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THE EFFECTS OF FR SCHEDULE SHIFT ON PHYSIOCHEMICAL INDICES OF STRESS IN THE ALBINO RAT.

PAUL M. VALLIANT
University of Windsor

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THE EFFECTS OF FR SCHEDULE SHIFT ON
PHYSIOCHEMICAL INDICES OF STRESS IN THE ALBINO RAT

BY
Paul M. Valliant
M.A. Lakehead University, 1976

A Dissertation
submitted to the Faculty of Graduate Studies
through the Department of Psychology
in Partial Fulfillment of the
Requirements for the Degree of
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1978
ABSTRACT

Male Wistar 80-100 day old rats maintained on a 23.75-hour water deprivation schedule were trained to respond on a CRF schedule. Subjects were then slowly shifted from the CRF schedule to an FR 20 schedule in increments of 2 responses per day, over a 10 day period. The subjects were maintained on the FR 20 schedule for 5 days, then suddenly shifted to either an FR 80 (20-80), FR 40 (20-40), or maintained on the FR 20 (20-20) schedule of reinforcement for up to seven days following the schedule shift. Three control groups were maintained throughout the experiment for later comparison. One group (WDNO) was water deprived and placed in the test chamber but not required to emit any responses for reinforcement (i.e., non operant). A second group (WD) was water deprived but not placed in the test chamber. A third group (Ad Lib) was not water deprived and also had no experience in the test chamber. At various intervals following the shift in operant schedule, subjects were sacrificed to determine the effect of the size of FR shift and duration of time on various physiochemical indices. An equal number of subjects from the experimental, and procedural control groups were sacrificed on Days 2, 4 and 8 following the shift in schedule, at the time of their usual exposure to the operant condition. Blood plasma (red blood cells, white blood cells and hemoglobin) relative circulating white leukocytes (lymphocytes, neutrophils, monocytes, basophils and eosinophils), and glandular weight (absolute thyroid,
thymus, spleen, adrenal and testicle) measures were collected for all subjects. Two-way ANOVAs demonstrated significant main effects between groups for white blood cells, hemoglobin, lymphocytes, neutrophils, absolute thyroid, thymus, and relative thymus and adrenal measures. Length of exposure to the FR schedule shift was found to have a significant effect on only the white blood cell measure. No significant interactions existed either between the groups or across the days of testing. It is quite evident from this investigation that certain physiochemical indices were affected by FR schedule shifts. The white blood cells, relative leukocytes, (i.e., lymphocytes and neutrophils), hemoglobin, absolute thyroid, thymus and relative thymus and adrenal measures responded quickly to the size of the FR shift. Since there were no significant trends which set the experimental and control subjects apart for red blood cells, relative leukocytes (i.e., monocytes, basophils and eosinophils) spleen and testicle weights, it is evident that investigators using a similar methodological approach in future experimentation need not be concerned with collecting these measures. Focus should be directed to those measures which did show significant differences between the groups. In addition, it would be of great importance to further examine the size of FR schedule shift over a longer amount of time.
ACKNOWLEDGEMENTS

The present paper emerged as a result of the efforts of those individuals who gave freely and unselfishly of their time and energy. To those people I dedicate this dissertation and would like to express my appreciation and gratitude.

Firstly, I extend my thanks to those professors who served as my dissertation committee. Dr. Ted Hirota, Chairman of the committee deserves a special note of thanks for his interest, comments and aid in developing the thesis of this paper. Dr. Dave Reynolds, was instrumental in providing a conceptualization and integration of the material. Dr. Byron P. Rourke, quite knowledgeable in this area of investigation, elucidated many of the issues needed to explain the theoretical model of stress. Lastly, Dr. Michael A. Persinger was instrumental in reinforcing my interest in this area of research, and providing feedback when problems arose. I am grateful to him for serving as my Mentor and encouraging me to study Psychology.

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CHAPTER 1

INTRODUCTION

Selye (1952, 1950) has maintained that organisms must have the ability to adjust to environmental situations otherwise they would quickly perish. Organisms, thus, can be viewed as adaptive mechanisms with the ability to adjust to a number of physical conditions (i.e. temperature) via their physiochemical response systems.

Selye's (1946) earlier research showed that organisms had the ability to adjust to noxious stimulus conditions (i.e. carcinogenic agents). Yet in some circumstances organisms exhibited exhaustion if the amount of the noxious stimulus was too intense. His research demonstrated that continued exposure to physical stimulus events would not only lead to physiological changes (i.e. endocrine gland atrophy) but also blood plasma changes would be displayed in terms of increased output of total white blood cell count. Others have also shown that organisms react to threatening conditions by exhibiting changes in blood measures (Palmblad, Cantell, Strander, Froberg, Karlsson, Levi, Granstrom & Unger, 1976). In fact Palmblad et al. (1976) have hinted at the interrelationship between endocrine gland atrophy and the production and release of white blood cells. Until this point, it has been consistently shown that physical conditions (i.e. noxious agents) affect an organism by causing total white blood cells to increase and endocrine glands to atrophy. Furthermore, recent investigators have suggested that sudden changes in life events can generate both immediate and long term changes in the probability of behavioral disorders and physiochemical immunological pathologies (Holmes and
Masuda, 1974; Rahe and Lind, 1971; Rahe and Paasikivi, 1971; Holmes and Holmes, 1970). These studies have suggested that changes in life events by evoking adaptation efforts which are faulty in degree and duration lower bodily resistance and enhance the probability of disease occurrences (Holmes and Masuda, 1974).

Therefore, the present study is concerned with utilizing both Selye's model of organismic response to physical stressors and Holmes and Masuda's model of life event changes and applying these to the model of FR schedule shift and physiochemical response. By suddenly increasing an FR schedule of reinforcement (ie. FR 20-40 or FR 20-80) to which an organism has previously adjusted will establish whether or not a "shift" from a previously predictable event (ie. FR 20) to an unpredictable event (ie. FR 40 or FR 80) will serve as a "stressor" condition and result in physiochemical changes in an organism. If predictability is a precursor of life change adaptation and FR schedules are suddenly shifted without prior warning, organisms may display this effect in terms of physiochemical disruption. In fact such an event may serve as a "psychological stressor" and consequently display organismic changes comparable to those shown by "physical stressors" as discussed by Selye (1952, 1946), since both stressors are known to cause similar physiochemical innervation.

Thus, the main objective of the following experiment is to investigate FR schedule shifts and the impact of these shifts on blood and organ systems in the rat. It is predicted that the size of FR schedule shift (ie. size of the stressor) and the amount of time which an organism is exposed to the event (ie. schedule change) will produce physiochemical changes.
A Review of the Area of Stress

Adaptation to the ever changing environment is one of the most important physiological adjustments an organism makes during its life (Selye, 1952; 1950 and 1946). Selye (1950) has pointed out that an organism must have the ability to adjust to external stimuli in order to survive. In fact organisms succumb to diseases as a result of their inability to adapt to environmental events (Selye, 1952). Studies have shown that diseases of adaptibility play the same unique role in pathology as noted in the General Adaptation Syndrome (GAS), whereby some animals are unable to adjust to stressful events and consequently perish (Selye, 1952; 1950; 1946).

Selye's initial investigation in the area of stress focused upon fabrication of a "model" which would elucidate the mechanisms responsible for organismic instability upon exposure to stressful situations. Thus, Selye (1950) suggested that stress should be viewed as a non-specific response of an organism's body to any physical demand. He assumed that under stressful stimulus conditions, organisms would respond in non-specific ways. This reaction could vary behaviourally, physiologically or chemically, depending upon the state of the organism when the stress was presented (Selye, 1952; 1950). Since little was known concerning the area of stress, Selye proposed the following questions concerning his model:

1. To what extent was this syndrome nonspecific?
2. What could one expect as the observable effects of stress?
3. How does stress develop in time; specifically is the
degree of its manifestation merely proportional to the magnitude of the damage at all times; or does the syndrome, like many infectious diseases, go through distinct stages in certain chronological order?

4. To what extent do specific actions by agents influence the manifestation of the non-specific syndrome?

5. To what extent could the stressor facilitate pathological insult to the organism?"

Selye (1946) conceded that one could not possibly study stress "per se" but merely investigate the effects of predisposition to certain physical conditions (e.g., exposure to cold, infections, and noxious chemical injections). Ruff and Korchin (1967) verified Selye's hypotheses that stress occurred when an organism was forced into a strenuous condition which required an increased rate and duration of responding. Furthermore, Bajusz (1969) expanded Selye's definition of stress to include the suggestion that stress was intended to express the emergency endocrine mobilization of organismic adaptation.

Due to the lack of knowledge concerning stress, Selye was encouraged to explain his model. Hence he utilized empirical data to show how an organism reacted when it was exposed to stress. Selye noted that the initial stage of "alarm" consisted of two phases: the first, described as the "shock phase" represented the immediate effect of the noxious agent's ability to facilitate physiological irritation of the cell tissue (Selye,
1952; 1946). The second, referred to as the "counter shock phase" represented the defensive effort of the organism to physiologically adjust (i.e., adrenal cortex enlargement) to noxious agents.

The second stage of Selye's model was aptly termed the "stage of resistance" because organisms would attempt to resist noxious stimuli. However, repeated exposure to these agents could only be resisted for a limited period after which the organism would succumb to the pathological effects induced by these stimulus conditions (Selye, 1975a; 1975b; 1952; 1948). "If the state of arousal continued", an organism would eventually succumb to a "stage of exhaustion" in which physical breakdown and/or death would occur. This triphasic response pattern was referred to by Selye as the General Adaptation Syndrome (GAS).

The Effect of Stress on the Endocrine and Nervous Systems

Many investigators have examined the various pathways of the Central Nervous System (CNS) in order to discover how stressors affect organisms. Bajusz (1969) has suggested that although the modes of operation for the CNS and the Endocrine system are different, these two major co-ordinating circuits are morphologically and physiologically integrated. Furthermore, he has suggested that experimental medicine has succeeded in demonstrating that the CNS is in a sense, a complex Physio-chemical system capable of producing chemical substances (neurohormones) that control the internal secretory functions of glandular organs and regulate a number of other physiological activities. Thus, the
Study of neuroendocrinology has evolved as an area of neurophysio-
chemical integration with added focus upon the physio-pathology
of diseases in the endocrine and nervous systems (Bajusz, 1969).

Activation of the sympathetic branch of the ANS facilitates
stimulation of the adrenal medulla which causes adrenaline
(epinephrine) to be released into blood plasma (Bassett & Cairncross, 1976). This allows for the spontaneous burst of energy
which is required during initial stress induction in "fight or
flight" like situations (Cannon, 1932). In response to long
term stress, the CNS activates the endocrine system to dissipate
the effects of noxious agents (Bajusz, 1969; Gray, 1971).

Gray (1971) has argued that stressful stimuli are mediated
throughout the organism via the CNS. These stressors eventually
make contact with the limbic, and reticular activating systems,
hippocampal and hypothalamic structures (Bajusz, 1969). Various
nuclei of the hypothalamus are indirectly stimulated and subse-
quently play an essential role in the release of adrenocortico-
trophin hormone (ACTH) (Oken, 1967). Although the hypothalamic
cells are not primary regulators of the CNS, they coordinate
and transmit impulses from higher centres of the brain via a
corticotrophin releasing hormone (CRH) (Hillhouse, Burden &
Jones, 1975) and relay them to the pituitary gland via the
neurohumoral and/or nervous system (Bajusz, 1969). Once stim-
ulated, the pituitary gland releases ACTH into the hypophyseal
portal vascular system. This substance then circulates through-
out the blood system, eventually making contact with the adrenal glands. Excitation of the adrenal cortex via ACTH causes glucocorticoids (GC) and mineralocorticoids (MC) to be released into the blood system (Haynes & Larner, 1975; Gray, 1971; Fortier, 1956; Selye, 1952).

The MC influence the anti-inflammatory processes in an organism whereas the GC (e.g., hydroxycorticosterone corticosterone and cortisone hormones) transform non-sugars into sugars and increase the deposition of sugar in liver (Gray, 1971). The GC continue the task initially undertaken by nor-adrenaline during the alarm stage of stress, thus providing the body with rapidly mobilized sources of energy. In effect the GC are long term 'energy stores' which continue physiological activation of the organism when nor-adrenaline is depleted during long term stress.

Conversely, the GC facilitate the peristaltic action of the blood vessels to quickly transport adrenaline and noradrenaline throughout the organism if a stressor persists for any period of time (Gray, 1971; Bajusz, 1969). Blood containing both MC and GC flows throughout the organism thereby completing a "feedback loop" to the brain, thus inciting the hypothalamus to either inhibit or mediate ACTH release from the pituitary when required (Haynes & Larner, 1975). However, in situations involving a high degree of stress the inhibitory feedback mechanism may be modified or by-passed via the activation of higher cortical centres.
such as the hippocampus (Bassett & Cairncross, 1975b). The severity of the stressor determines the extent of the humoral or neural involvement (Mekara, Stark, Marton, & Meszaros, 1972). Hence, continued stress triggers the release of ACTH when it is needed.

Selye (1975a, 1975b; 1948) has noted that the GC have the tendency to inhibit formation of antibodies, decrease white blood cells (lymphocytes) and cause involution of the thymus. Other physiological effects of stressors include atrophy of the thyroid, gonads, spleen, hypophysis decrease in body weight, nephrosclerosis of the kidney and gastro-intestinal ulcers (Selye, 1975a; 1948; Bajusz, 1969). With these considerations in mind Price, Thaler and Mason (1957) have posited that the pituitary-adrenocortical system is not only related to emotional processes associated with single specific emotional states such as anxiety and fear but more notably to emotional states with relatively undifferentiated components of distress.

**Neuroendocrinological & Physiological Stress Related Studies**

Research in the area of Neuroendocrinology has focused almost exclusively upon respondent conditioning situations and related physiological events (Brady, 1969). However, Brady has suggested that future investigation should emphasize the effects of instrumental conditioning experiments on the physiology of an organism.

More recently investigators have shown that the respiratory and cardiovascular systems can be easily affected by stressors
and to a greater extent the endocrine system can be influenced by instrumental performance (Weiss, 1972; Levi, 1969; Brady, 1966; Mason, Brady, Robinson, Taylor, Tolson & Mongey, 1961a; Mason, Mangan, Brady, Conrad & Rloch, 1961b). In fact a number of reports have called attention to somewhat transient physiological changes related to performance in instrumental conditioning experiments. Blood pressure, plasma peptinogen, and heart rate have all been operantly conditioned in animals, via electrical shock induction (Perez-Cruet, Tolliver, Dunn, Marvin & Brady, 1963; Wenzel, 1961; Hearst, Beer, Sheatz, & Galambos, 1960; Shapiro & Horn, 1955).

In effect, the application of operant methodology to the experimental analysis of psychophysiological relationships has provided a degree of control over the behavioural processes, especially those associated with neurophysiological investigations (Brady, 1966). Brady (1966) has reasoned that unlike most of the behaviourally produced respiratory and cardiovascular alterations which reportedly last only as long as the instrumental procedure is maintained, the endocrinological effects associated with behavioural conditioning are somewhat longer lasting.

**Short and Long Term Effects of Stress**

Recent investigation has shown that operant conditioning experiments can have an effect on the neuroendocrinology of an organism. In initial experiments by Mason, Brady, and Sidman (1957) monkeys were placed in three conditions which required
three different contingent responses. In the first condition, monkeys were reinforced with food on either a fixed ratio (FR) 20 or variable interval (VI) 60 second schedule. Blood samples were taken from animals and analyzed for elevation in plasma 17 hydroxycorticosteroid (17-OH-CS) levels one hour before and after sessions (Mason, et al. 1957). Blood samples were also taken following FR 20 schedules and after animals had been shifted to FR 100 schedules. In no cases were significant elevations in plasma 17 OH-CS levels found during the course of the experiment.

Animals were further tested by Mason et al. (1957) in anxiety conditioning and conditioned avoidance tasks. In a conditioned anxiety situation where animals were electrically shocked following presentation of a conditioned stimulus, (CS) (a clicking tone), was noted to facilitate an increase in 17 OH-CS levels in blood plasma. Mason et al. (1957) further noted that monkeys, required to bar press to delayed electric foot shock had increased levels of 17 OH-CS in plasma for up to one hour following exposure to this situation.

Elevation in 17 OH-CS levels via conditioning has been noted by many investigators. In fact when monkeys were required to lever press intermittently over a two hour period a significant increase in plasma 17 OH-CS occurred irrespective of whether the animals had avoided the shock. (Sidman, Mason, Brady, Thach, 1962). Further experimentation by the same investigators, using repeated exposures to this conditioned avoidance task caused a
significant rise in plasma 17 OH-CS levels with a return to baseline within a few hours following the task.

In an attempt to clarify these studies, Brady (1966) decided to investigate the effects of varying response-stimulus intervals during avoidance conditioning. Although the results of this study were not entirely clear, Brady noted that high rates of lever pressing were associated with a rise in plasma 17 OH-CS levels. Furthermore, experimentation not only revealed that the frequency of bar pressing to escape shock produced significant elevations in plasma 17 OH-CS but also the frequency of the shock presentation facilitated a significant increase of this chemical substance in blood samples. To control for the effects of shock and lever pressing, Brady (1966) decided to manipulate these independent variables in an attempt to get clearer results. He decided to present a CS prior to electrically shocking the animals. Results showed that lever pressing activated the pituitary-adrenocortical system to produce increases in blood steroids, irrespective of whether or not the animals had been shocked (Brady, 1966). Elevation in plasma 17 OH-CS level was noted to occur even when no shock was administered during the following two hour session (Brady, 1966).

The evidence presented up to this point suggests that animals placed in "emotionally arousing" situations showed elevations in steroid levels (17 OH-CS) irrespective of the avoidance conditioning task. However, this resulted only if electrical shock (CS)
produced anxiety. However, since these studies were only investigated over a short period, they may have tested for the short term effects of conditioning procedures.

Experiments controlling for long term changes in steroid levels have also been undertaken by Brady and his colleagues (1969). Mason, Brady, Polish, Bauer, Robinson, Rose & Taylor (1961c) have investigated corticosteroid and pepsinogen levels in monkeys subjected to a Sidman-avoidance conditioning procedure for a 72-hour period. Although there was an increase in plasma 17 OH-CS during this time, the plasma pepsinogen levels were consistently depressed below baseline levels. Yet, during the recovery period from conditioned-avoidance responding, the plasma pepsinogen levels recovered within 48 hours. In a follow-up experiment, Mason et al. (1961c) attempted to assess whether or not the Estes-Skinner conditioned suppression method of inducing stress in monkeys would produce contrasting effects from those previously noted in the Sidman-conditioning procedure. Animals tested in the Estes-Skinner conditioning situation were found to respond similarly in chemical-blood measure analysis (Mason et al. 1961c). Conversely, there appeared to be less epinephrine secretion increases (Mason et al. 1961c). While the elevation in 17 OH-CS level was high following conditioning, epinephrine levels were relatively low (Mason, et al. 1961c). However, animals that had been exposed to threatening situations consisting of either conditioned avoidance suppression techniques, reacted by showing an elevation in both chemical substances (17 OH-CS) and epinephrine).
Long term reactivity of the pituitary-adrenocortical system has also been investigated by Mason et al. (1961a). They subjected monkeys to Sidman avoidance sessions 72 hours in duration. Significant elevations were noted in urinary 17 OH-CS and epinephrine levels, with these returning to baseline levels within 6 or 3 days respectively (Mason et al. 1961a). Although the norepinephrine levels rose only slightly during the avoidance conditioning period they showed continued significant elevation and returned to normal within 6 days. Urinary androsterone and estrone levels dropped to below half the baseline value during the stress period, but showed significant increases from 3 to 6 days following the conditioning sessions. Thyroid hormone (thyroxine) levels as reflected in butanol extractable iodine (BEI) measurements also showed a significant slow rise to over twice the baseline value and reached a peak level early in the 6 day recovery period. In essence, these results demonstrated that the hormones tested by Mason et al. (1961a) underwent significant elevations during activation of the pituitary adrenocortical system.

Organisms show physiochemical responses during initial exposure to stress-like conditions. For example, during exposure to a stressor a certain amount of steroids may be required by the organism to dissipate noxious effects. Consequently, chemical pathways associated with the endocrine system must provide for this compensatory need (i.e., if ACTH is required in large amounts
to produce steroid synthesis and release; hypothalamic secretory
cells must facilitate the release of this substance from the
adenohypophysis (Bray, 1971; Bajusz, 1969). However, this causes
ramifying circuits from both the adenohypophysis (i.e., to the
gonads, adrenals and thyroids) and neurohypophysis (i.e., to the
kidneys and uterus) to show decreased hormonal outputs. As a
result decreased hormonal flow causes organs to atrophy and
initiates impairment of endocrine function.

Brady (1969; 1966) has contended that endocrinological-
behavioural investigation seems to represent one of the more
systematic applications of operant methodology to the experimental
analysis of psychophysiological relationships. Moreover, Brady
(1966) has reasoned that the emphasis upon neuroendocrinological
processes reflects the importance of these critical physiological
systems in the mediation of basic psychosomatic relationships.
Hence, he has cautioned that future attempts to validate the
nature of mediation processes in the production of altered physio-
logical states-and pathological somatic conditions build upon the
applications of behavioural analysis techniques to neuroendocrin-
ological processes.

Chemical and Morphological Effects of Stress

Until this decade most of the research concerned with physio-
chemical effects of learning was associated with electroshock
avoidance conditions. In addition, much of the research was
concerned with steady-state behaviour. Then Halasz (1968) intro-
duced a new concept into the behavioural testing of neuro-
pathological correlates that involve changes in new schedule of
reinforcement demands. Essentially Halasz viewed the organism
as a type of homeostatic response matrix to which systems theory
could be applied. If the organism was placed in a condition
which required adaptation to new demands such as a change in
schedule of reinforcement, Halasz (1968) hypothesized that changes
in physiology would occur. That such response-flexibility exists
in an organism which has been subjected to a schedule change, has
been demonstrated many times (Halasz, Hughes, Humphreys &
Persinger, 1971; Persinger, Lafreniere & Ossenkopp, 1974a;

Changes in the organism's physiology and chemistry would not
be unexpected, especially the more labile response systems at the
biological levels (i.e., blood chemistry). In fact significant
increases in plasma corticosterone have been found in operant
situations where rats were exposed to emotionally arousing situ-
atons (Davis, Memmott, McFadden & Levine, 1976; Goldman, Coover
& Levine, 1973; Levine, Goldman & Coover, 1972; Coover, Goldman
& Levine, 1971).

Although the area of stress has been investigated for the
past four decades, there is still a lack of information concerning
its effects on organisms (Levine et al. 1972). Most documented
cases report that during a stressful event, hormonal activation
is induced. Bajusz (1969) has pointed out that the pituitary-
adrenocortical response occurs over short and long-term periods as a result of exposure to stress. He has contended that adrenocortical hormones are necessary for the development of adaptation to stress-like situations (Bajusz, 1969).

Well documented studies (Levine et al. 1972; Brady, 1969; Brady, 1966; Mason et al. 1961a; 1961) support the notion that the pituitary-adrenocortical system may be activated by physical injuries, physiological events and aversive threatening stimuli of psychological origin. In fact Levine and his colleagues have reported that pituitary-adrenocortical activation may occur when an organism fails to receive some expected reinforcement (Levine et al. 1972).

In addition Coover et al. (1971) have demonstrated that rats trained to lever press for water on a continuous reinforcement schedule (CRF) showed a significant elevation in plasma corticosterone after being shifted to an extinction schedule. Conversely a control group did not display the same elevation of corticosterone. Seemingly the frustration which occurred as a result of withdrawal of reinforcement when such was expected was sufficient to activate the pituitary-adrenocortical system. Other investigators have also demonstrated that rats subjected to various reinforcement schedules, then suddenly shifted to either lower or higher reinforcement contingencies showed an increase in plasma corticosterone (Levine et al. 1973; 1972). Essentially these experimenters concluded that once expectancies were no longer
reinforced or available, activation of the arousal centres enhanced the endocrine system to respond in subjects (Levine et al. 1973; 1972; Coover et al. 1971).

More recent experimentation has shown that rats shifted from a stabilized FR 20 reinforcement schedule to either a higher response per lower reinforcement contingency (i.e., FR 40) or to a non-reinforcement (i.e., Extinction) schedule, caused a significant increase in plasma corticosterone. Yet, when the rats were required to emit less responses per reinforcement (i.e., being shifted from FR 20 to CRF) there was a notable decrease in plasma corticosterone (Goldman et al. 1973).

Many investigators have attempted to explain the mechanism involved in pituitary adrenocortical activation, however at present, the theory behind this model is only speculative (Bajusz, 1969). Levine et al. (1972) have utilized a unique idea to elucidate this mechanism. Based upon Sokolov's "habituation model", Levine et al. (1972) have suggested that a novel stimulus causes activation of an alerting mechanism and this produces physiological innervation of the organism's system. This includes activation of the brain, decreased blood flow to extremities, skin resistance changes and increased adrenal corticoid circulation. However, following repeated exposure to a novel or threatening condition, the organism finally habituates to the stimulus condition and physiological innervation no longer occurs.

A number of conditioning studies have demonstrated that the
effects of resistance and/or exhaustion are obvious in blood chemistry measures, (Selye, 1952; 1948; Douglas & White, 1947; 1944) and morphological changes in an organism (Persinger, Carrey, Lafrenier and Mazzuchin, 1978; Persinger, Ossenkopp, Kamaya & Pear, 1974b, Bassett & Cairncross, 1976; 1975; Bajusz, 1969; Selye, 1946).

Persinger et al. (1978) have demonstrated that rodents trained daily for two weeks on differential reinforcement of low rates (DRL6) second schedules and later shifted to DRL - 12 second schedules showed significant elevations in alkaline phosphatase, serum glutamate oxaloacetic transaminase (SGOT) and relative blood lymphocyte numbers, as compared to DRL - 6 second controls. Furthermore, highly significant differences existed between the 80% body weight of DRL tested rats and the non-tested ad. libitum controls for: glucose, blood urea, nitrogen, chloride, carbon dioxide, calcium, alkaline phosphatase, serum pyruvate oxaloacetic transaminase (SGOT), triglycerides and relative testicle weights.

Earlier research by Persinger et al. (1974) has also shown that rats slowly shaped up to, and maintained on a FR 50 schedule for 10 days and then suddenly shifted to an FR 125 schedule on Day 11 showed significant decreases in blood eosinophils (within 40 minutes) relative to the control animals maintained on the FR 50 condition. The "response strain" demonstrated by rats placed upon the FR 125 schedule showed the usual response dis-
rupture transients associated with pituitary-adrenocortical activation. Persinger et al. (1974) have argued that physio-
chemical organismic changes should occur as "long term" effects in addition to those found over a short term period. In fact they
have stated that organisms attempting to adjust to transient
shifted schedules of reinforcement without prior notice will show stressful reactions in their physio-chemistry (Persinger et al.
1974).

Bassett and Cairncross (1975a) have also noted physiological changes in rats exposed to stressful conditions. Rats placed in
a one-way active avoidance condition ranging from 1 to 75 Days with repeated pairing of irregular footshock from which they
could escape, showed significant morphological changes. Pronounced necrosis of the heart tissue and increases in plasma 11 hydroxy-
corticosteroid levels were noted. Furthermore, there was a sig-
nificant hypertrophy of the adrenals and a significant decrease in overall body weight. The noted changes in body weight and adrenal
glands were obvious effects of stress. Bassett and Cairncross
(1975a) have contended that the morphological changes were produced by increased activation of the GC, as a result of exposure to long-
term stress. Pollard, Bassett and Cairncross (1976) have further shown that by utilizing the same procedure as reported by Bassett
and Cairncross (1975a), caused significant morphological changes in rats adenohypophysis and generated elevations of 11 hydroxy-
corticosterone in blood plasma. However, unlike Bassett and
Cairncross' (1975a) study these changes only occurred up to Day 20 of the 60 day stress period. These results are important because the data suggests that animals have the ability to adapt to stressors within a short period of time (Pollard et al., 1976).

The findings previously discussed are of interest to those investigating the effects of stressors upon organisms. However, the results of lower animal studies are of further interest to human stress research. Recent investigations have demonstrated that sudden changes in an individual's life style can produce behavioural and physiological immunological disorders. Sudden changes in an organism's previous schedule of reinforcement is sufficient to generate stress-related manifestations in physiochemistry (Holmes & Masuda, 1974). In fact many of the symptoms reported involve psychosomatic-like profiles and marked similarities to general stress symptoms (Masuda & Holmes, 1974). The initial steps of organismic adjustment to life style changes are apparently manifested or correlated with dermatological difficulties and are followed by respiratory-immunological and endocrine genitourinary-cardiovascular effects (Holmes & Masuda, 1974). The major significance of the human data is that sudden life style changes, (i.e., comparable to shifts in reinforcement), evoke intra-organismic adjustment demands. If there are too many adjustments or if the adjustment is too severe, disease like syndromes begin to appear.
Rationale for Proposed Area of Research

Any homeostatic system exposed to relatively unstable conditions will show marked physio-chemical deviations during sudden intense changes in demand (Persinger et al., 1977; 1976). If the sudden change is transient and returns to previous levels then adjustment will occur relatively quickly and perturbations will decrease in the organism (Persinger et al., 1978; 1976). Essentially, sudden change in reinforcement schedule for a behavioural system (rat) that has been maintained for a period of time on a particular stimulus response pattern, may induce unstable stress-like conditions within the animal's physio-chemical system (Persinger, 1976 et al.). Following transient and immediate changes in labile short latency systems (i.e., blood chemistry) morphological changes would occur in organs in which stress (demand or activity) is greatest (Persinger, 1978; 1976; 1974a; 1974b; Bassett & Cairncross, 1976a; 1976b; 1975a; 1975b).

Animals exposed to stressful conditions consistently react in a similar way. As previously mentioned, Brady (1969; 1966) has shown that rats placed in emotionally arousing tasks tend to show elevations of 17 OH-CS in plasma. Other investigators have also found that changes in reinforcement schedule facilitates stress-like syndromes in organisms. Coover et al. (1973) have shown that shifting an organism from a CRF to an Extinction schedule caused increases of corticosterone in blood. Furthermore, Levine et al. (1972) and Goldman et al. (1973) have demon-
strated that rats maintained on a schedule of reinforcement for a stabilized period, then shifted to either a higher or lower response per reinforcement contingency expectation, showed increases in plasma corticosterone. These studies suggest that changes in reinforcement contingency initiate unstable states within a system as demonstrated by elevation in plasma steroids (17-OH-CS; corticosterone) (Goldman et al. 1973; Levine et al. 1972; Coover et al. 1971). In addition, Persinger et al. (1974b) have demonstrated that rats shifted from a FR 50 to FR 125 schedule of reinforcement after a stable 10 day period showed a significant decrease in blood eosinophils.

One would suspect, on the basis of the General Arousal Model as proposed by Holmes and Masuda (1974), that the specific tissue systems (i.e., Endocrine) influenced would vary as a function of the time of the stress-like (schedule change) application. In this case the time since the abrupt schedule change. Yet, according to Persinger et al. (1974b) blood chemical changes occur immediately and return to baseline or near baseline levels during the first two to three sessions after the change. Further, morphological changes in the related tissues begin shortly thereafter and display continued structural changes until the organism is able to adapt to the condition (Persinger et al. 1974b). These investigators have hypothesized that endocrine structures should demonstrate the largest changes within 3 to 7 days and then decrease to baseline conditions. Persinger (1978) has argued that
such patterns of stress induction should closely parallel the data presented by Holmes and Masuda (1974).

Thus, the objectives of the following study are to determine the immediate and long term behavioural and physiochemical effects of abrupt and transient changes in non-shock reinforcement schedules between groups of subjects following long term static situations. The organs of the Endocrine system (thyroid, adrenal, spleen, thymus and testicle), blood chemistry (red blood cells, white blood cells, and hemoglobin) and circulating white leukocytes (lymphocytes, neutrophils, monocytes, basophils and eosinophils) most affected by the schedule change will be examined for the effects of the shift in FR schedule. It is expected that the thyroid, thymus, spleen, and testicles weight will atrophy while the adrenal gland hypertrophies under the effects of FR schedule shift. Additionally the FR shift is expected to increase the number of white blood cells and percentage of hemoglobin in blood plasma. The circulating white leukocytes are expected to show some alteration as a result of FR schedule shift. It is hypothesized that the lymphocytes will decrease in number whereas the neutrophils will increase.
CHAPTER I

METHOD

Subjects

Ninety male Wistar 80-100 day rats were used in this experiment. All subjects were bred and reared in the University of Windsor Psychology Breeding Colony. Subjects were housed in single cages for the duration of the study. Temperature in the laboratory was maintained at 22°C ± 1.5°C. Lighting in the colony room was maintained by fluorescent lights which remained on between the hours of 08.00 to 16.00 hours.

Apparatus

Two 26 cm x 21 cm x 23 cm operant chambers constructed of Plexiglas sides and metal walls were used in this experiment (Figure 1). Each chamber contained a 1.5 cm x 2.0 cm lever (requiring a 7gm force to depress it) mounted 3 cm above the grid floor on the right side of the front wall. Three and a half cm to the left of the lever projected a Lehigh Valley Electronics (LVE) Model 1351 water dipper delivery system. Each operant chamber was illuminated by a 15 watt light bulb located behind a white translucent panel, positioned directly above the operant lever. White noise was delivered throughout each chamber by 5 cm diameter speakers connected to a LVE White noise generator. Both operant chambers were housed in sound attenuated units equipped with air circulation systems manufactured by the LVE company.
Figure 1: Operant chamber equipped with water dipper unit

A  operand chamber lever (hidden view)
B  water dipper delivery cup (hidden view)
C  water dipper unit
D  rubber grommet (attached to retracting metal rod)
E  operand chamber light (hidden view)
F  body of the solenoid
Operant chambers were connected to automatic programming equipment in an adjacent room, equipped to record responses and reinforcements.

**Procedure**

Subjects were placed on a 23.75 hour water deprivation schedule for two days. The following day subjects were trained to respond on a continuous reinforcement (CRF) schedule. At the beginning of the experiment each lever press activated the water dipper system to deliver 0.02cc of water. Subjects were tested 30 minutes per day, seven days a week. Each subject was tested in the same operant chamber throughout the entire experiment. The grids of the chambers were cleaned with a solution of 0.5% acetic acid and dried in order to attenuate olfactory cues (especially those later associated with schedule changes). Fecal boluses were counted and removed from each chamber following individual testing sessions. Subjects were returned to their home cages and given 15 minutes of water, 30 minutes following each daily session. Food (Purina rat chow) was available ad libitum.

On the first day of CRF training the subjects in all operant groups were not allowed to make more than 150 reinforced responses per day. This was done in order to allow for lever press acquisition and to attenuate the disruptive effects of schedule changes following excessive CRF training. The next day subjects were slowly shifted from CRF to FR 20 in increments of 2 responses per day, over a 10 day period. Next all operantly trained sub-
jects were divided into one of the following operant conditions:
1) Experimental FR 20-20 Experimental FR 20-40, and 3) Experimental FR 20-80. Essentially, an Experimental FR 20-20 group consisted of subjects who had been operantly trained to respond to a FR 20 schedule. They were maintained on the FR 20 condition for five days and on the sixth day, when a shift in reinforcement schedule was initiated for both experimental groups (i.e., FR 20-40; FR 20-80) this group was maintained on the FR 20 schedule. The FR 20-40 and FR 20-80 experimental groups, unlike the Experimental FR 20 group, were stabilized for five days on the FR 20 schedule, and on the "shift day", required to respond to FR 40 and FR 80 schedules, respectively. In addition to these groups of subjects, three "control groups" were maintained for later comparison. One group was given food and water ad libitum (Ad Lib) and maintained in individual cages in the colony room throughout the experiment. A second group with no operant training but water deprived (WDNO) was exposed to an operant chamber. A third group was water deprived (WD) and remained in the home cage throughout the experiment. At various intervals following the shift in operant schedule demand, subjects were sacrificed to determine the physiological effect of the shift. An equal number of subjects from the Experimental groups (i.e., FR 20-20, FR 20-40, FR 20-80) as well as the Procedural Control (AD LIBITUM; WDNO; WD) groups were killed on Day 2, 4 and 8 following the shift in schedule (e.g., 1) on Day 2 (i.e., one day following the shift to the new schedule 2), on Day 4
(three days following the shift) and 3) on Day 8 (seven days following the shift).

All subjects were killed at the time of their usual exposure to the operant chamber (about 23 hours since last testing). Instead of being taken to the operant room, the subjects were taken to a surgery laboratory and decapitated. Consequently all physiochemical changes which occurred would be correlated with the effects of the treatment (change in operant schedule demand) rather than acute post-sessional changes of the experiment.

Glandular Measurement. Following the experiment the subjects were weighed and then sacrificed by decapitation. A thyroid, thymus, spleen, adrenal and testicle were removed from the rats' body and wet weight taken within 15 minutes following death.

Blood Measurement. Whole blood samples (5cc.) were taken from each rat upon decapitation and placed in stoppered test tubes. Samples were transported to the Windsor-Western I.O.D.E. Hospital for analysis. A Coulter Counter Model F4 apparatus was used to analyze both red blood cell (RBC) and white blood cell (WBC) counts. (See Appendix A for specific blood analysis technique).

A .01 μl of blood was taken from the test tubes for each subject and smeared on standard microscope slides. A total of 100 white blood cells per slide were counted over a wide field for each rat's two best blood smears at 1000 x under oil immersion; the average number of each cell type was converted into percent of total population. Wright's staining technique was used to color the leukocytes and then the cells were counted for differential
leukocyte profiles (lymphocytes, neutrophils, monocytes, eosinophils, basophils).

**Behavioural Data.** The total number of responses and reinforcers for each individual rat in the operant chamber were recorded by counters and cumulative records. Operant chamber fecal boluses were counted daily after each session and water consumption recorded for home cage feeding.
CHAPTER III
RESULTS

The results for this experiment were evaluated by two way
(3 x 6 factorial design) analyses of variance (ANOVA), analyses
of covariance, a priori t-tests, means and standard deviations
trend analyses and Pearson Product R correlations, using the
statistical program for social sciences (SPSS). The minimal level
of statistical significance accepted for the measures was $p \leq .05$.

The main effects for the between factors (Groups) and the
within factors (Days) were analyzed for blood plasma measures
(mean number of red ($10^6$/mm$^3$) and white blood ($10^3$/mm$^3$) cells and
mean percentage of hemoglobin in grams per 100 milliliters (gm/dl)
of blood plasma); circulating white leukocytes (mean percentage of
lymphocytes, neutrophils, eosinophils, monocytes and basophils per
100 white blood cells); and glandular weights (mean weight for
thyroid, adrenal, thymus, spleen and testicle). Absolute and
relative glandular weight measurements were recorded and analyzed.
This was done to determine whether or not animal body weight was
a contributing factor to any significant difference between the
experimental and control groups. In addition, behavioural data
was analyzed for water consumption by groups (mean total consumed
over days of testing) and fecal boluses (mean total after shift
in schedule) deposited in the operant chamber over the days of
testing.
Blood Plasma and Circulating White Leukocyte Measures

Two way ANOVAs for the six conditions (20-20, 20-40, 20-80, Ad Libitum, WDNO and WD) and the three kill days (1, 3 and 7) for the blood plasma and circulating white leukocyte measures showed significant (p < .05) main effects of Groups and only one significant Day effect. No significant interactions were noted for any of the measures. Table 1 contains the means and standard deviations for these measures.

The two way ANOVAs demonstrated significant main effects for Groups in red blood cells (F = 3.14, df = 5,72, p < .001), white blood cells (F = 12.47, df = 5,72, p < .001), hemoglobin (F = 7.69, df = 5,72, p < .001), lymphocytes (F = 20.59, df = 5,72, p < .001) and neutrophils (F = 21.95, df = 5,72, p < .001). The above measures remained statistically significant when covariance for body weight was performed. There were no significant main effects of Groups for monocytes (F = 0.62, df = 5,72, p > .05), eosinophils (F = 2.17, df = 5,72, p > .05) and basophils (F = 0.39, df = 5,72, p > .05).

Two way ANOVAs for blood plasma and circulating white leukocyte measures further showed that only the white blood cells were significant over days of testing (F = 5.87, df = 2,72, p < .005) (Refer to Figure 3). There were no significant main effects for red blood cells (F = 1.17, df = 2,72, p > .05), hemoglobin (F = 1.80, df = 2,72, p > .05), lymphocytes (F = 0.60, df = 2,72, p > .05), neutrophils (F = 0.19, df = 2,72, p > .05), monocytes (F = 0.62,
Table 1.
Means and Standard Deviations (±) for Mean Number of Red and White Blood Cells, Percentage of Hemoglobin and Relative Leukocyte Counts for Rats Exposed to FR Schedule Shift Conditions (20-20, 20-40, and 20-80) or Control Conditions (Ad Libitum, WDHO, WD)

<table>
<thead>
<tr>
<th></th>
<th>FR 20-20 (n = 15)</th>
<th>FR 20-40 (n = 15)</th>
<th>FR 20-80 (n = 15)</th>
<th>Ad Libitum (n = 15)</th>
<th>WDHO (n = 15)</th>
<th>WD (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells (x 10^6)</td>
<td>5.9 ± 0.7</td>
<td>6.7 ± 1.4</td>
<td>5.8 ± 0.8</td>
<td>6.6 ± 1.2</td>
<td>6.0 ± 0.8</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>White Blood Cells (x 10^3)</td>
<td>7.7 ± 1.9</td>
<td>10.6 ± 2.5</td>
<td>11.5 ± 3.4</td>
<td>8.2 ± 1.4</td>
<td>7.4 ± 0.9</td>
<td>7.6 ± 1.4</td>
</tr>
<tr>
<td>Hemoglobin (%)</td>
<td>16.3 ± 0.6</td>
<td>16.9 ± 0.6</td>
<td>16.6 ± 0.7</td>
<td>15.5 ± 0.6</td>
<td>16.3 ± 0.7</td>
<td>16.1 ± 0.7</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>87.4 ± 3.2</td>
<td>79.6 ± 3.0</td>
<td>79.3 ± 2.4</td>
<td>87.7 ± 4.5</td>
<td>89.7 ± 4.4</td>
<td>87.9 ± 4.5</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>12.0 ± 3.2</td>
<td>18.7 ± 3.6</td>
<td>19.9 ± 2.3</td>
<td>11.0 ± 4.3</td>
<td>9.6 ± 3.7</td>
<td>11.5 ± 3.9</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>0.5 ± 0.8</td>
<td>1.3 ± 2.6</td>
<td>0.7 ± 1.3</td>
<td>0.6 ± 1.2</td>
<td>0.6 ± 1.2</td>
<td>0.4 ± 0.7</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.0 ± 0.1</td>
<td>0.1 ± 0.3</td>
<td>0.1 ± 0.2</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.6</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.1 ± 0.2</td>
<td>0.1 ± 0.2</td>
<td>0.1 ± 0.2</td>
<td>0.0 ± 0.1</td>
<td>0.0 ± 0.1</td>
<td>0.1 ± 0.2</td>
</tr>
</tbody>
</table>
df = 2.72, p > .05), eosinophils (F = 0.10, df = 2.72, p > .05) or basophils (F = 0.19, df = 2.72, p > .05) over the seven day testing period. (See Tables 7, 8 and 9 for significant differences on the individual days of testing.) Further analysis of the data was done to determine where the differences were in blood plasma and circulating white leukocyte measures for both the groups and across days. One way ANOVAs showed that significant differences remained for red blood cells (F = 3.47, df = 5.84, p < .01), white blood cells (F = 10.74, df = 5.84, p < .001), hemoglobin (F = 7.66, df = 5.84, p < .001), lymphocytes (F = 22.12, df = 5.89, p < .001) and neutrophils (F = 22.62, df = 5.84, p < .001).

In addition, a priori t-tests were performed on the data to determine where the significant contrasts were between groups for blood plasma and circulating white leukocyte measures (Refer to Figures 2 to 6 for a representation of the effects between groups and across days). The significant differences between groups as determined by a priori t-tests are presented on Table 6 (page 64). As can be seen in Figure 2 (page 34) there was a significant difference between groups for mean number of red blood cells but no significant difference across days of testing. In fact a close look at the data reveals that there was no trend in pattern between the groups in terms of the output of red blood cells as a result of an increase in the FR shift. Significant Groups and Day effects appeared for only the white blood cell measure. Figure 3 (page 36) shows that the greater shift in FR schedule condition between
Figure 2. Mean number of red blood cells per cu mm of blood plasma for groups of rats exposed to FR schedules or control conditions over a seven day testing period.

(Note: The scale for the 7 day testing period is not drawn on a continuum for Figs. 2-14.)
groups resulted in an increased output of white blood cells in the plasma of subjects. An a priori t-test contrast showed that there was a significant difference in white blood cells over days. The major day effects existed between Day 1 and Day 3 when compared to Day 7. As can be seen from Figure 3, there was a significant increased output of white blood cells 3 days after the schedule shift for the FR 20-20 & FR 20-40 groups. The total output of white blood cells asymptoted on Day 7 toward that value noted for Day 1 of testing. Conversely, the FR 20-80 schedule shift condition as can be seen from Figure 3 (page 36) showed an increased production of white blood cells over the 7-day testing period. With increased testing the FR 20-80 condition subjects showed no asymptote in output of white blood cells to the "stressful" task. It can probably be assumed that level of "stress" as reflected from shift in schedule of reinforcement was an important factor which contributed to white blood cell output. In fact, a priori t-tests showed that the severity of the increase in FR schedule condition (i.e., 20-80, 20-40, and 20-20) produced significant differences in the white blood cell measure (Refer to Table 6, page 64).

As previously mentioned there was a significant difference between groups for mean total percentage of hemoglobin output. As can be seen in Figure 4, (page 37), the schedule shifted groups (i.e., FR 20-80, FR 20-40) showed an increase in level of hemoglobin in blood plasma as compared to the experimental control group FR 20-20 and the relative control groups of subjects (i.e., Ad Lib,
Figure 3. Mean number of white blood cells per μm of blood plasma for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
Figure 4. Mean percentage of hemoglobin per cu mm of blood plasma for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
WDNO and WD). This is a significant finding, since "stress" is known to cause an increased consumption of oxygen (the oxygen molecule binds with hemoglobin, an oxygen-carrying pigment of the erythrocytes) (Friel, 1974). Thus one would expect this significant percentage of hemoglobin in the highly stressed groups. It can be argued that this effect is a direct result of the "stress" of the task and not merely the increased exposure to bar pressing, since subjects were killed approximately 23.5 hours after their last session of testing in the operant chamber.

As can be seen from Figures 5 (page 39) and 6 (page 40), with shifts in FR schedules the mean percentage of lymphocytes significantly decreased and neutrophils significantly increased. Lymphocytes were found to significantly decrease as a function of the intensity of the shift in FR schedule condition whereas neutrophils were noted to increase. This finding is in line with what others have found for this measure (Persinger, 1978). Essentially groups of subjects shifted to higher schedules of reinforcement (i.e., FR 20-40 and FR 20-80) were found to have significantly less lymphocytes and significantly more neutrophils in their blood plasma as compared to the experimental control (i.e., FR 20-20 condition) and other relative control conditions (i.e., Ad Lib, WDNO, WD).

Trend (polynomial) analyses for the 6 conditions over the seven days of testing showed that the red blood cell change approximated quadratic terms ($F = 5.02, df = 1, 84, p < .05$); white blood
Figure 5. Mean percentage of lymphocytes for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
Figure 6. Mean percentage of neutrophils for groups of rats exposed to FR schedules or control conditions over a seven-testing period.
cells approximated linear \((F = 9.01, df = 1.84, p < .005)\); quadratic \((F = 16.8, df = 1.84, p < .001)\) and cubic terms \((F = 23.8, df = 1.84, p < .001)\); percentage of hemoglobin approximated linear \((F = 6.99, df = 1.84, p < .001)\) cubic \((F = 11.11, df = 1.84, p < .005)\) and quartic terms \((F = 9.64, df = 1.84, p < .003)\); percentage of lymphocytes approximated linear \((F = 25.7, df = 1.84, p < .001)\), quadratic \((F = 19.11, df = 1.84, p < .001)\) and cubic terms \((F = 61.03, df = 1.84, p < .001)\); percentage of neutrophils approximated linear \((F = 25.60, df = 1.84, p < .001)\), quadratic \((F = 16.80, df = 1.84, p < .001)\) and cubic terms \((F = 61.60, df = 1.84, p < .001)\). Table 2 contains the means and standard deviations for these measures.

**Glandular Measures**

Table 3 (page 44) contains the means and the standard deviations of the glandular (both absolute and relative) and body weight measures. Two way ANOVAs demonstrated significant main effects between the six schedule conditions in absolute weight for thyroid \((F = 5.20, df = 5.72, p < .001)\), thymus \((F = 16.15, df = 5.72, p < .001)\) and spleen \((F = 8.83, df = 5.72, p < .001)\); and relative weight for thymus \((F = 13.50, df = 5.72, p < .001)\), adrenal \((F = 2.43, df = 5.72, p < .05)\) and testicle \((F = 4.10, df = 5.72, p < .005)\). There were no significant main effects between groups in absolute weight for adrenal \((F = 1.03, df = 5.72, p > .05)\) or testicle \((F = 2.04, df = 5.72, p > .05)\) and relative weights for thyroid \((F = 2.28, df = 5.72, p > .05)\) and spleen \((F = 0.94, df = 5.72, p > .05)\). Furthermore, two way ANOVAs showed no sig-
Table 2
Means and Standard Deviations (±) for Blood Plasma Measures and Relative Leukocyte Counts for Rats Exposed to an FR Schedule Shift Conditions (20-20, 20-40, 20-80) or Control Condition (Ad Libitum, WDN0, WD) over a 7 Day Period

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Days</th>
</tr>
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<td></td>
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<tr>
<td>Red blood cells</td>
<td>FR 20-20</td>
<td>6.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>FR 20-40</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>FR 20-80</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>(x 10^6)</td>
<td>Ad Lib</td>
<td>6.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>WDN0</td>
<td>5.9 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>FR 20-20</td>
<td>6.3 ± 1.8</td>
</tr>
<tr>
<td>White blood cells</td>
<td>FR 20-40</td>
<td>8.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>FR 20-80</td>
<td>9.7 ± 1.5</td>
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<tr>
<td>(x 10^3)</td>
<td>Ad Lib</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>WDN0</td>
<td>6.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>8.1 ± 1.5</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>FR 20-20</td>
<td>16.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>FR 20-40</td>
<td>16.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>FR 20-80</td>
<td>16.7 ± 0.8</td>
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Table 2 Continued

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<tr>
<th>Measure</th>
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<tbody>
<tr>
<td>Hemoglobin</td>
<td>Ad Lib</td>
<td>15.8 ± 0.4</td>
<td>15.8 ± 0.4</td>
<td>14.9 ± 0.7</td>
</tr>
<tr>
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<td>WDNO</td>
<td>16.3 ± 0.8</td>
<td>16.2 ± 1.0</td>
<td>16.4 ± 0.5</td>
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<tr>
<td></td>
<td>WD</td>
<td>16.5 ± 0.8</td>
<td>16.0 ± 0.6</td>
<td>15.8 ± 0.6</td>
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<tr>
<td></td>
<td>FR 20-20</td>
<td>86.0 ± 2.0</td>
<td>87.4 ± 3.2</td>
<td>88.7 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>FR 20-40</td>
<td>78.7 ± 4.0</td>
<td>80.2 ± 3.0</td>
<td>79.8 ± 2.3</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>FR 20-80</td>
<td>79.2 ± 3.0</td>
<td>78.5 ± 1.9</td>
<td>80.3 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Ad Lib</td>
<td>88.1 ± 5.3</td>
<td>88.9 ± 5.7</td>
<td>86.0 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>WDNO</td>
<td>91.2 ± 4.2</td>
<td>89.1 ± 3.0</td>
<td>88.8 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>89.0 ± 4.5</td>
<td>86.2 ± 4.5</td>
<td>88.9 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>FR 20-20</td>
<td>14.0 ± 2.0</td>
<td>11.6 ± 3.4</td>
<td>10.4 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>FR 20-40</td>
<td>19.1 ± 4.2</td>
<td>19.7 ± 3.1</td>
<td>17.3 ± 3.8</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>FR 20-80</td>
<td>20.1 ± 3.0</td>
<td>20.9 ± 0.9</td>
<td>18.7 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Ad Lib</td>
<td>11.0 ± 5.0</td>
<td>9.0 ± 4.8</td>
<td>13.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>WDNO</td>
<td>8.6 ± 4.1</td>
<td>10.0 ± 2.6</td>
<td>10.6 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>10.3 ± 3.9</td>
<td>12.8 ± 4.5</td>
<td>11.3 ± 3.6</td>
</tr>
</tbody>
</table>
Table 3

Means and Standard Deviations (±) for Absolute and Relative Glandular and Body Weights for Rats Exposed to FR Schedule Shift Conditions (20-20, 10-40 and 20-80) or control conditions (Ad Libitum, WDNO, WD)

<table>
<thead>
<tr>
<th></th>
<th>FR 20-20</th>
<th>FR 20-40</th>
<th>FR 20-80</th>
<th>Ad Libitum</th>
<th>WDNO</th>
<th>WD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid (mg)</td>
<td>1.23 ± 0.31</td>
<td>1.14 ± 0.30</td>
<td>1.23 ± 0.36</td>
<td>1.64 ± 0.33</td>
<td>1.59 ± 0.44</td>
<td>1.59 ± 0.44</td>
</tr>
<tr>
<td>Thyroid (µg/g)</td>
<td>3.48 ± 1.02</td>
<td>3.17 ± 0.88</td>
<td>3.40 ± 0.86</td>
<td>3.83 ± 0.94</td>
<td>4.19 ± 1.02</td>
<td>4.15 ± 1.29</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>0.32 ± 0.06</td>
<td>0.25 ± 0.05</td>
<td>0.24 ± 0.07</td>
<td>0.48 ± 0.13</td>
<td>0.35 ± 0.10</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>Thymus (mg/g)</td>
<td>0.88 ± 0.17</td>
<td>0.73 ± 0.12</td>
<td>0.66 ± 0.17</td>
<td>1.14 ± 0.22</td>
<td>0.93 ± 0.22</td>
<td>0.99 ± 0.14</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.58 ± 0.08</td>
<td>0.60 ± 0.06</td>
<td>0.59 ± 0.11</td>
<td>0.76 ± 0.08</td>
<td>0.62 ± 0.08</td>
<td>0.62 ± 0.06</td>
</tr>
<tr>
<td>Spleen (mg/g)</td>
<td>1.60 ± 0.16</td>
<td>1.64 ± 0.16</td>
<td>1.63 ± 0.24</td>
<td>1.75 ± 0.21</td>
<td>1.63 ± 0.22</td>
<td>1.63 ± 0.15</td>
</tr>
<tr>
<td>Adrenal (mg)</td>
<td>24.0 ± 2.0</td>
<td>24.0 ± 4.0</td>
<td>26.0 ± 3.0</td>
<td>26.0 ± 5.0</td>
<td>25.0 ± 4.0</td>
<td>25.0 ± 3.0</td>
</tr>
<tr>
<td>Adrenal (µg/g)</td>
<td>69.0 ± 6.0</td>
<td>66.0 ± 11.0</td>
<td>74.0 ± 8.0</td>
<td>61.0 ± 15.0</td>
<td>66.0 ± 11.0</td>
<td>64.0 ± 11.0</td>
</tr>
<tr>
<td></td>
<td>FR 20-20</td>
<td>FR 20-40</td>
<td>FR 20-80</td>
<td>Ad Libitum</td>
<td>WDNO</td>
<td>WD</td>
</tr>
<tr>
<td>------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>------------</td>
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<td>-----</td>
</tr>
<tr>
<td>Testicle (g)</td>
<td>1.70 ± 0.16</td>
<td>1.68 ± 0.13</td>
<td>1.63 ± 0.16</td>
<td>31.74 ± 0.14</td>
<td>1.77 ± 0.15</td>
<td>1.77 ± 0.14</td>
</tr>
<tr>
<td>Testicle (mg/g)</td>
<td>4.73 ± 0.48</td>
<td>4.64 ± 0.33</td>
<td>4.56 ± 0.56</td>
<td>4.03 ± 0.52</td>
<td>4.68 ± 0.46</td>
<td>4.64 ± 0.44</td>
</tr>
<tr>
<td>Body Wt (g)</td>
<td>360.0 ± 39.0</td>
<td>363.0 ± 33.0</td>
<td>356.0 ± 50.0</td>
<td>440.0 ± 73.0</td>
<td>379.0 ± 27.0</td>
<td>383.0 ± 37.0</td>
</tr>
</tbody>
</table>
significant effects over seven day period of testing for absolute glandular measures for thyroid ($F = 0.029$, $df = 2.72$, $p > .05$), thymus ($F = 0.78$, $df = 2.72$, $p > .05$), spleen ($F = 0.18$, $df = 2.72$, $p > .05$), adrenal ($F = 0.48$, $df = 2.72$, $p > .05$) and testicle ($F = 0.34$, $df = 2.72$, $p > .05$). No significant interactions existed for any of the measures.

Analysis of the glandular measures was further evaluated to determine where the significant differences existed between groups of subjects. One way ANOVAs between groups for glandular weights demonstrated significant differences in absolute measures for thyroid ($F = 5.81$, $df = 5.84$, $p < .001$), thymus ($F = 17.58$, $df = 5.84$, $p < .001$) and spleen ($F = 9.99$, $df = 5.84$, $p < .001$) and relative measurement for thymus ($F = 14.82$, $df = 5.34$, $p < .001$), adrenal ($F = 2.49$, $df = 5.84$, $p < .05$) and testicle ($F = 4.07$, $df = 5.84$, $p < .005$). No significant one way ANOVAs existed across days of testing for either absolute or relative glandular measures. A priori t-tests were performed on the data to show where the significant differences existed between groups for the glandular weights. The significant differences between groups are presented on Table 6 (page 64). As can be seen from Figure 7 (page 47) the FR schedule conditions (FR 20-20, 20-40, 20-80) significantly varied from the control conditions (Ad Lib, WDNO, WD). Yet, as shown from Figure 7 the FR 20-20 group of subjects varied in thyroid weight in the same direction as the other two FR schedule shift conditions (FR 20-40, 20-80). This seems to
Figure 7. Mean absolute thyroid gland weight for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
suggest that the FR 20-20 subjects may have been sufficiently
"stressed" by the task to cause an atrophy of their thyroid gland.
When the thyroid was further analyzed for relative weight no significant
differences were noted between groups. The differences noted in
Figure 7 were a result of large absolute body weights between groups.
However, as observed from the relative thyroid weight, Figure 8
(page 49), the intensity of the shift in schedule seemed to produce
a decrease in gland size of those groups subjected to this condition.
Figure 9 (page 50) shows that the schedule shift (i.e., FR 20-40,
20-80) significantly affected the size of the thymus gland. In fact,
there seems to be a basic trend showing up in the data. With increasing
intensity of the task (i.e., Ad Lib - a no stress group) to FR 20-80 -
(a greatly stressed group) the size of the absolute thymus gland signi-
ficantly atrophied.

Although there was a significant difference between groups for
mean absolute spleen weight as can be seen in Figure 10 (page 51),
it is evident that the significant differences occurred between most
groups (FR 20-40, 20-20, 20-80, WDN0 and WD) when compared with only
the Ad Lib control group. This finding seems to suggest that either
the Ad Lib group was the only group not subjected to a stressful
task or possibly that body weight differences between the groups may
have caused this significant difference. The second assumption may
indeed be more correct than the first due to the fact that mean
relative spleen gland weight was not significant.

Further analysis of the glandular measures using a priori t-test
comparisons for relative thymus, adrenal and testicle weight, showed
only the mean thymus weight (Figure 11, page 52) to consistently
vary as a function of the level of shift in schedule
Figure 8. Mean relative thyroid gland weight for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
Figure 9. Mean absolute thymus gland weight for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
Figure 10. Mean absolute spleen gland weight for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
Figure 11. Mean relative thymus gland weight for groups of rats exposed to FR schedules of reinforcement or control conditions over a seven day testing period.
condition. In fact with an increase in "stress", the thymus gland atrophied most significantly with the greatest FR shift in schedule. This data agrees with the earlier results for absolute thymus gland weight. As can be seen from Figure 12 (page 54) the shift in FR schedule condition from FR 20-80 produced a significant increase in relative adrenal weight. In fact a priori t-tests showed (Refer to Table 6, page 64) that the significant differences for this measure existed only between the FR 20-40 versus FR 20-80 group; FR 20-80 versus Ad Lib group, and Ad Lib versus WDNO and WD group. This suggests that with an increase in FR schedule shift the size of the adrenal gland hypertrophied as a direct function of the intensity of the stress associated with the task. This finding is in agreement with Selye (1975a, 1975b, 1952, 1946) and Bassett and Carrnecross (1975) who also showed that under "stress" the adrenal gland would hypertrophy as a result of the increased stimulation by ACTH.

Furthermore, as can be seen from Figure 13 (page 55) there seemed to be no general trend for relative testicle weight to vary as a function of the intensity of the shift in FR schedule condition. Although significant differences existed for this measure, it can be seen in Figure 13 as well as evidenced by a priori t-tests (Refer to Table 6, page 64) that the testicle weight significantly differed between the Ad Lib group and all other conditions (i.e., FR 20-20, FR 20-40, FR 20-80, WDNO and WD). However, as can be observed in Figure 14 (page 56) the FR
Figure 12. Mean relative adrenal gland weight for groups of rats exposed to FR schedules of reinforcement or control conditions over a seven day testing period.
Figure 13. Mean relative testicle weight for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
Figure 14. Mean absolute testicle weight for groups of rats exposed to FR schedules or control conditions over a seven day testing period.
schedule shifted groups (i.e., FR 20-40; FR 20-80) showed lighter absolute testicle weights than the experimental control (FR 20-20) and relative control groups (Ad Lib; WDNO, WD). Yet these differences were not significant.

Trend (polynomial) analyses for the six conditions over the seven day testing period demonstrated that the various glandular measures showed different trends. The absolute thyroid weight approximated linear terms ($F = 20.54$, $df = 1.84$, $p < .001$); relative thyroid weight approximated linear terms ($F = 9.77$, $df = 1.84$, $p < .005$); absolute thymus approximated linear ($F = 23.58$, $df = 1.84$, $p < .001$), cubic ($F = 22.41$, $df = 1.84$, $p < .001$) and quartic ($F = 7.40$, $df = 1.84$, $p < .01$) terms; relative thymus approximated linear ($F = 18.52$, $df = 1.84$, $p < .001$), cubic ($F = 19.86$, $df = 1.84$, $p < .001$) and quartic ($F = 4.13$, $df = 1.84$, $p < .05$) terms; absolute spleen approximated linear ($F = 6.93$, $df = 1.84$, $p < .01$), quadratic ($F = 9.42$, $df = 1.84$, $p < .005$), cubic ($F = 4.88$, $df = 1.84$, $p < .05$), quartic ($F = 4.74$, $df = 1.84$, $p < .05$) terms; the absolute testicle measure approximated linear terms ($F = 5.48$, $df = 1.84$, $p < .05$); lastly the relative testicle measure approximated quadratic ($F = 7.18$, $df = 1.84$, $p < .01$) and quartic terms ($F = 4.26$, $df = 1.84$, $p < .05$). Table 4 contains the means and standard deviations for these measures.

Blood Plasma and Glandular Measures Correlated

The blood plasma and glandular weight measures were further analyzed for correlation by using a Pearson Product Moment Corre-
Table 4
Means and Standard Deviations (±) for Absolute and Relative Glandular Measures for Rats Exposed to FR Schedule Shift Conditions (FR 20-20, 20-40, 20-80) or control conditions (Ad libitum, WDNO, WD) over a 7 Day Period

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>1</th>
<th>3</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FR 20-20</td>
<td>0.32 ± 0.05</td>
<td>0.34 ± 0.08</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>FR 20-40</td>
<td>0.23 ± 0.05</td>
<td>0.26 ± 0.04</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>Absolute Thymus</td>
<td>FR 20-80</td>
<td>0.25 ± 0.06</td>
<td>0.23 ± 0.06</td>
<td>0.23 ± 0.09</td>
</tr>
<tr>
<td>(g)</td>
<td>Ad Lib</td>
<td>0.50 ± 0.14</td>
<td>0.43 ± 0.06</td>
<td>0.50 ± 0.17</td>
</tr>
<tr>
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<td>WDNO</td>
<td>0.36 ± 0.11</td>
<td>0.36 ± 0.09</td>
<td>0.34 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>0.39 ± 0.07</td>
<td>0.36 ± 0.06</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>Relative Thymus</td>
<td>FR 20-20</td>
<td>0.91 ± 0.20</td>
<td>0.92 ± 0.16</td>
<td>0.81 ± 0.13</td>
</tr>
<tr>
<td>(mg/g)</td>
<td>FR 20-40</td>
<td>0.70 ± 0.17</td>
<td>0.73 ± 0.12</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>FR 20-80</td>
<td>0.69 ± 0.16</td>
<td>0.64 ± 0.13</td>
<td>0.64 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Ad Lib</td>
<td>1.14 ± 0.22</td>
<td>1.20 ± 0.22</td>
<td>1.07 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>WDNO</td>
<td>0.96 ± 0.24</td>
<td>0.96 ± 0.26</td>
<td>0.86 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>1.04 ± 0.19</td>
<td>0.97 ± 0.08</td>
<td>1.97 ± 0.15</td>
</tr>
<tr>
<td>Measure</td>
<td>Group</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Absolute Spleen</td>
<td>FR 20-20</td>
<td>0.58 ± 0.07</td>
<td>0.60 ± 0.10</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>FR 20-40</td>
<td>0.60 ± 0.04</td>
<td>0.59 ± 0.07</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>FR 20-80</td>
<td>0.61 ± 0.09</td>
<td>0.55 ± 0.11</td>
<td>0.59 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>Ad Lib</td>
<td>0.75 ± 0.11</td>
<td>0.75 ± 0.09</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>WDN0</td>
<td>0.62 ± 0.05</td>
<td>0.64 ± 0.10</td>
<td>0.60 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>0.62 ± 0.09</td>
<td>0.63 ± 0.09</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>Relative Testicle</td>
<td>FR 20-20</td>
<td>4.75 ± 0.36</td>
<td>4.58 ± 0.38</td>
<td>4.86 ± 0.68</td>
</tr>
<tr>
<td></td>
<td>FR 20-40</td>
<td>4.66 ± 0.55</td>
<td>4.37 ± 0.30</td>
<td>4.88 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>FR 20-80</td>
<td>4.54 ± 0.26</td>
<td>4.84 ± 0.95</td>
<td>4.30 ± 0.73</td>
</tr>
<tr>
<td></td>
<td>Ad Lib</td>
<td>4.08 ± 0.61</td>
<td>4.17 ± 0.63</td>
<td>3.83 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>WDN0</td>
<td>4.87 ± 0.54</td>
<td>4.69 ± 0.39</td>
<td>4.48 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>WD</td>
<td>4.37 ± 0.40</td>
<td>4.94 ± 0.53</td>
<td>4.61 ± 0.19</td>
</tr>
</tbody>
</table>
islation Coefficient SPSS program. Both white and red blood cells are known to be stored by the thymus and spleen glands, respectively (Bloom and Fawcett, 1962; Ham, 1957). Therefore it was assumed that either an increase or decrease in the number of these specific blood cells would be correlated with atrophy or hypertrophy of the specifically related endocrine gland. Analysis of the data using a Pearson r program showed that mean number of white blood cells was negatively and significantly correlated with both the absolute thymus gland weight \( r = -0.37, \text{df} = 88, p < 0.001 \) and relative thymus gland weight \( r = -0.31, \text{df} = 88, p < 0.005 \). No significant correlations existed for red blood cells when they were correlated with either absolute spleen gland weight \( r = 0.08, \text{df} = 88, p > 0.05 \) or relative spleen gland weight \( r = 0.03, \text{df} = 88, p > 0.05 \).

Selye (1975a and b; 1952; 1946) has put forth the hypothesis that "stressful" conditions increase the cortex size of the adrenal gland as a result of constant stimulation by ACTH. The adrenal gland consequently releases GC into the organism to allow for increased activity of the physiological system (Selye, 1975a and 1975b; 1952). An end result of this increased output of GC's is an atrophy of the thyroid, thymus, spleen and testicle measures. Therefore, correlation coefficients were performed between absolute and relative adrenal weights and similar respective weights of the thyroid, thymus, spleen and testicle because of the effect that the adrenal gland may have had on these glands. Significant positive correlations existed between absolute adrenal and absolute weights of
the thyroid \( r = 0.24, \text{df} = 88, p < .05 \), thymus \( r = 0.25, \text{df} = 88, p < .01 \) and spleen \( r = 0.30, \text{df} = 88, p < .005 \). No significant correlation was noted between absolute adrenal and absolute testicle weight \( r = 0.06, \text{df} = 88, p > .05 \). Furthermore, when measures were analyzed for relative weight of the subjects, the adrenal weight was positively and significantly correlated with relative weights of the thyroid \( r = 0.21, \text{df} = 88, p < .05 \) spleen \( r = 0.21, \text{df} = 88, p < .05 \) and testicle \( r = 0.28, \text{df} = 88, p < .005 \). No significant correlation existed between relative adrenal and relative thymus weight \( r = -0.12, \text{df} = 88, p > .05 \).

**Behavioural Measures**

The means and standard deviations for behavioural measures of those groups that showed defecation response (i.e., 20-20, 20-40, 20-80 and WDNO) in number of boluses deposited in the operant chamber following the initial "shift day" in FR schedule and secondary water consumption for all groups (FR 20-20, 20-40, 20-80, Ad Lib, WDNO and WD) are presented on Table 5 (page 62). Two way ANOVAs for mean number of boluses deposited in the operant chamber over the seven day testing period following the shift in FR schedule was not significant \( F = 1.09, \text{df} = 3, 48, p > .05 \) between the groups of subjects. However, there was a significant difference \( F = 4.66, \text{df} = 2, 48, p < .05 \) for this measure across days of testing. Further analysis showed that this measure was significant \( F = 4.72, \text{df} = 2, 57, p < .05 \) when evaluated with a one way ANOVA. An a priori
Table 5

Means and Standard Deviations (±) for Last Behavioural Measures
Before Decapitation From Rats Exposed to FR Schedule Shift Conditions
(20-20, 20-40, 20-80) or Control Conditions (Ad Libitum, WDNO, WD)

Over A Seven Day Testing Period

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day + 1</th>
<th>Day + 3</th>
<th>Day + 7</th>
</tr>
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<tbody>
<tr>
<td>20-20</td>
<td>0.14 ± 0.31</td>
<td>0.70 ± 0.63</td>
<td>0.98 ± 0.43</td>
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<tr>
<td>20-40</td>
<td>0.00 ± 0.00</td>
<td>0.34 ± 0.47</td>
<td>0.56 ± 1.09</td>
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<tr>
<td>20-80</td>
<td>0.00 ± 0.00</td>
<td>0.26 ± 0.24</td>
<td>0.78 ± 0.41</td>
</tr>
<tr>
<td>WDNO</td>
<td>0.40 ± 0.89</td>
<td>0.00 ± 0.00</td>
<td>0.40 ± 0.89</td>
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</tbody>
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<tbody>
<tr>
<td>20-20</td>
<td>17.8 ± 0.8</td>
<td>19.2 ± 1.1</td>
<td>18.4 ± 1.3</td>
</tr>
<tr>
<td>20-40</td>
<td>18.2 ± 1.5</td>
<td>18.2 ± 1.5</td>
<td>19.0 ± 1.7</td>
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<tr>
<td>20-80</td>
<td>18.4 ± 1.3</td>
<td>17.6 ± 1.1</td>
<td>18.6 ± 1.1</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Consumption</th>
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<tbody>
<tr>
<td>Ad Lib</td>
<td>34.6 ± 9.1</td>
<td>37.6 ± 10.6</td>
<td>32.2 ± 4.5</td>
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<tr>
<td>WDNO</td>
<td>19.6 ± 1.1</td>
<td>20.2 ± 2.2</td>
<td>18.8 ± 1.1</td>
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<tr>
<td>WD</td>
<td>20.4 ± 1.3</td>
<td>20.0 ± 2.2</td>
<td>20.4 ± 0.5</td>
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<tbody>
<tr>
<td>20-20</td>
<td>360.0 ± 42.0</td>
<td>371.0 ± 35.0</td>
<td>352.0 ± 47.0</td>
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<tr>
<td>20-40</td>
<td>364.0 ± 35.0</td>
<td>378.0 ± 15.0</td>
<td>350.0 ± 45.0</td>
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<tr>
<td>20-80</td>
<td>354.0 ± 41.0</td>
<td>352.0 ± 42.0</td>
<td>364.0 ± 73.0</td>
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<table>
<thead>
<tr>
<th>Weight</th>
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<tbody>
<tr>
<td>Ad Lib</td>
<td>440.0 ± 80.0</td>
<td>412.0 ± 66.0</td>
<td>469.0 ± 76.0</td>
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<tr>
<td>WDNO</td>
<td>378.0 ± 23.0</td>
<td>375.0 ± 23.0</td>
<td>301.0 ± 08.1</td>
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<tr>
<td>WD</td>
<td>383.0 ± 37.0</td>
<td>371.0 ± 52.0</td>
<td>394.0 ± 31.0</td>
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</table>
t-test was utilized to evaluate where the significant effect existed over the seven day testing period. The only significant difference noted ($t = 3.03$, $df = 57$, $p < .05$) was between Day 1 and Day 7 values.

A two way ANOVA for water consumption demonstrated that there was a significant difference ($F = 60.34$, $df = 5, 84$, $p < .001$) between the six groups. Further analysis of the data using an a priori t-test showed that the Ad Lib control group consumed a significantly greater ($F = 30.23$, $df = 57$, $p < .001$) amount of water than any of the other groups. There were no significant differences noted across days of testing for the water consumption measure ($F = 0.47$, $df = 2, 72$, $p > .05$). Also, there were no significant interactions for this measure.
Table 6. The levels of significance for a priori t-tests for schedule shift conditions (20-20, 20-40, 40-80) and control conditions (Ad Libitum, MD, 80).

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<th>20-MD</th>
<th>20-MO</th>
<th>40-80</th>
<th>40-Ad Lib</th>
<th>40-MD</th>
<th>40-MO</th>
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Level of significance:
- ⋆ p < .05
- ** p < .01
- *** p < .005
- **** p < .001
Table 7. The levels of significance for schedule shift (FR 20-20; 20-40; 20-80) and control conditions (Ad Lib, HFD, WD) on Day 1 of testing

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Level of significance:
- * p < 0.05
- ** p < 0.01
- *** p < 0.005
- **** p < 0.001
Table 8. The levels of significance for schedule shift ( FR 20-20; 20-40; 20-80 ) and control conditions (Ad Lib, HR, LD ) on Day 3 of testing

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Level of significance

- * p < .05
- ** p < .01
- *** p < .005
- **** p < .001
Table 9: The levels of significance for schedule shift (FR 20-20, 20-40, 20-80) and control conditions (Al Lib, MD, WD) on day 7 of testing.

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Level of significance:
* p < .05
** p < .01
*** p < .005
**** p < .001
CHAPTER IV
DISCUSSION

The results of this experiment showed that rats gradually shaped to respond to an FR 20 schedule and maintained on this for five days, then suddenly shifted to higher schedules (e.g., FR 20-40; FR 20-80) demonstrated major changes in their blood plasma, circulating white leukocytes, absolute and relative glandular weight measures. These significant findings were a function of the size of schedule change (i.e. relative shift in FR schedule condition) that the subjects were exposed to as compared to the length of exposure to the task.

Essentially, a shift in the FR schedule condition caused a significant increase in the number of white blood cells, hemoglobin, neutrophils and a decrease in red blood cells and lymphocytes. In addition, the FR schedule shifts caused a significant decrease in the absolute thyroid, thymus and relative thymus weights and a significant increase in the relative adrenal gland weight. Figures 2 to 7 inclusively and 9, 11 and 12 showed that as the intensity of the task greatly increased (i.e., FR 20-40; FR 20-80) these measures either increased or decreased in the direction that was previously hypothesized (see page 23).

With increased levels of stress, Selye (1975a; 1975b; 1952; 1946) has maintained that one would expect an increased output of white blood cells, hypertrophy of the adrenal gland and atrophy of the thyroid, thymus, spleen and testicle weights of subjects. This would occur within a period of a few minutes and continue for a number of
days or until the organism had adapted to the stressful condition. This is based on the fact that "stress" facilitates the production and release of glucocorticosteroids (GCs) into an organism's vascular system. The steroids are directly responsible for the atrophy of the glands. Although Selye (1975a; 1975b; 1952; 1946) has utilized pharmacological and carcinogenic agents to induce physiological stress in the organism, his basic assumption that blood plasma and glandular alterations occur as a function of these chemicals is imperative to our understanding of the sequence of events associated with physiological stress.

It is evident that the experimental design used by investigators is instrumental in determining the effects of certain conditions upon the physiochemical parameters of an organism. For example, Persinger et al. (1978) found that there was a significant decrease in lymphocytes and a significant increase in neutrophils and relative testicle weight in subjects exposed to a shift in DRL schedule (6-12). Furthermore earlier experiments conducted by Persinger et al. (1974b) showed that absolute thyroid and thymus-nodules significantly decreased in those subjects shifted from an FR 50-125 schedule of reinforcement condition. These results suggest that not only the type of schedule of reinforcement (DRL versus FR) but also the intensity of the shift in schedule demand affects the outcome of the changes in physiochemical measures.

The results of this experiment different somewhat from the findings reported by Selye (1975a; 1975b; 1952; 1946) and Persinger et al.
(1978; 1974b). For example, the present investigation was designed to study effects of FR schedule shifts upon the physiochemical indices of stress in the rat. Therefore, two different levels of schedule shifts were included in the experiment to test this assumption. Subjects were randomly assigned to an FR 20-20 (experimental control group), FR 20-40 or FR 20-80 condition. The experimental control subjects were required to continue responding to the FR 20 schedule. Conversely, experimental groups were shifted to either FR 20-40 or FR 20-80 conditions. These increased schedule demands required either twice or four times as much responding for a comparable amount of reinforcers as previously received in the FR 20 condition. It was predicted that these shifts in FR schedule would produce changes in blood plasma, circulating white leukocytes and glandular measures in the rat. This hypothesis was confirmed since the shifts in FR schedules did produce significant changes in the above measures (i.e. increase in white blood cells, hemoglobin, neutrophils and adrenals; and a decrease in lymphocytes; thyroid and thymus). Conversely, Persinger et al. (1974b; 1976; 1978) found only one measure significantly different, those subjects shifted from a DRL 6-12 schedule showed heavier testicle weights than controls.

Earlier investigation by Persinger et al. (1974b) only dealt with the short term effects of changes in an FR schedule (i.e., FR 50-125) upon physiochemical measures in the rat. The
present experiment attempted to demonstrate the immediate and long-term effects of shifts in FR schedule conditions on physiochemical indices. Although the results obtained from this experiment differ from Persinger's (1978, 1974) findings, they support his theory that morphological changes would be expected to occur in these glandular measures in which stress was greatest.

As previously discussed, the glandular weights significantly varied as a result of the effects of ACTH acting on these measures. The ACTH upon stimulating the adrenal generated a release of GCS into the blood system (Bajusz, 1969). Although the GCS provided the required energy, essential to maintaining homeostasis in the organism, this substance activated the immunological system, thus causing a release of white blood cells and atrophy of those glands associated with this response system.

Solomon et al. (1974) have suggested that "physiological and psychological stressors" have similar influence upon the immunological system via CNS and endocrine mediation. The results of this experiment support the assumption that a stressor such as "schedule shift" with its increased demand in activity had the ability to influence the blood chemistry, circulating white leukocytes and glandular weights of the subjects. Solomon et al. (1974) have advocated that increased stress can cause immunologic breakdown in the
organism. For example, once the immunologic system has been incapacitated by "stress like" manifestations such as schedule shifts it no longer produces macrophages to remove dead or damaged cells or releases interferon, a non-immunologic protein (produced by lymphocytes), to antagonize virus infections (Solomon et al. 1974). Thus one could expect to find an increase in the breakdown or death of an organism.

The findings in this experiment somewhat support the assumption that the immunologic system was activated by the shift in FR schedule. In fact the significant results obtained for the white blood cells and both absolute and relative thymus gland weights lend support to this assumption. White blood cells are manufactured in the bone marrow and stored in the thymus gland (Bloom & Fawcett, 1962). "Physiological" or "psychological stress" is known to activate the immunologic system and thereby cause a release of white blood cells from the thymus gland into the vascular system of the organism. That there was a significant increase in output of white blood cells and a decrease in the weight of the thymus gland suggests that these measures are more responsive to FR schedule changes which may be equated with "stress like" condition.

The significant increase in the white blood cells and the decrease in thymus gland weight substantiates the fact that these measures quickly responded to stressful changes in the organism, because of their immunologic association. The immunologic system is in a continual state of arousal in an organism in order to create homeostasis. Thus, the immunologic system must be capable of responding in a very short period
of time in order to offset "stresors". That both the white blood cells and thymus gland weights (absolute and relative) were negatively and significantly correlated, further supports the assumption that they are interrelated in terms of immunologic response.

Both absolute and relative body weights were taken for the thyroid, thymus, spleen, adrenal and testicle measures. This was done to insure that the animal body weight was not contributing to the significant difference between the groups. However, as shown from the data the glandular measures were not significant in all cases for both absolute and relative weights. For example, the thymus gland was the only measure that proved to be significant in both absolute and relative weights. These results imply the importance of collecting both absolute and relative weights since the body weight of the subjects did vary significantly between control and experimental groups.

Close inspection of the significant measures (absolute thyroid, thymus, spleen and relative adrenal, thymus and testicle) showed that the significant differences for the absolute spleen and relative testicle measures existed only between the Ad Lib group when compared with all other groups individually. (However, it should be noted that the FR schedule shift did produce a decrease in the absolute testicle measure but this difference was not significant). The significant difference between the Ad Lib group and all other groups in terms of the absolute spleen measure may be due to the fact that the Ad Lib group was not water deprived nor exposed to a stimulus change condition
(i.e., placed in an operant chamber containing white noise) or required to respond on an FR schedule. Statistical analyses using a priori t-test contrasts showed that neither the stimulus change condition nor the possible exposure to the schedule shift had any effect on the subjects. Evidence for these findings are supported by the results obtained from the WD and WDNO groups. First, the WD group was not exposed to a stimulus change condition nor required to respond to the FR schedule. Thus one would expect that these factors were not responsible for any of the changes in the WD group. Furthermore the WDNO group was exposed to the stimulus change condition but not required to respond to the FR schedule. Therefore one would suspect that the WDNO and WD groups would show contrasting changes in either blood plasma or glandular measures because of the different conditions that they were exposed to. However, a priori t-tests did not show any significant differences between the WDNO or WD groups. This suggests that the stimulus change condition could not have contributed to differences in glandular weight between groups.

Second, the FR 20-20 group was exposed to the stimulus change condition and required to respond to the FR schedule. Yet, a priori t-test contrasts did not show any significant differences for the FR 20-20 group when compared with either the WD or WDNO groups on the absolute spleen and relative testicle measures. This suggests that exposure to the stimulus change condition combined with the requirement to lever press for reinforcement did not contribute in any way to
the significant differences between the groups.

As previously explained the body weight of a subject affects the glandular weight measures. Therefore it is imperative that both absolute and relative weights be taken of the subjects to obtain a clearer outlook concerning significant differences between the groups in terms of glandular weights. As mentioned, the body weight of a subject can clearly affect the weight of a glandular measure. One example of this was observed for the testicle weight measure. The relative weight of the testicle showed that all groups significantly differed from the Ad Lib group. However, analysis of the absolute testicle weight showed that the FR schedule shifted groups had smaller testicles than the control groups. Such a difference in measures substantiates the fact that it is imperative to collect both absolute and relative measures in experiments dealing with glandular weights especially when the groups of animals are known to vary in body weight.

The results of this experiment showed that over a seven day period of testing only certain measures responded to the size of the shift in schedule. The white blood cells, relative leukocytes (i.e. lymphocytes, and neutrophils) hemoglobin, absolute thyroid, thymus and relative thymus and adrenal measures were affected by the size of the shift in FR schedule. Conversely, there were no significant trends which set the experimental and control subjects apart for red blood cells, relative leukocytes (i.e. monocytes, basophils and eosinophils)
spleen and testicle weights. Therefore it is evident that investigators interested in researching this area need not be concerned with collecting or analyzing the previously mentioned measures. Focus should be directed toward those measures which did show significance differences between the groups.

Implications and Suggestions for Future Research

It is evident from this experiment that significant differences existed between the FR schedule shifted and control groups of rats, in terms of (white blood cells, hemoglobin, lymphocytes, neutrophils and absolute thyroid, thymus and relative thymus and adrenal weights. However, only the white blood cell measure remained significant over the seven day period of testing. Inspection of this measure showed that the groups exposed to show shifts in FR schedule (e.g., FR 20-40) demands showed a peak release of white blood cells after 3 days of testing but by 7 day asymptoted in terms of total white cell output.

From this point of view it seems that the duration of responding was an important variable. However, the results of this experiment showed that the group of subjects exposed to a very high demand in activity (e.g., FR 20-80) did not show any asymptotic behaviour by Day 7 of testing as observed in white blood cell output. In fact the FR 20-80 group showed a greater increase in white blood cells as exposure to the days of testing continued. This implies that subjects exposed to a greater level of stress (e.g., FR 20-80) may require a longer period of time to habituate to the condition.
Conversely the results of this experiment showed that a moderate level of stress (e.g., FR 20-40) could be quickly adapted to. Subjects demonstrated a peak release of white blood cells by three days of exposure to the task.

These data imply that exposure to less intensive FR schedule shifts are easily adjusted to as compared to greater shifts in schedule. This suggests that organisms exposed to less intense FR shifts a greater number of times have an adaptive advantage. For example, if an organism is exposed to a moderately stressful condition (i.e., FR 20-40) quite frequently its physiochemistry may assume more "plasticity" in terms of adaptation. This is to say that the organism's system may undergo less severe physiochemical changes when exposed to a stressful condition because of its flexibility and ability to quickly regain homeostasis. Therefore, future investigations of this area should be concerned with two basic problems. First, there should be an attempt to focus upon heightened shifts in FR schedule of reinforcement and the amount of time required by an organism to asymptote to baseline conditions. Second, there should be an attempt to investigate whether or not constant exposure to moderately stressful conditions will facilitate quicker adaptive responses in an organism in terms of physiochemical stability.
APPENDIX A

BLOOD MEASUREMENT
Blood Measurement

Forty microlitres (μl) of blood plasma was removed from each test tube and placed in sample cups containing 20 millilitres (ml) of isotonic solution. From this preparation 0.1 cc solution was removed from each individual sample and placed in sample cups containing 10 ml of isotonic solution in order to derive RBC counts. For RBC count, the B attenuator and D aperture was set at 1 and 8 respectively on the Coulter Counter blood analyzer and the threshold control set at 20. A control sample previously equilibrated for RBC, WBC and Hemoglobin (by laboratory technicians) was analyzed prior to testing individual samples in order to insure that the Coulter Counter was functioning accurately. Once this was completed and the apparatus tabulating control samples correctly, the samples taken from each individual rat were analyzed for RBC count. Samples were positioned on the Coulter Counter, a glass aperture with an electrode attached, dropped into the solution and the scanner activated. Each sample was read four times and the average of the readings taken as the measurement of RBC. Once all samples were analyzed for RBC count, the WBC counts were taken.

For WBC analysis, the B attenuator and D aperture of the Coulter Counter apparatus were reset to .707 and 16 respectively, and the threshold at .09. It was important to allow the Coulter Counter a warm-up period of 5 minutes before doing the WBC count, in order that the new attenuator, aperture and threshold measurements cali-
brate. Prior to analyzing the 20 ml samples for WBC, 8 drops of Zapoglobin solution were placed in each sample cup of solution in order to lyse (destroy) the red cells in the samples. A WBC control was calibrated prior to analyzing the samples for WBC count to insure the apparatus was accurately functioning. The glass aperture was then inserted into the sample cups for individual subjects and the scanner activated. WBC counts were read three times and an average taken for each sample. The solution remaining in the WBC sample cups was then poured into a Coulter Counter Hemoglobin calibrator and readings taken to determine the percentage of hemoglobin in each sample.
References


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1951  Born in Sturgeon Falls, Ontario to John M. Valliant
       and Marion S. Ward.

1957-65  Elementary education obtained at Our Lady of Sorrows
         School, Sturgeon Falls, Ontario.


1970    Attended 1st year of college at Nipissing University,
        North Bay, Ontario.

1971-74  Attended Laurentian University, Sudbury, Ontario.
         Graduated Cum Laude.
         Received Governor General's Gold Medal.

1974-75  Enrolled in Master of Arts Program in the Department
         of Psychology, Lakehead University, Thunder Bay, Ontario.

1975-78  Registered in the Ph.D. program at the University of
         Windsor, in the Department of Psychology, Windsor, Ontario.