such as gastric acidity returned to control levels.

(2) The removal of the prolonged stress in Experiment 1 led to a rapid return of the acidity levels to the level of the control animals. This lack of persistence of this measure indicates that disassociation from stressful conditions might aid the therapy of disease brought on by stress-induced changes in gastric acid secretion.

(3) The pattern of acid-ulcer relationships in Experiment 1 are similar to those found in humans suffering from gastric ulceration. While the volume of gastric secretion did not change under stress, the concentration of the acid in the secretion was markedly reduced and ulceration increased. Thus the volume of gastric secretion showed no correlation with ulceration while free acidity and free acid output showed a significantly negative relationship with ulcer extent.

Although it appears that the gastric ulcer in the rat is an analogue of the human gastric ulcer, a word of caution is in order about the use of the pylorus ligation technique in the study of the ulcerogenic effects of stress. As noted in the results of Experiment 1, the ulcers occurred in the forestomach. This area is covered with a malpighian mucosa similar to the esophagus and contrasts with the corpus of the stomach which is thicker and contains acid and mucous secreting apparatus. Because of its structure, lesions in the forestomach cannot be considered true gastric ulcers directly equivalent in comparative pathology to human peptic ulcers. Only when comparable results in humans and in the glandular region of rat stomachs are exhibited can the findings be generalzied with any confidence. This view is also expressed by Lambert (1968). Therefore, although rat ulceration may

be studied using the pylorus ligation technique as a measure of the stress reaction in the rat or for pharmacological studies on the effects of antacid chemicals, its use as an analogue of human ulceration may be inappropriate.

(4). The results of Experiment 2 indicate that water consumption was not severely interfered with by the prolonged stress. Contrary to Pare's (1965) results, the stressed rats showed a decreased water intake only during the first 24-hour period and returned thereafter, to the level of the control animals for the remaining six 24-hour periods of stress. It appears, then, that the influence of this variable on the physiological measures in prolonged stress studies is negligible.

(5) In Experiment 2 the author noticed no overt signs of distress displayed by the animals receiving a rest period. Since some chronic effects of stress on certain autonomic variables were demonstrated, the findings indicate support for Gantt's (1944, 1962) principle of schizokinesis; namely that a discrepancy develops between the general autonomic and the specific or somatic components of an animal's adaptation to a stressful environment.

SUMMARY

Two experiments were conducted to assess the effects of prolonged stress on gastric acidity, ulceration, blood eosinophil count, blood sugar level, body, adrenal and thymus weight, and water consumption; and to determine whether any effects persisted beyond the stress period. A total of sixty-eight albino rats were exposed to a prolonged session of CS (light) presentations every five minutes, 50% of which were randomly paired with unavoidable shock.

The results indicated that: (a) in the pylorus ligated rats gastric acidity decreased during stress. Animals that received a rest period following stress did not differ from control animals. Ulceration occurred only in the rumen of the rat's stomach; (b) adrenal weight and blood sugar level did not change in response to stress; (c) eosinophil count, thymus weight, and body weight decreased both during and following stress; and (d) water intake was suppressed during the first 24 hours of the stress period only.

It was concluded that:

(1) The autonomic aspects of emotion show a non-uniform pattern of activity both during stress or during a post-stress period of rest;

(2) Removal of stressors may aid in the therapy of human ulceration;

(3) Because of the location of the ulceration in the rat's stomach, extrapolation of ulcerogenesis in pylorus-ligated rats to human ulceration may be inappropriate;

(4) The results support the principle of schizokinesis since overt signs of anxiety were not noted in animals receiving a post-stress rest period although some autonomic effects of stress persisted; and

(5) Water consumption was not severely interfered with, and the influence of water intake on other measures in prolonged stress studies may be minimal.

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APPENDIX A

Treatment of Blood For Eosinophil Counts - Miale Procedure

An acid dye (phloxine) is used to stain the eosinophils, propylene glycol is used to lyse the erythrocytes, and sodium carbonate is used to accelerate staining.

Diluting fluid:

Propylene glycol	50 ml.
Distilled water	40 ml.
Phloxine (1% aqueous)	10 ml.
Sodium carbonate (10% aqueous)	1.0 ml.

Filter and store at room temperature. Discard after one month.

Procedure

1. Draw blood to the 1.0 line in a white blood cell diluting pipet.
2. Fill to the 11 line with diluting fluid.
3. Shake very briefly and let stand for 15 minutes.
4. Shake for 30 seconds. (In Experiment 2 shaking was done by hand in a figure eight motion.)
5. Fill a standard counting chamber and count.

APPENDIX B

Blood Sugar Analysis - Folin-Wu Procedure

Chemical Reagents

(a) Alkaline copper reagent

Dissolve 40 gm. of anhydrous sodium carbonate in 400 ml. of distilled water, and transfer to a 1 liter volumetric flask. Add 7.5 gm. of tartaric acid, and when this has dissolved add 4.5 gm. of crystallized copper sulfate ground to a fine powder and make up to the mark with distilled water.

(b) Phosphomolybdic acid color reagent

Place 35 gm. molybdic acid and 5 gm. sodium tungstate in a liter beaker. Add 200 ml. of a 10% sodium hydoride solution and 200 ml. of distilled water. Boil vigorously for 20 - 40 minutes. Cool, dilute to about 350 ml. and add 125 ml. of 85% phosphoric acid. Dilute to 500 ml. and mix well.

(c) Standard glucose solution

Stock Standard: Dissolve 1.0 gm. of the highest purity anhydrous dextrose in 50 ml. of a filtered saturated solution of benzoic acid in water, and make up to the mark of a 100 ml. volumetric flask with more of the saturated benzoic acid solution. This solution keeps indefinitely.

Working Standard: Transfer 2.0 ml. of the stock standard solution to a 100 ml. volumetric flask and make up to the mark with water. Mix well. Of this solution, 1.0 ml. corresponds to a 1:10 filtrate of blood containing 100 mg. % of blood sugar.

Procedure

(a) Blood filtrate. In a 25 ml. volumetric flask place 1 ml. of the 1:10 protein-free blood filtrate and 1 ml. of alkaline copper reagent. Mix by light lateral shaking and place in a boiling water bath for 6 minutes. Remove (without shaking) and cool in cold water for 3 minutes. Add 1 ml. of phosphomolybdic acid color reagent. Let stand for 4 minutes, then dilute to the 25 ml. mark with distilled water. Mix well by 10 repeated inversions, and allow to stand for 10 minutes. Transfer a portion of the colored solution to a clorimeter tube and read with a blue filter against a blank solution set at 0.

(b) Blank. Run a parallel determination as described for the blood filtrate using 1 ml. of distilled water in place of the blood filtrate. Transfer a portion of the final solution to a colorimeter and set the colorimeter to its 0 reading against this solution.

(c) Standard. Run a determination as described for the blood filtrate, but use 1 ml. of the working standard solution of glucose instead of the blood filtrate. Read a portion of the final colored solution in a colorimeter against the blank set at 0.

Calculations

(a) Folin-Wu blood sugar = $\frac{100}{\text{reading for standard}}$

factor

(b) Blood sugar level in sample = reading for unknown X Folin-Wu

(mg.%) blood sugar factor

APPENDIX C

EXPERIMENTAL DATA

Table 1

Experiment 1 - Ulceration Ratings

Subject/Group	1	2	3	4
1	2	3	2	1
2	3	2	2	1
3	2	1	3	2
4	0	3	2	0
5	4	3	1	1
6	3	4	2	1
7	3	3	4	1
8	2	3	3	1
9	4	0	1	3
10	1	2	1	2
11	4	3	3	died
Mean:	2.55	2.45	2.18	1.3

Table 2

Free Acidity
(meq./ml.)

Subject/Group	1	2	3	4
1	.0983	.0388	.0701	.1253
2	.0518	.0540	.0875	.0724
3	.0942	.0832	.1037	.1096
4	.0684	.0396	.1015	.1059
5	.0000	.0761	.0984	.0932
6	.0430	.0580	.0789	.1128
7	.0456	.0782	.0743	.0636
8	.0902	.0911	.0327	.0604
9	.0221	.1030	.1105	.0803
10	.0124	.0470	.0188	.0495
11	.0000	.0134	.0961	
Mean:	.0478	.0620	.0793	.0873

Table 3

Free Acid Output (meq)

Subject/Group	1	2	3	4
1	1.1206	.4268	.6239	2.5937
2	.7304	.6480	1.0413	.6806
3	1.1021	.9318	1.6903	1.8742
4	.6361	.4158	1.3398	1.0378
5	.0000	.8599	1.1611	.8574
6	.4472	.5220	.6707	1.9966
7	.5472	1.1026	1.1145	.6551
8	1.0012	.8928	.5265	1.0026
9	.1746	1.4935	1.2266	1.3410
10	.1203	.5828	.2162	.5643
11	.0000	.1809	1.4127	
Mean:	.5345	.7324	1.0021	1.2603

Table 4

Total Acidity
(meq./ml.)

Subject/Group	1	2	3	4
1	.1231	.1004	.1059	.1415
2	.0869	.1024	.1069	.0972
3	.1198	.1070	.1588	.1361
4	.1065	.0921	.1257	.1329
5	.0679	.0960	.1599	.1164
6	.0993	.0912	.1149	.1349
7	.0791	.1069	.0996	.0892
8	.1130	.1139	.0804	.0817
9	.0740	.1278	.1412	.1280
10	.0515	.0792	.0495	.0817
11	.0431	.0525	.1233	
Mean:	.0877	.0972	.1151	.1140

Table 5

Total Acid Output (meq)

Subject/Group	1	2	3	4
1	1.4033	1.1044	.9425	2.9291
2	1.2253	1.2288	1.2721	.9137
3	1.4017	1.1984	2.5884	2.3273
4	.9905	.9671	1.6592	1.3024
5	.6722	1.0848	1.8868	1.0709
6	1.0327	.8208	.9767	2.3877
7	.9492	1.5454	1.4940	.9188
8	1.2543	1.1162	1.2944	1.3562
9	.5846	1.8531	1.5673	2.1376
10	.4996	.9821	.5693	.9314
11	.1207	.7088	1.8125	
Mean:	.9213	1.1464	1.4603	1.6275

Table 6

Volume of Gastric Secretion
(ml.)

Subject/Group	1	2	3	4
1	11.4	11.0	8.9	20.7
2	14.1	12.0	11.9	9.4
3	11.7	11.2	16.3	17.1
4	9.3	10.5	13.2	9.8
5	9.9	11.3	11.8	9.2
6	10.4	9.0	8.5	17.7
7	12.0	14.1	15.0	10.3
8	11.1	9.8	16.1	16.6
9	7.9	14.5	11.1	16.7
10	9.7	12.4	11.5	11.4
11	2.8	13.5	14.7	
Mean:	10.03	11.75	12.64	13.89

Table 7

Thymus Results
(mg.)

Subject/Group	1	2	3
1	400.60	396.41	694.57
2	306.70	257.43	459.45
3	176.01	268.07	312.47
4	424.81	438.57	513.48
5	356.46	271.80	568.26
6	465.11	406.35	515.46
7	342.50	348.12	451.03
8	403.62	195.33	409.03
Mean:	359.48	322.76	490.97

Table 8

Thymus/Body-Weight Ratio

Subject/Group	1	2	3
1	1.284	1.247	1.957
2	1.400	1.287	2.042
3	0.867	1.025	1.467
4	1.550	1.381	2.140
5	1.635	1.890	2.378
6	1.077	1.720	2.786
7	1.796	2.012	2.425
8	1.336	0.723	1.623
Mean:	1.370	1.411	2.1023

Table 9

Adrenal Results
(mg.)

Subject/Group	1	2	3
1	35.75	26.84	23.03
2	35.03	39.17	42.56
3	31.41	39.95	38.58
4	4.95	20.95	17.78
5	22.65	20.71	24.81
6	20.06	31.21	29.93
7	30.74	25.96	34.76
8	33.97	20.46	22.60
Mean:	26.82	28.16	29.26

Table 10

Adrenal/Body-Weight Ratio

Subject/Group	1	2	3
1	.1146	.0844	.0649
2	.1645	.1959	.1766
3	.1547	.1988	.1811
4	.0181	.0812	.0708
5	.1039	.0903	.1038
6	.1558	.1311	.1618
7	.0775	.1545	.1869
8	.1017	.0961	.0897
Mean:	.1114	.1290	.1295

Table 11

Eosinophil Results*
Per Cent Change in Eosinophil Counts

Subject/Group	1	2	3
1	-45.6	10.8	170
2	48.3	2.2	41.8
3	-1.9	104.3	101.4
4	-10.9	61.3	176.8
5	11.2	3.3	426.8
6	67	-41.1	162.4
7	-13.3	-73.8	13.8
8	138	-41.8	82
Mean	24.1	3.15	146.8

*Negative sign indicates decrease during stress period.

Table 12

Blood Sugar (Value at Death)
(mg.%)

Subject/Group	1	2	3
1	118.6	98.0	99.2
2	94.6	87.8	104.9
3	77.5	91.2	149.3
4	90.1	92.3	149.3
5	94.6	84.4	78.7
6	86.6	91.2	91.2
7	106.0	79.8	93.5
8	67.3	102.6	92.3
Mean	91.9	89.5	107.3

Table 13

Per Cent Blood Sugar Change*

Subject/Group	1	2	3
1	166.7	60.4	222
2	27.7	-3.6	58.6
3	-17.1	3.6	70.1
4	25.4	19.1	92.6
5	-19.4	57.1	-11.5
6	-28.5	21.2	27
7	-52.4	-33.3	49.1
8	-5	-31.8	-13.8
Mean	12.175	11.59	61.75

*Negative sign indicates decrease during stress period.

Table 14

Per Cent Body Weight Change*

Subject/Group	1	2	3
1	-5.13	2.20	7.61
2	-10.33	0.50	7.11
3	-2.46	-3.98	8.45
4	-1.46	-9.92	9.58
5	0.00	9.05	8.37
6	0.00	5.06	7.57
7	5.41	5.45	6.99
8	0.00	-11.11	6.75
Mean	-1.75	-0.34	7.80

*Negative sign indicates decrease during stress period.

Table 15

Water Consumption
(ml.)

Subject/Day		1	2	3	4	5	6	7
Group I Continuous Shock	1	5	28	26	21	23	21	32
	2	8	9	24	21	25	23	33
	3	2	6	0	36	36	26	38
	4	8	29	23	23	25	27	24
	5	11	31	20	29	20	24	24
	6	7	30	26	21	20	27	30
	7	6	33	26	35	25	30	29
	8	7	38	30	34	30	31	30
Mean:		6.75	25.5	21.875	27.5	25.5	26.125	30

Table 15 (Cont'd)

	Subject/Day	1	2	3	4	5	6	7
Group II Recovery	1	8	27	27	30	32	27	38
	2	5	16	29	25	23	21	25
	3	6	22	20	19	21	15	23
	4	10	22	21	30	25	27	25
	5	17	32	26	31	26	30	30
	6	12	23	18	24	12	24	23
	7	20	28	22	32	20	24	23
	8	11	35	26	28	27	27	29
Mean:		11.125	25.625	23.625	27.375	23.25	24.375	27

Table 15 (Cont'd)

	Subject/Day	1	2	3	4	5	6	7
Group III Control	1	29	33	33	34	34	30	40
	2	42	33	35	29	44	42	46
	3	25	22	23	24	27	24	27
	4	31	39	33	33	31	29	37
	5	28	32	30	29	23	30	30
	6	23	12	29	38	25	26	25
	7	17	24	25	26	22	25	24
	8	30	33	29	28	30	35	34
Mean		28.125	28.5	29.625	30.125	29.5	30.125	32.875