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Title of Thesis: Endocrinological Responses to the Administration of Nicotine: Interactions with Drug Initiation, Conditioned Effects, and Conditions of Stress

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Abstract

Title of Dissertation: Endocrinological Responses to the Administration of Nicotine: Interactions with Drug Initiation, Conditioned Effects, and Conditions of Stress

David Edward Morse, Doctor of Philosophy, 1984

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The present study used a within subjects (rabbits) design to investigate the effects of the chronic intermittent intravenous administration of nicotine on several endocrinological and biochemical parameters which may contribute to the physiological determinants and health consequences of cigarette smoking. Specifically, measurements were made of plasma concentrations of catecholamines (norepinephrine, epinephrine and dopamine), corticosterone, glucose and insulin. These hormones and their respective control mechanisms have previously been discussed as contributing to: a) the reinforcement of drug taking behaviors during the development and maintenance of cigarette smoking, b) decreases in body weight and the increased incidence of cardiovascular disorders among cigarette smokers, and c) responses induced by noxious environmental stimuli (stress responses). The experiment was divided into three major sections each of which examined a different aspect of the role of nicotine in tobacco use, including: a) the effects during drug initiation (Phase I), b) conditioned drug responses (Phase II), and c) the reduction of stress induced responses (Phase III).

The results of Phase I of the experiment indicated that the administration of nicotine resulted in increases in the plasma

concentrations of catecholamines (norepinephrine, epinephrine and dopamine), corticosterone and glucose, and in decreased concentrations of insulin. The magnitude of the norepinephrine and epinephrine responses decreased during the first week of testing, while all other responses were found to be relatively similar in the "naive" and nicotine "experienced" (tolerant and/or habituated) animals. These nicotine induced responses may act as sources of reinforcement in the development and maintenance of cigarette smoking behavior (and may mediate the lower body weight which is apparent among smokers when compared to non-smokers) by altering the level of physiological and/or endocrinological activity (i.e., level of arousal) or the state of hunger/satiety being experienced by the smoker.

It was also observed that increasing doses of nicotine resulted in increasing plasma concentrations of catecholamines and corticosterone which have been implicated as potential mediators of several cardiovascular disorders. Preliminary evidence suggested a positive relationship between nicotine and histological indications of myocardial degeneration and failure.

Phase II demonstrated that the repetitive administration of nicotine in conjunction with the simultaneous presentation of a non-drug stimulus (flashing lights) may result in the development of conditioned physiological responses. The results of this study indicated the presence of both conditioned opponent and primary process responses. Conditioned norepinephrine responses were similar to those elicited by nicotine (i.e., an increase in plasma concentration) although approximately one-half the size, while the conditioned dopamine responses were opposite to those produced by

nicotine administration. Conditioned responses, such as those observed in the present study, may contribute to the tobacco abstinence and withdrawal syndromes. It is possible that the appropriate presentation of the conditioned stimuli (and subsequent elicitation of the conditioned response) may be of benefit in reducing the discomfort associated with abstinence and the withdrawal syndrome by substituting for the administration of the drug.

The results of the final phase of the experiment indicate that the presentation of noxious stimulation (physical restraint was used in the present study as an example of the broader class of noxious environmental stimuli) produced increases in the plasma concentrations of norepinephrine, epinephrine and corticosterone. The administration of nicotine to nicotine-experienced animals did <u>not</u> reduce the intensity of physiological responses induced by the application of noxious stimulation. In fact, the administration of nicotine concurrent with the application of stress resulted in a somewhat greater intensity of responding than was induced by application of the stressor alone. These results suggest that the stress ameliorating effects of smoking are <u>not</u> based on the reduction of stress related peripheral physiological processes.

Endocrinological Responses to the Administration of Nicotine: Interactions with Drug Initiation, Conditioned Effects, and Conditions of Stress

by

David Edward Morse

Dissertation

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FOREWORD

The opinions or assertions contained herein are the private views of the author and should not be construed as official or necessarily reflecting the views of the Uniformed Services University of the Health Sciences or Department of Defense.

DEDICATION
To my Parents

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TABLE OF CONTENTS

Volume I

Appendix A

Volume II

Photograph and Figures

Appendix B

Volume II

Detailed Procedures

Appendix C

Volume II

Statistical Tables

Appendix D

Volume II

Graphs

Cigarette smoking is the single most important preventable cause of premature mortality and morbidity in the United States (U. S. Dept. of Health, Education and Welfare, 1979). It was estimated that in 1979 more than 30% of the adult (18 years or older) male and female population of the United States were current users of cigarettes, representing nearly 55 million individuals. In addition, it was estimated that there were approximately 30 million former cigarette smokers in 1979. Therefore, the consequences of current cigarette smoking or a prior history of cigarette use may affect up to 85 million people, approximately one-half of the adult population. There is extensive evidence that the administration of nicotine in animals and cigarette smoking in humans produces a variety of biochemical, endocrinological and physiological effects. However, despite years of research, the role which many of these responses play in the development and maintenance of cigarette smoking and in the etiology of the pathologies associated with tobacco use have not been established.

Diseases Associated with the Use of Tobacco

It has been clearly demonstrated that cigarette and tobacco use are related to increased mortality due to ischemic heart disease, cancers of the respiratory tract, and the chronic obstructive pulmonary diseases (U.S. Dept. of Health, Education and Welfare, 1979). The overall mortality ratio for all smokers of cigarettes is about 1.7 compared to non-smokers of similar age, the ratio increasing with the quantities smoked. The primary contributors to the increased mortality are coronary heart diseases, including myocardial

infarction, sudden cardiac death and atherosclerosis. The secondary and tertiary factors involved in the elevated mortality among smokers include: cancers of the lung, oral and buccal mucosa, pancreas and urinary bladder, and the chronic obstructive pulmonary disorders, emphysema and chronic bronchitis. The increase in morbidity among cigarette smokers is primarily due to an elevation in the incidence of respiratory system disorders, although there is an increase in all forms of acute conditions (i.e., colds and bronchitis). The increases in the mortality and morbidity values are most evident for long term (years) users of tobacco and also among former long term smokers.

Based on this information it is apparent that the health consequences of cigarette and tobacco smoking are severe and these consequences may affect a very large proportion of the population. Therefore, it is important that we investigate and understand the mechanisms involved in the cigarette smoking habit and the development of its health consequences.

Theories of Cigarette Smoking

Several theories have been proposed to explain cigarette smoking behavior. These theories have been reviewed by Jaffe and Kanzler (1979) and Ashton and Stepney (1982), and are described briefly in the following paragraphs. In general, the explanations may be grouped into three major categories: social and psychosocial, psychological-developmental, and psychobiological-constitutional theories. While the majority of the theories are relevant to all of the stages of cigarette smoking (i.e., development, maintenance and cessation) they frequently emphasize only one or two stages and

disregard the remainder. However, it is very likely that each of the theories has some contribution to all stages of cigarette use (Jaffe & Kanzler, 1979).

It has been argued that the development of cigarette smoking behavior is primarily governed by social and psychological factors, although some recent work (Kozlowski & Harford, 1976; Silverstein, Feld & Kozlowski, 1980; Silverstein, Kelly, Swan & Kozlowski, 1982) has proposed an important role for biological and pharmacological factors in early cigarette use. (Kozlowski and associates argue that the initial sensitivity of the smoker to the effects of nicotine will determine whether it is rewarding or aversive both upon initial exposure and on subsequent occasions. More specifically, as the sensitivity of the individual increases then the aversive effects produced by nicotine administration will become more intense and/or more prominent and will decrease the likelihood of continued nicotine use.) The social and psychosocial theories relating to the development of smoking argue that early cigarette use is a response to social pressures as may be evident in peer groups or, as proposed in Bandura's Social Learning Theory, it is an imitation of adult or peer behaviors. The psychological-developmental explanations of smoking include psychoanalytic theory, Erikson's Theory of psychosocial development (U. S. Dept. of Health, Education and Welfare, 1979) and the Jessors' (Jessor, 1978) model of adolescent deviant behavior. In these explanations it is proposed that smoking is a reflection of an underlying state of psychological turmoil due to an arrest in

psychological development, or it is a normal outcome of adolescent rebellion.

The psychobiological and biological-constitutional theories of tobacco use primarily emphasize explanations for the maintenance of cigarette smoking and the withdrawal syndrome. The major psychobiological theories state that cigarette use and its associated activities (i.e., removing the cigarette from the pack, lighting the cigarette, puffing and inhaling the smoke) become conditioned secondary reinforcers through repeated association with primary reinforcers. (The identity of the primary reinforcers involved in cigarette smoking are not clearly established, however it has been proposed that changes in plasma glucose concentrations [Hickey & Horner, 1973] or changes in autonomic nervous system activity [Eysenck, 1973; c.f., Schachter, 1977] may subserve this function.) The development of the association between the primary and conditioned reinforcer occurs during the early phases of cigarette use. Following the establishment of its reinforcing characteristics, the cigarette and/or the act of smoking may be substituted for the primary reinforcement in order to satisfy desires or drive states, off-set the discomfort of withdrawal, or decrease the effect of noxious stimuli (i.e., anxiety, pain, stress, etc.) (Jarvik, 1973; Russell, 1971). (Several explanations have been proposed to account for the <u>apparent</u> stress reducing effects of smoking: a) smoking may reduce withdrawal discomfort by replenishing the body content of nicotine which has been depleted by physiological changes concomitant with stress (Schachter, 1977), b) smoking may reduce the physiological arousal associated

with stressful and/or noxious stimuli, or c) the act of smoking may focus attention away from the stressor and thereby decrease its impact on the individual [Ashton & Stepney, 1982].)

The biological explanations for the maintenance of smoking behaviors are probably best exemplified by the "titration" model proposed by Schachter (1977). This theory argues that the smoker is physically addicted and/or dependent upon some component of tobacco smoke. More specifically, in this theory it is proposed that the smoker is addicted to nicotine or to a metabolite of nicotine (i.e., cotinine). The smoker then adjusts the rate of cigarette intake to a level which maintains the concentration within the body of the desired smoke component. Insufficient doses result in withdrawal and craving, whereas excessive concentrations result in illness and toxicity (Herman & Kozlowski, 1979). While it is widely accepted that exposure to high doses of nicotine will result in illness it has recently been proposed that other components of cigarette smoke, in particular carbon monoxide and various aldehydes, may contribute to the toxic response (U.S. Dept. of Health, Education and Welfare, 1979).

Eysenck (1973) has also proposed a model of cigarette smoking, arguing that cigarette use reflects an attempt by smokers to adjust their degree of physiological and psychological arousal to a level which is optimal for the situation. Eysenck's model is based on the assumption that for any task or situation there is an ideal level of arousal which results in optimal performance or responding to the situation. Cigarette smoking is known to have a variety of stimulant (sympathomimetic) effects upon the autonomic nervous system (U.S.

Dept. of Health, Education and Welfare, 1979; Larson, Haag & Silvette, 1961; Larson & Silvette, 1968; 1975). Thus, Eysenck (1973) argues that the use of cigarettes by habitual smokers reflects an attempt by the smokers to manipulate their level of physiological (as indexed by autonomic nervous system activity) and psychological (as measured by electroencephalogram) arousal to a level which is optimal for the stimulus condition.

Both the biological and psychobiological explanations of smoking primarily emphasize reasons for the maintenance of smoking behavior. However, each of these theories has implications regarding smoking cessation and the withdrawal syndrome. According to the biological theories, cessation of smoking results in the removal of the addictive agent thus initiating a noxious physical state of withdrawal. In the case of the psychobiological (and to some extent the psychosocial) explanations, abstinence from smoking, while not resulting in aversive physical consequences, results in the removal of a frequent and thus highly desirable source of reinforcement. In summary, it may be argued that both of these groups of theories propose that smoking behavior is maintained by its reinforcement characteristics (i.e., smoking is maintained by some positively valued outcome effect or because of the alleviation and/or avoidance of noxious stimulation), and that smoking cessation results in a noxious condition (physical and/or psychological) of withdrawal.

The Role of Nicotine in Cigarette Smoking

A number of the components of cigarette smoke have been investigated for their possible contribution to the addictive

characteristics of smoking (U. S. Dept. of Health, Education and Welfare, 1979; Larson, Haag & Silvette, 1961; Larson & Silvette, 1968; 1975). The major emphasis in these studies has been on the role of nicotine. While the experimental results are somewhat inconsistent and contradictory, it appears that nicotine is the primary pharmacological agent involved in cigarette addiction. A short review of studies on the contribution of nicotine in smoking behaviors is presented in the following paragraphs.

Chemistry and Pharmacology of Nicotine. Nicotine is an alkaloid contained in the leaves of tobacco (Nicotiana tabacum). It is a colorless, volatile base (pk_a = 8.5) which is readily soluble in water (Taylor, in Gilman, Goodman & Gilman, 1980). Nicotine is rapidly absorbed from the respiratory tract, buccal mucosa and the skin. The half life of nicotine following inhalation or parenteral administration is approximately 30-60 minutes.[1] Approximately 80-90% of nicotine is metabolized in the body, primarily by the liver but also in the kidneys and lungs. Nicotine and its metabolites (cotinine and nicotine-1'-N-oxide) are eliminated by the kidneys, the rate of elimination being dependent upon urinary pH (excretion is increased with elevation of urinary acidity and is decreased under conditions of urinary alkalination; Beckett, Rowland & Triggs, 1965; Beckett & Triggs, 1967; Feyerabend & Russell, 1978; Matsukura, Sakamoto, Takahashi, Matsuyama & Muranaka, 1979; Russell & Feyerabend, 1978).

Nicotine when administered in low to moderate doses in man (Taylor, in Gilman et al., 1980; U.S. Dept. of Health, Education and Welfare, 1979) and many animals (Larson, Haag & Silvette, 1961; Larson

& Silvette, 1968; 1975) has sympathomimetic effects (i.e., effects similar to those produced by activation of the sympathetic autonomic nervous system); high dosages have depressant and/or lethal effects. The complex responses produced by the administration of nicotine are due not only to its action at many neuroeffector and chemosensitive sites, but also to the fact that it has both stimulant and depressant effects. The overall response to nicotine may be viewed as the summation of nicotine effects at each of the effector sites. For example, "nicotine may increase heart rate and blood pressure via stimulation of sympathetic ganglia and/or paralysis (blockade) of parasympathetic ganglia. Conversely, nicotine may decrease heart rate and blood pressure by paralysis of the sympathetic system and/or stimulation of parasympathetic nerves" (Taylor, in Gilman et al., 1980). It is probable that the dose of nicotine received by the average smoker has primarily sympathomimetic effects which are evident in increases in heart rate, blood pressure (systolic and diastolic) and cardiac output (U. S. Dept. of Health, Education and Welfare, 1979).

In general, procedures which have investigated the possible role of nicotine in cigarette addiction may be grouped into three categories: a) animal self-administration, b) human self-administration (of cigarettes), and c) human self-administration following the exogenous administration of nicotine or modification of nicotine bioavailability. While the results obtained in these experiments are somewhat contradictory, the majority of studies support the position that nicotine plays an important role in the

regulation and maintenance of smoking. A short review of representative studies is presented below.

Self-Administration of Nicotine in Animals. Several studies have investigated the self-administration of nicotine in rats (Clark, 1969; Hanson, Ivester & Morton, 1977, 1979; Lang, Latiff, McQueen & Singer, 1977; Latiff, Smith & Lang, 1980) and primates (Deneau & Inoki, 1967; Yanagita, 1973). Hanson and associates report that following an initial "priming" period of drug exposure, rats maintain a relatively constant rate of responding (bar press) for the intravenous administration of nicotine. Priming is a procedure during which repetitive drug administration is independent of the responding of the organism and may act to induce a condition of physiological dependence or tolerance to the drug. The rate of responding was dependent upon the dose of nicotine administered in each infusion and the level of initial exposure (i.e., the rate of self-administration tended to be positively related to the dose of nicotine which was used in the priming period). Goldberg and Spealman (1982) found that the intravenous administration of nicotine had both reinforcing and punishing characteristics depending on the schedule of presentation and the reinforcing event. The results of this study indicate that the characteristics of the response to the administration of nicotine are not invariant; they are dependent in part upon the schedule of presentation and concurrent environmental factors.

Lang et al. (1977) demonstrated that nicotine self-administration was increased by food deprivation (animals were maintained at 80% of free feeding body weight), and when injections

were adjunctive (Latiff et al., 1980) to a food presentation schedule. In the latter study it was found that the rate and pattern of nicotine self-administration under the food delivery schedule (food presentation was dependent on responding) were higher and more stable than under any other condition tested. Deneau and Inoki (1967) found that a "priming" period (a period during which drug administration was independent of responding) was necessary to induce stable patterns of self-administration. However, research by Hanson et al. (1977, 1979) and Cox (personal communication, 1983) indicates that priming is not necessary for the development of nicotine self-administration in rats. At the present time the evidence regarding the necessity of drug priming procedures in the study of nicotine self-administration is somewhat equivocal and warrants further investigation.

Self-Administration of Cigarettes in Humans. Investigation of the self-administration of cigarettes in humans has taken several forms: a) studies of the frequency and quantity of cigarettes smoked following changes in the nicotine content of the cigarettes, b) changes in smoking behavior after modification of the bioavailability of nicotine, and c) studies of cigarette use following the exogenous administration of nicotine (i.e., nicotine delivery by some means other than smoking). In a number of studies, subjects were supplied with high or low nicotine cigarettes and asked to maintain a record of how much they smoked. It was expected that if the smoker is smoking for nicotine then there would be an increase in the consumption of the low nicotine cigarettes as compared to those containing higher levels of nicotine. Goldfarb, Jarvik and Glick (1970) found no difference in

the consumption of high and low nicotine cigarettes among a group of habitual smokers. However, the majority of studies have found some degree of regulation, ranging from the extremely precise (Ashton & Watson, 1970) to moderate adaptation (Goldfarb, Gritz, Jarvik & Stolerman, 1976; Kozlowski, Jarvik & Gritz, 1975; Kumar, Cooke, Lader & Russell, 1977; Russell, Wilson, Patel, Cole & Feyerabend, 1973; Schachter, 1977). In addition, it should be noted that Ashton, Stepney and Thompson (1979) found that when plasma nicotine concentrations were measured it gave evidence that the degree of compensation by smokers was more exact than was expected on the basis of the number of cigarettes smoked (i.e., it was found that plasma nicotine concentrations were closer to the subject's normal baseline value than would have theoretically been predicted based on their consumption of cigarettes with various nicotine deliveries).

In a recent investigation (Benowitz, Hall, Herning, Jacob, Jones & Osman, 1983), it was reported that nicotine consumption (as measured by the plasma concentration of cotinine, the primary metabolite of nicotine) among a group of habitual smokers was unrelated to the Federal Trade Commission reported nicotine yield (high or low) of the cigarettes they smoked. Further, it was found that plasma nicotine (cotinine) concentrations were correlated positively only with the daily total consumption of cigarettes. These findings provide additional evidence that the consumption of nicotine in the habitual smoker is highly dependent on the manner in which the cigarette is smoked and is not necessarily dependent on the nicotine delivery of the cigarette. In addition, these findings indicate the

necessity that in future research the dose of administered nicotine be experimentally controlled, or alternatively, that plasma nicotine concentrations be measured.

A somewhat different approach to the study of smoking has incorporated investigation of the topography (i.e., the number, size, depth and rate of puffing) of cigarette use. Henningfield and Griffiths (1979) reported that when the availability of cigarettes was restricted subjects increased the number of puffs taken on each cigarette and the length of abstinence was inversely related to the latency to puffing when cigarettes were again available. Griffiths and Henningfield (1982) found that dilution of the cigarette smoke (by ventilation of the filter system) resulted in subjects increasing the rate and total number of puffs taken on each cigarette. However, subjects were allowed only four minutes to smoke each cigarette, and the increased filter ventilation resulted in a decrease in the quantity of the cigarette which was burned during each puff when compared to non-ventilated cigarettes. Therefore, if the proportion of the ventilated cigarette which was consumed during each puff was decreased then the smoker would have to take an increased number of puffs in order to consume equal quantities of the ventilated and non-ventilated cigarettes, thus offering an alternate explanation for the experimental results (i.e., if an individual smoker consumes a relatively constant amount of each cigarette, then they would have to increase the number, duration or depth of puffs taken on a slow burning ventilated cigarette). Herning, Jones, Bachman and Mines (1981) found that smokers increased the volume of each puff when

smoking cigarettes delivering lower nicotine yields than their normal brand.

Taken as a whole, the group of studies cited above indicate that a regulatory mechanism is involved in cigarette smoking and the probable regulating agent is nicotine. The conclusion is most strongly supported by the studies of Goldfarb et al. (1976) and Schachter (1977) in which the nicotine content of cigarettes was manipulated independent of their tar delivery. However, the role of tar and associated taste factors may not yet be dismissed as changes in cigarette smoking may not be completely accounted for by changes in cigarette nicotine delivery. A recent investigation (Stepney, 1981), which independently manipulated cigarette tar and nicotine delivery, indicates that cigarette smoking may not be explained by nicotine yield alone.

Studies of Cigarette Use Following Administration of Nicotine From a Non-Tobacco Source. The final category of studies includes those procedures in which nicotine bioavailability was altered or nicotine was administered other than via cigarette use. Schachter, Kozlowski and Silverstein (1977) demonstrated that increases in urinary acidity (a condition which increases the excretion of unmetabolized nicotine in the urine and thus decreases the nicotine content of the body) resulted in significant increases in cigarette consumption. Lucchesi, Schuster and Emley (1967) conducted a study in which nicotine bitartrate (2-4 mg/hr for 6 hours) was administered by intravenous infusion to a group of smokers. The results indicated that the intravenous nicotine produced a significant decrease

(approximately 29%) in the frequency of smoking and the amount of each cigarette consumed as compared with saline control trials. Jarvik, Glick and Nakamura (1970) using nicotine capsules, and Russell, Wilson, Feyerabend and Cole (1976) using nicotine chewing gum produced significant (although small, approximately 15%) decreases in smoking among groups of habitual users. Unfortunately, in each of these procedures the subjects were aware that the experimental manipulations were in some manner related to cigarette smoking -- knowledge which may have influenced the experimental results.

Based upon these studies it appears safe to conclude that nicotine plays an important role in the regulation of smoking behavior and may be the primary pharmacological agent involved in cigarette addiction. Whether or not nicotine is the sole or most important addictive pharmacological component of tobacco, there is no doubt that nicotine is present in the smoke of cigarettes and that the smoker is exposed to it. Therefore, further investigation is warranted regarding the contribution of nicotine to tobacco use and addiction and to the pathological conditions associated with smoking.

Physiological, Endocrinological and Biochemical Effects of Nicotine Administration

As was stated earlier in the paper, nicotine produces a complex pattern of responses which are dependent upon the dose of nicotine and its site of action. Nicotine is recognized as having effects on the central nervous system (e.g., the chemoreceptor trigger zone), on the peripheral nervous system (especially the sympathetic and parasympathetic autonomic ganglia), and at peripheral effector

organ sites (such as the neuromuscular junction) (Gilman et al., 1980). The response produced by the administration of nicotine may be viewed as the summation of its stimulant and depressant effects at each of the effector sites.

An immense quantity of research has been conducted over the past century to examine the physiological and endocrinological effects of nicotine and cigarette smoking. The reader is referred to the excellent reviews of Larson, Haag and Silvette (1961) and Larson and Silvette (1968; 1975) for comprehensive presentations of the available material. Selected references will be used in the following discussion.

Peripheral Nervous System Effects. Su and Bevan (1970) and Westfall and Brasted (1974) found that nicotine has direct releasing effects upon norepinephrine in peripheral post-ganglionic sympathetic neurons. The release of norepinephrine appears to be due to the binding of nicotine to a "nicotinic type receptor" on the adrenergic nerve terminal (Westfall & Brasted, 1972). Maengwyn-Davies and Thoa (1973) and Stewart, Booker and West (1973) reported that nicotine administration produced an increase in norepinephrine turnover in cardiac and arterial tissue.

Central Nervous System Effects. The administration of nicotine has been shown to produce a significant increase in the release of radioactively labelled norepinephrine (H^3) from brain tissue in the cat (Hall & Turner, 1972) and rat, while producing no change in the total brain norepinephrine concentration (Bhagat, Kramer & Seifter, 1967). Bhagat (1970) found that the chronic administration

of nicotine (0.5 mg/kg, five times a day for six weeks) accelerated the rate of disappearance of labelled norepinephrine (i.e., increased the turnover rate) from rat brain without affecting tissue concentration. In the hypothalamus and pituitary of rats, nicotine has been reported to increase norepinephrine release (Hall & Turner, 1972) and inhibit the release of Prolactin, probably by the activation of hypothalamic dopaminergic neurons (Eneroth, Fuxe, Gustafsson, Hokfelt, Lofstrom, Skett & Agnati, 1977 a & b; Fuxe, Agnati, Eneroth, Gustafsson, Hokfelt, Lofstrom, Skett & Skett, 1977). The activation of hypothalamic dopaminergic neurons results in an increase in the release of Prolactin Inhibitory Factor into the hypothalamic-hypophysial portal blood system, thus resulting in a decreased release of Prolactin from the anterior pituitary.

Effects on the Adrenal Medulla. In adrenal tissue it has been reported that chronic (up to nine months) exposure to nicotine produces hypertrophy of medullary tissue, without producing discernible histologic changes in adrenal cortical cells. In addition, it was found that the medullary hypertrophy was due primarily to an increase in the number and size of norepinephrine containing cells (Eranko, 1955; Eranko, Hopsu & Raisanen, 1959). Biscardi and Wassermann (1966) and Kovacic and Robinson (1970) found that nicotine promoted the release of norepinephrine and epinephrine from the isolated adrenal medulla of snakes and dogs, respectively. The release of catecholamines increased with increasing doses of nicotine to a maximum level beyond which responding decreased. This pattern of responding

exemplifies the biphasic stimulant-depressant (activation-blockade) effects produced by the administration of nicotine.

Slotkin and Seidler (1975) demonstrated that the acute and chronic effects of nicotine on adrenal medullary tissues are different. Acutely, high doses of nicotine depleted adrenal catecholamines and decreased the number of functional secretory vesicles. However, chronic administration at all doses had no effect on resting catecholamine and vesicle concentrations, but resulted in increased levels of tyrosine hydroxylase and dopamine β hydroxylase (both of these enzymes are involved in the synthesis of norepinephrine and epinephrine). The elevation of enzyme concentrations was found to continue for several days following the cessation of nicotine administration. It was observed that the increase in the activity of catecholamine synthesizing enzymes might reflect a compensatory response to the secretory effects of chronic or frequent nicotine administration (i.e., there was an increase in the activity or quantity of catecholamine synthesizing enzymes, such that the increase in synthetic activity counterbalanced the release of catecholamines which was induced by nicotine).

Effects on Plasma Hormone Concentrations. Several studies have reported that the inhalation of cigarette smoke in dogs (Westfall & Watts, 1962, 1963) and nicotine injection in dogs and other species (Kiser, Booher & Watts, 1956; Narita, Shibata, Waki, Egashira & Suzuki, 1973) produce an increase in the plasma concentration of epinephrine. In addition, nicotine was found to induce moderate increases in plasma norepinephrine concentration, although these

effects were not always statistically significant. Watts (1960) argues that an inverted U shaped curve best explains the relationship of nicotine effects on epinephrine secretion. Increasing doses of nicotine were found to promote increasing plasma epinephrine concentrations up to a maximum response beyond which the concentration of epinephrine tailed off (towards baseline). These results are similar to those reported by Biscardi and Wassermann (1966) and Kovacic and Robinson (1970) using isolated adrenal glands. The fact that Watts (1960) found no increase in plasma norepinephrine may reflect a difference in the peripheral plasma half life of epinephrine and norepinephrine. In adrenalectomized animals, the intra-aortic injection of nicotine was found to produce no effects on cardiac function or plasma catecholamine concentrations (Woods, Richardson, Richardson & Bozeman, 1956), thus indicating that the release of catecholamines from the adrenal glands probably accounts for the changes in cardiac function and plasma concentrations of the biogenic amines following nicotine administration.

Tsujimoto, Nishikawa, Dohi and Kojima (1974) reported that acute nicotine injections in dogs and monkeys resulted in increased levels of plasma catecholamines, glucose and free fatty acids. In both species, nicotine induced a rise in catecholamines that slightly preceded the increase in plasma glucose. Using human smokers, several studies have reported that smoking acutely elevates the plasma concentrations of epinephrine and cortisol (Hill & Wynder, 1974) and norepinephrine and free fatty acid (Carruthers, 1976). Cryer, Haymond, Santiago and Shah (1976) demonstrated that cigarette smoking produced

an increase in catecholamine and plasma glucose concentrations, and that these effects were decreased by adrenergic blocking agents. In addition, it was reported that smoking resulted in increased growth hormone and cortisol levels, which were not altered by adrenergic blockage. In another study (Spohr, Hofman, Steck, Harenberg, Walters, Hengen, Augustin, Morl, Koch, Horsch & Weber, 1979), smoking was found to increase plasma cortisol, glucose, lactate and free fatty acids.

Effects on Urinary Excretion of Epinephrine and Norepinephrine. Cigarette smoking has been found to increase the urinary excretion of epinephrine in smokers when compared with non-smoking periods (Frankenhaeuser, Myrsten, Waszah, Neri & Post, 1968) and when compared with non-smokers (Westfall & Watts, 1963; Turnbull & Kelvin, 1971). The injection of high doses of nicotine in rats was found to increase urinary concentrations of epinephrine and norepinephrine while low doses produced increases in epinephrine excretion only (Westfall, 1965). Myrsten, Post, Frankenhaeuser and Johansson (1972) found no difference in the urinary catecholamines of subjects allowed to smoke and those not smoking while engaged in a hand-eye coordination task; the authors propose that the stressful requirements of the coordination task overshadowed the effects of smoking on catecholamine excretion.

Adrenal Cortical Effects. Cigarette smoking and nicotine administration have also been reported to affect adrenal cortical functioning, steroid release, plasma glucose concentration and hormone levels, such as insulin, growth hormone and β endorphin. Several

relevant articles have been cited in preceding sections; additional studies are discussed below.

Rubin and Warner (1975) using isolated cat adrenal cortical cells found that the application of nicotine (6-600 umoles) increased the synthesis of the adrenal steroids and increased the action of adrenocorticotrophic hormone (ACTH) on steroidogenesis. It was also reported that nicotine had both direct and indirect effects on increasing the secretory activity of cortical cells. In rats, it has been reported that nicotine (0.4 mg nicotine base/kg) administration results in an increase in the plasma concentration of corticosterone within sixty minutes (Balfour, Khullar & Longden, 1975). Turner (1975) reported that in rats which had been demedullated, the increase in plasma corticosterone and free fatty acids following nicotine administration was blunted (i.e., the steroid and lipid concentration increased but to a lesser extent than in animals which had not undergone demedullation). It was also reported that demedullation did not affect the increase in corticosterone levels when test animals were stressed by being placed on an elevated platform, thus leading to the conclusion that the release of corticosterone under conditions of stress is controlled by a separate mechanism than controls its release by nicotine. It was proposed that the steroid response to the administration of nicotine was primarily mediated via activation of the sympatho-adrenal medullary system.

In conscious and anesthetized dogs (Suzuki, Ikeda, Narita, Shibata, Waki & Egashira, 1973), it was found that intravenous nicotine (0.1 mg/kg) produced an increase in the secretion of 17-OH

corticosteroids as measured in vena cava blood. Further, it was apparent that hypophysectomy eliminated the response, indicating that pituitary adrenocorticotrophic hormone is necessary to promote the release of adrenal glucocorticoids in response to the administration of nicotine. In human subjects, Hokfelt (1961) found that the smoking of two cigarettes caused an increase in plasma cortisol levels, with the maximal response occurring approximately two hours following the initiation of smoking. However, no significant differences were found in the plasma cortisol levels of smokers and non-smokers following several hours of smoking.

The Effects of Nicotine on Glucose and Insulin. The effects of nicotine or cigarette smoking on plasma glucose concentrations in animals and human subjects has been reviewed elsewhere (Larson, Haag & Silvette, 1961; Larson & Silvette, 1968; 1975). Larson et al. (1961) reviewed thirty studies which had used animals, including dogs, rabbits and rats, and found that in 28 studies the administration of nicotine had produced increases in plasma glucose. Out of a total of 23 studies using human subjects, 17 were reported as having demonstrated increases in plasma glucose following cigarette use. Several more recent studies using humans (Sandberg, Roman, Zavodnick & Kupers, 1973) and rats (Bizzi, Tacconi, Media & Garattini, 1972) have reported increases in plasma glucose concentration following cigarette smoking or nicotine injection, respectively. Glauser, Glauser, Reidenberg, Rusy and Tallarida (1970) found no differences in the fasting blood glucose concentrations of seven male smokers before and one month after stopping smoking. However, it was found that before

the cessation of cigarette use the subjects displayed elevated plasma concentrations of glucose thirty minutes following a glucose challenge -- a response that indicates that smoking may interfere with the normal homeostatic regulation of plasma glucose.

Tjalve and Popov (1973) found that nicotine had a biphasic effect upon the release of insulin from isolated pancreatic islet cells extracted from rabbits. Nicotine in some concentrations (5×10^{-5} - 5×10^{-4} Molar) produced stimulation of insulin release, while at higher concentrations (10^{-3} Molar) it inhibited the release of insulin. In human subjects, Sandberg et al. (1973) found that the smoking of three cigarettes resulted in an increase in plasma insulin within sixty minutes; the nicotine effect of increasing plasma glucose was evident within thirty minutes. Winternitz and Quillen (1977) reported that cigarette smoking had no effect on insulin concentration in human subjects following a four hour fast. Both of the preceding studies reported that cigarette smoking induced increases in the plasma concentration of growth hormone -- a peptide hormone released from the adenohypophysis which in part regulates the utilization of glucose in conjunction with insulin. In an epidemiological study involving over 500 Jamaican adults it was found that smokers displayed a decreased insulin response to a glucose challenge, indicating that chronic smoking may interfere with insulin responses (Florey, Milner & Miall, 1977).

To summarize the preceding information, it is evident that the administration of nicotine in animals and cigarette smoking in humans produces a variety of biochemical, endocrinological and physiological

effects. Specifically it has been demonstrated that nicotine and smoking promote the release and synthesis of norepinephrine and epinephrine in a variety of tissues, including: the adrenal medulla, cerebral cortex and hypothalamus, autonomic ganglia and cardiovascular tissues. In addition, it has been shown that the acute administration of nicotine results in an increase in the plasma concentration of glucocorticoids (cortisol and corticosterone), free fatty acids and glucose and that these effects may be mediated by release of catecholamines from the sympathoadrenal medullary system. The effect of nicotine on insulin, growth hormone and others remains unclear. Despite years of psychological and biological research, the role which these responses play in the development and maintenance of cigarette smoking and in the etiology of the pathologies associated with tobacco use has not been established.

New Directions for Research

One theory of cigarette smoking has proposed that nicotine through its stimulatory effects on the release of catecholamines, cortisol and/or glucose has a positive reinforcement value for the smoker, which contributes to the development and maintenance of the behavior. Recently it has been demonstrated that cigarette smoking promotes the release of vasopressin and β endorphin -- central peptides which may play a role in the modulation of central nervous system activity and cognitive functioning (Pomerleau, Fertig, Seyler & Jaffe, unpublished 1982). Additional information pertaining to these effects, and if and how they change over time, may yield much in understanding the development and maintenance of cigarette smoking.

It has been widely recognized and accepted that elevation of plasma lipids (free fatty acids, triglycerides, cholesterol) is implicated in premature cardiac and vascular disorders which are evident among smokers. Further, it has been suggested that increases in plasma cortisol and catecholamines, acting through a sensitizing effect on the myocardium, promote the incidence of sudden coronary death (Eliot, Todd, Clayton & Pieper, 1978). A better understanding of the mechanisms underlying these effects would have important implications for treatment and prevention.

Several problems exist in the manner in which earlier investigations have attempted to study the endocrinological effects of nicotine and cigarette smoking. Almost without exception previous studies have employed one of the following methodologies: a) comparison of human habitual cigarette smokers under smoking and non-smoking conditions, b) comparison of smoking and non-smoking individuals, c) investigation of the acute effects of nicotine in animals subsequent to only one exposure, d) comparison of the effects of chronic nicotine exposure using different groups of animals for each point in time, and e) examination of nicotine effects in isolated tissues. Unfortunately, the majority of these procedures do not allow for the careful examination of responding and changes in responding over time on a within subject basis during the initiation and maintenance of drug use. It has recently been proposed that the initial exposure to nicotine (and other drugs of abuse; Heartzen, Kocher & Miyasoto, 1983) and the severity of the effects produced, may influence the pattern of responding induced by subsequent exposure to

nicotine and thus determine the probability of continued self-administration (Silverstein, Kelly, Swan & Kozlowksi, 1982; Silverstein, Feld & Kozlowski, 1980; Kozlowski & Harford, 1976). More specifically, it has been argued that the more severe and unpleasant the consequences of the initial exposure to nicotine then this results in subsequent exposure also having negative attributes and therefore would decrease the probability of continued self-administration. The severity of the effects would be dependent upon the dose of nicotine administered and on the sensitivity of the individual to nicotine effects (i.e., the intensity of the aversive effects induced by nicotine will increase as the dose of nicotine administered or the sensitivity of the individual increases). Because these effects may be specific to individuals it is important that future research incorporate within subjects designs which will allow for the careful examination of individual drug exposure and response histories.

Purpose of the Study. The present study used a within subjects design to investigate the effects of the chronic intermittent intravenous administration of nicotine on several stress and possibly disease related endocrinological and biochemical parameters. Specifically, measurements were made of plasma catecholamines (norepinephrine, epinephrine and dopamine), corticosterone, insulin and glucose. The experiment was divided into three major sections each of which examined a different aspect of the role of nicotine in tobacco use: a) the effects during drug initiation, b) conditioned drug responses, and c) the reduction of stress responses. A short

explanation of each phase of the experiment is presented in the following paragraphs.

Phase I of the procedure examined the effects of the administration of nicotine on endocrine functioning during drug initiation in naive animals (rabbits). Subsequent experimental sessions examined any changes in endocrinological function which occurred as the test animals acquired increasing "experience" with nicotine. The results of this phase of the study relate to possible biological mechanisms involved in the initiation and maintenance of cigarette smoking and to the development of the cardiovascular pathologies associated with tobacco use. In particular, this phase of the study investigated the effects of chronic nicotine exposure on the response of catecholamines and corticosterone which may serve as reinforcers of the drug taking behavior, and both of which have been proposed as mediators of the elevated incidence of cardiovascular diseases among smokers (Eliot, Todd, Clayton & Pieper, 1978; U. S. Dept. of Health, Education and Welfare, 1979).

The second and third phases of the procedure made use of the nicotine "experienced" animals which were developed in Phase I of the experiment.[2] Phase II of the study examined whether the nicotine induced endocrinological and biochemical responses may be conditioned to non-drug stimuli (flashing lights were used in the present experiment). The occurrence of conditioning and the intensity and direction of the effects (i.e., similar to the unconditioned response or an opponent process) have important interpretations relating to the maintenance of cigarette smoking and reactions to abstinence. It is

possible that the conditioned drug response may serve as a discriminative stimulus for the drug taking behavior, and in the absence of the drug may contribute to craving and the aversive withdrawal syndrome.

Solomon and Corbit (1973; 1974), Solomon (1980) and Hurvich and Jameson (1974) have proposed opponent process models of motivation-affect and neural organization, respectively. These theories are based upon the concept of negative feedback systems -- there exist systems that reduce or counteract all deviations from the baseline value (the normal homeostatic value) whether the deviation is positive or negative in direction. The presentation of a stimulus (unconditioned stimulus) results in a deviation from baseline which in turn initiates the development of a counteracting or opponent process. The opponent process is postulated to be sluggish in its rate of development, latency and rate of decay when compared with the primary process. The summation of the primary and opponent processes yields a value equal to or approximating the baseline. Following repeated presentation of the unconditioned stimulus the opponent process may become a conditioned response to cues associated with the unconditioned stimulus. The opponent process will manifest its quality and intensity when the unconditioned stimulus input is suddenly terminated or when the conditioned stimuli are presented.

Siegel (1975; 1977); Poulos, Hinson and Siegel (1981); and Pomerleau (1981) using an opponent process model, have proposed an explanation for the maintenance of self-administration and the development of tolerance effects in drug use. The frequent

presentation of a drug results in the development of physiological-biochemical and/or behavioral opponent processes which counteract the primary drug effects. Over a period of time the opponent process will increase in intensity and duration, requiring the continued use of the drug in order to maintain normal functioning. Thus, the motivation for continued drug use is the avoidance of the opponent process which becomes predominant during abstinence. Under special conditions, the presentation of conditioned stimuli may elicit the opponent response independent of drug administration.

Phase II of the procedure examined the conditionability of the endocrinological and biochemical effects induced by nicotine administration. In this aspect of the study the test animals were exposed to stimuli (flashing lights) previously associated with the administration of nicotine -- however, no drug was actually administered. The intensity and direction of the conditioned responses have implications regarding the maintenance of tobacco use and reactions to abstinence. As stated previously, the conditioned drug responses may serve as discriminative stimuli for the drug taking behavior, and in the absence of the drug may contribute to the craving associated with abstinence and withdrawal. Alternatively, if the conditioned drug response is reinforcing to the smoker then it is possible that elicitation of the conditioned response may decrease the frequency and quantity of, or craving for, continued drug use.

Phase III of the procedure originates with the "psychological tool" model (Ashton & Stepney, 1982) of cigarette smoking and the reports of smokers that under stressful situations cigarette smoking

has a calming effect (Nesbitt's Paradox; Schachter, 1973). In this model it is proposed that smoking can have beneficial short-term psychological effects: the maintenance of performance under conditions of monotony and fatigue, increasing the selectivity of attention, and attenuating the effects of stress. The model is consistent with the view that nicotine is a central aspect of cigarette use because the psychic effects of smoking are attributable to nicotine.

In several studies Schachter and his associates found that when smokers were exposed to a series of painful electric shocks they consumed more cigarettes than when under a less stressful condition (Schachter, Silverstein, Kozlowski, Herman & Liebling, 1977; Schachter, Silverstein & Perlick, 1977). Silverstein (1976) reported that in a group of habitual smokers, allowed to smoke high nicotine, low nicotine or no cigarettes, the intensity of shock which subjects were willing to endure was positively related to the nicotine content of the cigarettes. In this procedure it was assumed that the more anxious the subjects the less pain they would be willing to tolerate. Thus, if smoking has an anti-anxiety effect the smoker should tolerate greater intensity shocks. Perlick (1977) found that subjects smoking high nicotine cigarettes were less irritated by a series of simulated jet over-flights than were subjects not allowed to smoke or allowed only low nicotine cigarettes. In both studies it was found that the behavior of the control non-smoking subjects was similar to that of the smokers allowed to smoke high nicotine content cigarettes indicating that continued smoking may act only to alleviate the

discomfort associated with abstinence. The similarity of responding between non-smokers and smokers allowed to smoke indicates that smoking has no beneficial stress reduction effects other than the possible reduction of noxious withdrawal symptoms.

Only a few studies have examined the endocrinological effects of nicotine under conditions of stress. Myrsten et al. (1972) reported no difference in the urinary excretion of catecholamines among a group of subjects allowed to smoke and those not smoking while engaged in a hand-eye coordination task; the authors proposed that the stressful requirements of the task overshadowed the effects of nicotine. Benwell and Balfour (1982) found that the chronic administration of nicotine (0.4 mg/kg for 40 days) in rats had no effect on adrenocortical responses to acute stress. However, in contrast to the no-drug control condition, it was found that repeated exposure to the stressful procedure did not result in adaptation of the adrenocortical response in animals receiving nicotine.

The role of nicotine in the stress ameliorating effects of cigarette smoking is not clear. During Phase III of the procedure, test animals were mildly stressed (by physical restraint) for short periods of time. The simultaneous effects of the presentation of the stressor and of nicotine were evaluated on various stress related physiological parameters: plasma catecholamines and corticosterone. The results of this procedure speak to the question of whether the stress ameliorating effects of cigarette use are physiologically based.

To summarize, the present study used a within subjects design to investigate the effects of the chronic intermittent intravenous administration of nicotine on several stress related (and possibly disease related) endocrinological and biochemical parameters. Specifically, measurements were made of plasma catecholamines, corticosterone, insulin and glucose. The experiment may be divided into three major sections each of which examined a different aspect of the role of nicotine in tobacco use: a) the effects during drug initiation, b) conditioned drug responses, and c) the postulated reduction of stress induced responses.

Methods

Overview of Design and Procedures

The study investigated the effects of a) drug initiation, b) conditioning of nicotine induced responses, and c) the effects of mild physical restraint stress on several hormonal and biochemical responses induced by nicotine administration and changes in these responses with chronic exposure to nicotine. The study included four experimental conditions: three dosages of nicotine and physiological saline solution as a control. Experimental animals (see section on Subjects, page 37) were randomly assigned to one of the conditions and received daily administrations of 0.025 mg, 0.050 mg or 0.100 mg nicotine/kg of body weight or physiological saline solution (see section on Drug Administration, page 45). Nicotine was administered by indwelling intravenous catheters four times a day for thirteen consecutive days. A complete description of nicotine injection is presented in the following section on drug administration.

The experiment consisted of several phases which were conducted over a period of approximately three weeks. A time-line detailing the sequence of experimental events is presented in Appendix A (page A2, Volume II). Details of each of the experimental manipulations are presented below. The procedure may be roughly separated into four phases: a) pre-experimental manipulations and, b) Phases I - III of nicotine administration.

The pre-experimental manipulations included a four day gentling period, followed by four days on which body weights were measured (see section on Pre-Surgical Procedures, page 38). The body

weight values were used to compute drug dosages. Antibiotic therapy was instituted on the first day of body weight measurements as a precaution to decrease the probability of infection following surgical or other experimental procedures. All animals were surgically prepared with chronic indwelling intravenous catheters (see section on Surgical Procedure, page 40), surgical procedures being conducted after a minimum of four days of antibiotic therapy, and two days prior to the initiation of nicotine administration. The catheters, one intra-arterial and the other intravenous were used for the acquisition of sample fluids (arterial catheter) and for the administration of nicotine or saline (venous catheter) solutions.

Phase I of the study investigated the effects of nicotine administration on endocrinological function in previously nicotine naive animals. This aspect of the experiment encompassed Days 1-7 of nicotine administration. Blood (plasma) samples were taken on Days 1, 3 and 7. Subsequent experimental sessions (i.e., on Days 3 and 7) evaluated changes in the nicotine induced endocrinological responses as the test animals acquired increasing "experience" with nicotine.

Plasma samples acquired during Phase I of the experiment were analyzed for their content of catecholamines (epinephrine, norepinephrine and dopamine), corticosterone, insulin and glucose. An outline of the methods used for each assay is presented in Appendix B pages B8-B30, Volume II). It has been demonstrated in previous research that catecholamines, glucocorticoids and glucose are affected by the administration of nicotine (see Introduction section for discussion of effects). However, it has not been clearly demonstrated

whether the nicotine induced responses are altered by increasing prior exposure to nicotine (i.e., whether there are changes in the drug induced responses due to the development of pharmacological and/or behavioral tolerance or sensitization).[3]

Phase II of the investigation examined conditioning of the endocrinological effects (Siegel, 1977) induced by nicotine administration. The procedure was based on the work of Siegel (1972, 1975, 1977) which investigated the conditioning of insulin and morphine induced responses. Throughout Phases I and II of the study, a flashing light was paired with nicotine administration as a conditioning stimulus. To investigate the conditioning of endocrinological responses, physiological saline solution was substituted for nicotine and was administered to all test animals in a manner identical to previous nicotine exposure. The test for conditioning of endocrinological responses was conducted in the morning on Day 9 of drug administration, with data from Day 7 used for comparison of effects.

As in Phase I, plasma samples were analyzed for catecholamines, corticosterone, insulin and glucose. Conditioning of the endocrinological responses, and the intensity and direction of the effects (i.e., similar to the unconditioned response or an opponent process; cf. Siegel, 1975, 1977) may be related to the maintenance of cigarette smoking and the abstinence syndrome (it is possible that the conditioned drug response may be aversive to the smoker, or it may serve as a discriminative stimulus in the maintenance of cigarette smoking or for the development of craving in the abstinence syndrome).

The final phase (Phase III) of the experiment originated with the "psychological tool" model (Ashton & Stepney, 1982) of cigarette smoking. In this model it is proposed that cigarette smoking (possibly due to the nicotine content of the cigarette) may have an ameliorating effect on responses to stress. During this aspect of the procedure, test animals were stressed by being placed under partial physical restraint (Rabbit Restrainer, Model XPL-502-AR, Plas Labs, Plastics Manufacturing and Supply, Inc., Lansing, MI 48906) concurrent with the period of drug administration. The effects of the simultaneous presentation of the stressor and nicotine were evaluated on several stress related physiological parameters, including plasma catecholamines and corticosterone. When compared with appropriate control conditions (i.e., presentation of the stressor without nicotine) the results speak to the question of whether the stress ameliorating effects of nicotine, and possibly cigarette use, are physiologically based. The test sessions were conducted on Days 11 and 13 of nicotine administration, with the order of presentation counterbalanced (animals in the saline control condition were stressed on only one day; data for the alternate day were used as No Stress/No Nicotine control values).

Following termination of all experimental sessions a post-mortem examination was performed on each test animal. Euthanasia was performed by the intravenous administration of a lethal dose of Pentobarbital. Determinations were made of catheter placement to assess the exact site of nicotine infusion and sample withdrawal and the condition of surrounding vessels. Additionally, observations were

made on all major internal organs to determine the presence of any pathological condition which might influence any of the variables being studied. Specific attention was given to any sites of infection, hemorrhage, emboli or infarct. Histologic examination of kidney, liver or other tissues was made if warranted by macroscopic determinations (i.e., indications of infection or of thrombus formation). Body weight determinations were made on all animals at the time of autopsy.

All plasma samples were collected between 0900 and 1230 hours, coinciding with the first and second of the four daily administrations of nicotine. Samples were collected immediately before nicotine administration (time zero) and 30 minutes following the initiation of drug administration during the first of the daily drug sessions. During the second of the daily drug sessions, samples were collected immediately before drug administration (time zero) and at 15, 45 and 90 minutes following initiation of drug infusion. The acquisition of multiple samples during each test session permitted investigation of the time-course of drug induced effects on each of the hormones being studied (i.e., time of maximum response or percent of response at specific intervals). The collection of samples at a constant time of day was intended to decrease variance in hormone levels due to diurnal changes, particularly for catecholamines and corticosterone.

Summary of Design

The study examined the effects of the intravenous administration of nicotine on several biochemical and endocrinological variables: plasma glucose, insulin, catecholamines and

corticosterone. Four drug dose conditions were included in the experiment: 0.025 mg, 0.050 mg and 0.100 mg nicotine/kg of body weight, or physiological saline (control). The initial phase of the procedure examined the effect of nicotine on endocrine responses as a function of the extent of prior nicotine exposure. Phase II examined whether there was conditioning of the nicotine induced biochemical and endocrine effects. Phase III examined the endocrinological responses to stress with and without the simultaneous administration of nicotine.

Subjects

Sixteen[4] male New Zealand White rabbits[5] (Oryctolagus cuniculus[6]; obtained from Hazelton-Dutchland Laboratory Animals, Inc., Denver, PA) were used in the experiment. Twelve animals successfully completed the two weeks of nicotine administration, three animals in each of the drug dosage conditions. The pre-experimental body weight for each animal was approximately 3.0-3.5 kg, corresponding to an age of approximately 8 months. Animals were randomly assigned to one of the four experimental conditions (i.e., three different nicotine dosages and saline control), with the stipulation that the initial mean body weight for each of the conditions be approximately equivalent.[7,8]

All animals were individually housed in stainless steel wire cages (61 cm x 61 cm x 37 cm height, Lab-Care Caging Systems, Research Equipment Co., Inc., Bryan, TX) and maintained in an independent animal vivarium separate from other laboratory facilities. The floor of each cage consisted of a stainless steel grid (with openings

approximately 1 cm x 1 cm) suspended over a removable bedding tray filled with absorbent material (Lab-Sorb, Red River Commodities, Fargo, ND). The vivarium was maintained on a 12 hour light/dark cycle beginning at 0600 hours (EST), with a temperature of $20^{\circ}\pm1^{\circ}$C and relative humidity of approximately 50%.

Animals were allowed unrestricted access to water (supplied in 300 ml bottles equipped with a sipper tube) throughout the experiment. Rabbit diet (Charles River Rabbit Formula, Country Foods-Div. of Agway, Inc., Syracuse, NY) was available ad libitum, except that food was withdrawn 8 hours (between midnight and 0100 hrs.) preceding all surgical and experimental manipulations. Supplementary diet, including carrots and green vegetables, was provided at least twice each week.

An overnight fast (beginning between midnight and 0100 hrs.) prior to experimental sessions was necessary in order to achieve baseline physiological levels (i.e., to reduce intra- subject variance) for the plasma concentrations of glucose and insulin, both of which are highly variable depending upon the recency of feeding. A period of fasting prior to surgery decreased the likelihood of emesis induced by the anesthetic.

Pre-Surgical Procedures

A gentling period of four days duration was conducted prior to the initiation of the experiment. During this period, all animals were handled twice each day by the experimenter to acclimate them to human contact and experimental manipulation. Subsequent to gentling, there was a four day period during which daily measurements were made

of body weight for each animal. Body weight measurements were made using a Sartorius electronic scale (Model 3801 MP-BCD) equipped with a universal programmer/printer (Model 7080). Body weight was computed as the average of ten individual weighings. The electronic scale was programmed to automatically make ten sequential weighings (approximately 1/second) and to average the values. The average weight obtained by this procedure tends to be more accurate than individual weighings which may be influenced by body movement of the animal.

Three days prior to the scheduled date of surgery all animals began receiving antibiotics. Antibiotic therapy consisted of the intramuscular injection of 30,000 units Bicillin/kg of body weight and the intramuscular injection of 4 milligrams Gentamicin Sulfate/kg. Gentamicin (Gentavet, Burns-Biotec Laboratories, Inc., Omaha, NE 68103) was administered as a single dose injection on a daily basis, while Bicillin (Bicillin Fortified, Wyeth Laboratories, Inc., Philadelphia, PA 19101) was administered every third day. Bicillin is a suspension of 150,000 units Penicillin G Benzathine and 150,000 units Penicillin G Procaine/ml, for intramuscular injection. Administration of Gentamicin was continued for the duration of the experiment. Bicillin was administered only during the pre-operative phase of the study (i.e., three days prior to surgery and immediately before surgery).

The antibiotic regimen was intended to reduce the probability of post-operative infections and/or infections promoted by later experimental manipulations. In particular, the implantation of indwelling chronic catheters may provide an excellent route of

invasion for pathogenic micro-organisms, and therefore necessitates the use of precautionary measures. Bicillin acts by interfering with the synthesis of bacterial cell wall constituents during cellular replication and is most effective against gram positive bacteria. Gentamicin interferes with protein synthesis in susceptible organisms, and is most effective in combatting infections produced by gram negative micro-organisms. In particular, Gentamicin is effective against infections due to Klebsiella, Proteus and Psuedomonas which are frequent pathogens of human patients equipped with chronic intravenous or urinary catheters (Physicians Desk Reference, 1980; Gilman et al., 1980). The administration of the antibiotics by intramuscular injection was necessary because Bicillin and Gentamicin may not be mixed in vitro with solutions containing heparin (which was used to flush the intravenous catheters). All injections were administered at the end of the day in order to decrease interference with experimental variables (i.e., conditioning of stress related responses). Neither Bicillin (personal communication with Drs. Sylvario and Lewis, Wyeth Laboratories, Inc., Philadelphia, PA 19101) nor Gentamicin (personal communication with Drs. Bigbee and Boraski, Schering, Co. for Burns-Biotec Laboratories, Inc., Omaha, NE 68103) are known to exert direct effects upon any of the endocrinological systems being investigated.

Surgical Procedure

Animals were anesthetized with a combination of Ketamine HCl (Vetalar, 45 mg/kg body weight, Parke, Davis and Co., Detroit, MI) and Xylazine (Rompun, 2.5 mg/kg body weight, Cutter Laboratories, Inc., Shawnee, KS). Ketamine and Xylazine were administered by

intramuscular injection in the region of the gastrocnemius muscle. Additional doses of Ketamine (approximately 5-10 mg/kg) were administered during the surgical procedure as required to maintain analgesia.

Ketamine is a rapid acting non-narcotic, non-barbiturate anesthetic agent for veterinary use. It produces an analgesic state which has been termed "dissociative," in that it appears to selectively interrupt association pathways to the brain. The pharmacological action of Ketamine is characterized by profound analgesia, mild cardiac stimulation and respiratory depression, and the maintenance of normal pharyngeal-laryngeal reflexes (Marshall & Wollman, in Gilman et al., 1980). Xylazine is a sedative and analgesic agent as well as a muscle relaxant. Its effects are based on the inhibition of intraneural transmission of impulses in the central nervous system. Ketamine and Xylazine when administered in combination by intramuscular injection result in the induction of surgical analgesia in 5-10 minutes, with a duration of 20-50 minutes. Pre-test data demonstrated that administration of the doses listed above resulted in satisfactory analgesia being maintained for approximately 35-45 minutes. Selection of the combination of Ketamine and Xylazine for use as surgical anesthetics was based on the following characteristics: a) they have minimal effects on blood pressure and heart rate and therefore would not compromise tissue perfusion, b) they do not significantly decrease respiration or pharyngeal reflexes (i.e., did not require the use of respiratory support), and c) they have a wide range of effective doses without toxicity.

Following sedation of the animal, the fur on the ventral surface of the neck and dorsal cervical regions was removed with electric shears and depilatory cream (Neet Cream Hair Remover, Whitehall Laboratories, Inc., New York, NY). The skin surface was then cleansed with Povidone-Iodine topical antiseptic solution (Camall Co., Washington, MI 48094) and 70% alcohol solution. The animal was then transferred to the operating table and restrained in a supine position with the neck hyperextended. All surgical procedures were conducted under aseptic conditions in designated university facilities (Veterinary Surgery Division, Department of Laboratory Animal Medicine, USUHS).

A two centimeter incision was made in the skin covering the carotid notch left of the midline from the caudal end of the larynx toward the suprasternal notch. Blunt dissection was used to separate the muscles ventral to the trachea and expose the left common carotid artery (see Fig. 1, Appendix A, page A3, Volume II). Retraction was used to maintain visibility in the surgical field between the sternomastoid and sternothyroid muscles. The surgical field was periodically irrigated with sterile saline solution to prevent dehydration of the exposed tissues.

The fascia from the carotid sheath was separated to expose approximately 1.5 centimeters of the artery. Dissection was performed using mosquito (fine tipped) forceps to prevent damage to the vagus nerve and internal jugular vein which accompany the carotid artery. Two ligatures (surgical silk, 3-0 gauge, Ethicon, Inc., Summerville, NJ) were placed on the cephalic portion of the artery, leaving long

ligature ends. A bulldog clamp was used to compress the artery near the caudal site of exposure. Several additional loosely placed ligatures were situated between the two constricted areas of the artery.

Slight tension was put on the cephalic ligatures to prevent movement of the vessel while a cut was made in the artery. Fine tipped scissors (Vannas Ultra Micro Scissors, Roboz Surgical Instrument Co., Inc., Washington, DC) were used to make a V-shaped cut approximately 1/3 the diameter of the artery, the points of the scissors being directed toward the heart. A catheter (Intramedic Polyethylene Tubing, PE-60, ID 0.58 mm, Clay Adams, Div. of Becton, Dickinson and Co., Parsippany, NJ) was inserted in the artery and directed toward the heart. The bulldog clamp was then removed and the catheter advanced approximately 4-6 centimeters or until a region of adequate blood flow had been reached (i.e., a point at which blood could be easily withdrawn via the catheter). The ligatures around the caudal aspect of the artery were tightened and tied off. In addition, the long ends of the cephalic ligatures were tied around the catheter to further anchor it in place.

Cannulation of the left external jugular vein was conducted in a manner similar to that used for the carotid artery. The jugular catheter was inserted approximately 5-6 centimeters and was directed toward the heart. During surgery the catheters were periodically (once every 15 minutes) flushed with sterile physiological saline solution containing Heparin (50 units/ml).

The catheters were extended subcutaneously to a point overlying the cervical spine where they were exteriorized. Underlying tissue and fascia at the incision sites were closed with GI chromic gut sutures (3-0 gauge, Half Circle Tapered Needle, Ethicon, Inc., Summerville, NJ) using a continuous appositional suture pattern. Cutaneous tissues were closed with Ethilon sutures (3-0 gauge, Half Circle Needle with reverse cutting edge, Ethicon, Inc., Summerville, NJ) using a simple discontinuous pattern. A purse-string everting suture was placed around the point of exit of the catheters. Nitrofurazone 0.2% topical antibiotic ointment (Wendt Laboratories, Minneapolis, MN 55437) was applied to all incision sites for three days following surgery.

Following completion of surgery each animal was fitted with a rabbit equipment vest, equipped with a stainless steel catheter harness (Model 410-M, Spalding Medical Products, Arroyo Grande, CA 93420) designed to protect the incisions and catheters. Animals were then returned to their home cage. The arterial and venous catheters were connected to a three-channel fluid swivel (Model 310, Spalding Medical Products, Arroyo Grande, CA 93420) attached to the roof of the cage (see Fig. 2, Appendix A, page A5, Volume II). The fluid swivel was in turn connected to intravenous fluid reservoirs located external to the cage. A slow infusion of the test drug or of physiological saline solution with Heparin was maintained in each catheter at all times. For a more complete description of drug infusion and catheter maintenance please refer to the section on Drug Administration.

The post-surgical recovery of all animals was monitored every four hours between 0800 and 2330 hours. Assessments were made of pupillary and eyeblink reflexes, superficial tactile and deep tendon pain responsivity, righting response and general locomotor function (i.e., limb movement). These measurements were made to determine if any peripheral or central nervous system damage resulted from surgery (i.e., from thrombus formation). Observations were also made to determine if excessive or unusual bleeding was evident, thus requiring adjustment of anticoagulant therapy. A minimum of 48 hours was allowed for post-surgical recovery prior to the initiation of nicotine administration.

Drug Administration

Nicotine and physiological saline solution containing Heparin (Lypho-Med, Inc., Chicago, IL 60651) were administered via the chronic indwelling intravenous and/or intra-arterial catheters. The dosage of each drug and the volume of fluid infused was controlled by two Manostat Cassette Peristaltic Pumps (Manostat Corp., New York, NY), see Figure 3, Appendix A (page A6, Volume II). Operation of one of the pumps was regulated by a Chrontrol 24 hour multiple programmable automatic timer (Lindburg Enterprises, Inc., San Diego, CA 92111).

A pair of 7 watt light bulbs was suspended 4-5 cm above the roof of each cage and blinked at a rate of approximately 1 Hz whenever the timing device activated the peristaltic pump. The lights were turned on simultaneously with the activation of the drug infusion pump and were maintained in the activated (blinking) condition during the

entire 20 minute drug administration period (under these conditions it was expected that the activation of the flashing lights would slightly precede the delivery of nicotine to its site of effect). The lights were operated by a Gerbrands model G4660 28 volt DC Power Supply and a model E1100H Electronic Timer. The flashing lights were used as a conditioned stimulus associated with the administration of nicotine. In Phase II of the study, the flashing lights were paired with saline (drug) administration to examine whether there was conditioning of the biochemical responses produced by nicotine.

Nicotine hydrogen tartrate (FW=498.44 with 2 H_2O, BDH Chemicals Ltd., Poole, England) was dissolved in sterile physiological saline solution to make the following dosages: 0.025 mg, 0.050 mg and 0.100 mg/kg of body weight (computed as nicotine base)[9]. Solutions were adjusted to pH 7.4 by the addition of NaOH and then filtered (Sterifil D, 0.22 umeter pore size, Millipore, Nihon Millipore Kogyo K.K., Yonezawa, Japan) to remove undissolved or other particulate material. All nicotine doses were administered in a volume of 1.5 ml; physiological saline was administered as a control. These doses of nicotine were selected on the basis of reviews of research pertaining to the pharmacological and toxicological effects of nicotine (Larson, Haag & Silvette, 1961; Larson & Silvette, 1968; 1975) and also on pre-test data.

The nicotine and saline solutions were administered by constant rate intravenous infusion over a twenty minute period (0.075 ml/min, total volume 1.5 ml). Drug administration occurred four times each day between 0900 and 1600 hours. Drug sessions were scheduled to

begin every two hours during the specified period (i.e., sessions began at 0900, 1100, 1300 and 1500 hours). This mode of administration allowed for investigation of the acute biochemical effects of nicotine in animals receiving chronic intermittent exposure, and changes in responding with increasing prior exposure. In addition, the pattern of administration is analogous to that of the human cigarette smoker; repetitive self-administration during the day followed by an overnight period of abstinence. Because of the similarity to the human condition, this method may be a more appropriate model for examining cigarette/nicotine effects on humans than are procedures using single bolus injections or continuous exposure.

The nicotine and saline solutions were administered through the venous catheters. Between 1600 and 0900 hours, when the venous catheters were not in use for nicotine administration, the catheters were maintained on a slow infusion of physiological saline containing Heparin to prevent clotting (flow rate = 0.50 ml/hour, concentration of Heparin = 25 units/ml). A two-way valve located in the infusion tubing allowed for alternation between infusion of each of the solutions. The venous catheter allowed for the administration of nicotine at a constant rate of delivery throughout the 20 minute infusion period.

Throughout the experiment the arterial catheters were used exclusively for the acquisition of blood samples. Except when samples were being taken, the arterial catheters were maintained by infusion of physiological saline solution containing Heparin (flow rate = 0.50

ml/hour, concentration of Heparin = 25 units/ml). Slight adjustments in the infusion of Heparin solutions were made as required if excessive hemorrhaging or clotting became evident.

Sample Collection

Blood samples were obtained at six times during individual drug sessions. Samples were taken at time zero (immediately prior to nicotine administration) and 30 minutes following drug initiation during the first drug infusion of the day. In addition, samples were obtained at time zero and at 15, 45, and 90 minutes following the initiation of the second of the daily drug infusions. All samples were approximately 3 ml of whole blood. The hematocrit (percent packed red blood cells) for every animal was determined using blood from the pre-nicotine, time zero sample.[10] If the hematocrit was below 20%, testing procedures involving the affected animal(s) were discontinued until recovery occurred.[11]

The samples were collected in syringes containing 100 units of Heparin to prevent clot formation. Following collection, samples were immediately transferred to 10 ml polypropylene test tubes and maintained at $4^{\circ}C$ (on ice). Within 90 minutes of collection, all samples were centrifuged at 1500 x g for 10 minutes at $4^{\circ}C$ to separate the cells and plasma. Aliquots of plasma were then transferred to 1 ml polypropylene sample tubes which were stored at $-70^{\circ}C$ (or $-20^{\circ}C$) until needed in the appropriate assay (see Design and Appendix B regarding specific assays, pages B8-B30, Volume II).

Results

In order to most effectively and concisely present the experimental findings, they have been organized into sections corresponding to Phases I-III (Phase I: Drug Initiation, Phase II: Conditioning of Endocrinolgoical Responses, and Phase III: Effects of Nicotine on Stress Induced Endocrine Responses) of the experiment. Within each of these sections the experimental results are further organized and discussed by individual assay procedures (i.e., catecholamines, corticosterone, glucose and insulin). Detailed tables corresponding to each of the statistical analyses are provided in Appendix C, pages C32-C107, Volume II. Detailed figures presenting the experimental data are contained in Appendix D, pages D108-266, Volume II.

Data for Phases I and II of the experiment were analyzed by repeated measures Analysis of Variance (ANOVA) using the General Linear Models (GLM) subroutine of the SAS Statistics package (SAS Users Guide: Statistics, 1982 Edition, SAS Institute Inc., Cary, NC 27511). (Only the data collected from those animals which completed the study were used in the ANOVA procedure, however all available data were included in all subsequent analyses, tables and figures.) In each of the analyses Drug Condition (i.e., Nicotine dose: 0.025, 0.050, 0.100 mg base/kg or physiological saline) was considered a between subjects variable, while the Day (i.e., the day of drug administration and testing) and Sample (i.e., sample 0 [baseline]-5 on each test day) were treated as within subjects variables. Subjects were treated as a random effect in the analyses. (The examination of the experimental data using a repeated measures ANOVA may be more

conservative than some other possible analyses, such as an analysis of covariance using the sample 0 baseline value as a covariate.) Post-hoc comparisons of individual main effects were performed using Tukey's Studentized Range (HSD) statistic.

Data from Phase III of the experiment were analyzed using the Student's t statistic for related and unrelated samples, dependent upon the comparison being performed. Comparisons were performed within each drug condition between the two testing days (i.e., Stress with Nicotine and Stress without Nicotine) and for the sequence of samples obtained within each day (i.e., the change from baseline [sample 0]). In addition, the responses of animals in each of the nicotine conditions were compared with those of control (saline) animals with and without the application of physical restraint stress. A more complete description of the individual comparisons accompanies the text of "Phase III: Stress Induced Responses" presented later in this section.

Phase I: Drug Initiation

Catecholamines: Statistically significant increases in plasma concentrations were observed for Norepinephrine and Dopamine, while Epinephrine displayed similar although non-significant trends (see Tables 1, 2 and 3, Appendix C, pages C32, C37 and C41, for the ANOVA tables, and Figures 1-15, Appendix D, pages D111-D140 for the detailed graphic presentation of the data). The norepinephrine, epinephrine and dopamine responses for Phase I of the experiment are presented in the figures on the following pages.

Phase I: Norepinephrine

Figure Legend

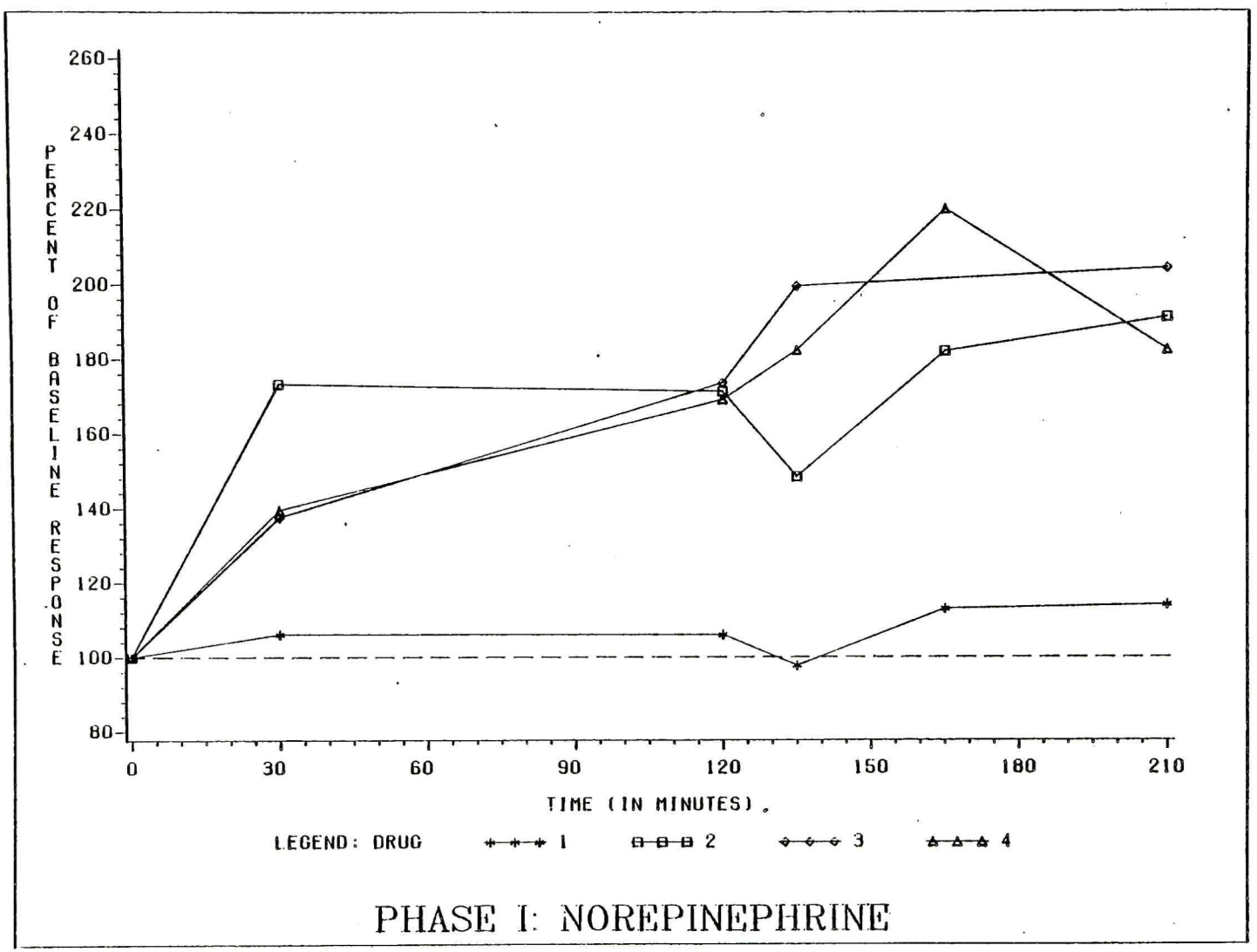
Drug Condition 1 = Saline Control

Drug Condition 2 = 0.025 mg Nicotine/kg

Drug Condition 3 = 0.050 mg Nicotine/kg

Drug Condition 4 = 0.100 mg Nicotine/kg

(In order to simplify the presentation of data from Phase I the data from Days 1, 3 and 7 have been combined. If the reader wishes a more detailed presentation of the day by day changes in responding he is referred to the detailed figures presented in Appendix D.)



PHASE I: NOREPINEPHRINE

Phase I: Epinephrine

Figure Legend

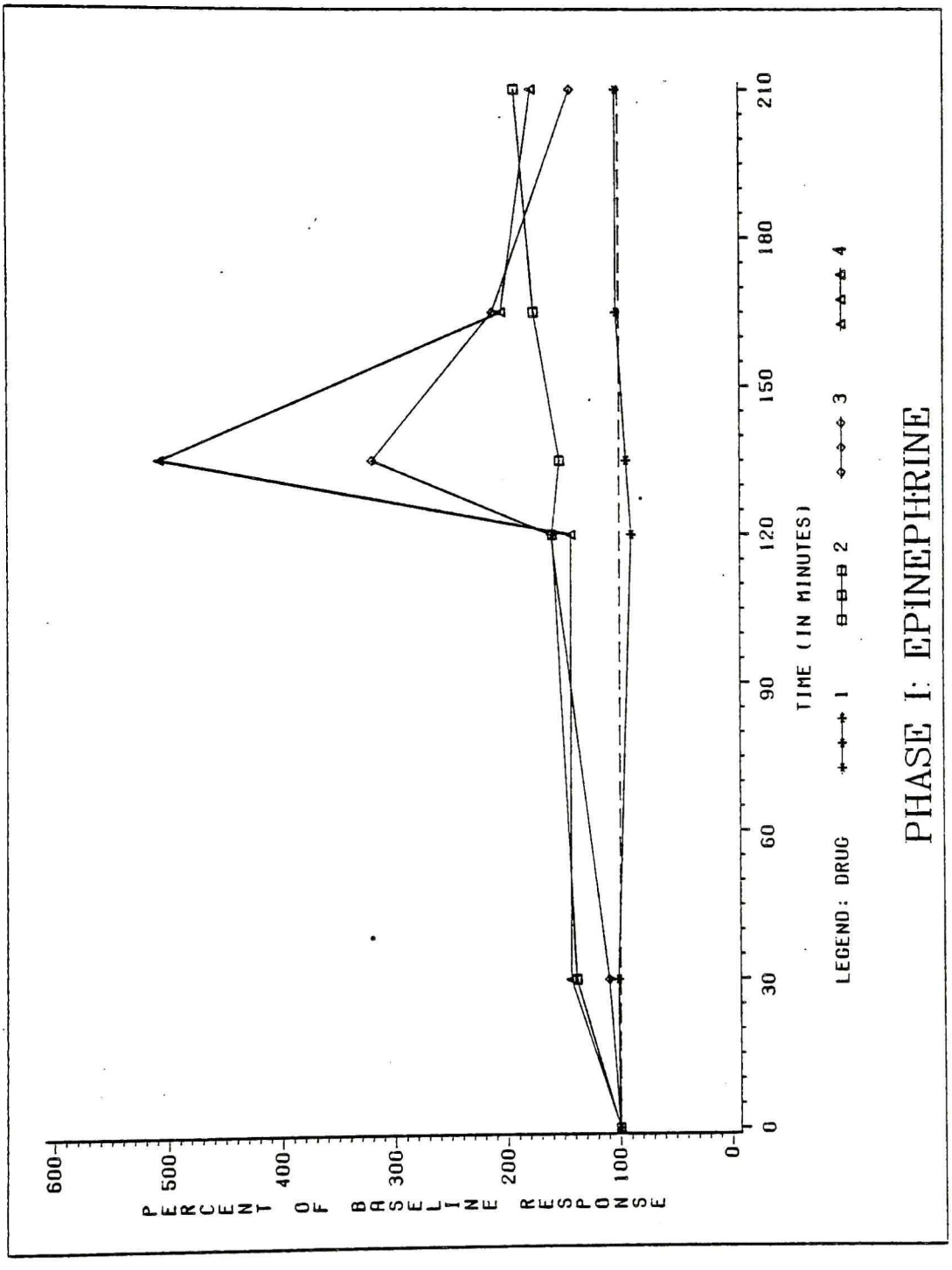
Drug Condition 1 = Saline Control

Drug Condition 2 = 0.025 mg Nicotine/kg

Drug Condition 3 = 0.050 mg Nicotine/kg

Drug Condition 4 = 0.100 mg Nicotine/kg

(In order to simplify the presentation of data from Phase I the data from Days 1, 3 and 7 have been combined. If the reader wishes a more detailed presentation of the day by day changes in responding he is referred to the detailed figures presented in Appendix D.)


PERCENT OF BASELINE RESPONSE
600
500
400
300
200
100
0
0
30
60
90
120
150
180
210
TIME (IN MINUTES)
LEGEND: DRUG 1 2 3 4
PHASE I: EPINEPHRINE

Phase I: Dopamine

Figure Legend

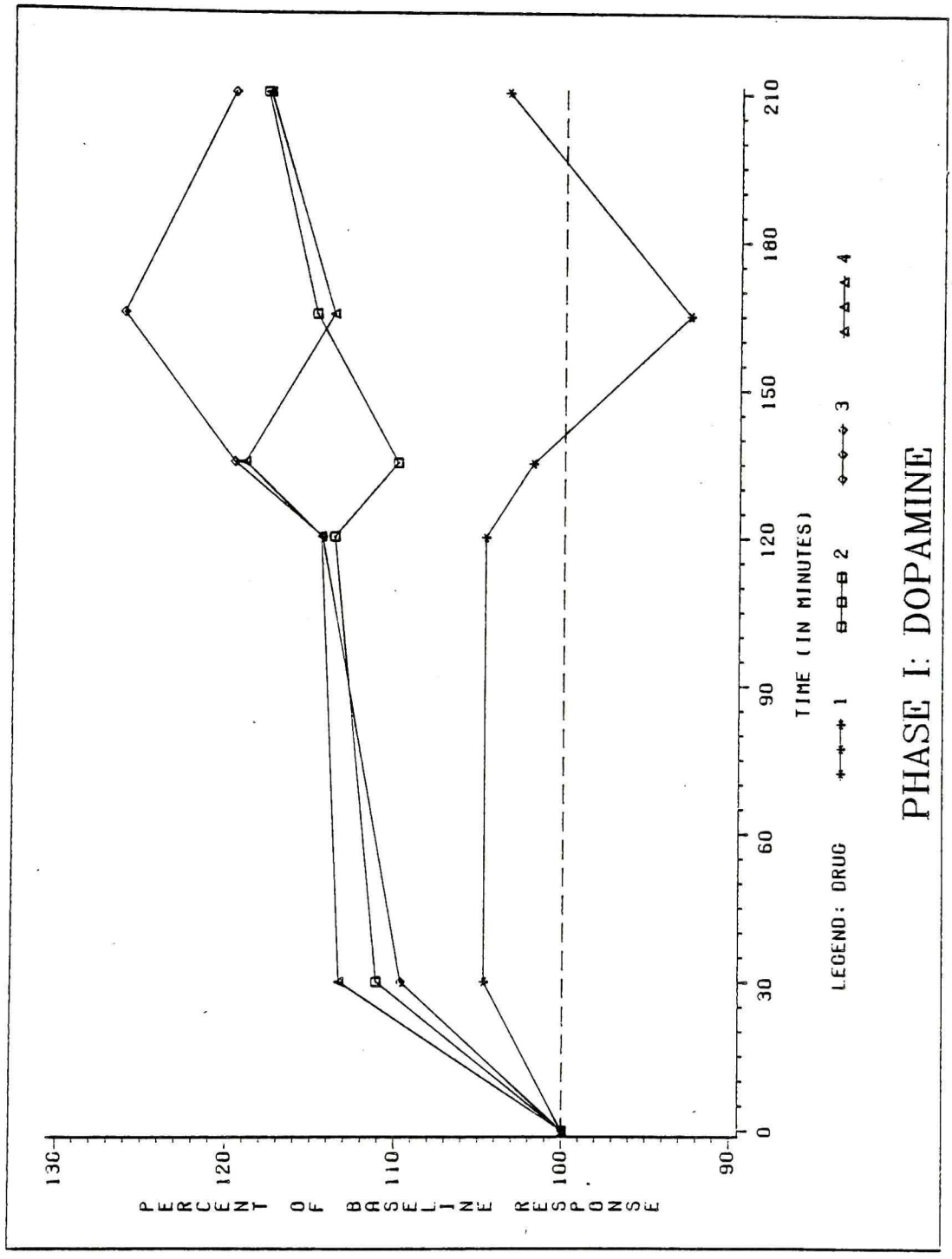
Drug Condition 1 = Saline Control

Drug Condition 2 = 0.025 mg Nicotine/kg

Drug Condition 3 = 0.050 mg Nicotine/kg

Drug Condition 4 = 0.100 mg Nicotine/kg

(In order to simplify the presentation of data from Phase I the data from Days 1, 3 and 7 have been combined. If the reader wishes a more detailed presentation of the day by day changes in responding he is referred to the detailed figures presented in Appendix D.)



PHASE I: DOPAMINE

Significant main effects in the analyses for Norepinephrine and Dopamine were found for the Sample ($F=11.50$, $df=5,40$, $p<0.0001$ and $F=3.50$, $df=5,40$, $p<0.0102$, respectively), while a non-significant trend for the Sample was observed in the analysis of Epinephrine responses ($F=1.67$, $df=5,40$, ns). A significant effect of the Testing Day was found in the analysis of Epinephrine ($F=4.29$, $df=2,16$, $p<0.0323$), while Norepinephrine and Dopamine displayed similar although non-significant effects ($F=2.97$, $df=2,16$, ns and $F=2.53$, $df=2,16$, ns, respectively). A significant main effect for the Drug Condition was not found in any of the analyses.

The interaction of the Drug Condition and the Sample was significant for Norepinephrine ($F=1.62$, $df=15,40$, $p<0.1124$), while Epinephrine and Dopamine displayed similar tendencies but these were not significant ($F=1.06$, $df=15,40$, ns and $F=1.39$, $df=15,40$, ns, respectively). No additional interactions were found to be significant (see Tables 1, 2 and 3, Appendix C, pages C32, C37, and C41).

Comparison of the group mean responses for each effect was performed using Tukey's Studentized Range Test. Examination of the Drug x Sample interaction in the analysis of Norepinephrine responses indicates that the administration of nicotine in the medium (0.050 mg/kg) and high (0.100 mg/kg) doses resulted in significant increases in plasma norepinephrine by the third or fourth post-nicotine infusion sample, while similar non-significant effects were evident in the low nicotine (0.025 mg/kg) condition; the administration of saline solution had no significant effect on plasma norepinephrine

concentrations. There were no significant differences in the baseline plasma norepinephrine concentrations for any of the drug groups. Similar patterns of responding were evident for epinephrine and dopamine following the administration of nicotine (these effects can be seen in the figures presented on the subsequent pages), however these effects were not significant probably due to the small number of subjects used in the study and the very high variability in baseline hormone levels which were observed.

Although no significant main effect for the drug condition was found in any of the analyses of catecholamine responses, it may be useful to discuss the pattern of group relationships. Increased plasma concentrations of norepinephrine and epinephrine were observed for all nicotine doses when compared with the saline control group (see Tables 1 and 2, Part II, Appendix C, pages C33 and C38, and Figures 5 and 10, Appendix D, pages D120 and D130).

A somewhat different pattern of responding was evident for dopamine (see Table 3, Part II, Appendix C, page C42, and Figure 15, Appendix D, page D140), with increased plasma concentrations of dopamine among animals in the high and medium nicotine conditions when compared with those animals receiving the low nicotine dose. The mean dopamine response for the animals in the saline control group fell between the responses of the high and low nicotine groups and was not significantly different from either extreme.

For all forms of catecholamines measured in this procedure, it was observed that a higher mean plasma concentration occurred on the first day (Day 1) of drug administration than was evident on Day 7

(see Tables 1, 2 and 3, Part II, Appendix C, pages C33, C38 and C42). The differences in the mean plasma concentration of epinephrine were significant, while the differences in norepinephrine and dopamine were not. For norepinephrine and dopamine the decrease in the mean plasma concentration was evident by the third day (Day 3) of drug administration, there being no detectable additional decrement in responding on Day 7. The decrease in epinephrine responsivity was somewhat more gradual, with the mean value for Day 3 falling between and not significantly different from those on Days 1 and 7 (the mean plasma concentration of epinephrine on Day 7 was significantly lower than that which was measured on Day 1).

Tables 1, 2 and 3, Part IV, subpart A (Appendix C, pages C36, C40 and C44) present the group mean responses of norepinephrine, epinephrine and dopamine for each drug condition on each of the observation days during Phase I of the experiment. On Day 1 those animals which were administered nicotine (in all doses) displayed increased mean plasma concentrations of all three catecholamines when compared to the saline control group. There was a general diminution in responsivity over the remaining observation days of Phase I (see the detailed Figures 5, 10 and 15, Appendix D, pages D120, D130 and D140). Some exceptions to this pattern are evident, as is apparent for norepinephrine in the high nicotine condition and for epinephrine and dopamine in the medium dose condition. No large or consistent pattern of changes (increases or decreases) in the mean plasma concentration of catecholamines was evident for animals in the control group. It should be noted that differences in the baseline values

between the three test days may in part explain some of the inconsistancies evident in these tables (no explanation for these differences was evident).

Comparison of the mean response for each of the six samples (Samples 0 [baseline] - 5) indicates that nicotine administration resulted in consistently increased plasma concentrations of all three forms of catecholamines as compared to the baseline pre-nicotine infusion levels, as is evident in the graphs of Phase I responses on the preceding pages (see also the detailed Figures 2-4, 7-9 and 12-14, Appendix D, pages D113-D118, D123-D128 and D133-D138). The administration of nicotine resulted in increased plasma concentrations of norepinephrine, with significant differences for the comparison of the group means for samples 2-5 with the baseline value. Epinephrine also displayed increased plasma concentrations subsequent to nicotine infusion, however the mean response for samples 1-5 was not significantly larger than the sample 0 (baseline) value. There was a general increase in plasma concentrations of dopamine over the period of observation (samples 0-5) although none of the comparisons were significant.

Tables 1, 2 and 3, Part III (Appendix C, pages C35, C39 and C43) present the mean response data for the saline control condition and the combined data for all nicotine doses subdivided by sample for Day 7. In Part IVB of each table (see Appendix C, pages C36, C43 and C47) are presented the group mean responses for each drug condition and sample, using the combined data for all observation days during Phase I of the procedure. Examination of the mean values and the

change scores presented in these tables indicates that animals receiving nicotine displayed increased (compared to baseline) plasma concentrations of norepinephrine, epinephrine and dopamine, and the increases were generally larger (although nonsignificant) than those occurring in the saline control condition (see the Figures of the Phase I catecholamine responses which are included in the text on preceding pages). In contrast, the typical response of animals in the saline condition was a pattern of fluctuation around (above and below) the baseline value for each of the catecholamines. In addition, the tables indicate that the plasma concentration of catecholamines was influenced by the intravenous infusion of saline solution (this conclusion being drawn from the cyclic rise and fall in catecholamine levels coinciding with the two "drug" infusions occurring during the observation period), however, these effects were considerably smaller than those induced by nicotine.

In summary, the intravenous administration of nicotine resulted in significant increases in the plasma concentration of norepinephrine (in the medium and high nicotine conditions) and nonsignificant increases in epinephrine and dopamine -- the increases were generally dose related (i.e., the magnitude of the response increased as the dose of nicotine increased). In addition, the initial exposure to nicotine (Day 1) was found to result in significantly higher mean plasma catecholamine concentrations than were induced by subsequent infusions of nicotine (Day 3-7). A stable level of responding was evident for all three catecholamines within 3-7 days of the initial exposure to nicotine, providing an indication

of the rapid development of tolerance for nicotine induced effects. Finally, while the administration of nicotine frequently did not result in statistically significant increases in catecholamines, it did result in a pattern of elevated plasma concentrations which were distinctly different from the apparently more random variation of control values.

Corticosterone: Significant increases were observed in the analysis of corticosterone responses for the main effect of the Sample variable ($F=4.90$, $df=5,40$, $p<0.0014$) and the Drug x Day x Sample interaction ($F=1.74$, $df=30,80$, $p<0.0263$; see Table 4, Part I, Appendix C, page C45). Non-significant trends were found for the Drug Condition ($F=2.08$, $df=3,8$, ns), and the Drug x Sample interaction ($F=1.42$, $df=15,40$, ns). No other main effects or interactions were found to be significant. A graph which presents the mean corticosterone response during Phase I for each drug condition is presented on the following page.

Comparison of the group means (see Table 4, Part II, Appendix C, page C47) for each condition in the Drug x Day x Sample interaction (in conjunction with the Drug x Sample interaction) yields the following conclusions. The administration of the high (0.100 mg/kg) and medium (0.050 mg/kg) doses of nicotine resulted in significantly increased plasma concentrations of corticosterone when compared to the daily pre-nicotine (Sample 0) baseline value. Unfortunately these effects are somewhat unclear, as the significant increases in corticosterone were not consistently time-locked to the period of drug infusion (i.e., the peak increases in corticosterone did not occur at

Phase I: Corticosterone

Figure Legend

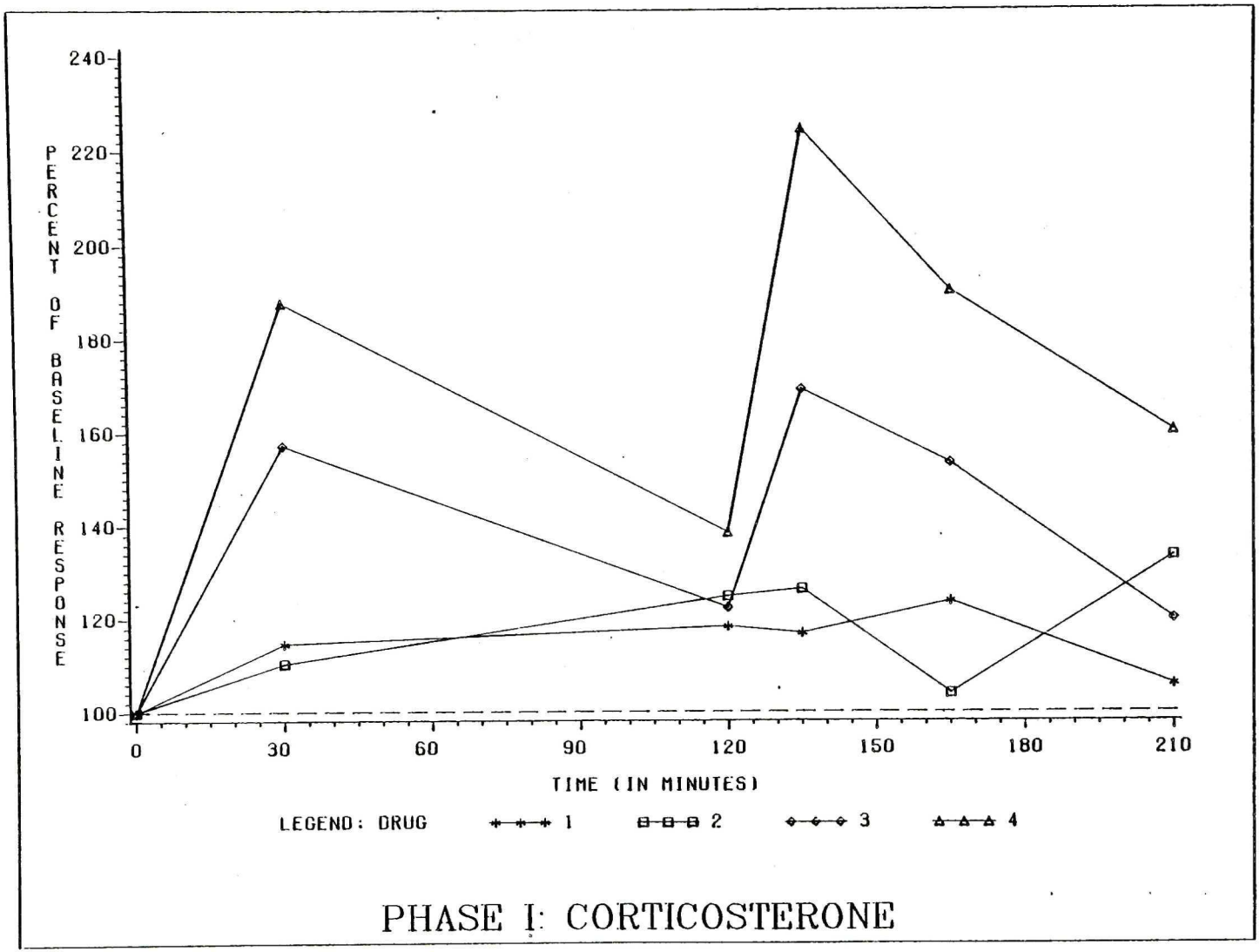
Drug Condition 1 = Saline Control

Drug Condition 2 = 0.025 mg Nicotine/kg

Drug Condition 3 = 0.050 mg Nicotine/kg

Drug Condition 4 = 0.100 mg Nicotine/kg

(In order to simplify the presentation of data from Phase I the data from Days 1, 3 and 7 have been combined. If the reader wishes a more detailed presentation of the day by day changes in responding he is referred to the detailed figures presented in Appendix D.)



PHASE I: CORTICOSTERONE

a consistent time interval following the administration of different doses of nicotine or the administration of nicotine on different days). However, based on the interaction effects, in conjunction with the graph of Phase I: Corticosterone responses, it seems fair to conclude that a) the administration of physiological saline solution had no significant or consistent effect on circulating plasma corticosterone levels, b) the administration of nicotine (in all doses) resulted in a consistent pattern of increased plasma concentrations of corticosterone (samples 1-5) when compared to baseline (sample 0) values, and c) the administration of the medium and high doses of nicotine resulted in significant increases in plasma corticosterone when compared with pre-drug levels.

While no main effects were found for the Drug Condition or Testing Day, the examination of the group means for these variables may be useful in understanding the procedural effects. Comparison of the group means (see Table 4, Part II, Appendix C, page C46) for each of the Drug Conditions indicated that for all doses of nicotine administered there was an increased mean plasma concentration of corticosterone when compared with the saline control group (see Figures 16-20, Appendix D, pages D141-D150). However, the elevation of plasma corticosterone levels was not significant when comparing the nicotine conditions with the saline control group. Comparison of the group mean values for each of the three Test Days showed no significant alteration (increase or decrease) in responding during Phase I of the experiment.

Examination of the mean response for each of the experimental Samples (i.e., baseline 0-5) indicated that the administration of nicotine resulted in a two phase response: a) a rapid and statistically significant increase in the plasma concentration of corticosterone within 15-30 minutes of the initiation of each drug infusion (there was a significant increase in the plasma concentration of corticosterone following the first [compare Samples 0 and 1] and second [compare Samples 2 and 3, or 0 and 3] of the daily drug infusions), b) followed by a rapidly decelerating return to near baseline plasma levels of corticosterone. In addition, there was a gradual increase (over baseline) in plasma corticosterone concentrations during the four hour observation period (see Table 4, Part III, Appendix C, page C48). This latter effect may be ascribed to the fact that the rate of decay for the drug induced responses was not sufficiently rapid to reinstate baseline values during the two hours interspersed between individual drug infusions. Thus, subsequent responses induced by the administration of nicotine were superimposed on the elevated levels remaining from prior exposure, and therefore resulted in further increases in corticosterone levels.

In summary, it may be stated that: a) the administration of the medium and high doses of nicotine resulted in elevated plasma concentrations of corticosterone when compared to individual daily baseline levels (sample 0), b) mean plasma concentrations of corticosterone for animals receiving all doses of nicotine were higher than for the saline control condition (although the differences were not significant), c) the increase in plasma corticosterone levels

over the baseline values was generally dose related (i.e., an increase in the dose of nicotine resulted in an increase in the size of the corticosterone response (see Table 4, Parts II and IVB, Appendix C, pages C47 and C49), and d) the administration of nicotine resulted in a rapid increase and subsequent gradual decrease in plasma corticosterone levels within 15-30 minutes of the initiation of each infusion. There was no diminution in corticosterone responses during Phase I of the experiment, suggesting that tolerance and/or habituation to the effects of nicotine did not develop.

Glucose and Insulin: The administration of nicotine resulted in significant increases in plasma glucose concentrations, and decreases in plasma insulin levels[12] (see Tables 5 and 6, Part I, Appendix C, pages C50 and C55). See the graphs of glucose and insulin responses which are presented on the following pages. Significant main effects were found in the analysis of glucose responses for the Drug Condition ($F=5.58$, $df=3,8$, $p<0.0231$) and Sample ($F=7.10$, $df=5,40$, $p<0.0001$). In addition, non-significant trends were observed for the effects of the Test Day ($F=2.88$, $df=2,16$, ns) and for the interaction of the Drug Condition by Sample ($F=1.49$, $df=15,40$, ns); no other interactions were found to be significant. The analysis of insulin responses showed main effects due to the Drug Condition x Day x Sample interaction ($F=1.60$, $df=30,80$, $p<0.0505$) and the Day x Sample interaction ($F=1.91$, $df=10,80$, $p<0.0556$). An interaction of the Drug Condition by Sample ($F=2.06$, $df=15,40$, $p<0.0352$) was also evident.

In the analysis of insulin responses statistically significant effects were observed in the Drug Condition x Sample interaction

($F=2.06$, $df=15,40$, $p<0.0352$). Non-significant trends were also found for the Drug Condition x Day x Sample interaction ($F=1.60$, $df=30,80$, ns) and the Day x Sample interaction ($F=1.91$, $df=10,80$, ns). Examination of the group means for the Drug Condition x Sample interaction (see Table 6, Part II, Appendix C, page C57) indicates that there were significant decreases in the plasma concentration of insulin for animals in the saline control group (the most probable explanations for the decrease in plasma insulin levels as seen in the control condition are that a) insulin decreased as a function of the duration of the experimentally imposed period of fasting, or b) the "drug" administration procedure per se was sufficient to induce an increase in autonomic sympathetic nervous system activity which then suppressed the release of insulin). No significant decreases in the mean plasma insulin levels were seen for animals which were receiving nicotine (however, this conclusion may be misleading and is discussed in greater detail in Footnote 12)(see also Figures 26-30, Appendix D, pages D161-D170 for a more detailed presentation of these data).

Comparisons of the group mean responses for each of the drug conditions (see Table 5, Part II, Appendix C, page C51) indicated that nicotine administration resulted in significantly elevated mean levels of plasma glucose when comparing the medium nicotine dose condition (0.050 mg/kg) to the saline control group (see also the detailed Figures 21-25, Appendix D, pages D151-D160). The group means for the remaining drug conditions fell within the range encompassed by the significant nicotine condition - saline control group comparison.

Phase I: Glucose

Figure Legend

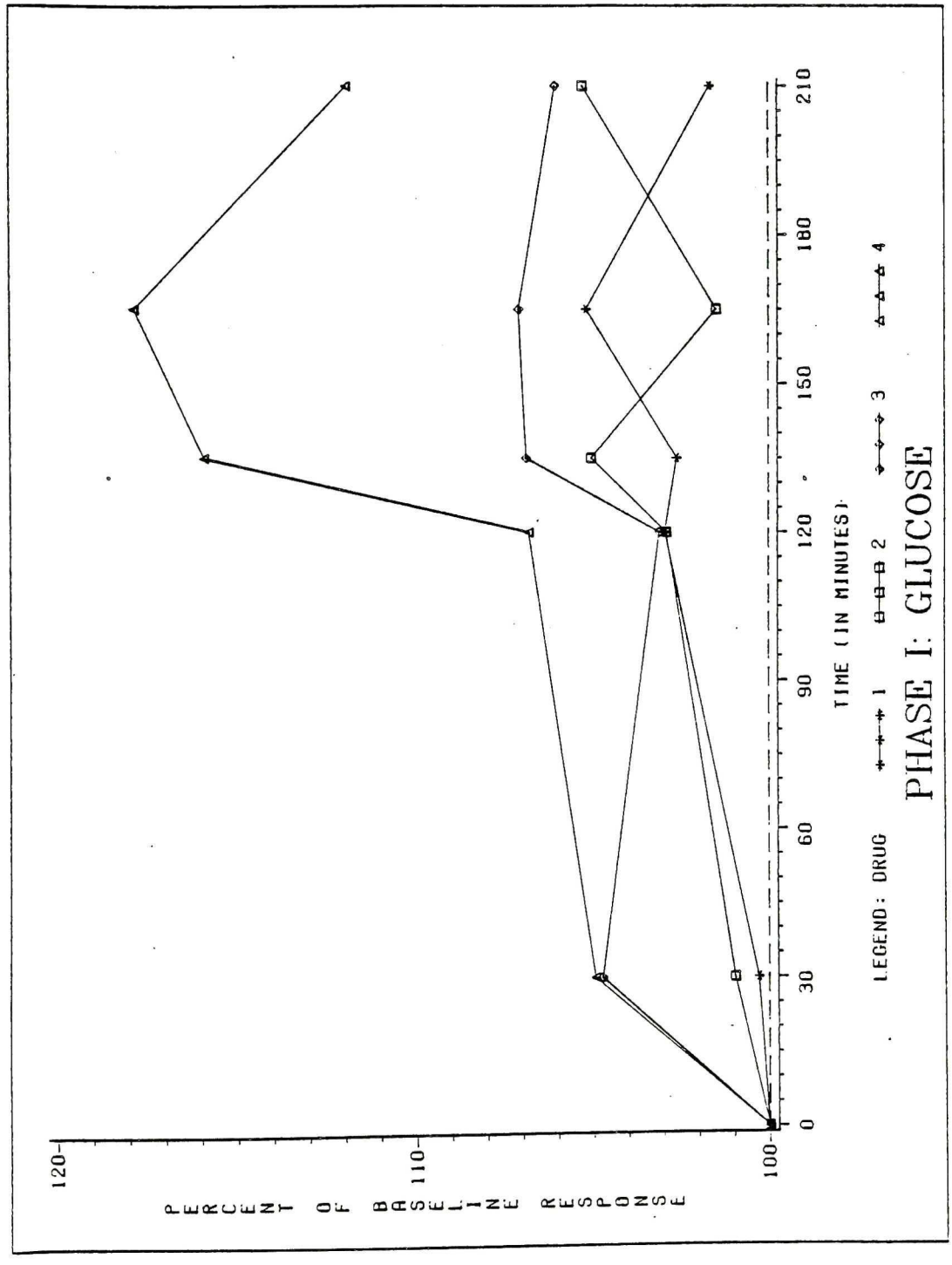
Drug Condition 1 = Saline Control

Drug Condition 2 = 0.025 mg Nicotine/kg

Drug Condition 3 = 0.050 mg Nicotine/kg

Drug Condition 4 = 0.100 mg Nicotine/kg

(In order to simplify the presentation of data from Phase I the data from Days 1, 3 and 7 have been combined. If the reader wishes a more detailed presentation of the day by day changes in responding he is referred to the detailed figures presented in Appendix D.)



PHASE I: GLUCOSE

Phase I: Insulin

Figure Legend

Drug Condition 1 = Saline Control

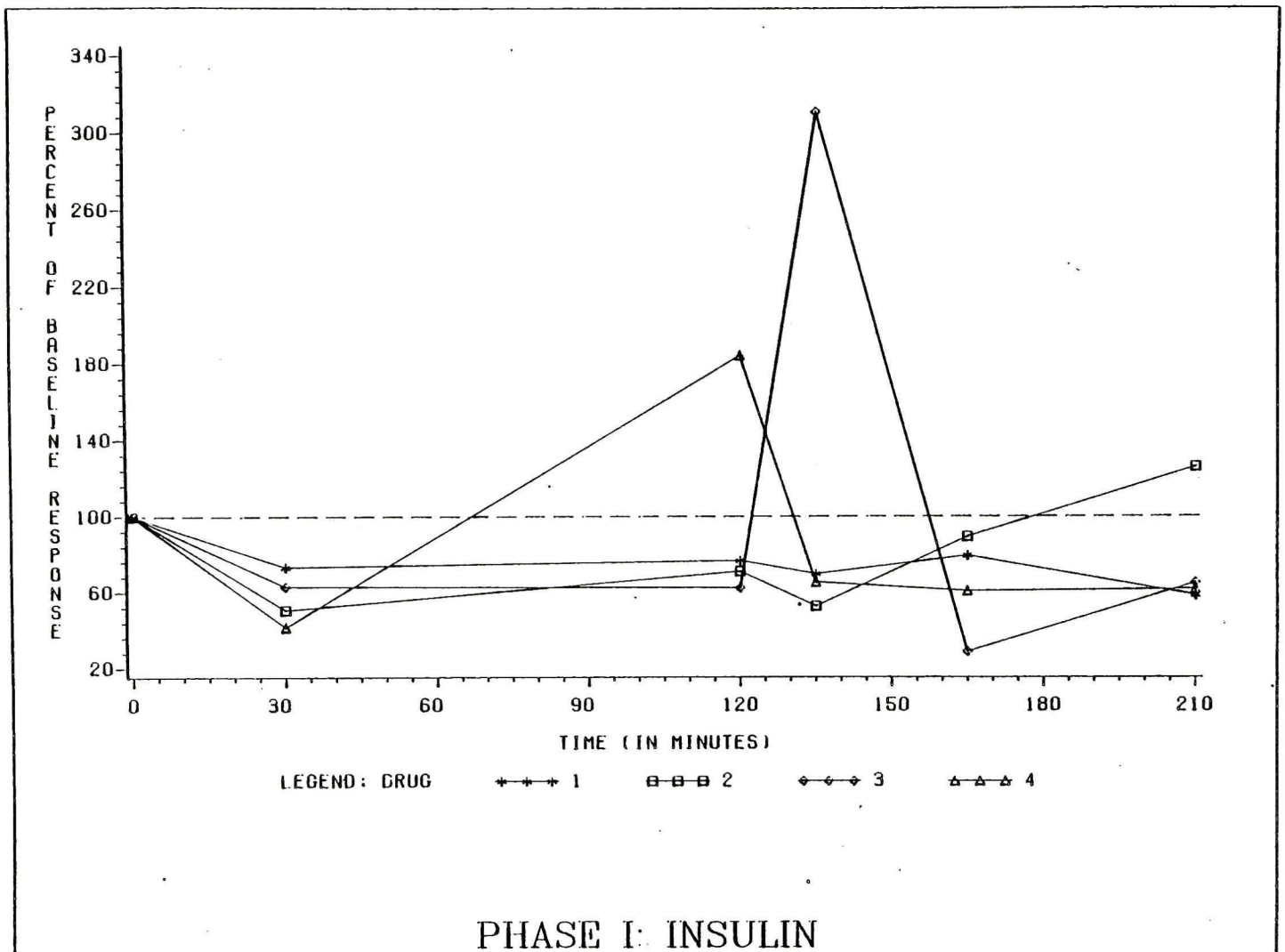
Drug Condition 2 = 0.025 mg Nicotine/kg

Drug Condition 3 = 0.050 mg Nicotine/kg

Drug Condition 4 = 0.100 mg Nicotine/kg

This graph presents data for individual representative animals from each of the drug conditions.

(In order to simplify the presentation of data from Phase I the data from Days 1, 3 and 7 have been combined. If the reader wishes a more detailed presentation of the day by day changes in responding he is referred to the detailed figures presented in Appendix D.)



PHASE I: INSULIN

Mean plasma levels of glucose were lower (although the effect was not statistically significant) on Day 7 than on Test Days 1 and 3, although there were no apparent differences in the magnitude of nicotine induced responses on any of the Test Days (the decreased mean glucose value for Day 7 was apparently due to an initial lower baseline level which was most apparent amongst animals in the high nicotine condition). Comparison of the Sample mean values indicated that the administration of nicotine resulted in a general increase in plasma glucose levels, the effect reaching statistical significance approximately two hours following drug administration (see Table 5, Part II, Appendix C, page C51). The magnitude of glucose responses induced by the administration of nicotine was positively related to the dose of nicotine administered (see Table 5, Parts II and IVB, Appendix C, pages C52 and C54).

To summarize the findings for glucose and insulin responses: a) the administration of nicotine resulted in increased plasma glucose levels, b) changes in plasma glucose were evident within 30 minutes of the initial drug infusion of each day and continued throughout the four hour observation period, and c) the administration of nicotine resulted in decreased concentrations of plasma insulin. No changes in glucose or insulin responses were evident during Phase I of the experiment.

Summary of Phase I: The experimental results suggest that a) the administration of nicotine resulted in a positive dose-related increase in the plasma concentrations of catecholamines (norepinephrine, epinephrine and dopamine), corticosterone and

glucose, b) nicotine administration resulted in generally decreased levels of plasma insulin, and c) partial habituation to the effects of nicotine was evident in catecholamine responses but was not apparent in corticosterone, glucose or insulin responses.

Phase II: Conditioning of Endocrinological Responses

Data from Phase II of the experiment were analyzed by repeated measures Analysis of Variance (ANOVA) using the General Linear Models (GLM) subroutine of the SAS Statistics package (SAS Users Guide: Statistics, 1982 Edition, SAS Institute, Inc., Cary, NC 27511). The results of the statistical analyses for Phase II were similar to the findings of Phase I. (The similarity of the statistical findings is not unexpected considering that one-half of the data used in the analyses of Phase II effects was also included in the analyses of Phase I of the experiment.) The results of the statistical procedures are presented in Tables 7-12 (Appendix C, pages C60-C77); the statistical findings are not discussed in the text of this section as this would be redundant with the presentation of Phase I. Presented in Tables 7-12, Part III (Appendix C, pages C62, C65, C68, C71, C74 and C77) are the comparisons of responses for the saline control and nicotine groups on Day 7 (nicotine administration) and Day 9 (Test of Conditioned Responses); these comparisons are discussed below. In the analysis of Phase II the data for all nicotine conditions have been combined because the results of Phase I indicated that the administration of all dosages of nicotine resulted in either increased or decreased plasma concentrations of each hormone compared to saline administration.

Catecholamines: As described previously, the administration of nicotine (Day 7) resulted in increased (although the effects were not necessarily statistically significant) plasma concentrations of catecholamines (norepinephrine, epinephrine and dopamine) (see the Graphs of the Phase II responses on the immediately following pages) when compared to baseline (Sample 0) levels or the saline control condition (see Tables 7-9, Appendix C, pages C60-C68, and the detailed Figures 31-42, Appendix D, pages D171-194). Mean norepinephrine and epinephrine responses for the saline control condition displayed only small fluctuations around the baseline value. On Day 9 (Test of Conditioned Response) the pattern of the norepinephrine response was similar (i.e., an increase in plasma concentrations) to the effect produced by the administration of nicotine (Day 7) although the magnitude of the effect was approximately one-half that which was induced by the drug. The epinephrine response of nicotine habituated animals on Day 9 was similar to that displayed by subjects in the control condition, suggesting that there were no conditioned epinephrine effects.

Dopamine responses displayed cyclic changes during the daily four hour observation period. For control subjects plasma dopamine concentrations were found to rise gradually during the first two and one-fourth hours of observation (i.e., Samples 0-3), then <u>decrease</u> sharply to near baseline levels (at approximately two and three-fourths hours following the initiation of testing procedures), followed by a rapid <u>increase</u> in plasma concentrations (corresponding to Sample 5). Similar cyclic changes in plasma dopamine

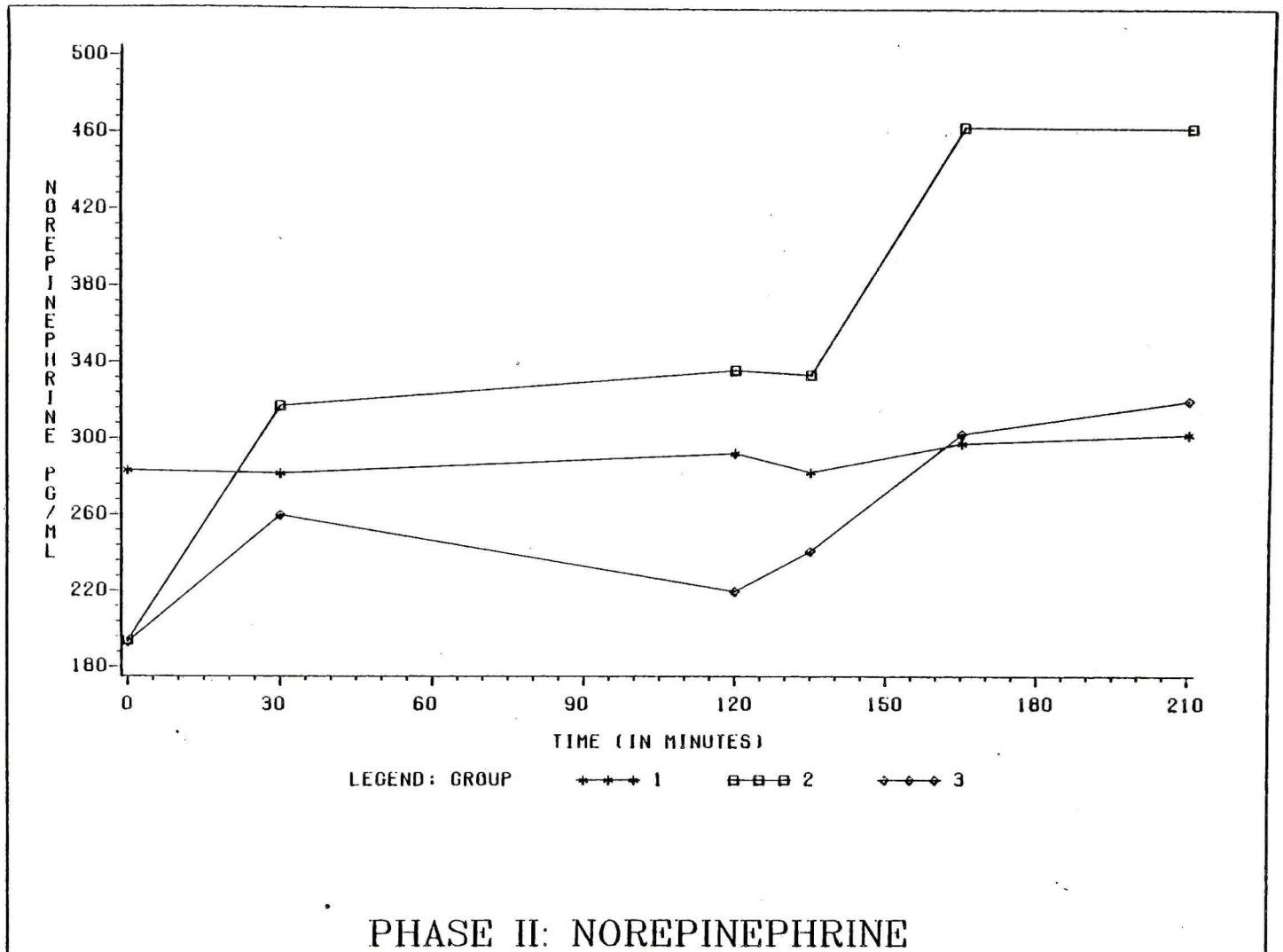
Phase II: Norepinephrine

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: NOREPINEPHRINE

Phase II: Norepinephrine

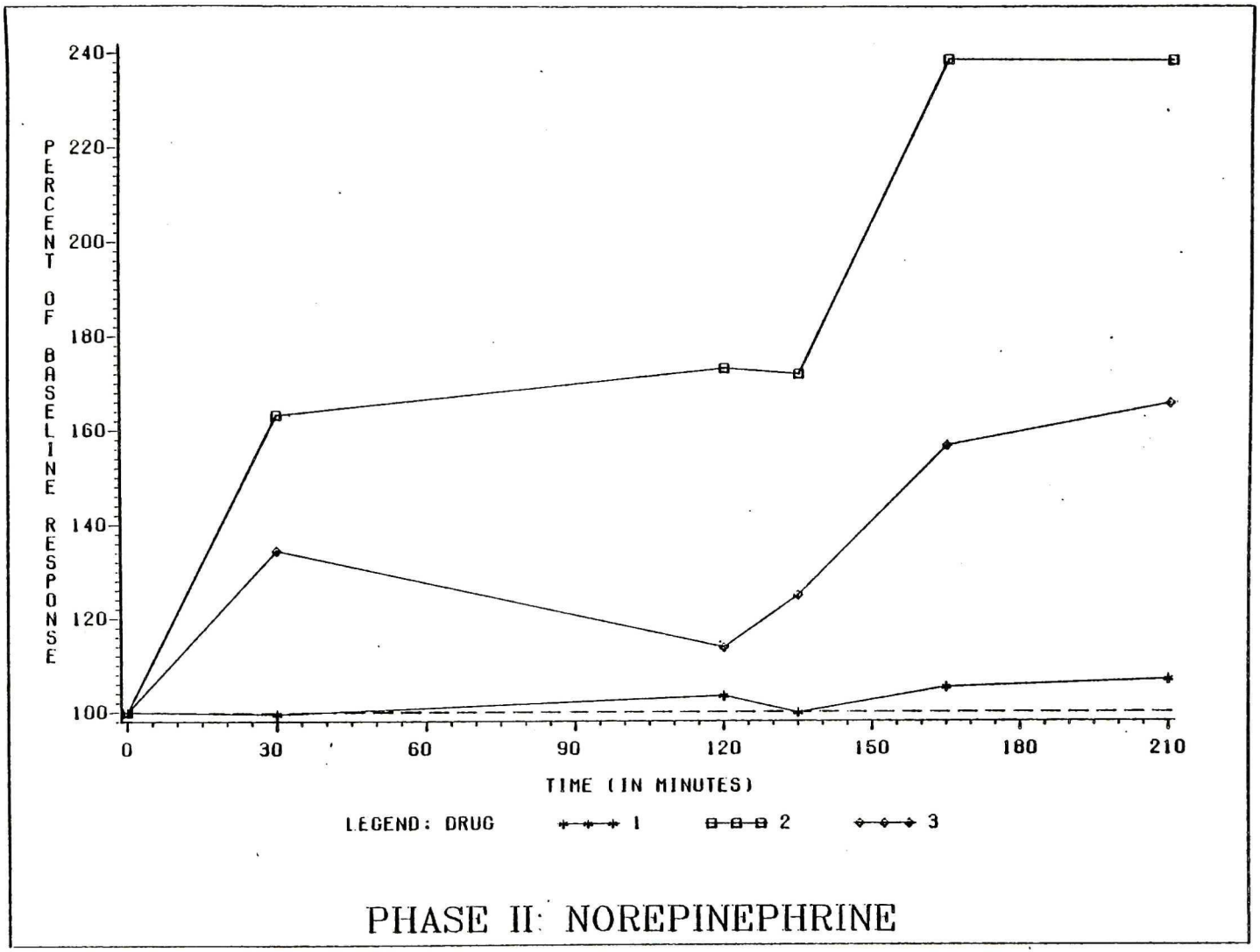
PERCENT OF BASELINE RESPONSE

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: NOREPINEPHRINE

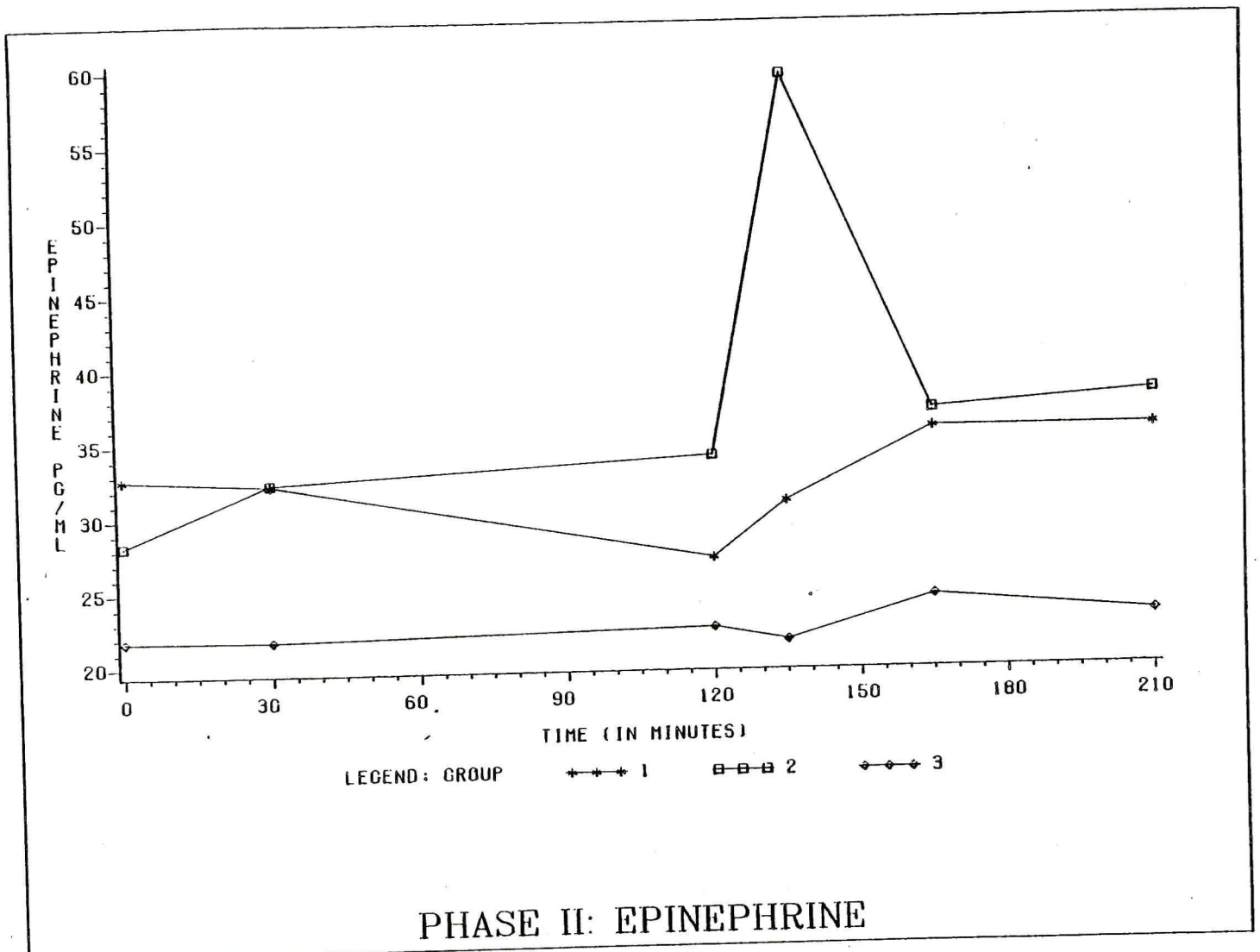
Phase II: Epinephrine

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: EPINEPHRINE

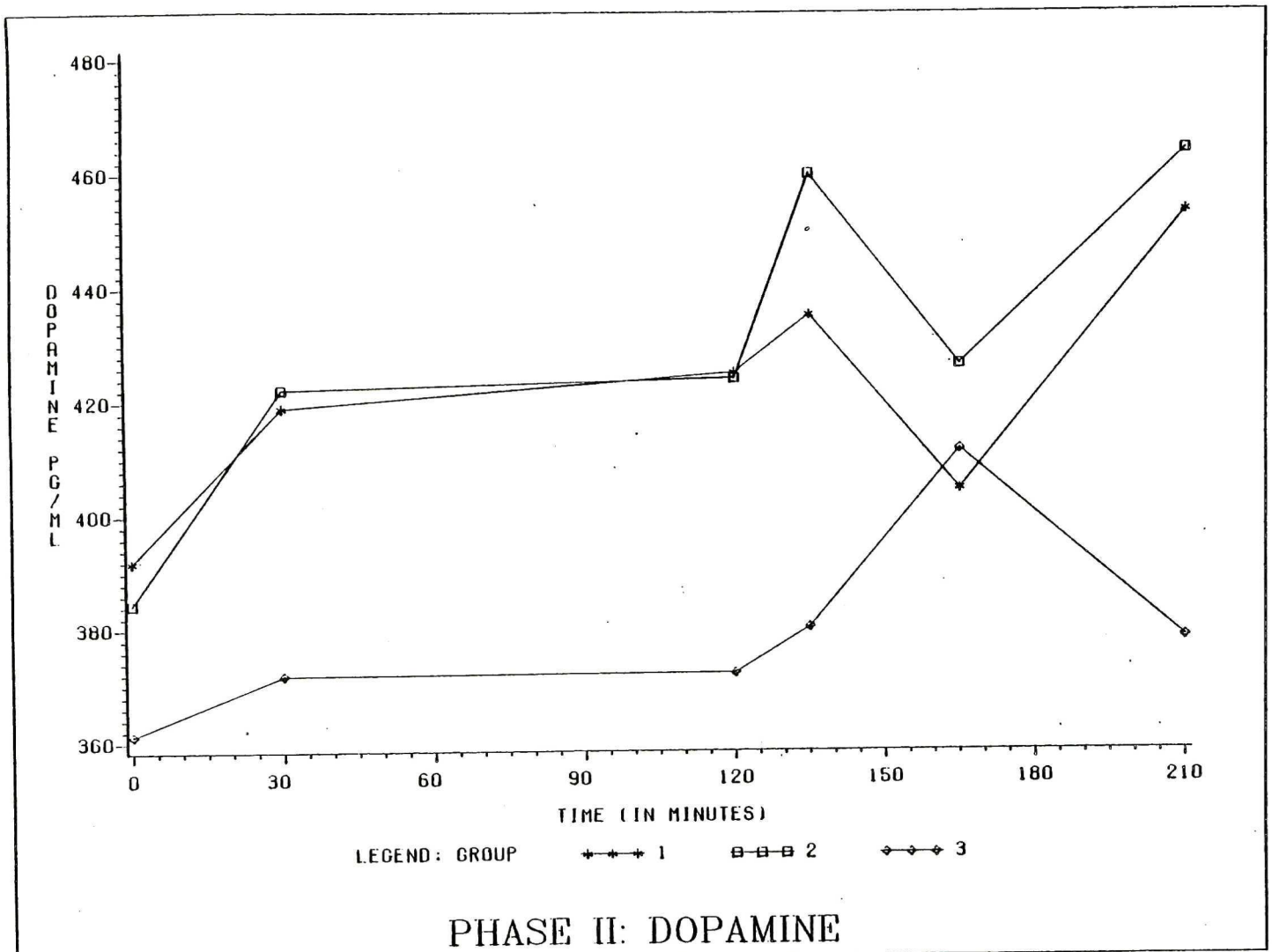
Phase II: Dopamine

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: DOPAMINE

Phase II: Dopamine

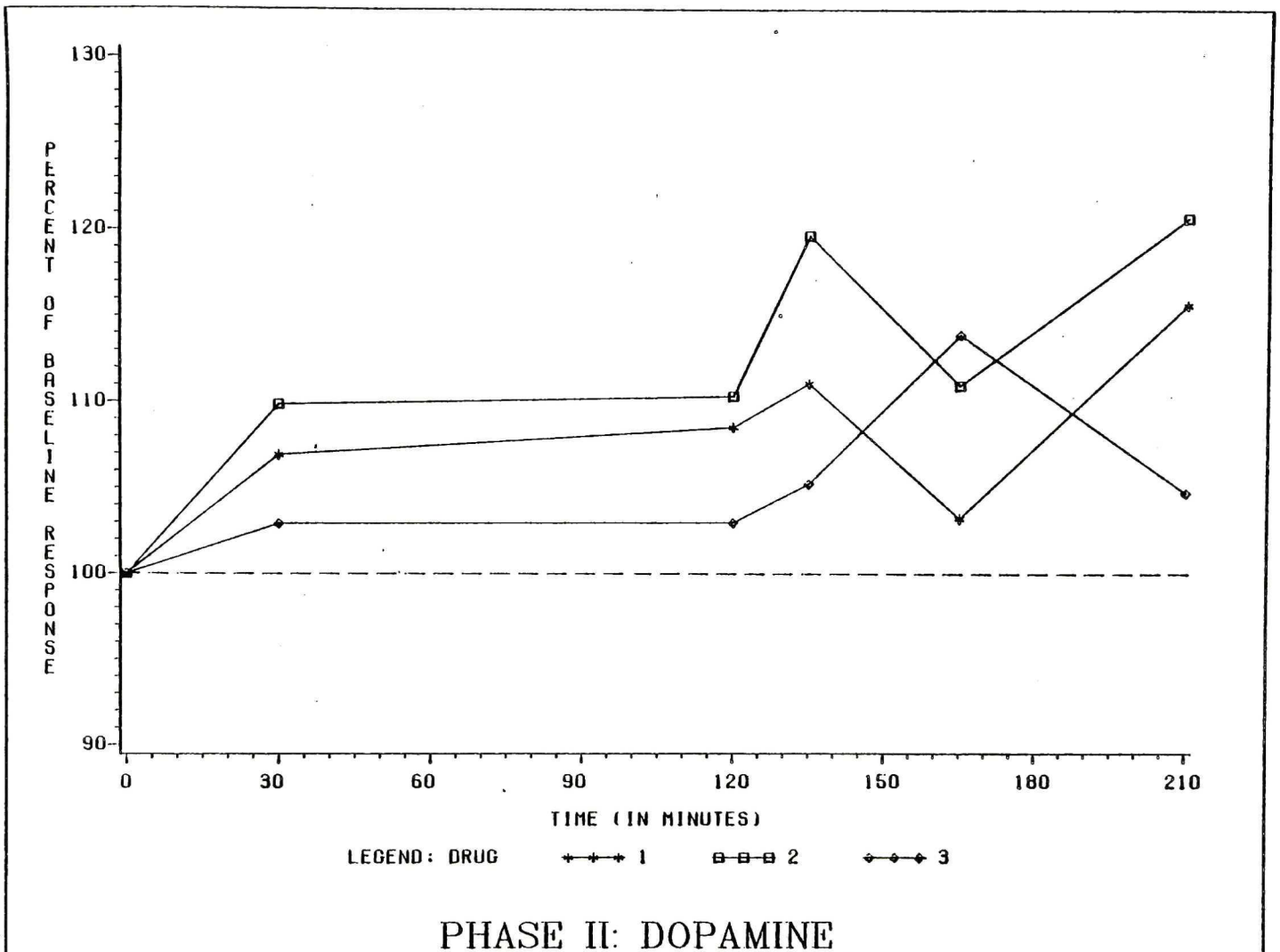
PERCENT OF BASELINE RESPONSE

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: DOPAMINE

concentrations were apparent among animals receiving nicotine (i.e., nicotine administration resulted in elevated plasma levels of dopamine but did not alter the cyclic changes). An inverse pattern of responding was evident on Day 9 for animals which had previously been exposed to nicotine. In the conditioned response there was a small increase in plasma dopamine levels during the initial two and one-fourth hours of testing (i.e., Samples 0-3), which was followed by a large increase and subsequent decrease in plasma concentrations as measured in Samples 4 and 5 respectively. It would appear that the cyclic changes in plasma dopamine levels in the conditioned drug response were the reverse (opposite) of the effects induced by the administration of nicotine (however, it is also possible that the "conditioned response" may have been due to a shift in the time-course of the normal cyclic variation in plasma dopamine levels).

In summary: a) the administration of nicotine resulted in increases in the plasma concentrations of norepinephrine, epinephrine and dopamine, b) the repetitive administration of nicotine in conjunction with the simultaneous presentation of environmental stimuli resulted in the development of conditioned norepinephrine and dopamine responses (however there was no evidence of the development of conditioned epinephrine responses), c) conditioned norepinephrine responses were similar (although of a smaller magnitude) to the effects produced by the administration of nicotine, and d) conditioned changes in plasma dopamine were opposite (opponent) to effects produced by nicotine.

Corticosterone, Glucose and Insulin: As described previously, the administration of nicotine (Day 7) resulted in increased plasma concentrations of corticosterone and glucose when compared to responses of the saline control group (see the Graphs on the following pages, and Tables 10 and 11, Part III, Appendix C, pages C71 and C74). In addition, the administration of nicotine resulted in small decreases in the plasma concentration of insulin (see the Graph which follows; and Table 12, Part III, Appendix C, page C77). The drug administration procedure (as evaluated in the saline control condition) had no consistent effect on plasma concentrations of corticosterone during the observation period, but resulted in gradual increases and decreases in plasma glucose and insulin levels respectively. Responses induced by the administration of nicotine were somewhat larger than the effects produced by the drug infusion procedure itself. The pattern of corticosterone, glucose and insulin responses observed on Day 9 (Test of Conditioned Responses) was similar to the responses of the saline control group, thus suggesting that conditioned drug effects did not develop for these systems (the reader may refer to the detailed Figures 43-54, provided in Appendix D, pages D195-D218).

In summary, the results of Phase II suggest that: a) the repetitive administration of nicotine in conjunction with the appropriate presentation of environmental stimuli resulted in the development of conditioned norepinephrine and dopamine responses (conditioned responses were not observed for epinephrine, corticosterone, glucose and insulin), and b) the conditioned responses

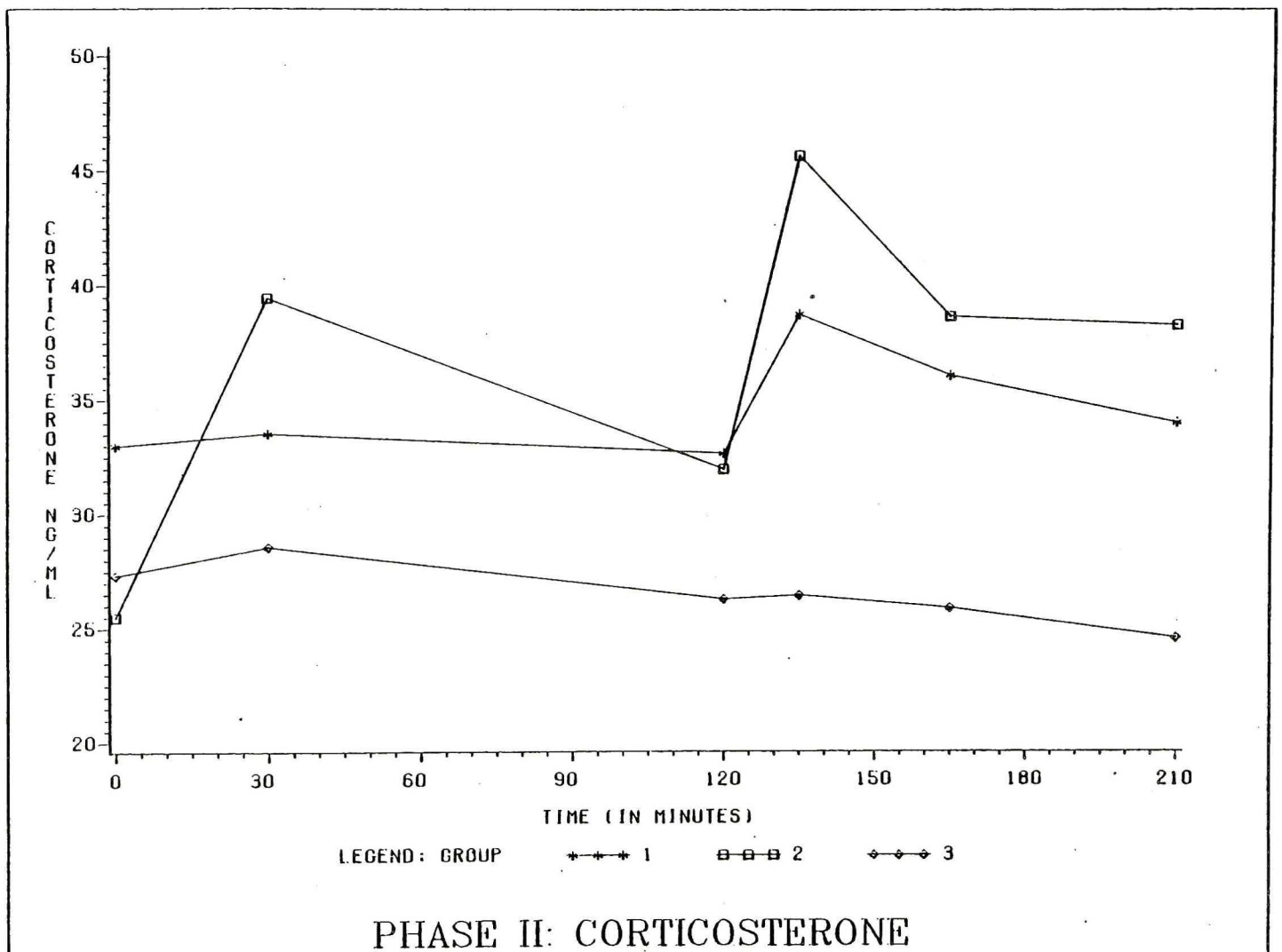
Phase II: Corticosterone

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: CORTICOSTERONE

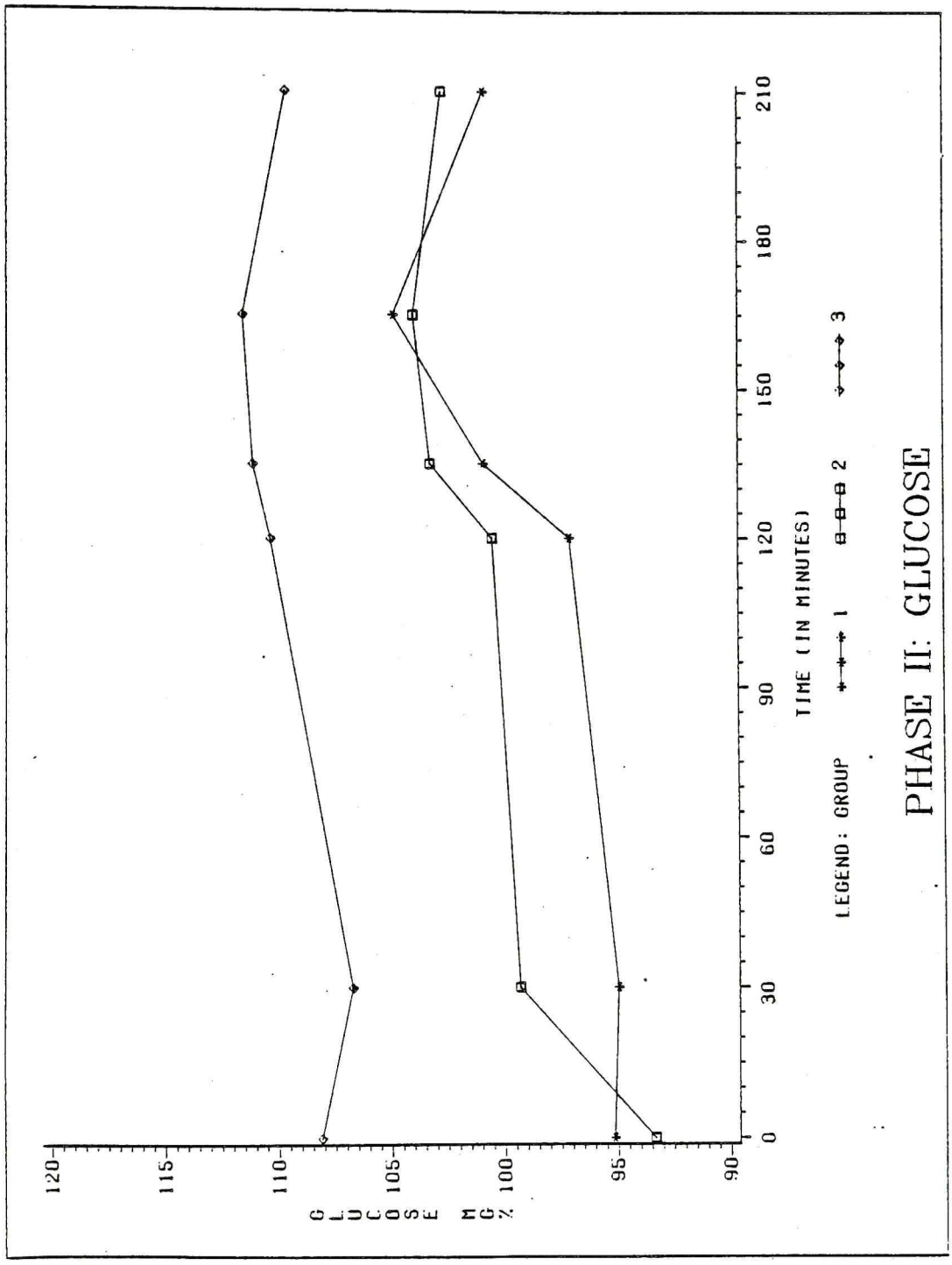
Phase II: Glucose

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: GLUCOSE

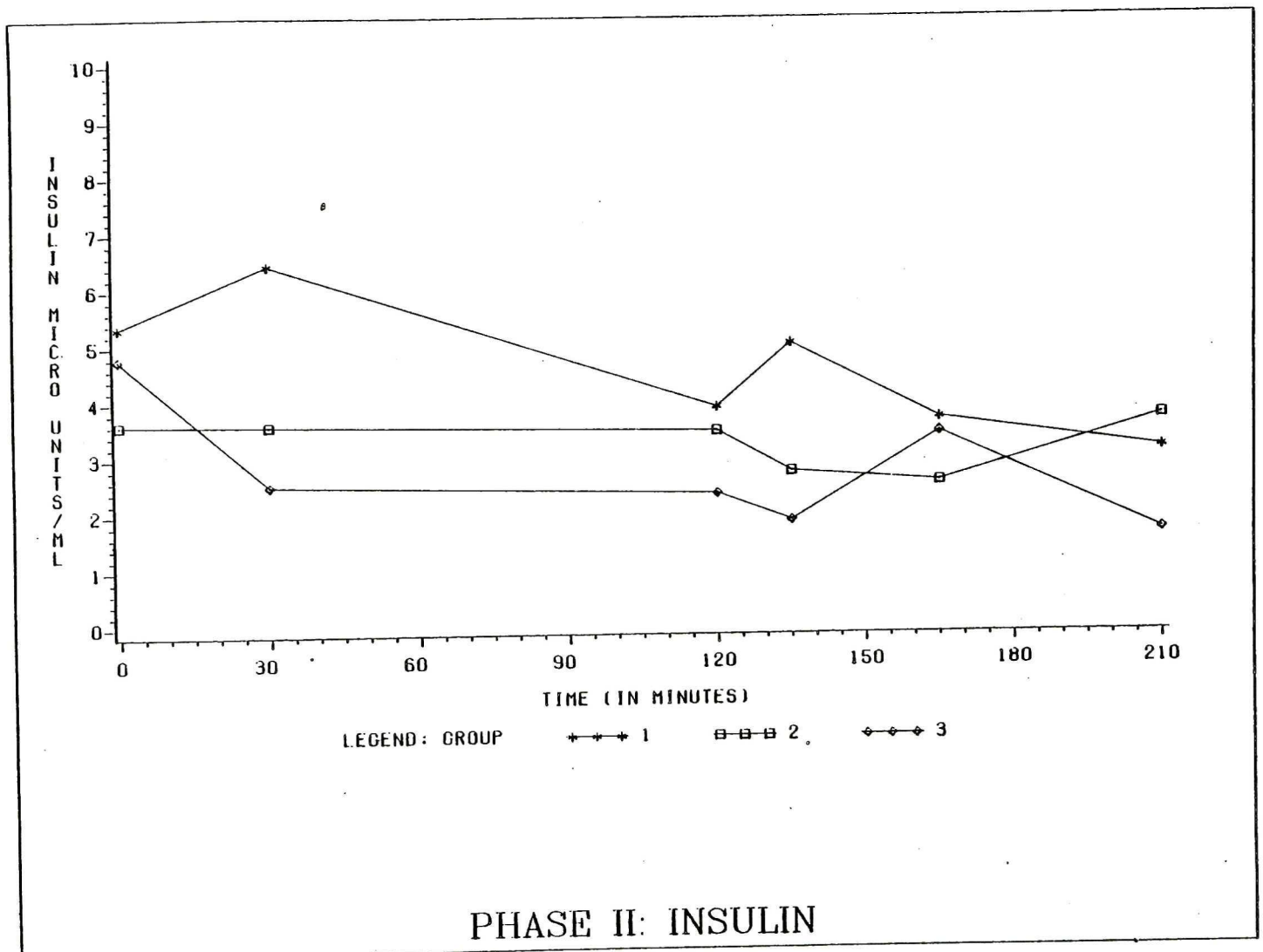
Phase II: Insulin

Figure Legend

Drug Condition 1 = Saline Control condition,
Combined data for Days 7 and 9

Drug Condition 2 = Combined data for All Nicotine Doses,
Day 7 - Nicotine Administration

Drug Condition 3 = Combined data for All Nicotine Doses,
Day 9 - Test of Conditioned Drug
Response (No Nicotine Administered)



PHASE II: INSULIN

were similar or opposite to the effects produced by nicotine administration (i.e., conditioned norepinephrine responses were similar to the changes induced by nicotine, while conditioned dopamine responses were opposite to the effects of nicotine).

Phase III: Effects of Nicotine on Stress Induced Endocrine Responses

Data from Phase III of the experiment were analyzed using the Student's t statistic for related and independent samples (the selection being dependent on the comparison being performed). Analyses were performed on catecholamine and corticosterone responses.[13] Comparisons were performed within each Drug Condition between the two Test Days (i.e., Stress with Nicotine and Stress without Nicotine) (see Tables 13-16, Part II, Appendix C, pages C79, C84, C89 and C94) and for the sequence of Samples taken during each Test Day (i.e., comparisons of samples 1-5 with the baseline [Sample 0] value) (see Tables 13-16, Part I, Appendix C, pages C78, C83, C88 and C93). In addition, responses of animals in each of the nicotine conditions were compared with the responses of the control (saline condition) animals, when the control subjects were and were not being stressed by the application of physical restraint.

The majority of the statistical procedures failed to demonstrate significant differences between the experimental conditions -- this effect apparently due to the small number of subjects used in the experiment and the large variability in the magnitude of intersubject responses and differences in baseline hormone levels. The statistical findings (including group mean response values and standard errors) are presented in Tables 13-16

(Parts I-IV, Appendix C, pages C78-C97) for the use of the reader, however, these materials will not be discussed in the text of this section.

Examination of the Graphs presented on the following pages and Tables 13-16, Part IV (Appendix C, pages C82, C87, C92 and C97) clearly indicates that for the control subjects the application of physical restraint resulted in large and sustained increases in the plasma concentrations of norepinephrine, epinephrine and corticosterone (compare the responses of control subjects on the No Stress and With Stress test days) (see also the detailed Figures 55, 59, 63 and 67, which are provided in Appendix D, pages D220, D228, D236 and D244). Plasma norepinephrine and epinephrine concentrations attained levels as high as 300% of the initial baseline, while maximum responses for corticosterone were approximately 450% of baseline. Plasma dopamine concentrations did not change in any consistent manner (dopamine levels increased and decreased approximately 5% during the observation period) as a function of the application of the physical restraint stressor.

In animals which had previously been exposed to nicotine (i.e., chronic intermittent nicotine administration) the application of the stressor alone (i.e., physical restraint without the simultaneous administration of nicotine) resulted in increases in the plasma concentrations of norepinephrine, epinephrine and corticosterone which were approximately equal to (the same order of magnitude) the responses observed in the control subjects. Catecholamine and corticosterone responses for these animals (i.e.,

Phase III: Norepinephrine

(The graph presents the data only for that portion of the test session during which the animals were being stressed by physical restraint.)

Figure Legend

Drug Condition 1 = Saline Control, No Stress

Drug Condition 2 = Saline Control with Restraint Stress

Drug Condition 3 = 0.025 mg Nic/kg experimental group, Restraint Stress without Nicotine

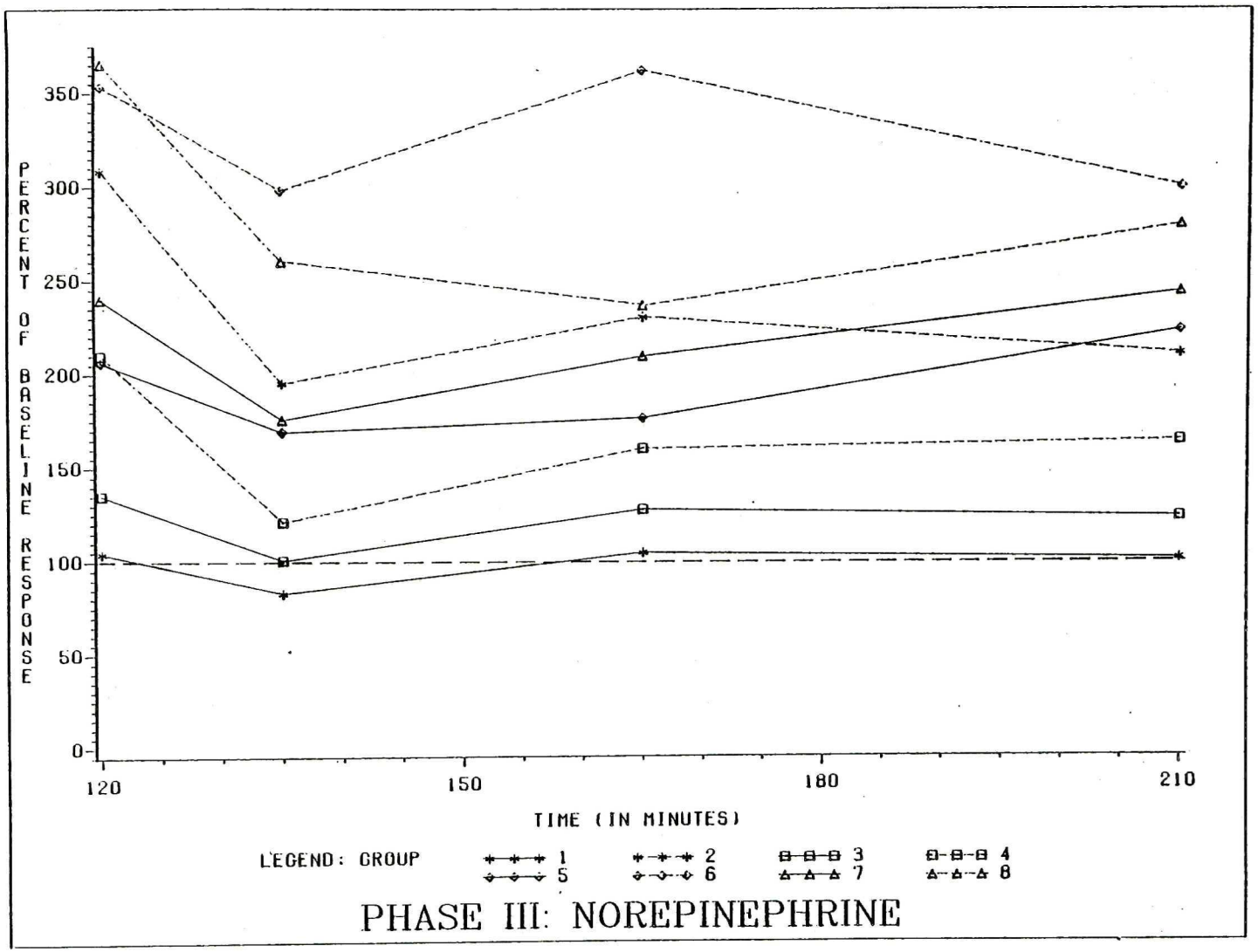
Drug Condition 4 = 0.025 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 5 = 0.050 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 6 = 0.050 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 7 = 0.100 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 8 = 0.100 mg Nic/kg experimental group, Restraint Stress with Nicotine



PHASE III: NOREPINEPHRINE

Phase III: Epinephrine

(The graph presents the data only for that portion of the test session during which the animals were being stressed by physical restraint.)

Figure Legend

Drug Condition 1 = Saline Control, No Stress

Drug Condition 2 = Saline Control with Restraint Stress

Drug Condition 3 = 0.025 mg Nic/kg experimental group, Restraint Stress without Nicotine

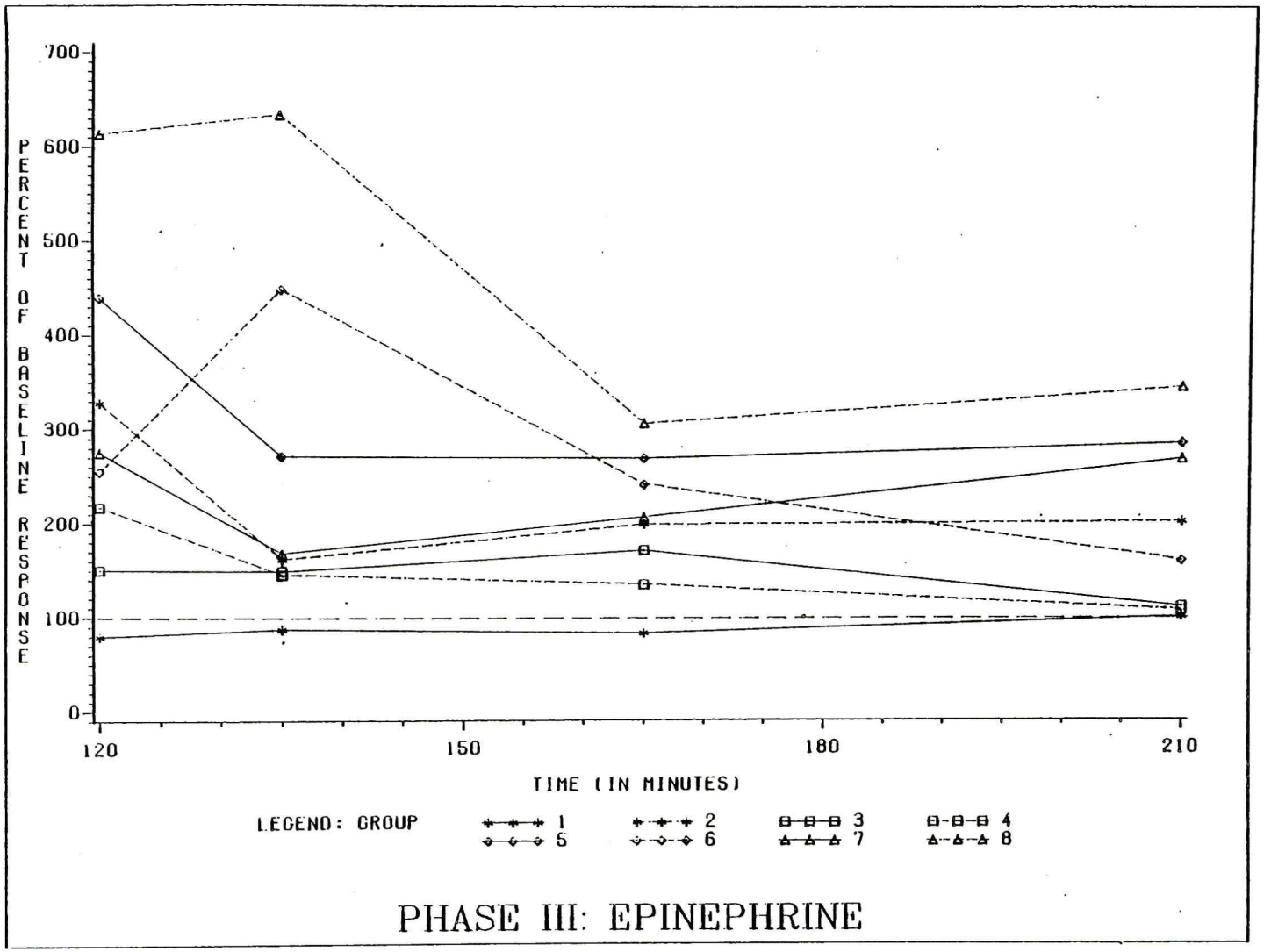
Drug Condition 4 = 0.025 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 5 = 0.050 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 6 = 0.050 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 7 = 0.100 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 8 = 0.100 mg Nic/kg experimental group, Restraint Stress with Nicotine



PHASE III: EPINEPHRINE

Phase III: Dopamine

(The graph presents the data only for that portion of the test session during which the animals were being stressed by physical restraint.)

Figure Legend

Drug Condition 1 = Saline Control, No Stress

Drug Condition 2 = Saline Control with Restraint Stress

Drug Condition 3 = 0.025 mg Nic/kg experimental group, Restraint Stress without Nicotine

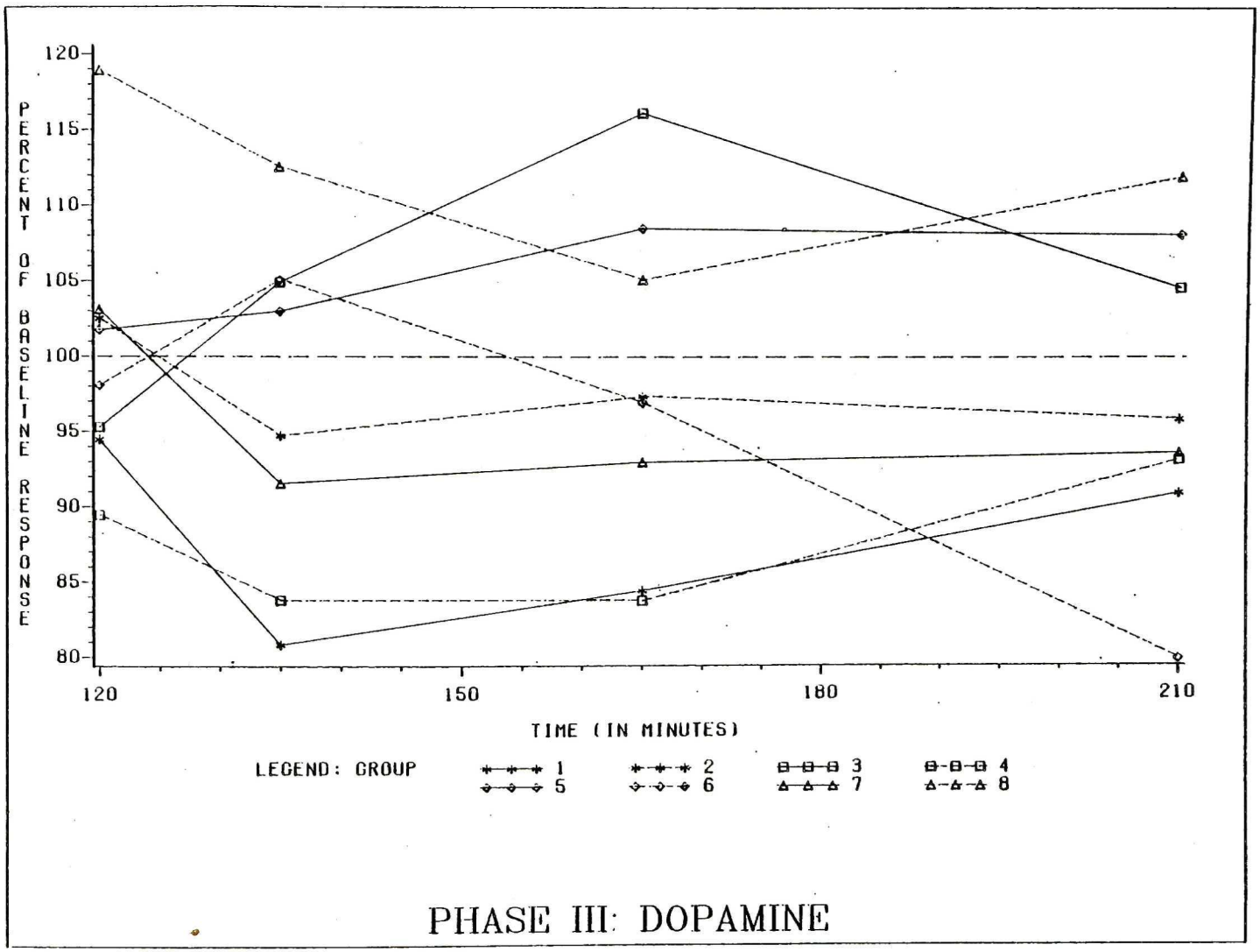
Drug Condition 4 = 0.025 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 5 = 0.050 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 6 = 0.050 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 7 = 0.100 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 8 = 0.100 mg Nic/kg experimental group, Restraint Stress with Nicotine



PHASE III: DOPAMINE

Phase III: Corticosterone

(The graph presents the data only for that portion of the test session during which the animals were being stressed by physical restraint.)

Figure Legend

Drug Condition 1 = Saline Control, No Stress

Drug Condition 2 = Saline Control with Restraint Stress

Drug Condition 3 = 0.025 mg Nic/kg experimental group, Restraint Stress without Nicotine

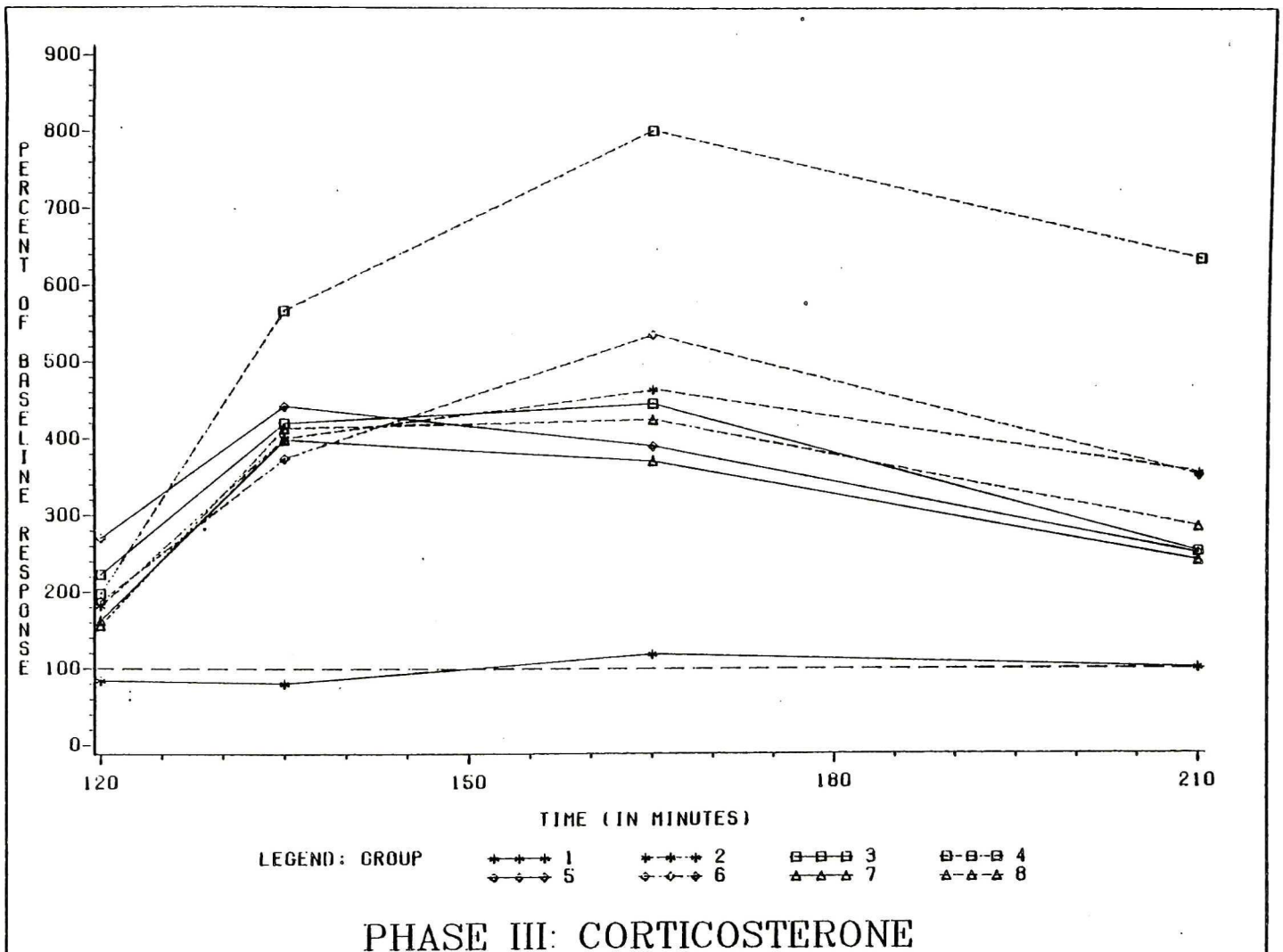
Drug Condition 4 = 0.025 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 5 = 0.050 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 6 = 0.050 mg Nic/kg experimental group, Restraint Stress with Nicotine

Drug Condition 7 = 0.100 mg Nic/kg experimental group, Restraint Stress without Nicotine

Drug Condition 8 = 0.100 mg Nic/kg experimental group, Restraint Stress with Nicotine



PHASE III: CORTICOSTERONE

the nicotine "habituated" animals) were similar or somewhat larger (than the responses promoted by the stressor alone) when nicotine was administered simultaneously with the application of the physical restraint stressor (the reader may wish to refer to the detailed Figures 55-70, provided in Appendix D, pages D219-D250). Clearly, the administration of nicotine in any of the three doses did not reduce the magnitude of the endocrine responses induced by the stressor.

The results of Phase III suggest that a) the application of physical restraint stress in the control animals resulted in large (although statistically non-significant) and sustained (continuing for a minimum of 90 minutes) increases in plasma concentrations of norepinephrine, epinephrine and corticosterone when compared with the no stress control trials, b) the effects produced by physical restraint in animals which were "experienced" (habituated) with the effects of nicotine were similar to responses observed in the control subjects, and c) the simultaneous presentation of nicotine and the stressor (in nicotine habituated animals) resulted in similar or somewhat larger responses than were induced by the stressor alone. These findings suggest that nicotine does not reduce the intensity of peripheral autonomic activation induced by stress.

Discussion

This study used endocrinological and biochemical measures to examine the effects of nicotine on several processes that may provide a better understanding of the biological bases of reinforcement in cigarette smoking (Phases I and II) and the potential role of nicotine as a mediator of stress reduction (Phase III). Phase I of the experiment examined the response of catecholamines, corticosterone, glucose and insulin to the intravenous administration of three doses of nicotine in naive animals and changes in responding as the test animals acquired increasing experience with nicotine. The second phase of the procedure examined whether it is possible to condition the nicotine induced responses to previously non-drug related stimuli. As in Phase I, catecholamines, corticosterone, glucose and insulin were measured. The results of this aspect of the study (i.e., whether conditioned responses are similar to the effects induced by nicotine or are an opponent process) have implications regarding abstinence and therapies used in smoking cessation as discussed in the introduction to this article. The third phase of the experiment investigated the effects of nicotine administration on the reduction of endocrinological indices of stress. This aspect of the study originated with the "psychological tool" model of Ashton and Stepney (1982) and the reports of smokers that under stressful conditions cigarette smoking relaxes them.

Phase I

The administration of nicotine resulted in increases in the plasma concentration of catecholamines (norepinephrine, epinephrine and dopamine), corticosterone and glucose. Nicotine administration

resulted in more rapid decreases in plasma concentrations of insulin, but there were short-lived increases in plasma insulin after the floor value was reached. In general, these effects were dose related (i.e., the magnitude of the response increased with increases in the dose of nicotine being administered). The pattern of nicotine induced responses of corticosterone, glucose and insulin did not change significantly during the first week of drug administration (i.e., these responses did not display indications of habituation or sensitization).

Increases in the plasma concentration of catecholamines[10] (particularly norepinephrine and epinephrine) were greatest on the first day of nicotine administration. On subsequent testing days (Days 3 and 7) the pattern of catecholamine responses was similar to that found on the first day of drug administration, but the responses were somewhat reduced in amplitude (i.e., the maximum and/or total response induced by nicotine administration was less than on Day 1). Stable levels of catecholamine responding (i.e., unchanging in pattern or magnitude) were achieved within three to seven days of the initiation of nicotine administration.

The change in catecholamine responses suggests the development of a partial tolerance and/or habituation to the effects of nicotine. The rapid development of tolerance and of constant levels of responding (within 3-7 days of the initiation of drug administration) coincides with expectations based on the results of the majority of the available literature (Larson, Haag & Silvette, 1961; Larson & Silvette, 1968, 1975). As stated previously, no changes were evident during the first week of testing in the pattern or magnitude of the nicotine induced

responses of corticosterone, glucose or insulin. Within the context of the present experiment these findings have two important implications: a) having reached a stable and unchanging degree of responsivity to nicotine it might be expected that responding would continue at this level for a long period of time, and b) the attainment of a stable level of responding suggests that the test animals were "experienced" (i.e., acclimated, tolerant and/or habituated) with the effects of nicotine and had probably reached a state of physiological, endocrinological and biochemical responding similar to that of the habitual cigarette smoker. In addition, the continued sensitivity of these systems to the effects of nicotine increases the likelihood that these responses are involved in the development and maintenance of cigarette smoking.

It is a well accepted fact that cigarette smokers tend to weigh less than do non-smokers of comparable age and height (Grunberg, 1982; Wack & Rodin, 1982). Exactly why this weight difference occurs is not known. In a series of experiments conducted by Grunberg and associates (Grunberg, 1982; Grunberg & Morse, in press; Grunberg, Bowen & Morse, in press) it has been reported that cigarette smoking in humans and nicotine administration in animals results in a decreased preference for and consumption of sweet tasting/high calorie foods. Abstinence from cigarettes or the cessation of nicotine administration was found to result in increased consumption of sweet tasting/high calorie foods while not affecting the consumption of other types or categories of foods (i.e., bland, salty, etc.).

It has been proposed that hunger and eating behavior may (at least in part) be regulated by a glucose sensitive mechanism (Friedman & Stricker, 1976; LeMagnen, 1980; LeMagnen, Devos & Larue-Achagiotis, 1980; Woods, Decke & Vasselli, 1974). Decreases in the plasma concentration of glucose result in hunger and eating, whereas increases in plasma glucose result in satiation and the suppression of eating behaviors. The suppression of hunger and the induction of a state of satiation may act as a highly effective reinforcer for the maintenance of behavior. It has been proposed that nicotine via its effect of increasing plasma glucose may be a powerful reinforcer involved in the development and maintenance of cigarette smoking behavior (Hickey & Harner, 1973).

The results of the present experiment suggest that a) the administration of nicotine resulted in increased concentrations of plasma glucose, b) increases induced by nicotine administration were larger than increases observed in control subjects (the increased concentrations of plasma glucose among the control animals were apparently mediated either by the duration of the fasting period or as a result of some aspect of the "drug administration" procedure), and c) there was no substantial diminution in glucose responding during the first week of drug administration. Considering that glucose responses were similar in "naive" (Test Day 1) animals and in nicotine "experienced" (Test Days 3 and 7) subjects there appears to be no reason to predict a change (decrease or increase) in the response with continued exposure to nicotine.

Considering the findings of Grunberg et al. and the hypothesized role of glucose in the regulation of eating, together with the present results suggests how cigarette smoking (nicotine administration) may increase glucose availability and thereby change patterns of eating (i.e., taste preferences, food consumption) and body weight (i.e., due to changes in nutritive need and caloric intake), and act as a reinforcer in the development and maintenance of smoking behavior.

Measurements were made of plasma insulin levels in an attempt to determine the sequence of events involved in nicotine induced increases in plasma glucose (i.e., do increases in plasma glucose precede changes in insulin concentration or are they subsequent to changes in plasma insulin levels). If nicotine has a direct effect on increasing plasma glucose then it would be expected that homeostatic mechanisms would promote a compensatory increase in plasma insulin levels. However, if nicotine acts indirectly on glucose through the insulin regulatory system then it would be expected that increases in glucose concentration would be subsequent to, or coincide with, decreases in plasma insulin. In the present experiment it was found that the administration of nicotine resulted in decreased concentrations of plasma insulin concurrent with increased levels of glucose. These findings, in conjunction with several studies presented earlier (Florey et al., 1977; Sandberg et al., 1973; Tjalve & Popov, 1973) suggest that nicotine (cigarette smoking) interferes with the regulation of insulin homeostatic response mechanisms and thereby

indirectly results in increases in plasma glucose. However, these effects were not clearcut and require further investigation.

It was found that the administration of nicotine resulted in increased concentrations of plasma catecholamines and corticosterone. The measurement of catecholamines and corticosterone served two functions within the context of this experiment: a) together these measures provide an indication of the level of autonomic nervous system activity (Baum, Grunberg & Singer, 1982; Baum, Grunberg, Lundberg & Singer, in press), and b) responses involving these systems have been implicated as potential mediators of cardiovascular damage (Eliot, Todd, Clayton & Pieper, 1978) and increased mortality among cigarette smokers (U.S. Dept. of Health, Education and Welfare, 1979).

Nicotine has been proposed as playing a major role in several of the biological and psycho-biological theories of cigarette smoking (see the Introduction section for a discussion of the major theories of smoking). Eysenck (1973) has proposed that cigarette smoking (the self administration of nicotine) reflects the attempt of the smoker to alter his/her level of arousal. This theory proposes that (at least in part) the motivation of habitual smoking is due to the continued ability of cigarettes (nicotine) to affect a change in the level of arousal being experienced by the individual. The findings of the present study suggest that the administration of nicotine, even in the drug "experienced" (habituated and/or tolerant) organism, results in changes in the level of autonomic nervous system activity. The changes in plasma catecholamines and corticosterone (indices of autonomic nervous system activity) in response to the administration of nicotine may be

interpreted as indicating changes in arousal levels. Thus, it appears that nicotine may subserve the role of the modification of arousal as is required in the theory. However, it is also possible that the changes in autonomic nervous system activity induced by the administration of nicotine may act as reinforcers (or as indices of changes in other physiological systems which have reinforcing characteristics) in the development and maintenance of cigarette smoking behaviors.

Cigarette smokers display an increased incidence of premature morbidity and mortality due to cardiovascular disorders when compared to non-smokers (U.S. Dept. of Health, Education and Welfare, 1979). The development of these disorders in human smokers becomes apparent following years or even decades of cigarette use. Any physiological or endocrinological response which habituates within a short time of the initiation of cigarette (nicotine) use would be an unlikely candidate for mediating (causing or promoting) the cardiovascular diseases associated with smoking. However, this does not exclude the possibility that compensatory changes (i.e., changes in metabolism, rate of turnover, synthesis, storage and release, etc.) induced by nicotine use may be involved in the pathophysiologic processes of cardiovascular disease.

It has been proposed that elevated levels of glucocorticoids (e.g., corticosterone) may increase the sensitivity of cardiovascular tissues to the effects catecholamines (Eliot, et al., 1978). The increased sensitivity of the tissues to the effects of circulating catecholamines may result in frequent over-stimulation (i.e., stimulation which exceeds optimal levels) which leads to tissue damage

and eventual failure. This model has been proposed as an explanation of the increased incidence of premature death among smokers due to several cardiovascular disorders, including sudden coronary death syndrome, ischemic heart disease, and myocardial infarction. As described previously, the results of this experiment indicate that the administration of nicotine in the naive or nicotine habituated animal resulted in increased plasma concentrations of corticosterone and catecholamines (norepinephrine, epinephrine and dopamine). In addition, while norepinephrine and epinephrine responses displayed partial habituation, a stable and continuing level of responsivity for catecholamines and corticosterone was achieved within the first week of nicotine administration. The rapid development of tolerance, with the subsequent establishment of stable levels of responding, leads to the conclusion that responding might continue at a similar level with continued exposure to nicotine. Preliminary findings indicated a positive dose related relationship between nicotine and histologic evidence of myocardial degeneration and failure.[15] Thus, it would seem that the administration of nicotine (to nicotine naive or habituated animals) induces a series of endocrinological responses which may mediate and/or promote cardiovascular damage.

Further research is needed to confirm these observations and to assess the extent to which these effects may potentiate the cardiovascular disorders prominent among cigarette smokers. These findings have several important implications pertaining to methods of reducing cigarette related cardiovascular damage, for example: a) the reduction or elimination of the nicotine content of tobacco products,

and b) the use of pharmacologic blocking agents (either added to the tobacco content of the cigarette or taken separately) to reduce the intensity of responses induced by the administration of nicotine (cigarette smoking).

In summary, the findings of Phase I suggest that exposure to nicotine, in the naive or habituated organism, results in increased plasma concentrations of catecholamines, corticosterone and glucose, and a more rapid decrease in the plasma concentration of insulin. All responding was maintained at similar or slightly reduced levels with the continuation of nicotine administration (i.e., responses induced by nicotine on Day 7 were similar to those produced on Days 1 and/or 3). Continued responsivity to nicotine has many implications related to a) theories of reinforcement in the development and maintenance of smoking behaviors, and b) explanations of differences in body weight (i.e., taste preferences, food consumption, caloric intake) and the incidence of cardiovascular disorders between smokers and non-smokers.

Phase II

Solomon and Corbit (1973; 1974), Solomon (1980) and Hurvitch and Jameson (1974) have proposed opponent process theories of motivation-emotion and neural organization, respectively. These theories are founded upon the assumption of negative feedback -- the presentation of a stimulus (unconditioned stimulus) which results in a deviation (primary process response) from baseline (an increase or decrease from the normal homeostatic value) will in turn initiate a counteracting or opponent process (secondary process response). The summation of the primary and opponent process responses yields a value

equal to or approximating the baseline. Following repeated presentation of the unconditioned stimulus, the opponent process may become conditioned to stimuli (conditioned stimuli) previously associated with the presentation of the unconditioned stimulus. The opponent process will manifest its quality and intensity when the unconditioned stimulus is terminated or when the conditioned stimulus is presented.

Siegel (1975; 1977), Poulas, Hinson and Siegel (1981) and Pomerleau (1981), using an opponent process model, have proposed an explanation for the maintenance of self-administration and the development of tolerance effects in habitual drug use. The frequent presentation of a drug results in the development of physiological-biochemical and/or behavioral opponent processes which counteract the primary effects of the drug (Crowell, Hinson & Siegel, 1981; Mansfield & Cunningham, 1980; Siegal, 1972; 1975). Over an extended period of drug use the opponent process will gain in strength and duration, ultimately requiring the continued use of the drug in order to maintain normal functioning. Thus, the motivation for continued drug use is the avoidance of the opponent process (whether it be physiological-biochemical, behavioral, cognitive or affective) which manifests its characteristics during periods of drug abstinence. Under appropriate circumstances the presentation of conditioned drug-related stimuli may elicit the opponent process response.

Solomon and Corbit (1973; 1974) argue that under all circumstances a conditioned response will be opposite in nature to the primary unconditioned response. When the model is extended to a discussion of physiological processes involved in drug use, it would be

expected that conditioned responses would be opposite to the unconditioned drug induced physiological effects. While many studies have demonstrated the presence of conditioned opponent physiological responses following repetitive drug administration, many other studies have reported conditioned responses similar to those resulting from drug administration (Crowell et al., 1981; Eikelboom & Stewart, 1979; 1981; Mansfield & Cunningham, 1980). The existence of conditioned primary process responses can not be explained by the opponent process theory.

Many volumes of research are available regarding the physiological (endocrinological, biochemical, etc.) and behavioral effects of the common drugs of abuse (e.g., Gilman et al., 1980). Extensive research has also been conducted on conditioning of drug induced physiological processes (see reviews by Eikelboom & Stewart, 1982 and Woods & Kulkosky, 1976) with many studies having successfully demonstrated these effects. Unfortunately, the findings of many studies have been conflicting and quite contradictory, and can not be accounted for by the relatively simplistic opponent process model.

Eikelboom and Stewart (1982) have reviewed the literature on conditioned physiological responses and have proposed a new theory to account for the apparently paradoxical existence of conditioned opponent and primary process responses. The theory, as does the opponent process model, requires the existence of negative feedback loops in the control (regulation) of physiological processes. The authors argue that failure to properly identify the site of action of a drug (i.e., afferent or efferent systems) has led to the confusion

regarding previous findings. The model allows for two types of conditioned responses (i.e., similar to the primary process or an opponent process) depending on the site of action of the drug. In addition, it is argued that conditioning may occur only for those responses mediated and controlled by neural integration systems.

A drug which acts upon the afferent (input) system of a feedback loop results in a change in one or more aspects of the input to the system. This change in input (the unconditioned stimulus) will have an effect on the feedback system integrator similar to that which would be induced by a change in the regulated variable. The drug induced change in afferent input results in activation of the integrator and of the efferent system, which then mediates the drug response (unconditioned response). The presentation of a conditioned stimulus alters the activity of the afferent, integrator and efferent systems (effects being similar to those produced by the drug), resulting in a conditioned response similar to the drug-induced effect.

In contrast, a drug which acts upon the efferent system of a feedback loop, or on effector organs, may directly induce changes in the internal milieu. The alteration in physiological parameters will result in changes in afferent input (unconditioned stimulus) which in turn results in the activation of the integrator and efferent systems to oppose (unconditioned response) the drug induced effects. Thus, when a drug acts upon the efferent system of a negative feedback loop, conditioned responses will oppose the direct effect of the drug (i.e., conditioned opponent process).

In the present study evidence was found suggesting the development of conditioned responses for two of the three catecholamines being measured. Conditioned norepinephrine responses were similar in pattern (i.e., increases in plasma concentration) although of smaller magnitude than responses induced by the administration of nicotine. In contrast, dopamine displayed a conditioned opponent process response (i.e., the cyclic changes in the plasma concentration of dopamine were opposite to those observed for the control and nicotine conditions) when compared to the drug induced effect. (An alternative explanation for the conditioned dopamine opponent process response is available: presentation of the conditioned stimulus may have resulted in a phase shift in the time-course of the usual daily cyclic variation in plasma dopamine thus resulting in increasing concentrations during a time period when decreases would typically be evident.)

Applying the theory of Eikelboom and Stewart (1982), one only needs to know the primary site of action of a drug in order to predict the nature of the conditioned response. Unfortunately, such information is frequently not available. In addition, the situation does not appear to be as simple as the theory implies, as has been demonstrated in a study in which conditioned responses were found to be partially dependent on environmental factors (Eikelboom & Stewart, 1981). Other studies (compare Siegel, 1972, 1975; Woods & Shogren, 1972) have reported different conditioned drug responses, the effect apparently depending on the dose of the drug used during conditioning trials.

Neither the opponent process model nor the model of Eikelboom and Stewart is fully capable of explaining the pattern of conditioned responses observed in the present experiment. The opponent process theory can not account for the fact that the conditioned norepinephrine response was similar to the primary nicotine induced unconditioned response. Further, it is extremely difficult for the model proposed by Eikelboom and Stewart to parsimoniously account for the presence of conditioned opponent and primary process responses within two such closely related autonomic nervous system transmitters as norepinephrine and dopamine (or to account for the apparent lack of conditioned epinephrine effects).

Based on the current state of knowledge, the expected sources of norepinephrine and dopamine measured in plasma would be the peripheral autonomic nervous system, adrenal medulla and those structures of the central nervous system (e.g., hypothalamus - pituitary, chemoreceptor trigger zone, pineal, etc.) which have direct hormonal or chemical communication with blood (i.e., an incomplete blood-brain barrier). Traditionally, measurements of plasma and/or urinary concentrations of catecholamines have been used as indices of autonomic nervous system activity; a change detected in one measure would be expected to correspond to a change (although not necessarily an identical change) in the other measures.

If dopamine were being released as a byproduct of the activity of norepinephrine containing cells then it would be expected that norepinephrine and dopamine responses would be similar. The fact that the observed responses were opposite to each other (i.e., one an

opponent conditioned response and the other a primary process conditioned response) suggests that peripheral plasma norepinephrine and dopamine originate from distinct and independent sources. (However there is an alternative explanation for this effect: the presentation of the conditioned stimulus may have induced a change in the activity of the catecholamine synthetic enzymes, thus altering the intracellular ratio of norepinephrine and dopamine and thereby changing the quantities of each being released during synaptic transmission. Thus it is possible that norepinephrine and dopamine are being released from the same cells, but that the ratio and/or quantity in which each is released changes as a function of the catecholamine synthetic enzymes.) One possible source of dopamine is the hypothalamic-pituitary system. Changes in the activity of this system could account for the effects observed in this experiment.[16]

In order to account for the experimental results using the model proposed by Eikelboom and Stewart (1982) it is necessary to postulate that nicotine acts on both afferent and efferent aspects of catecholamine control systems. Following from Eikelboom and Stewart (1982), norepinephrine responses (i.e., increases in plasma norepinephrine subsequent to the adminstraton of nicotine or the presentation of conditioned drug-related stimuli) may be explained as being mediated by the effects of nicotine on afferent systems, whereas dopamine responses (i.e., increases in plasma dopamine subsequent to the administration of nicotine and decreases following the presentation of conditioned stimuli) may be accounted for by the actions of nicotine on the efferent arm of the feedback mechanism. To summarize, these

findings suggest the following conclusions: a) nicotine acts on both afferent and efferent feedback systems in its effects on peripheral hormone levels, and b) norepinephrine and dopamine measured in peripheral plasma originate from spearate and independent sources. In addition, these findings suggest that response patterns for all forms of catecholamines must be analyzed separately because these measures may not necessarily covary as indices of autonomic arousal.

The results of Phase II have several implications regarding treatment in abstinence from cigarettes and the accompanying withdrawal syndrome. As mentioned in the discussion of the results of Phase I, glucose and perhaps catecholamines have been proposed as agents involved in reinforcement during the development and maintenance of cigarette smoking behavior. The present results suggest that the repetitive administration of nicotine (as occurs in habitual cigarette smoking) may result in the development of conditioned responses. If the drug induced response were a positive reinforcer and the conditioned response were similar to the unconditioned response, then the presentation of the conditioned stimulus with the subsequent elicitation of the conditioned response would be expected to decrease the discomfort (withdrawal syndrome) associated with the absence of the drug. In contrast, if the conditioned response were an opponent process, then removal of all conditioned drug related stimuli would decrease the severity of the withdrawal syndrome (i.e., if the conditioned response were an opponent process then the repetitive presentation of the conditioned stimulus would increase the intensity of the withdrawal syndrome). In addition, if the conditioned drug

response serves as a discriminative stimulus for the drug taking behavior, or if it serves as a physiological substrate which is labelled as craving and/or withdrawal during periods of abstinence, then it would be that removal of all conditioned drug related stimuli would decrease the severity and/or discomfort associated with withdrawal.

To summarize the findings of phase II: a) the repetitive administration of nicotine (and possibly cigarettes) in conjunction with the appropriate presentation of environmental stimuli resulted in the development of conditioned physiological responses for norepinephrine and dopamine, b) the conditioned norepinephrine responses were similar to responses induced by the administration of nicotine while conditioned dopamine responses were opposite (or opponent) to the effects of nicotine, and c) conditioned stimuli might help to reduce the discomfort associated with nicotine abstinence and withdrawal.

Phase III

The final phase of the experiment examined the potential contribution of nicotine to the reduction of stress induced responses. This aspect of the experiment used measurements of plasma catecholamines and corticosterone as indicators of autonomic arousal.

Cigarette users frequently report that when under conditions of environmental stress they smoke more and that smoking relaxes them. Ashton and Stepney (1982) have proposed that smoking may have beneficial short-term psychological effects, including a) the maintenance of performance under conditions of monotony and fatigue, b) increasing the selectivity of attention, and c) attenuating the

effects of stress. Further, these investigators hold that nicotine is a central factor involved in cigarette use because the psychic effects of smoking are attributable to nicotine. As discussed in the introduction section, nicotine is a sympathomimetic which elicits a number of physiological responses similar to those resulting from activation of the sympathetic autonomic nervous system. How the self-administration of a stimulant apparently results in a calming or quieting of stress induced responses is not clear. This set of circumstances appears most perplexing and has been termed "Nesbitts Paradox" by Schachter (1973).

Many smokers report increased smoking when under conditions of stress. Schachter and his associates (Schachter, Silverstein, Kozlowski, Herman, & Liebling, 1977; Schachter, Silverstein & Perlick, 1977) found that when smokers were subjected to a highly stressful situation (the repeated applicaton of painful electric shocks) their consumption of cigarettes was greater than when under a low stress condition (the application of mild electric shocks). Both studies indicate that for the habitual smoker the application of a stressor (noxious stimulation) leads to increased cigarette smoking.

Several studies have examined the role of cigarette smoking in stress reduction. Silverstein (1976) reported that in a group of habitual smokers allowed to smoke high nicotine (1.1 mg/cigarette), low nicotine (0.3 mg/cigarette) or no cigarettes, the intensity of shocks which subjects were willing to endure was positively related to the nicotine content of the cigarettes (i.e., as nicotine increased the subjects endured greater intensity electric shocks). It was assumed

that the greater the level of anxiety experienced by the subjects, the less pain they would be willing to endure. Thus, if smoking has anti-anxiety effects the smoker (allowed to smoke) should tolerate shocks of greater intensity. Perlick (1977) found that smokers allowed to smoke high nicotine (1.3 mg/cigarette) cigarettes reported less irritation in response to simulated jet overflights than did subjects not allowed to smoke or those allowed only low nicotine (0.3 mg/cigarette) cigarettes. In both studies it was found that control non-smoking subjects displayed similar responses to those of smokers allowed to smoke high nicotine cigarettes. Therefore, these studies indicate that a) smoking may not have any beneficial effects other than to reduce withdrawal symptoms produced by conditions (e.g., stress) that somehow decrease the bioavailability of nicotine (c.f., Grunberg, Morse & Barrett, 1983; Schachter et al., 1977), b) smoking under conditions of stress results in responses similar to those of non-smoking control subjects (i.e., failure to consume adequate quantities of nicotine while under conditions of stress results in an increased sensitivity and/or responsivity to the stressor), and c) these effects are directly related to the nicotine content of the cigarette. There are two main possibilities for the mode of action of smoking (nicotine administration) in the reduction of stress induced responses. Smoking and/or nicotine may alter psychological process involved in the stress response (e.g., cognitive labelling of the stressor and its effects; c.f., Schachter & Singer, 1962) or it may reduce the physiological activity associated with stress.

In the present study, it was observed that the application of physical restraint stress induced large and sustained increases in the plasma concentrations of catecholamines (particularly norepinephrine and epinephrine) and corticosterone (see the Results section for a more detailed presentation of these effects). In animals that had been given nicotine for 10 or more days, the administration of nicotine concurrent with the application of the restraint stressor resulted in the induction of endocrinological responses similar to or of greater intensity than those effects produced by the stressor alone. Using the measures of catecholamines and corticosterone as indices of autonomic activity (arousal) (c.f., Baum, Grunberg & Singer, 1982) these findings suggest that the reported stress ameliorating effect of cigarette smoking (nicotine administration) probably is not mediated by a reduction of stress related physiological responses.

The present results do not suggest the contribution of a physiological component in the reported stress ameliorating effects of nicotine, however they do not address various psychological explanations for the phenomenon. Ashton and Stepney (1982) proposed that the act of smoking allows the smoker to focus attention away from the stressor and thereby reduce its effect. Schachter and Singer (1962) demonstrated that the interpretation of an undifferentiated state of autonomic arousal was determined by cognitive and environmental factors. Storms and Nisbett (1970) found that under appropriate conditions it was possible to convince subjects to misattribute the source of their physiological arousal. Possibly the smoker, accustomed to the mild autonomic stimulation (arousal)

associated with the consumption of cigarettes, may misattribute the stress induced arousal as being caused by cigarettes. The misattribution of the stress induced arousal from the stressor to the cigarette would thereby reduce the intensity of the cognitive component of the stress response. Each of these alternative explanations of smoking and stress effects warrants further research.

Summary and Conclusions

The results of Phase I of the experiment suggested that the administration of nicotine resulted in increases in the plasma concentrations of catecholamines (norepinephrine, epinephrine and dopamine), corticosterone and glucose, and in more rapid decreases in the concentrations of insulin. While norepinephrine (and possible epinephrine) responses displayed partial habituation during the first week of testing, all other responses were found to be consistent in the "naive" and nicotine "experienced" (habituated) organism. These responses are discussed as possible sources of reinforcement in the development and maintenance of cigarette smoking, and also as mediators of the changes in specific food consumption that contribute to lower body weight among smokers when compared to non-smokers.

It also was observed that increasing doses of nicotine resulted in increasing plasma concentrations of catecholamines and corticosterone which have been implicated as potential mediators of several cardiovascular disorders. Preliminary evidence suggested a dose related relationship between the quantities of nicotine administered and histological indications of myocardial degeneration and failure.

Phase II demonstrated that the repetitive administration of nicotine in conjunction with the appropriate presentation of environmental stimuli may result in the development of conditioned physiological responses. The observations suggest the presence of opponent and primary process conditioned responses. Appropriate presentation of the conditioned stimuli (with the subsequent elicitation of the conditioned response) may help to reduce the discomfort associated with abstinence and the withdrawal syndrome.

The results of Phase III of the experiment suggest that the administration of nicotine to habituated animals does not reduce the intensity of physiological responses induced by the stress of physical restraint. In fact, the administration of nicotine concurrent with the application of a physical stressor resulted in a somewhat greater intensity of responding than was induced by the application of the stressor alone. These results suggest that the stress ameliorating effects of cigarette smoking are not based on a reduction of the intensity of peripheral physiological stress responses.