1968

Differential Effects of Guanethidine and Reserpine on Norepinephrine in the Brain and Heart of Male Albino Rats Subjected to Restraint

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DIFFERENTIAL EFFECTS OF GUANETHIDINE AND RESERPINE ON NOREPINEPHRINE IN THE BRAIN AND HEART OF MALE ALBINO RATS SUBJECTED TO RESTRAINT

BY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PHARMACOLOGY

UNIVERSITY OF RHODE ISLAND

1968

DOCTOR OF PHILOSOPHY THESIS

OF

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UNIVERSITY OF RHODE ISLAND

1968

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ACKNOWLEDGMENT

The author wishes to thank Dr. John J. DeFeo for his patience and guidance and under whose supervision this study was conducted; Dr. Anthony M. Guarino, presently of the National Institutes of Health, Bethesda, Md., for helpful discussions and for editing this manuscript; Mrs. Karen Knapp Mauger for her very fine and extensive technical assistance throughout the daily and terminal protocols and for her help in the preparation of this manuscript; Miss Nancy Anne Tucker for her help in the preparation of this manuscript; and Mr. Angelo B. Mendillo for his practical assistance.

The author also gratefully acknowledges the American Foundation for Pharmaceutical Education for a predoctoral fellowship which made this investigation possible.

The author would especially like to thank his wife, Donna R. Blaszkowski for her patience and understanding during the preparation of the dissertation.

ABSTRACT

The effect of guanethidine (1.20 mg/kg, i.p.) or reserpine (0.2 mg/kg, i.p.) has been compared on various indices of pituitary-adrenal stimulation in rats subjected to chronic forced restraint for 24 days. This was accomplished by analyzing the serum corticosterone, the adrenal ascorbic acid (AAA) and various organ weights such as the adrenals, brain and heart. Peripheral and central norepinephrine (NE) was also measured.

Chronically stressed, control rats or those treated with guanethidine demonstrated both behavioral and neurochemical adaptation. Initial excitation associated with restraint was related to changes in NE in the brain and heart. As the experiment progressed, the stressed, guanethidine-treated animals showed changes in peripheral NE that were similar to those of the stressed, vehicle-treated animals; whereas, the non-stressed guanethidine-treated animals showed a progressive decline in peripheral NE over the 24 day study. This change in the peripheral NE in the stressed, guanethidine-treated animals was attributed to increased production of NE, perhaps by the adrenals, since low doses of guanethidine do not affect adrenal catecholamines.

Reserpine-treated, stressed animals showed 40% mortality over the 24 day period, thus indicating non-adaptation. This increased mortality was not due to starvation or decreased water intake. It is suggested that non-adaptation in these animals may be due to a chemical sympathectomy, and the animals were unable to respond to severe changes in the environment.

Changes in behavior, in the different groups of animals, were correlated with changes in brain NE. Evidence is also presented indicating that reserpine and guanethidine do not deplete heart NE by the same mechanism(s).

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LIST OF ABBREVIATIONS

AAA	adrenal ascorbic acid
ACTH	adrenocorticotrophic hormone
alpha MMT	alpha methyl-meta-tyrosine
ANS	autonomic nervous system
AR	alarm reaction
ATP	adenosine triphosphate
CA	catacholamine(s)
COMT	catechol-O-methyl transferase
CNS	central nervous system
CRF	corticotrophin releasing factor
CRS	chronic restraint stress
CS	cortical steroids
DD	depleting dose
DPP	1,1-dimethyl-4-phenylpiperazinium
E	epinephrine
FFA	free fatty acid
5-HT	5-hydroxy tryptamine; serotonin
GAS	general adaptation syndrome
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
M	metanephrine
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
MHPG	3-methoxy-4-hydroxyphenylglycol
MPA	metaphosphoric acid
NE	norepinephrine
NM	normetanephrine
s.c.	subcutaneous
SD	standard deviation
SE	standard error
TCA	trichloroacetic acid
TP	tryptophan pyrrolase
VMA	vanillyl-mandelic acid

I. INTRODUCTION

The life of an animal depends upon its ability to adapt to changes in both internal and external environment; for example, one of Claude Bernard's classical experiments demonstrated that adaption to cold exposure involved heat production from two main sources: muscular activity and chemical processes other than those concerned with muscular activity. Research in this field has shown that adaptation to changes in environment or to a stimulus (stressor) involves an interplay between the central nervous system (CNS), the autonomic nervous system (ANS) and the endocrine system. In many species, including man, every type of stress activates the anterior pituitary-adrenocortical system (Tepperman, 1962). A discharge of sympathin, thought to be a mixture of epinephrine (E) and norepinephrine (NE), from adrenergic nerve endings, has also been shown to occur during systemic stress. Inasmuch as adaptation involves nervous activity, numerous studies have been carried out to determine the effects of various drugs on the central and peripheral nervous systems.

The major function of NE in the body is as the dominant transmitter released from sympathetic adrenergic neurons of mammals (von Euler, 1956). In view of the presence of both NE and dopamine in brain neurons having the same characteristics as sympathetic adrenergic neurons, it is probable that NE also serves as the transmitter in the CNS (Hillarp et al., 1966a). Maynert and Klingman (1961) discussed the possibility that brain NE functions as part of the sympathetic nervous system and that its release is accompanied by arousal or excitement. Trendelenburg (1963) reported that the nerve terminals of adrenergic fibers have functions

other than just those concerned with the synthesis and release of NE. The NE stores are important sites of action for various drugs, and the ability of the stores to take up various sympathomimetic agents has been the subject of much important physiological and pharmacological research.

Leblanc and Nadeau (1961) and Leduc (1961) showed that exposure of an animal to cold produced a significant and immediate increase in urinary excretion of NE. These observations drew attention to the important role of both the adrenergic nervous system and the adrenal glands in cold adaptation and survival. Others have shown that catecholamine (CA)-depleting drugs, such as reserpine, prevented the normal physiological responses to cold, leading to poor adaptation and death (Zilberstein, 1960; Taylor, 1961); a close relationship between cold resistance and urinary excretion of NE was obtained in such treated animals (Johnson, 1963). Previous work in this laboratory (Rosecrans and DeFeo, 1965) showed first, that reserpinized animals subjected to chronic restraint stress (CRS) had a 50% greater mortality than did similarly treated rats which were not stressed, and second, that the reserpinized animals subjected to stress showed a decreased food and water intake. The question therefore arose whether this increased mortality might be due either to depletion of the central and/or peripheral NE or to starvation. Since non-adaptation may be associated with interference with some physiological mechanism(s), the question was further refined to include a study of the relationship between the apparent exhaustion of NE with non-adaptation (Rosecrans, 1963).

These reports and others indicated the importance of NE in the survival of rats subjected to stress. However, it is difficult from studies such as these to determine whether the decreased resistance was

due mainly to a peripheral or central action of reserpine, since this drug has important depleting effects on both brain CA and indolamines (Sheppard and Zimmerman, 1960a), on peripheral CA (Orlans et al., 1960), and produces marked sedation.

In the present study guanethidine was selected to evaluate the importance of the peripheral liberation of CA. Guanethidine is a potent sympatholytic drug, which blocks nervous transmission in the noradrenergic postganglionic fibers (Maxwell et al., 1960a) and depletes various peripheral organs of their CA content without affecting amine stores in the brain (Kuntzman et al., 1962). This drug does not cross the blood-brain barrier to any appreciable extent (Kuntzman et al., 1962). Further, guanethidine, contrary to reserpine, does not impair the resistance of rats exposed to very low temperatures (Pouliot and Leblanc, 1963).

An additional purpose of the present investigation was to evaluate more precisely the importance of peripheral secretion and action of NE vs the central secretion and action of NE in the defense against stress and the involvement in adaptation.

In summary, this work was designed to test the following hypotheses:

1. Increased mortality of reserpine-treated rats subjected to chronic restraint may be due to:
   a. depletion of central NE,
   b. depletion of peripheral NE,
   c. depletion of both central and peripheral NE,
   d. starvation.
2. Non-adaptation of rats subjected to chronic restraint is due at least in part to exhaustion of central and/or peripheral NE.

II. LITERATURE SURVEY

A) STRESS

1. Introduction

The term "stress" is undoubtedly one of the most impressive terms in the lexicon of science; but like many words, it means different things to different people. The term probably originated in the field of engineering; and to the engineer it means an external force directed at some physical object, with the result of "strain" and temporary or permanent alteration in the structure of the object. The word has also been borrowed for use in literature and conversation to indicate a special force or emphasis exerted on some word or idea in speaking or writing.

The concept of stress was first introduced into the life sciences by Hans Selye in 1936 and elaborated in successive papers, leading to a full theoretical statement in book form in 1950. Today, many writers in physiology and psychology have adapted the engineering convention; stress being the external agent or stimulus and strain being the resultant. This usage is probably appealing because of the ease with which it seems to fit into the concept of homeostasis.

From the homeostatic point of view, a stress is some stimulus condition that results in the disruption of equilibrium in a system and produces changes in that system against which mechanisms of equilibrium are activated. One example of such mechanisms at the physiological level is Selye's adaptation syndrome, which is an elaborate series of neural-hormonal reactions against the effects of noxious agents on the tissue system. However, Selye (1956) has been instrumental in

stimulating a reversal of the engineering convention for the language of stress. He refers to the "noxious" stimulating condition which produces stress reactions as the "stressor"; and the reaction is called the stress. Sometimes Selye uses the term "stress" to refer to the initial impact of the stressor on the tissue, sometimes to the adaptive mechanisms whose function it is to restore homeostasis, and sometimes to the wear and tear, damage, or disease consequences of prolonged homeostatic processes. There are at least three meanings here to the term "stress", even though the word always refers to the state or reaction of the animal's tissue systems to the stressor or noxious stimulus.

It makes little difference, however, if the external force is called a stress or stressor, or whether the effect on the animal is called stress or strain. It is important that we are consistent in our terminology, that the definition of terms is clear, and that the connotations of terms come as close as possible to the analogy intended by theory. For example, "strain" is a poor analogy to adaptive or homeostatic mechanisms, but "adaptive syndrome" is more suitable.

2. History

It would be impossible to do justice to Selye's concept of stress in the few short paragraphs to follow. His own work on this subject has extended over four decades and has resulted in over a thousand publications. Selye (1936) found antecedents for his work in the concept of Hippocrates, that disease is not only suffering but also toil; that is, the fight of the body to restore itself to normal. Claude Bernard's (1859) description, that one of the most characteristic features of all

living beings is their ability to "maintain the constancy of their internal milieu", despite changes in the surroundings, was an acknowledged base for Selye (1950) and also for Cannon (1932) in the development of his concept of homeostasis.

Cannon suggested that the ANS is the initiator of adaptive mechanisms. He proposed that under environmental changes, ANS stimulation, especially plasma adrenalin, could initiate cellular preparedness and permit adaptation. His main experimental approach was total or partial sympathectomy where homeostatic responses were observed under normal and stress conditions.

Selye, dissatisfied with Cannon's theory, did a series of experiments demonstrating that any animal presented with a noxious stressor would respond in a very characteristic manner elicited by adrenalcortical hyperfunction due to stimulation of the anterior pituitary.

The theories of Cannon and Selye do not necessarily conflict but together tend to present the overall picture of the concept of stress. While the classical observations of Cannon clearly pointed to the adrenal medulla as an important reactor in response to disturbing factors, Selye studied the adrenal cortex and placed more emphasis on the pituitary-adrenocortical system. However, both of these systems are operating in stress conditions, and the type of reaction depends on the nature of the stressing factors.

There is little doubt of the central position of the pituitary-adrenal axis in adaptation; however, ANS activity is also important. George Sayers (1950) compared these relationships. He stated that the adrenal-cortical hormones play a generally supportive role rather than an initiating role in bodily processes; whereas the adrenal medulla

initiates cellular and metabolic changes in response to an emergency. Also, the adrenal cortex plays a passive role and makes it possible for various regulatory systems to expend the additional effort necessary for homeostatic adjustment.

3. General Adaptation Syndrome

Selye pointed out that, although different disease syndromes have unique properties and symptoms, they have many features in common, and it is these common features that constitute stress. Disease is not just suffering, but a fight to maintain the homeostatic balance of our tissues, despite damage. Selye's definition of stress is as follows: "Stress is a state manifested by a specific syndrome, which consists of all the non-specifically induced changes within a biological system." Thus stress has its own characteristic form and composition but no particular cause. It may be described more simply as the rate of wear and tear in the body. It is increased during nervous tension, physical injury, infection, muscular exertion or any other strenuous activity, and is connected with a nonspecific defense mechanism which increases resistance to stressor agents. An important part of this defense mechanism is the increased secretion by the hypophysis of adrenocorticotrophic hormone (ACTH), which in turn stimulates the adrenal cortex to produce corticoids (gluco- and mineralocorticoids).

Selye postulated that organisms subjected to alarming stimuli will respond in a given manner, which he termed the "stress syndrome" or "general adaptation syndrome" (GAS). The GAS evolves in three stages: the initial stage, or the "alarm reaction" (AR) during which defensive forces are mobilized; a secondary stage, the "stage of resistance",

which reflects full adaptation to the stressor; and, finally, the "stage of exhaustion" which follows when the stressor is severe and applied for a prolonged period.

The AR is associated with the discharge of ACTH, cortical steroids (CS), and CA, plus various other physiological changes such as autonomic excitability, heart rate, muscle tone, blood content changes and gastrointestinal ulceration. During the second stage, there is an adaptation to the stressor which results in a diminished reaction and thus increased resistance. Depending on the nature and intensity of the stressor and the condition of the organism at the time of exposure, the period of resistance may be short or prolonged. Finally, when the animal can no longer adapt to or compensate for the prolonged overexposure to the stressor, it will pass into the exhaustive stage and death ensues.

Berry and Buckley (1966) reviewed the physiological responses to stress as suggested by Selye (1955). The responses are as follows: the stressor acts on the body or some part of it directly by way of the pituitary and adrenals. An immediate discharge of ACTH stimulates the release of corticoids from the adrenal cortex. If the stress is extremely severe, the adrenal cortex shows morphological changes characteristic of hyperactivity. Simultaneously, the animal's corticoid requirement markedly increases, and there is an increase in the blood concentration and urinary excretion of corticoids and their metabolites. There is a general stimulation of the sympathetic division of the ANS; and the splanchnics induce the adrenal medulla to discharge E and NE, thus increasing the discharge of NE at various peripheral receptor sites and causing the cardiovascular responses of vasoconstriction and hypertension. Other physiological changes include alterations in water and

electrolyte metabolism, gluconeogenesis and increased blood sugar levels, alteration in both red and white blood cell counts, and increased renin production by the kidney.

Selye (1950) reported that almost all the changes caused by systemic stressors follow a characteristic triphasic course, in which the direction of the deviation from the normal is the same in the AR (stage one) and the stage of exhaustion (stage three) but is reversed in the stage of resistance (stage two). For example, the adrenals lose lipids in stages one and three, but store lipids in stage two; the thymus discharges thymocytes in stages one and three, but stores them in stage two; there are hypoglycemia and hypochloremia in stages one and three, but hyperglycemia and hyperchloremia in stage two. This explains many of the apparently contradictory findings reported in the literature concerning the effects of exposure to systemic stressor agents. Hence, the changes produced by stressors in the various targets cannot be discussed without consideration of the time-relations as stated above. There are also organ weight changes which characterize each particular stage of the stress syndrome. In general, there is adrenalcortical enlargement and hyperactivity; there is atrophy of the thymus, the spleen and lymph nodes and of other lymphatic structures in the body during the AR. Gonadal weight is also decreased during this stage. In the latter stages of the syndrome these changes will persist or return to the normal.

Throughout the stress syndrome, numerous biochemical patterns change, an indication of specific homeostatic adjustments. Changes in adrenal weight provide some information on endogenous ACTH secretion; however, adrenal weight alone does not reflect rapid modifications in

ACTH secretion. Other changes that are attributed to the release of ACTH are: a fall in adrenal ascorbic acid (AAA); a rise in plasma corticosterone; an increase in liver tryptophan pyrrolase (TP) activity; and an excessive mobilization of free fatty acids (FFA) from body fat depots (Westermann, 1962). Increases of adrenocorticosteroid output and decreases in adrenal cholesterol (Fortier et al., 1950) and ascorbic acid (Sayers and Sayers, 1947) have been reported as good indexes of adrenocortical hyperactivity. Within the past decade numerous analytical methods have been developed for the direct measurement of ACTH (Lipscomb and Nelson, 1962; Munson and Toepel, 1958) and for the direct analyses of plasma and adrenal corticosteroids (Guillemin et al., 1958; Zenker and Bernstein, 1958). These methods have greatly enhanced recent investigations concerning the pituitary-adrenal axis.

4. Pituitary-Adrenal Axis and Stress

Selye (1950) reported that, before a stressor will elicit a normal GAS response, it must first reach the centers of the two coordinating systems responsible for setting this defense reaction into motion. These are the hypothalamus, as a center of the ANS, and the anterior pituitary, as the chief coordinator of the endocrine glands. Smith (1927), one of the first researchers in this area to study the relationship between adrenocortical activity and the pituitary gland, demonstrated a rapid adrenal cortical atrophy due to hypophysectomy. Selye (1936) reported that a number of "nocuous" treatments, which varied greatly in their nature and specific effects, possessed a common property: the ability to produce hypertrophy of the adrenals and involution of the thymus in the intact rat. Following hypophysectomy, however, although

the specific effects were again produced, the nonspecific effects on the adrenals and thymus failed to appear. Since that time, it has been demonstrated that, every type of stressor activates the anterior pituitary-adrenocortical system.

Selye (1950) reported that, upon exposure to numerous types of stress, the anterior pituitary responds with an increased production of ACTH, thus causing a release of the adrenal cortical hormones. He also reported that there is some evidence to indicate that certain stressors may affect the anterior pituitary by way of the hypothalamic center. Selye's discovery initiated a great deal of research in the many aspects of endocrinology of the anterior pituitary-adrenocortical axis. One of the most important aspects, which is still being investigated, is the CNS control of the anterior pituitary function. Because the activation of the anterior pituitary-adrenocortical axis occurs in response to practically all experimental procedures in intact animals, it has been extremely difficult to analyze the mechanism of activation. This problem became more complex after it was demonstrated that the secretion of ACTH from the anterior pituitary is increased by neurohumors from hypothalamic areas (DeGroot and Harris, 1950). This development was preceded by a large number of publications assigning the role of "activator" of anterior pituitary to many substances occuring in the body. However, the work of Guillemin (1958, 1959b), Saffran (1959a; 1959b) and others, demonstrated that the hypothalamus exerts its effect on the pituitary via a hormonal substance, which when brought to the anterior pituitary through the hypophyseal portal vessels, stimulated the secretion of ACTH. This polypeptide, which is probably related to vasopressin, has been named the corticotrophin releasing factor (CRF).

Another physiological possibility involved in the anterior pituitary regulation is that the CNS exerts some chronic inhibitory influence over the hypothalamic mechanisms which stimulate ACTH release (Porter, 1954; Egdahl, 1961). The basic role played by stress in the activation of the pituitary-adrenal axis has been reviewed by numerous investigators, such as Fortier (1962) and Reichlin (1963). The mechanism of activation of ACTH is discussed in detail by Hilf (1965). A more detailed discussion on the synthesis and control of ACTH secretion in normal and stress conditions is given by Ganong (1963) and Mangili et al. (1966).

Ganong (1963) concluded that there is an increase in ACTH secretion to meet emergency situations. The ACTH is carried along the pathways that funnel through the median eminence. He states that there is a basal level of ACTH secretion that is independent of these pathways but not necessarily independent of neural control. He further states that the hypothalamus and the pituitary function as a unit in the response to stress and that the ACTH secretion rate in stressed animals is determined by the balance struck between the hypothalamic "drive" and the degree to which circulating corticoid levels inhibit ACTH secretion. Corticoids inhibit ACTH secretion by an effect on its synthesis and probably by an additional acute blocking effect. Similar conclusions were reported by Chowers et al. (1967). They stated that corticosteroids act directly on the hypothalamus to decrease CRF release and content and to influence storage and release of ACTH. Their findings support the view that, under certain conditions, corticosteroids exert an inhibitory effect on the hypothalamus as well as on the anterior pituitary. Mangili et al. (1966) discuss data clearly indicating that

the CNS plays an essential role in the control of the pituitary-adrenal axis, both in basal conditions and during stress-induced activation. They discussed the fact that it is not yet clear whether the same nervous pathways which control ACTH secretion through the steroid feedback mechanism are also involved in the activation of the pituitary during stress. They cited evidence to indicate a certain degree of independence between these two systems and supported the hypothesis that two seperate mechanisms are involved in the control of the pituitary-adrenal axis. This does not mean, however, that the two mechanisms are operating independently. There is ample evidence to prove the contrary, that is, that the final adjustment of the secretory activity of the pituitary results from a close interplay between the activating impulses (stress) which reach the pituitary and the feedback effect of the steroids.

5. Cathecholamines and Stress

E is discharged from the adrenal medulla during various types of systemic stress. A discharge of NE from adrenergic nerve-endings also accompanies systemic stress. The presence of some NE-like entity is thought to participate in the adrenergic response to stress. This discharge of its adrenergic hormones by the adrenal medulla during the GAS appears to be mainly, if not entirely, a result of splanchnic stimulation (Selye, 1950).

Stressing factors involving the CA-producing systems may either induce a reaction from the adrenal medulla, chiefly provoking an increased release of E, or activating the NE-producing nerves, or both. The E-release is the most common response to a variety of stressing factors, particularly those which involve a certain degree of emotional discomfort.

From this point of view, the E-secretory response is in a sense unspecific. Activation of the NE system appears to result from more specific stimuli, which bring blood pressure and temperature homeostasis into action (von Euler, 1964).

When NE is considered, the concern is not with the adrenal medulla but rather with the ANS and, more specifically, with its sympathetic branch. NE is a chemical transmitter of the postganglionic sympathetic nerves (von Euler, 1951). Very little NE comes from the adrenals in man, as revealed by the slight effect of total adrenalectomy (von Euler, 1955). The stimulus for adrenomedullary discharge travels via preganglionic sympathetic pathways from the hypothalamic nuclei which are in close anatomical relation to the autonomic "centers" (Folkow and von Euler, 1954). Ordinarily, sympathetic activation accompanies adrenomedullary discharge.

Leduc (1961) reported a constant and considerable increase in the NE excretion in rats exposed to cold. There was also an increase in E secretion. However, if the NE release in these rats was prevented by pharmacologic agents, the animals died; an indication of the importance of this reaction as a homeostatic mechanism in the metabolic sector. Other investigators have reported the importance of NE in cold adaptation (LeBlanc and Pouliot, 1964; LeBlanc et al., 1967).

Gutman and Weil-Malherbe (1967) studied the subcellular distribution of NE in the heart and spleen in rats after exposure to cold. Exposure to -15° for 90 minutes induced release of NE from the coarse, particulate, and soluble fraction of heart muscle. Chang and Su (1967) exposed rats to cold for two hours to study the effect of increased sympathetic activities on the subcellular distribution of NE in the heart. Cold

exposure caused a 30% decrease of total NE content in both auricles and ventricles of normal or adrenalectomized rats. They suggested that the NE in the particulate fraction is the functional part of the amine available for release by nerve impulses. Ingenito (1968) reported an increase in brain NE following 30 days of cold exposure. He suggested that this increase is derived from a source similar to that involved in the increases in peripheral CA on prolonged exposure to cold.

Hsieh and Carlsson (1957) showed that in cold-adapted rats the calorigenic effect of NE was potentiated and exceeded that of E. This calorigenic effect of NE in cold-adapted rats is considered to be dependent on an increased oxidation of lipids (Hanmon et al., 1963). Brodie et al. (1966) showed that the emergency mobilization of FFA from fat depots is under the precise and direct control of NE released from the sympathetic nerves and that E from the adrenal medulla is not needed for this function. They suggested that moderate exercise may be fueled by an increased release of FFA through the action of NE.

Ganong and Lorenzen (1967) reported that most stressful stimuli and drugs which increase ACTH secretion also decrease brain NE. Conversely, the monoamine oxidase inhibitors (MAOI) increase NE content, and some of these drugs decrease ACTH secretion. Levi and Maynert (1964), however, claimed that the stress-induced decrease in brain NE is the result of a decline in NE that is outside the nerve endings, pointing out that changes in NE content do not always accompany changes in ACTH secretion. They also reported that stressful stimuli cause increased ACTH secretion, and that adrenalectomy has the same effect on ACTH secretion. One might expect that brain NE would be decreased by adrenalectomy; however, adrenalectomy had no effect on brain NE.

In conclusion, stress and exposure to cold are mainly associated with an increase in the NE excretion, indicating the importance of this hormone in circulatory and temperature controlling homeostatic mechanisms. Mental stress involving exhilaratory or aggressive reactions is also associated with an increase in the NE excretion. The types of emotional stress characterized by apprehension, anxiety, pain, or general discomfort are regularly accompanied by an increase in the E excretion (von Euler, 1964).

B) CATECHOLAMINES

1. Catecholamine Metabolism

Research on the physiology and pharmacology of the CA during the past few years has introduced into the literature many excellent reports concerning the syntheses and metabolism of the CA in the brain and heart and also the role of these amines as neurochemical transducers. Review articles (Axelrod, 1963; 1965; Kopin, 1964; Glowinski and Baldessarini, 1966); two excellent symposia (Krayer, 1959, Achenson, 1966); and two texts (von Euler, 1956; Wurtman, 1966) are available for information on other aspects of CA function.

The CA have been recognized as hormones, and it is now known that NE plays a dual role: it is the immediate precursor of E; and it is also considered by many investigators to be the neurotransmitter substance at adrenergic nerve endings. The steps in the formation of the CA; E, and NE from phenylalanine were first proposed by Blaschko in 1939. This pathway was established by Gurin and Delluva (1947) who administered radioactive phenylalanine to rats and isolated radioactive E from the adrenal gland. Udenfriend and Wyngaarden (1956) demonstrated

that, in addition to radioactive phenylalanine, radioactive tyrosine and dopa also form radioactive E in the adrenal medulla. It is now accepted that this pathway represents the actual biosynthetic transformations. Sympathetic nerve preparations are also capable of converting radioactive tyrosine or dopa to radioactive E (Goodall and Kirshner, 1958).

Some CA are synthesized in the brain (Udenfriend and Zaltzman-Nirenberg, 1963) and in chromaffin tissue such as the adrenal medulla, but most of it is synthesized in the sympathetic nervous system (von Euler, 1956). The starting material is the amino acid L-phenylalanine, which is converted by a hydrolase enzyme to L-tyrosine (Gurin and Delluva, 1947). L-tyrosine can also be taken up by the blood and is converted by L-tyrosine hydroxylase to L-dopa. This hydroxylation is the rate-limiting step (Levett et al., 1964; Udenfriend et al., 1966; Neff and Costa, 1966; Spector et al., 1967). Another step is the conversion of L-dopa to L-dopamine via the enzyme dopa decarboxylase (Holtz, 1939; Lovenberg, 1962). Dopamine is then converted to L-NE by the enzyme dopamine-beta-oxidase (Levin et al., 1960). The properties of this enzyme were reviewed by Schoot and Creveling (1965). NE is then converted to E by the enzyme, phenylethanolamine-N-methyl transferase (Axelrod, 1962). This enzyme is highly localized in the adrenal medulla. The biosynthesis of E in the adrenal medulla appears to be regulated by the pituitary-adrenocortical system (Wurtman and Axelrod, 1965).

Blaschko and Welch (1953) showed that E is stored in chromaffin tissue and that NE is stored in both chromaffin tissue and in certain neurons, including the sympathetic nerves. The storage complex contains

adenosine triphosphate (ATP) and a protein in vesicles or granules (Hillarp and Nelson, 1954). The neural storage mechanisms can store not only locally synthesized by also circulating CA (Axelrod et al., 1959c), as well as structurally related substances (Crout and Shore, 1964); these mechanisms can also restore a portion of the CA released from their granules (Mendlowitz et al., 1964).

The uptake of circulating E and NE by various tissues was studied in animals after the intravenous administration of physiological amounts of tritium-labeled compounds (Axelrod et al., 1959c; Whitby et al., 1961). Within two minutes, heart, spleen, lung, and adrenal gland took up the largest amounts of the circulating amines, while muscle and brain took up the least. The large amount of circulating CA taken up by the heart suggests that these amines discharged from the adrenal medulla into the blood stream could serve to stock the myocardial stores. Kopin and Gordon (1963b) observed that 20% of the CA stores in the heart are derived from the circulating NE. Only negligible quantities of the circulating CA were taken up by the brain (Weil-Malherbe et al., 1959; 1961a), a result of the presence of a blood-brain barrier toward these amines. The hypothalamus took up small amounts of CA, while the pituitary gland and the pineal gland contained larger concentrations of radioactive CA, probably because the blood-brain barrier is not in the immediate area of these structures. The large amounts of CA that are present endogenously in the brain are presumably synthesized from precursors that are capable of crossing the blood-brain barrier. Glowinski and Iversen (1966) show that there are also differences in NE turnover rates in specific areas of the brain. The rapid uptake and binding of CA occurs mainly in the

sympathetic nervous system (Hertting and Axelrod, 1961) and is a major means of CA inactivation (Kopin et al., 1962). The amount of labeled CA taken up into a tissue is related to the fraction of the cardiac output which perfuses the organ and the density of sympathetic nervous tissue in the organ. The heart, which has a rich sympathetic nerve supply and which receives a relatively large proportion of the cardiac output, takes up a large proportion of the administered CA. Other organs which have a rich sympathetic nerve supply also bind NE (Kopin, 1966). NE is not only taken up by tissues from the circulation but it is also retained for long periods of time (Whitby et al., 1961). Evidence that bound NE is present in more than one pool has been derived both from pharmacologic and from biochemical sources. After the administration of labeled NE, there is a multiphasic decrease in the radioactive NE remaining in the heart both in vivo (Axelrod et al., 1961a) and in vitro (Kopin et al., 1962), indicating that there is more than one compartment for storage of the amine. Trendelenburg (1961b) suggested a division of tissue NE stores into a "bound" and an "available" store. The binding protects NE from enzymatic attack (Whitby et al., 1961).

As the CA are released for alpha or beta stimulation (Ahlquist, 1948), they are quickly degraded by enzymes, chiefly monoamine oxidase (MAO) (Blaschko et al., 1937; Zeller et al., 1955) and catechol-O-methyl transferase (COMT) (Axelrod, 1957). The physiological activity of the deaminated or the O-methylated metabolites is only a fraction of that of the parent compound, indicating that both types of transformation are inactivating. After the administration of NE or E, the main urinary metabolites are vanillyl-mandelic acid (VMA) (Armstrong

et al., 1957), normetanephrine (NM), metanephrine (M) (Axelrod, 1957) and 3-methoxy-4-hydroxyphenylglycol (MHPG) (Axelrod et al., 1959a). The following minor metabolites have also been identified. 3,4-dihydroxymandelic acid (Kershaw et al., 1958); 3,4-dihydroxyphenylglycol (Kopin and Axelrod, 1960); N-acetylnormetanephrine (Smith and Wortis, 1962); N-methyladrenaline and N-methylmetanephrine (Axelrod, 1960).

Some of these metabolites are conjugated in the liver to form sulfates and glucuronides; and most of them, as well as E and NE, can be detected in the urine and blood under certain circumstances (Manger et al., 1959; Armstrong and McMillan, 1957; Sjoerdsma et al., 1959).

MAO is a nonspecific enzyme; it deaminates alkyl and aromatic amines that have an amine group attached to the terminal carbon atoms (Blaschko et al., 1937). This enzyme has been found in all mammalian tissues, highly localized in the mitochondria (Blaschko et al., 1957). MAO is inhibited by hydrazine derivatives in vitro and in vivo (Zeller and Borsky, 1952). Relatively large quantities of MAO are present in sympathetic nerves (Snyder et al., 1965). Tyramine, dopamine and serotonin are much better substrates for MAO than NE and E (Kopin, 1964). When MAO activity is markedly inhibited in the intact animal, there is a rise in the tissue levels of several monoamines, including NE and serotonin (Shore et al., 1957). MAO inhibition also depresses the spontaneous release of stored radioactive NE from sympathetic nerve endings (Axelrod et al., 1961a) as well as the release of amine following the administration of reserpine (Shore et al., 1957).

COMT has been partially purified and its properties studied (Axelrod and Tomchick, 1958). It catalyzes the transfer of the methyl group of S-adenosylmethionine to the 3-hydroxy group of catechols. The

enzyme is relatively nonspecific and O-methylates a wide variety of endogenous catechols such as E, NE, dopamine, dopa, 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxymandelic acid as well as many foreign catechols. It does not O-methylate monophenols. COMT is widely distributed in all organ tissues including sympathetic and parasympathetic nerves, glands, blood vessels and all areas of the brain; but it is concentrated chiefly in the liver and kidney (Axelrod and Tomchick, 1958). The enzyme is localized in the cytoplasm, in contrast to MAO which is found in the mitochondria. COMT is inhibited by pyrogallol (Axelrod and Laroche, 1959b), catechols (Carlsson et al., 1962) and tropolones (Belleau and Burba, 1961). When given to intact animals, COMT inhibitors prolong the action of administered CA or CA discharged from nerves.

In conclusion then, a hypothetical picture of the metabolism and storage of CA, as shown by Bertler (1961) is as follows.

1. tyrosine is the precursor of dopa;

2. dopa is decarboxylated in the cytoplasm by dopa decarboxylase;

3. the dopamine formed is immediately transferred to granules, which probably are of the same type as the E and NE containing particles. In these granules dopamine can be stored unchanged as in the caudate nucleus;

4. dopamine can also be transformed to NE;

5. the amines are liberated from their storage sites by nerve impulses and then reach their receptors in relatively high concentrations;

6. outside the granules a continuous inactivation of the CA is going on by means of MAO and COMT;

7. most of the circulating NE is metabolized by O-methylation; the remainder is taken up and bound in dense core vesicle in sympathetic nerves;

8. when NE is released, it is metabolized by MAO within the nerve or by COMT outside the nerve;

9. a large amount of the CA is inactivated by being bound again or by diffusing into the circulation.

2. Norepinephrine and the Concept of Neurochemical Transducer

Background information on the role of NE as a neurotransmitter is included in papers by Brodie and Bevane (1963) and Maynert and Kuriyama (1964). The sympathetic or adrenergic nervous system is recognized as one of the fine regulatory mechanisms in animals. It is involved in regulation of blood pressure, carbohydrate and fat metabolism, temperature, eye movement, and many other functions both central and peripheral. The concept that the sympathetic nerves secrete a physiologically active compound was first proposed by Elliott (1905). He described the action of E on smooth muscle organs and on the heart muscle and suggested that sympathetic nerves release a pressor substance which could then act on an effector organ. Many years later, the classic experiments of Loewi (1921) and of Cannon and Uridil (1921) revealed that a chemical substance secreted by the sympathetic nerves is the actual regulatory agent. Loewi provided experimental evidence that stimulation of sympathetic nerves resulted in the release of an adrenalin-like substance. These workers established the concept of neurotransmission. The identification of the sympathetic neurotransmitter as NE was established by von Euler in 1949. Once the neurotransmitter was identified, it became

obvious that its formation involved several intermediates and tissue enzymes. The demonstration by Goodall and Kirshner (1958) of the formation of NE from tyrosine in homogenates of sympathetic nerve tissue provided final evidence that the nerve trunk and terminals can manufacture their own neurotransmitter from the dietary precursor, tyramine.

Brodie and Beaven (1963) described a theoretical model of the biophysical units at nerve endings that are responsible for the formation, storage, inactivation and physiological release of NE and other biogenic amines. These units have been termed "neurochemical transducers", since they translate elctrical impulses into a quantity of free hormone; the free amine, in turn, acts on a target organ to produce mechanical or chemical energy or on an adjacent neuron to produce more electrical impulses. The theoretical model of the neurochemical transducer is based on kinetic data which describe the movement of H^3-NE within compartments in sympathetic nerve endings after the amine is taken up in tracer amounts. Mathematical analysis of the kinetic data has shown that endogenous NE is localized in an open two-compartment system and that the content of stored amine is in a dynamic balance between rates of synthesis and disappearance (Montanari et al., 1963). One compartment is a mobile or readily available pool from which NE is released by nerve impulses. The amine formed in this pool is maintained at a high concentration by a transport mechanism or pump which resists its free outward diffusion. This motile NE pool is in chemical equilibrium with a larger pool of NE in the second compartment, held in granules, presumably as a complex. The NE in granules is considered to act as a reserve pool of amine. Hence, if NE is released by a

primary action on the mobile pool, some of the amine in the reserve pool will also be mobilized in order to maintain chemical equilibrium.

According to the neurochemical transducer model, MAO controls the amount of NE in the nerve endings so that at the steady-state level the amine does not freely diffuse onto receptor sites. In the absence of sympathetic tone, NE can leave the storage compartments by simple diffusion through the lipoid membrane onto MAO. After nerve stimulation the amine is released directly onto the receptor sites and reaches the blood stream as the free base.

The possibility that the sympathetic nerves may be involved in the selective uptake and retention of CA has been previously discussed. Circulating NE and E are selectively taken up and retained by sympathetic nerves. This uptake and binding serves as an important mechanism for the rapid inactivation of these hormones. The action of the transmitter released at the adrenergic terminals may also be terminated by localized enzymatic destruction and by escape into the circulation.

A number of investigators have reported that during sympathetic nerve stimulation endogenous NE is released into the circulation (von Euler, 1956; Rosell et al., 1963; Boullin, 1966b). The fate of this transmitter substance depends upon the fraction of the cardiac output to the tissue and the density of the sympathetic innervation (Kopin et al., 1965). Leduc (1961) postulated the expectation that steady state levels of the transmitter within adrenergic nerves could be maintained by a mechanism of local synthesis, coupled in some way to the state of nervous activity and hence to the rate of secretion of NE.

There is acceleration of CA biosyntheses due to increased nervous

activity in the adrenal medulla (Bygdeman and von Euler, 1958; Bygdeman et al., 1960). The rate-limiting step for the biosynthesis of NE in heart tissue (Levitt et al., 1965) and in salivary glands (Musacchio and Weise, 1965) has been shown to be the conversion of tyrosine to dopa. Roth et al. (1966) using an isolated hypogastric nerve-vas deferens preparation, also reported a 3-fold increase in the amount of NE synthesized from exogenous tyrosine. They concluded that the isolated vas deferens preparation is capable of synthesizing NE from exogenous tyrosine and that local NE synthesis is in some way regulated by nervous activity and is not a steady state phenomenon independent of impulse traffic. Austin et al. (1967) confirmed these results, showing that on electrical stimulation of the nerve an increased synthesis and release of NE and dopamine was observed. They postulated that nerve stimulation increased production of some step in the pathway subsequent to tyrosine hydroxylase. Sedvall and Kopin (1967), using the rat submaxillary gland preparation, suggested an impulse-induced acceleration of NE synthesis in the adrenergic neurons and an apparently stimulated synthesis at or before the tyrosine hydroxylation step. They concluded that the rate of NE synthesis might be controlled by tyrosine transport, product inhibition, endogenous inhibitors or cofactors of tyrosine hydroxylase.

Kopin (1967) stated that the events that occur following the arrival of the sympathetic nerve impulse at the nerve terminals and that lead to release of NE are essentially unknown. Acetylcholine can induce the release of NE from sympathetic nerves; however, the importance of acetylcholine as an obligatory intermediary in release of the adrenergic transmitter is not established. There is evidence

that, at least at some sites in some species, acetylcholine may play a facilitory role in the release of NE from sympathetic nerve endings.

C) GUANETHIDINE

1. History and Pharmacology

Guanethidine, (2-(octahydro-1-azocinyl)-ethyl)-guanidine sulfate is a potent antihypertensive agent which is purely a synthetic compound. Its synthesis was first described in 1959 by Maxwell, Mull and Plummer. Maxwell et al. (1959; 1960a; 1960b) have comprehensively investigated the pharmacology of guanethidine. Guanethidine elicits a biphasic response: an initial sympathomimetic effect (hypertension and contraction of the nictitating membrane in the cat), followed by the failure of postganglionic nerve stimulation. Guanethidine also potentiates the response of effector systems to NE, and after a single dose the potentiation may be observed for up to five days. Maxwell et al. suggested that the initial sympathomimetic responses were due to direct adrenergic effects and that the later sympatholytic actions were due to a reserpine-like depletion of CA stores.

Page and Dustan (1959) gave five to 15 mg/kg i.v., of guanethidine to normotensive dogs and cats. They found an initial transient fall in the arterial pressure, followed by a prominent rise and tachycardia that persisted from 30 minutes to two hours. The increase in pressure was associated with vaso-constriction and increase in cardiac output; its degree and duration were directly related to the dose. The pressor response was accompanied by some sympathomimetic effects, such as pilo-erection and contraction of the nictitating membrane, probably the result either of the release of the endogenous stores of NE or of a

more direct sympathomimetic action of the compound. The initial sympathomimetic action was followed by a prolonged period of sympathetic paralysis, accompanied by a decrease of arterial pressure (which was more prominent in experimentally produced hypertensive animals than in normal), bradycardia, decrease of pulse pressure and relaxation of the nictitating membrane. These effects persisted to some degree for four to ten days. Page et al. (1961) tentatively concluded that guanethidine caused a decrease in blood pressure by initially interfering with the release of NE at the myoneural junction. The pressor response was associated with the initial depletion of endogenous amine stores, especially in the heart and blood vessels. The chronic hypotensive effect was due to the depletion of peripheral stores of NE.

McCubbin et al. (1961) reported similar effects in anesthetised dogs. They showed that during the sustained pressor response, i.v. NE responses were augmented in dogs with the vagus nerve severed. No signs of augmentation were observed in dogs with the nerve intact. They concluded that the increase in pressure suggests the release of bound endogenous CA stores.

Cass and Spriggs (1961) suggested that guanethidine has a dual mechanism in exerting its sympatholytic actions. They proposed that the sympatholytic action is secondary to its primary bretylium-like action. (Bretylium is a unique compound among the sympatholytic drugs, since it is a quaternary ammonium compound that acts at nerve terminals to prevent the physiological release of the adrenergic transmitter and neither releases appreciable amounts of NE in vivo nor antagonizes the effects of administered CA (Boura and Green, 1959)). Their conclusion was based on the finding that guanethidine blocks the effects of

sympathetic stimulation in rats long before it produces an appreciable decline in the amount of NE in sympathetic nerve endings. This mechanism of action of guanethidine was reinforced by the findings of Hertting et al. (1962). Bein (1960), however, has expressed doubt that guanethidine action includes a mechanism similar to that suggested for bretylium.

2. Effects on Catecholamines

Sheppard and Zimmerman (1959; 1960b) showed in rats that guanethidine reduced the CA concentration of the spleen and heart, and in dogs it lowered the CA concentration of the heart and arteries. Cass et al. (1960) reported that guanethidine depleted the NE in the heart of rabbits and cats. They suggested that the compound produced its chemical sympathectomy through depletion of NE at the peripheral nerve endings. Cass and Spriggs (1961) observed that a single dose of guanethidine in rats produced a considerable and longlasting depletion of the CA. Other workers have reported depletion of CA in peripheral tissue with guanethidine (Butterfield and Richardson, 1961; Stone and Beyer, 1962; Sanan and Vogt, 1962; Kuntzman et al., 1962).

Hertting et al. (1961b) showed in male cats that guanethidine markedly decreased the uptake of H^3-NE in the heart and spleen, but not in the adrenals, and only moderately decreased it in the liver. These workers suggested that guanethidine appeared to act by preventing entry and/or binding of H^3-NE in the tissues. Dengler et al. (1961) demonstrated that guanethidine inhibited the uptake of isotopic NE by cat tissue and depleted the peripheral tissues of NE but not of serotonin. They suggested that the depletion of the NE stores in cells may depend on inhibition of the uptake mechanism. Bisson and Muschell (1962) suggested that guanethidine abolishes the ability of

the tissue to bind CA.

Lindmar and Muschell (1961) used isolated rabbit hearts perfused with Tyrode's solution to study the effect on the NE output. Tyramine and 1,1-dimethyl-4-phenylpiperazinium iodide (DPP) increased the output of NE. Guanethidine decreased the NE release by tyramine and the NE release induced by DPP was also blocked. Similar results were also reported by Kroneberg and Schumann (1962). They showed that inhibition of the tyramine response was due to a direct action of guanethidine and not to a depletion of CA stores. However, guanethidine has also been shown to potentiate the response to NE and to inhibit the pressor response to tyramine (Bhagat and Shideman, 1963a). Bhagat (1963a) showed that guanethidine had no effect on the depleting action of tyramine in the rat heart.

Kuntzman et al. (1962) reported that guanethidine produces a longlasting depletion of heart NE; however, it does not lower the amine in the brain, presumably owing to the low rate of penetration across the blood-brain barrier. The investigators concluded that guanethidine releases NE from sympathetic nerve endings by an action which seems to differ from that of reserpine. Evidence is also given for the view that guanethidine acts oppositely to bretylium and activates the process involved in the normal release of NE by nerve impulses.

Day and Rand (1963) showed that guanethidine acts on stores of NE at sympathetic nerve endings. Abercrombie and Davies (1963) discussed the nature of the action of guanethidine and concluded that it blocks the effect of postganglionic sympathetic nerve stimulation by interfering with the syntheses of transmitter and that it also has a direct sympathetic effect. DaVanzo et al. (1964) showed that guanethidine

decreased the effectiveness of sympathetic nerve stimulation by causing a depletion of CA stores in peripheral nerves. They concluded that this depletion occurs by a mechanism different from that of the ganglionic and adrenergic blocking agents.

Chang et al. (1964) reported that guanethidine does not release NE by a simple one-to-one displacement. Guanethidine must first be taken up before appreciable amounts of CA are released. Initially, the drug shares occupancy of the nerve endings with the endogenous amine, then, after entering the sympathetic neurons, it releases NE, perhaps by enhancing the permeability of the terminal membranes. At first, guanethidine releases at a rapid rate the more available pool of NE; in fact, large doses of guanethidine can elicit a pronounced sympathomimetic effect. Guanethidine also remains localized in adrenergic neurons for a considerable time and causes a steady loss of CA after the period of rapid release.

The action of guanethidine in blocking adrenergic function is not a direct result of NE loss; in fact, the amine levels are not yet reduced when sympathetic blockade is complete and are still low when it is terminated. However, the intensity of the adrenergic blockade ultimately produced by single doses of the drug is directly proportional to the initial rate of NE depletion and is closely related to the amount of guanethidine taken up by NE storage depots. These results suggest that the drug increases the porosity of the nerve membrane, thereby releasing NE and making nerve terminals unresponsive to incoming stimuli (Brodie et al., 1965).

Shore and Giachetti (1966) presented evidence that guanethidine at 10^{-4}M inhibited both a membrane amine pump and an intracellular

amine-concentrating mechanism. At a low concentration of guanethidine (5×10^{-6}M) the intracellular mechanism is preferentially inhibited. The action of guanethidine on the membrane pump is readily removed by washing. They concluded, that at clinical doses, guanethidine acts only on the intracellular mechanism, thus leading to depletion of NE.

Lundborg and Stitzel (1967) showed that guanethidine inhibits both the transport of amines through the nerve cell membrane and an uptake mechanism present in amine-storage granules. They suggested that the membrane pump-inhibiting ability possessed by guanethidine may account for the sympathomimetic activity and exogenous amine potentiation which often accompanies guanethidine administration.

Fielden and Green (1967) did a study on the NE-depleting and sympathetic-blocking action of guanethidine. They concluded that the sympathetic-blocking action of guanethidine is distinct from its NE-depleting action. The early action of guanethidine may be due, then, to adrenergic neuronal blockade, and in light of the Burns and Rand (1962) hypothesis, the drug may act by preventing the release of acetylcholine and its ability to release, in turn, NE.

Guanethidine, like alpha-methyl NE, metaraminal, and octopamine, is taken up and retained by adrenergic neurons; it causes the release of NE and is itself released by reserpine (Chang et al., 1964; 1965; Brodie et al., 1965). Boullin et al. (1966) reported that the term "false transmitter" has been applied to substances that replace NE in sympathetic nerve endings and that on nerve stimulation are themselves released onto adrenergic receptors to evoke sympathetic responses. Guanethidine is also taken up by adrenergic neurons, displaces the transmitter, and is discharged by nerve stimulation; but it fails to

exert a sympathomimetic action of its own.

Boullin (1966a; 1966c) showed that calcium is also involved in the storage and release of guanethidine, but not in guanethidine uptake. Boullin (1968) showed that the removal of calcium ions causes release of guanethidine primarily from nonspecific cellular binding sites and not from the intra-neuronal stores. He suggested that the action of guanethidine is to destroy the intra-neuronal store, and that its action may be particularly evident under calcium-free conditions.

Dixit et al. (1961) showed that guanethidine can also produce neuromuscular blockade, possibly by preventing acetylcholine release. Costa et al. (1962a) suggested that guanethidine might actually mimic the action of acetylcholine in adrenergic fibers, thus leading to a prolonged release of NE. Agarwal et al. (1965) found that chronic administration of guanethidine increased the acetylcholine content of brain, intestines and heart of albino rats. Rand and Wilson (1967) proposed receptors for adrenergic neuron-blocking activity allied to the acetylcholine receptors at other sites, and that interaction by guanethidine with these receptors interferes with cholinergic transmission at the neuromuscular junction and the ganglionic synapse. However, Chang et al. (1967b) in studies on the neuromuscular-blocking action of guanethidine, reported that its main effect appeared to be on muscle fibers. Contractile responses to direct muscle stimulation were reduced, and the form of muscle action potentials were changed.

There is also conflicting evidence for the effect of guanethidine on brain CA. Pfeifer et al. (1962; 1967) reported that guanethidine produced some effect on the CNS and decreased the NE in the brain. Dagirmanjian (1963) showed that single injections of guanethidine did

not lower the hypothalamic NE in the cat, but daily injections over a period of seven days consistently produced a decrease in the hypothalamic NE. Attempts to lower the hypothalamic NE in rats after single injections or daily injections of guanethidine were unsuccessful. Sanan and Vogt (1962) observed a fall in NE of the hypothalamus in cats after guanethidine and suggested that the effect was due to a reflex stimulation of the sympathetic centers, rather than to a direct effect on the hypothalamus. Studies on the disposition of guanethidine in the body show that the drug does not readily cross the blood-brain barrier. This seems to explain the lack of effect on brain amines, since intracisternal injections of the drug can lead to depletion of brain NE (Kuntzman et al., 1962).

Cass et al. (1960) reported that guanethidine did not affect the CA in brain and adrenal glands. Stone and Beyer (1962) reported similar results. Kuntzman et al. (1962) reported the resistance of the CA in the rat adrenal medulla to depletion by guanethidine. (See page 48.) Athos et al. (1962) demonstrated that direct perfusion of the isolated denervated adrenal gland in situ in the dog with guanethidine had no effect on the CA secretion. Intravenous dose in intact dogs caused no adrenal medullary stimulation; in fact, the adrenal secretion rate was markedly lowered. Garrett et al. (1965) did studies on isolated bovine adrenal glands but reported that both E and NE were released in varying proportions.

Superstine and Sulman (1966) found that guanethidine also influenced the output of pituitary hormones. Guanethidine suppressed the secretion of follicle-stimulating hormone, luteinizing hormone and somatotropin, while it stimulated the secretion of the luteotropic, adrenocortico-

tropic and antidiuretic hormones. Although the altered hormone secretion did not bear directly on the mode of action of guanethidine, the suggestion was made that the hypothalamus might be intermediary in the drug's useful physiological effects.

3. Miscellaneous

Kuntzman et al. (1962) reported that after the administration of guanethidine, 35 mg/kg, i.v., to rats, about half of the drug disappears from the animal in the first hour. After two hours, the rate of disappearance is sharply reduced and the level in the body declines exponentially with a half-life of about seven hours. At this time the drug is highly localized in various tissues; however, brain and plasma levels were barely measurable.

According to Chang et al. (1964), guanethidine is localized in rat tissues by two kinds of binding sites: nonspecific sites, present in all tissues and analogous to those which reversibly bind most drugs with tissue components; and specific sites; present mainly in tissues containing NE in high concentration. They found guanethidine taken up into heart slices by two processes, only one of which is readily saturated and which is inhibited by anaerobic conditions and by amphetamine.

In accord with this view are the results of Schanker and Morrison (1965), who administered guanethidine to rats (3.5 - 28 mg/kg, i.v.) and found that it became localized in a number of tissues. After two hours, the tissue/plasma concentration ratios were about 20 for heart, 10-12 for lung and intestines, 5-6 for skeletal muscle, spleen and kidney and 2 for liver. The general pattern of distribution changed with time. Localization was greatest in liver and kidneys in the beginning,

but later became greatest in heart and skeletal muscle.

Furst (1968) reported that after the injection of guanethidine sulfate (20 mg/kg, i.p.) the drug was rapidly and extensively metabolized. Approximately 70% of the drug was eliminated in the urine within 24 hours, mainly as a polar metabolite. Schanker and Morrison (1965) reported similar results, citing rapid metabolism and excretion in urine during the first hour after administration.

4. Summary

Kopin (1968) briefly summarized the effect of guanethidine in the following manner: Guanethidine depletes NE stores in tissues (Sheppard and Zimmerman, 1959; Cass et al., 1960), is bound to some extent in the same particulate fraction as NE (Chang et al., 1965), and can be released by sympathetic nerve stimulation (Boullin et al., 1966). Although this guanidine derivative may displace NE and have a role as a false transmitter (Boullin et al., 1966), it interferes with sympathetic neuronal function prior to any decrease in CA stores (Cass and Spriggs, 1961). Guanethidine may, therefore, diminish release of NE by interfering with the process of transmitter release rather than by replacing the transmitter.

D) RESERPINE

1. History and Pharmacology

Rauwolfia preparations employed clinically are obtained primarily from the root of Rauwolfia serpentina (Benth), a climbing shrub of the Apocynaceae family. Although preparations of the Rauwolfia alkaloids have been used for centuries in India, their widespread use in Western medicine began only after the isolation of reserpine in 1952 (Mueller

et al.). The principal pharmacological actions of reserpine are: 1. mild CNS depression and blockade of conditioned avoidance behavior, 2. hypotension, 3. hypothermia, and 4. marked peripheral ANS effects consisting of an increase in parasympathetic and a decrease in sympathetic activity (DiPalma, 1965; Goodman and Gilman, 1965).

Plummer et al. (1954) reported that reserpine inhibited the hypothalamus, initiating sympatholytic or parasympathomimetic responses. Weiskrantz (1957), in studying the behavioral pattern of reserpine, observed that it appeared to inhibit sensory input to the brain. Jacobson (1959), studied reserpine under psychic stress and found that it produced nonspecific effects. Domino (1962) concluded that reserpine either stimulates or depresses most areas of the brain, except the limbic system.

Holzbauer and Vogt (1956) found that reserpine depleted NE from its normal brain stores and concluded that reserpine acts as a sympatholytic agent. Brodie and Shore (1957) theorized that reserpine produces its tranquilization effect by releasing serotonin from its normally bound form.

Carlsson et al. (1957) demonstrated that dopa, a precursor of NE, restored the normal activity of reserpinized animals. Carlsson et al. (1958) demonstrated that reserpine also depleted dopamine, a precursor to NE and it is now believed that this latter compound has an action of its own. Carlsson theorized that reserpine produced its effects by depriving central synapses of accessible NE and dopamine.

Brodie et al. (1961) and Burns and Shore (1961) presented evidence that reserpine produces its effect through serotonin depletion. They showed that reserpine causes equal depletion of NE and serotonin from

their normal bound states in the brain. They observed that serotonin produced sedative effects similar to those of reserpine; that alpha-methyl-m-tyrosine (alpha MMT), which depletes the brain of its NE, had no sedative effect; and that cold stress four hours prior to the administration of reserpine prevented its sedative effects and also inhibited the depletion of serotonin induced by reserpine, but not that of NE. Revizin et al. (1961) demonstrated a correlation between CNS depression and lowered serotonin; however, they could demonstrate no relationship between NE depletion and evoked potentials.

Giarman and Schanberg (1959), working with serotonin, and Weil-Malherbe et al. (1961b), working with NE, found that in brain fractions from reserpinized animals there was an increase in the free/bound ratio of the concentration of both amines, an indication that reserpine has the ability to increase the "free" form of NE and serotonin. It is assumed that it is this "free" form of the amine that is accessible to central synaptic sites.

2. Effect on Catecholamines

In spite of the numerous attempts to explain the sometimes dramatic effect of reserpine, its mode of action is still largely unknown. The ability of reserpine to deplete tissue stores of their CA is well established and has been reviewed by Shore (1963) and Costa et al. (1966). Reserpine has been found to cause a depletion of the CA of various tissues and the extent of this depletion is found to be both species (Collengham and Mann, 1958) and tissue dependent (Carlsson et al., 1958). Very small, single doses of reserpine deplete the heart of NE (Brodie et al., 1957), while somewhat higher doses are necessary to affect brain

NE (Brodie et al., 1957; Holzbauer and Vogt, 1956) and dopamine (Carlsson et al., 1958). The depletion of CA from the adrenal medulla is relatively resistant to reserpine and still higher doses are required (Carlsson et al., 1957); this depletion in the adrenal medulla appears to be dependent, in part, on an intact nerve supply to the gland (Holzbauer and Vogt, 1956; Mirkin, 1961). Other investigators confirmed the ability of reserpine to deplete the CA in peripheral tissues other than the heart and adrenal medulla, these include: arterial tissue (Burn and Rand, 1957); sympathetic ganglia (Muscholl and Vogt, 1958); and fat (Paasonen and Pletscher, 1960).

Carlsson et al. (1957), Paasonen and Krayer (1958), and Bhagat and Shideman (1964) all reported that a minimum of two weeks time was required for the complete dissipation of the effects of reserpine upon cardiac CA depletion. In arriving at an explanation for this delayed reversibility of the effect of reserpine on CA stores, different workers have ruled out such possibilities as: 1. prolonged retention of reserpine at the site of action (Shore and Olin, 1958); 2. inhibition of CA biosynthesis (Paulson and Hess, 1963); or 3. slower turnover of amines in the heart (Bhagat, 1963b). However, there is now some acceptance of a possible reason for this long-lasting effect on CA stores as being related to an effect on a storage mechanism. Pretreatment of animals with reserpine impairs the uptake of adrenalmedullary granules of newly formed CA after dopa administration, or of administered NE and E by various tissues (Schaepdryver, 1959).

Axelrod et al. (1961b) and Hertting et al. (1961a) showed that in reserpinized tissues exogenous NE and E do not accumulate when the tissues are exposed to NE and E. Dengler et al. (1961) found that the

presence of reserpine in the incubation medium prevented the accumulation of NE in tissue slices. This ability of reserpine to inhibit the tissue uptake of CA has been widely accepted and theories have been elaborated in an attempt to explain the action of reserpine on amine storage on this basis (Brodie and Beaven, 1963). However, studies by Kopin et al. (1962) and Lindmar and Muschall (1964) present evidence that casts doubt upon this interpretation. Lindmar and Muschall measured the uptake of NE in the perfused rat heart. An analysis of the NE content of the heart at the end of the perfusion revealed that in normal animals the NE taken up from the medium could be accounted for almost entirely as unchanged NE which accumulated in the tissue. In reserpinized hearts the amount of NE taken up from the perfusing medium was the same as in normal hearts, but in this case the accumulated material did not appear as unchanged NE in the heart. These results suggest that the uptake of NE is normal in reserpinized hearts but that the accumulated NE cannot be retained within the tissue and is rapidly lost by metabolism. It follows then, that in reserpinized animals the tissues are able to accumulate exogenous CA, but they are no longer able to retain the CA thus accumulated. Kopin et al. showed that in the reserpinized rat heart the initial rate of accumulation of H^3-NE did not differ from that in normal hearts. They also reported that the kinetics of release of NE from the isolated reserpinized heart suggest that this drug effects the firmly bound reserve pool, but not the readily available pool. Kopin and Gordon (1962; 1963a) showed that the NE released from rat tissues by reserpine left the tissues largely in the form of MAO metabolites. Kopin et al. (1962) and Axelrod et al. (1962) showed that reserpinized rat hearts were unable to retain accumulated H^3-NE. These

results also indicate that the NE accumulated in reserpinized hearts did not leave the tissue as unchanged NE but was rapidly degraded by MAO to acid and alcohol metabolites which then left the tissue. Bhagat (1964b) also suggested that the main action of reserpine might be its interference with the storage mechanism and not with the uptake of CA. There appeared to be little change in the initial rate of uptake of CA after reserpine; but the tissue was unable to store it, so that which was taken up was rapidly lost through destruction by MAO. This suggestion could explain the CA-depleting effect of reserpine. Tissue whose ability to respond to nerve stimulation or to tyramine has been reduced by reserpine, can have that responsiveness temporarily restored by an infusion of NE (Burn and Rand, 1958; 1960; Rosell and Sedvall, 1961), further evidence in support of the view that CA uptake is normal in reserpinized tissues.

The conclusion that reserpine does not affect CA uptake thus appears to be a reasonable one. How reserpine affects the intracellular storage of CA remains obscure, though reports that reserpine affects the uptake and storage of NE in isolated NE storage particles (von Euler and Lishajko, 1963; von Euler et al., 1964) suggest that this may be the site of action of reserpine. Dahlstrom et al. (1965) concluded that the primary action of reserpine in producing a long-lasting block of storage function is not to block the mechanism for amine uptake (reabsorption) localized in the cell membrane of the entire adrenergic neuron, but to block the storage mechanism in the amine granules. They also concluded that the cell membrane is not the primary site of action of reserpine.

3. Effects on the Pituitary-Adrenal Axis

A survey of the literature concerning the action of reserpine on the pituitary-adrenocortical system reveals a conflicting picture. Several workers have shown that reserpine reduces the reactivity of the pituitary-adrenocortical system, probably by depressing the response of the hypothalamic regions that control the secretion of ACTH. This view was based on experiments which demonstrated that reserpine pretreatment of rats and monkeys prevented the decline in AAA or the rise in plasma corticosteroids ordinarily evoked by ether, histamine, surgical trauma, cold exposure, or emotional stress (Wells et al., 1956; Mahfouz and Ezz, 1958). Investigation of this possible mechanism was complicated by the fact that reserpine stimulates ACTH secretion. This has been shown in the rat (Khanzan et al., 1961; Maickel et al., 1961; Saffran and Vogt, 1960; Wells et al., 1956), in the dog (Egdahl et al., 1956) and in the monkey (Harwood and Mason, 1957). The dose levels found to stimulate ACTH secretion in the rat were 1 mg/kg, i.v. or 2.5 mg/kg, i.p.; changes in AAA, blood corticosterone or both were used as indicators of an increase in ACTH secretion.

The endocrine aspects of reserpine were first noted by Gaunt et al. (1954) in normal animals; changes in organ weight data indicated that reserpine caused mild stimulation of the adrenal cortex. Egdahl et al. (1956) showed that single injections of large doses of reserpine in dogs caused maximal ACTH release as judged by corticoid secretion rates. Work done in the monkey by Haward and Mason (1957) was in agreement with these findings. However, Mason and Brady (1956) found that reserpine, given for one week in monkeys, completely

suppressed the expected rise in plasma corticosteroids usually associated with anxiety states.

Wells et al. (1956) resorted to repeated daily injections of reserpine, counting on "adaptation" to the drug, to eliminate the stimulating effect on ACTH secretion. In contrast to the marked decrease in AAA seen four hours after the first injection, the values were at the control level 24 hours after the last injection. These observations were confirmed by Kitay et al. (1959) and Maickel et al. (1961). Wells et al. (1956) concluded that reserpine in a single initial injection caused marked ACTH release; however, after continued injections the pituitary became refractory to subsequent ACTH-releasing stimuli, and the workers concluded that reserpine in some way inhibited the secretion of the hypothalamic secretory factor for ACTH.

Guillemin (1957) attempted to determine whether any of the proposed neuro-humoral agents (5-HT, NE, etc.) were identical to CRF. He did not agree with previous findings regarding the ability of tranquilizers to inhibit acute stressors. Guillemin used a psychological stressor (forced restraint) in his study. He observed that, while reserpinized rats did not resist being restrained, the animals still displayed adrenocortical hyperfunction as indicated by the depletion of AAA. He also found that reserpine itself is a potent stressor, even though the animals were preinjected with the drug seven days prior to the restraint.

Mahfouz and Ezz (1958) used very small doses of reserpine, 8 ug/kg, i.m., before imposition of stress. They reported that the decrease in AAA seen in controls after exposure to heat, exposure to cold, or blood-letting under ether anesthetic did not occur in rats injected with

reserpine. Furthermore, reserpine was not able to inhibit the usual depletion of AAA by various doses of ACTH, but did inhibit the stressors mentioned above. Therefore, these workers concluded that reserpine inhibited the pituitary-adrenal axis by inhibiting some central regulatory mechanism, possibly the hypothalamus.

Kitay et al. (1959), in confirming the experimental data of previous investigators in this field, added a new dimension which led to different interpretation. They observed that when reserpine was given for a three-day period, the adrenal hypertrophy implied a continuous, high rate of ACTH secretion rather than a refractoriness after an initial stimulation. The essential new fact was that during this period, pituitary ACTH remained low, about one-third of normal. A presumably"non-specific" stressor, E, had similar effects; after either reserpine or E had been administered over a period of three days, the stress of ether anesthesia failed to cause a depletion of AAA. They suggested that the refractoriness of the ACTH response to stressful stimuli after previous administration of a depressant drug might be due to the reduction of pituitary ACTH provoked by the drug. The fact that E had no effects similar to reserpine provided additional support for this hypothesis. These observations were confirmed by Brodie et al. (1961) and Maickel et al. (1961). They further showed that exposure of rats to cold also reduced pituitary ACTH content and that this was followed by a period during which imposition of a second stressful experience (reserpine or additional cold) failed to stimulate increased ACTH secretion

In a series of papers, Maickel et al. (1961), Westerman et al. (1962) and Westerman (1965), by using the decrease in AAA, elevation

of plasma corticosterone and FFA, increase in the activity of TP and tyrosine transaminase in the liver, and increase in adrenal weight as indices, studied the action of reserpine and cold exposure (4°) on the anterior pituitary-adrenocortical system in rats. Their results indicated that reserpine depleted brain serotonin and NE, produced sedation, and induced a sustained stimulation of the anterior pituitary-adrenocortical system. Cold exposure had similar effects on the pituitary; however, no data on brain amines were given. Demonstration that these effects of reserpine were not seen in hypophysectomized or adrenalectomized rats while the drug still depleted brain amines indicated that the increased activity of the adrenal cortex was induced by a hypersecretion of ACTH. These investigators contended that the long duration of the pituitary activation was not evoked by reserpine *per se*, which disappeared from the body within a few hours (Hess *et al.*, 1958), but by the amine-depleting action of the drug. In these studies, it was shown that reserpine (5.0 mg/kg, i.v.) or a prolonged exposure to cold (4° for 20 hours) lowered the ACTH content of the pituitary to such an extent that the animals were unable to respond to an additional stressful stimulus or to another dose of reserpine by increasing plasma corticosterone.

Eechaute *et al.* (1962) failed to confirm these observations. They found that after pretreatment with a single dose of reserpine or after a series of four daily injections, reserpine did not block the adrenocortical response to stress of acute cold exposure. Montonari and Stockham (1962) presented similar results in different types of experiments. However, no pituitary ACTH or brain amine levels were reported in either of these studies.

Since CA injected in very low doses will stimulate ACTH secretion (Harris, 1955), the possibility was considered that the reserpine-induced release of CA from the adrenal medulla and sympathetic nerve endings might mediate ACTH hypersecretion. However, Maickel et al. (1961) reported that administration of reserpine to adrenodemedullated rats induced the same degree of pituitary activation as in the intact rat.

Syrosingopine, a carbethoxy analogue of reserpine, in small doses, released only peripheral NE (Orlans et al., 1960), e.g., from the heart, but did not stimulate the pituitary adrenal system. Only large doses which also depleted brain amines and produced sedation caused a sustained pituitary-adrenal stimulation. Therefore, it was suggested that the action of reserpine on the pituitary might be related to the depletion of brain amines.

Costa et al.(1962b) showed that administration of RO-4-1284, a benzoquinolizine derivative having reserpine-like action, produced marked sedation, a depletion of brain amines, and an elevation of the plasma corticosterone. This compound, in contrast to reserpine, was very short-acting. After about eight hours the animals were no longer sedated, and the brain amines returned to normal. A corresponding short-lasting elevation of plasma corticosterone indicated a correlation between brain amines and ACTH hypersecretion. Whether the reserpine-induced activation of the pituitary was more closely related to changes in the brain CA or to the depletion of brain serotonin was studied by using alpha-MMT, which in rats produced a long-lasting depletion of brain NE without significant changes in brain serotonin (Hess et al., 1961). There was no stimulation of the pituitary-adrenocortical

system in these rats, as indicated by unchanged TP activity. However, administration of reserpine caused a marked sedation and increased plasma corticosterone similar to those in the animals not pretreated with alpha-MMT. Westerman et al.(1962) also concluded that discharge of ACTH was related to serotonin depletion.

Giuliani et al. (1966) presented evidence to show that reserpine is able to enhance synthesis and release of ACTH even after both of these processes have been largely suppressed by the administration of potent adrenocortical steroids. They suggested that the main effect of reserpine on the pituitary-adrenal axis was that of enhancing rather than depressing ACTH secretion, and that this effect was achieved by the suppression of the midbrain inhibitory action. The reserpine-induced block of stress reactions was thought to be a result of the feedback effect of the enhanced blood levels of adrenal steroids.

4. Miscellaneous

Reserpine has been shown to leave tissues rapidly after injection, so that at the time of maximal NE depletion, little or no reserpine can be detected in tissues (Hess et al., 1956). However, experiments with tritiated reserpine have revealed that the drug persists in tissues in very low concentrations for at least 48 hours after injection (Plummer et al., 1957).

5. Summary

Bloom and Giarman (1958) concluded that reserpine, which reduces amine storage by acting on the storage particles, releases amines earliest from unidentified structural elements present in the supernatant fraction, and does so with increased catabolism by MAO. Recovery

from reserpine-induced behavioral and autonomic symptoms correlates with recovery of the accumulation process but not with the slower recovery of endogenous amine levels. Reserpine does not appear to affect either the synthetic enzymes (Glowinski and Baldessarini, 1966; Glowinski et al., 1966) or uptake of amines into the neuronal cytoplasm (Glowinski and Baldessarini, 1966; Dahlstrom et al., 1965; Carlsson et al., 1965; 1966; Malmfors, 1965). Overall synthesis could be impaired by accelerated catabolism of unbound dopamine before the latter is converted to NE.

E. POINTS OF SIMILARITY AND DIFFERENCES REGARDING GUANETHIDINE AND RESERPINE EFFECTS ON CATECHOLAMINES

Guanethidine and reserpine have several properties in common, but there is much evidence to suggest that their mechanisms of action are different. In the first place, the chemical structures of the two compounds are entirely different. Guanethidine is a derivative of guanidine and an extremely strong base with low lipid solubility; in contrast, reserpine is a polycyclic, weakly basic structure having a high lipid solubility. It seems unlikely, therefore, that the two compounds release NE by the same mechanism.

The biphasic response to guanethidine (Maxwell et al., 1959; 1960a; 1960b) and its potentiation of the response of effector systems to NE (Boura and Green, 1962) are similar to those following reserpine administration and this similarity is extended since the pressor action of tyramine is reduced after guanethidine administration (Bhagat and Shideman, 1963b). Sheppard and Zimmerman (1960b) showed that guanethidine partially depleted the CA of rat heart and spleen, an action not entirely analogous to that exhibited by reserpine, because it did

not deplete the CA of the brain or adrenal medulla (Cass et al., 1960).

The effects of guanethidine on biogenic amines differ from those of reserpine in a number of respects: guanethidine depletes heart NE more slowly than does reserpine; guanethidine does not lower the serotonin content of platelets or intestines; and large doses of guanethidine produce a brief sympathomimetic effect which precedes the adrenergic neuronal-blocking action and appears to depend on the availability of stored NE (Bein, 1960; Burn, 1961).

Kuntzman et al. (1962) showed that over a four-hour period guanethidine (35 mg/kg) lowered heart NE to about ten percent of normal. The NE remained low for 24 hours and, during the next 24 hours, gradually started to rise. Coincident with the rise in NE, the guanethidine had virtually disappeared from the body. These effects are in contrast to those of reserpine which produces a depletion of heart NE that persists long after the drug is no longer detectable (Hess et al., 1956).

Kuntzman et al. also reported that in doses of 150 mg/kg (i.p.) guanethidine did not reduce the content of CA in the adrenal medulla. However, doses of 400 mg/kg, or 80 times those which reduce the content of heart NE by 50%, did lower the content of medullary amines by about 50%. Similarly, doses of reserpine necessary to reduce medullary amines by one-half were about 80 times those which caused a 50% decline in heart NE. Kuntzman et al. further showed that heart NE was lowered by 50% one hour after giving reserpine in doses large enough to deplete heart NE completely. In contrast, the rate of release by guanethidine was slower: about 50% in two hours. As expected, guanethidine did not lower the content of brain NE since it does not enter the brain in appreciable amounts.

Kuntzman et al. stated that although the action of guanethidine in reducing heart NE is readily reversible, that of reserpine is relatively nonreversible; however, they emphasized that this does not necessarily mean that the two drugs affect NE storage by different mechanisms. The drugs could act on the same storage process, with reserpine having a high affinity for the storage sites. However, guanethidine and reserpine appear to release NE by different mechanisms, since bretylium counteracts guanethidine but not reserpine in releasing NE. They concluded that guanethidine releases NE from sympathetic nerve endings by an action which seems to differ from that of reserpine; guanethidine may release NE by activating the normal process of physiological release.

Harrison et al. (1963) showed that in three hours guanethidine produced an initial loss of some 24% of the CA of the dog heart, while reserpine produced a 65% loss in four hours. These findings suggest a different mechanism of NE depletion in the period immediately after administration of the drugs. Also, the amine-depleting action of guanethidine was accompanied by a sympathomimetic response which was not seen with reserpine. Apparently guanethidine releases CA on to the receptor sites, but the CA released by reserpine do not gain access to the receptor (Kuntzman et al., 1962; Bhagat, 1964a).

Reserpine releases NE onto MAO by inhibiting nonreversibly an active transport mechanism or pump that maintains the amine in a mobile pool at nerve endings (Spector et al., 1960). In contrast, guanethidine has been postulated to release NE onto receptor sites (Kuntzman et al., 1962). Kopin and Gordon (1963a) claimed that CA released by guanethidine are inactivated by MAO and COMT. Nash et al.

(1964) has shown that NE is released onto the receptor site and is subsequently O-methylated. Their results showed that reserpine releases NE almost entirely in the form of deaminated products whereas guanethidine releases the amine as the free base.

Gaffney et al. (1963) suggested that guanethidine's interference with adrenergic transmission is independent of changes in the level of stored adrenergic-transmitter. The reserpine-induced blockade of adrenergic transmission may ultimately be dependent upon the depletion of adrenergic transmitter, but almost complete depletion of stored adrenergic transmitter must occur before reserpine-induced adrenergic blockade occurs.

Chang et al. (1964) stated that guanethidine is localized in rat tissue by two kinds of binding sites. (See page 34). Reserpine was shown to release considerable amounts of guanethidine from tissues containing high levels of NE; however, reserpine released little or no guanethidine from tissues having only small amounts of CA. It was also inferred that reserpine had completely blocked the uptake of guanethidine into adrenergic neurons, since amphetamine produced no additional inhibition of guanethidine uptake.

Muskus (1964) presented evidence of different sites of action of guanethidine and reserpine. His results were discussed on the basis of a two-compartment theory of the NE stores. He indicated that guanethidine differs from reserpine in its site of action, because guanethidine appears to act more strongly than reserpine on the small compartment of tyramine-sensitive NE. This was true for both the depleting and the releasing action of guanethidine.

Lundborg and Stitzel (1967) suggested that the membrane pump-inhibiting ability possessed by guanethidine, but not reserpine may account for the sympathomimetic activity and exogenous amine potentiation which often accompanies guanethidine but not reserpine administration.

Burns and Rand (1958; 1960) postulated that reserpine impairs adrenergic function by its action in depleting the stores of the transmitter, NE. Guanethidine was also found to deplete CA from peripheral organs (Sheppard and Zimmerman, 1959), and it was suggested by Cass et al. (1960) that the loss of adrenergic function might be attributed to the loss of transmitter substance from the nerve endings. However, it was later shown that the initial blocking action of guanethidine was not related to depletion of the NE stores (Cass and Spriggs, 1961; Gaffney et al., 1963). Chang et al. (1967a) concluded that the adrenergic neuron blocking effect of guanethidine is consequent on the rapid depletion of the particulate NE which is available for release by nerve impulse.

III. METHODS AND MATERIAL

A) ANIMALS AND ANIMAL HOUSING

Male, albino rats of the Sprague-Dawley strain, weighting between 75-100g, obtained from Charles River[1], were used throughout the investigation. All animals were kept at room temperature in uncrowded community cages (51 x 56 x 38 cm)[2] for two to three weeks after arrival in the laboratory. Purina[3] rat chow and tap water were provided ad libitum. At a period beginning two weeks prior to their use, the rats were transferred to suspended metabolism cages[2], two per cage, where they were kept throughout the study. The metabolism cages were placed in an air-conditioned animal room where the temperature was maintained at 21.1 $\pm$ 0.5°. Following the transfer, each animal was given 20g of Purina rat chow daily at about 2:30 p.m., and tap water was provided ad libitum.

The animal room was illuminated artifically for a twelve hour period beginning at 6:00 a.m. In an effort to minimize any effect of movement into and out of the animal room, only authorized personnel were allowed admittance. When not undergoing restraint, the experimental animals were housed in the same room as the control animals. Control rats were not restrained, but were kept in the suspended metabolism cages without food and water during the restraint period.

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1. Charles River Breeding Farms, North Wilmington, Massachusetts.
2. Wahmann Manufacturing Co., Baltimore, Maryland.
3. Ralston Purina Co., St. Louis, Missouri.

B) RESTRAINT STRESSOR PROCEDURE

Because immobilization has been shown to produce considerable stress due to the increased neuromuscular exertion involved (Selye, 1946), a restraint stressor procedure was used. Rats were removed from the animal room, about one hour after vehicle or drug injection, and transferred to a second room where they were restrained. The illumination conditions and temperature in this room were identical to those in the animal room. The animals were placed on restraining boards, similar to the ones used by Renaud (1959).

Each board was designed to restrain four rats simultaneously by securing their paws to each of four vertical metal posts. Strips of adhesive tape, two cm wide to allow circulation, were wrapped cuff-wise about the paws of the restrained animals, and size one safety-pins were then pinned to the tape. The tape and pins were left on the animals throughout the entire period of the experimentation. The rats were restrained in a supine position simply by slipping the closed safety-pins over the metal posts. With this method, a rat could be immobilized in a few seconds. The restrained animals were held in this position for five hours.

The studies of Renaud (1959), recommended that the incisors of rats restrained in such a position be cut to prevent them from biting their paws. This problem was solved by immobilizing the head by placing it between two metal posts.

C) EXPERIMENTAL DESIGN

1. General Consideration and Daily Protocol

Drugs. Aqueous solutions of guanethidine sulfate[1] (Ismelin™; A2448)[2] and reserpine phosphate[1] (Serpasil™; 65-293)[2] were prepared daily and administered intraperitoneally (i.p.). The doses were calculated in terms of the free base content of both drugs.

To permit the animals to adapt to the injection procedure, all rats were weighed to the nearest gram and injected i.p. with the drug vehicle, glass distilled water (1.0 ml/kg) every other day for 14 days prior to the experiments. The animals were kept undisturbed for 24 hours prior to their use. Experiments were begun on the 15th day. From 7:00 to 9:00 a.m. daily the animals were weighed and injected by the same person with the vehicle or with the appropriate drug. Animals weighed between 150-215g at the beginning of the experimental period. Water and food consumption were measured and the water jars refilled during this period.

2. Dose-Response Studies

The purpose of this study was to determine that dose of reserpine effecting a 50% depletion of brain NE and that dose of guanethidine effecting a 50% peripheral depletion of NE as measured in the heart.

One hundred and twenty rats were divided into twelve groups of ten animals each. Housing and daily preparatory protocol were as described above. Each day for six days all rats in each group were injected i.p. with one of the following doses (mg/kg): reserpine,

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1. Both drugs graciously supplied by Mr. Jack Cooper, Ciba Pharmaceutical Products, Inc., Summit, N.J.
2. Lot number.

0 (vehicle), 0.05, 0.1, 0.25, 1.0, and 2.5; guanethidine, 0 (vehicle), 0.5, 1.0, 2.5, 5.0, and 10.0. Approximately eight hours after the last injection the animals were removed from the animal room and within one minute were sacrificed by decapitation (manual guillotin)[1]. The brain was removed from the skull, rinsed with saline at 0^{o} and blotted; the heart was removed from the body cavity, cut in half, rinsed with cold saline and blotted. These operations were accomplished in less than a minute after decapitation. Each organ was immediately placed into a separate aluminum mesh basket and immersed into liquid nitrogen contained in a wide-mouthed, vacuum-insulated jar. When the nitrogen stopped boiling, the tissue was removed, wrapped individually in aluminum foil, coded and stored in a freezer at -40^{o} until assay.

3. Chronic Restraint Studies

The dose of reserpine and of guanethidine established in the dose-response studies were used throughout this phase of experimentation. The animal handling, the injection and the feeding were as described above. A total of 300 animals were used in this portion of the study, according to the following procedure: Individual experiments of 1, 3, 6, 12, and 24 days duration were conducted on animals that were randomly divided for each experiment into six experimental groups of ten rats each. The daily protocols for the various groups were as follows:

1. Vehicle control. These animals received 1 ml/kg, i.p., of the drug vehicle daily.

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1. Harvard Apparatus Co., Inc., Dover, Massachusetts.

2. Guanethidine control. These animals received 1.20 mg/kg, i.p., of guanethidine daily.
3. Reserpine control. These animals received 0.2 mg/kg, i.p., of reserpine daily.
4. Vehicle restrained. These animals received 1 ml/kg, i.p., of the drug vehicle and were subjected to restraint daily.
5. Guanethidine restrained. These animals received 1.20 mg/kg, i.p., of guanethidine and were subjected to restraint daily.
6. Reserpine restrained. These animals received o.2 mg/kg., i.p., of reserpine and were subjected to restraint daily.

Restrained animals were subjected daily to the immobilization procedure described above. At the termination of the above experiments, the restrained animals were sacrificed by decapitation within one minute of their removal from the stressing room. Control animals were decapitated within one minute of their removal from the animal room.

After decapitation, blood was collected from the trunk of each animal into twelve ml centrifuge tubes and allowed to stand until a clot formed. The samples were then centrifuged[1] for ten minutes at 2000 rpm. The serum was separated by decantation into four ml Kimax[2] test tubes that were then stoppered, coded, and stored for assay at -40°. The adrenal glands were removed from the body cavity, freed of fatty tissue, blotted and immediately frozen in liquid nitrogen; this procedure took less than one minute. The frozen adrenals were then placed in four ml Kimax[2] test tubes, stoppered, coded and stored at -40° until assay. Brain and heart were removed and stored as described above.

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1. International Clinical Centrifuge, Model Cl 21890M, International Equipment Co., Needham Heights, Massachusetts.
2. Owens-Illinois Glass Co., Toledo 1, Ohio.

4. Blood Pressure Studies

The object of this portion of the study was to determine whether the dose of reserpine and of guanethidine used in the chronic restraint studies had any effect on blood pressure. Fifteen animals were divided into three groups of five animals each. Daily drug doses (i.p.) were as follows: group one, vehicle, 1 ml/kg; group two, reserpine, 0.2 mg/kg; group three, guanethidine, 1.20 mg/kg. Daily protocols, injecting and feeding procedures were as described above; however, there was a slight variation in the housing: in each group, one animal was individually housed. This modification proved to have no effect on the blood pressure recording. In order to permit the animals to adapt to the handling, the container, the tail cuff and the recording apparatus, blood pressures were determined on four separate days prior to drug treatment. During the actual determination, the animals were removed from the animal room, and blood pressure was determined daily from about 11:00 a.m. - 1:00 p.m. for twelve days and then every other day until day 24.

The tail cuff method for indirect systolic blood pressure determination was used. The animals were placed in an incubator box at 40^{o} for 15 - 20 minutes to cause peripheral vasodilation to facilitate the determination of blood pressure. After incubation each rat was allowed to enter a clear plastic container (23 x 7 x 5 cm). The internal length of the container was regulated with a movable piston so that the animal is snugly but not uncomfortably restrained. The rear door at the open end of the chamber was then placed in position, leaving only the rat's tail protruding. The rat generally becomes calm after a few minutes in the container.

An inflatable, blood pressure cuff[1], connected to a Physiograph monometer system[2] was slipped on the rat's tail as far proximally as possible. The monometer system was connected to a hand bulb for manual cuff inflation. The end of the tail distal to the cuff was passed through a small plastic holder, so designed that when a screw clamp was tightened, the tail came into contact with a Beckman microphone transducer.[3] The transducer lead was connected to a Beckman Infraton[3] signal divider set for maximum pulse. The Infraton output lead was connected to the direct current input of an HP oscilloscope (Model 130B)[4] set at a sweep time of 20 msec/cm.

This system converts pressure pulses in the rat's tail to peaks on the oscilloscope screen. Squeezing the rubber bulb of the manual monometer system increases pressure in the system, causes the cuff to be inflated with the consequence that the peaks on the oscilloscope screen disappear as tail circulation distal to the cuff is cut off. By allowing the air in the cuff to escape slowly, pressure in the monometer system is reduced and tail circulation is restored as indicated by the reappearance of peaks on the screen. Because it is assumed that for distal circulation to be restored the pressure in the tail arteries must be slightly greater than the pressure in the manometer system, the pressure, as read on the monometer dial, at which pulsation first appears on the screen is a measure of systolic blood pressure of the animal. Diastolic pressures cannot be observed with this technique. The recorded

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1. Harvard Instruments, Cambridge, Massachusetts.
2. E. and M. Instruments, Houston, Texas.
3. Beckman Co., Palo Alto, California.
4. Hewlett-Packard Co., Palo Alto, California.

blood pressure of a rat on a given day is reported as the average of at least three successive determinations. Once the animal was quiet, successive determinations fell into a range not exceeding a few mm Hg.

D) ANALYTICAL METHODS

1. Estimation of Brain and Heart Norepinephrine

The extraction procedure here is a modification of the method described by Shore and Olin (1958), and the quantities were reduced to one-third. This rapid and simple procedure for the chemical estimation of NE in animal brain and heart tissue involves an extraction with n-butanol from salt-saturated, acidified homogenate. The amine is unstable at alkaline pH. Reduction of the polarity of the butanol by the addition of n-heptane and extraction with dilute acid (0.01N HCl) returns the NE to the aqueous-acid phase. An oxident is then added to the aqueous-acid solution to form the aminochrome, which on the addition of alkaline ascorbic acid, yields the trihydroxyindole. The oxidation products of NE in alkaline solution shift the fluorescence emission to the visible region (520 mu). The solutions were then assayed for the development of fluorescence by a modification of the method described by Maynert and Klingman (1962).

Solvents and Reagents:

Ascorbic acid (reagent grade, Matheson, Coleman and Bell)

Acetic acid, glacial (reagent grade, Baker and Adamson)

n-Butanol (reagent grade, Baker and Adamson)

Ethylenediamine (reagent grade, Eastman Organic Chemicals)

Hydrochloric acid (reagent grade, Baker and Adamson)

n-Heptane (reagent grade, Matheson, Coleman and Bell)

Norepinephrine (Nutritional Biochemical Corp.)

Potassium ferricyanide (AR grade, Mallinckrodt)

Sodium acetate, anhydrous (AR grade, Mallinckrodt)

Sodium bisulfite (AR grade, Mallinckrodt)

Sodium chloride (reagent grade, Baker and Adamson)

Sodium hydroxide pellets (AR grade, Mallinckrodt)

Reagent grade n-butanol and n-heptane were purified by the method of Shore and Olin (1958). Excess water was removed by decanting off the butanol after about 24 hours.

Acetate Buffer, 2M, pH 6.0: One volume of 2M acetic acid is added to 22 volumes of 2M sodium acetate. The pH was checked with a Radiometer, pH meter 28[1].

Alkaline Ascorbate Solution: This solution was prepared fresh daily prior to its use by mixing six ml of 10N NaOH (Anton and Sayre, 1962) with one ml of one percent sodium bisulfite and 0.16 ml of ethylenediamine (von Euler and Lishajko, 1960), then adding one ml of a one percent ascorbic acid solution. All solutions used in the preparation of this alkaline ascorbate solution were prepared fresh daily.

Norepinephrine standard: NE.HCl was used for preparing the standard solution, the concentration was calculated in terms of the base. Because it was observed that a solution of NE (100 ug/ml) in 0.01N HCl deteriorated even when refrigerated, the solution was made fresh daily and a working standard of suitable concentration (1 ug/ml to 5 ug/ml) in 0.01N HCl were prepared prior to the start of each assay.

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1. Landon Co., Westlake, Ohio.

Glass-distilled water was used in the preparation of all solutions. All glassware and polyethylene tubes were washed three times with distilled water followed by two rinsings with glass-distilled water.

Method: The method described here was used to determine NE from whole rat brain and heart. At the time of the assay, each brain or heart was removed from the aluminum foil, weighed and immersed into liquid nitrogen as described above. The frozen tissue was then placed into a steel tablet die of suitable size and crushed by striking the upper punch with a hammer (Cullingham and Cass, 1963).

A steel tablet die and punch set with an internal diameter of 15 mm, was placed in a wooden block. One punch (lower) served as the base; the other punch (upper) was movable. Prior to placing the tissue into the die, the two punches and die were immersed into the liquid nitrogen for 15 - 20 seconds to prevent local heating and destruction of the NE.

Each brain was reduced to three pellets by carefully breaking the frozen tissue into three approximately equal segments and compressing each segment into a pellet. These pellets of still frozen tissue were transferred to a 15 ml homogenizing tube[1] containing five ml of acidified butanol. Each heart was reduced to two pellets by a similar procedure and transferred to another homogenizing tube containing acidified butanol. The butanol was chilled (Chang, 1964) to 0^{o} prior to its use.

The brain or heart was homogenized within two minutes in the chilled butanol by a motor-driven Teflon homogenizer[2]. The homogenizing

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1. Catalogue number s35, TriR Instruments, Inc., Jamaica, New York.
2. Catalogue number s21, TriR Instruments, Inc., Jamaica, New York.

tube was maintained in an ice bath during this procedure. The homogenate was transferred to a 50 ml, polyethylene test tube (29 x 105 mm)[1] containing four gm of NaCl. Three successive washings of the homogenizing tube with five ml portions of chilled butanol effected quantitative transfer of the homogenate.

One-half or one ml of the NE working standard (1 ug/ml), with volume adjusted to five ml with 0.01N HCl was simultaneously carried through the assay procedure with the tissue homogenate. Five ml of 0.01N HCl was used for the blank.

The polyethylene tubes were capped and then shaken for 20 minutes on a reciprocating shaker. It was observed that the extraction of NE was virtually complete after a 10 to 15 minute period and that shaking carried on for periods up to one hour had no further effect on the amount extracted. Following centrifugation at 10,000 rpm for ten minutes, 13 ml of the butanol phase was decanted into a 25 ml polyethylene cylindrical graduate and poured into a second set of 50 ml, polyethylene tubes containing 23 ml of n-heptane and two ml of 0.01N HCl. The tubes were capped and then shaken for five minutes on a reciprocating shaker and then centrifuged at 10,000 rpm for five minutes. The organic phase (upper) was removed by aspiration.

One-half ml of the aqueous-acid phase from each sample was then carefully transferred to twelve ml polyethylene test tubes (16 x 100 mm)[1]. The transfer of any trace of the organic phase was avoided by using 0.5 ml

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1. Ivan Sorvall Inc., Norwalk, Conn.
2. Lourdes Model AB centrifuge, Lourdes Instruments Corp., Brooklyn, N.Y.

Kimax[1] volumetric pipettes. Two-tenths ml of water and 0.1 ml of 2M acetate buffer (pH 6) were added to each tube. The contents of each tube were mixed by manual inversion. To this mixture was added 0.1 ml of freshly prepared potassium ferricynide (40 mg%, w/v) and the contents were mixed. Two minutes later 0.2 ml of freshly prepared alkaline ascorbate solution was added and the contents were mixed. The solutions were then transferred to quartz cuvettes and their fluorescence intensities were read by means of an Animco-Bowman spectrophotofluorometer[2] at an excitation wavelength of 400 mu, and fluorescence wavelength of 520 mu (uncorrected). The source of emission was xenon arc lamp.

A standard curve for NE was prepared daily prior to the tissue assay. Blank, 0.5, 1, 2, 3 and 4 ml portions of the NE working standard (1 ug/ml) were prepared and adjusted to five ml with 0.01N HCl. Five ml of 0.01N HCl was used for the blank. These samples were extracted as were the tissue samples; however, these tissue-free solutions were shaken for only a few minutes because prolonged shaking caused partial loss of the NE (Shore and Olin, 1958). Standard curves were also prepared by adding known amounts of NE to separate aliquots of tissue homogenate and by carrying these aliquots through the extraction procedure. The slope of the line was approximately equal to that of the tissue-free NE samples.

Six samples of brain or heart tissue homogenate were extracted at one time and assayed fluoreometrically along with an internal or external (tissue-free) working standard plus the blank. Periodically, tissue

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1. Owens-Illinois Glass Co., Toledo, Ohio.
2. American Instrument Co., Silver Springs, Maryland.

homogenates from control animals were extracted and assayed to check the procedure.

The spectrophotofluorometer was turned on about 15 minutes before the time of taking the readings to obtain a stable zero setting. The "zero-adjust" and "dark current" were balanced in the photo multiplier as described in the manual. A sensitivity setting of 30 was used for all assays. Eight cuvettes were calibrated while containing a known amount of a NE (1 ug/ml) and were found to give approximately the same relative fluorescence readings. These cuvettes were used for all assays. The fluorescence readings for the extracted samples were taken from five to ten minutes after the addition of the alkaline ascorbate solution. Two to three readings were taken of each sample or were continued until the solution stabilized as indicated by consecutive readings which were constant. After about 30 - 45 minutes the fluorescence began to fade. The samples were then allowed to sit for a period of about 24 hours, and the fluorescence was read again. This "tissue-blank" reading, which was equal to or very close to the "reagent blank" fluorescence, was then subtracted from the corresponding reading obtained for that sample the day before, and the difference was taken as the actual fluorescence contributed by NE. In actual practice, however, it was not necessary to take "tissue-blank" readings for each sample. Tissue-blank readings, taken at random daily during the assays, were not substantially different from "reagent-blank" readings. Therefore, "reagent-blank" fluorescence reading was subtracted from the fluorescence reading of each tissue sample, and this difference was considered to be the actual fluorescence contributed by NE.

All the conditions established in this assay were repeated precisely every time the assay was done, since there are many parameters which can alter the ultimate fluorescent readings. Duration of shaking, centrifugation, type of test tubes, etc., were kept constant once they were established. Exposure of NE to different reagents for different times may cause a variable rate of destruction of the NE. This is particularly important when one is dealing with very dilute solutions. It was also considered necessary to run a set of standards with each set of unknowns.

Distribution of NE between the salt saturated aqueous phase and n-butanol was such that, with the volumes used in this procedure, only about 65% of the amine was extracted. This partition ratio is independent of the CA concentration (Shore and Olin, 1958) and is used because lesser amounts of interfering substances are extracted. When n-heptane is added to the water-saturated n-butanol, water separates out from the solvent and increases the volume of the aqueous-acid phase. It was for these reasons that standards were prepared by carrying known amounts of NE through the entire extraction procedure.

NE added to the tissue homogenates (heart and brain) was extracted to the extent of 97 to 103% compared with aqueous solutions not containing tissue homogenates.

The hydrochloric acid and sodium hydroxide solutions were standardized according to the method in United States Pharmacopea XVII.

2. Estimation of Serum Corticosterone

The predominant adrenal corticosteroid present in the rat plasma is corticosterone (Bush, 1953). The method described here utilizes

0.5 ml of rat serum and is a modification of the method described by Guillemin et al. (1959a). Corticosterone was extracted with $CHCl_3$ and the fluorescence was developed using 30N H_2SO_4.

Solutions and Reagents:

Absolute ethyl alcohol (reagent grade, U.S. Industrial Chemical Co.)

Chloroform (spectrophotometric grade, Mallinckrodt)

Corticosterone (alcohol free, Nutritional Biochem. Corp.)

Iso-octane (2,2,4-trimethylpentane, practical grade, Matheson, Coleman, and Bell)

Sodium hydroxide (AR grade, Mallinckrodt)

Sulfuric acid (AR grade, Mallinckrodt)

Fluorescence reagent: The 30N H_2SO_4 was prepared as described by Guillemin et al. (1959) (420 ml concentrated H_2SO_4 up to 500 ml with glass-distilled water). The water was added slowly and with constant stirring. The bottle containing the H_2SO_4 was kept at 0^o in an ice bath. It was observed that this fluorescence reagent was stable for at least four weeks under refrigeration.

Stock standard: A solution containing 1 mg/ml of corticosterone (free alcohol) was prepared in absolute ethanol. This solution was stable for at least two months when refrigerated. Another solution containing 10 ug/ml of corticosterone was made in ethyl alcohol and was used for preparing the working standard.

Working standard: A working standard containing 0.4 ug/ml of corticosterone was made by diluting one ml of an ethanolic solution containing 10 ug/ml of corticosterone to 25 ml with glass-distilled water. The working standard was stored in a refrigerator and was found to be stable for at least a month. However, a working standard was

prepared fresh daily.

Method: At the time of assay, the serum samples were removed from the freezer and thawed out. One-half ml of each serum sample was placed in an 18 ml glass-stoppered Kimax (Cat. #45100) test tubes. Two-tenths and 0.4 ml aliquots of the corticosterone working standard were placed in similar tubes. The volume in all tubes was adjusted to two ml with glass-distilled water. Two ml of glass-distilled water was used for the reagent blank. Two blanks were used in each assay. A standard curve was prepared daily, prior to the serum assay. Four ml of iso-octane were added to all the tubes via a 50 ml buret, and the contents were mixed for 15 seconds with a Vortex[1] mixer. Following centrifugation[2] at 2500 rpm for three minutes, the iso-octane layer was removed by aspiration. This step removed the neutral steroids. Two ml of glass-distilled water, followed by five ml of chloroform, were added to each tube, and the contents were mixed vigorously for 30 seconds on a Vortex mixer and then centrifuged for five minutes at 3000 rpm. In the case of standards and "reagent blanks", the aqueous layer was aspirated off by a capillary tube (connected to a water vacuum pump) until the water-chloroform interface was broken. The tip of the capillary was then moved around inside the tube above the liquid in order to remove all the water in contact with the sides of the tube. In the serum samples, water was removed to just within the protein layer, the film was broken, and the rest of the water removed along with the protein precipitate.

One-half ml of 0.1N NaOH was added, and the tubes were shaken

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1. Vortex Model K-500 J, Scientific Industries Inc., Queens Village, N.Y.
2. International Model HN, International Equipment Co., Needham Hts.,Mass.

for 15 seconds and centrifuged for three minutes at 3000 rpm. This step, which removed phenolic estrogens, was carried out rapidly, since prolonged exposure to alkali destroys corticosteroids. The aqueous layer was removed by aspiration. A four ml aliquot of the chloroform extract was then transferred to another set of glass-stoppered test tubes containing 1.5 ml of the fluorescence reagent (30N H_2SO_4). The time was noted and the contents were mixed on a Vortex mixer for 30 seconds and centrifuged for five minutes, at 3000 rpm. The chloroform layer (upper) was removed by aspiration, and one ml of the acid extract was transferred to a fluorometer quartz cuvette. Fluorescence was determined in about 35 - 45 minutes after the last mixing step (excitation 470 mu and fluorescence 520 mu - uncorrected). The values for the unknowns were obtained from the standard curve.

The exact time for reading the fluorescence was determined to be that time (about 40 minutes) at which the reading of the standards was stable and at a maximum. Figure 7 shows that stability and maximum fluorescence were of relatively long duration; therefore, consistent timing was not absolutely necessary. The intensity of fluorescence was a linear function of the concentration of the corticosterone over a range of 0.05 mg to 0.40 mg (Figure 8). The recovery of corticosterone added to the serum was about 100% over the range of 0.1 mg to 0.4 mg.

Chloroform (spectrophotometric grade) and iso octane (practical grade) were found suitable for the assay. Further purification was not necessary. Sulfuric acid obtained from Baker and Adamson gave the highest "blank fluorescence" values. Mallinckrodt sulfuric acid gave the lowest "blank" values. It was not necessary to redistill the ethyl alcohol (analytical grade).

The sensitivity of any fluorescence assay is usually limited by the fluorescence contributed by the reagents used. It is always necessary to determine the fluorescence of each reagent at the wavelength used in the particular assay. Table 1 gives the fluorescence (fluoreometer readings) values of each reagent used for corticosterone and norepinephrine assays. The readings were taken under experimental conditions of the respective assay procedures. It can be easily seen that analytical grade reagents made by different manufacturers show a wide variation in their fluorescence.

3. Estimation of Adrenal Ascorbic Acid

The Sullivan and Clark (1955) method for assaying ascorbic acid in urine is based on the reduction of ferric to ferrous ion by ascorbic acid and the colorimetric measurement of the ferrous ion through formation of a red-orange-colored complex with alpha - alpha - dipyridyl. Other reducing material was inhibited by the addition of orthophosphoric acid and by maintaining a low pH (pH 1.0). Maickel (1960), adapted this reaction for the determination of ascorbic acid in the adrenal gland. The procedure described here is a modification of this method.

Solutions and Reagents:

Trichloroacetic acid (reagent grade, Baker and Adamson)

Metaphosphoric acid (AR grade, Mallinckrodt, HPO_3 approximately 35%, the remainder being sodium metaphosphate)

Orthophosphoric acid (reagent grade, Baker and Adamson)

Alpha -Alpha-dipyridyl (Calbiochem.)

Ascorbic acid (reagent grade, Matheson, Coleman and Bell)

Ferric chloride (AR grade, Mallinckrodt)

5% Trichloroacetic acid + 2% metaphosphoric acid reagent: Fifty grams of trichloroacetic acid (TCA) was dissolved in about 500 ml of glass-distilled water. Twenty grams of metaphosphoric acid (MPA) dissolved in about 200 ml of glass-distilled water was added to the TCA solution, and the resulting solution was diluted to one liter. The solution was stored in a refrigerator at 0^{o}. A fresh solution was prepared each week.

1% Ferric chloride solution: One gram of ferric chloride was dissolved in glass-distilled water; about 0.1 ml of concentrated HCl was added, and the solution was diluted to 100 ml. A fresh solution was prepared daily.

0.5% aqueous alpha -alpha- dipyridyl solution: Five grams of crystalline material was dissolved in about 500 ml of hot double-distilled water and diluted to one liter. A fresh solution was made each week.

Ascorbic acid standards.

Stock standard: One hundred mg of ascorbic acid were dissolved in 5% TCA + 2% MPA reagent, and this was diluted to 100 ml with 5% TCA + 2% MPA. A fresh solution was prepared daily.

Working standard: A working standard containing 100 ug/ml of ascorbic acid was made from the stock solution each day, just prior to the assay, by diluting 2.5 ml of the stock standard to 25 ml with 5% TCA + 2% MPA.

Method: At the time of assay, the adrenals were removed from the test tube, weighed and transferred to a 15 ml homogenizer tube containing five ml of 5% TCA + 2% MPA reagent. The homogenizing tube was placed in an ice bath and the tissue was homogenized in less than a

minute by a motor-driven Teflon homogenizer. The contribution of the adrenal tissue to the total volume of the homogenate was negligible. The homogenate was transferred to a twelve ml polypropylene test tube.[1] The homogenate was centrifuged[2] for ten minutes at 2000 rpm and the supernatant liquid was used for the assay. One ml of the supernatant (containing 10 - 15 mg of ascorbic acid) was then placed in a 1.25 cm colorimeter test tube (cat. #33-29-27)[3].

Two-tenths, 0.3, 0.4, 0.5, and 0.6 ml aliquots of the ascorbic acid working standard were placed into 1.25 cm colorimeter test tubes and the volume was adjusted to one ml with the 5% TCA + 2% MPA reagent. One ml of the 5% TCA + 2% MPA was used for the reagent blank.

Five ml of 0.5% (w/v) alpha - alpha-dipyridyl solution was added to each tube, followed by the addition of 0.2 ml of 85% orthophosphoric acid and one ml of one percent ferric chloride solution. The contents were thoroughly mixed after each addition. Tubes were kept for 25 minutes at 37^{o} - 38^{o} and the absorbance of each sample was determined at 525 mu on a Bausch and Lomb, Spectronic 20[3], colorimeter. If the tubes were kept at room temperature, readings were taken after 70 minutes. The final readings were the same whether the reaction was run at room temperature or at $37\text{-}38^{o}$. The concentration of unknown was obtained from a standard curve prepared daily prior to the assay. Absorbance readings of standards were reproducible from day to day. The recovery of the ascorbic acid added to the homogenate was about 95%.

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1. Ivan Sorvall Inc., Norwalk, Conn.
2. International Model HN, International Equip. Co., Needham Hts.,Mass.
3. Bausch and Lomb, Rochester, N.Y.

The color developed was stable for some time. The colorimeter was allowed to warm up for about 15 minutes and was adjusted to zero with the reagent blank. The optical density followed Beer's law over the range of 5.0 to 70.0 mg in a 7.2 ml final volume (Figure 10).

E) STATISTICAL ANALYSIS

All values reported herein were analyzed statistically; the significant difference between means was calculated using the Student's "t" test. The level of significance is reported at the 0.05 level. Standard error (SE) of the mean was calculated according to the following formula:

$$SE = \sqrt{\frac{\sum (x-\bar{x})^2}{n(n-1)}}$$

Analysis of variance was done according to the Aardvark program used by the University of Rhode Island computer laboratory (Hemmerle, 1967, p. 177).

All statistical calculations were done by means of an IBM 360 computer.

IV. RESULTS

Tables and figures are contained in this section.

TABLE I

RELATIVE FLUORESCENCE OF THE REAGENTS USED IN FLUORESCENCE ASSAYS OF SERUM CORTICOSTERONE AND BRAIN AND HEART NOREPINEPHRINE

Reagent	Relative Fluorescence	
	Norepinephrine Assay A=400 F=520 Sensitivity=30	Corticosterone Assay A=470 F=520 Sensitivity=30
Tap water	0.000	0.000
Distilled water	0.000	0.000
Glass distilled water	0.000	0.000
Heptane (not washed)	0.002	---
Heptane (washed)	0.002	---
n-Butanol (not washed)	0.010	---
n-Butanol (washed)	0.009	---
n-Butanol (spec. grade)	0.002	---
0.01N HCl (AR-Baker Adamson)	0.000	---
10N NaOH (AR-Mallinckrodt)	0.001	---
Potassium ferricyanide (AR grade)	0.000	---
Alkaline ascorbic acid solution	0.004	---
Chloroform (AR grade)	---	0.000
Chloroform (spec. grade)	---	0.000
Iso octane (practical)	---	0.001
Iso octane (spec. grade)	---	0.000
Ethyl alcohol (AR)	---	0.000
Fluorescence reagent (AR Baker Adamson)	---	0.015
Fluorescence reagent (AR Mallinckrodt)	---	0.009

A = activation wavelength
F = fluorescence wavelength

TABLE II

THE EFFECT OF RESTRAINT ON MEAN CUMULATIVE DAILY WEIGHT GAINS OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE.

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
2	7 ± 6[a] (10)[b]	4 ± 4 (10)	2 ± 2 (10) A[c]	-2 ± 4 (10) A	5 ± 6 (10) B	4 ± 3 (10) BD
3	7 ± 5 (10)	9 ± 4 (10)	5 ± 4 (10)	-2 ± 5 (10) A	3 ± 9 (10)	0.4 ± 4 (10) AD
4	11 ± 5 (10)	9 ± 5 (10)	7 ± 5 (10)	-3 ± 5 (10) A	2 ± 7 (10) AC	-2 ± 5 (10) AD
5	18 ± 9 (10)	14 ± 6 (10)	10 ± 6 (10) A	-3 ± 6 (10) A	-1 ± 10 (10) AC	-3 ± 6 (10) AD
6	19 ± 7 (10)	15 ± 7 (10)	8 ± 7 (10) A	-8 ± 8 (10) A	1 ± 7 (10) ABC	-7 ± 9 (10) AD
7	20 ± 8 (10)	17 ± 8 (10)	9 ± 9 (10) A	-7 ± 7 (10) A	0.1 ± 9 (10) AC	-10 ± 8 (10) AD
8	23 ± 8 (10)	21 ± 6 (10)	7 ± 10 (10) A	-8 ± 8 (10) A	0.6 ± 8 (10) AC	-14 ± 9 (10) AD
9	27 ± 7 (10)	23 ± 8 (10)	8 ± 11 (10) A	-5 ± 8 (10) A	0.3 ± 8 (10) AC	-11 ± 12 (9) AD
10	27 ± 7 (10)	24 ± 10 (10)	10 ± 12 (10) A	-5 ± 8 (10) A	2 ± 9 (10) AC	-14 ± 10 (9) ABD

TABLE II - continued

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
11	32 ± 9 (10)	29 ± 8 (10)	13 ± 14 (10) A	-4 ± 13 (10) A	3 ± 12 (10) AC	-6 ± 9 (9) AD
12	33 ± 8 (10)	29 ± 8 (10)	7 ± 13 (10) A	-4 ± 10 (10) A	0.7 ± 9 (10) AC	-13 ± 9 (9) ABD
13	33 ± 8 (10)	28 ± 9 (10)	9 ± 12 (10) A	-5 ± 12 (10) A	0.2 ± 10 (10) AC	-16 ± 14 (9) AD
14	37 ± 9 (9)	31 ± 8 (10)	10 ± 13 (10) A	-5 ± 12 (10) A	0 ± 13 (10) AC	-14 ± 21 (9) AD
15	37 ± 9 (9)	33 ± 9 (10)	11 ± 12 (10) A	-6 ± 12 (10) A	-0.8 ± 11 (10) AC	-16 ± 25 (9) AD
16	41 ± 10 (9)	33 ± 8 (10)	12 ± 13 (10) A	-6 ± 13 (10) A	0.5 ± 11 (10) AC	-10 ± 21 (8) AD
17	39 ± 9 (9)	35 ± 10 (10)	11 ± 12 (10) A	-7 ± 14 (10) A	-3 ± 10 (10) AC	-14 ± 26 (8) AD
18	41 ± 11 (9)	36 ± 10 (10)	12 ± 16 (10) A	-7 ± 14 (10) A	-0.8 ± 11 (10) AC	-14 ± 29 (8) AD
19	43 ± 10 (9)	35 ± 10 (10)	12 ± 20 (10) A	-7 ± 15 (10) A	-5 ± 11 (10) AC	-6 ± 21 (7) A

TABLE II - continued

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
20	43 ± 11 (9)	36 ± 10 (10)	12 ± 16 (10) A	-8 ± 14 (10) A	-7 ± 14 (10) AC	-14 ± 21 (7) AD
21	45 ± 13 (9)	40 ± 9 (10)	13 ± 15 (10) A	-7 ± 15 (10) A	-4 ± 14 (10) AC	-8 ± 20 (7) A
22	47 ± 12 (9)	38 ± 7 (10)	14 ± 23 (10) A	-4 ± 15 (10) A	-2 ± 14 (9) AC	-14 ± 26 (7) AD
23	46 ± 15 (9)	41 ± 10 (10)	15 ± 26 (10) A	-4 ± 14 (10) A	-8 ± 14 (9) AC	-9 ± 27 (7) A
24	49 ± 13 (9)	44 ± 9 (10)	17 ± 21 (10) A	-5 ± 15 (10) A	-4 ± 14 (9) AC	2 ± 15 (6) A

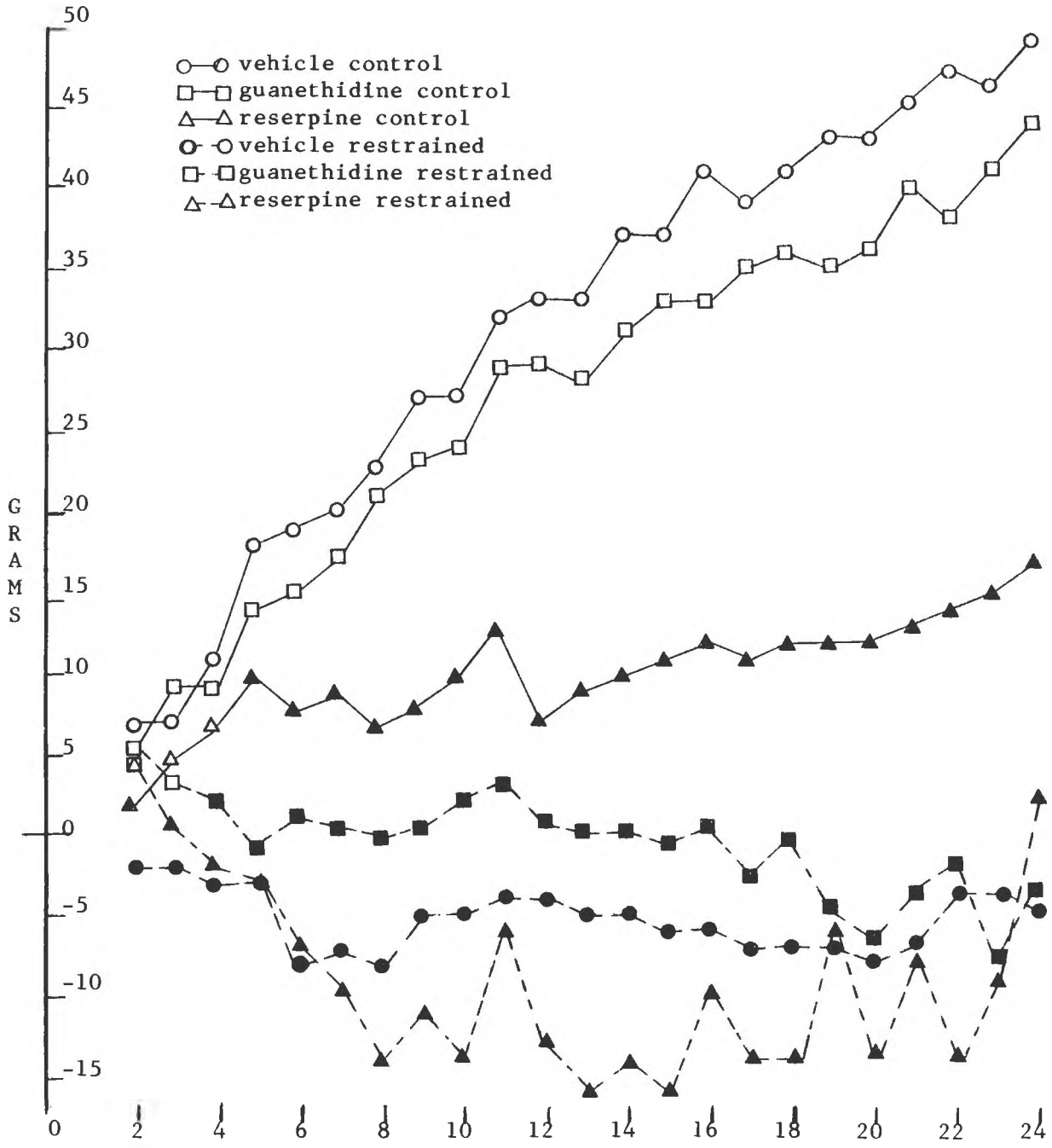
a: mean ± S.E., cumulative weight gains (grams) from day one.
b: number of rats.
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle- control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily for 24 days: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

Figure 1

THE EFFECT OF RESTRAINT ON MEAN CUMULATIVE DAILY WEIGHT GAINS ON MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) daily for 24 days. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg. Vehicle, glass-distilled water, 1 ml/kg.

TABLE III

THE EFFECT OF RESTRAINT ON MEAN DAILY WATER CONSUMPTION
OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	32 + 1.71[a] (10)[b]	34 + 1.23 (10)	36 + 1.93 (10)	31 + 0.95 (10)	30 + 3.01 (10)	37 + 1.52 (10)
2	36 + 1.65 (10)	32 + 1.33 (10)	36 + 0.66 (10)	35 + 1.42 (10)	41 + 5.35 (10)	42 + 1.23 (10) C[c]
3	33 + 1.27 (10)	32 + 1.17 (10)	32 + 0.73 (10)	36 + 1.84 (10)	38 + 1.08 (10) B	44 + 0.79 (10) C
4	34 + 1.17 (10)	31 + 1.96 (10)	32 + 1.84 (10)	37 + 1.61 (10)	36 + 0.95 (10) B	40 + 0.92 (10) C
5	33 + 0.76 (10)	31 + 0.85 (10)	32 + 0.98 (10)	36 + 1.39 (10) A	42 + 3.73 (10) B	41 + 1.99 (10) C
6	35 + 1.17 (10)	33 + 1.49 (10)	34 + 1.36 (10)	39 + 1.68 (10) A	43 + 1.99 (10) B	46 + 2.41 (10) C
7	35 + 0.85 (10)	35 + 1.74 (10)	33 + 1.17 (10)	36 + 1.93 (10)	39 + 1.61 (10)	43 + 1.11 (10) C
8	31 + 2.15 (10)	36 + 2.25 (10)	32 + 1.27 (10)	37 + 1.42 (10) A	40 + 0.76 (10)	42 + 1.17 (9) C
9	37 + 1.39 (10)	35 + 1.80 (10)	31 + 1.61 (10)	39 + 1.84 (10)	38 + 1.01 (10)	41 + 1.00 (9) C

TABLE III - continued

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
10	31 ± 1.20 (10)	30 ± 1.63 (10)	31 ± 1.04 (10)	35 ± 1.61 (10)	38 ± 0.54 (10) B	40 ± 0.70 (9) C
11	36 ± 1.52 (10)	36 ± 2.22 (10)	32 ± 1.93 (10)	38 ± 1.36 (10)	37 ± 1.14 (10)	39 ± 0.83 (9) C
12	34 ± 0.66 (10)	33 ± 1.90 (10)	25 ± 1.52 (10)	38 ± 2.18 (10)	36 ± 1.23 (10)	36 ± 1.53 (9) C
13	36 ± 1.93 (10)	34 ± 2.47 (10)	28 ± 1.14 (10)	33 ± 1.30 (10)	34 ± 1.42 (10)	31 ± 0.90 (9) C
14	36 ± 1.80 (10)	33 ± 2.97 (10)	26 ± 1.65 (10)	35 ± 1.39 (10)	35 ± 1.49 (10)	29 ± 1.47 (9)
15	35 ± 0.60 (9)	32 ± 1.33 (10)	21 ± 1.49 (10)	34 ± 1.61 (10)	36 ± 1.08 (10) B	37 ± 3.70 (9) C
16	32 ± 1.00 (9)	31 ± 1.65 (10)	26 ± 2.12 (10)	33 ± 1.58 (10)	35 ± 1.42 (10) B	32 ± 2.08 (8)
17	46 ± 1.87 (9)	35 ± 2.06 (10)	24 ± 1.90 (10)	37 ± 2.22 (10) A	39 ± 1.80 (10)	30 ± 4.95 (8)
18	43 ± 1.87 (9)	34 ± 1.96 (10)	33 ± 2.97 (10)	37 ± 1.80 (10) A	40 ± 0.89 (10) B	33 ± 4.42 (8)
19	38 ± 0.97 (9)	32 ± 1.42 (10)	28 ± 1.23 (10)	38 ± 2.69 (10)	36 ± 1.39 (10)	33 ± 2.98 (7)

TABLE III - continued

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
20	37 ± 0.47 (9)	31 ± 1.23 (10)	27 ± 1.14 (10)	33 ± 1.68 (10)	37 ± 1.17 (10) B	37 ± 7.77 (7)
21	37 ± 0.80 (9)	34 ± 1.01 (10)	29 ± 1.49 (10)	39 ± 1.55 (10)	38 ± 0.60 (10)	35 ± 2.83 (7)
22	40 ± 1.47 (9)	33 ± 2.34 (10)	27 ± 1.87 (10)	36 ± 2.18 (10)	38 ± 1.47 (9)	32 ± 1.51 (7)
23	38 ± 1.57 (9)	34 ± 1.87 (10)	25 ± 2.12 (10)	38 ± 1.84 (10)	41 ± 1.37 (9) B	29 ± 1.96 (7)
24	37 ± 1.70 (9)	34 ± 1.71 (10)	28 ± 0.79 (10)	32 ± 1.55 (10)	39 ± 1.37 (9) B	25 ± 0.87 (7) C

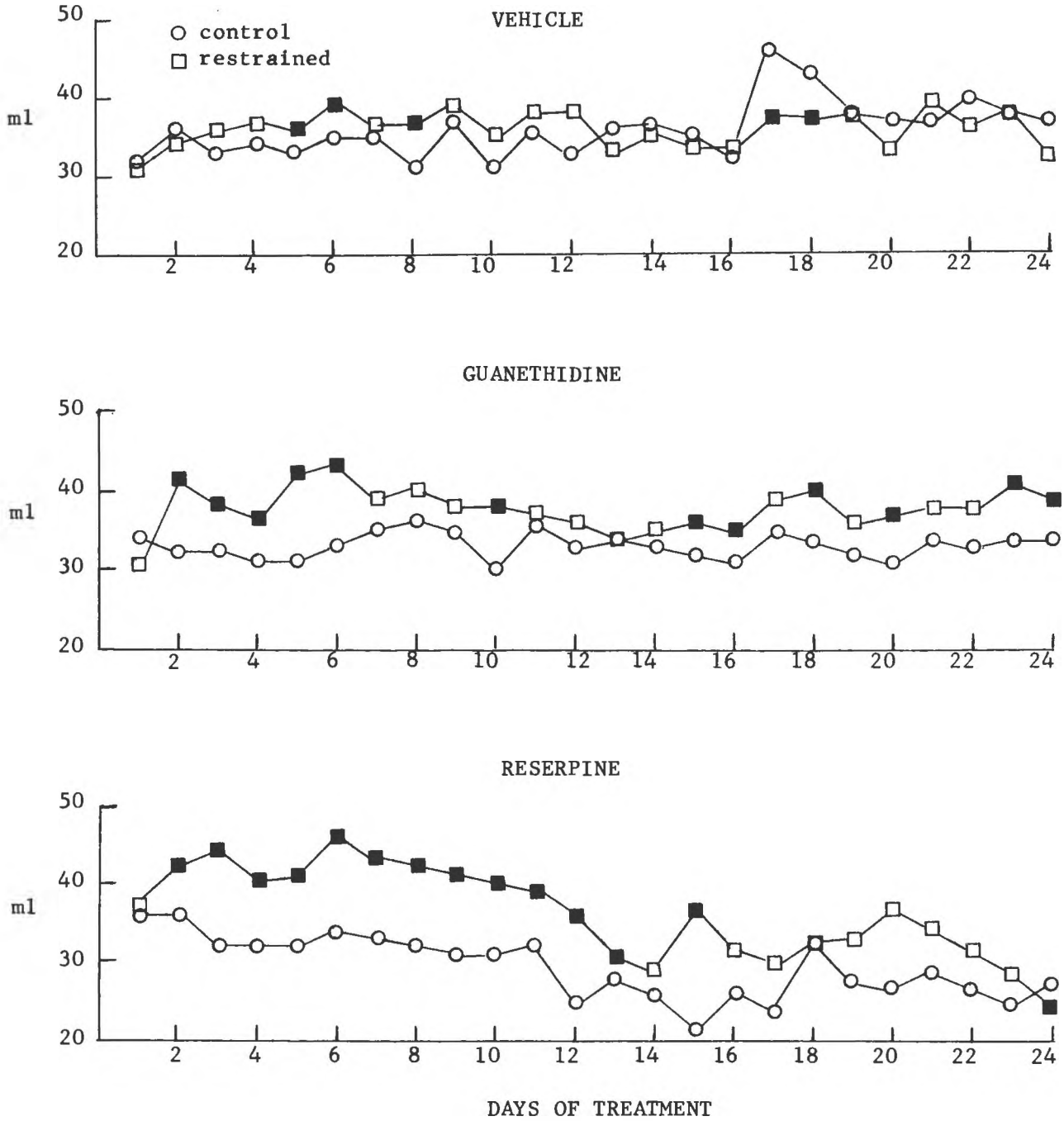
a: mean ± S.E., ml of water consumed/rat/day.
b: number of rats.
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from guanethidine-control group.
C - significantly different from reserpine-control group.

Drug doses, (i.p.) daily for 24 days: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

Figure 2

THE EFFECT OF RESTRAINT ON MEAN DAILY WATER CONSUMPTION OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated animals. Drug doses (i.p.) daily for 24 days: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg. Vehicle, glass-distilled water, 1 ml/kg.

TABLE IV

THE EFFECT OF CHRONIC GUANETHIDINE OR RESERPINE TREATMENT ON THE MEAN SYSTOLIC BLOOD PRESSURE OF MALE ALBINO RATS

Days of Treatment	Control Animals		
	Vehicle	Guanethidine	Reserpine
1	114 ± 3.1[a][b]	115 ± 2.2	100 ± 1.7 AB[c]
2	117 ± 1.7	118 ± 0.8	91 ± 3.5 AB
3	119 ± 0.8	115 ± 1.7	95 ± 3.1 AB
4	117 ± 3.5	119 ± 4.4	108 ± 1.3 AB
5	114 ± 3.1	113 ± 1.7	98 ± 4.4 AB
6	118 ± 1.7	120 ± 2.6	97 ± 2.6 AB
7	114 ± 4.0	116 ± 2.6	100 ± 2.6 AB
8	117 ± 3.1	121 ± 0.8	104 ± 4.9 AB
9	115 ± 1.3	112 ± 1.7	96 ± 2.2 AB
10	119 ± 2.6	122 ± 2.6	96 ± 2.2 AB
11	118 ± 3.1	117 ± 2.2	100 ± 4.9 AB
12	116 ± 2.6	114 ± 2.2	99 ± 4.0 AB
14	113 ± 2.2	123 ± 2.6 A[c]	97 ± 4.4 AB
16	112 ± 3.5	119 ± 3.1	101 ± 3.5 B
18	114 ± 2.2	116 ± 2.6	96 ± 4.9 AB
20	111 ± 4.4	112 ± 4.9	94 ± 3.5 AB
22	126 ± 3.1	124 ± 4.0	93 ± 5.3 AB
24	124 ± 3.5	125 ± 2.6	88 ± 4.0 AB

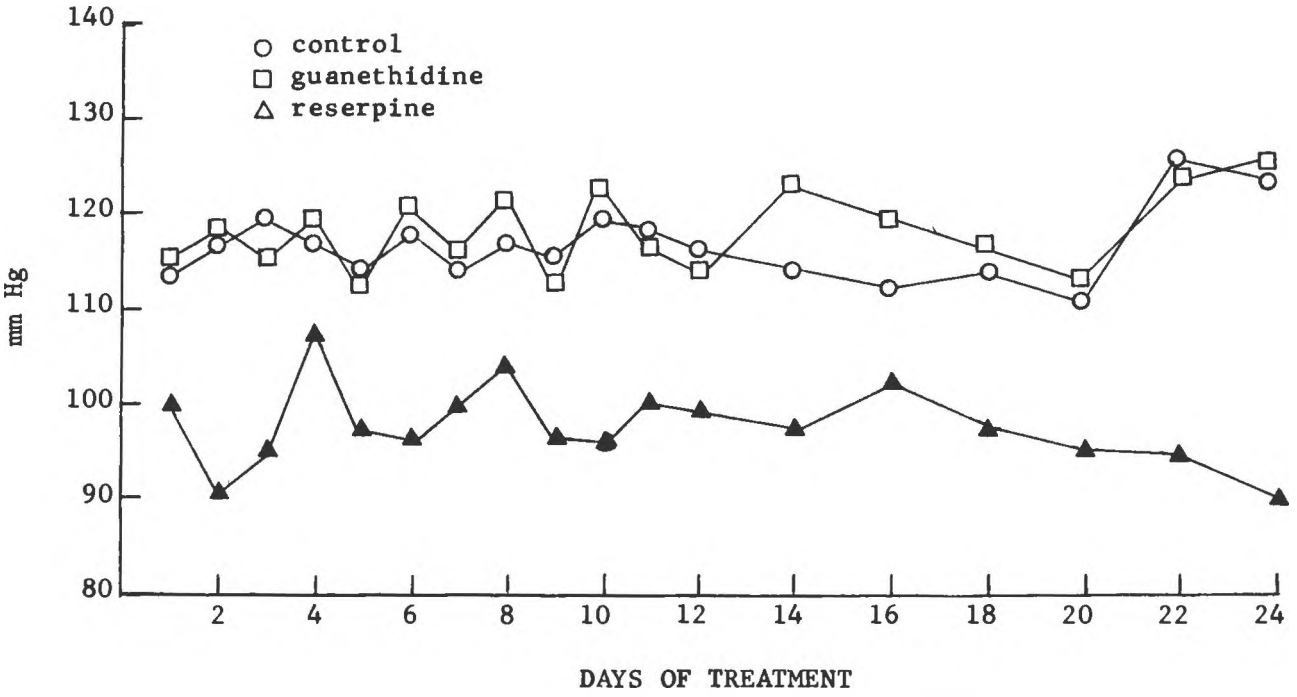
a: mean systolic blood pressure (mm Hg) ± S.E.
b: N = 5 animals/group
c: statistical comparison at probability; $P < 0.05$.

A - significantly different from vehicle-control group
B - significantly different from guanethidine-control group

Drug doses, (i.p.) daily for 24 days: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg. Vehicle, glass-distilled water, 1 ml/kg.

Figure 3

THE EFFECT OF CHRONIC GUANETHIDINE OR RESERPINE TREATMENT ON THE MEAN SYSTOLIC BLOOD PRESSURE IN MALE ALBINO RATS



Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group. Drug doses, (i.p.) daily for 24 days: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg. Vehicle, glass-distilled water, 1 ml/kg.

TABLE V

THE EFFECT OF RESTRAINT ON ABSOLUTE ADRENAL WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	0.022 ± .002[a] (10)[b]	0.020 ± .001 (10)	0.022 ± .001 (10)	0.021 ± .002 (9)	0.022 ± .002 (10)	0.021 ± .001 (10)
3	0.023 ± .002 (10)	0.024 ± .001 (10)	0.025 ± .002 (10)	0.025 ± .002 (10)	0.024 ± .002 (10)	0.030 ± .002 (9) ABD[c]
6	0.017 ± .001 (10)	0.015 ± .002 (10)	0.016 ± .002 (10)	0.020 ± .002 (10)	0.024 ± .001 (10)	0.022 ± .001 (10) AD
12	0.025 ± .001 (10)	0.025 ± .001 (10)	0.026 ± .002 (10)	0.030 ± .002 (10)	0.033 ± .003 (9) AC	0.027 ± .002 (9)
24	0.028 ± .002 (10)	0.024 ± .002 (10)	0.024 ± .002 (10)	0.031 ± .002 (10)	0.031 ± .003 (9)	0.035 ± .004 (6) D

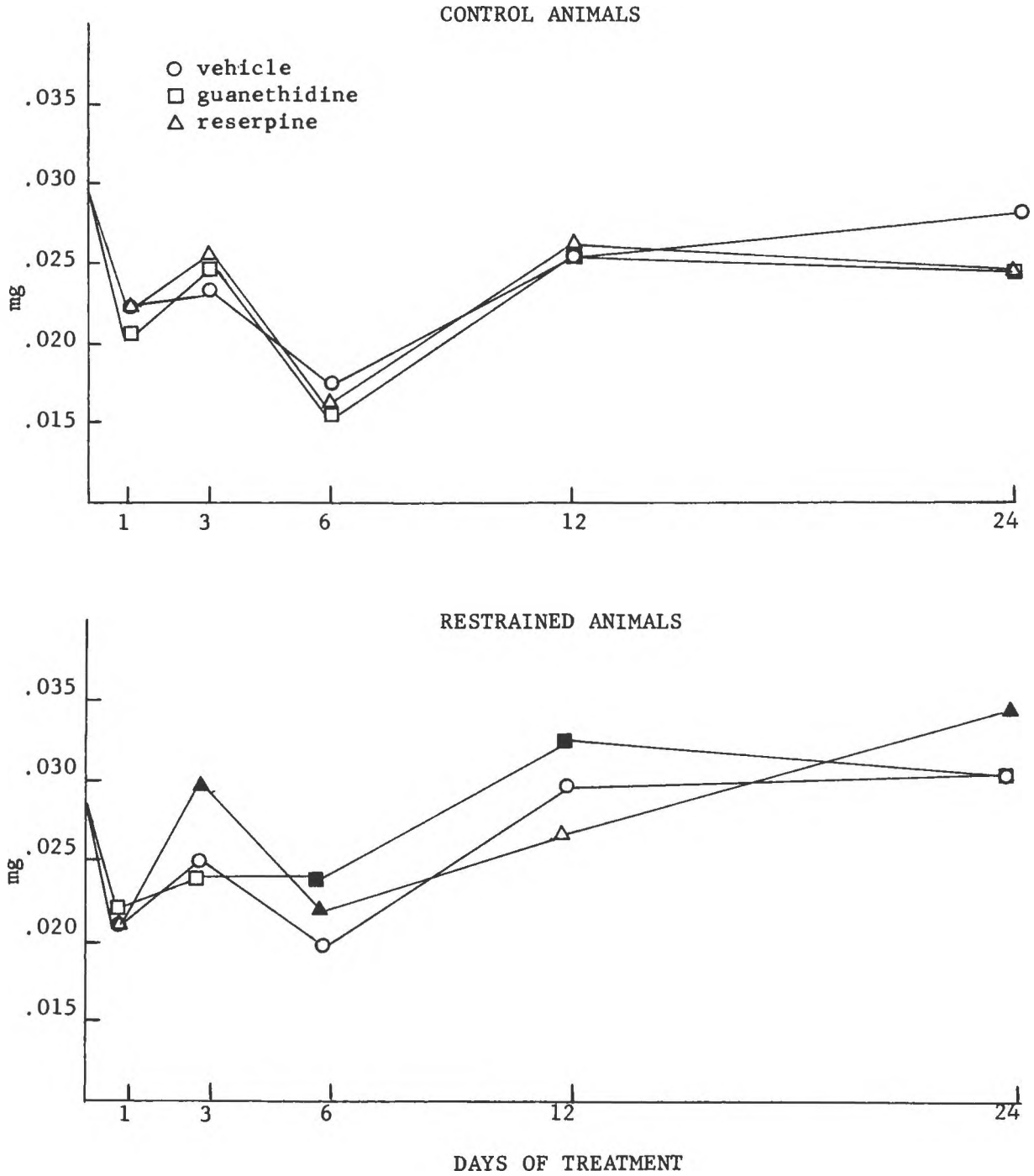
a: mean ± S.E. (mg).
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Note: Twenty animals selected at random, no treatment, had adrenal weights of 0.029 ± 0.002 mg.

Figure 4

THE EFFECT OF RESTRAINT ON ABSOLUTE ADRENAL WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Solid symbols designate a significant difference ($P < 0.05$).
See Table V for statistical comparison.

TABLE VI

THE EFFECT OF RESTRAINT ON ABSOLUTE HEART WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	0.62 ± .02[a] (10)[b]	0.65 ± .01 (10)	0.62 ± .02 (10)	0.63 ± .01 (9)	0.59 ± .02 (10) BC[c]	0.61 ± .02 (10)
3	0.68 ± .02 (10)	0.66 ± .02 (10)	0.64 ± .02 (10)	0.75 ± .02 (10) A	0.70 ± .02 (10)	0.69 ± .02 (9) B
6	0.69 ± .02 (10)	0.71 ± .02 (10)	0.71 ± .01 (10)	0.68 ± .02 (10)	0.68 ± .03 (10)	0.67 ± .01 (10)
12	0.78 ± .03 (10)	0.72 ± .02 (10)	0.76 ± .02 (10)	0.69 ± .02 (10) A	0.71 ± .02 (9)	0.61 ± .02 (9) ABD
24	0.80 ± .03 (10)	0.72 ± .02 (10) A	0.76 ± .03 (10)	0.66 ± .02 (10) A	0.67 ± .02 (9) A	0.69 ± .03 (6) A

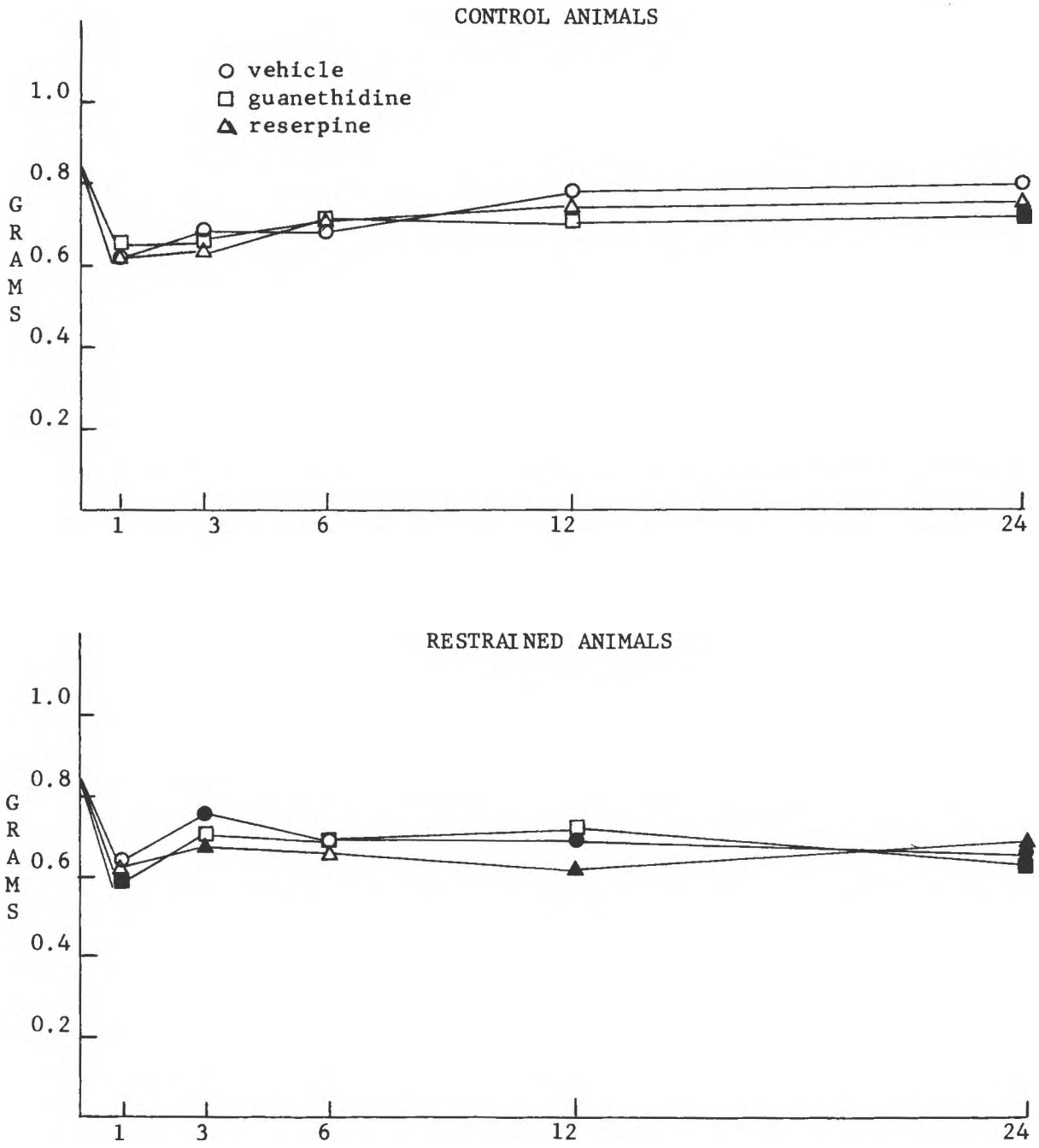
a: mean ± S.E. (grams).
b: number of rats.
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Note: Ten animals selected at random, no treatment, had heart weight of 0.84 ± 0.03 grams.

Figure 5

THE EFFECT OF RESTRAINT ON ABSOLUTE HEART WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Solid symbols designate a significant difference ($P < 0.05$).
See Table VI for statistical comparison.

TABLE VII

THE EFFECT OF RESTRAINT ON ABSOLUTE BRAIN WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	1.62 ± .02[a] (10)[b]	1.69 ± .02 (10)	1.67 ± .02 (10)	1.60 ± .02 (9)	1.61 ± .02 (10)	1.66 ± .02 (10)
3	1.70 ± .04 (10)	1.66 ± .02 (10)	1.65 ± .03 (10)	1.64 ± .03 (10)	1.64 ± .04 (10)	1.62 ± .03 (9)
6	1.59 ± .03 (10)	1.63 ± .03 (10)	1.64 ± .02 (10)	1.56 ± .03 (10)	1.55 ± .02 (10) C	1.59 ± .03 (10)
12	1.58 ± .05 (10)	1.66 ± .03 (10)	1.70 ± .02 (10) A[c]	1.57 ± .04 (10)	1.52 ± .02 (9) C	1.53 ± .02 (9) D
24	1.68 ± .04 (10)	1.66 ± .03 (10)	1.62 ± .04 (10)	1.59 ± .03 (10)	1.65 ± .04 (9)	1.58 ± .06 (6)

a: mean ± S.E. (grams).
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

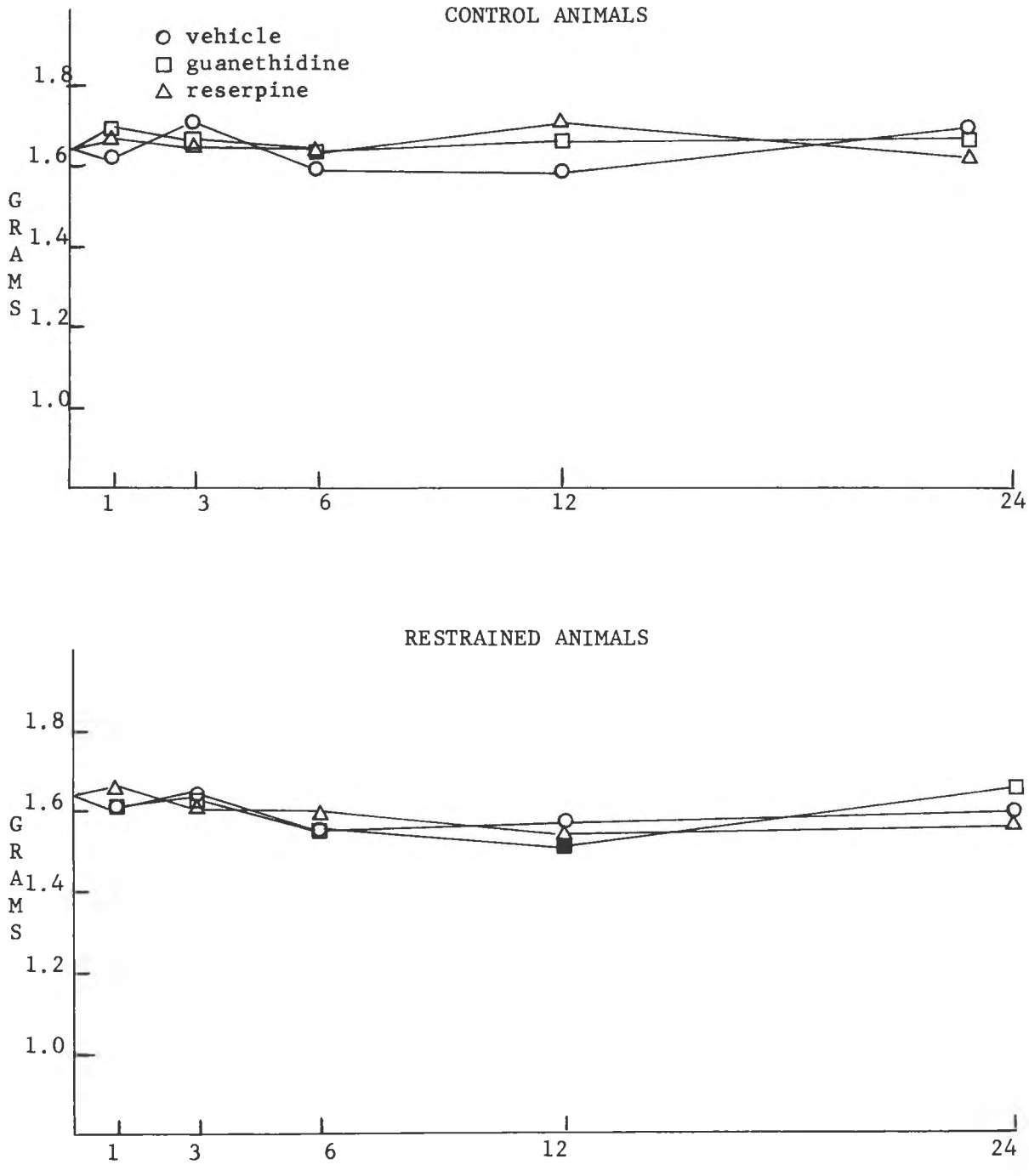
A - significantly different from vehicle-control group.
B - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

Note: Ten animals selected at random, no treatment, had brain weights of 1.64 ± 0.03 grams.

Figure 6

THE EFFECT OF RESTRAINT ON ABSOLUTE BRAIN WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Solid symbols designate a significant difference ($P < 0.05$).
See Table VII for statistical comparison.

Figure 7

TIME COURSE FOR THE DEVELOPMENT OF CORTICOSTERONE FLUORESCENCE IN 30 N H_2SO_4

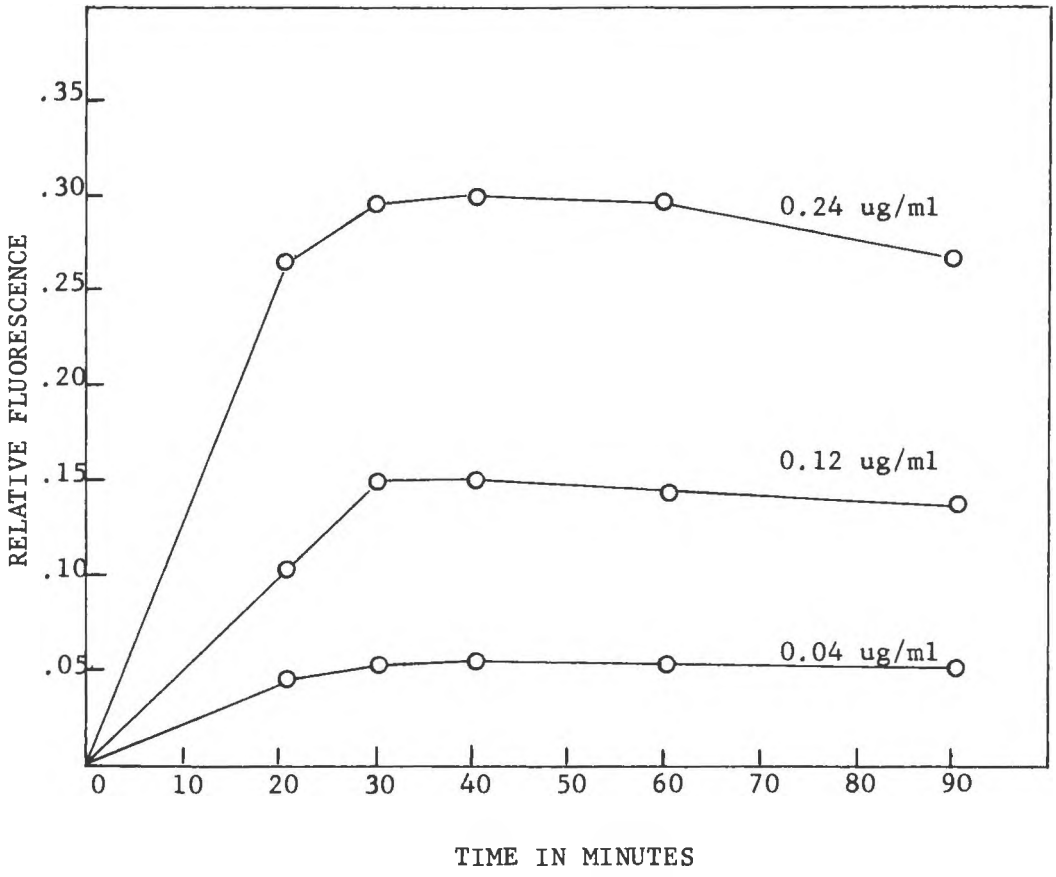
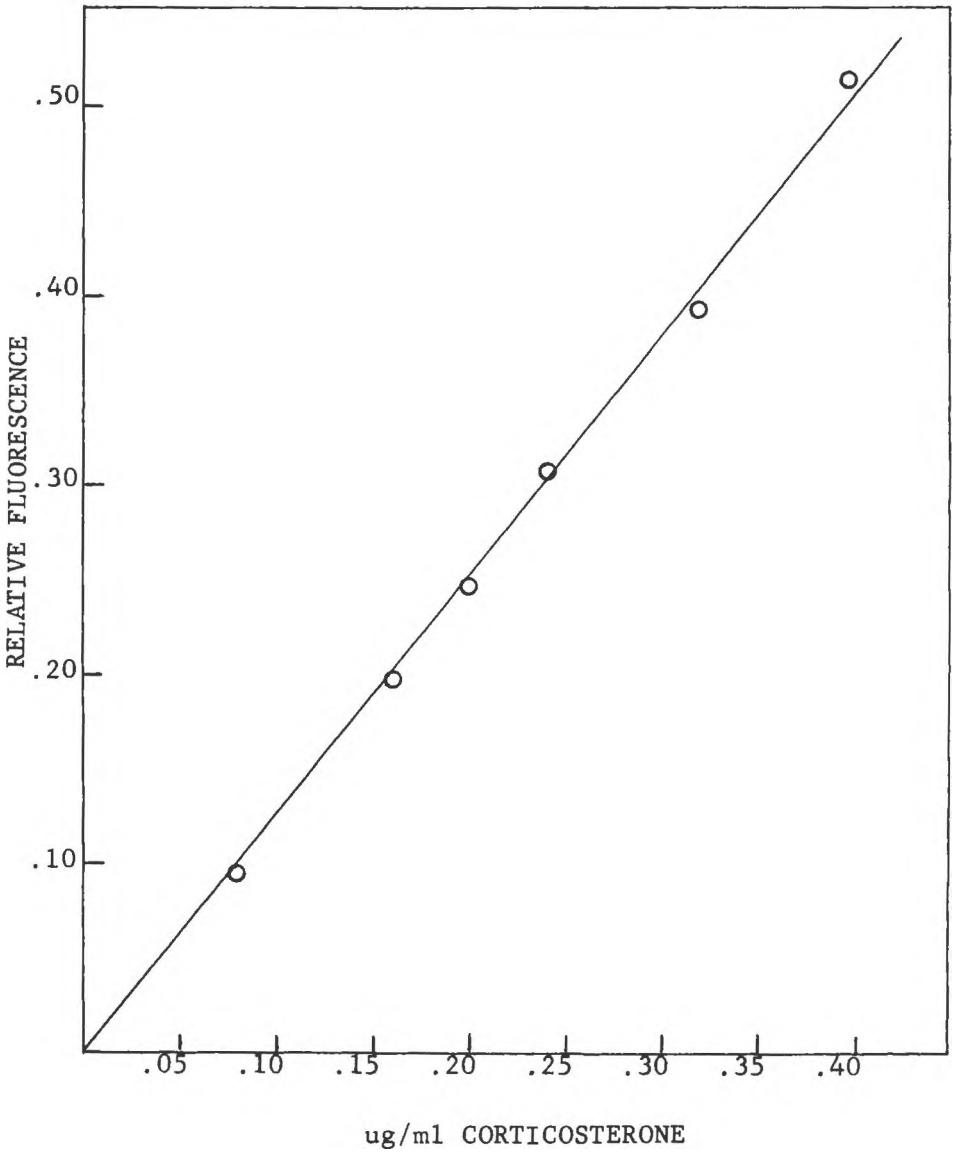


Figure 8

TYPICAL STANDARD CURVE FOR CORTICOSTERONE



Fluorescence intensity at varying concentrations of corticosterone.
Fluorescence is given in arbitary units,
(meter multiplier x % transmission x 100)
Activating wave length, 470 mu.
Fluorescence wave length, 510 mu.

TABLE VIII

THE EFFECT OF RESTRAINT ON SERUM CORTICOSTERONE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	22 ± 3.2[a] (10)[b]	23 ± 2.5 (10)	19 ± 2.8 (10)	30 ± 1.7 (9) A[c]	33 ± 1.9 (10) AC	31 ± 1.3 (10) AD
3	19 ± 2.2 (10)	19 ± 2.2 (10)	16 ± 1.3 (10)	24 ± 2.2 (10)	25 ± 1.9 (10)	25 ± 2.0 (9) D
6	19 ± 3.5 (10)	25 ± 1.6 (10)	29 ± 3.2 (10) A	24 ± 2.2 (10)	26 ± 2.2 (10)	25 ± 1.6 (10)
12	21 ± 2.2 (10)	20 ± 2.8 (10)	15 ± 1.6 (10) A	20 ± 0.9 (10)	23 ± 1.7 (9)	28 ± 2.5 (9) ABD
24	23 ± 2.2 (10)	25 ± 2.8 (10)	25 ± 2.2 (10)	21 ± 3.2 (10)	27 ± 2.3 (9)	33 ± 3.7 (6) B

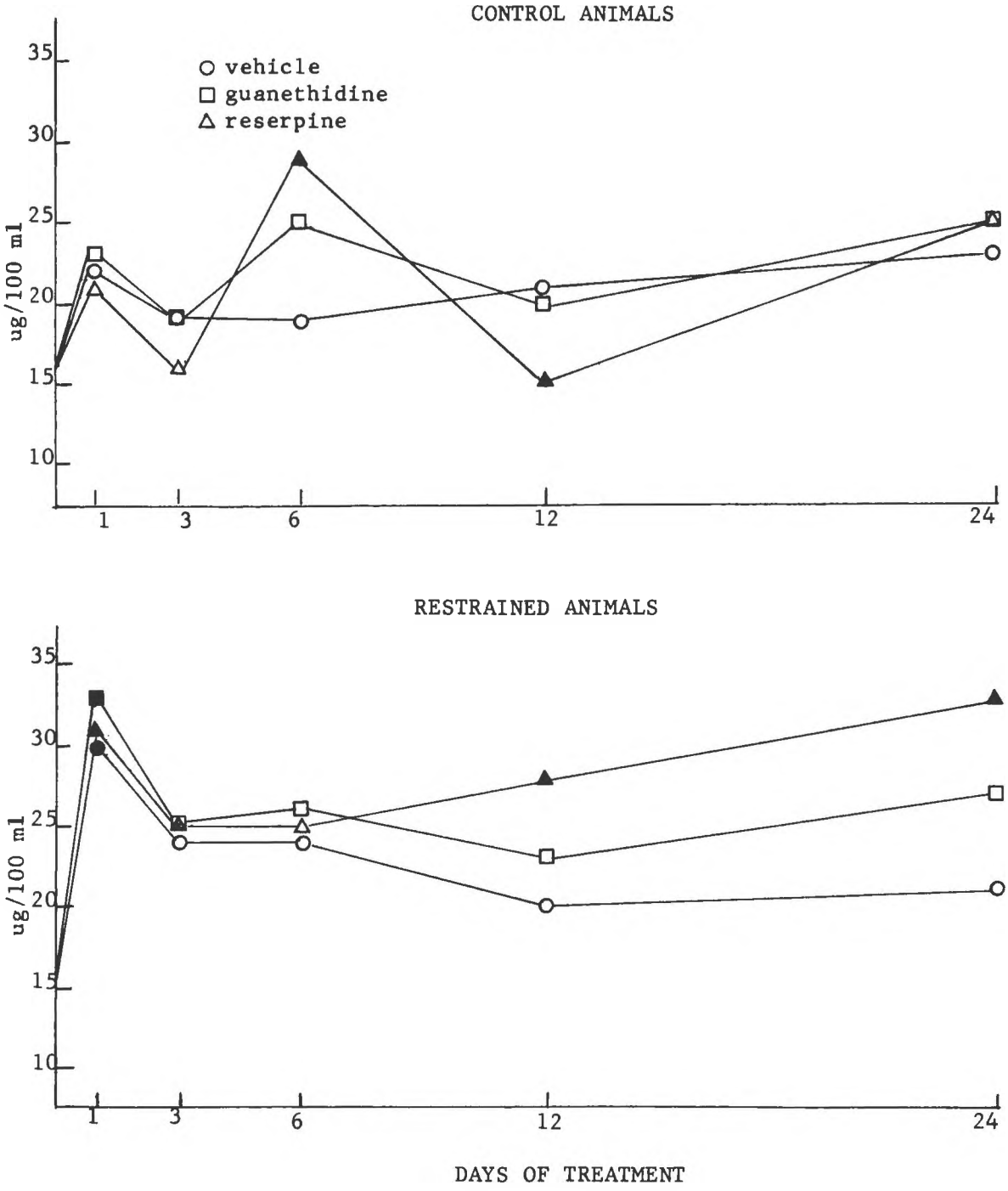
a: mean ± S.E. (mg/100 ml)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Note: Ten animals selected at random, no treatment, had serum corticosterone levels of 16 ± 3.0 mg/100 ml.

Figure 9

THE EFFECT OF RESTRAINT ON SERUM CORTICOSTERONE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg. Vehicle, glass-distilled water, 1 ml/kg.
Solid symbols designate a significant difference ($P < 0.05$).
See Table VIII for statistical comparison.

Figure 10

TYPICAL STANDARD CURVE FOR ASCORBIC ACID

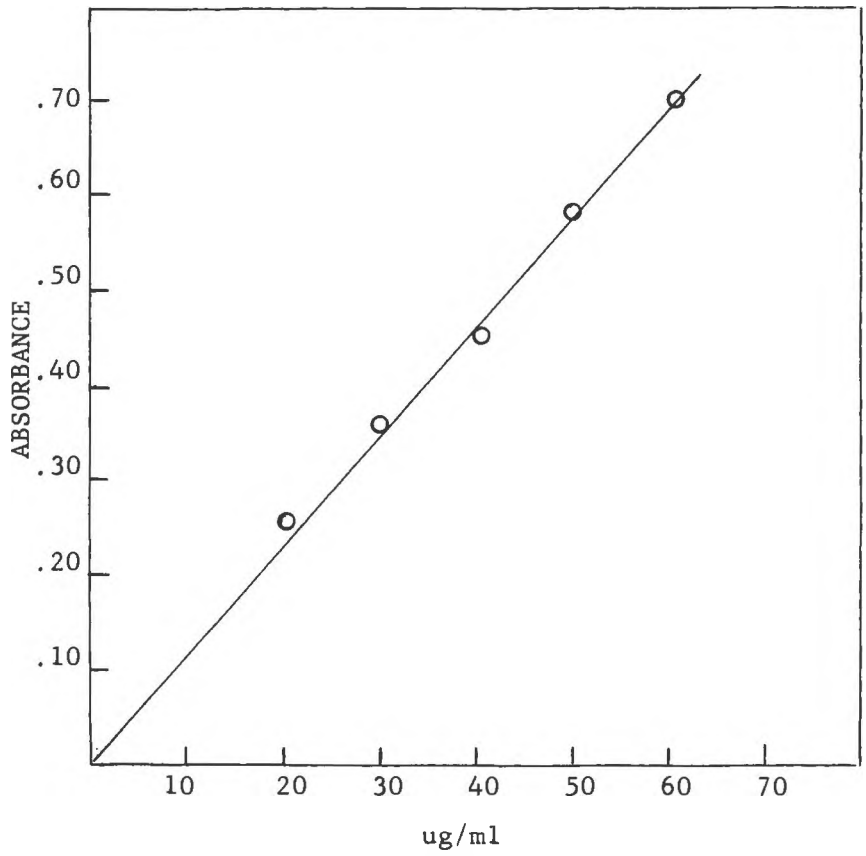


TABLE IX

THE EFFECT OF RESTRAINT ON ADRENAL ASCORBIC ACID OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	570 ± 30[a] (10)[b]	492 ± 23 (10)	503 ± 29 (10)	371 ± 41 (9) A[c]	359 ± 23 (10) AC	367 ± 29 (10) AD
3	551 ± 32 (10)	462 ± 47 (10)	468 ± 42 (10)	519 ± 31 (10)	490 ± 27 (10)	464 ± 35 (9)
6	750 ± 59 (10)	743 ± 77 (10)	651 ± 50 (10)	646 ± 48 (10)	555 ± 57 (10) A	690 ± 47 (10)
12	585 ± 39 (10)	518 ± 27 (10)	593 ± 44 (10)	521 ± 39 (10)	580 ± 39 (9)	551 ± 27 (9)
24	560 ± 28 (10)	572 ± 40 (10)	623 ± 50 (10)	581 ± 53 (10)	596 ± 34 (9)	595 ± 58 (6)

a: mean ± S.E. (mg/100g)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

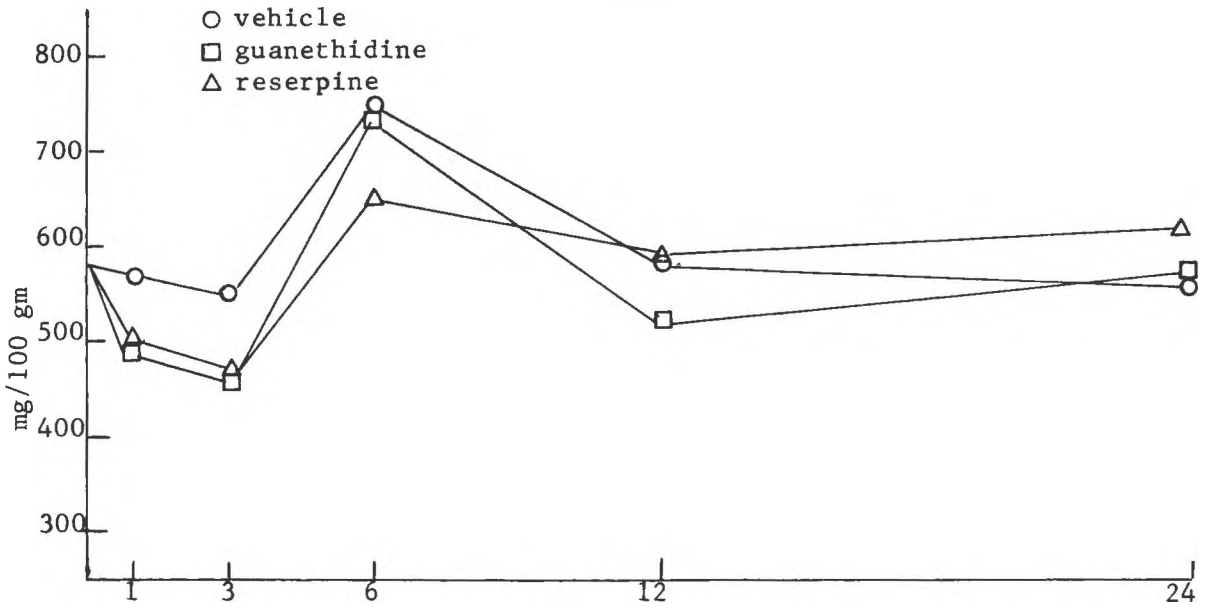
A - significantly different from vehicle-control group.
B - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Note: Twenty animals selected at random, no treatment, had adrenal ascorbic acid levels of 579 ± 35 ug/100g.

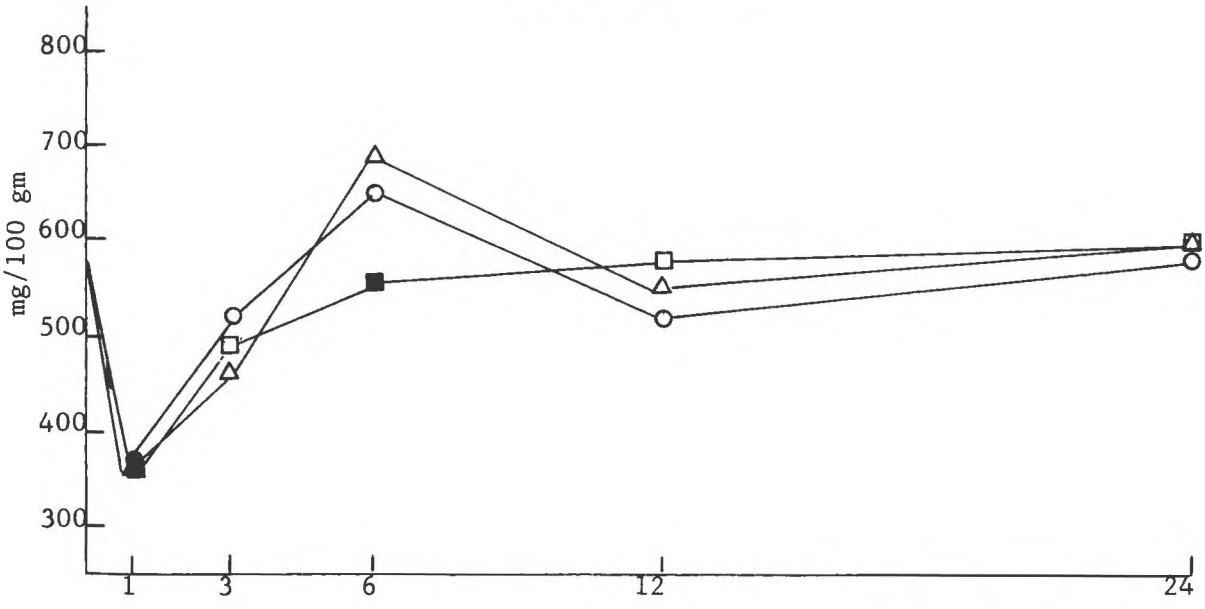
Figure 11

THE EFFECT OF RESTRAINT ON ADRENAL ASCORBIC ACID OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

CONTROL ANIMALS



RESTRAINED ANIMALS

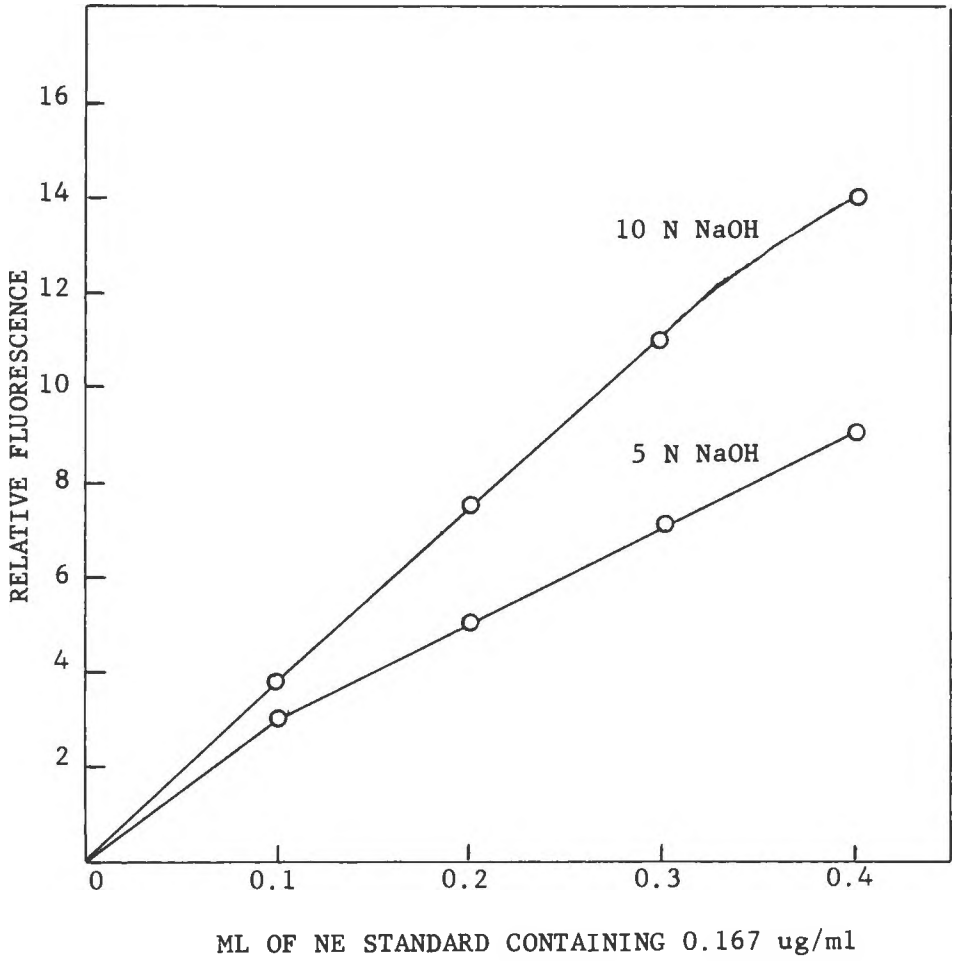


DAYS OF TREATMENT

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Solid symbols designate a significant difference ($P < 0.05$).
See Table IX for statistical comparison

Figure 12

A TYPICAL GRAPH OF THE EFFECTS OF 10 N NaOH AND 5 N NaOH ON THE RELATIVE FLUORESCENCE OF NOREPINEPHRINE



1. After Anton, A.H. and Sayre, D.F.: J. Pharmac. exp. Ther. 138: 360, 1962.

Figure 13

A TYPICAL GRAPH OF THE EFFECT OF ETHYLENEDIAMINE (EDA)
ON THE RELATIVE FLUORESCENCE OF NOREPINEPHRINE

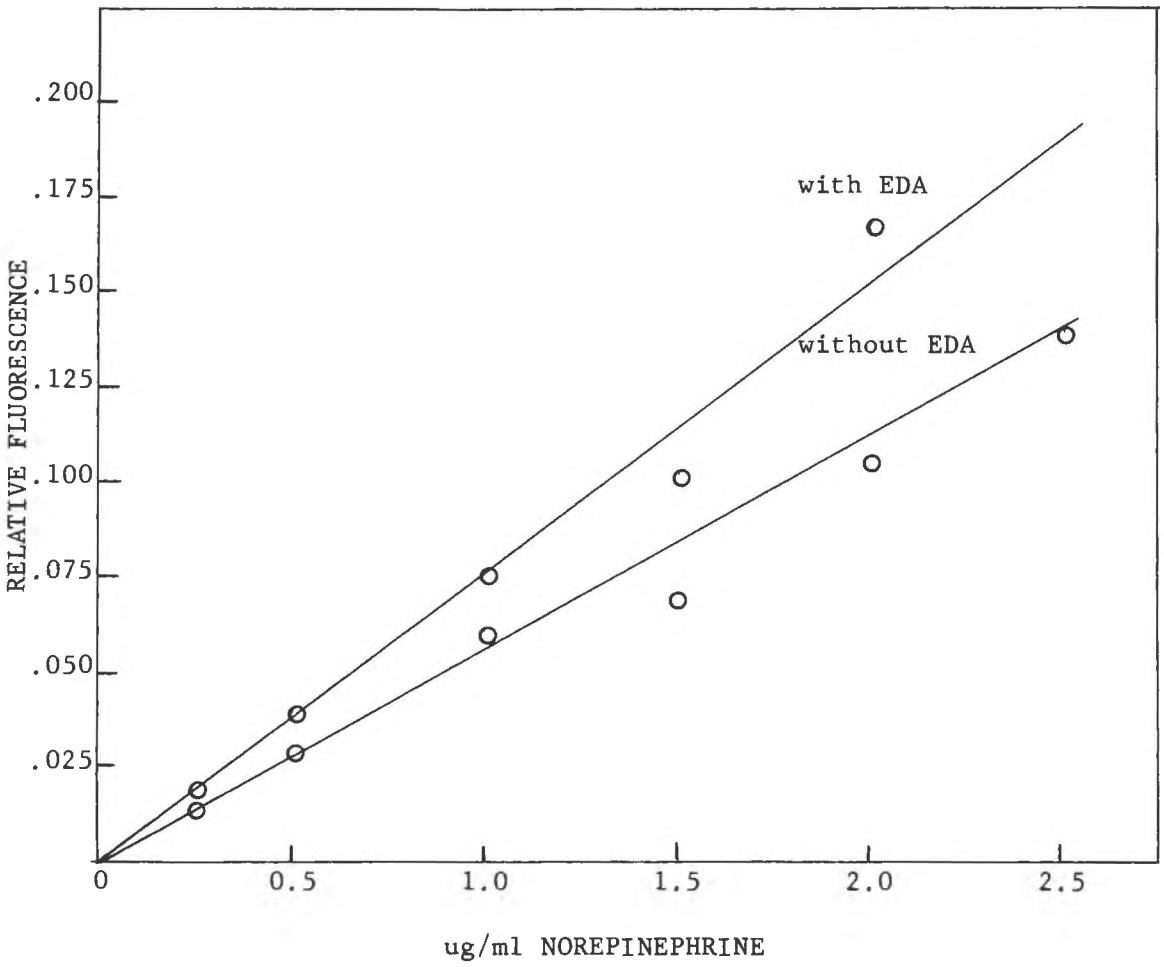


Figure 14

TYPICAL STANDARD CURVES OF NOREPINEPHRINE (NE) AND EPINEPHRINE (E)

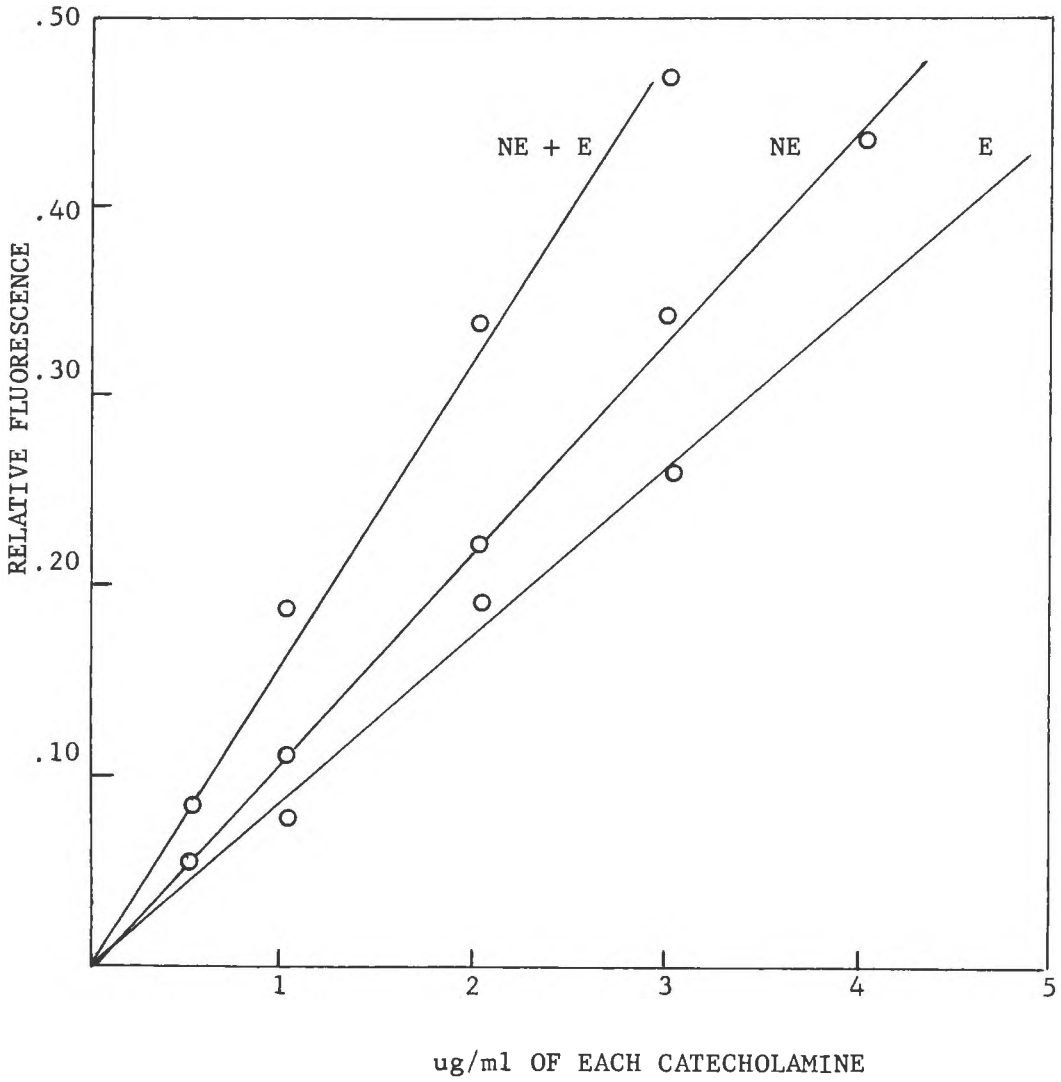
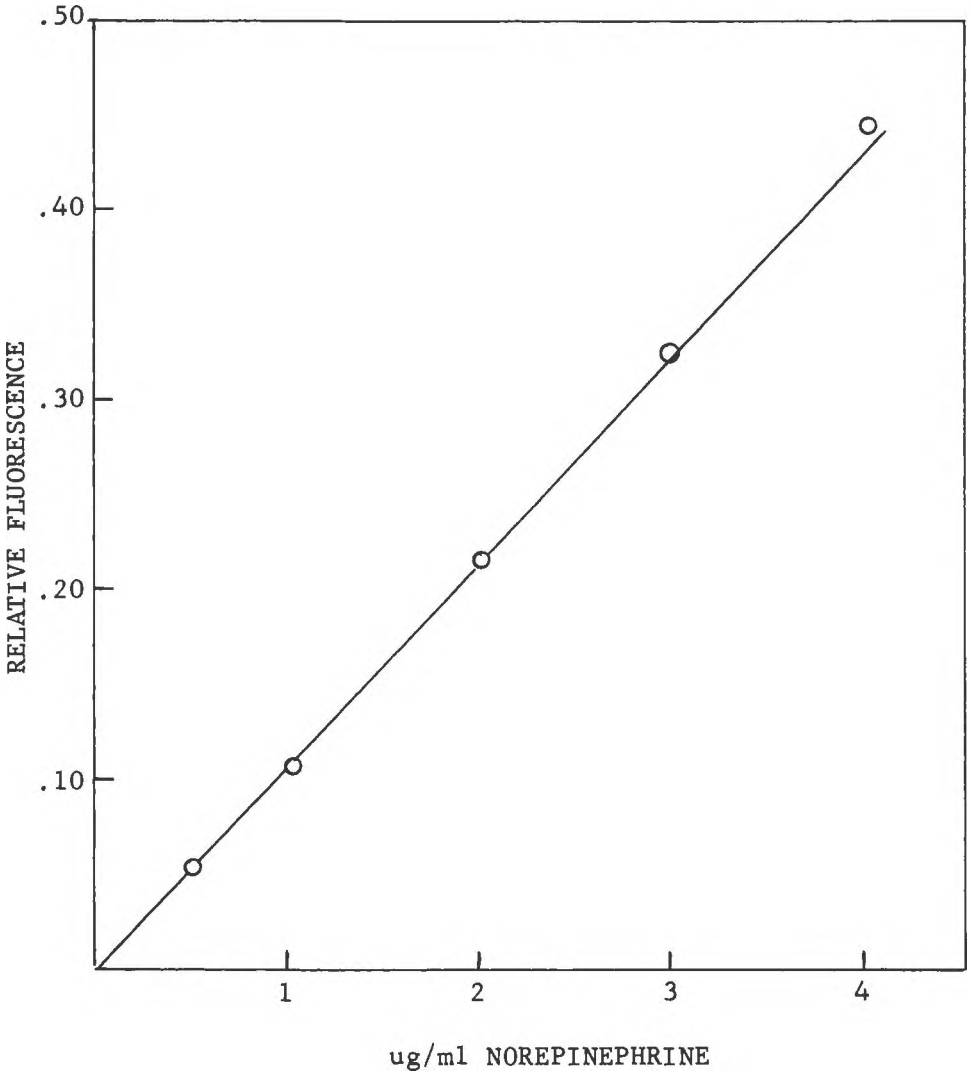


Figure 15

TYPICAL STANDARD CURVE FOR NOREPINEPHRINE



Fluorescence intensity at varying concentrations of norepinephrine.
Fluorescence is given in arbitrary units,
(meter multiplier x % transmission x 100)
Activating wavelength, 400 mu.
Fluorescence wavelength, 520 mu.

TABLE X

COMPARISON OF THE CONTROL TISSUE NOREPINEPHRINE (NE) ASSAY WITH PREVIOUSLY REPORTED VALUES USING THE SHORE AND OLIN (1958) METHOD

Tissue	NE Conc. (ug/g)	Source of Data
Rat brain	0.237 ± 0.008[a]	This report
	0.236 ± 0.010[b]	This report
	0.238 ± 0.023 (SD)	Porter et al. (1961)
	0.243 ± 0.011	Green et al. (1962)
	0.255 ± 0.026 (SD)	Green and Sawyer (1960)
Rat heart	1.032 ± 0.049[a]	This report
	1.022 ± 0.041[b]	This report
	1.060 ± 0.030	Bhagat (1967)
	1.020 ± 0.040	Bhagat and Gillman (1960)
	0.990 ± 0.060	Kuntzman and Jacobson (1964)

a: 10 rats selected at random, no treatment
b: 70 rats, vehicle-control, glass-distilled water, 1 ml/kg, i.p.

All values are expressed as the mean ± S.E., except where indicated.

TABLE XI

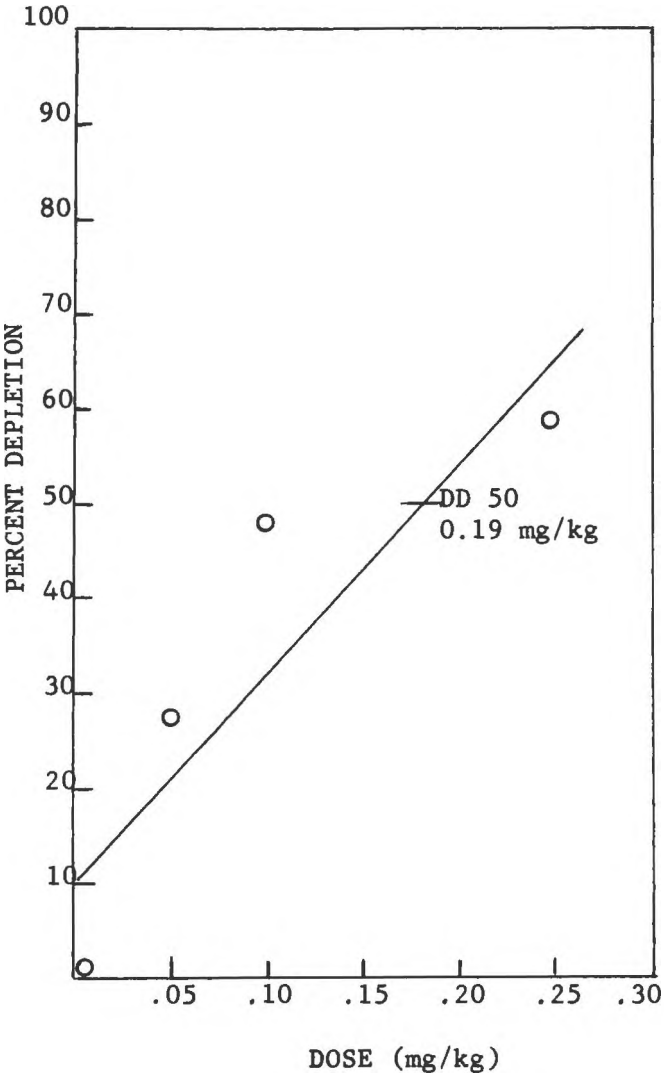
DOSE-RESPONSE EFFECT OF RESERPINE OR GUANETHIDINE ON BRAIN AND HEART NOREPINEPHRINE (NE) OF MALE ALBINO RATS INJECTED (IP) DAILY FOR SIX DAYS

Treatment	Dose	BRAIN NE[a]		HEART NE[a]	
		ug/gm	percent depletion	ug/gm	percent depletion
	ml/kg				
Vehicle	1.00	0.224 ± 0.005	00.0	1.017 ± 0.044	00.0
	mg/kg				
Reserpine	0.05	0.171 ± 0.008	23.7[b]	0.036 ± 0.005	96.5[b]
	0.10	0.140 ± 0.008	37.5[b]	0.018 ± 0.004	98.2[b]
	0.25	0.092 ± 0.003	58.9[b]	0.009 ± 0.003	99.1[b]
	1.00	0.013 ± 0.003	94.2[b]	0.005 ± 0.003	99.5[b]
	2.50	0.004 ± 0.001	98.2[b]	0.002 ± 0.002	99.8[b]
	ml/kg				
Vehicle	1.0	0.236 ± 0.014	00.0	1.016 ± 0.028	00.0
	mg/kg				
Guanethidine	0.5	0.274 ± 0.007	00.0	0.714 ± 0.046	29.7[b]
	1.0	0.238 ± 0.016	00.0	0.358 ± 0.037	64.8[b]
	2.5	0.242 ± 0.014	00.0	0.198 ± 0.016	80.5[b]
	5.0	0.247 ± 0.016	00.0	0.096 ± 0.008	90.6[b]
	10.0	0.226 ± 0.016	00.0	0.063 ± 0.006	93.8[b]

a: Concentration of NE expressed as mean ± S.E. for 10 animals in each group.
b: significant difference from controls ($P < 0.01$).

Figure 16

CALCULATION OF THE DD[1] 50 FOR CENTRAL NOREPINEPHRINE DEPLETING ACTION OF RESERPINE

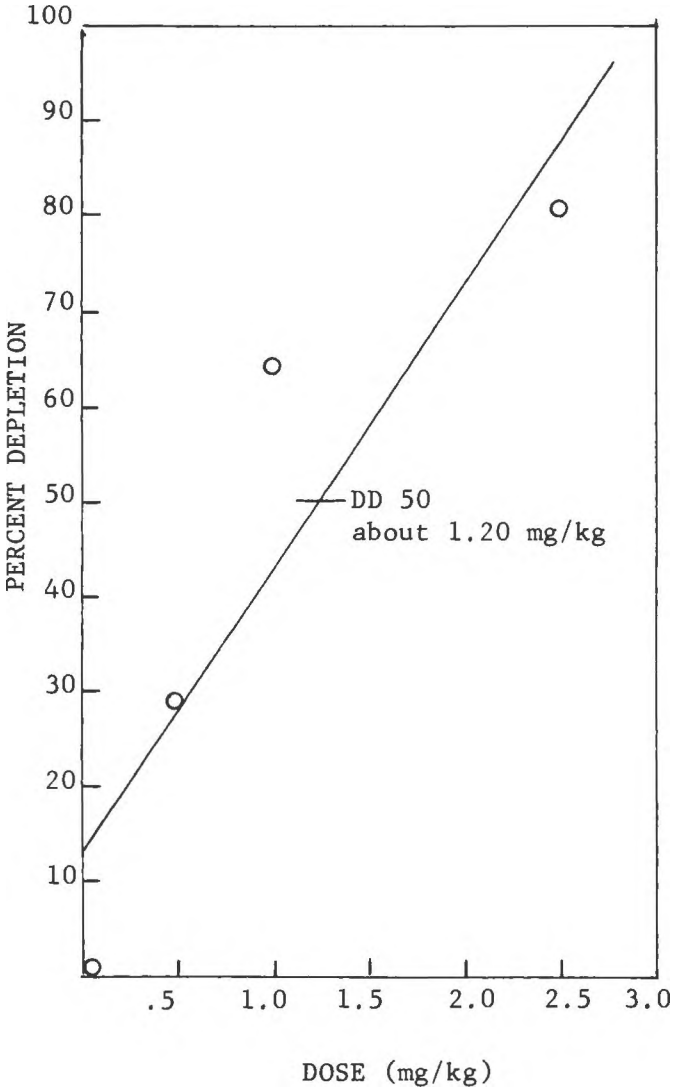


A plot of the six day dose-response curve for norepinephrine depleting effect of reserpine in the brain tissues of male albino rats. (o) are the original points. Line was fitted by the method of least squares (Snedecor, 1956).

1. DD - depleting dose.

Figure 17

CALCULATION OF THE DD[1] 50 FOR PERIPHERAL NOREPINEPHRINE DEPLETING ACTION OF GUANETHIDINE



A plot of the six day dose-response curve for epinephrine depleting effect of guanethidine in the heart tissues of male albino rats. (o) are the original points. Line was fitted by the method of least squares (Snedecor, 1956).

1. DD - depleting dose.

TABLE XII

THE EFFECT OF RESTRAINT ON BRAIN NOREPINEPHRINE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	0.257 ± .007[a] (10)[b]	0.228 ± .009 (10) A[c]	0.252 ± .022 (10)	0.229 ± .010 (9) A	0.243 ± .009 (10)	0.188 ± .008 (10) ABD
3	0.227 ± .014 (10)	0.221 ± .007 (10)	0.169 ± .017 (10) A	0.209 ± .007 (10)	0.231 ± .004 (10) B	0.150 ± .017 (9) AB
6	0.220 ± .009 (10)	0.214 ± .006 (10)	0.110 ± .012 (10) A	0.220 ± .009 (10)	0.249 ± .013 (10) C	0.064 ± .008 (10) ABD
12	0.226 ± .008 (10)	0.221 ± .015 (10)	0.060 ± .007 (10) A	0.246 ± .014 (10)	0.300 ± .013 (9) ABC	0.061 ± .006 (9) AB
24	0.259 ± .014 (10)	0.238 ± .019 (10)	0.025 ± .006 (10) A	0.275 ± .010 (10)	0.254 ± .015 (9) C	0.028 ± .005 (6) AB

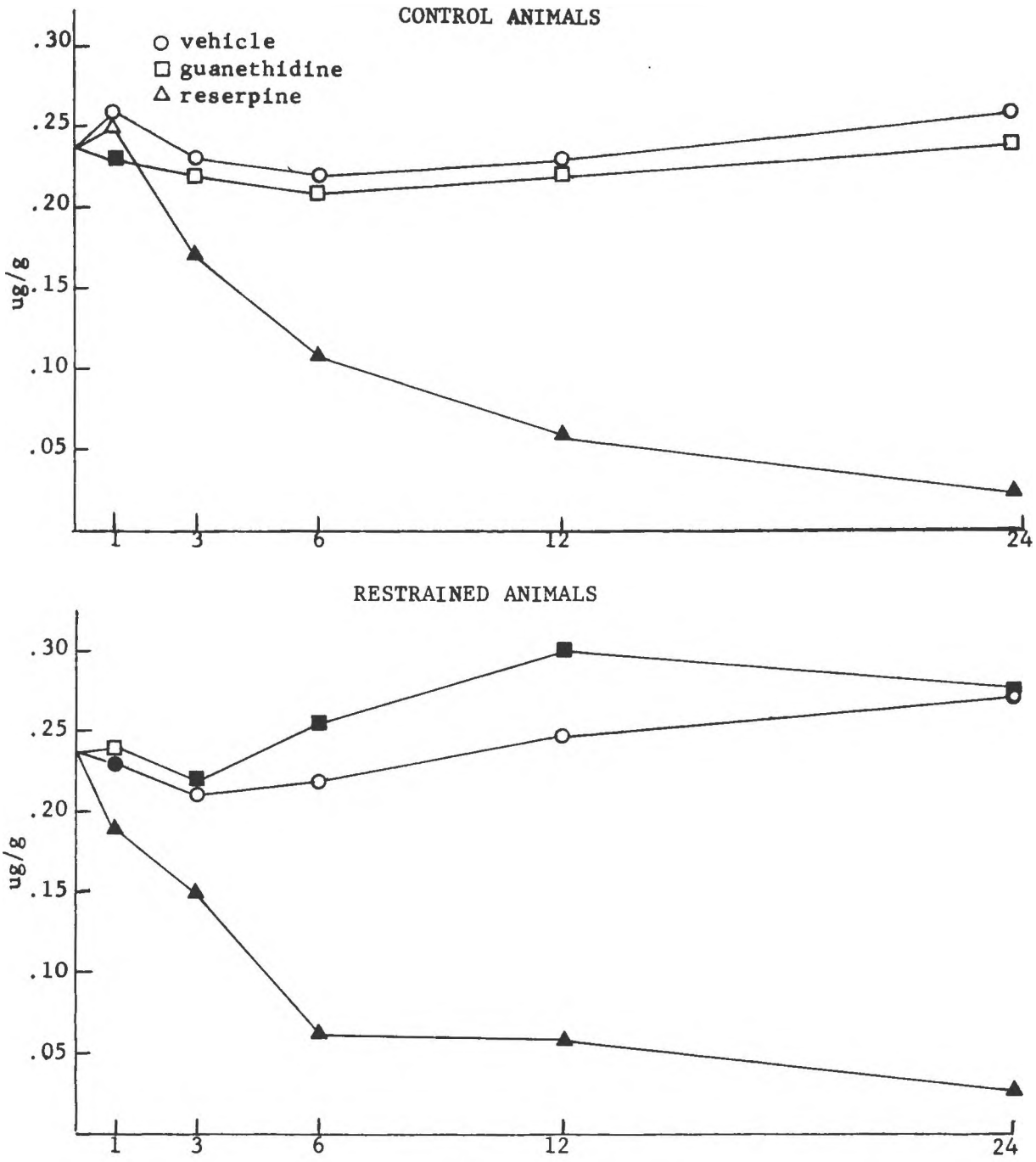
a: mean ± S.E. (ug/g)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Note: Ten animals selected at random, no treatment, had brain norepinephrine levels of 0.237 ± .008 ug/g.

Figure 18

THE EFFECT OF RESTRAINT ON BRAIN NOREPINEPHRINE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Solid symbols designate a significant difference ($P < 0.05$).
See Table XII for statistical comparison.

TABLE XIII

THE EFFECT OF RESTRAINT ON HEART NOREPINEPHRINE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	1.003 ± .033[a] (10)[b]	0.759 ± .040 (10) A[c]	0.071 ± .013 (10) A	0.942 ± .034 (9)	0.844 ± .038 (10) A	0.075 ± .018 (10) AB
3	1.035 ± .051 (10)	0.756 ± .089 (10) A	0.011 ± .005 (10) A	0.702 ± .034 (10) A	0.545 ± .047 (10) AB	0.007 ± .004 (9) AB
6	0.967 ± .032 (10)	0.528 ± .025 (10) A	0.014 ± .004 (10) A	0.635 ± .022 (10) A	0.698 ± .045 (10) AC	0.012 ± .004 (10) AB
12	1.091 ± .037 (10)	0.638 ± .041 (10) A	0.020 ± .005 (10) A	1.034 ± .046 (10)	0.864 ± .029 (9) ABC	0.018 ± .009 (9) AB
24	1.023 ± .055 (10)	0.453 ± .042 (10) A	0.022 ± .009 (10) A	1.128 ± .046 (10)	0.845 ± .055 (9) ABC	0.047 ± .017 (6) AB

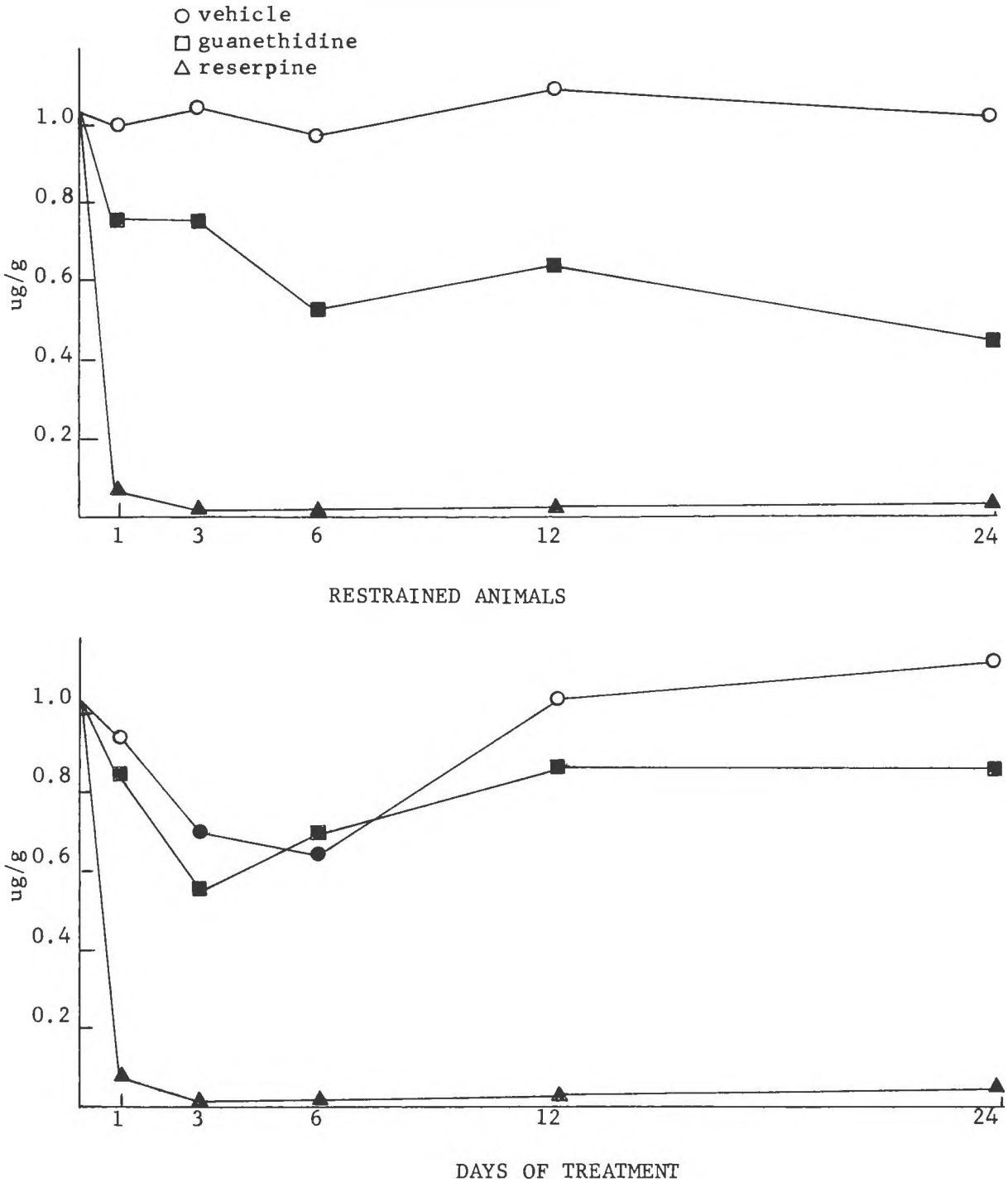
a: mean ± S.E. (ug/g)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from guanethidine-control group.
C - significantly different from reserpine-control group.

Drug doses, (i.p.) daily. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Note: Ten animals selected at random, no treatment, had heart norepinephrine levels of 1.032 ± .049 ug/g.

Figure 19

THE EFFECT OF RESTRAINT ON HEART NOREPINEPHRINE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE



Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.
Solid symbols designate a significant difference ($P < 0.05$).
See Table XIII for statistical comparison.

TABLE XIV

THE EFFECTS OF RESTRAINT ON THE FOOD INTAKE[a], WATER INTAKE, MEAN WEIGHT GAIN AND MORTALITY RATE OF RATS TREATED WITH GUANETHIDINE OR RESERPINE

Groups	Mean Water Intake (ml/rat/day)	Mean Weight Gain/Day (g)	Mortality (percent)
Vehicle Control	36 ± 0.71	2.0 ± 0.2	10[b]
Guanethidine Control	33 ± 0.35	1.8 ± 0.1	0
Reserpine Control	30 ± 0.76	0.7 ± 0.3	0
Vehicle Restraint	36 ± 0.49	0.2 ± 0.2	0
Guanethidine Restraint	38 ± 0.57	0.0 ± 0.2	10
Reserpine Restraint	37 ± 1.06	0.1 ± 0.3	40

All values are expressed as the mean ± S.E. for the 24 day study.

a: food intake; 20 g/day.
b: accidental death.
N = 10 animals/group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XV

THE EFFECT OF RESTRAINT ON ADRENAL WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	100 ± 9[a] (10)[b]	100 ± 5 (10)	100 ± 5 (10)	95 ± 10 (9)	100 ± 9 (10)	95 ± 5 (10)
3	100 ± 9 (10)	104 ± 4 (10)	109 ± 8 (10)	109 ± 8 (10)	104 ± 8 (10)	130 ± 7 (9) ABD[c]
6	100 ± 6 (10)	88 ± 13 (10)	94 ± 13 (10)	118 ± 10 (10)	141 ± 4 (10) AC	129 ± 5 (10) AD
12	100 ± 4 (10)	100 ± 4 (10)	104 ± 8 (10)	120 ± 7 (10)	132 ± 9 (9) AC	108 ± 7 (9)
24	100 ± 7 (10)	86 ± 8 (10)	86 ± 8 (10)	111 ± 6 (10)	111 ± 10 (9)	125 ± 11 (6) D

a: values are expressed as the percent of vehicle-control group. (mean ± S.E.)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XVI

THE EFFECT OF RESTRAINT ON HEART WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	100 ± 3[a] (10)[b]	105 ± 2 (10)	100 ± 3 (10)	102 ± 2 (9)	95 ± 3 (10) BC	98 ± 3 (10)
3	100 ± 3 (10)	97 ± 3 (10)	94 ± 3 (10)	110 ± 3 (10) A	103 ± 3 (10)	101 ± 3 (9) B
6	100 ± 3 (10)	103 ± 3 (10)	103 ± 1 (10)	99 ± 3 (10)	99 ± 4 (10)	97 ± 1 (10)
12	100 ± 4 (10)	92 ± 3 (10)	97 ± 3 (10)	88 ± 3 (10) A	91 ± 3 (9)	78 ± 3 (9) ABD
24	100 ± 4 (10)	90 ± 3 (10) A[c]	95 ± 4 (10)	83 ± 3 (10) A	84 ± 3 (9) A	86 ± 4 (6) A

a: values are expressed as the percent of vehicle-control group (mean ± S.E.).
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XVII

THE EFFECT OF RESTRAINT ON BRAIN WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	100 ± 1[a] (10)[b]	104 ± 1 (10)	103 ± 1 (10)	99 ± 1 (9)	99 ± 1 (10)	102 ± 1 (10)
3	100 ± 2 (10)	98 ± 1 (10)	97 ± 2 (10)	96 ± 2 (10)	96 ± 2 (10)	95 ± 2 (9)
6	100 ± 2 (10)	103 ± 2 (10)	103 ± 1 (10)	98 ± 2 (10)	97 ± 1 (10) C	100 ± 2 (10)
12	100 ± 3 (10)	105 ± 2 (10)	108 ± 1 (10) A[c]	99 ± 3 (10)	96 ± 1 (9) C	97 ± 1 (9) D
24	100 ± 2 (10)	99 ± 2 (10)	96 ± 2 (10)	95 ± 2 (10)	98 ± 2 (9)	94 ± 4 (6)

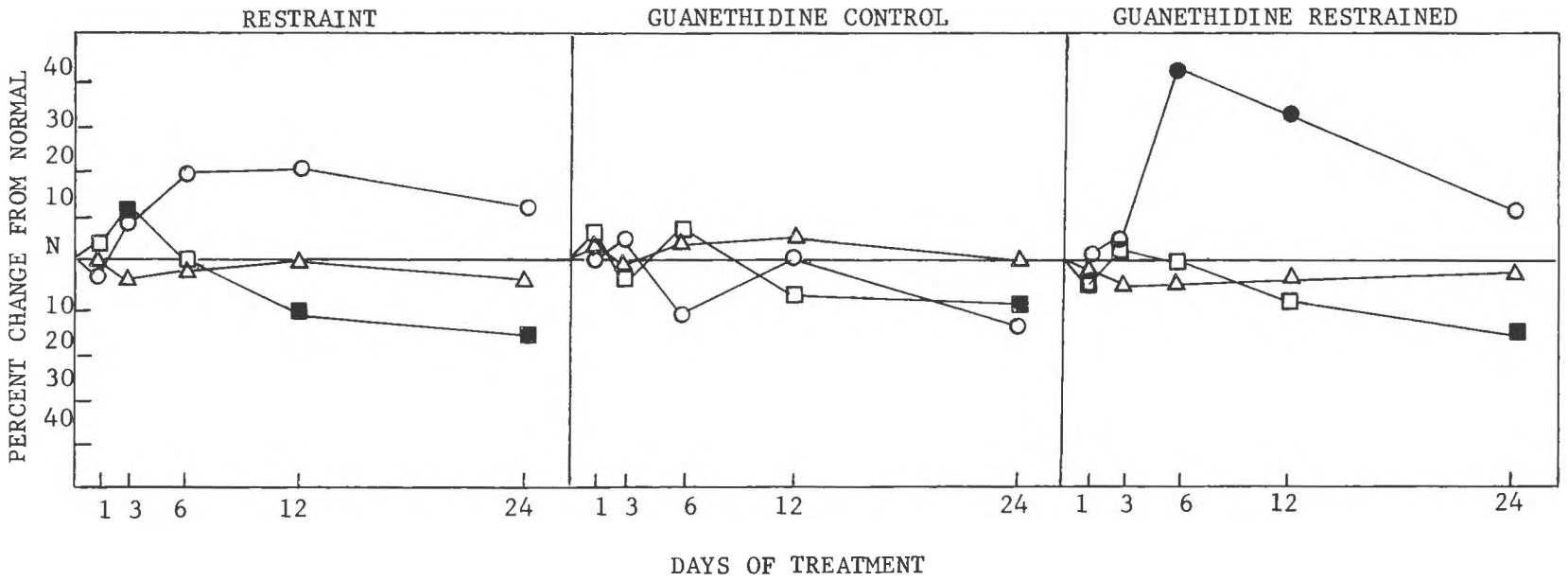
a: values are expressed as percent of vehicle-control group (mean ± S.E.)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from guanethidine-control group.
C - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

Figure 20

A COMPARISON OF THE EFFECTS OF RESTRAINT ON VARIOUS ORGAN WEIGHTS OF NORMAL AND GUANETHIDINIZED MALE ALBINO RATS

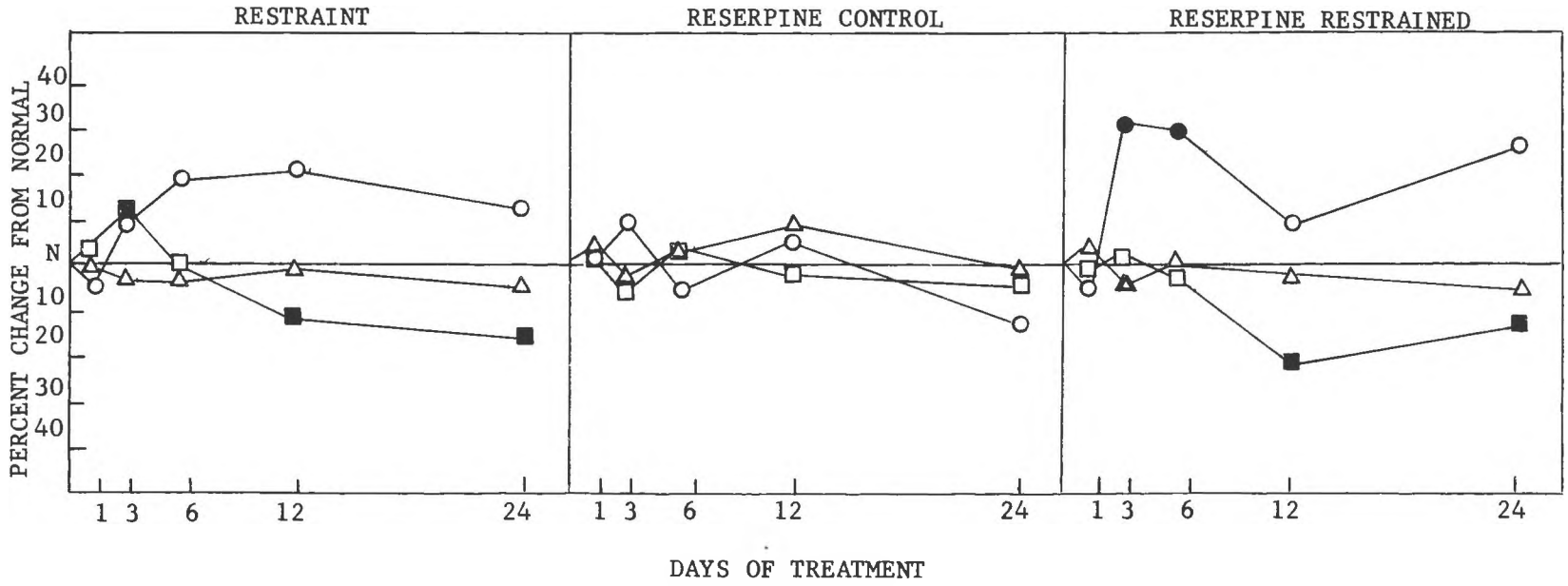


○ adrenal weight
△ brain weight
□ heart weight

Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group.
Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Vehicle, glass-distilled water, 1 ml/kg.

Figure 21

A COMPARISON OF THE EFFECTS OF RESTRAINT ON VARIOUS ORGAN WEIGHTS OF NORMAL AND RESERPINIZED MALE ALBINO RATS



○ adrenal weight
△ brain weight
□ heart weight

Solid symbols designate significant difference ($P < 0.05$) from vehicle-treated group.
Drug doses, (i.p.) daily: Reserpine, 0.2 mg/kg. Vehicle, glass-distilled water, 1 ml/kg.

TABLE XVIII

THE EFFECT OF RESTRAINT ON ADRENAL ASCORBIC ACID OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	100 ± 5[a] (10)[b]	86 ± 5 (10)	88 ± 6 (10)	65 ± 11 (9) A[c]	63 ± 6 (10) AC	64 ± 8 (10) AD
3	100 ± 6 (10)	84 ± 10 (10)	85 ± 9 (10)	94 ± 6 (10)	89 ± 6 (10)	84 ± 8 (9)
6	100 ± 8 (10)	99 ± 10 (10)	87 ± 8 (10)	86 ± 7 (10)	74 ± 10 (10) A	92 ± 7 (10)
12	100 ± 7 (10)	89 ± 5 (10)	101 ± 7 (10)	89 ± 7 (10)	99 ± 7 (9)	94 ± 5 (9)
24	100 ± 5	102 ± 7	111 ± 8	104 ± 9	106 ± 6	106 ± 10

a: values are expressed as the percent of vehicle-control group. (Mean ± S.E.)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XIX

THE EFFECT OF RESTRAINT ON SERUM CORTICOSTERONE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	100 ± 14[a] (10)[b]	105 ± 11 (10)	86 ± 15 (10)	136 ± 6 (9) A[c]	150 ± 6 (10) AC	141 ± 4 (10) AD
3	100 ± 12 (10)	100 ± 12 (10)	84 ± 8 (10)	126 ± 9 (10)	132 ± 8 (10)	132 ± 8 (9) D
6	100 ± 18 (10)	132 ± 6 (10)	153 ± 11 (10) A	129 ± 9 (10)	137 ± 8 (10)	132 ± 6 (10)
12	100 ± 10 (10)	95 ± 14 (10)	71 ± 10 (10) A	95 ± 5 (10)	110 ± 7 (9)	133 ± 9 (9) ABD
24	100 ± 10 (10)	109 ± 11 (10)	109 ± 9 (10)	91 ± 15 (10)	117 ± 9 (9)	144 ± 11 (6) B

a: values are expressed as the percent of vehicle-control group (mean ± S.E.).
b: number of rats.
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XX

THE EFFECT OF RESTRAINT ON HEART NOREPINEPHRINE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	100 ± 3[a] (10)[b]	76 ± 5 (10) A[c]	7 ± 18 (10) A	94 ± 4 (9)	84 ± 5 (10) A	7 ± 28 (10) AB
3	100 ± 5 (10)	73 ± 12 (10) A	1 ± 45 (10) A	68 ± 5 (10) A	53 ± 9 (10) AB	0.5 ± 57 (9) AB
6	100 ± 3 (10)	55 ± 5 (10) A	1 ± 29 (10) A	66 ± 3 (10) A	72 ± 6 (10) AC	1 ± 33 (10) AB
12	100 ± 3 (10)	6 ± 6 (10) A	2 ± 25 (10) A	95 ± 4 (10)	79 ± 3 (9) ABC	2 ± 50 (9) AB
26	100 ± 5 (10)	44 ± 9 (10) A	2 ± 41 (10) A	110 ± 6 (10)	83 ± 7 (9) ABC	5 ± 36 (6) AB

a: values are expressed as the percent of vehicle-control group. (mean ± S.E.)
b: number of rats.
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.

Drug doses, (i.p.) daily. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XXI

THE EFFECT OF RESTRAINT ON BRAIN NOREPINEPHRINE OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	100 ± 3[a] (10)[b]	89 ± 4 (10) A[c]	98 ± 9 (10)	89 ± 4 (9) A	95 ± 4 (10)	73 ± 4 (10) ABD
3	100 ± 6 (10)	97 ± 3 (10)	74 ± 10 (10) A	92 ± 3 (10)	102 ± 2 (10) B	66 ± 11 (9) AB
6	100 ± 4 (10)	97 ± 3 (10)	50 ± 11 (10) A	100 ± 4 (10)	113 ± 5 (10) C	29 ± 13 (10) ABD
12	100 ± 4 (10)	98 ± 7 (10)	27 ± 12 (10) A	109 ± 6 (10)	133 ± 4 (9) ABC	27 ± 10 (9) AB
24	100 ± 5 (10)	92 ± 8 (10)	10 ± 24 (10) A	106 ± 4 (10)	98 ± 6 (9) C	11 ± 18 (6) AB

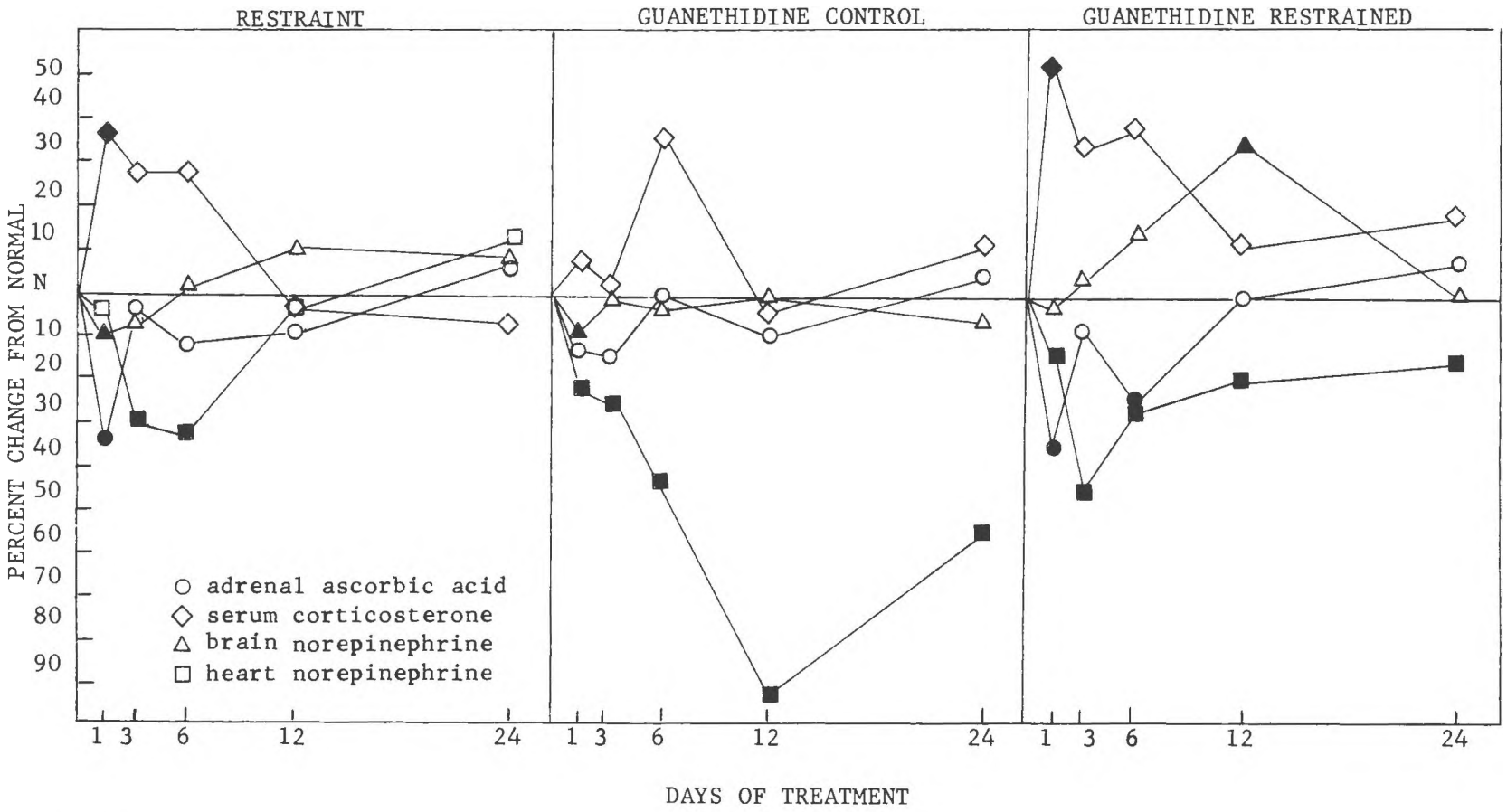
a: values are expressed as the percent of vehicle-control group (mean ± S.E.)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

Figure 22

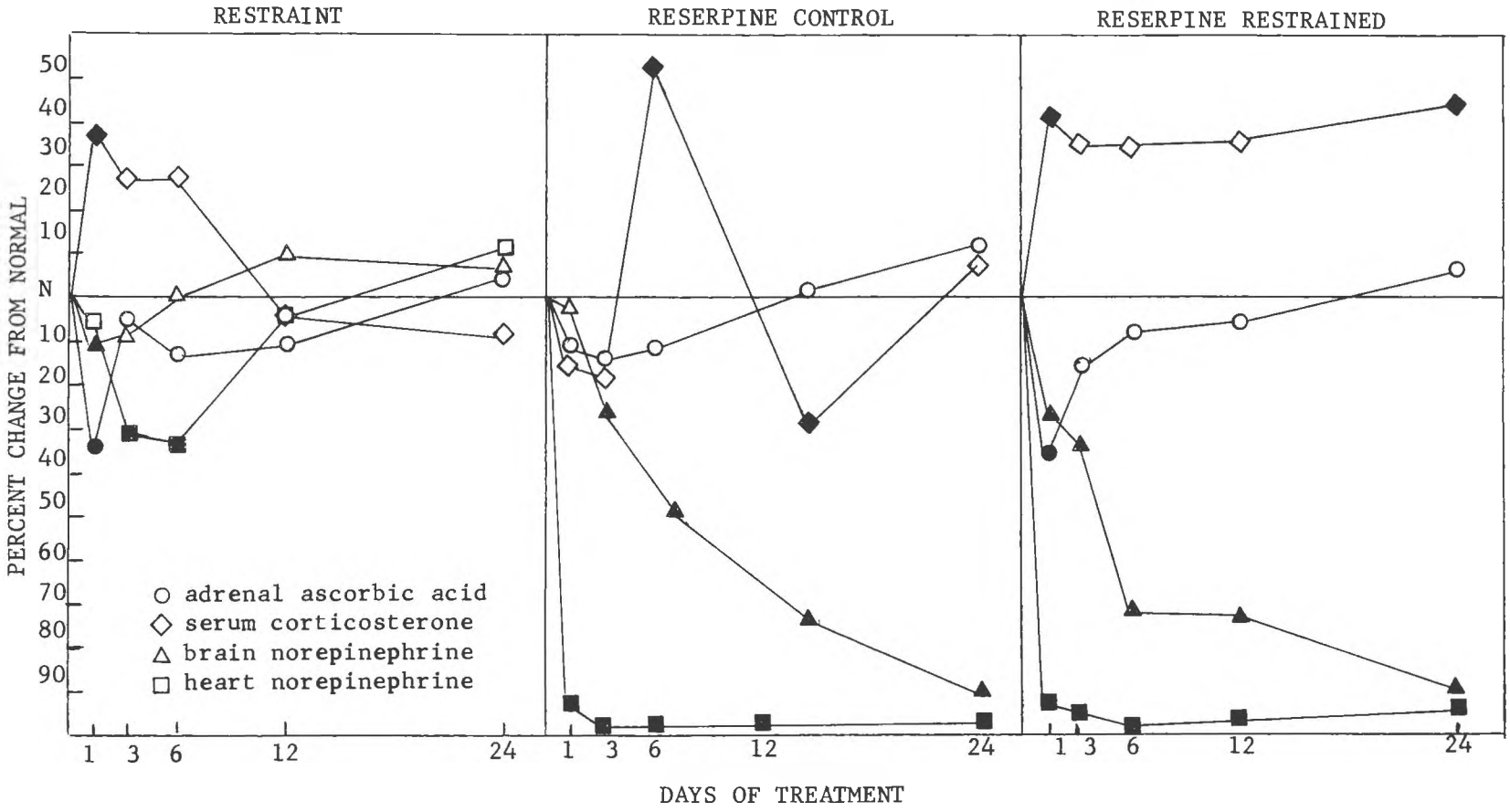
PITUITARY-ADRENAL RESPONSES AND CHANGES IN BRAIN AND HEART NOREPINEPHRINE AFTER DAILY ADMINISTRATION OF SMALL DOSES (1.20 mg/kg, i.p.) OF GUANETHIDINE. A COMPARISON OF THE EFFECTS OF RESTRAINT ON ADRENAL ASCORBIC ACID, SERUM CORTICOSTERONE, BRAIN AND HEART NOREPINEPHRINE LEVELS



Solid symbols represent values differing significantly from normal ($P < 0.05$). Each point represents the mean of 6 - 10 animals except normal values which represent the mean of 60 animals.

Figure 23

PITUITARY-ADRENAL RESPONSES AND CHANGES IN BRAIN AND HEART NOREPINEPHRINE AFTER DAILY ADMINISTRATION OF SMALL DOSES 0.2 mg/kg, i.p.) OF RESERPINE. A COMPARISON OF THE EFFECTS OF RESTRAINT ON ADRENAL ASCORBIC ACID, SERUM CORTICOSTERONE, BRAIN AND HEART NOREPINEPHRINE LEVELS



Solid symbols represent values differing significantly from normal ($P < 0.05$). Each point represents the mean of 6 - 10 animals except normal values which represent the mean of 60 animals.

TABLE XXII

ANALYSIS OF VARIANCE ("F" VALUES)

Source of variation	Adrenal Ascorbic Acid	Serum Corticosterone	Brain Norepinephrine	Heart Norepinephrine	Weight Gains
Days	7.81a	1.44	261.70a	686.90a	13.65a
	(4.26)[d]	(4.26)	(4.26)	(4.26)	(4.26)
Drug	0.00	0.60	14.17a	65.30a	0.91
	(2.26)	(2.26)	(2.26)	(2.26)	(2.26)
Restraint	10.89a	33.23a	0.00	0.00	316.54a
	(1.26)	(1.26)	(1.26)	(1.26)	(1.26)
Day vs Drug	10.31a	4.44a	9.92a	37.79a	14.17a
	(8.26)	(8.26)	(8.26)	(8.26)	(8.26)
Day vs Restraint	1.26	1.25	7.80a	20.57a	5.61a
	(4.26)	(4.26)	(4.26)	(4.26)	(4.26)
Drug vs Restraint	0.68	2.90c	2.84c	0.21	4.41b
	(2.26)	(2.26)	(2.26)	(2.26)	(2.26)
Interaction	2.74a	2.98a	2.84a	11.69a	19.50a
(Day vs Drug vs Restraint)	(8.26)	(8.26)	(8.26)	(8.26)	(8.26)

a - significant at $P<0.01$.
b - significant at $P<0.05$.
c - significant at $P<0.10$.
d - degrees freedom

Note: took into account unequal number of animals/group due to deaths.

Drug doses, (i.p.) daily. Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

V. DISCUSSION

A. Circadian Rhythms and Lighting Schedule

Wurtman (1967) stated that there are two major cycles governing the amount of time for which a particular geographic locus is exposed to sunlight: an on-off, 24-hour cycle of day and night, whose components are usually of unequal length but average twelve hours per day in the course of a year; and a continuous, annual cycle of change in the length of the daily light period. These cycles are accompanied by parallel cycles in the intensity of the sunlight.

Isomorphic with, and frequently dependent upon, these two physical cycles are two major endocrine phenomena: diurnal and annual rhythms. Many endocrine functions in animals demonstrate a 24-hour periodicity. In all cases where these diurnal endocrine rhythms have been studied, they have been found to be naturally synchronized with the ambient lighting schedule. The adrenal-dependent rhythms tend to respond to experimental lighting regimens, where they rapidly resynchronize to new lighting schedules (Wurtman, 1967).

The studies of Hemmingsen and Krarup (1937) demonstrated that rats were able to adapt to a complete reversal of the light-dark cycle in eight to ten days. Pittendrigh (1960) reported that in some animals a repeated light-dark phase shift of a few hours may synchronize the physiological rhythms after several days. Pauly and Scheving (1967) showed that circadian rhythms are synchronized by the light-dark cycle to which the animals are subjected.

To minimize the annual variations also known to occur in an animal (Leduc, 1961), the chronic restraint phase of the study was completed

in approximately two months, from October 16th to December 19th.

B. Stressing Procedure

The safety-pin method, described in the "methods and material" section, was used to secure the animals during the stressing period, as an alternate to the more usual method (Renaud, 1959) of taking the rats' four paws directly to the metal posts of the restraining board. This safety-pin modification was employed for two reasons. First, direct taping of the rats' four paws to the metal posts each day required excessive handling of the animals; this extra handling affects behavior (Weltman et al., 1961) and also introduced delays into the stress procedure. Second, the daily removal of the tape from the chronically restrained animals caused inflammation, local edema, and general irritation to the paws. All of these reactions were eliminated by leaving the tape intact on the paws throughout the 24 days. Preliminary studies showed that most of the animals adapted to the tape and pins in two to three days as was evidenced by the fact that they no longer tried to remove them.

C. Weight Gain Studies

This phase of the investigation was initiated to provide information on the effects of restraint on the individual body weight of rats treated with guanethidine or reserpine. All animals used in this study were routinely weighed to the nearest gram, daily, using an Ohaus[1] small-animal balance. Individual records were kept of the daily weights. The rats were housed, fed and watered as previously described.

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1. Ohaus Seale Corp., Union, New Jersey.

Steinberg and Watson (1959, 1960) showed that a variety of stressful procedures brought about a decrease in growth rate and a reduction in the animals' intake of food and water. Scott (1955) and Imms (1965) ruled out a connection between the growth rate decrease in rats caused by stress, and the reduction of either food or water intake. Imms (1965), however, did report some changes in water excretion which varied with the types of stress. He concluded that the data could be explained by an increase in the rate of oxidative metabolism, since food consumption was unchanged during stress while the rate of growth decreased. Similar results were noted in this study.

Jackson (1962) reported that comparing gross weights of the treated animals to the gross weights of the untreated, control animals is valid only when starting weights of both groups do not differ. Weight can be quantified either in terms of animal weight on the first day of drug treatment compared percentage-wise with animal weight at the end of the study, (first day weight/final day weight X 100), or by subtracting beginning weight from final weight at the end of the study. Two methods of analysis of the body weight data were employed in this study:

a) Daily weights. For each day of the study, the individual daily weights of all animals in each group were compared to the individual daily weights of the control groups.

b) Cumulative daily weight gains. These were determined by subtracting the weight of each animal on day one from its weight on each of the remaining days of the study.

Daily comparisons were made between the control groups and each treatment group. Mendillo (1965) and Guarino (1966) observed that the second method is the most sensitive in a study such as this.

The total weights of the control (vehicle-treated) group were compared to the total weights of each experimental group on each day of the study (Table XXIII, appendix). There were no significant differences on days 1, 2 or 3; however, a significant difference between the control group and the stressed (vehicle-treated) group occured on day 4 and continued until the end of the experiment. This same observation was noted with the reserpine-treated, stressed animals. However, the guanethidine-treated, stressed animals showed no significant change in total weight from the total weight of the control animals until day 5. The drug-treated, non-stressed animals showed no difference from the control animals on any day of the study.

When the weights of drug-treated, stressed animals were compared to those of the stressed, vehicle-treated animals, there was no significant difference. However, when the weights of the control, guanethidine-treated animals were compared to those of the stressed, guanethidine-treated animals, a significant decrease occured on day 5 and continued throughout the remainder of the study. Further, when the weights of the control reserpine-treated group were compared to those of the reserpine-treated stressed group, a significant decrease occured on day 1 and continued throughout the study.

The cumulative weight gains from day 2 were calculated for each animal. The mean $\pm$ SE of these values was determined. The cumulative weight gains of the control group were compared to the gains of each of the experimental groups for each day of the study. This method of presenting the data is very sensitive both to small changes in food consumption and to altered metabolic rates (Mendillo, 1965).

Data presented in this manner are found in Table II and Figure 1, which demonstrate that the reserpine-treated, control animals showed a significant decrease in weight gains from day 2 to the end of the study, except for days 3 and 4. These values are negligible; however, in view of the overall pattern. The weight loss in these animals was completely obscured when total weights were considered. Guanethidine-treated, control animals had weight gains that were as stable as those of the vehicle-treated control animals.

All stressed animals showed a decrease in weight gains. Beginning on day 2 and continuing throughout the study, weight changes in the vehicle-treated, stressed animals were significantly lower than values for the control group. These animals exhibited negative weight changes; that is, they lost weight. At the end of the treatment these rats were -5 ± 15 grams lighter than their individual starting weights, while the control rats were 49 ± 13 grams heavier than their individual starting weight.

Reserpine-stressed rats showed significant weight loss on day 3, whereas the guanethidine-stressed rats showed a significant weight loss on day 4. These significant changes continued throughout the study.

When the cumulative daily weight gains of the stressed drug-treated animals were compared to the stressed, vehicle-treated animals, a different daily pattern was seen; however, there was no significant difference in the majority of the daily comparisons.

When comparing the cumulative daily weight gains of the control, guanethidine-treated animals to the stressed, guanethidine-treated animals, there was a significant difference from day 4 on to the end of the study. A similar comparison between the two reserpinized groups revealed

a significant difference on day 3 and on to the end of the study.

D. Food and Water Intake Studies

This phase of the study involved the observation of daily food and water intake of normal and of stressed rats treated with guanethidine or reserpine. A similar study done in this laboratory (Rosecrans, 1963) showed a 50% mortality in reserpine-treated, restrained rats. He stated that reserpinized animals subjected to restraint appeared to die from starvation which seemed to stem from their inability to adapt to the stress, since normal reserpinized animals had a mortality rate one-fifth that of the stressed animals. The decreased water and food balance caused by reserpine would attest to this nonadaptation.

Animals were housed, fed and watered as previously described. During the 5-hour period that test animals were being restrained, the control animals were kept without food and water. At the end of the study the rats were sacrificed as previously described.

Analysis of the food intake data during the 24-day study showed that the rats ate all the food offered to them. Analysis of the water intake data during this 24-day period showed that the mean total ml intake per control animal per day was 36 ± 0.71. In comparison to the vehicle-treated, control animals, the control, guanethidine-treated animals drank 8% less water per day and the control, reserpine-treated animals drank 17% less water per day.

The water intake data for the stressed animals showed that there were no significant differences in the mean total ml intake per animal per day. The vehicle-treated, stressed animals drank 36 ± 0.49 ml/day; the stressed, guanethidine-treated animals drank 38 ± 0.57 ml/day; and

the stressed, reserpine-treated animals drank 37 ± 1.06 ml/day. However, when data for the control, guanethidine-treated animals were compared to those for the stressed, guanethidine-treated animals, and when the data for the control, reserpine-treated animals were compared to those for the stressed, reserpine-treated animals, there appeared to be a significant difference. A summary of the mean daily intake per rat per day for water and for food is found in Table XIV. The daily variations in water intake are shown in Table III and Figure 2. The restrained, drug-treated animals drank more water per day than did the control, drug-treated animals.

E. Blood Pressure Studies

Since both guanethidine and reserpine have the ability to lower blood pressure in rats, it was thought that a reduction in blood pressure might affect the ability of the rats to adapt to the chronic (24-day) restraint procedure. Therefore, the object of this study was to determine whether the dose of guanethidine or of reserpine used in the chronic restraint studies had any effect on the blood pressure of the test animals. Guanethidine (1.20 mg/kg) and reserpine (0.2 mg/kg) were administered i.p., daily for 24 days. Drug vehicle, glass-distilled water (1 ml/kg), was administered daily to the control animals. There were five animals per group. Housing, handling and daily protocol were all as previously described. Blood pressures were recorded daily for the first 12 days and then every other day until day 24. The pressure was measured by means of the tail cuff (indirect) method. Blood pressure was not recorded in the rats that were restrained for the 5-hour period, because the blood-pressure method requires incubation of the

rat at about 40^{o} for 15 to 20 minutes. This incubation would add to the daily protocol, another stressor procedure that might obscure the results (Mendillo, 1965).

The results from this series of experiments showed that the systolic blood pressure of the guanethidine-treated rats did not significantly differ from that of the control rats during the 24 days of treatment. The mean systolic pressure in the guanethidine-treated animals ranged from 112 $\pm$ 1.7 to 125 $\pm$ 2.6 mm Hg and the mean systolic pressures in the control animals ranged from 111 $\pm$ 4.4 to 126 $\pm$ 3.1 mm Hg. However, in rats receiving reserpine, the systolic blood pressure was decreased by some 17%, a significant difference from both the control rats and the guanethidine-treated rats. The systolic pressure range was 88 $\pm$ 4.0 to 108 $\pm$ 1.3 mm Hg in the reserpine-treated rats. The results are summarized in Table IV and Figure 3.

F. General Observation Studies

Throughout the chronic phase of the study, all the control animals appeared to be sedated. The onset of maximal sedation was not observed in these animals because of the daily protocol; however, the onset of sedation appeared about one to two hours following the administration of reserpine and about one hour following guanethidine administration. Recovery from sedation was evident after 24 hours in all animals.

The sedation observed in the vehicle-treated animals was not as pronounced as in the drug-treated animals. Reserpine-treated animals were less responsive to noise and mild probing than were guanethidine-treated and vehicle-treated animals. The vehicle-treated animals were most responsive. The degree of sedation with reserpine seemed to be progressive: less response to noise was observed by the twelfth day,

and it decreased even more by day 17.

All drug-treated animals exhibited some degree of ptosis. Mild ptosis was observed daily in the guanethidine-treated animals, but it was more pronounced from the fifth day to the completion of the study. The daily onset of ptosis in the guanethidine-treated animals was earlier than in the reserpine-treated animals; however, ptosis was more pronounced in the reserpine-treated animals.

All control animals were defensive; also, the reserpine- or the guanethidine-treated animals were more irritable than the vehicle-treated animals. Vehicle-treated animals subjected to daily restraint demonstrated an extreme degree of excitability during the first six days: a behavioral effect that progressively decreased with the duration of the experiment, indicating some degree of adaptation. Rosecrans and DeFeo (1965) reported similar findings.

The excitability of the vehicle-treated, stressed animals was greatest at the onset of the restraining procedure: they struggled excessively when being tied down, concentrating their efforts on release from the restraining boards. When released, they were exhausted and easy to handle. The guanethidine-treated, stressed animals were moderately active when being tied down and during restraint. On release from the restraining boards these animals, although they were more active than their vehicle-treated counterparts, could nevertheless be handled without difficulty. The reserpinized-stressed animals were less excitable at the onset of the experiment; however, they became more excitable and difficult to handle as the experiment progressed. More specifically, throughout the experiment, the reserpine-treated animals were very easily tied down and lay still on the boards; but,

as the experiment progressed, they became increasingly vicious upon release from restraint. These animals appeared to exhibit a type of "sham rage", attacking, biting and clawing anything that came within their reach. They frequently fought amongst themselves after being placed in their cages. These symptoms would indicate a type of non-adaptation.

Generally, by day 5 all the stressed animals were noisy, but not aggressive. The next day all the animals struggled harder on the boards and were more difficult to handle when released; particularly, the reserpine-treated animals were very aggressive when released. Day 7, all the animals became more restless when tied down. By day 10 the animals were hard to handle when released from stress; in addition they took on the appearance of exhaustion, a condition that increased slowly to day 12. On that day the reserpine-treated animals showed decreased activity when restrained. Day 13, the vehicle-treated, stressed animals were gasping during the restraint procedure and looked lethargic. On day 18 the reserpine-treated animals appeared semiconscious and by day 24 there was 40% mortality in the reserpine-treated, stressed animals.

All the stressed animals showed increased activity, compared to the control animals. All stressed animals defecated and urinated when being tied down and also when they were tied down. Diarrhea was noted in the stressed animals but not in the control animals. Priapism was noted in all stressed animals; however, it did not occur daily in every animal. There was 10% mortality in the guanethidine-treated stressed animals.

When the animals were sacrificed, the hyperreflexia associated with decapitation was absent only in the reserpine-treated animals.

Chromodacryorrhea was noted in all stressed animals at some time during the restraining procedure. This varied from 10 to 40% in vehicle-

treated animals, 30 to 50% in the guanethidine-treated animals and 30 to 80% in reserpine-treated animals. This response was occasionally observed in some of the drug-treated control animals.

In summary, the vehicle-treated, stressed animals were excessively active and struggled when being tied down but were easy to handle when released. The guanethidine-treated stressed animals were moderately active and somewhat harder to handle when released from the stress. The reserpine-treated stressed animals became progressively excited when released from restraint, although the tie-down was initiated with greater ease then with the control animals.

G. Effects of Restraint on the Pituitary-Adrenal Axis and Various Organ Weights of Normal, Guanethidine or Reserpine Treated Rats

1. Organ Weight Studies

Organ weight relationships, expressed as absolute weights, are shown in Tables V, VI, VII and in Figures 4, 5, 6; the relationships, expressed as the relative weight (g/100g of the body weight), are shown in Tables XXIV, XXV, XXVI in the appendix. These values are given in both forms to demonstrate the relationship of the organ weights, both when compared to each other and when compared to body weight.

Although the most direct index of ACTH hypersecretion would be the elevation of the plasma level of the hormone, a routine method for its estimation is not available. Therefore, indirect indices that reflect the action of ACTH on the adrenal cortex were sought. However, no single test adequately defines the time-course of ACTH release (Maickel et al., 1961). The classical biochemical response evoked by cold stress is a decrease in AAA, a rise in plasma corticosterone, an increase in liver TP activity, an increase in the weight of adrenal glands, and an

excessive metabolization of FFA from body fat depots, (Westermann et al., 1962). These various responses are also indices of pituitary-adrenal stimulation in the rat (Maickel et al., 1961). In the present report, three of the preceding responses were used to indicate pituitary-adrenal activation: increase in adrenal weight; decrease in AAA; and increase in plasma corticosterone.

The adrenal hypertrophy is a measure of the cumulative effect of ACTH, since maximal increases are attained only after several hours of pituitary stimulation. This index is particularly useful in describing a prolonged pituitary stimulation, because adrenal hypertrophy can persist long after corticosterone have returned to almost normal (Maickel et al., 1961). The AAA test indicates the approximate intensity of an acute discharge of ACTH; but it is of little value in determining the time-course, since ascorbic acid does not return to normal for some hours after the pituitary stimulus has stopped (Long, 1947). The plasma corticosterone in the rat is an accurate barometer of the intensity of the ACTH discharge, and it is also a good indicator of its duration, since the adrenal glucocorticoid secretion returns to normal shortly after the stimulus is discontinued (Schonbaum et al., 1959).

The application of these tests in this study indicate that restraint, guanethidine and reserpine elicit similar signs of pituitary-adrenal activation: adrenal hypertrophy; decreased AAA; and elevated plasma corticosterone. Maickel et al.(1961) reported that similar responses in hypophysectomized rats, exposed to cold (4^{o}) and given reserpine, do not occur and inferred that they are caused by hypersecretion of ACTH.

According to Selye's theories regarding chronic stress, animals respond differently to individual stressors only in a guantitative way,

the responses all being qualitatively the same. Therefore, one of the objectives of this experiment was to characterize the effect of CRS in normal animals in terms of GAS. Since the brain, heart, and adrenals had to be weighed for the analysis of NE and AAA, respectively, it became of interest to see if the stress procedure or drug treatment had any effect on the weight of these organs. Selye (1950) stated that, in the case of GAS, the AR might be subdivided into two, more or less distinct phases: 1. the phase of shock, which might vary from a few minutes to about 24 hours, depending upon the intensity of the damage inflicted; and 2. the phase of counter-shock. Here, Selye observed an increase in the adrenal weight, indicating adrenal hyperfunction. In general, he maintained that the reversal of most of the changes seen during the shock phase occurred in the phase of counter-shock.

Gray and Munson (1951) reported that the responses of the hypothalamic-pituitary-adrenocortical system to stress are extremely rapid. Virnikos-Danelles, (1964) and Stockham, (1964), demonstrated an increase in plasma and adrenal corticosteroid in the rat as early as 100 seconds after application of a stimulus. Jones and Stockham (1966) showed that a second stress maintained the high levels of adrenocortical synthesis induced by the first stress for a longer period of time, rather than immediately causing an increase in the plasma levels.

Because of experimental design and since the pituitary-adrenal axis response to stress is rapid, it was not within the scope of these studies to characterize the AR completely, or to characterize it in terms of different shock-phases. Nevertheless, appearance of the shock-phase might be suggested to some degree by the slight decrease in the absolute adrenal weight in stressed animals treated with vehicle

or with reserpine on day 1 as compared to the non-stressed animals (Table V and Figure 4). Guanethidine-treated, stressed rats showed a slight increase. Also, appearance of the counter-shock phase might be suggested by the enlargement observed in the adrenals in all groups of animals on day 3. The maximal increase in relative adrenal weight was noted on day 24 for stressed rats treated with vehicle or with reserpine and on day 12 for the guanethidine-treated, stressed rats. Control rats treated with the vehicle or with the drugs showed similar responses.

In general, there was an increase in the absolute adrenal weight in all the groups studied, but the stressed animals showed a greater variation in the adrenal weight than did the control animals. Vehicle-treated control animals showed an increase of 0.006 mg from day 1 to day 24, whereas the vehicle-treated, stressed animals showed an increase of 0.010 mg over the same period of time. Similar results were obtained from the drug-treated animals. The reserpine-treated stressed animals showed the largest variation in weight, 0.014 mg. Significant differences, however, appeared only on days 6 and 12 with the guanethidine-treated animals and on days 3 and 6 with the reserpine-treated group. (See Table V for a statistical comparison.) The data in Table V shows an increase in the absolute adrenal weight, in the vehicle-treated control animals suggesting that the injection of water also induced a form of stress; however, the data in Table XXIV (appendix) shows that there was no change in the relative adrenal weight in these animals over the 24-day study, except for day 6. These data also show that, in the vehicle-treated, stressed animals, there was an increase in the relative adrenal weight, reaching a maximum on day 12. In fact, all of the stressed animals showed the maximum increase on day 12. Table V,

representing the absolute adrenal weight, failed to demonstrate this point. The control animals did not show a significant change when the adrenal weight was represented in this manner. Comparison of the relative adrenal weight, Table XXIV, makes clear that the control animals treated with vehicle or with drug showed no significant increase in the adrenal weight; however, there was an increase in adrenal weight in the stressed animals.

With the possible exception of biological variation, the low figures observed on day 6 are impossible of a valid explanation. In nearly all parameters tested, day 6 gave an unusual response.

It is difficult to analyze glandular activity from a weight change only, but on the basis of the severity of restraint, the increase in weight of the adrenals could be due to ACTH stimulation and associated chemical depletion during extreme activation.

Selye (1950) reported that congestion, edema, and hemorrhage in the brain are often particularly conspicuous in men who die from heatstroke. Acute swelling, shrinkage, or pyknosis of cells have also been reported, but some investigators regard these as postmortem artifacts. All these changes are so similar to those produced by other types of intense acute stress that they may well be nonspecific manifestations of the GAS. Also, experimental evidence has shown that stress and overdosage with various hormones which are produced during stress can cause characteristic morphologic changes in the heart. Mineralocorticoids can elicit a type of myocarditis very similar to that seen in acute rheumatic fever. Irritation of animals with an electric current is also effective in causing cardiac lesions. Shapiro and Melkado, in 1957, reported an increase in heart size in stressed animals. However, these same

investigators, in 1958, reported that there was no significant change in heart weight due to stress.

In this study, analysis of heart weights varied according to the method of data treatment. The absolute heart weights of the stressed animals treated with vehicle or with drug varied from day to day, perhaps an indication of the effect of stress. The absolute heart weights in the control animals treated with vehicle or with drug (Table VI and Figure 5) showed an overall increase from day 1 to day 24, perhaps an indication of growth. However, when the relative heart weights (Table XXV; appendix) were compared, an inverse relationship for these data appeared in the control animals (vehicle and drug treated); whereas the data for the stressed animals all showed an increase. All groups reached a maximum relative heart weight on day 12, and again, there was an unusual response on day 6. Because the relative heart weights were higher for the stressed animals than for the control animals (See Tables VI and XXV for a statistical comparison), there is some indication that stress had an effect on the total heart weight.

Absolute brain weights likewise varied from day to day to the extent that no correlation could be made in all the groups studied. (Table VII and Figure 6). However, when the brain weights were expressed as the relative weights, Table XXVI (appendix), an inverse relationship was noted as with the heart weights. There was also a change in the brain weight on day 12 of all the stressed animals, except the guanethidine-treated animals, that corresponded to the changes seen with adrenal and heart weight, when expressed in this manner. (See Tables VII and XXVI for a statistical comparison.) In summary, the most significant statement that can be drawn from the

brain weight data is that expressing brain weights as percentage of body weight seems to be the most sensitive method for analysis.

The effects of daily restraint on the organ weights of normal rats make evident to a degree their ability to adapt. Following the initial AR, the tendency of the adrenal weight to return to the original values is a good example of animal adaptation to external environment, as is perhaps the stabilization of the heart and brain weights on day 12.

2. Serum Corticosterone Studies

To establish a reliable method of assay for corticosterone, the method described by Guillemin et al., 1959a was used. Two modifications of the fluorescence reagent were tried and checked for their stability and fluorescence ability. A mixture of sulfuric acid and 50% aqueous ethanol in a ratio of 2.4:1 (Zenker and Bernstein, 1958) was tested, as was a mixture of sulfuric acid and absolute ethanol in a 65:35 ratio (Peterson, 1957). However, it was found that Guillemin's original fluorescence reagent (30N H_2SO_4) gave maximal fluorescence, sufficient to be measured in a final volume of four ml in an Aminco-Bowman spectrophotofluorometer. A comparison of the control tissue corticosterone values with previously reported values (Maickel et al., 1967) using the Guillemin et al (1959a) method was also carried out.

A statistical comparison of the effects of restraint on serum corticosterone in the rat treated with guanethidine or reserpine is given in Table VIII and Figure 9. There was an increase on day 1 from day 0 in the vehicle-treated, control animals, but it was not as great as in the vehicle-treated, stressed animals. There were similar increases in all the other groups, but the stressed group values on day 1

were approximately double those on day 0. There was a return to lower levels on day 3 in the drug-treated, control animals, followed by an even greater increase on day 6. By day 12 and day 24, the cycle appeared to repeat itself.

3. Adrenal Ascorbic Acid Studies

The reliability of the method for the assay of AAA (Maickel, 1960) was determined. It was observed by Dixit (1965) and in this laboratory that, when a stock solution of ascorbic acid (10 mg/ml) in 5% TCA was prepared in double-glass-distilled water, it oxidized very rapidly, even when refrigerated. The rate of oxidation was found to be related to the concentration of TCA (Dixit, 1965). Dixit also observed that the addition of 2% MPA protected the ascorbic acid from oxidation, and that the solution was stable for at least five days in the refrigerator. However, the addition of 2% MPA increased the time required for the development of maximal color intensity at room temperature (24-25^{o}), and consequently increased the time required to complete the assay. This difficulty was overcome by completing the reaction at 27-38^{o}; under these conditions, maximal color intensity was obtained within 20 minutes.

Musulin and King (1936) showed that the oxidation of MPA serves to protect ascorbic acid in solution against oxidation in the presence of TCA. They suggested that the high acidity could protect the ascorbic acid from oxidation; and in this respect, MPA (5.0 to 8.0%) was rated highest. In the present study, it was found that the pH of 5% TCA and of 5% TCA + 2% MPA were practically the same, pH 1.0 and 0.95 respectively, an indication that high acidity, as such, is of little importance in preventing the oxidation of ascorbic acid. A comparison of the control AAA values with previously reported values (Dixit, 1965),

both using the Maickel (1960) method, was carried out.

The AAA values are presented in Table IX and Figure 11. Values for vehicle-treated, control rats were fairly stable throughout the study, except for day 6, which showed an unusual response. Control animals treated with guanethidine or reserpine showed similar responses throughout the study; however, on day 6, the response with the reserpine-treated animals was less than that with the guanethidine-treated animals. Both these groups showed higher values on day 24 than those for the vehicle-treated, control animals.

The restrained animals, vehicle and drug treated, showed initially the same response; a decrease on day 1, followed by increases on days 3 and 6. Guanethidine-treated animals showed less of a gain on day 6 than did either the vehicle- or reserpine-treated animals. The values from day 12 to day 24 were not significantly different from controls.

These tables and figures show that there was a fall in AAA and a rise in serum corticosterone on day 1 in the vehicle-treated, stressed animals: an indication of an AR and an activation of the pituitary-adrenal system. These responses were reversed by day 3: an indication of physiological adaptation. By day 6 the responses were fairly constant, a confirmation of the animals' adaptation to the external environmental changes.

Control animals, treated with guanethidine or reserpine, exhibited variations in AAA and serum corticosterone that were similar to the stress reaction seen with the vehicle-treated, stressed animals; reserpine-treated rats were less reactive to the stress than were the other animals. The response varied somewhat in each group of animals, but the guanethidine-treated, control animals appeared to adapt by day 12,

whereas the reserpine-treated, control animals never seemed to adapt. This was not unexpected; Maickel et al. (1961) had shown that reserpine produces a biochemical picture similar to that produced by cold stress. Nor was the response seen with the guanethidine-treated, control animals unexpected; however, it has not been reported in the literature. The vehicle-treated, control rats also exhibited a slight AR. Day 6 again showed the unusual response in the control animals.

In general, the drug-treated, stressed animals exhibited a greater AR as compared with the vehicle-treated, stressed animals. The responses reversed themselves by day 3 in both groups of animals; however, the reserpinized animals did not appear to adapt until about day 12, another indication that reserpine tends to prevent adaptation.

The pituitary-adrenal system was apparently active throughout the period of chronic stress even though serum corticosterone returned to normal by day 12. The fact that the adrenal weight did not return to control values, but showed a general trend of returning toward normal, may indicate an increase in adrenal efficiency or responsiveness. Similar observations were noted by Rosecrans and DeFeo (1965). This effect may be the result of a progressive response of the adrenals to ACTH as demonstrated by Stark et al. (1963). AAA, however, returned to normal by day 24.

Although an apparent decrease in pituitary and adrenal function prevailed during adaptation to stress, the serum corticosterone exhibited relative adaptation and appeared to be maintained at high levels throughout the experiment, especially in the guanethidine- and reserpine-treated animals. If the adrenal weights can be considered an indication of corticosteroid synthesis and release, then the concurrent maintenance

of both serum corticosterone and increased adrenal weight would indicate an increased adrenal efficiency during adaptation to stress. These indices are also good indications of the duration of ACTH discharge and prolonged pituitary stimulation respectively.

Reserpine appears to have produced a definite effect of its own on the pituitary-adrenal axis. At the beginning, the effects of reserpine on organ weights of normal animals were almost identical to the effects of restraint in the vehicle-treated animals. However, on day 3, the heart weights in the vehicle-treated, stressed animals showed an increase, while the reserpine-treated, non-stressed animals showed a decrease in weight. Organ weight changes, Figures 20 and 21, were divergent, in con-contrast to untreated animals under stress; however, there were some signs of adaptation in the organ weight values.

The serum corticosterone values would indicate that reserpine does not prevent animals from achieving adrenal adaptation, since these values are approximately the same as those of untreated stressed animals. Reserpine evoked adrenocortical responses similar to those seen in the restrained animals (Figure 23), but it apparently did not inhibit CRS to any degree. In fact, it seemed to act as an acute stimulator of the pituitary-adrenal axis and prevented the animal from adapting to the stress. How reserpine prevented adaptation has not been determined.

Guanethidine also produced a definite effect on the pituitary-adrenal axis. Guanethidine-treated, control animals showed responses in organ weights almost identical to those for the reserpine-treated, control animals. Similar responses were also noted in the stressed drug-treated animals with regard to organ weights. Guanethidine also appeared to prevent adaptation to stress, as indicated by its dissimilar

effects on the various organ weight changes. However, by day 24, the adrenal weights were returning toward normal, an indication of a type of adaptation.

Serum corticosterone and AAA in guanethidine-treated, nonstressed rats were identical to those of the reserpine-treated, nonstressed rats. Similar responses were also noted in the vehicle-treated, nonstressed animals. These responses lead to the suggestions, first, that the injection *per se* had some effect on the pituitary-adrenal axis, second, that a drug having a systemic response stimulates the pituitary-adrenal axis to a greater degree, and, third, that the response observed may be identical in most if not all drug studies. While these suggestions are admittedly based on mere speculation at this point, it is fact with the two drugs used in this study and may not apply to other drugs. It can also be seen in Figure 22 that the guanethidine-treated, stressed rats appeared to adapt to the stress, as indicated by the tendency of serum corticosterone and AAA to return to normal. Reserpine-treated, stressed rats showed quite a different picture, Figure 23.

H. Effect of Restraint on the Brain and Heart Norepinephrine Levels in Normal, Guanethidine or Reserpine Treated Male Rats

1. Norepinephrine Assay Studies

The method used was designed to permit a rapid evaluation of the ability of certain compounds to deplete both brain and heart of NE; it is a modification of existing procedures, principally the Shore and Olin (1958) organic extraction method. According to Anton and Sayre (1962), this method is probably the most convenient one to determine CA in tissue; however, it can be used only for tissue, and it may be further restricted to certain tissue, such as the brain and heart.

They could not completely recover added CA from rat and guinea pig liver, and occasionally recoveries from brain decreased from about 60% to about 25% for no apparent reason. This variability was also noted by other investigators (Green and Erickson 1960).

Shore and Olin (1958) reported that the partition coefficient of NE between the butanol and the aqueous-acid phase (10:1) was 0.65. In this investigation about 65% of NE added to tissue homogenates was recovered. These recoveries were not consistant; however, replicate determinations carried out on homogenates without adding NE gave values for the endogenous CA that were reproducible within 10% ($\pm$3 SEM).

Since the present investigation was concerned with the effect of drugs on tissue levels of NE, the absolute amount of NE in the tissue was of consequence, only if, under rigidly controlled analytical conditions, the replicate determinations of NE were constant. Hence, all the results reported are in terms of micrograms of endogenous NE per gram of tissue, without correction for the partition coefficient of added amine, between the organic phase and the aqueous-acid phase.

It was observed in this laboratory that the alkaline ascorbic acid solution prepared by the method of Maynert and Klingman (1962) tends to age rapidly, turning pink in less than an hour. Anton and Sayre (1962) observed that the use of 10N NaOH made the ascorbate solution more stable and less subject to discoloration. They also found that in their oxidation procedure, as the volumes of a standard CA solution increased, an almost linear response was obtained with alkaline ascorbate made with 10N, but not with 5N NaOH. A similar response was noted in the present investigation (Figure 12). However, it was observed that the mixture of ascorbic acid and the 10N NaOH was

also somewhat unstable, discoloring after a few hours. This change was usually accompanied by increased relative fluorescence values in the blank sample and sometimes by a decrease of the net fluorescence. These effects were minimized by the addition of 0.16 ml of ethylenediamine to the alkaline ascorbate solution, which stabilized the solution by delaying the development of the pink color in the alkaline ascorbate solution for 3 - 4 hours (von Euler and Lishajko, 1961). These workers also observed that the net fluorescence values for NE were markedly higher if transformation of lutines was made with alkaline ascorbate solution to which ethylenediamine had been added. Similar results were obtained in this study (Figure 13). The increased fluorescence action of this reagent is attributed to condensation of the CA with ethylenediamine (Weil-Malherbe and Bone, 1952).

Since other CA can be extracted and may therefore interfere with this method, standards were prepared using E, NE and dopamine. Dopamine did not fluoresce at the particular wavelength used, but there was some interference from E as is seen in Figure 14. According to Udenfriend (1962), however, there is little or no measurable E in the brain or heart tissue of the rat.

The relative fluorescence at varying concentrations of NE is shown in Figure 15.

The reliability of the modified Shore and Olin (1958) NE assay method was established by performing numerous assays on untreated animals selected at random from those present in the laboratory. The results were then compared with accepted literature values and appear in Table X. The data for brain and heart NE fall within an accepted range and are consistent throughout the study.

Udenfriend (1962) reported an average of 0.44 ug/g of NE in wet brain tissue of rats. Other investigators, including Maynert and Klingman, 1961; Merrills, 1962; Anton and Sayre, 1964 and Grabarits and Harvey, 1966, reported similar values. In this study, the modified Shore and Olin (1958) NE assay method gave values that were approximately half those reported by the other workers. Nevertheless, other authors, those listed in Table X, all using the Shore and Olin (1958) method or a modification of it, reported values similar to those seen in this report. The heart NE values were similar to those reported by Udenfriend (1962) and others.

2. Dose-Response Studies

The purpose of this experiment was to determine what dose of reserpine would effect a 50% depletion of brain NE and what dose of guanethidine would effect a 50% depletion of heart NE. These two doses, as determined here, were then used in the chronic restraint studies.

The results obtained from these experiments show that reserpine (Figure 16) exhibited a typical dose-response relation to the NE concentration in the rat brain, when given over a suitable range, and that guanethidine (Figure 17) has a similar effect on the NE concentration in rat heart. The results are summarized in Table XI.

Reserpine had a gradual, depleting effect on brain NE and exerted a maximal or near-maximal effect when given at a dose of 1.0 mg/kg. However, the effect of reserpine on heart NE showed near-maximal depletion following the administration of 0.05 mg/kg and larger doses exhibited no significantly greater effect.

Since this phase of the study was done to determine which dose of reserpine gave a 50% depletion of brain NE, and since 1.0 mg/kg showed

a near-maximal effect, only the data from rats given the first three doses of reserpine, and from rats given the vehicle were used to plot the dose-response curve. Figure 16 shows that the dose of reserpine causing a 50% depletion of brain NE was calculated to be 0.19 mg/kg; therefore, a reserpine dose of 0.2 mg/kg was used throughout the chronic restraint phase of the study.

The results in Table XI show that increasing amounts of guanethidine, up to 5 mg/kg, led to progressively greater decrease in heart NE. However, when 10 mg/kg of guanethidine were administered, the decrease obtained was about the same as that from only 5 mg/kg.

Guanethidine showed no depletion of brain NE at any dose tested. At the 0.5 mg/kg dose level, however, guanethidine effected a slight increase in brain NE and this value was significantly different from the control values ($P < 0.05$), whereas none of the other doses altered brain NE. It is difficult to draw any significance from this finding and it may well be an artifact. Inasmuch as the action of guanethidine on cardiac NE was the principal aim of the study, and since the increase in brain NE was not related to the investigation, the finding was not followed up at this time.

As with reserpine, the data from rats given the first three doses of guanethidine, and from rats given the vehicle, were used to plot the dose-response curve. The dose of guanethidine effecting a 50% depletion of cardiac NE was 1.20 mg/kg (Figure 17). This dose was used throughout the chronic restraint phase of the study.

The method of least squares was used to plot the dose-response curve for both drugs (Snedecor, p. 124, 1956), with the aid of an IBM 360 computer.

3. Chronic Restraint Studies

Brain and heart NE was calculated and fluctuations were noted. NE is released from neurons at accelerated rates by stimuli (Glowinski and Baldessarini, 1957) and its decline after pharmacological inhibition of its biosynthesis is dependent on the presence of adequate stimuli (Hillarp et al., 1966b). In some instances its concentration in brain (Maynert and Levi, 1963) and in heart (Chang and Su, 1967) has been reported to be lowered by stress.

Comparative determinations for brain NE are presented in Table XII and Figure 18. There does not appear to be a significant change in brain NE in vehicle and guanethidine control rats. Reserpine control animals showed a progressive decline in brain NE from day 1 to day 24. Vehicle-treated, stressed animals showed a decline in brain NE on day 3, followed by a gradual increase. The guanethidine-treated, stressed animals also showed an increase in brain NE on day 1 and then a significant decrease on day 3, followed by a gradual increase on days 6 and 12 and then a decline again on day 24. The reserpine-treated, stressed animals showed a significant decrease in brain NE on day 1; it decreased even further on the consecutive days of study.

Usually brain NE is resistant to decline, even under conditions of a highly intensified stimulus (Paulsen and Hess, 1963); in some circumstances, a stressor, which under certain circumstances or in different degrees might have reduced NE, may actually raise it (Welch and Welch, 1968). This resistance to reduction of brain NE was noted in these studies, especially in the vehicle- and guanethidine-treated, stressed animals.

The exact mechanism whereby stress causes the release of brain NE is unknown (Maynert and Levi, 1964); however, Welch and Welch (1968) postulated a mechanism to explain the rapid elevation of brain NE that may be induced by stress. They suggested that a mechanism is activated which conserves NE by inhibiting its normal intraneuronal catabolism concurrent with the increased requirement for it to maintain neurotransmission.

A statistical comparison of the heart NE values is presented in Table XIII and Figure 19. Heart NE was lower on day 6, as compared to the other animals in the vehicle-treated, control group. Guanethidine-treated, control animals showed a significant decrease on day 1, and a further significant decline on the following days. Reserpine-treated, control animals showed almost no cardiac NE from day 1, and it fell even lower as the experiment progressed. Vehicle-treated, stressed animals showed a low level of NE on day 1, compared either with the vehicle-treated, control animals or to day 0. This level fell even further by day 6; however, the values on day 12 compared favorably to those of vehicle-treated, control animals and to those of non-treated animals on day 0. By day 24, the heart NE rose even higher in these animals. The guanethidine-treated, stressed animals showed a significant decrease in heart NE on day 1; it fell even lower by day 3, but by day 12, it returned to values comparable to those on day 1. Reserpine-treated, stressed animals had values comparable to those of the reserpine-treated, control animals. Control brain and heart NE was fairly stable throughout the experiment.

Chang and Su (1967) demonstrated that increased sympathetic activities in the rat heart, induced by exposure to cold, primarily release

NE. Others (Leduc, 1961; Feller and Hales, 1964) postulated that the increased excretion of CA during exposure of animals to cold stress might indicate a greater activity of the sympathoadrenal system.

4. Adaptation Studies

Along with behavioral adaptation, there also appeared to be a neurohumoral adaptation. The problem in analyzing these results was the fact that the brain NE of stressed rats was not significantly different from control rats, except for day 1. Some correlation can be seen among the differences between these two groups of animals and their heart NE. The decrease in brain NE, shown in Figure 22 corresponds fairly well to the general activity of the stressed animals during the first week of the study. The significant decrease in heart NE during this period is perhaps an indication of increased sympathetic activity and non-adaptation at this stage of the study. Graham (1966) obtained increased E and NE excretion rates in restrained rats and suggested that the stress of physical restraint caused marked increases in both adrenomedullary and neurogenic sympathetic activity.

The importance of a possible correlation between behavioral adaptation and brain and heart NE is not to be denied, although it is difficult to quantify the behavioral parameters studied here. The fact that stress brought about a decrease in brain NE at first and then an increase on day 12 appeared to associate this amine with the behavioral activity associated with this group of animals. Guanethidine-treated, stressed animals demonstrated an intermediate behavioral effect as compared with vehicle-treated, stressed animals (exhausted) and reserpine-treated, stressed animals (hyperactive), upon their release from restraint. In the guanethidine-treated, stressed animals, the heart NE

was identical with the vehicle-treated, stressed animals but not to the guanethidine-treated, control animals. There appeared to be no depletion of the heart NE due to the action of guanethidine. This effect may be due to the ability of the stress situation to stimulate the drug's metabolism by mediating a rapid induction of the liver microsomal enzymes responsible for its metabolism (Driever and Bousquet, 1965). Reserpine treatment led to dissimilar response effects on heart NE. The deviation may be due to the fact that reserpine action is seen long after there are no traces of the drug. This evidence also suggests that these two drugs do not deplete heart NE by the same mechanism.

J. General Discussion

There were no deaths attributable to the experimental design in any of the control groups (vehicle or drug-treated). That no deaths were noted in the vehicle-treated, stressed rats in the 24-day study indicated that the animals adapted to this procedure. Similarly, the one death in the guanethidine-treated, stressed group also indicated adaptation in this group of animals. The mortality of the reserpine-treated, stressed rats was very high; 40% in 24 days clearly indicated the inability of reserpinized animals to adapt to the chronic, forced restraint stress (Table XIV).

Other investigators, using other forms of stress, reported a high mortality in reserpinized animals. Rosecrans and DeFeo (1965), using restraint for three hours, reported 50% mortality in reserpinized rats. They stated that the high mortality may have resulted in part from starvation (they noted a decreased food and water intake); but starvation does not appear to be the answer, since in this study and in others (See page 124) the animals ate all the food offered to them. Buckley

et al. (1964), using flashing lights, autogenic stimulation, and cage oscillation stressors, showed that reserpine not only failed to protect the rats from chronic stress but potentiated the lethal effect of the stressors. The mortality of reserpine-treated animals under stress was over 200% at the end of 27 weeks. They concluded that under prolonged chronic stress the reserpine produced a potentiating or an additive effect on the pituitary-adrenal response to stress, thus producing adrenal insufficiency that led to the death of the animals. Westermann et al. (1962) stated that failure of rats to respond to stressful stimuli after large doses of reserpine might be attributed partly to a pituitary-adrenal system which is already under maximal stimulation and partly due to exhaustion of the pituitary ACTH. If the animals were then exposed to cold, the plasma corticosterone did not rise further (Maickel et al., 1961) and the inability of the animal to respond to cold-exposure was reflected in a higher death rate, resulting from the stress. Other investigators (Leduc, 1961; Johnson, 1963) suggested that failing NE excretion in animals exposed to cold could be due to the inhibition of NE synthesis by reserpine despite the report by McDonald and Weise (1961), who demonstrated that reserpine does not alter the biosynthesis of CA. Johnson (1966) reported that survival of cold-exposed animals appeared to be related to the liberation of NE, with E release serving as an important secondary mechanism of defense against cold.

Pouliot (1966) stated that a drug should be free of central effects if the correlation between NE secretion from peripheral tissue stores of CA and resistance to cold stress is to be considered. He employed guanethidine, whose action is largely peripheral and without effect on

brain NE, in adrenodemedullated rats, all of which died when exposed to cold stress. The failure of the guanethidine-treated rats to increase their NE excretion led him to conclude that guanethidine prevents either the increase in release or the biosynthesis of NE, or both, normally induced by cold exposure. He also concluded that the sympathetic blockade by guanethidine could be partly responsible for the lack of acceleration of CA liberation. His results corroborated those of previous investigators who showed that cold resistance is dependent upon the liberation and action of NE.

Since non-adaptation may be associated with the inhibition of some physiological mechanism, it would be interesting to speculate on the relationship between apparent exhaustion of central and peripheral NE with non-adaptation. Many data are available in the literature to suggest that NE plays an important role in enabling animals to respond to pituitary-adrenal stimulation induced by environmental stress. Animals without a functioning peripheral sympathetic nervous system would not be able to adapt to a chronic stress, even though the pituitary-adrenal system might be functioning normally (Rosecrans and DeFeo, 1965).

It is not possible to correlate completely non-adaptation in reserpinized, stressed animals and CA depletion. In Figure 23, the heart NE patterns are similar to those in the brain; hence, the depletion of peripheral NE alone does not explain the toxicity in the stressed, reserpinized rats. Further, the heart NE is virtually the same in the stressed, reserpinized rats as in the nonstressed, reserpinized rats. Guanethidine and its principal action on peripheral NE also offers no clue to the cause of death in reserpinized, stressed rats because here

the heart picture and mortality are different and no correlation can be seen. However, when speculations on the increased toxicity in reserpinized, stressed rats are set forth, it should be kept in mind that reserpine affects both peripheral and central CA and indolamines and that it produces marked sedation, whereas, guanethidine affects only peripheral CA and at the low dose used here it probably does not affect the CA in the adrenal medulla. Pouliot (1966) suggested the dependency of survival of cold-stressed adrenodemedullated animals upon a peripheral secretion of NE, Bygdeman et al. (1960) reported an acceleration of CA synthesis in the adrenal medulla following an increased nervous stimulation and Pouliot (1966) assumed that the same mechanism occurs in noradrenergic fibers. The work of Leduc (1961) also suggests increased synthesis of NE occurs as a result of increased nervous activity.

The rats in the present study were not demedullated; hence, the guanethidine-treated animals were able to respond to the stress and adapt perhaps by increased NE synthesis. The stress seemed to override the NE-depleting action of guanethidine (Figure 22). Since reserpine affects total CA content in the rat, then, in a sense, they were chemically demedullated and could not adapt to the stress by increasing their NE content. However, it is not certain that the increased toxicity in reserpinized, stressed rats was due solely to CA depletion per se, either central or peripheral. That CA play an important role in adaptation can be seen from the work of Necinu and Kregei (1961), who observed that the usual peptic ulcer produced by reserpine and cold stress could be completely inhibited by the administration of DOPA, a NE precursor, and that the usual 50% mortality produced by cold stress was prevented by DOPA in reserpinized rats.

The decrease in central sympathetic outflow in reserpine-treated animals (McCubbin and Page, 1958) may also be an important factor in non-adaptation with these animals. However, numerous other parameters (mentioned both by other investigators and throughout this report) may also come into play, and CA depletion is but one factor in the total picture of adaptation and reserpine toxicity.

The work of Welch and Welch (1968) should also be kept in mind, when attempts are made to correlate stress and CA depletion. They state that during stress, mice, which differ in behavioral reactivity because of differences in their previous environmental conditions, metabolize brain biogenic amines at different rates and also activate to different degrees temporal mechanisms which control the availability of the amine during stress.

Neurochemical adaptation which appears to parallel behavioral adaptation for the most part has been characterized in normal drug-treated rats. The increased behavioral activity associated with the initiation of restraint followed the changes in brain NE and it tended to return to normal when behavioral adaptation occurred. Serum corticosterone also appeared to be a good index of behavioral adaptation. The results from previous investigations support the validity of these observations. Pfeifer and Galambos (1967) suggested that NE has a more important role in the change of susceptibility to seizures than serotonin or dopamine. Smith (1963) showed that enhancement of the activity-increasing effect of d-amphetamine after alpha-methyl dopa followed changes in brain NE more closely than changes in either brain serotonin or dopamine. Maynert and Klingman (1962) discussed the possibility that brain NE functions as part of the sympathetic nervous system

and that its release is accompanied by arousal or excitement.

Grundfest (1957), summarizing research from previous years, concluded that central nervous transmission is accomplished via neurochemicals. He was unable to demonstrate electrically excitable central dendrites. Rothboller (1959) and Brodie and Shore (1957) contended that changes in brain NE represent fluctuations of excitatory activity initiated by an adrenergic system. Rosecrans (1963) and Guarino et al. (1967) also showed that NE was associated with increased CNS excitation.

It should be pointed out again that reserpinized, stressed animals demonstrated abnormal behavior after release from restraint, although they were quiet while under restraint, an important observation because it cannot be concluded that stress inhibited the reserpine-induced sedation. At the same time, reserpine produces chemical sympathectomy (Trendelenburg, 1961a), a fact that may account for the decreased activity in these rats under restraint. Reserpine may be stimulating the excitatory brain mechanisms that are controlled by NE, since restraint caused variations in NE and overt activity was noted when brain NE was decreased. Reserpine also, by producing its exhaustive depletion of the central sites of NE, may have produced a central hypersensitivity similar to nerve postsynaptic hypersensitivity following denervation. Such an apparent hypersensitivity may be responsible for the behavioral excitation observed in reserpinized, stressed rats (Rosecrans, 1963).

The ability of reserpine to prevent adaptation was again demonstrated beyond doubt. The means by which it was accomplished, however, are debatable, for it should be remembered that reserpine has important depleting effects on both brain CA and indolamines (Sheppard and Zimmerman, 1960a). The guanethidine-treated, stressed animals appeared to adapt

to the stress, and their behavioral pattern, similar to the vehicle-treated, stressed animals, also indicates that perhaps NE plays a role in behavior patterns.

VI. SUMMARY AND CONCLUSIONS

A. A modification of the Shore and Olin (1958) method for the extraction of tissue NE has been described, which was applied to the routine estimation of brain and heart amines of rats subjected to restraint and treated with guanethidine or reserpine.

B. Serum corticosterone was estimated by the method of Guillemin et al. (1959a) and AAA was estimated by the method of Maickel (1960).

C. Dose-response studies were carried out over a period of six days, to establish what dose of reserpine would effect a 50% depletion of central CA and what dose of guanethidine would effect a 50% depletion of peripheral CA.

D. The dose of reserpine (0.2 mg/kg, i.p.) and of guanethidine (1.20 mg/kg, i.p.), established in the dose-response studies were used throughout the chronic (24 day) phase of the study.

1. Reserpinized, control animals gave adrenalcortical responses similar to those of the stressed animals. That reserpinized, stressed rats failed to adapt to the stress was indicated by the high mortality, 40% in 24 days. Starvation was eliminated as a causative factor in the increased mortality, since all animals ate all of the food offered to them. The high mortality could not be attributed either to the peripheral depletion or to the central depletion of CA per se, since reserpine depletes both areas of their CA. There was some evidence to indicate that depletion of both central and peripheral CA may account in some degree for the increased mortality. A mechanism is postulated to explain why reserpine prevented adaptation. Some correlation was noted between the brain NE levels and behavior. The abnormal behavior

noted in the reserpinized, stressed rats may have been due to the depletion of brain NE.

2. Guanethidine also produced adrenalcortical responses similar to those evoked by restraint; however, the responses were not as great. All values tended to return to normal by day 6, except heart NE which showed a continuous depletion until day 12 and then a gradual return toward normal. The guanethidine-treated, stressed rats showed the characteristic adrenalcortical responses: increased adrenal weight; increased serum corticosterone; and decreased AAA. The stress appeared to overcome the peripheral NE depletion caused by guanethidine and values started to return to normal by day 12. This may have been the result of increased syntheses of CA, particularly from the adrenal medulla, since it has been reported that low doses of guanethidine do not affect adrenal E and NE. This may also account for the adaptation of these animals to restraint, since there was only 10% mortality. These animals displayed behavioral patterns similar to those of the vehicle-treated, stressed animals; their brain NE patterns were also similar.

E. Evidence is presented indicating that reserpine and guanethidine affect CA by different mechanisms.

F. Adaptation to CRS was demonstrated by organ weight changes and by the analyses of the interrelationship among serum corticosterone, AAA, brain and heart NE. After an initial AR, all of the parameters measured tended to return to control levels: an indication of physiological adaptation. The pituitary-adrenal system was apparently active throughout the period of chronic stress. Control animals, subjected to stress, exhibited some degree of behavioral adaptation, passing from an extreme

degree of excitability at the initiation of the stress to a moderate degree of docility when released from it. Brain NE showed an initial decrease, followed by an increase on day 12. Heart NE tended to return to normal also by day 12. The vehicle-treated, control animals showed some indication of pituitary-adrenal stimulation which was due probably to the handling and to the injection procedure.

Non-adaptation of rats subjected to chronic restraint may be due at least in part to exhaustion of both central and peripheral NE since the reserpine-treated, stressed animals showed an increased mortality (40%) in 24 days and the guanethidine-treated, stressed animals had 10% mortality over the same time period. The results from this study suggest the possible significance of the sympathetic division of the ANS and adrenal CA in adaptation of the animals to chronic stress.

VIII. APPENDIX

TABLE XXIII

THE EFFECT OF RESTRAINT ON MEAN DAILY BODY WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	215 ± 17[a] (10)[b]	218 ± 25 (10)	230 ± 24 (10)	209 ± 22 (10)	212 ± 14 (10)	208 ± 17 (10) C
2	222 ± 20 (10)	222 ± 25 (10)	231 ± 24 (10)	207 ± 23 (10)	217 ± 14 (10)	212 ± 16 (10) C
3	222 ± 21 (10)	227 ± 23 (10)	235 ± 23 (10)	207 ± 21 (10)	214 ± 15 (10)	209 ± 17 (10) C
4	226 ± 20 (10)	227 ± 24 (10)	237 ± 21 (10)	206 ± 21 (10) A[c]	214 ± 14 (10)	207 ± 17 (10) AC
5	233 ± 23 (10)	232 ± 25 (10)	240 ± 23 (10)	206 ± 22 (10) A	211 ± 16 (10) AB	206 ± 17 (10) AC
6	235 ± 22 (10)	233 ± 23 (10)	238 ± 23 (10)	201 ± 21 (10) A	213 ± 14 (10) AB	202 ± 18 (10) AC
7	235 ± 22 (10)	235 ± 23 (10)	238 ± 22 (10)	203 ± 23 (10) A	212 ± 14 (10) AB	198 ± 17 (10) AC
8	238 ± 20 (10)	239 ± 23 (10)	237 ± 23 (10)	201 ± 20 (10) A	211 ± 17 (10) AB	196 ± 19 (9) AC
9	242 ± 21 (10)	241 ± 22 (10)	238 ± 23 (10)	204 ± 22 (10) A	211 ± 18 (10) AB	198 ± 24 (9) AC

TABLE XXIII - continued

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
10	242 ± 20 (10)	242 ± 23 (10)	240 ± 25 (10)	205 ± 23 (10) A	213 ± 16 (10) AB	196 ± 23 (9) AC
11	247 ± 22 (10)	247 ± 24 (10)	243 ± 26 (10)	205 ± 20 (10) A	215 ± 20 (10) AB	203 ± 21 (9) AC
12	248 ± 20 (10)	247 ± 23 (10)	237 ± 26 (10)	206 ± 20 (10) A	213 ± 17 (10) AB	196 ± 22 (9) AC
13	248 ± 18 (10)	246 ± 27 (10)	239 ± 24 (10)	204 ± 19 (10) A	212 ± 18 (10) AB	193 ± 23 (9) AC
14	255 ± 20 (9)	249 ± 23 (10)	240 ± 26 (10)	204 ± 22 (10) A	212 ± 21 (10) AB	195 ± 30 (9) AC
15	256 ± 20 (9)	251 ± 23 (10)	241 ± 26 (10)	203 ± 20 (10) A	211 ± 19 (10) AB	193 ± 32 (9) AC
16	260 ± 20 (9)	251 ± 24 (10)	242 ± 25 (10)	203 ± 16 (10) A	212 ± 19 (10) AB	201 ± 28 (8) AC
17	258 ± 20 (9)	253 ± 23 (10)	241 ± 20 (10)	202 ± 17 (10) A	209 ± 18 (10) AB	197 ± 32 (8) AC
18	260 ± 22 (9)	254 ± 24 (10)	242 ± 22 (10)	202 ± 18 (10) A	210 ± 20 (10) AB	197 ± 32 (8) AC
19	262 ± 21 (9)	254 ± 24 (10)	243 ± 25 (10)	202 ± 18 (10) A	207 ± 20 (10) AB	202 ± 33 (7) AC

TABLE XXIII - continued

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
20	262 ± 22 (9)	254 ± 23 (10)	242 ± 24 (10)	201 ± 20 (10) A	205 ± 22 (10) AB	194 ± 32 (7) AC
21	264 ± 24 (9)	258 ± 25 (10)	243 ± 26 (10)	202 ± 17 (10) A	208 ± 23 (10) AB	201 ± 33 (7) AC
22	266 ± 23 (9)	256 ± 25 (10)	244 ± 34 (10)	205 ± 20 (10) A	213 ± 18 (9) AB	194 ± 39 (7) AC
23	265 ± 26 (9)	259 ± 26 (10)	245 ± 38 (10)	205 ± 18 (10) A	213 ± 19 (9) AB	200 ± 39 (7) AC
24	268 ± 25 (9)	262 ± 26 (10)	247 ± 31 (10)	204 ± 18 (10) A	215 ± 19 (9) AB	215 ± 21 (6) AC

a: mean ± S.E., daily weights (grams).
b: number of rats.
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from guanethidine-control group.
C - significantly different from reserpine-control group.

Drug doses, (i.p.) daily for 24 days: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XXIV

THE EFFECT OF RESTRAINT ON RELATIVE ADRENAL WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	0.011 ± 0.001[a] (10)[b]	0.011 ± 0.001 (10)	0.011 ± 0.001 (10)	0.011 ± 0.001 (9)	0.011 ± 0.001 (10)	0.011 ± 0.001 (10)
3	0.011 ± 0.001 (10)	0.011 ± 0.000 (10)	0.012 ± 0.001 (10)	0.012 ± 0.001 (10)	0.012 ± 0.001 (10)	0.014 ± 0.001 (9) ABD[c]
6	0.008 ± 0.001 (10)	0.007 ± 0.001 (10)	0.008 ± 0.001 (10)	0.010 ± 0.001 (10) A	0.012 ± 0.001 (10) AC	0.011 ± 0.001 (10) AD
12	0.010 ± 0.001 (10)	0.011 ± 0.000 (10)	0.011 ± 0.001 (10)	0.016 ± 0.001 (10) A	0.017 ± 0.001 (9) AC	0.016 ± 0.001 (9) AD
24	0.011 ± 0.001 (10)	0.009 ± 0.001 (10)	0.010 ± 0.001 (10)	0.015 ± 0.001 (10) A	0.015 ± 0.002 (9) C	0.016 ± 0.002 (6) A

a: mean ± S.E. (g/100 g of body weight)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XXV

THE EFFECT OF RESTRAINT ON RELATIVE HEART WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	0.322 ± .008[a] (10)[b]	0.316 ± .006 (10)	0.308 ± .009 (10)	0.324 ± .004 (9)	0.314 ± .007 (10)	0.306 ± .007 (10) B[c]
3	0.325 ± .006 (10)	0.316 ± .002 (10)	0.309 ± .005 (10)	0.357 ± .011 (10) A	0.339 ± .007 (10) C	0.334 ± .005 (9) D
6	0.319 ± .004 (10)	0.324 ± .005 (10)	0.343 ± .006 (10) A	0.358 ± .007 (10) A	0.351 ± .011 (10) AC	0.347 ± .006 (10) A
12	0.329 ± .014 (10)	0.301 ± .005 (10)	0.335 ± .005 (10)	0.374 ± .011 (10) A	0.372 ± .010 (9) AC	0.350 ± .006 (9)
24	0.305 ± .010 (10)	0.276 ± .003 (10) A	0.309 ± .007 (10)	0.323 ± .006 (10)	0.314 ± .005 (9) C	0.320 ± .006 (6)

a: mean ± S.E. (g/100 g of body weight)
b: number of rats
c: statistical comparison at probability, $P < 0.05$

A - significantly different from vehicle-control group.
B - significantly different from vehicle-restrained group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses; (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

TABLE XXVI

THE EFFECT OF RESTRAINT ON RELATIVE BRAIN WEIGHT OF MALE ALBINO RATS TREATED WITH GUANETHIDINE OR RESERPINE

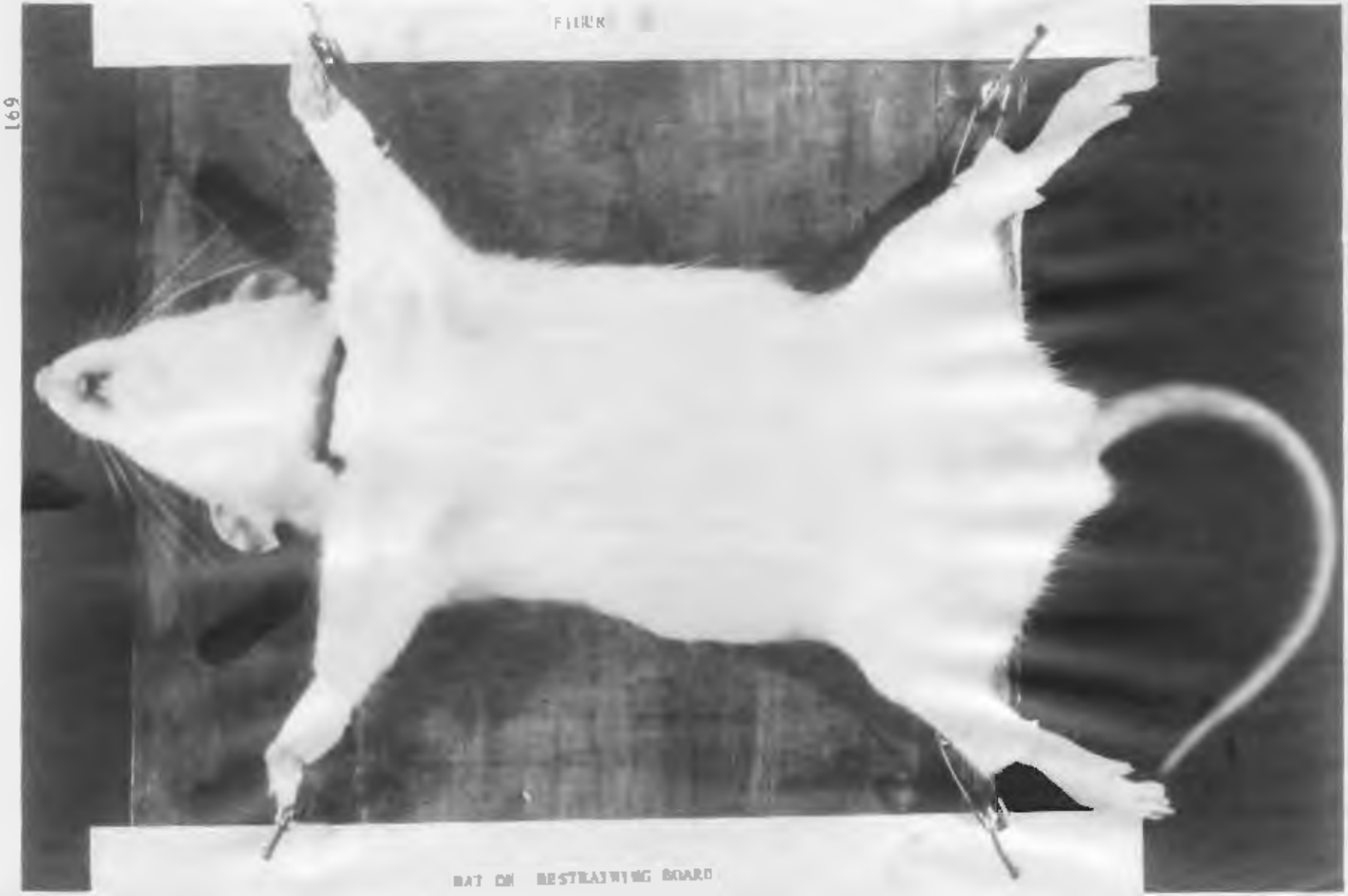
Days of Treatment	Control Animals			Restrained Animals		
	Vehicle	Guanethidine	Reserpine	Vehicle	Guanethidine	Reserpine
1	0.838 ± .025[a] (10)[b]	0.822 ± .016 (10)	0.831 ± .012 (10)	0.820 ± .019 (9)	0.839 ± .024 (10)	0.834 ± .028 (10)
3	0.813 ± .027 (10)	0.806 ± .026 (10)	0.802 ± .028 (10)	0.784 ± .023 (10)	0.793 ± .019 (10)	0.792 ± .024 (9)
6	0.740 ± .018 (10)	0.744 ± .017 (10)	0.799 ± .020 (10) A[c]	0.824 ± .015 (10) A	0.801 ± .027 (10)	0.827 ± .015 (10) A
12	0.672 ± .022 (10)	0.698 ± .015 (10)	0.757 ± .024 (10) A	0.847 ± .029 (10) A	0.802 ± .022 (9) AC	0.883 ± .028 (9) AD
24	0.643 ± .025 (10)	0.641 ± .022 (10)	0.663 ± .016 (10)	0.783 ± .018 (10) A	0.771 ± .025 (9) AC	0.743 ± .048 (6)

a: mean ± S.E. (g/100 g of body weight)
b: number of rats
c: statistical comparison at probability, $P < 0.05$.

A - significantly different from vehicle-control group.
C - significantly different from guanethidine-control group.
D - significantly different from reserpine-control group.

Drug doses, (i.p.) daily: Guanethidine, 1.20 mg/kg. Reserpine, 0.2 mg/kg.
Vehicle, glass-distilled water, 1 ml/kg.

FIGURE



BAT ON RESTRAINING BOARD

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