DOCUMENT RESUME

ED 276 564 SE 047 537

AUTHOR Molinaro, Jane Anne
TITLE The Relationship of Test Anxiety to Serum Beta-endorphin.
INSTITUTION Ohio State Univ., Columbus. Graduate School.
PUB DATE 86
NOTE 186p.; Ph.D. Dissertation, Ohio State University. Correlation tables contain small print.
PUB TYPE Dissertations/Theses - Doctoral Dissertations (041) -- Reports - Research/Technical (143)

EDRS PRICE MF01/PC08 Plus Postage.
DESCRIPTORS Anxiety; Biochemistry; College Science; Higher Education; *Human Body; *Physiology; *Premedical Students; Science Education; *Test Anxiety; *Test Wiseness
IDENTIFIERS Endorphins; Hormones; Medical College Admission Test; *Science Education Research

ABSTRACT

This study attempted to determine if test anxiety is manifested in pre-medical students as a result of the Medical College Admission Test (MCAT) and if Beta-endorphin similarly responds to that type of situational stress. Seventeen participants completed the Test Anxiety Inventory (TAI) by Spielberger et al. (1980) and donated 30 ml of blood for Beta-endorphin analysis two days prior to the MCAT and also three days after the test. Student grade point average (GPA) and number of science courses were other variables analyzed. Results indicated that TAI were more elevated pre-MCAT and Beta-endorphin was higher after the examination. Total TAI was significantly inversely related to GPA and there were positive correlations between GPA and MCAT scores. Regression results indicated that select items from TAI, GPA, and Beta-endorphin could be used to predict performance on particular MCAT subsets and that MCAT scores were influenced by test anxiety. Appendices include copies of the TAI, procedures used in processing blood samples for Beta-endorphin, and complete correlation matrices. A 14-page bibliography is included. (ML)

THE RELATIONSHIP OF TEST ANXIETY TO SERUM BETA-ENDORPHIN

By

Sister Jane Anne Molinaro, Ph.D.

The Ohio State University, 1986

Professor Robert W. Howe, Adviser

The purpose of this study was to test pre-medical students before and after the Medical College Admission Test (MCAT) to determine the presence of test anxiety and its effect on MCAT scores.

Seventeen participants reported two days prior to the MCAT to complete the Test Anxiety Inventory (TAI) by Spielberger et al., 1980, and to donate 30 ml of blood. Subjects returned three days post-MCAT to repeat the procedure. Results of the TAI (with subscales Worry, Emotionality, Total) were tabulated. Plasma was extracted from pre to post-MCAT serum samples and subjected to Radioimmunoassay (RIA) to measure the amount of Beta-endorphin.

Student GPA and number of Science courses were other variables analyzed.

It was hypothesized that the TAI scores and Beta-endorphin levels would be significantly elevated as a result of stress associated with the MCAT, and test anxiety (as measured by TAI and Beta-endorphin) would be significantly related to MCAT scores. It was further hypothesized that TAI scores would be related to Beta-endorphin and that GPA and Science courses would relate to MCAT and test anxiety measures.

Results indicated that TAI (subscales) were more elevated pre-MCAT and Beta-endorphin was higher after the examination. Test anxiety (TAI Total and subscales) was significantly inversely related to performance on MCAT. Pre-MCAT Beta-endorphin was negatively correlated with MCAT subsets of Chemistry and Science Problems. TAI subscales and serum Beta-endorphin were not significantly correlated; several items of the TAI were significantly related to Beta-endorphin.

Total TAI was significantly inversely related to GPA. There were positive correlations between GPA and MCAT scores.

Pre-MCAT Beta-endorphin results showed a significant positive relationship with undergraduate Biology; a significant negative correlation with undergraduate Chemistry courses. Post-MCAT Beta- endorphin data was significantly inversely correlated with graduate Chemistry courses.

Regression results indicated that select items from the TAI, GPA and Beta-endorphin could be used to predict performance on particular MCAT subsets and that MCAT scores were influenced by test anxiety.

Other conclusions and recommendations for further research are presented. The Appendices include copies of the TAI, procedures used in processing blood samples for Beta-endorphin, and complete correlation matrices.

Sister Jane Anne Molinaro

The Relationship of Test Anxiety to Serum Beta-endorphin

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Sister Jane Anne Molinaro, A.B., M.Sci.Ed.

* * * * *

The Ohio State University

1986

Dissertation Committee:

Robert W. Howe

Stanley L. Helgeson

Gopi A. Tejwani

Approved by

Robert W. Howe

Robert W. Howe, Adviser
College of Education: Theory and Practice

DEDICATION

To my deceased parents

ACKNOWLEDGEMENTS

Heartfelt gratitude is extended to the dissertation commitee members: Dr. Robert W. Howe, whose competence and intelligence was augmented by patience; Dr. Gopi A. Tejwani, who generously shared laboratory facilities and extensive knowledge about serum Beta-endorphin research; Dr. Stanley L. Helgeson who never complained about reading "still another copy."

Special thanks to Jim Wagner from the Baker Computer Center for his technical assistance.

I am deeply indebted to all my friends who said, "You will make it!"

I gratefully acknowledge my natural and religious families whose prayers and support have sustained me throughout this endeavor.

Above all, I praise and thank God for His many blessings and pray that I will use this accomplishment for His greater Honor and Glory.

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CHAPTER I

INTRODUCTION

Testing is a reality both in education and society. A view of educational practice reveals that teachers subject students to frequent examinations. They test to determine interest, achievement, aptitudes and intelligence. Credence is given to scores received from these tests and used to screen, admit or reject, place and select, judge, grade, and evaluate students. Research on "test use" points out that whenever results are used in important decisions that affect individual life changes, pupils modify their behavior accordingly (Madeus, 1984).

Psychological variables have been suggested to influence test anxiety. Some are: self pre-occupying worry, insecurity and self-doubt (Sarason, 1984), negative thoughts during actual testing (Galassi et al., 1981), and fear of failure (Gaudry and Spielberger, 1971). Whatever the purported causes, when tests are used at crucial decision-making points for students, the associated stress may be sufficient to interfere with authentic output. This is a factor that educators, who are interested in the academic well-being of their students, must address attentively.

Personal concerns for students who are veritably test anxious has led to the discriminate investigation, through literature, of the actual state. Advanced speculation suggests a possible physiological involvement.

As an educator who is interested in substantiating the findings on test anxiety, careful efforts have been made in selecting the particular population of pre-medical

students. This group has the potential to demonstrate test anxiety since they are required to successfully complete an instrument which will screen their entry into a field of choice. Findings, involving pre-medical students, in particular may elucidate key information on test anxiety which may then be applied to this population and lead to further research with other populations of students.

NEED FOR THE STUDY

Test Anxiety in General

Many students are unable to deal with anxiety and are forced to "dropout" of college (Lusk, 1979). Furthermore, anxiety may become so intense that students not only contemplate suicide but actually execute it (C. H. Brown in Spielberger, et al., 1979 p. 167). These realities cause Head and Lindsey (1983) to submit, that (test) anxiety and its effect on college students must become a major concern for all higher education personnel.

According to Tryon (1980) there was a consistent moderate negative correlation between test anxiety and total achievement. Other studies have shown that test anxiety has also interfered with students' ability to profit from instruction, thus having consequential negative effects on grade point average (Spielberger & Katzenmayer, 1959; Culler & Holahan, 1980; Benjamin et al., 1981).

Earler findings (Mandler and Sarason, 1952) suggested that test anxiety was mainly cognitive in nature, i.e., individuals experienced feelings of inadequacy and helplessness, anticipation of punishment or diminished self-esteem. Therefore, when high-test-anxious persons were placed in evaluative settings, a class of task-irrelevant or interfering responses was evoked. More recent findings substantiate this conception. Wine (1982) claimed that highly test anxious individuals performed more poorly on

cognitive tasks than less anxious individuals, especially if the tasks were difficult and were given in evaluatively stressful circumstances (Wine, 1982, p. 209).

It seems evident therefore, that student achievement can be suppressed when students are in this state. This fact alone makes it worthy of consideration and investigation.

Physiological Aspects of Test Anxiety

Work done by Leibert and Morris (1967) suggested that there are two distinguishable components of test anxiety, one cognitive which they classified as "Worry" (e.g. thinking about the consequences of failure, expressing doubts about one's ability) and the other "Emotionality" which refers to autonomic or physiological reactions that become evident in a test situation.

Modern day analysis of test anxiety has preserved the carefully differentiated factors. The distinction allows one to categorize "Worry" as the more psychological, cognitive oriented member and "Emotionality" as the affective component (Morris et al., 1981).

This notion of the autonomic arousal aspect (Sarason, 1984) has generated personal interest to re-examine physiological ramifications of test anxiety. And since test anxiety is a situation-specific form of general anxiety (Sieber, 1980) or stress, serum β-endorphin surfaces as a possible associated contender in the affective response.

The relationship between stress and endorphins was first described by Guillemin (1977), although initial isolation of the endogenous opioids were conducted in 1975 by Hughes et al. Using pharmacological bioassays, the researchers demonstrated the presence of two pentapeptides with opiate-like activity in animal brain tissue. Beta-lipotropin (β-LPH) a 91-amino-acid peptide (Fig. 1) was isolated as a minor component of pituitary extracts. Soon after, the discovery was made that the long chain

peptide contained methionine enkephalin as a sequence of amino acid within the longer chain. Subsequently, several endorphins were identified from porcine pituitary extracts and characterized.

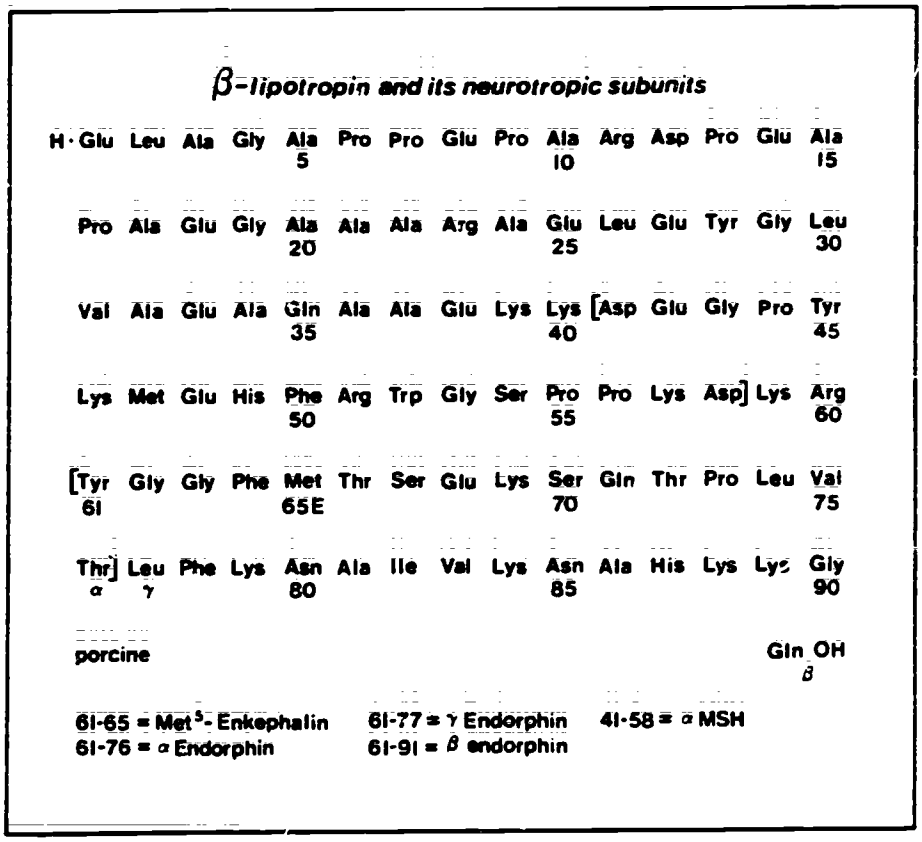

Figure 1: Structure of Beta-lipotrophin and related opioid peptides. (Cooper, Bloom, & Roth, 1982) Reproduced with special permission of the Publisher, Oxford University Press, 200 Madison Avenue, New York, 10016.

Since their isolation, endorphins have been implicated as having both euphoric (Bloom et al., 1980) and analgesic (Devon, 1984) effects.

Studies have shown that chronic pain patients have a lower than normal level of serum Beta-endorphin (β-endorphin). Consistent with this hypothesis, numerous other reports indicate that pituitary endorphins are released under conditions of stress or pain (see Lewis et al., 1984).

When testing pre-surgical stress, Miralles et al. (1983), found that endorphin plasma levels increased significantly ($p < 0.05$) during stress. The researchers posited that the observed increase was correlated directly with the emotional stressful situation of pre- surgery. Stress seems to be a natural stimulus triggering pain suppression thereby releasing β-endorphin to serve as the analgesia (Terman et al., 1984).

Cohen et al. (1983), state that the endogenous opioid system has been clearly implicated as an important component in the adaptation in human pain perception involved in the stress response (p.466).

The evidence that the endogenous opiate, β-endorphin is elevated during stress-induced activity could be applied to the individual who requires analgesia to cope with the pressured condition of examinations.

The premise may be forwarded therefore, that the human body physiologically adapts to the anxious student who is being evaluated. Furthermore, a corroborating assumption may be that serum β-endorphin level is elevated in response to anxiety associated with tests.

Theoretical Model of Test Anxiety

General attention about the adverse reactions that stress and anxiety inflict on academic achievement has led to numerous methodologies for study. Models have been proposed to describe the stress cycle (Selye, 1956; McGrath, 1982; Molberg, 1985). An adaptation of these has been prepared to characterize the presumable effects of test anxiety.

Stage A of the model (Fig. 2), suggests that a stress situation begins with some set of circumstances in the sociophysical environment. It becomes a stimulus for a given individual if he or she perceives it as leading to some undesirable state of affairs if left unmodified or to some desirable state of affairs if modified (McGrath, 1982, p. 21).

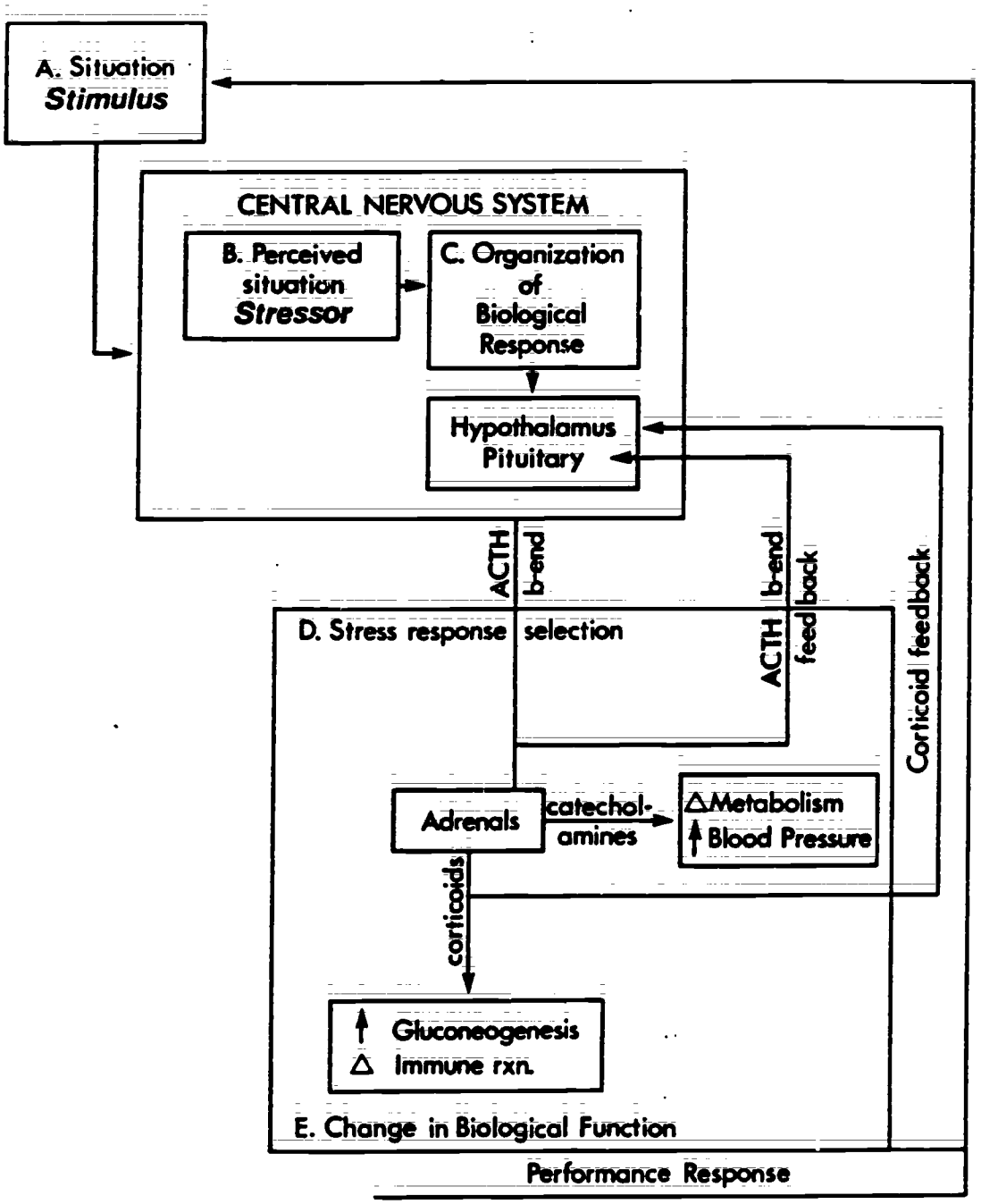

Figure 2: Theoretical model of test anxiety

Stage B in the model represents a perceived situation. The link between Stage A and Stage B is what Lazarus (1966) has called "cognitive appraisal;" that which can result in the experience of stress as a subjective state. Note that the stressor is processed in the central nervous system (CNS).

Adopting the premise that pre-medical students are prime candidates for test anxiety, this model may then be used as a filter. The Medical College Admission Test (MCAT) could be appropriately adapted to the objective situation of Stage A. Contingent upon the results of this situation (examination), students optimize their chances of being accepted into medical school. While not the only component of selection, it is a key one. Moreover, the discrimination is such that it is not unreasonable to believe that some students who would succeed as doctors will never have the opportunity because their less than favorable MCAT scores may have omitted them from initial consideration. Hence, the intense competition of the instrument presents itself as a situation that the student perceives as leading to some desired state if modified, namely, to be eligible for medical school.

Stage B or the subjectively experienced stress depends on an individual's "perception" and interpretation of the objective or external (Stage A) stress situation (Head & Lindsey, 1983).

Pre-medical students are aware of the distinguishing aspect of the MCAT. Medical schools are crowded and can admit only a certain number of new students. For example, each year approximately 36,000 individuals apply to almost 120 medical schools in the United States for about 16,000 available openings (AAMC, 1986).[1] Most schools only accept applicants if scholastic ability, personal and social adjustment, and involvement in extracurricular activities meet a certain criteria. The MCAT

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[1] Statistics for 1984-85 specify that 35,944 individuals filed 331,937 applications for 16,395 places in medical schools (AAMC, 1986).

scores may be weighted heavily in determining intellectual ability, particularly for students whose grades are minimally acceptable for medical school admission or for students from colleges or universities without an established track record (Boyles, et al., 1982).

It is assumed, therefore, that not only the decisive testing requirement itself but also the perception of its implications will elevate test anxiety.

The stressor stimulates a variety of biological responses (Fig. 2, C.) one of which is the secretion of adrenocorticotropic hormone (ACTH) and β-endorphin concomitantly from the pituitary (Guillemin et al., 1977).

The **response selection** (Fig. 2, D.) when activated involves specific physiological activity that is precipitated by test anxiety (Holroyd et al., 1978; Mandler and Kremen, 1958; Obrist et al., 1978). The ACTH reaching the adrenal cortex triggers the secretion of corticoids, mainly glucocorticoids such as cortisol or corticosterone. Through gluconeogenesis, these compounds supply a readily available source of energy for the adaptive reactions necessary to meet the demands of the agent. The corticoids also facilitate various other enzyme responses and suppress immune reactions (Golberger et al., 1982). Cortisol excretion (Tennes & Kreye, 1985) and metabolic modification by elevation of systolic blood pressure (Kermis, 1983) have been known to accompany test anxiousness. Furthermore, DuBois et al. (1981) detected a strong correlation between plasma β-endorphin and cortisol.

The chain of events is cybernetically controlled by several feedback mechanisms. For instance, if there is a surplus of ACTH, a short-loop feedback returns some of it to the hypothalmus-pituitary and this shuts off further ACTH/ β-endorphin production. In addition, through a long-loop feedback, a high blood level of corticoids or peptides similarly inhibits too much secretion (Goldberger et al., 1982).

Changes in the biological function (Fig. 2, E.) affect the response or performance process. McGrath (1982) refers to this as "behavior." He states that performance depends on ability, task difficulty and the standards against which it is compared. It has been shown that increases in arousal would degrade performance, presumably because of the interference of fear, anxiety or disorganization (p. 23).

Differentiating between adaptive and maladaptive behavioral responses is addressed in Chapter II.

It is important to emphasize that the present model is only one way of attempting to incorporate both the psychological and physiological systems as activated by the perception and execution of the MCAT.

PURPOSE OF THE STUDY

This study is designed to analyze the association between psychometric and physiological outcomes. Therefore, the ultimate purpose is to empirically elucidate significant information about the relationship between test anxiety as measured by the Test Anxiety Inventory (TAI) and serum β-endorphin.

Aware of the delicate evaluation resulting from the examination, it may be argued that perception and execution of the required Medical College Admission Test (MCAT), becomes a situation-specific event which has the potential to cause elevated levels of test anxiety and serum β-endorphin. The objective is to determine if the presence of test anxiety has disfavorably altered MCAT results. A relationship , if detected, between test anxiety (psychological and physiological) and achievement will hopefully encourage further research. Thus in the future, (if) when causal factors can be experimentally perceived, constructive intervention measures may be taken.

STATEMENT OF THE PROBLEM

Results of this investigation will point to a number of questions whose answers are necessary for an adequate understanding of the effects of the psychological/ physiological relationship. They are as follows:

1. Will test anxiety as measured by the TAI subscales (Worry, Emotionality, and Total) be significantly elevated as a result of stress associated with the MCAT-test:
    a. Pre-MCAT?
    b. Post-MCAT?
2. Will test anxiety as measured by serum β-endorphin be significantly elevated as a result of the stress associated with the MCAT:
    a. Pre-MCAT?
    b. Post-MCAT?
3. Will test anxiety as measured by TAI (Worry, Emotionality, Total) be significantly related to subset scores of the MCAT- Biology, Chemistry, Physics, Science Problems, Reading and Quantitative:
    a. Pre-MCAT?
    b. Post-MCAT?
4. Will test anxiety as measured by serum β-endorphin be significantly related to subset scores of the MCAT- Biology, Chemistry, Physics, Science Problems, Reading and Quantitative:
    a. Pre-MCAT?
    b. Post-MCAT?
5. Is test anxiety as measured by the TAI (Worry, Emotionality, Total) significantly related to serum β-endorphin:

a. Pre-MCAT?

b. Post-MCAT?

6. Will GPA be a significant predictor of MCAT scores?

a. Will GPA be significantly related to TAI (Worry, Emotionality, Total) results?

b. Will GPA be significantly related to serum β-endorphin (Pre-MCAT) (Post-MCAT)?

c. Will GPA be significantly related to MCAT scores?

7. Will the number and nature of science courses be significantly related to test anxiety?

a. Will content or total science courses be related to TAI (Worry, Emotionality, Total)?

b. Will content or total science courses be related to serum β-endorphin?

The problem of this study therefore, is to investigate the relationship between test-anxiety, serum β-endorphin and related variables in pre-medical students who take the required Medical College Admissions Test (MCAT). Choosing the MCAT as the treatment creates the strategic situation whereby anxiety level may be tested. Literature supports the fact that pre-medical students, who will be selected on the basis of their performance on the MCAT, are presumably potential candidates for test anxiousness (Feletti and Neame, 1981).

DEFINITION OF TERMS

Analgesia signifies a specific absence of pain in a certain cutaneous area. All other modalities of sensation are otherwise intact (Afifi & Bergman, 1986). The endorphins function as excitatory transmitter substances that activate portions of the brain's analgesic system.

Test anxiety is a psychological/physiological reaction; sometimes called evaluation anxiety (Wine, 1982) to distinguish it from general anxiety because of its situation-specific nature. Its uniqueness often corresponds to the cognitive appraisal of the test.

Emotionality refers largely to a person's awareness of bodily tension (Sarason, 1984). It involves the autonomic arousal aspect of anxiety (Wine, 1971).

Endogenous means produced or arising from within a cell or organism. The endogenous secretion of neuropeptides such as β-endorphin from the pituitary is presumed to occur during the time of stress (Akil et al., 1984).

Opium is the substance obtained by air-drying of the juice from the unripe capsule of the poppy, Papaver somniferum. It contains a number of important alkaloids such as morphine, codeine, and papaverine, all of which demonstrate some measure of analgesic activity (Thomas, 1981). Opium is probably the oldest known medication and morphine has been known since the 19th century to be the major alkaloid responsible for most of its pharmacological and medicinal effects (Malick & Bell, 1982).

Opiate is a substance containing or derived from opium. Powerful drug (s) such as morphine, for example, are widely used as analgesics pharmacologically. β-endorphin is the body's endogenous opiate (Akil, 1984).

Radioimmunoassay (RIA) is a very sensitive method of determining the concentration of substances, particularly the protein-bound hormones in blood plasma. This

procedure is based on competitive inhibition of radioactively-labeled hormones to specific antibody. Concentrations of protein in the picogram (10^{-12} gram) range can be measured by using this technique (Thomas, 1981). It is one of the biochemical processes that tests for the presence of β-endorphin in plasma.

Worry has been described as "preoccupation with performance (Doctor & Altman, 1969, p. 564)," "cognitive concern about the consequences of failing, the ability of others relative to one's own (Liebert & Morris, 1967, p. 975)."

DELIMITATIONS

Sampling restrictions preclude random selection, as volunteer pre-medical students (N = 17) taking the MCAT, only, were included in the study. Two blood samples were obtained, one pre-MCAT and the other post-MCAT.

LIMITATIONS

The distinct selection of this group was made because of possible meaningful results and propensity toward replication, however this does provoke inherent limitations.

Timing also is a limitation factor because the test is offered (only) twice annually. Soliciting participation of subjects in April and September imposed a temporal constraint on data collection.

Due to the fact that two blood samples were taken, it is impossible in this study to determine a β-endorphin curve.

HYPOTHESES

The following major hypotheses were developed and tested in order to confer information pertinent to the purpose of the study.

Hypothesis 1: Test anxiety as measured by the TAI subscales (Worry, Emotionality, and Total) will be significantly elevated as a result of stress associated with the MCAT-test:

a. Pre-MCAT.

b. Post-MCAT.

Hypothesis 2: Test anxiety as measured by serum β-endorphin will be significantly elevated as a result of the stress associated with the MCAT:

a. Pre-MCAT.

b. Post-MCAT.

Hypothesis 3: Test anxiety as measured by TAI (Worry, Emotionality, Total) will be significantly related to subset scores of the MCAT- Biology, Chemistry, Physics, Science Problems, Reading and Quantitative:

a. Pre-MCAT.

b. Post-MCAT.

Hypothesis 4: Test anxiety as measured by serum β-endorphin will be significantly related to subset scores of the MCAT- Biology, Chemistry, Physics, Science Problems, Reading and Quantitative.

a. Pre-MCAT.

b. Post-MCAT.

Hypothesis 5: Test anxiety as measured by the TAI (Worry, Emotionality, Total) will be significantly related to serum β-endorphin.:

a. Pre-MCAT.

b. Post-MCAT.

Hypothesis 6: GPA will be a significant predictor of MCAT scores.

a. GPA will be significantly related to TAI (Worry, Emotionality, Total) results.

b. GPA will be significantly related to serum β-endorphin (Pre-MCAT) (Post-MCAT).

c. GPA will be significantly related to MCAT scores.

Hypothesis 7: Number and nature of science courses will be significantly related to test anxiety.

a. Content or total Science courses will be related to TAI.

b. Content or total Science courses will be related to to serum β-endorphin.

Consequent performance on the independent variable (MCAT), within a specific range of results, is one of the components of a list of requirements for admission into medical school. Because of its qualifying nature, the test will presumably effect elevated levels in the dependent variables such as test anxiety and serum β-endorphin.

It is important to clarify that correlation does not imply causality (Best, 1977). Replication studies are necessary to test the following: if test anxiety (Total) and/or either of its components: Worry or Emotionality and level of serum β-endorphin are positively correlated then it may be cautiously assumed that the endogenous substance may act as nature's response to a stressful situation. If test-anxiety (Total) and/or either of its components: Worry or Emotionality and serum β-endorphin levels are negatively correlated, another assumption could then be judiciously forwarded, i.e.

elevation of endorphin during physiological stress may not be applied to the test-anxiety form of stress. Numerous correlations could represent a causal influence but an experimental study with manipulation and control would be necessary to substantiate results.

PLAN OF THE REPORT

The report is organized into five chapters. The first is the introduction. This includes the need for the study (of) test anxiety in general and the physiological aspects of test anxiety. The theoretical model in Chapter I, while appearing to be a cursory representation of the literature review is an effort to clarify the interface between psychological and physiological factors. Chapter II contains the literature review. The material presented is an embellishment of the discussions on test anxiety and serum β-endorphin (physiological) which were introduced in Chapter I. Detailed definitions, descriptions, research reviews and studies underscore the theoretical framework from which this investigation evolved. In Chapter III the sample of the study is defined, treatment and research procedures are described, and biochemical methods used for analyses are discussed. Chapter IV synthesizes the findings. Chapter V summarizes, presents conclusions, and offers recommendations and implications for subsequent research.

CHAPTER II

REVIEW OF THE LITERATURE

The sections in this chapter are divided into two main categories. In the first, test anxiety literature is reviewed and discussed in terms of: definitions, measurement and effects; Worry and Emotionality components; and physiological response. The second main category deals with serum β-endorphin research. The theoretical literature discussed involves their possible role in stress and analgesia- characteristics which suggest their presumable association with test anxiety. In general, the theories and studies cited are salient to the problem being addressed and are responsible for generating the questions and hypotheses presented in Chapter I.

CHARACTERISTICS OF TEST ANXIETY

Definitions, Measurement and Effects

In an attempt to develop a construct of test or evaluation anxiety, researchers have proposed conceptual definitions then formulated instruments to test them. An initial challenge was to clarify the distinction between anxiety in general and anxiety that is situation- specific in an evaluative setting. The developmental process described led to the most compelling definition, model and instrument selected specifically for this study.

General Anxiety

Sieber (1980) maintains that test anxiety is a special case of general anxiety. It incorporates phenomenological, physiological and behavioral responses that accompany concern about possible failure.

General anxiety permeates our lives. Adaptive mechanisms forewarn man and higher mammals of possible danger which triggers innate and learned coping responses in the quest for survival. Anxiety may become a stimulus for effective problem-solving in one individual but a confounding stimulus for another.

Effective problem solvers perceive anxiety as part of a positive experience. Thus for them, anxiety can produce healthy adaptation and development. However, individuals who view anxiety as a confounding stimulus react in a maladaptive manner. In this case, a problem may evoke emotional responses of panic, illness, worry, anger, resignation, shame or the desire to escape physically or mentally through defensive acts of repression or rationalization (Sieber, 1980, p. 18).

A strategic look at test anxiety must be taken from the vantage point of general anxiety. Figure 3 represents a scientific chain which reflects the conceptions of test anxiety that somewhat support this perspective.[2]

Darwin (1872) made anxiety and fear subjects of scientific inquiry. He claimed that each phenomena was indistinguishable and both were manifested in higher mammals in the same manner. For example, rapid heart beat, perspiration, dilation of pupils, dryness of mouth, trembling etc. occurred as adaptive mechanisms- enabling the organism to cope with or flee from sources of danger for survival.

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[2] The idea for producing the chart stemmed from J. E. Sieber (1977). The list (Fig. 3) is by no means complete. It includes the most frequently cited self-report instruments in the studies examined.

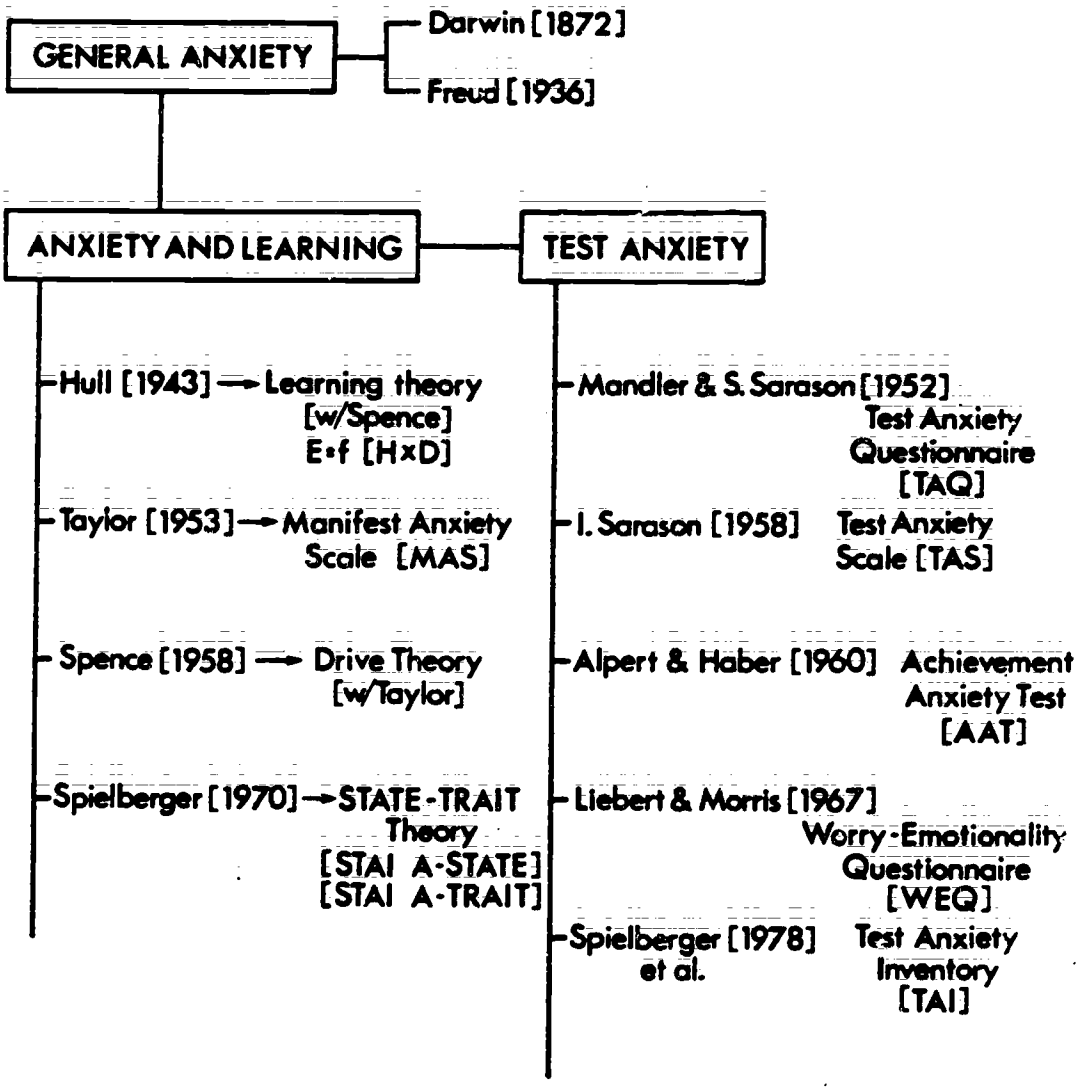

Figure 3: Evaluation Anxiety: Theories and Measurement. (NOTE: Dates conform to publication of psychometric instruments. In some cases, theories appeared in the literature previous to the dates indicated).

Later research by Freud (1936) emphasized the distinction between anxiety and fear. He believed that both objective and neurotic anxiety exist. Objective anxiety, more complex than fear, is based on a history of learning about danger in the external environment. Neurotic anxiety is similar to fear in that it is a complex internal reaction to some perceived danger based on an individual's own history of traumatic experiences, often repressed. Generally, persons who exhibit maladaptive responses need therapy to bring repressed material into consciousness.

Darwin and Freud developed the physiological and psychological rudiments of test anxiety research.

Anxiety and Learning

Research on anxiety and learning influenced the field of test anxiety.

Hull's theory (1943) diverged from those of Darwin and Freud and significantly contributed to anxiety's role in learning. In an attempt to explain and predict learning of new responses, Hull and his associate Spence, proposed that there are certain factors effecting the probability of the learning response, such as:

$$E = f\ (H \times D)$$

(E) is the excitatory potential or probability of response. It is a function (f) of (H) which represents the strength of the learned habit, and (D) which signifies the person's drive state. Emotional responsiveness (E) is Hull's term for anxiety. According to his theory, highly emotional persons respond more intensely to stressful and inimical stimuli and can exhibit more forceful escape responses.

Early investigators adopted the assumption that anxiety level was equivalent to emotional arousal.

Emotionality became the personality variable that led Taylor (1953) to develop a self-report instrument for assessment: the Taylor Manifest Anxiety Scale (MAS).

The measure has been widely used in learning laboratories as well as in the study of abnormal psychology and personality. The MAS reflects trait anxiety- a subjects propensity to respond anxiously under specified stressful circumstances (Rapaport & Katkin, 1972). It is useful as an index of the drive level evoked by psychological not physiological stress.

The Taylor-Spence Drive Theory (K. W. Spence, 1958; Taylor, 1956) with its roots in Hullian principles influenced test anxiety research. In essence, it predicted that high anxiety will facilitate performance in learning easy materials, but it will lead to performance decrements on difficult tasks.

Spielberger (1966) later extended the Drive Theory when he proposed that:

1. For subjects with superior intelligence, high anxiety will facilitate performance on most learning tasks. While high anxiety may initially cause performance decrements on very difficult tasks, it will eventually facilitate the performance of bright subjects as they progress through the task and correct responses become dominant.

2. For subjects of average intelligence, high anxiety will facilitate performance on simple tasks and, later in learning, on tasks of moderate difficulty. On very difficult tasks, high anxiety will generally lead to performance decrements.

3. For low intelligence subjects, high anxiety may facilitate performance on simple tasks that have been mastered. However, performance decrements will generally be associated with high anxiety on difficult tasks, especially in the early stages of learning. (Heinrich & Spielberger, 1982, p. 147).

As a supplement to the Drive Theory, Spielberger (1970) differentiated between anxiety state (A-State) and anxiety trait (A-Trait). He declared that the A-State is a transitory, emotional condition which varies in intensity and fluctuates over time. The individual perceives stimuli of a (real or imagined) situation and responds with certain emotions or behavior; therefore tension, apprehension and activation of the autonomic nervous system occur. A-trait on the other hand, refers to the relatively

stable personality characteristic. The A-trait is the disposition to perceive as threatening a wide range of stimuli and the tendency to respond with extreme A-State reactions, thus may be called "anxiety proneness." It appears that those with high A-trait have performance decrements associated with A-state events because of worrying and self-centeredness.

Spielberger, Gorsuch and Lushene (1970) developed the State-Trait Anxiety Inventory (STAI) as a determiner of these elements. Some argue that the STAI is not strictly a test anxiety device because it does not measure evaluative stimulus situations (Sieber, 1977; Wine, 1980). Nonetheless, the model/instrument has been used to measure evaluation anxiety. For example, proponents believe that high A-state individuals perceive "tests" as the stimulus which signals danger and evokes consequent autonomic response. Furthermore, individuals who have elevated A-trait are more concerned with the evaluation of their performance on tests than with the details that are intrinsic to the performance itself.

The STAI has been used to deliver pertinent information on the perception of "test difficultness" resulting in anxiety (Head and Lindsey, 1983; 1984), and in physiological response research (O'Neil et al., 1969; Kermis, 1983; Davis, 1985).

Instruments presented in the anxiety and learning section of Figure 3 have been used by test anxiety factfinders who concur with the theory implied.

Test Anxiety

Test anxiety investigation "officially" began three decades ago when George Mandler and Seymour Sarason (1952) presented pioneer research in the field. In their effort to distinguish the concept from general anxiety, the authors proposed a definitional construct that included two factors of test anxiety: cognitive (a feeling of inadequacy, helplessness...anticipation, punishment or loss of status and esteem and

implicit attempts at leaving the test situation) and somatic (autonomic arousal) components (p. 166).

Mandler and Sarason believed that two kinds of drives are evoked by the testing situation: 1) the learned task drives which are subsided by responses which lead to the completion of the task, and 2) learned anxiety drives- those related to task completion (which reduce anxiety), and those which interfere with task completion.

The latter were considered in the construction of the Test Anxiety Questionnaire (TAQ). The 39-item instrument was designed to measure self-oriented responses, those readily evoked in a test situation, that interfere with the learning of task relevant reponses. Using the TAQ with college students, they demonstrated that those with high test anxiety performed more poorly in evaluative situations than low test-anxious students. It was proposed that decrements in performance were attributable to the arousal of task-irrelevant responses in test situations. Furthermore, test-anxious individuals react to evaluative stressful situations by emitting negative self-centered responses. These effects of test anxiety reverberated in the reports of subsequent researchers (Sarason, 1960; Benjamin et al., 1981).

I. Sarason's Test Anxiety Scale (TAS) (1958) was spawned by the TAQ. The device demonstrated that high test-anxious individuals elicit heightened autonomic arousal and a tendency to ruminate about failure in circumstances of evaluation stress. It was noted that subjects scoring high on the TAS obtain lower scores on aptitude and classroom tests than subjects scoring low on the TAS. Reiterating the earlier theory, Sarason charged that low-anxious subjects turn their attention to the task while high-anxious individuals focus on internal, self-oriented responses. The plausible assumption therefore, is that performance deterioration could be interpreted in terms of selective attention.

The "attentional phenomenon" conception of test anxiety has been underscored by prominent theorists (see Wine, 1971; 1980). Wine maintains that the debilitating effect on task performance is a result of the highly test anxious person responding to evaluative testing conditions with ruminative, self-evaluative worry and thus cannot direct adequate attention to task-relevant variables. She states that:

> The low-test-anxious person is focused on task relevant variables while performing tasks. The highly test-anxious subject is internally focused on self-evaluative, self-deprecatory thinking and the perception of his autonomic performances...he cannot perform adequately while dividing his attention between internal cues and task cues (Wine, 1971, p. 92).

Incorporating Sarason's theories, Wine as well as others have studied test anxiety from the perspective of cue utilization. These investigators define test anxiety as a variable associated with individual differences in cognitive activities such as attention, appraisal, storage and retrieval of information (see Geen, 1980).

Test anxiety research investigations employing the TAS are quite numerous. Pertinent ones were selected to steer the direction of this study, however, some conflicting results were found.

Galassi et al. (1984) administered the TAS at the beginning, middle and end of an in-course examination. Little relationship occurred between test anxiety and performance. In their earlier study however (1981) the TAS detected an increase of negative thoughts ten minutes before the completion of a test from students (with higher GPA's) who reported elevated bodily sensations indicative of arousal.

In 1982, Bentley used the TAS to conclude that students who react with physiological symptoms more than usual under stress received higher grades. Elevated arousal also was a greater predictor of GPA's. Bentley purported that high anxious students turn their stress effects inward instead of outward- using themselves as a scapegoats, rather than society. Contrary to Sarason's theory. the attention inward in this case was not deprecatory.

The results indicate that test anxiety as measured by the TAS is a significant determinant of performance on problem solving tasks involving the manipulation of responses (Harleston, 1962). It is not surprising, therefore, that high test anxious students make more item-response changes on tests than do low anxious ones (Green, 1981).

While Schmitt and Crocker (1984) proposed that test anxiety can contribute to erratic performance of an examinee, Klinger (1984) through the TAS measure, detected little indication that test anxiety is debilitating for those less prepared.

A recent study by Sarason (1984) seems to synthesize TAS findings. He determined that test-anxious persons experience self-preoccuping worry, insecurity, and self-doubt in evaluative situations. On this basis, the TAS remains a popular assessment device for exploring test anxiety.

In (1960), Alpert and Haber proposed that anxiety may facilitate or impair performance in test-taking situations depending on its nature. They prepared a 28-item questionnaire: the Achievement Anxiety Test (AAT) in which two subscales, debilitating and facilitating anxiety in examination situations, are ascertained. Though not derived from the TAQ, its widespread use merits attention.

A typical facilitating-anxiety item is: " Before a test, I become excited and alert and this helps me to organize what I know." The debilitating-anxiety example is: "When I am about to take a test, I get upset and forget a lot of the things I studied." Items such as these were selected on the basis of their correlations with academic performance measures. McKeachie (1969) argued that the facilitating-anxiety scale may be more closely related to achievement motivation than to traditional concepts of anxiety. Using the AAT, Tobias and Abramson (1971) concluded that it is reasonable to assume: students with high motivation should achieve more than those

with low motivation scores. Therefore it appears that the AAT measures confound the anxiety experienced in test situations with actual test performance (Spielberger et al., 1978).

Plake et al. (1981) performed a validity investigation of the AAT on the basis of its extended use in psychological and sociological research. They caution against using the two parts of achievement anxiety (facilitating and debilitiating) as independent measures, claiming that the underlying structure of the instrument is more complex than the one originally hypothesized.

In general, research on anxiety and learning through use of the TAQ and its counterparts, suggested that when evaluative stress is high, the highly anxious perform at lower levels than the low-anxious. For example, high test anxious individuals tend to score lower on classroom and aptitude tests (Alpert & Haber, 1960, Harper, 1974; Mandler & Sarason, 1952; I. Sarason, 1975, 1963; Spielberger et al, 1978). Evaluative stress seems to elicit some type of "state anxiety" which interferes with performance of the highly anxious. Commonly, "state" test anxiety has been tested as a univariate condition that interferes with performance as it is increased.

Worry and Emotionality Components

The univariate distinction of test anxiety was challenged in 1967 by Liebert and Morris. They suggested that state anxiety has two components: Worry (W) and Emotionality (E). This prospective guided the formulation of the Worry-Emotionality Questionnaire (WEQ). Items were specifically selected from the TAQ on the basis of their content validity for assessing emotional reactions (Emotionality) and cognitive concerns about performance (Worry) during examinations.

WEQ studies have shown that Worry is inversely related to performance expectations of high school and college students taking classroom exams (Doctor & Altman,

1969; Liebert & Morris, 1967); Emotionality is unrelated to performance expectations in some samples (Liebert & Morris, 1967; Spiegler, Morris & Leibert, 1968); and negatively related in others (Doctor & Altman, 1969; Morris & Liebert, 1970).

In a review of W and E research by Deffenbacher (1980), the following pattern of results were reported:

1. Worry and Emotionality are significantly correlated.

2. Worry consistently forms a negative or inverse relationship with performance expectations.

3. Findings for Emotionality are inconsistent and mixed. Emotionality was either unrelated, related only within certain strata of Worry, or negatively related to performance measures.

4. Worry is the more important variable of the two, accounting for more variance in relationships with performance or performance expectations. Furthermore, studies controlling the common variance show that Worry forms a negative correlation with performance, whereas Emotionality no longer correlates significantly with performance (p. 118).

Morris and Liebert (1970) when replicating earlier work (1969) found that the effects of anxiety on academic and intellectual performance is accounted for by the effects of the Worry component, while Emotionality is unrelated to this type of performance. Furthermore, the negative correlations in both studies between grade and anxiety were shown by means of partial correlation to be due to the relationship between Worry and grade. When the variance due to Emotionality is eliminated, the correlations remain about the same, however, when Worry is partialed out, the correlation drops to nonsignificance. They stressed that a distinction should be made between the two factors when designing studies to investigate the relationship between (test) anxiety and performance (Morris & Liebert, 1970, p. 337).

On the whole, the W and E concept materializes as a cogent premise for test anxiety research. Instruments using them as subscales would perhaps be better detectors of test anxiousness.

The Test Anxiety Inventory (TAI) by Spielberger et al. (1978) appears to be an enhancement of earlier instruments while underscoring the Worry and Emotionality distinction. The subscales W and E were factor analyzed to yield high internal consistency and reliability for the TAI (Morris et al., 1981; Thyer & Papsdorf, 1982).

The TAI total scores are equivalent to the widely used TAS. The Emotionality subscale has been the best predictor of state anxiety scores (as measured by the STAI-A-State) and the Worry subscale has correlated significantly with GPA's (Spielberger et al., 1978).

A substantiating study by Minor (1985) used the TAI to conclude that students who scored high on the Worry subscales were likely to have negative thoughts during an exam. Small but significant partial correlations between Worry and GPA were found for both males and females.

Another interesting result was obtained by Van der Ploeg and Hulshof (1984) who administered a Dutch adaptation of the TAI to secondary school students. They found that the debilitating effects of high test anxiety and especially the Worry component, on performance were nested in the upper range of intelligence. Boys and girls with lower intelligence achieved less and were less influenced by the impairing effects of test anxiety (p.343).

Having shed new light on the concept, the TAI appears to have excellent potential for use in the assessment of test anxiety as a situation-specific personality trait (Spielberger et al., 1978). The properties and correlates of the TAI are discussed in detail in Chapter 3.

Physiological Response

Test anxiety is a "state" that is situation-specific to the evaluative setting. The more difficult an examination and the more important the consequences that are attendant upon successful performance, the more likely the situation will be perceived as threatening by most students (Sarason & Spielberger, 1978, p. 172). The threatening circumstances evoke cognitive (Worry) and physiological (Emotionality) reponses.

The conception that test anxiety incorporates both cognitive and physiological factors has been widely accepted. However, provocative reviews have been forwarded regarding the role of physiological arousal in test anxiety. Sieber (1980) contended that the role of physiological arousal in effecting anxiety is not well understood. In concurrence, Holroyd and Appel (1980) indicated that most research offers no information about the role of physiological changes in test anxiety and in how the test anxious perform (p.132). They suggested that physiological and cognitive components are poorly correlated with each other even though common sense would expect it to be otherwise.

From a substantial survey of empirical findings associated with test anxiety and physiological response (see Holroyd & Appel, 1980), suitable studies have been selected for discussion in effort to elucidate reasons for their (Sieber, 1980; Holroyd & Appel, 1980) claims. A close examination reveals some possible problems in the methodology that may have led to the above conclusions. For example, using the TAS at the beginning of the quarter, Holroyd et al. (1978) separated high test anxious (HA) from low test anxious (LA) females. Stroop color-word tasks and anagram tests were used in an experimental setting. Responses and physiological activity were monitored. A Grass preamplifier with Beckman electrodes was used to measure changes in autonomic response. Results showed that differences in reported anxiety and test per-

formance were not accompanied by corresponding differences in autonomic activity. Heart rate only appeared to reflect variability. All other tonic and phasic electrodermal activity were virtually identical in HA/LA females. It was concluded that deficits in information processing associated with test anxiety did not result from maladaptive levels of autonomic arousal.

In 1983, Kermis designed a study to explore the association between psychometric and physiological results. Kermis used the STAI A-Trait instrument to separate high test anxious from low test anxious. In an experimental setting using imagery, script Reading he alternated disruptive and relaxation cues while monitoring systolic blood pressure and heart rate. Pulse rate was reduced after relaxation cues; that is, it was greatest after disruptive cues and and lowest after helpful ones.

A legitimate skeptical view may be taken of the contrived settings and results of heart rate variablity (Holroyd et al., 1978) and pulse rate changes (Kermis, 1983)- each of which notoriously reflects diurnal changes.

The following appear to show some relationship between cognitive and autonomic arousal.

Davis et al. (1985) administered the STAI A-trait to separate high and low test anxious students. Using a middle-term examination, they tested serotonin levels of individuals scheduled to take the test. Baseline levels of serotonin were higher among high A-trait individuals. Serotonin increased in both HA and LA in the situational stress of exams. A 3-day post examination measure of serotonin level would have perhaps provided strength for this study.

Tennes & Kreye (1985) modified the Test Anxiety Scale for children (TASC). Using second graders in the natural setting of school days, they measured cortisol levels in the urine of subjects during two morning hours on regular school days and

compared them with levels on days that achievement test were administered. Results showed no correlation with students answers in terms of accuracy. Children who were slightly above average in intelligence and children who were low achievers were found to have elevated cortisol levels. The fact that no relationship was found between the children's free cortisol excretion levels and scores on the TASC may be attributable to the questionable accuracy of self-reporting in the case of the low achievers. The better students may have perceived the importance of optimal response to the situation of tests.

For participants in a study, Morris et al., (1981) selected TAQ items that concentrated on Worry and Emotionality. Normal class periods and regular classroom examinations were used to obtain data. Pulse-rates were self-obtained at the begining of a normal class period and four different times thereafter. The process was repeated on test days. After the pulse rates were taken students completed a modified TAQ with W and E components only. Emotionality was positively related to pulse rate change, whereas Worry was not. While this result would appear to conform to theory, self-reported pulse rates are suspect.

A study by O'Neil et al. (1969) presents a strong argument against the above mentioned generalizations (Sieber, 1980; Holroyd & Appel, 1980). The methodology called for the use of the STAI, A-State, in natural Computer-Assisted Instruction (CAI). After completing difficult computer instructions, students responded to the STAI and systolic blood pressure (SBP) was obtained with a Baumanometer, Model 300. The same procedure followed an easy computer task. Results of t-tests revealed that A-State scores were significantly higher in the difficult task periods and on easy tasks were significantly lower than the difficult task period. Furthermore, SBP increased during difficult and decreased during easy periods.

A relationship was found between the psychometric and physiological aspects in this study. Replicated results such as these would qualify the positions of the test anxiety learning theorists.

Summary

Definitions, theories, and instruments vary, however recurring themes of "what anxiety does" to the test-anxious student dominate the literature. Some general effects of test anxiety have been cited to be as follows: 1) testing situations evoke both learned drives and learned anxiety drives. Some anxiety drives are task-relevant while others are task- irrelevant (Mandler & Sarason, 1952; Sarason, 1960; Wine, 1971; 1980); 2) high test-anxious people are more self-preoccupied and self- dissatisfied than the low test-anxious individual (Sarason, 1960); 3) self-oriented, interfering Worry (cognitive) and affective Emotionality (physiological) are both intrinsic to the phenomenon of test anxiety (Liebert & Morris, 1967; Spielberger, 1978; Deffenbacher, 1980).

The Liebert and Morris conception as refined by Spielberger et al. (1978) has been adopted as the most plausible definition, measurement and effect of test anxiety. The theory has provided the underpinning for choice of instrument and design in this study.

Firmly establishing a correlation between the cognitve and affective would hasten measures for intervention in the test-taking process.

CHARACTERISTICS OF SERUM BETA-ENDORPHIN

Discovery of Endogenous Opiates

The phenomenon of pain is an adaptive mechanism which has a protective function. However, it causes unpleasant sensory and emotional experiences (Devor, 1984). A traditional pain management practice has been the use of morphine and other opiates. Efforts to determine the mechanism of how morphine produces analgesia led to the discovery of receptors which were specific for opiates.

The direct demonstration of their existence was a difficult process (see Simon, 1982), nonetheless, opiate receptors were found in all vertebrates suggesting a long history of evolutionary survival. LeRoith et al. (1982) claimed an even older (unicellular) origin. This important fact led scientists to believe that the opiate receptors were not present for the sole purpose of binding plant-like substances. A search began for an endogenous ligand "the binding of which was the real reason for the existence of the receptor (Simon, 1982, p. 4)". The substance would have high affinity for the receptor and opiate-like activity. Scientific surveys determined that no known neurotransmitters and hormones could bind in that manner. Therefore investigators began probing for a "natural" substance that serves as an endogenous ligand.

A research team headed by Hughes (1975) was responsible for the breakthrough. Classical pharmacological bioassays confirmed the presence of two pentapeptides known as methionine-enkephalin and leucine-enkephalin with opiate-like activity from porcine brain. A year later, Guillemin (1976) isolated longer peptides called endorphins. These are the natural ligands that mimic morphine in circumstances of stress and pain.

The analgesic action of β-endorphin is the subject of interest and discussion.

Location and Function of Endorphins

In 1982, recombinant DNA techniques aided neuroscientists to refine the classification of the endogenous opiates into three genetically distinct peptide families: the β-endorphin /ACTH precursor (known as proopiomelanocortin- POMC), the enkephalin precursor (known as proenkephalin or proenkephalin A), and the dynorphin/neo-endorphin precursor (also known as prodynorphin or proenkephalin B) (Akil et al., 1984) (see Fig. 4).[3]

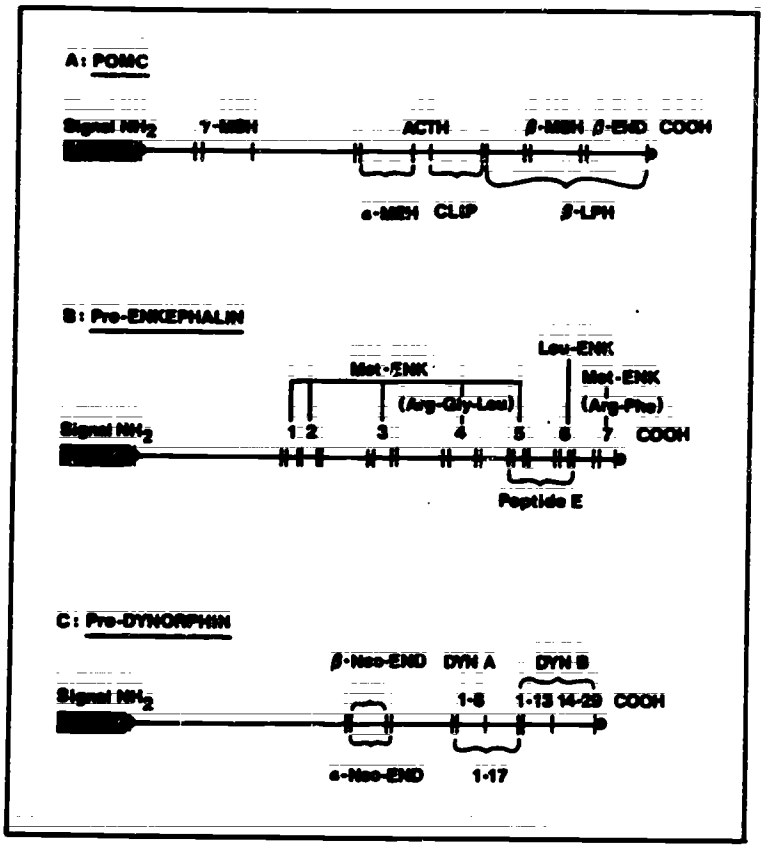

Figure 4: Schematic representation of protein precursor families. Annual Review of Neuroscience, Vol. 7, 1984. Reproduced with permission of Annual Reviews,Inc. 4139 El Camino Way, Palo Alto, CA, 94306.

[3] Consideration will be confined to the peptides from POMC.

Bloom et al. (1977) proposed that the location of POMC are in the intermediate and anterior lobes of the pituitary gland. Further proof was obtained when removal of the pituitary gland in experimental animals resulted in a reduced amount of plasma β-endorphin and β-lipotropin activities (Guillemin et al., 1977; Ghazarossian et al., 1979). Furthermore, decreases in pituitary β-endorphin was followed by a reciprocal increase of this peptide in blood (Guillemin et al., 1977; Hollt et al., 1978).

There appear to be two cell groups that produce β-endorphin/ ACTH peptides. The main cell group found in the region of the arcuate nucleus of the medial basal hypothalamus, has fibers which project and include many areas of the limbic system and brain stem. High levels of opiate receptors have been found in the limbic system, a region presumably associated with human behavior and emotion. The second group, in the nucleus of the solitary tract and nucleus commissuralis is not well described in terms of its projections (Akil et al. 1984).

Knowledge of its origin directs attention to β-endorphin as a modulator of the stress response.

POMC, as all precursors, are biologically modified for use in the system. Post-translational processing involves cutting specific peptides out of the precursor protein molecule. The portions may be modified by acetylation, amidation, phosphorylation, methylation, glycosylation and further cleavage as part of the biological program of the cell. Akil et al., (1984) explained that post-translational events determine the exact mix of peptides in a given neuron. They vary from tissue to tissue in spite of their common origin. The peptides then take on differing potencies, pharmacological profiles and receptor selectivities. They become critical in determining function and constitute a crucial step in the regulation and homeostasis of a given opioid system in a particular region of the CNS (p. 227).

β-endorphin is cleaved in vivo from β-lipotropin. The main method of modification occurs by alpha-N- acetylation on the tyrosine side (Flg. 1).

The function of β-endorphin is mainly involved in the stress response. Akil et al. (1985) found: (1) that the intermediate lobe releases its products (one of which is N-ac- β-endorphin) into the bloodstream more readily after repeated stress, (2) N-ac-β-endorphin (1-31) accumulates with stress and appears as the dominant form in plasma, and (3) with repeated activation there is induction of biosynthesis and acceleration of processing.

Similar to catecholemines, the endorphins may have a basic multisystem function essential to the homeostasis of the survival of the organism.

Endorphins as Hormones

The high concentration of β-endorphin in the pituitary gland suggests that the peptide functions as a hormone. β-endorphin would qualify as such if it could be shown that: (1) it is released from the pituitary gland by specific stimuli, (2) it is transported in blood, and (3) it acts on some distant target organ that is adequately sensitive to the concentrations of free peptide in blood which result from the application of releasing stimuli (Cox & Baizman, 1982, p. 145).

Pituitary Release of Beta-endorphin

A linkage in the neuroendocrine control mechanisms for the concomitant release of β-endorphin and ACTH from the pituitary has been suggested (Guillemin et al., 1977; Weidemnann et al. 1979). ACTH and β-endorphin are secreted in response to known ACTH-releasing stimuli such as metyrapone and hypoglycemia (Nakao et al., 1978; Wardlaw & Franz, 1979).

The role of the endogenous opioid system in response to stress has been carefully investigated. The intermediate lobe releases its products into the bloodstream more readily after repeated stress.

In humans, it has been shown that endorphins are secreted when subjected to mild stress (Boarder et al., 1982), pre-surgical (Miralles et al., 1983) and surgical stress (Cohen et al., 1981; 1983; DuBois et al., 1981; Hargreaves et al.,1983), and during pregnancy and labor (Cahill & Akil, 1982). It was also found that the pain of exercise may stimulate a general stress response similar to the many and varied stimuli that result in increased secretion of ACTH/ β-endorphin into peripheral and venous blood. This was observed in trained individuals by easy and strenous running (Colt et al., 1981; Farrell et al., 1982; Fraioli et al., 1980), and in untrained participants (Gambert et al., 1981).

Although a cause and effect relationship between endorphin secretion and analgesic response is not firmly established, findings such as these lend credibility to the concept.

Beta-endorphin in Blood

β-endorphin has been observed in the plasma of humans, therefore confirming that its presence is not artifactual (Nakao et al., 1978; Wardlaw & Franz et al., 1979; Hollt et al., 1979; Ghazarossian et al., 1980; Wiedemann et al., 1983). Lypka et al., (1983) directly determined and monitored 24-hour changes in β-endorphin levels.

The process of detection of β-endorphin is sensitive and time- consuming. Before it can be measured, β-endorphin must be extracted from plasma. There are several sophisticated methods employed for extraction however, two were considered for the study: use of silicic acid and use of talc. Each has been well-documented in the literature (see citations in Chapter III).

In 1978, Suda et al. reported that β-endorphin could not be detected after an extraction method using silicic acid and elution with acid/acetone. However, replicated studies have challenged this finding. For example, using silicic acid in the extraction procedure, Farrell et al., (1982) found increased levels of β-endorphin after treadmill exercise in well-trained athletes and Gambert et al., (1981) showed that mild exercise revealed levels of β-endorphin in blood which were greater in men (51 pg/ml) than in women (9.0 pg/ml). In 1979, Hollt and his team attempted to quell the "controversy in the literature concerning the existence of β-endorphin in the plasma of normal subjects". Using the silicic acid method of extraction, they obtained 11.33 to 21.67 pg/ml of β-endorphin in the blood of healthy participants under baseline conditions.

Reputed success with the talc extraction method also refutes the Suda et al. study (1982). Levels of β-endorphin were detected by Wardlaw & Franz (1979), the mean was 21 pg/ml. Inturissi et al., (1982) using talc observed 30 pg/ml of β-endorphin in blood of psychiatric patients. Further confirmation of detected levels were obtained by Colt et al. (1981) who found 17.6 pg/ml after an easy exercise run and 28.0 pg/ml following strenous activity by well-trained athletes.

It appears from the above results that β-endorphin is authentically present and may be calculated after it has been extracted onto silicic acid or talc.

Once removed from plasma, further biochemical procedures are necessary to measure the levels of β-endorphin present in blood. The Radioimmunoassay (RIA) technique has been popularized for measurement of opiate peptides in bodily fluids or extracts. It has been called a competitive protein binding technique because it uses radio- actively labeled hormone as the tracer and antisera (prepared against a specific hormone) as a binding site. Competition betwe unlabeled hormone in patient (sub-

jects) sample and the added labeled hormone for a limited number of binding sites forms the basis of the assay. For β-endorphin in general, the process involves:

1. Recovering serum β-endorphin from plasma (some use synthetic) and purifying it. The β-endorphin is injected into a foreign species (such as rabbit). This stimulates the production of specific antibodies which are recovered from blood plasma.

2. The purified β-endorphin may be radiolabeled in the laboratory or purchased. When labeled β-endorphin is added to anti-endorphin (antibody) a reversible complex is formed. The radioactivity of the complex can be measured.

3. When unlabeled hormone (from sample-participant blood) is added to the complex some of the radioactive endorphin will be displaced by the unlabeled substance. The greater the quantity of unlabeled hormone, the greater the displacement.

4. The complex is then precipitated and its radioactivity measured. Standard curves are constructed relating the amount of unlabeled hormone added to the loss of radioactivity on the complex.

A problem inherent in all RIA is that of crossreactions. Since neuropeptides generally derive from post-translational processing of protein precursors, a larger number of peptide fragments may have common antigenic determinants and show crossreaction. It is important to note therefore, that every RIA depends on the specificity and sensitivity of the antiserum (Ab) used and the purity and standards tested for crossreactivity. In the case of β-endorphin research, this becomes even more critical. It appears that any Ab recognizing part of the β-endorphin amino acid sequence will also recognize β-lipotropin (Fig. 5). Because of this phenomenon, it is more accurate to refer to " β-endorphin- like immunoreactivity" to the measured β-endorphin in analysis. The term implies crossreaction with β-lipotropin on a molar basis.

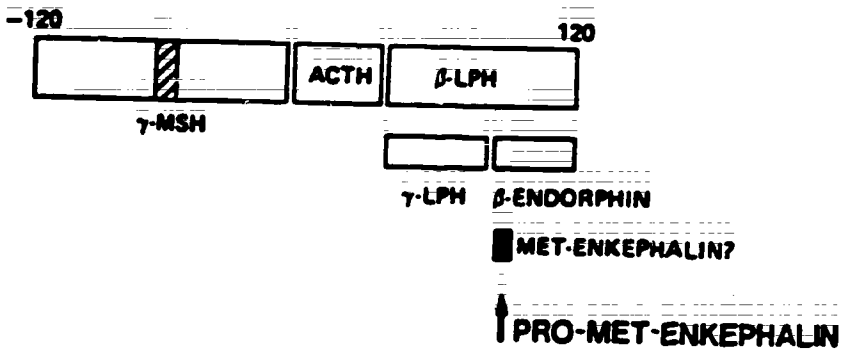

Figure 5: Cleavage products of the pro-opiomelanocortin (POMC) precursor. Reproduced with special permission of the Publisher, Williams & Wilkins, 428 E. Preston St., Baltimore, MD 21201.

The intrinsic problem of cross-reactivity is prevalent in β-endorphin research. The percentage is reported in major studies. It can range from 5% (Wlkes et al., 1980); 10-25 % (Colt et al., 1980; Hollt et al., 1979; Wardlaw & Franz, 1979; Inturissi et al. 1980); approximately 30% (Ross et al., 1979; Ghazarossian et al., 1980); 50 % (Cohen et al., 1981; Lypka et al., 1983; Miralles et al., 1983); to 100% (Matthews et al., 1982). The β-endorphin antibody used in this study cross-reacted 36 % with β-lipotropin (Tejwani et al., 1983).

The phenomenon of crossreactivity figures in the varied accounts of baseline levels of β-endorphin in the blood. Conflicting findings of "normal human plasma levels" emerge. For example, in a revised protocol from the Immunonuclear Corporation (1980), the normal mean value of plasma β-endorphin is given as 21.28 pg/ml.

However, there is also 50% crossreactivity reported. Other studies indicating the normal values of approximately 20 pg/ml are: Hollt et al. (1979) and Wardlaw & Franz (1979) who each admit crossreactivity of 10-20%. Cohen et al. (1984) and Wilkes et al. (1980) designated 113.00- 115.00 pg/ml at 50 % and 5% crossreactivity respectively. A 100% crossreactivity yielded 1024.00 pg/ml of β-endorphin in blood of normal subjects (Ho et al., 1980).

Designing protocols that measure β-endorphin levels pre and post treatment appear to be a common and perhaps circumventing solution to the discrepancies. The changes reflected are cautiously examined.

McLaughlin et al. (1980) argue that no method of direct measurement of β-endorphin in blood is available because of the shared amino acid sequence of β-endorphin with β-LPH, suggesting that the detection of such a peptide requires chromatographic characterization in conjunction with the use of RIA (p. 288). While this appears to be tenable, Colt et al. (1981) found the recovery of β-endorphin and β-LPH from plasma averaged 75% after extraction and 68% after extraction and chromatography. Many of the studies reviewed did not perform follow-up chromatographic procedures.

Though crossreactivity and variety of baseline level remain puzzles to be solved, research on β-endorphin detection in the blood continues to show progress.

Target Organs

In order to establish a genuine hormonal designation to β-endorphins, more research is needed on this third criterion. Most reports claim that the possible targets of β-endorphin are yet unknown.

Specific areas of opiate research such as consummatory and cardiovascular responses, gastrointestinal functions, respiratory effects, thermoregulation, and so on are being

conducted (see review, Olson, 1983). Studies are new and inconclusive. It is believed with some degree of certainty however, that the autonomic nervous sytem contains endorphins (Schultzberg et al., 1978) and opioid receptors (Young et al., 1980). These observations point to a powerful role of the opioids in environmental demands, including physical and psychological stress.

Summary

There is a compelling body of evidence linking the endogenous opioid, β-endorphin to the regulation of responsiveness to pain. Pharmacological studies are needed to determine the potential clinical usefulness of endorphins as strong analgesics with hopefully low side effects.

The discovery of opioid receptors and of the endogenous opioid peptides which are ligands of the receptors have influenced studies in the pain of surgery and the stress of exercise. It appears that:

> Opioids may have evolved more complex and integrated functions, recruiting higher levels of the neuroaxis serving in the control of affect and mood, drive and reinforcement or in the process of filtering information controlling attentional mechanisms (Akil et al., 1984 p. 235).

A focal point of this investigation is to determine if β-endorphin which is elevated in the stress and pain of surgery and exercise will reflect similar behavior in the process of filtering information and controlling attentional mechanisms in the test-taking process. Specific methods will be used to determine if the MCAT activates the intermediate lobular cells of the pituitary to secret levels of β-endorphin that are measurable and significant.

CHAPTER III

RESEARCH DESIGN AND PROCEDURES

Procedures followed before, during and after data collection are discussed in this chapter. Section one describes the population used in the study. The next section highlights the characteristics of the instrument which has led to its selection as the psychological assessment of test anxiety and how data were analyzed. Section three discusses the non-manipulated treatment that played a role in determining if and to what extent students' test anxiety effected the outcome scores. Section four describes the procedures and manner of data collection. Discussions of extraction of β-endorphin from plasma and the radioimmunoassay technique complete the chapter. The last two methods of research design were used to analyze physiological results.

POPULATION OF STUDY

Non-probability sampling of students who were scheduled to take the Medical College Admission Test (MCAT), a necessary component for admission into medical school, was used in the study. The purposive investigation required volunteers. These were solicited through posted announcements, school newspaper (Lantern), contact with the Stanley H. Kaplan Educational Center (preparation program), and pre-med honor society meetings before the target date of the MCAT.

Persons interested returned post cards indicating their willingness to become involved. Subjects were notified about the time and place of subsequent data collection. Those who agreed to participate received a twenty-dollar gratuity.

Seventeen pre-medical students, 11 male and 6 female, with a mean age of 21.5 agreed to participate. None had taken the MCAT previously. All had equivalent to third-year undergraduate status. Six members of the group attended the Kaplan Educational preparation program.

The subjects were their own controls; that is, data accumulated before the MCAT were compared with post MCAT results. Literature reviews provided baseline data for serum β-endorphin and the TAI Professional Manual (Spielberger et al., 1980) provided baseline comparisons for the TAI.

INSTRUMENTATION

Nature of the Test

Meeting a variety of criteria for the study, the Test Anxiety Inventory (TAI), obtained from the Consulting Psychologists Press, Inc. (Spielberger,1980) was used as the paper- pencil measure of test anxiety. It was developed to determine individual differences in test anxiety as a situation-specific personality trait. On this instrument, respondents report how often they experience specific symptoms of anxiety before, during and after administration.

The TAI may be given individually or in groups but is also designed for self-administration. Clarity of directions and the 8 to 10 minute time for execution made it suitable for the study.

The one-page response form (Appendix A) consists of 20 objective items which are weighted on a scale of 1 to 4. The four choices are: (1) almost never, (2) sometimes, (3) often, and (4) almost always. For example, in responding to item 4, "I freeze up on important tests," students select how they generally feel during tests. All items except the first follow this pattern of response. In item number 1, "I feel

confident and relaxed during tests, the "almost never" response is an indicator of high anxiety while "almost always" represents low anxiety.

A minimum TAI total score is 20 and the maximum is 80. The larger scores indicate higher anxiety.

Subscale Measures

Spielberger's (1980) goals in developing the TAI were: (1) to construct a brief, objective, self-report scale that was highly correlated with other measures of test anxiety and (2) to employ factor analysis to identify subscales measuring Worry and Emotionality as major components of test anxiety (p. 2). Subscales which measure Worry (TAI/W) and Emotionality (TAI/E) each consist of eight items and therefore, are weighted in a range from 8 to 32. The items on the TAI/W subscale are: 3, 4, 5, 6, 7, 14, 17, and 20. TAI/E subscale items are: 2, 8, 9, 10, 11, 15, 16, and 18.

On the TAI Test Form (Appendix A) items which represent "Total" are those which were not clearly factor analyzed into Worry and Emotionality when the test was devised. The items are: 1, 12, 13, and 19. The weighted values range from 4 to 16. These scores are combined with Worry and Emotionality to obtain a Grand Total TAI Total score. (W and E combined scores = 64. Overall score for TAI = 80).

Templates were provided to facilitate hand-scoring of Worry, Emotionality and Total components.

The added dimension of subscales has helped to characterize the instrument as "probably the strongest in its theoretical and psychometric development (DeVito, 1983)."

Developing and Norming of Test

Normative data for the TAI is based on: college students (1,449 undergraduates: 654 males, 795 females), incoming freshmen (1,129 : 533 males, 596 females), community college students (320: 136 males, 184 females), high school students (1,118 ninth - through twelfth - grade: 527 males, 591 females)[4] and Navy recruits (190 males).

In the four samples that included both sexes, the TAI Total scores for females were consistently 3 to 5 points higher. The females also scored consistently higher on the Worry and Emotionality subscales. Mean TAI Total scores for males across all samples were similar with high school and Navy recruits scoring slightly higher than the college sample. Tables of percentile were generated from the resultant scores of the normative samples. These tables were consulted when analyzing pre-medical student responses.

Reliability

Test-retest reliability coefficients for the TAI total scores were derived from administration of the test twice during a two to six week time period. Three investigators were selected to administer the TAI to graduate students, college students and high school students. Time lapse and reliability coefficients are presented in Table 1.[5] The author attributes the drop in reliability of the high school group to the longer-lapse period and also to a possible clarification of college and career plans made by individual students.

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[4] Students were from the following: college- University of South Florida, high school- Community College in Tampa Florida, and public high school- Jacksonville and Pinellas County (St.Petersburg and Clearwater), Florida.

[5] All TAI tables are reproduced by special permission of the Publisher: Consulting Psychologists Press, Inc., P.O. Box 60070, Palo Alto, CA 9436, from Manual for the Test Anxiety Inventory by Charles D. Spielberger, 1980.

Table 1

Test-Retest Reliabilty for TAI Scores

Investigator	N	Subjects	Time Lapse	R
A	31	Graduate Students	2 weeks	.80
B	159	College Students	3 weeks	.80
C	42	H. S. Students	1 month	.81
	42	H. S. Students	6 months	.62

The alpha coefficients for the five normative samples of: college undergraduates, college freshmen, community college, high school and Navy recruits evidence the internal-consistency reliability of the TAI and its subscales. Computed by Kuder-Richardson Formula 20, as modified by Cronbach (1951), the alphas for the TAI Total scale were uniformly high for both males and females (.92 or higher). The median alphas for the TAI/Worry and TAI/Emotionality subscales, .88 and .90, indicate satisfactory internal consistency for the 8-item subscales (Spielberger et al., 1980, p. 8).

Validity

Correlations of the TAI with six other measures beginning with the updated Sarason's (1978) Test Anxiety Scale (TAS) and Liebert and Morris's (1967) Worry and Emotionality Questionnaire (WEQ) are presented in Table 2.

The relatively high correlations of the TAI scales with the WEQ Worry and Emotionality scales suggest the concurrent validity of the TAI as a measure of test anxiety. Each TAI subscale was more highly correlated with its WEQ subscale counterpart than with other WEQ scales for males or females. These data provide evidence of the concurrent and discriminant validity of the TAI/W subscale for both

Table 2

Correlations of TAI Scores/Measures of Anxiety for College Grads

	Males (n=115)			Females (n=185)		
Measure	TAI	W	E	TAI	W	E
TAS	.82	.79	.73	.83	.77	.69
WEQ-Worry	.73	.74	.59	.69	.70	.58
WEQ-Emotionality	.77	.71	.71	.85	.66	.84
STAI, A-Trait	.54	.51	.46	.48	.44	.41
STAI, A-State	.67	.54	.67	.34	.31	.28
Exam A-State	.86	.70	.86	.77	.61	.76

sexes; they also indicate that the TAI/E and WEQ-Emotionality scales are essentially equivalent for females.

The moderate positive correlations between the TAI scales and the STAI A-Trait and STAI and A-State scales were generally lower than the correlations of the TAI scales with other test anxiety measures. On the basis of these correlations, the TAI can not be classified as a measure of either trait or state anxiety. Since neither of these parameters were to be investigated in the study per se, it did not eliminate the instrument from consideration. Further directing the choice of this test was a study by Thyer and Papsdorf (1982) who demonstrated discriminant and concurrent validity of two commonly used measures of anxiety, one of which was the TAI. It was concluded that the test:

> seems to possess sufficiently high discriminant validity to justify its continued use as a screening instrument for research purposes and as a dependent variable in clinical outcome studies (p.1202).

The instrument was also correlated with measures of personality, study skills, intelligence, aptitude measures and academic achievement. Data resulting from corre-

lations with GPA are pertinent to the study. Table 3 reflects norming student sample correlations with GPA.

Table 3

Correlations of TAI Scores with Academic Achievement

	Males				Females			
Subjects	N	TAI	W	E	N	TAI	W	E
GPA H. S.	177	-.22	-.34	-.07	196	-.11	-.21	-.02
GPA College 1	115	-.31	-.47	-.13	185	-.18	-.35	.00
GPA College 2	445	-.12	-.17	-.07	538	-.12	-.21	-.05

Note: Table 3 is a portion of the one presented in the manual, p. 6.

There were negative correlations between TAI Total and TAI/W scales and the GPA. Most of the correlations were statistically significant and were slightly higher for males. Correlations of GPA's with TAI/W were consistently higher than those with the TAI Total scale; however, the TAI/E subscale and grades essentially did not correlate (Spielberger, 1980, p. 6). These findings are consistent with those of Liebert and Morris (1967).

The characteristics just described contributed to the measure of appropriateness and consequent selection of the test for this study. Research-based reliability and validity of an instrument has the potential to increase the credibility of resultant scores derived from it.

TREATMENT

The New Medical College Admission Test (MCAT) which was designed to evaluate the quality of academic preparation for the study of medicine was used as the treatment. The test was developed under the sponsorship of the Association of American Medical Colleges (AAMC) and is administered by the American College Testing Program twice annually: spring and fall (Interpretive Manual, 1977). Medical school admission officers usually suggest that the test is taken in the calendar year before the year in which candidates want to enter medical school. Volunteer participants fell into this category.

Scores on the subsets of the new MCAT have been used by a majority of American and Canadian Medical Schools as one of the criteria for selecting applicants since 1978. MCAT scores on six subsets or assessment areas are provided for each candidate. They test understanding of Science Knowledge: Biology, Chemistry and Physics; Science Problems; Skills Analysis: Reading and Skills Analysis: Quantitative.

The MCAT takes one whole day to complete. Morning sessions are divided into the Science Knowledge Subtest (135 minutes maximum) and Science Problems Subtest (85 minutes). Following a lunch break, afternoon sessions are devoted to skills Analyses: Reading and Quantitative (85 minutes each).

The scores are reported on a scale ranging from 1-15; with the standard error of measurement for each subset calculated as one scaled score point (Hojat et al., 1985).

Because of the comprehensive assessment of ability and endurance, the MCAT was selected as the appropriate treatment to analyze test anxiety. Its mandatory nature underscored the choice.

Assured of the provisions of the Buckley Amendment and the Right to Privacy Act, students unanimously signed authorization for the release of their MCAT scores.

Arrangement was made with the College of Arts and Sciences to provide results when available in exchange for individual permission slips.

Included on the release form was license to obtain the participant Grade Point Average (GPA) to date. Number and nature of Science courses (undergraduate and graduate) that had been completed prior to the MCAT were also verified (with permission) at this time. It was believed that the added knowledge of classwork ability would further elucidate resultant MCAT scores and perhaps determine whether or not test anxiety affected the outcome.

The Medical College Admission Test results were used as the independent variable of analysis to determine psychological and physiological measures of test anxiety.

PROCEDURES OF DATA COLLECTION

Volunteer participants were asked to report to the Clinical Pharmacology Laboratory, Means Hall, at University Hospital, two days prior to the MCAT examination. The choice of "two days before" was meant to encourage more participants to volunteer for the study as one day before would perhaps be used for last minute preparations. Discussions with pre-medical students in general substantiated the likelihood of test anxiety at this time. Upon arrival, a consent form (Appendix B) was carefully read and signed by individuals before submitting to the project requirements.

After participants completed the 20-item objective TAI to determine their level of test anxiety, a professional phlebotomist withdrew 30 ml of blood into 2 - 15 ml heparinized tubes. Samples were transferred to 50 ml polypropylene tubes containing 3 ml Bacitracin (0.5%) a known antibiotic which also inhibits proteolysis and ethylene-di-glycol-tetraacetic acid (EDTA) (80 mM) an anticoagulant. Blood samples were refrigerated until centrifugation at 10,000 rpm at 4^{0} C in a Sorvall Centrifuge.

The supernatant plasma was transferred to 15 ml polypropylene tubes using disposable polyethylene (β-endorphin adheres to the surface of glass) pipettes. Plasma was acidified with 0.5 ml 1N HCl per 5 ml of plasma to further inhibit protease activity. These samples were frozen at -70° C until the time of assay.[6]

Each of these procedures was repeated three days following the execution of the MCAT examination. Timing suggested that scores on the paper-pencil instrument would reflect a modification in test anxiety level.

Pharmokinetic studies indicate that during the administration of dexamethasone (0.5 mg 6 hourly for 48 hours) endogenous β-endorphin levels can fall significantly in 24 hours and are (even) undetectable at 48 hours (Smith et al., 1981). Foley et al. (1979) examined the effects on cancer patients who were given several doses of β-endorphin intravenously and to one intracerebroventricularly. They found that the β-endorphin is rapidly cleared from human plasma (more slowly in the CSF).

It was theorized therefore, that blood level β-endorphin of test anxious subjects, too, would show some change.

EXTRACTION OF BETA-ENDORPHIN FROM PLASMA

A linkage in the neuroendocrine control mechanisms for the release of ACTH and β-endorphin has been suggested (Guillemin et al., 1977; Wiedemann et al., 1979). Conditions of stress have been shown to induce marked cortisol rises and increases in plasma β-endorphin (Cohen et al., 1981; DuBois et al., 1981; Mirales et al., 1983).

Having established (Chapter II) that anxiety over test-taking situations may be sufficiently stressful, it appeared that levels of β-endorphin would be altered in the peripheral blood of participants sometime during the test-taking process.

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6 The effects of freezing and thawing were investigated by Wilkes et al. (1980) who found that when protease inhibitors are used there appears to be no change in the level of β-endorphin.

Samples had been collected and preserved as described previously.

Common Methods of Extraction

Prior to the actual extraction, it was necessary to choose a technique that would yield maximum recovery of β-endorphin from plasma. Two methods appearing in the literature have been used with reasonable success. One method uses procedures involving the adsorption of β-endorphin onto silicic acid (Farrel et al., 1982; Gambert et al., 1981; Ghazarossian et al., 1979; Ho et al., 1980; Hollt et al., 1979; Lypka et al.,1983; Mirales, 1983), the other involves the use of talc (Colt et al., 1981; Inturissi et al., 1982; Nakao et al, 1978; Wardlaw et al.,1979).

In order to test for best recovery, 47 ml of blood taken from a normal volunteer was drawn into a 50 ml polypropylene tube containing 3 ml bacitracin. After centrifugation, 30 ml of supernatant plasma was placed in two 15 [illegible]. Tube 1 was treated with talc using the method described by Wardlaw et al. (1979) with some modifications (Appendix C), and to tube 2, silicic acid was added following the methods of Hollt (1979) with adaption at the elution stage (Appendix D).

The extracted plasma was subsequently subjected to radioimmunoassay (discussion to follow). The 78% recovery of adsorbed β-endorphin from talc compared to the negligible amount recovered from silicic acid, made this the method of choice.

RADIOIMMUNOASSAY

Radioimmunoassay (RIA) was the technique used for the measurement of β-endorphin in extracted plasma. The process involves competition between a radiolabeled antigen (Ag*) and its unlabeled counterpart (Ag) for binding to a limited amount of specific antibody (Ab) (Felber, 1975). The reaction proceeds to equilibrium, thus we have:

$$Ag^* + Ag + 2AB \quad <--> \quad Ag^* Ab + AgAb + Ag^* + Ag$$

The basic assumption is that the labeled hormone will act in exactly the same way as the unlabeled form and compete for the specific site on the antibody. Aliquots of standard β-endorphin were incubated with a fixed amount of ^{125}I ("tracer"). The fixed amount of antibody and the amount of tracer bound to the antibody becomes inversely proportional to the standard. In this study, ^{125}I was used as the labeled marker (Ag^*) or tracer substance to indicate the presence of ligand (Ag) or β-endorphin in subjects' blood. The amount of bound β-endorphin/antibody complexes were precipitated. The gamma count of the precipitate (due to tracer) was inversely proportional to the concentration of the standard. A standard curve was produced. Unknown concentrations of β-endorphin were calculated (Beckman Apparatus) by interpolation along the standard curve.

Materials

The specific materials discussed below are described in Appendix E. All reagents were prepared according to Tejwani, et al. (1983).

The antibody is the immunoglobulin produced in response to an antigen having a specific binding affinity for it. Once the antigen is bound the avidity or strength of attachment should be high. Besides avidity, the specificity is also a key desired factor. Specificity will ensure binding only to the desired substance (Bishop et al., 1985). The antibody was prepared with bovine serum albumin, a procedure which greatly increases the binding of labeled β-endorphin to the specific antibody and increases the sensitivity of the assay (Guillemin et al., 1977). The crossreactivity of β-endorphin with β-lipotropin which occured in the sample results was 36% (Tejwani et al., 1983; Vaswani, 1984).

The isotope ^{125}I was used as the radiolabled antigen because of its high gamma emitters. It has a half-life of 60 days.

The unlabeled antigen is known as the standard, this non-radioactive ligand was chemically and immunologically indistinguishable from the radioactive counterpart.

The RIA buffer was prepared to ensure the stability of all the reagents.

Prepared as discussed in Appendix E, charcoal dextran was the substance used in nonspecific adsorption of the antigen. Coating it with dextran created a molecular sieve that passed only unbound antigen molecules for retention by the charcoal.

Analysis of Samples

Tubes were labeled as indicated (Appendix F). The specific binding tube contained all the reagents present in the standard tubes except standard. These are called the zero standard concentration tubes (Early et al., 1985). The non-specific binding, or blank tube contained all the reagents except antibody. It was used to determine binding not resulting from the specific antigen-antibody reaction. Minus the antibody, the total tube contained only the total activity representative of the total activity used in each assay tube. Control tubes contained 1.9 - 1000 pg of antigen. They were used to determine the precision of the assay.

A calibration curve was made by adding a known amount of standard or unlabeled Ag (10 μl of 1 μg/ ml) to the 1000 pg tube. After vortexing, 500 μl were added to the 500 pg tube and so on until all tubes (1.9 - 1000) were serially diluted. These were incubated for 24 hours at 4^{0} C with 100 μl of antibody (dilution 1: 4000). The following day, 100 μl of radioactive label (^{125} I) was added to every tube and incubated for 24 hours at 4^{0} C. On day three, 500 μl of charcoal solution were added to all except tube # 3, the Total tube. After centrifugation, the supernatant bound peptides were counted on the Beckmann Gamma Instrument with errors of less than 5% at 95% confidence level.

Note: The samples (subjects blood) which contained an unknown amount of natural (unlabled) antigen were mixed with the same amounts of antigen and antibody as in the standard curve mixture, the antigen-antibody complex was separated and the ratio of radioactivity was determined when compared with that of the original amount of labeled antigen. The ratio was expressed in a percentage and when referred to the calibration curve, gave the amount of unlabeled (natural) antigen in the sample.

The curve fitter computer program generated values of percent bound of beta-endorphin (Ag*/Ab). Using a logarithmic scaling process, points were averaged and smoothed by interpolating values from the fitted, standard curve.

Binding Assay Precision

The details of quality-control parameters of RIA's may be found in Chard (1978). He suggests that standard curve statistics, that is "0" standard blank, mid-point and slope are appropriate measures on which to base internal quality control. For example, the zero standard and assay blank should always be noted. If they fall outside limits set by previous experience then the fault must be identified before further runs are performed. A common cause of deviation is the use of outdated tracer which in all systems leads to a fall in the zero standard, and in some to a simulataneous increase in the assay blank.

The commonest example of an intercept on the standard curve is the midpoint. This represents the standard dose at which (percentage bound/percentage bound in "0" standard) is equal to 0.5 (Chard, 1978, p. 215).

Chard cautions that a substantial number of assays have to be done before there is a firm basis for the analysis. He suggests that the variation may not be normally distributed and that logarithmic transformation may be necessary.

When a number of assays have been performed, the mean and standard deviations are calculated for the serial values. In subsequent work any assay for which the control result lies more than 2 standard deviations from the mean is rejected and repeated. The aim of rejection is to eliminate outliers (Chard, 1978, p. 218).

Each of these criteria were met in this study.

Experimental Error

Participants' blood was delivered into 2, 15 ml Becton Dickinson Vacutainer (Rutherford, NJ) heparinized tubes. Each of the tubes' contents was transferred to a 50 ml polypropylene tube which was already charged with 3 ml Bacitracin/EDTA. The tranferral was done to facilitate handling of tubes for centrifugation and aspiration. While care was taken in the transferral process, a minute amount of sample blood adhered to the original vacutainers. The tubes are non-calibrated, therefore a conservative estimate of the amount of residual blood appeared to be less than 1.0 ml.

Following centrifugation (10,000 x g) in Sorvall, the supernatant plasma was precipitated. Disposable polyethylene pipettes were used in this process. An average of 15-17 ml of plasma was removed. The variety in amount is due to the individual's normal hematocrit. Since β-endorphin is evenly distributed in the plasma, the possibility of neglecting a decanted amount of β-endorphin per se was less than 1%.

The plasma was frozen at -70^0 C until use.

Frozen plasma was thawed and 5 ml were removed from each sample in preparation for extraction and RIA. The remaining amount was refrozen. An Eppendorf adapter with a pipette tip accommodating 1 ml was used five times to obtain the amount necessary for extraction. Relying on the efficiency of the instrument, pipetting error is estimated at less than 1 %.

The repeater pipette was used to assist the process of adding antigen, antibody, buffer and charcoal to the tubes prepared for RIA. Pipetting error is this case, is presumed to be less than 1 %.

It is important to note that while appropriate measures were taken to ensure that simple technical errors were reduced to a minimum, experimental error in the collection, transfer, extraction and RIA procedures should have no more than 5 %.

Three extraction/standard curve and evaluation procedures of samples were conducted. Each assay was calculated using a concurrently extracted standard curve. In assuming that extracted β-endorphin behaved like β-endorphin standard no correction was necessary for the efficiency of the extraction.

Data obtained will be explained in Chapter IV.

Data Analysis

The instrument was distributed to the participants who responded to the objective TAI pre and post-MCAT. An ex post facto method of analysis was conducted. The SPSS Program (Nie et al., 1975) was used to generate descriptive statistics: means, standard deviations and frequencies. Correlation coefficients and stepwise multiple regression through the use of the SPSS Program (Nie et al., 1975) were used to determine relationships between independent and dependent variables.

Establishing a comparative norm using RIA for serum β-endorphin levels is a difficult process as the literature attests. Wardlaw and Frantz (1979), Inturissi et al. (1982) and Colt (1981) extracted β-endorphin onto talc (as was done in this experiment) and reported 10-25% crossreactivity of the antisera with β-lipotropin. They published basal values for serum β-endorphin as 21 pg/ml, 30 pg/ml, and 17.6 pg/ml respectively. The latter result occured after an easy run by well-trained athletes with the level elevated to 28.0 pg/ml after a strenuous run.

Based on these findings, an arbitrary serum β-endorphin value of less than 40 pg/ml is guardedly assigned as the normal, base level for presumable non-stressed individuals.[7]

Statistical data of β-endorphin (pg/ml) were subjected to the SPSS Program (Nie et al., 1975) for analysis.

Pilot Study

A pilot study using pre-medical students (N = 5) was conducted under the same circumstances as those described and using the talc method of extraction. Three of the five subjects had taken the MCAT previously. Results from these participants confirmed the feasibility of the use of talc in assaying participants' blood. Pre-MCAT measures of β-endorphin were compared to amounts found in samples obtained post MCAT (Appendix G). The modification of β-endorphin levels before and after MCAT was correlated with scores from the TAI to determine the presence of a relationship between psychological and physiological factors.

Paper-pencil results from Pre to Post-MCAT showed no significnant change. RIA of blood samples indicated that serum β-endorphin was elevated Pre-MCAT.

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[7] It is important to mention that none of the above studies differentiated between male and female levels of serum β-endorphin. The same pattern of analyses was employed in this investigation.

CHAPTER IV

RESULTS

Chapter IV is comprised of four sections. The first includes descriptive statistics for all variables examined in the study. Section two contains analyses of correlation coefficients between independent and dependent variables. The degree of relationships among MCAT scores as influenced by sets of TAI results and levels of β-endorphin are interpreted and discussed. As an addendum to the explanation of findings, and for the purposes of future research design, regression results and supplementary analyses are included and discussed. The last section deals with testing hypotheses of the study.

SAMPLE CHARACTERISTICS

Frequencies, Means and t-Tests were obtained for the TAI and serum β-endorphin pre and post-MCAT in order to answer questions and test hypotheses concerning the status of the subject of the study.

The sample consisted of 17 pre-Medical students. Characteristics are described in Table 4.

Table 4

Frequency Chart for all Subjects

	Sex	Mean AGE	Mean GPA
Males	11	22	3.47
Females	6	21	3.55
Total	17		

Note: None had taken the MCAT previously. Six attended the Kaplan preparation program.

Test Anxiety Inventory Results

The means and standard deviations of TAI scores pre and post-MCAT are found in Table 5.

All participants completed the self-report instrument, the Test Anxiety Inventory (TAI) before and after taking the Medical College Admission Test (MCAT).

Mean values for the Worry subscale were 11.29 pre-MCAT and 10.65 after the completion of the test. Norm values for Worry found in the TAI Professional Manual (Spielberger, 1980) placed participants in this study in the 39th percentile pre-MCAT and and in the 34th percentile post-MCAT.

Emotionality scores reflected a mean of 14.76 pre-MCAT and 13.82 post-test. The test anxiety percentile table identified subjects in the 36th percentile in the Emotionality subscale pre-MCAT. The Mean of 13.82 post-MCAT, compared to norm sample means indicated that students were in the 35th percentile.

Total results pre-MCAT (Mean = 33.53) when compared to norms were in the 38th percentile of the test anxiety scale. A post-test value (Mean = 31.47) was found in the 33th percentile of norm samples.

Table 5

Means and SD of TAI Scores Pre and Post-MCAT

	TAI						
	Worry			Emotionality		Total	
	N	M	SD	M	SD	M	SD
Pre-MCAT	17	11.29	2.41	14.76	3.50	33.53	5.33
Post-MCAT	17	10.65	2.26	13.82	2.94	31.47	5.13

Note: Total score included a possible (16 points) from 4 items which did not factor analyze into W or E, plus scores of W and E subscales (32 points each). T (above) = a possible 80 points, the highest level on the test anxiety scale.

With subscale values less than the 40th percentile on the scale of test anxiety, subjects did not evidence undue paper-pencil measured stress but anxiety was higher pre-MCAT. On the whole, the students appeared to have low anxiety related to the MCAT. This was also the case in pilot study findings (Appendix G).

Student's t-Tests were applied to obtain a ratio which would determine whether the observed difference was sufficiently larger than a difference expected by chance.

Table 6 presents the differences between means of TAI before and after the MCAT.

Overall mean values of the subscales indicated that subjects, in general, did not appear to reflect high test anxiety however, there were decrements from pre to post-MCAT.

Table 6

Differences Between Means of TAI Pre and Post-MCAT

TAI	Pre-MCAT			Post-MCAT			t-value	p<
	N	M	SD	N	M	SD		
W	17	11.29	2.41	17	10.65	2.24	1.89*	0.077
E	17	14.76	3.50	17	13 82	2.94	2.22**	0.041
T	17	33.53	5.33	17	31.47	5.13	4.03***	0.001

* p <.10
** p <.05
*** p <.01

Test anxiety results in terms of subscales showed that the the t-value for Emotionality (t = 2.22, df = 16) was significant at p < .05. Because upper scores were indicative of higher anxiety, results suggested that students were more anxious before taking the MCAT (Mean = 14.76) than after (Mean = 13.82) the test.

Test anxiousness was expressed in the manner of responses on the pre-MCAT Worry items as the mean pre- test (11.29) was more elevated than the mean post-test (10.65).

Incorporating W and E, Total score results pre-MCAT (Mean = 33.53), when compared to post-MCAT (Mean = 31.47) data were significant at the p <.01 level.

Beta-endorphin Results

The statistical summary of means and standard deviations of serum β-endorphin from three separate RIA's are presented in Table 7.

Table 7

Means and SD of B-e Pre and Post MCAT

		b-endorphin	
	N	M	SD
Pre-MCAT	12	52.08	9.95
Post-MCAT	12	60.83	7.93

n = 12 cases, outliers removed

Note: b-endorphin = picograms/milliliter (pg/ml)

The means represent the sample "without outliers." Subjects whose values were skewed were omitted from parts of analyses where levels of pre and post-MCAT measures of serum β-endorphin were necessary to test hypotheses.

Standard curves were conducted concurrently with each assay. The internal quality control criteria were met with each experiment as reflected in the midpoint of the slopes (Appendix I). However, close examination of the three means (Appendix J) revealed some spurious results which necessitated elimination.

Subjects "B," "G," "I," "J," and "O," were omitted because of questionable values in one or more of the assays conducted. The calculation of the mean for each of the participants resulted in prohibitive values (from the criteria specified) exceeding

100 pg/ml. Examination of Tables 28 and 29 in Appendix J will disclose the approriateness of using the "Mean of Three Assays Without Outliers" for purposes of correlation in these analyses.

Outliers consisted of 4 males and 1 female. TAI scores for these subjects were significant in the subscale of Emotionality ($p < .01$) only, with elevation pre-MCAT.

The mean values obtained for the participants in this study, with the 36% crossreactivity by the antiserum with β-LPH was 52.08 pg/ml pre-MCAT and 60.83 pg/ml post-MCAT. Since 40 pg/ml had been cautiously designated as the base-line level of β-endorphin, a Student's t-test was applied to determine if mean levels observed by subjects were significantly different from those in published literature. Results are found in Table 8.

Table 8

Differences Between Means of B-e (W/O Outliers) and Baseline Values

	Mean of Sample	Baseline Mean+	t-Test	p‹
Pre-MCAT	52.08	40.00	1.23	0.179
Post-MCAT	60.03	40.00	-2.74	0.019**

* p ‹.05
n = 12 cases, outliers removed

Note: *b*-endorphin = picograms/milliliter (pg/ml)
+ Value assigned from published research.

These data appear to suggest that individuals experienced moderate concern over the MCAT in general. And contrary to pilot study results (Appendix G), the physiological parameter of β-endorphin of main study subjects is elevated post-MCAT.

The mean difference between serum β-endorphin levels pre and post-MCAT may be found in Table 9.

Table 9

Differences Between Means of B-e (W/O Outliers) Pre and Post-MCAT

	N	M	SD	t- value	p<
Pre-MCAT	12	52.08	9.59	-2.30*	0.042
Post-MCAT	12	60.83	7.30		

* p < .05
n = 12 cases, outliers removed

Note: b-endorphin = picograms/milliliter (pg/ml)

Serum β-endorphin values obtained after completion of the MCAT were significant as seen by the t value at the .05 level (t = 2.30, df = 11). The negative attribute underscores the fact that the β-endorphin was elevated after the MCAT examination.

Paper-pencil results, unlike those of serum β-endorphin reflect higher anxiety pre-MCAT. Hence, there is a difference in the test as measured by the TAI (higher pre-test) and the physiological indicator as measured by β-endorphin (higher post-test).

CORRELATIONAL ANALYSES

Pre-MCAT coefficients for the TAI subscales, β-endorphin and GPA are presented in Table 10.

Table 10

Correlation Matrix for TAI (W,E,T), B-e, GPA (Pre-MCAT)

	Worry (W)	Emotionality (E)	Total (T)	PRESAM	GPA
W	1.000				
E	-0.087[a]	1.000			
T	0.298[a]	0.880[a***]	1.000		
PRESAM	0.398[b]	-0.069[b]	0.150[b]	1.000	0.088
GPA	-0.307[a]	-0.353[a]	-0.472[a*]	0.088[b]	1.000

* p <.10
*** p <.01
a n = 17 cases
b n = 12 cases, outliers removed
PRESAM = b-endorphin values before MCAT.

Note: Selected variables are included in this table. A complete correlation matrix is in Appendix L.

A highly significant correlation was found between Emotionality and Total ($r = .880$, $p < .01$).

The significant correlation between Total and GPA suggested that when GPA was high, Total test anxiety as measured by the TAI was low.

The negative coefficients in Table 10 between Emotionality and Worry, PRESAM (β-endorphin) and Emotionality, GPA and Worry, GPA and Emotionality were below the .48 level needed to approach significance with a sample of 17 with a 5% chance of statistical error (.57 is needed for significance with a sample of 12). The negative signs however did imply inverse relationships. There were positive correlations between PRESAM and Worry (which approached significance) and PRESAM and Total.

Table 11 presents the correlational results of the Worry, Emotionality, Total, β-endorphin and GPA parameters post-MCAT.

Table 11

Correlation Matrix for TAI (W,E,T), B-e, GPA (Post-MCAT)

	Worry (W)	Emotionality (E)	Total (T)	POSTSAM	GPA
W	1.000				
E	0.140[a]	1.000			
T	0.581[a**]	0.843[a***]	1.000		
POSTSAM	0.098[b]	-0.258[b]	0.200[b]	1.000	0.464
GPA	-0.329[a]	-0.438[a]	-0.486[a**]	0.464[b]	1.000

** p < .05
*** p < .01
a n = 17 cases
b n = 12 cases, outliers removed
POSTSAM = b-endorphin values after MCAT.

Sex variables were not significant; therefore are not reported in this table. They are included in the overall matrix in Appendix L.

Correlation of Total and Emotionality was again highly significant ($r = .843$, $p < .01$) after completion of the examination. The relationship between Total and Worry was significant post-MCAT.

Correlations between GPA and TAI Total pre and post-MCAT (see Tables 10 and 11) were negative and significant at the $p < .05$ level. This pattern resembles that of the norm samples. In this study, GPA had a stronger correlations with Emotionality than did the norm samples (see Chapter 3). Post-MCAT β-endorphin and GPA had a stronger positive correlation than pre- MCAT and approached the $p < .05$ level of significance.

Correlations with MCAT Performance

Pre and post-MCAT data for Correlations of TAI subscales, GPA, and β-endorphin with performance are presented in Tables 12 and 13.

Table 12

Correlation Matrix for TAI (W, E, T), GPA, B-e, and Performance on MCAT (Pre-MCAT)

	Worry (W)	Emotionality (E)	Total (T)	GPA	b-endorphin PRESAM
W	1.000				
E	-0.087	1.000			
T	0.298	0.880***	1.000		
GPA	-0.307	-0.353	-0.472*	1.000	0.088
PRESAM	0.398	-0.069	0.150	0.088	1.000
BIO	-0.391	-0.613***	-0.793***	0.578**	-0.355
CHEM	-0.491**	-0.401	-0.671***	0.659***	-0.525**
PHY	-0.386	-0.378	-0.567**	0.372	0.188
SPROB	-0.589**	-0.269	-0.563**	0.715***	-0.509*
READ	-0.432*	-0.415*	-0.602**	0.503**	-0.040
QUAN	-0.360	-0.172	-0.408	0.325	-0.045

* p <.10
** p <.05
*** p <.01

Note: TAI and MCAT scores = 17 cases.
b-endorphin and MCAT scores = 12 cases, outliers removed.

GPA Correlations with MCAT

Overall patterns show that GPA correlations found in Tables 12 and 13 were all positive with high significance for Biology ($r = .578$, $p < .05$), Chemistry ($r = .659$, $p < .01$), Science Problems ($r = .715$, $p < .01$), and Reading ($r = .503$, $p < .05$).

Table 13

Correlation Matrix for TAI (W, E, T), GPA, B-e and Performance on MCAT (Post-MCAT)

	Worry (W)	Emotionality (E)	Total (T)	GPA	b-endorphin POSTSAM
W	1.000				
E	0.140	1.000			
T	0.581	0.843***	1.000		
GPA	-0.329	-0.438	-0.486**	1.000	0.464
POSTSAM	0.098	0.258	0.200	0.464	1.000
BIO	-0.580**	-0.640***	-0.831***	0.578**	-0.088
CHEM	-0.388	-0.403	-0.600**	0.659***	0.424
PHY	-0.261	-0.380	-0.526**	0.372	0.071
SPROB	-0.515**	-0.308	-0.581**	0.715***	0.182
READ	-0.371	-0.466*	-0.627**	0.503**	-0.192
QUAN	-0.445*	-0.163	-0.414*	0.325	0.190

* p < .10
** p < .05
*** p < .01
Note: TAI and MCAT scores = 17 cases.
b-endorphin and MCAT scores = 12 cases, outliers removed

Subjects with high GPA performed better in all subset areas especially the ones indicated. The correlation of GPA with subscales of the TAI, were all negative; significance was attained between GPA and Total score pre and post-MCAT suggesting a logical pattern: high GPA, less test anxiety pre and post-MCAT.

TAI Subscale Correlations with MCAT

A general outcome of the Pre-MCAT correlations of TAI subscales with performance indicated all negative correlations with the subject areas. The inverse relationships suggested that with: lower Worry there was higher performance in all subsets; lower Emotionality (not as strong as Worry), higher performance in all; lower Total score (lower anxiety), higher performance in all.

Significant correlations were observed between Worry and Chemistry ($r = .491$, $p < .05$) Worry and Science Problems ($r = .589$, $p < .05$), Worry and Reading ($r = .432$, $p < .10$). Students with course content backgrounds indicated (as will be seen later) were low. The correlation between Emotionality and Biology was highly significant ($r = -.613$, $p < .01$). As in the Worry results, Reading surfaced as a correlate with Emotionality ($r = -.415$, $p < .10$).

All Total score coefficients were negative, with 5 of 6 subset areas being highly significant and the 6th, Quantitiative, also approached significance. The negative correlations indicate that students with higher MCAT scores had less test anxiety as (measured by the TAI) than those who had lower MCAT scores.

Post-MCAT data demonstrated a change in profile of test anxiety subscales as related to performance. Results of the TAI indicated significance between Worry and Biology ($r = -.580$, $p < .05$), Worry and Science Problems ($r = -.515$, $p < .05$), Worry and Quantitative ($r = -.445$, $p < .10$).

The correlation between Emotionality and Biology remained highly significant ($r = -.640$, $p < .01$). The Emotionality - Reading relationship ($r = -.466$, $p < .10$) continued to reflect a negative relationship between the TAI and MCAT.

Emotionality was highly correlated with Biology ($p < .01$) pre and post-MCAT suggesting anxiety over the subset area. The relationship between Emotionality and

Reading increased in "strength" from pre-MCAT (r= -.415) to post-MCAT (r= -.466) which indicated an association possibly not due to chance.

The correlations of post-MCAT TAI Total score measures of test anxiety with the MCAT subsets were all significant. The negative coefficients suggested that lower Total scores (therefore lower test anxiety) were related to higher scores on the MCAT.

Serum Beta-endorphin Correlations with MCAT

An analysis of Table 12 for Pre-MCAT β-endorphin results points to significance between PRESAM and Chemistry ($r = -.525$, $p < .10$) and PRESAM with Science Problems ($r = -.509$, $p < .10$). With the exception of Reading, the same correlations were evident (with higher significance), between Worry and the corresponding subsets. The higher the test anxiety (elevated β-endorphin), the greater the possible negative effect on performance in the areas of Chemistry and Science Problems.

Post-MCAT serum β-endorphin (Table 13) results showed no significant correlations.

Correlations with Number of Courses

Students were asked to indicate the number of undergraduate (1) and graduate (2) courses completed by them. Coding the subject areas as shown in Tables 14 and 15 facilitated the arrangement of the correlation computer program.

The Total Science (TS1 and TS2) designation incorporates all courses that may overlap areas, such as Genetics and Microbiology in Biology. Quantitative and Reading scores were not included because there were no courses that treated them as such.

Table 14 describes the relationship between TAI and related science courses pre and post-MCAT.

Table 14

Correlation Matrix for TAI (W,E,T) and Related Science Courses (Pre and Post-MCAT)

Number of Courses	Worry		Emotionality		Total	
	PREW	POSTW	PREE	POSTE	PRET	POSTT
Bio-TB1	0.355	0.190	-0.340	-0.207	-0.147	-0.066
Bio-TB2	-0.210	-0.063	-0.164	-0.213	-0.188	-0.121
Chem-TC1	-0.260	-0.116	0.317	0.128	0.141	0.052
Chem-TC2	-0.301	-0.250	-0.259	-0.367	-0.241	-0.321
Phys-TP1	-0.073	0.234	0.302	0.183	0.199	0.242
Phys-TP2	0.000	0.000	0.000	0.000	0.000	0.000
TS-TS1	0.263	0.233	-0.129	-0.139	-0.031	-0.114
TS-TS2	-0.266	-0.120	-0.214	-0.285	-0.231	-0.190

n = 17 cases

Note: 1 & 2 = Undergraduate, Graduate courses respectively.

TAI Subscale Correlations with Number of Courses

No significant correlations were detected between TAI subscale results and number of courses taken pre or post-MCAT (see Table 14).

Serum Beta-endorphin Correlations with Number of Courses

Table 15 synthesizes correlational results of serum β-endorphin and related science courses pre and post-MCAT.

Pre-MCAT findings for serum β-endorphin and undergraduate Biology ($r = .651$, $p < .05$) were significant at $p < .05$ level. Participants with more undergraduate Biology courses appeared to be more test anxious as indicated by elevated β-endorphin.

Table 15

Correlation Matrix for B-e and Related Science Courses (Pre and Post-MCAT)

Number of Courses	*b*-endorphin	
	PRESAM	POSTSAM
Bio-TB1	0.651**	-0.303
Bio-TB2	0.298	0.225
Chem-TC1	-0.517*	-0.046
Chem-TC2	0.117	-0.605**
Phys-TP1	-0.061	0.073
Phys-TP2	0.000	0.000
TS-TS1	0.574**	-0.409
TS-TS2	0.299	0.041

* p < .10
** p < .05
n = 12 cases, outliers removed

Note: 1 & 2 = Undergraduate, Graduate courses respectively.

This was also the case with Total Science courses at the undergraduate level (r = .574, p <.05).

The negative attribute attached to the correlation between undergraduate Chemistry (TC1) courses and serum β-endorphin level (r = -.517, p <.10) pre-MCAT may be interpreted as the greater number of undergraduate Chemistry courses, the lower the serum β-endorphin level and less physiological stress.

A post-MCAT correlation between graduate courses in Chemistry (TC2) and β-endorphin (r = -.605, p < .05) related that students who were more prepared in the subject area were probably less stressed physiologically after the examination.

Unlike TAI subscale results, pre-MCAT serum β-endorphin showed significant correlations with number of courses. Table 15 suggested that students with undergraduate Biology (TB1) and fewer Total Science courses were more test anxious as indicated by elevated serum β-endorphin. Participants with more undergraduate Chemistry courses pre-MCAT had less test-anxiety.

Post-MCAT data show that more graduate courses in Chemistry apparently reduced stress after taking the test.

REGRESSION ANALYSES

This section considers identifying significant predictor variables that relate to the MCAT subset areas, namely: Biology, Chemistry, Physics, Science Problems, Reading and Quantitative. Separate analyses were done for each variable using a stepwise procedure. Free entry of individual items of the TAI, serum β-endorphin values, GPA, and nature and number of courses, were permitted access as predictor variables. The purpose of this procedure was to further qualify relationships, identify the variance explained and assist with future research design.

Correlational analyses demonstrated relationships between Worry, Emotionality, and Total as composites, the regression analysis program (Nie et al., 1975) was arranged to enter each of the 20 items in a stepwise fashion to determine the best test anxiety (TAI items, β-endorphin, and other) predictors of performance in the subject area.

Codes for the variables in Tables 16-20 resemble those already described in the correlation matrices. Individual items on the TAI are designated as PREV because they represent variable scores before taking the MCAT.

Subjects with unacceptable serum β-endorphin values (5) were removed from these analyses. Post data were not considered in the predictive analysis.

Table 16 presents the results of regression analysis with MCAT Biology used as the dependent variable. The program that was used stopped entry when no item had a t-value significant at the .05 level or higher.

Table 16

Regression Analysis for MCAT-Biology

Variable	Multiple R	R^2	Beta	F(1,10)	p <
PREV 9 (E)	0.607	0.369	-0.607	5.840	.036**

** p < .05
n = 12 cases, outliers removed

One TAI item entered the stepwise analysis as a significant predictor of Biology. Categorized as an Emotionality expression, PREV 9 reads: "Even when I'm well-prepared for a test, I feel very nervous about it." The negative Beta indicated that students selecting this item had lower scores on the MCAT-Biology variable.

Pre and post-MCAT Emotionality was inversely correlated with Biology (see Appendix L). The strong significance of PREV 9 implied that this item may have played a major role in that relationship.

Table 17 presents results when Chemistry was used as the dependent variable, all predictor variables remained unchanged.

Table 17

Regression Analysis for MCAT-Chemistry

Variable	Multiple R	R^2	Beta	F(1,10)	p <
Prev 15 (E)	0.647	0.418	-0.647	7.181	.023**
PRESAM	0.891	0.794	-0.726	17.312	.000***

** p <.05
*** p <.01
n = 12 cases, outliers removed

The strongest single variable was PREV 15: "I feel very panicky when I take an important test." As an Emotionality item with a negative Beta, the entry of the variable suggested that higher test anxiety (autonomic arousal) may have influenced lower Chemistry scores.

PRESAM appeared as the second predictor variable for Chemistry and added to the variance of PREV 15. The two variables together accounted for 79 % of the variance; this is a high explained variance for two variables. Students selecting the item (PREV 15) and those with high pre-MCAT β-endorphin had lower Chemistry scores.

Physics, entered as the criterion variable with the same predictors, is found in Table 18.

Item 2 of the TAI measures Emotionality, "While taking examinations, I have an uneasy, upset feeling." Item 6 on the other hand, suggested Worry, "The harder I work at a test the more confused I get." Together these accounted for 67 percent of the variance explained in predicting performance in Physics.

Table 18

Regression Analysis for MCAT-Physics

Variable	Multiple R	R^2	Beta	F(1,10)	p <
Prev 2 (E)	0.695	0.483	-0.695	9.336	.012**
Prev 6 (W)	0.819	0.671	-0.638	9.180	.006***
TB2 (G-Bio)	0.901	0.824	-0.638	12.508	.002***
PRESAM	0.952	0.906	-0.649	16.813	.001***

** p <.05
*** p <.01
n = 12 cases, outliers removed

TB2 contributed to the prediction of performance in Physics. The priority entry is possibly due to the uniqueness of the sample.

PRESAM accounted for significance ($p < .01$) as a predictor of Physics and together with the above mentioned variables accounted for 90 percent of the variance in the criterion variable; this is a high amount of explained variance.

MCAT-Science Problems was entered into the regression equation as the dependent variable (Table 19) in effort to determine which variables would effect the outcome. GPA ($p < .05$) and PRESAM ($p < .01$) were the best predictors of performance in the sub- of Science Problems on the MCAT. The positive Beta suggested that the higher the GPA and serum β-endorphin values pre-MCAT, the better the performance in Science Problems.

Resultant data from the dependent variable Reading are presented in Table 20.

Table 19

Regression Analysis for MCAT-Science Problems

Variable	Multiple R	R^2	Beta	F(1,10)	p <
GPA+	0.620	0.385	0.621	6.270	.031**
PRESAM	0.840	0.705	0.671	10.792	.004***

** p <.05
*** p <.01

Note:GPA value is for n = 12; Tables 12 and 13 n = 17

Table 20

Regression Analysis for MCAT-Reading

Variable	Multiple R	R^2	Beta	F(1,10)	p <
Prev 4 (W)	0.787	0.619	-0.787	16.260	.002
Prev 13 (T)	0.881	0.777	-0.427	15.688	.001

*** p <.01
n = 12 cases, outliers removed

Item 4, a Worry item states : "I freeze up on important exams." PREV 13, on the other hand is an item in the "Total" category, "During important tests I am so tense my stomach gets upset." A correlation between Reading and Worry was significant (p <.10) as indicated in Table 16. The significance of item 4 (p <.01) in the regression analysis (p <.01) possibly accounted for much of the relationship that occured.

No independent variables entered the stepwise regression analysis to predict performance in Quantitative skills.

Supplementary Analyses

Due to physiological interest, suplementary analyses were conducted to investigate the impact of β-endorphin on test taking and paper-pencil measures on test-taking. Upper and lower PRESAM were examined (Appendix K) to determine if levels of serum β-endorphin would relax those who were "worriers" or those who had a tendency to become "emotionally, autonomically aroused." No conclusive pattern emerged.

Upper and lower β-endorphin values (PRESAM) were compared to Worry scores pre-MCAT to determine if there was an implied influence of one on the other, that is to see if high PRESAM levels were associated with low Worry scores and low PRESAM values were associated with high Worry scores and if those presumable "opposite" relationships effected better MCAT results. No pattern on PRESAM or no difference on tests was evident.

A similar comparison was made with Emotionality scores, PRESAM and MCAT results. No pattern was detected.

HYPOTHESIS AND PROBLEM TESTING

Hypothesis 1

Test anxiety as measured by the TAI subscales (Worry, Emotionality, and Total) will be significantly elevated as a result of stress associated with the MCAT-test:

a. Pre-MCAT.
b. Post-MCAT.

Pre-MCAT test anxiety level as measured by the TAI, in general, was below the 40th percentile range of norm samples listed in the TAI Professional Manual, therefore, the students did not appear to be unduly stressed. However, the values of subscales were higher than the standard error of the instrument and evidenced decrements from pre to post-MCAT in the subscales. Hypothesis 1 is supported.

Hypothesis 2

Test anxiety as measured by serum β-endorphin will be significantly elevated as a result of the stress associated with the MCAT:

a. Pre-MCAT.
b. Post-MCAT.

The 40 pg/ml criterion from published literature for normal, non-stressed individuals, indicated that participants were moderately stressed pre-MCAT.

Post-MCAT RIA data not only were significantly different from the normal values specified, but showed an increase from pre-MCAT levels. Pre and post-MCAT results support Hypothesis 2.

Hypothesis 3

Test anxiety as measured by TAI (Worry, Emotionality, Total) will be significantly related to subset scores of the MCAT- Biology, Chemistry, Physics, Science Problems, Reading and Quantitative:

a. Pre-MCAT.
b. Post-MCAT.

Pre-MCAT TAI data (Table 12) showed negative correlations with the MCAT scores. Total Score which incorporates Worry and Emotionality was significant with all subset areas except Quantitative. Regression Tables 16, 17, 18, and 20, indicated that selected items from the TAI showed significant prediction and accounted for a

substantial amount of the variance of MCAT subset area scores. Since the Beta was negative, this indicated that higher scores in TAI resulted in lower MCAT scores. On this basis, Hypothesis 3 is supported with a significant negative relationship.

TAI Total scores for post-MCAT data were significantly correlated with the six subset areas. Hypothesis 3 is strongly supported.

Hypothesis 4

Test anxiety as measured by serum β-endorphin will be significantly related to subset scores of the MCAT- Biology, Chemistry, Physics, Science Problems, Reading and Quantitative:

a. Pre-MCAT.
b. Post-MCAT

Pre-MCAT data found in Table 12 indicated that all areas except Physics were negatively correlated with serum β-endorphin. This implied that the higher the β-endorphin level the lower the scores on the subset areas. Significance was noted in Chemistry and Science Problems which indicated that participants with higher β-endorphin values scored lower in Chemistry and Science Problems. The correlation between Biology and β-endorphin approached significance. Regression Tables 17, 18, and 19, indicated that serum β-endorphin showed significant prediction and accounted for a substantial amount of the variance of MCAT subset area scores. Since the Beta was negative (Tables 17 and 18), higher levels of β-endorphin indicated lower MCAT scores. Table 19 had a positive Beta. Pre-MCAT results support Hypothesis 4.

Table 13 revealed that correlations between β-endorphin and the MCAT subsets were mostly positive. Higher β-endorphin are related to higher MCAT scores in all areas except Biology and Reading, two areas which lend support to Hypothesis 4.

Hypothesis 5

Test anxiety as measured by the TAI (Worry, Emotionality, Total) will be significantly related to serum β-endorphin:

a. Pre-MCAT.
b. Post-MCAT.

Pre-MCAT data found in Table 12 suggested a mixed relationship. Serum β-endorphin and Worry approached significance.

Post-MCAT data indicated all positive correlations. Based on the data Hypothesis 5 is not supported. Trend data indicate positive correlations with the exception of pre-Worry.

Hypothesis 6

GPA will be a significant predictor of MCAT scores.

a. GPA will be significantly related to TAI (Worry, Emotionality, Total) results.
b. GPA will be significantly related to serum β-endorphin (Pre-MCAT) (Post-MCAT)
c. GPA will be significantly related to MCAT scores.

Tables 12 and 13 showed that GPA is inversely related to TAI scores. This indicated that subjects with high GPA had low TAI scores and hence, low paper-pencil test anxiety. Significant correlations were found between Total and GPA pre-MCAT ($p < .10$), Total and GPA post-MCAT ($p < .05$). The negative correlations indicated that when GPA was low, self- reported test anxiety was high. This pattern supports Hypothesis 6.

GPA was not related to pre-MCAT β-endorphin values implying that no physiological pattern was detected. Serum β-endorphin level correlations with GPA were non-significant. Pre and Post-MCAT results were positive with POSTSAM approaching significance. High GPA with higher serum β-endorphin suggests high GPA students

were more stressed as measured physiologically after testing than before. This however, was not apt to influence test performance.

The significant, positive correlations of GPA with all subset areas except Physics, indicate that GPA was a significant variable and predictor of MCAT performance. Hypothesis 6 is supported.

Hypothesis 7

Number and nature of science courses will be significantly related to test anxiety.
a. Content or total science courses will be related to TAI.
b. Content or total science courses will be related to serum β-endorphin

Pre and Post-MCAT correlations between TAI and Science courses, found in Table 14 had no significant relationships. This suggested that nature and number of courses taken in preparation were not related to self-reported test anxiety. Part 1 of Hypothesis 6 was not supported.

Correlations with serum β-endorphin pre-MCAT (Table 15) however, did show significance in undergraduate Biology and Total Science ($p < .05$). The positive nature indicated that students with more undergraduate preparation had a higher level of stress. Chemistry on the other hand, was negatively correlated. Students with more undergraduate Chemistry courses had lower stress.

Post-MCAT data revealed a significant negative correlation with TC2, graduate level Chemistry. Serum β-endorphin results support Hypothesis 7.

CHAPTER V

SYNTHESIS, CONCLUSIONS AND RECOMMENDATIONS

Statistical analyses of test anxiety as measured by the TAI and test anxiety as measured by serum β-endorphin were conducted in this investigation. The study was undertaken because literature which is available on test anxiety and its negative effect on achievement has not considered the association of serum β-endorphin, the body's endogenous opiate, with evaluation stress. The present study attempted to determine if test anxiety, with its subscale components Worry and Emotionality, is truly manifested in pre-medical students as a result of the competitive event of the Medical College Admission Test (MCAT) and if β-endorphin similarly responds to that type of situational stress. An important aspect of the analyses was to determine if test anxiety is inversely related to performance.

Correlational and multiple correlational studies are a first step in this line of investigation. If replicative findings occur then causal-comparative research through manipulation and control may be accomplished. In this case, the control would be an intervention measure to assuage anxiety in the test-taking process in order to effect optimal performance.

SYNTHESIS OF FINDINGS AND DISCUSSION

Data from this investigation must be viewed in light of the volunteer (small) sample which had distinctive characteristics. Participants as pre-medical students were

above the mean in ability and preparedness for the test situation and instrument used as the non-manipulated treatment. The competitive nature of the Medical College Admission Test (MCAT) may not have provided as strong a stimulus for test anxiety as some other test taken by a different sample. With these caveats in mind results are reviewed as follows:

1. Based on norm samples, TAI data indicated that students in general were not extremely stressed, however, self-report measures of the TAI were more elevated pre-MCAT. Students appeared to be more test-anxious in anticipation of the key evaluative event. TAI Total score, which had the highest significance showed decrements from pre to post-MCAT.

2. When compared to the criterion set for normal, non-stressed individuals, participants' serum β-endorphin values (pg/ml) were higher in general. There were significant elevations post-MCAT. Rumination about performance may have caused test anxiety.

3. Serum β-endorphin was not correlated with any of the TAI subscales. However, the positive correlation with Worry pre-MCAT approached significance.

4. Test anxiety as measured by the TAI was significantly inversely related to GPA. There were significant positive correlations between GPA and MCAT scores.

5. Test anxiety was significantly inversely related to performance.

   a. Worry correlated negatively with Chemistry, Science Problems and Reading pre-MCAT and with Biology, Science Problems and Quantitative post-test.

b. Emotionality reflected a high negative correlation with Biology before and after the MCAT. A correlation with Reading before and after the test occured.

c. Pre and post-MCAT TAI Total scores, with the exception of Quantitative pre-test, were all significantly, negatively correlated with performance. The higher the self-reported test anxiety, the lower the scores in the MCAT subset areas.

d. Pre-MCAT β-endorphin results showed negative correlations with Chemistry and Science Problems.

e. On five of six MCAT subset areas, one or both test anxiety measures was a significant predictor of performance.

6. The nature and number of science courses did show a relationship to β-endorphin. Subjects with more undergraduate Biology had higher serum β-endorphin values pre-MCAT. Subjects with more undergraduate Chemistry had lower serum β-endorphin values pre-MCAT. And participants with more graduate Chemistry had lower serum β-endorphin values after the examination.

7. Results from Regression Analyses demonstrated that:

a. Select items from the Test Anxiety Inventory (TAI) could be used to predict performance on the subsets of the MCAT.

b. GPA predicted performance in the Science Problems subset of the MCAT.

c. β-endorphin values pre-MCAT were significant predictors for three of the six areas of the MCAT: Chemistry, Physics and Science Problems.

TAI Results: The instrument measured test anxiety in terms of Worry, Emotionality and Total. As seen in the overall mean values of the subscales, subjects self-reported test anxiety was moderate to low; however, there were decrements from pre to post-MCAT. Significant elevations were found in Emotionality before the test. These data corroborate those already documented which found that "Emotionality scores dropped significantly following completion of the examination irrespective of inititial level of test anxiety or performance expectancy (Doctor and Altman, 1969, p. 563)."

The change in Worry scores from pre to post-MCAT corresponds to patterns found in the literature. Spielber et al. (1968) established that among students facing a very important examination Worry scores are elevated pretest (as much as five days before). Consonant with these data, Worry scores obtained by subjects two days before the MCAT showed significant elevation ($p < .10$). The strength of the significance is believed to correspond to the nature of the participants and their better-than-average ability. Less academically capable and prepared students would perhaps evidence higher Worry. Nonetheless, significance was observed to substantiate the claim.

Doctor & Altman (1969) claimed that both Worry and Emotionality scores dropped significantly from pre to post test periods although the absolute change in Worry was less than that of Emotionality. The present study has replicated this finding.

Beta-endorphin Results: Mean β-endorphin assay results suggested that individuals were moderately concerned over the MCAT with significant elevation ($p < .05$) after completion of the examination.

Literature findings propose that test anxiety (Worry and Emotionality) is elevated in anticipation of an evaluative event, it was believed that β-endorphin would act

as nature's response to the pain of test anxiousness and would also manifest higher values pre-MCAT. The pilot study adhered to this assumption.

Key scientific findings, however, indicate that increased values of β-endorphin are observed after a stressful event. For example, Wardlaw and Frantz (1979) found elevated levels post administration of metyrapone.[8] Fraioli et al. (1980) obtained higher values of β-endorphin post-physical exercise. Colt et al. (1981) obtained higher values following easy and strenous runs suggesting that the stress of running stimulates secretion of β-endorphin, β-lipotropin. DuBois et al. (1981) observed that post-surgical stress produced a significant increase in plasma β-endorphin reactivity.

It is conceivable that the theory held by Cohen et al. (1983) may be applied in this case, that is, elevated plasma β-endorphin may be considered a Biological marker of the human stress response (to evaluation anxiety)[9] much as plasma cortisol levels have previously been used (p. 463). And that stress could easily occur after a challenging test.

Subjects approached the MCAT with some degree of confidence. It is not unreasonable to assume that the enormity of the situation may have caused distress over perpective less-than-optimal performance and its unfavorable consequences.

In reviewing the outcome of mean comparisons, it may be cautiously assumed that self-report measures point to anticipation of evaluative events as potential stimuli for test anxiety and the physiological parameter of β-endorphin places the emphasis of examination stress on the response. Nevertheless, these findings suggest that anxiety played a role in the test-taking process.

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8 A drug to determine ability of the pituitary gland to increase secretion of corticotropin.

9 Parentheses mine.

TAI Related to MCAT Scores

Literature has proposed that Worry forms a consistent negative or inverse relationship with performance (Deffenbacher, 1980). In general, findings from this investigation strongly support this position.

Deffenbacher (1980) and Tryon (1980) showed that although Worry is related to performance decrements in the presence of evaluative stress, Emotionality is not. Their finding may be qualified by results obtained from this correlational review. Regression analysis (Emotionality items) indicated high predictiblity of performance decrements.

Relationship of TAI with Serum Beta-endorphin

Contributing to the knowledge of evaluation anxiety patterns is the influence of β-endorphin in the test-taking process. Serum β-endorphin was found to be inversely correlated in the specific areas of the MCAT before the examination.

Morris et al. (1981) found that Emotionality was related to pulse rate change. O'Neill et al. (1969) obtained higher A-state scores on difficult tasks with a concomitant increase of systolic blood pressure during difficult tasks. It was theorized therefore that because Emotionality is the physiological component of the TAI which measures autonomic arousal, a significant correlation would be found with serum β-endorphin. Significance was not attained, however there were positive correlations between serum β-endorphin and Worry pre-MCAT. Correlations with the subscales of the TAI were all positive post-MCAT. The size of the sample may have been a factor in the less than significant values.

In the regression results, certain item responses had higher β-endorphin therefore, selected items were significantly different from PRESAM.

Significance of GPA in the Study

Spielberger & Katzenmayer (1959), Culler & Holahan (1980), and Benjamin et al. (1981) found that test anxiety interferes with student's ability to profit from instruction thus having negative effects on grade point average (GPA).

In this study, GPA was significantly negatively correlated with Total TAI pre and post-MCAT. However, the test anxiety was not extreme. It is necessary to re-emphasize the uniqueness of the volunteer sample. In general, they were exemplary students (Mean GPA = 3.5, see Appendix K) who had qualified for an elite pre-medical program. Participant's overall standing in achievement may have influenced the less than average amount of stress due to the MCAT examination.

Effects of Course Background on Test Anxiety

The study looked at the nature and number of science courses to monitor their contribution to test anxiety. It was hypothesized that the more courses (more pre-paredness) the less test anxiety before the MCAT.

There were no significant correlations between number of courses and TAI. Serum β-endorphin results however, suggested that those who took undergraduate Biology courses appeared to be more test anxious as indicated by elevated β-endorphin. Subjects seemed "worried" about Biology (as seen in the subscale correlations) and the physiological response of β-endorphin increase tends to substantiate the stress.

The negative correlation between undergraduate Chemistry (TC1) and serum β-endorphin pre-MCAT brought out a unique charactieristic of this sample. It seems as though subjects prepared in basic Chemistry were less physiologically stressed before the test.

A correlation between Chemistry (TC2) and β-endorphin post-MCAT could indicate that students who were more prepared in the subject area were less stressed physiologically after the examination.

These findings too, may be peculiar to the small sample size, however, they will be a source of interest for subsequent study.

RECOMMENDATIONS

The most prominent recommendation is that the investigation should be replicated with a larger sample if possible.

Follow-up studies should repeat paper-pencil measurement of test anxiety with an instrument that differentiates between subscales. This would adhere to the insight by Sarason (1984) who states: correlations that relate to the Worry-Emotionality distinction define more reliably the reactions people have when placed in evaluative situations. Certain items on the subscales appeared to have more strength for this type of population.

Future research correlating the effects of serum β-endorphin with evaluation anxiety should refrain from categorically assigning at what stage of the test-taking process elevated stress will occur. The phenomenon may be related to the unique characteristics of the participants and/or the content of the examination. This type of study should be applied to other samples, types of tests, and lower-level students. RIA methodology should be reviewed to circumvent the problem of "outliers."

Significance was found in the correlation of GPA with TAI Total; there was a high positive relationship with MCAT scores. Therefore, this should not be omitted from the problem statement of subsequent studies. The relationship has been well-documented in literature. Other areas with low GPA students should be explored.

An important finding which concurs with reputable literature sources is that test anxiety (Worry and Emotionality) is negatively correlated with performance. Serum β-endorphin activity in a test-anxious situation, cautiously adds to the body of knowledge concerning evaluation stress. The stress of tests may elevate the endogenous opiate in anticipation or response to the stimulus of examinations. Elevations before the test may predict performance. Increase in (pg/ml) value after the test may be reactive but no less significant.

An embellished look at courses of study as factors in test anxiety is recommended. Some findings in these data were unexplainable.

Future research should identify a potential curve by collecting data more than two times (pre and post).

The TAI (especially selected items from regression analysis) could be used with large samples to "screen" those students who are potentially test-anxious. They would be invited to volunteer for serum β-endorphin analyses.

It is important to re-emphasize replication of this type of study. Perhaps, students with low GPA's or those highly "nervous" should be selected as the sample. Repeated research would elucidate patterns of responses on the instrument and values of β-endorphin to help determine (when test scores are analyzed) if the hormone secretion is primarily facilitative.

If it is established that test anxiety causes elevated β-endorphin with debilitative effects on performance then experimental research should be conducted which would introduce an intervention measure, such as relaxation exercises to see if this would neutralize the stress associated with tests.

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Appendix A

TAI TEST FORM

TEST ATTITUDE INVENTORY

Developed by Charles D. Spielberger

in collaboration with

H.P. Gonzalez, C.J. Taylor, G.R. Ross and W.D. Anton

NAME_____DATE_____SEX M F

T_____W_____E_____

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
1. I feel confident and relaxed while taking tests	①	②	③	④
2. While taking examinations I have an uneasy, upset feeling	①	②	③	④
3. Thinking about my grade in a course interferes with my work on tests	①	②	③	④
4. I freeze up on important exams	①	②	③	④
5. During exams I find myself thinking about whether I'll ever get through school	①	②	③	④
6. The harder I work at taking a test, the more confused I get	①	②	③	④
7. Thoughts of doing poorly interfere with my concentration on tests	①	②	③	④
8. I feel very jittery when taking an important test	①	②	③	④
9. Even when I'm well prepared for a test, I feel very nervous about it	①	②	③	④
10. I start feeling very uneasy just before getting a test paper back	①	②	③	④
11. During tests I feel very tense	①	②	③	④
12. I wish examinations did not bother me so much	①	②	③	④
13. During important tests I am so tense that my stomach gets upset	①	②	③	④
14. I seem to defeat myself while working on important tests	①	②	③	④
15. I feel very panicky when I take an important test	①	②	③	④
16. I worry a great deal before taking an important examination	①	②	③	④
17. During tests I find myself thinking about the consequences of failing	①	②	③	④
18. I feel my heart beating very fast during important tests	①	②	③	④
19. After an exam is over I try to stop worrying about it, but I just can't	①	②	③	④
20. During examinations I get so nervous that I forget facts I really know	①	②	③	④

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Appendix B

CONSENT TO SPECIAL TREATMENT OR PROCEDURE FORM

THE OHIO STATE UNIVERSITY

Protocol No. 85H0253

8/27/85

CONSENT TO SPECIAL TREATMENT OR PROCEDURE

I, _____, hereby authorize or direct Sister Jane Anne Molinaro, M.Sci.Ed., Gopi A. Tejwani, Ph.D., Nicholas Gerber, M.B., B.S., Jeffrey A. Houck, M.D., Robert M. Guthrie, M.D. or associates or assistants of his or her choosing, to perform the following treatment or procedure (describe in general terms) I understand that I have been asked to participate in a research program to evaluate the effect of stress on serum β-endorphin. β-endorphin is an endogenous opiate secreted by the pituitary gland; therefore, present in my serum. I will have blood drawn from the vein in my arm prior to and one-week following the MCAT (Medical College Admission Test). I will, also, take the Test Anxiety Inventory prior to the MCAT and one-week following the MCAT examination. A stress profile level will be obtained.

Upon MYSELF.
(myself or name of subject)

The experimental (research) portion of the treatment or procedure is: I will take the Test Anxiety Inventory (paper-pencil test) and have drawn from my arm 30cc of blood to be analyzed for β-endorphin levels. This will be done one-day prior to the MCAT. One-week following the MCAT, I will return to take the test and have drawn from my arm another 30cc of blood . I will be given the results of my stress profile before and after the MCAT.

This is done as part of an investigation entitled: Radioimmunoassay of the endogenous opiate, β-endorphin as Related to Anxiety Induced by the Administration of the MCAT Test.

1. Purpose of the procedure or treatment: To compare the β-endorphin levels prior to and following a stressful situation. Also, to compare the different stress levels.

2. Possible appropriate alternative methods of treatment: Not to participate.

3. Discomforts and risks reasonably to be expected: Local pain, fainting, bleeding into the tissues (bruising), and rarely, an infection might occur at the site of the needle stick into the vein in my arm. The total amount of blood drawn during this study period will be 60cc (2oz, one-fourth of a cup) including two (2) needlestick over a period of one week.

4. Possible benefits for subjects/society: No benefit to me, the volunteer subject, but accumulation of data for the evaluation of β-endorphins related to anxiety/stress. I will be provided with a profile of my stress level.

5. Anticipated duration of subject's participation: Thirty (30) minutes over a period of one week (prior to and following the MCAT examintion).

I hereby acknowledge that Sister Jane Anne Molinaro, M.Sci.Ed, Gopi A. Tejwani, Ph.D., Nicholas Gerber, M.B., B.S., Jeffrey A. Houck, M.D. and Robert M. Guthrie, M.D. has provided information about the procedure described above, about my rights as a subject, and that he/she answered all questions to my satisfaction. I understand that I may contact him/her should I have additional questions. He/She has explained the risks described above and I understand them; he/she has also offered to explain all possible risks or complications.

I understand that, where appropriate, the U.S. Food and Drug Administration may inspect records pertaining to this study. I understand further that the records obtained during my participation may be made available to the sponsor of this study and that the records will not contain my name or other personal identifiers. Beyond this, I understand that my participation will remain confidential.

I understand that I am free to withdraw my consent and participation in this project at any time after notifying the project director without prejudicing future care. No guarantee has been given to me concerning this treatment or procedure.

In the unlikely event of injury resulting from participation in this study, I understand that immediate medical treatment is available at University Hospital of The Ohio State University. I also understand that the costs of such treatment will be at my expense and that financial compensation is not available. Questions about this should be directed to the Human Subject Review Office at 422-9046.

I have read and fully understand the consent form. I sign it freely and voluntarily. A copy has been given to me.

Date:_____ Time:_____ AM PM Signed:_____

Witness(es) if Required _____

_____
(Person Authorized to Consent for Subject -- if Required)

I certify that I have personally completed all blanks in this form and explained them to the subject or his/her representative before requesting the subject or his/her representative to sign it.

Signed: _____
(Signature of the Project Director or his/her Authorized Representative)

Form HS-028A (Rev. 12/83)

Appendix C

TALC TREATMENT

After thawing acidified plasma, 5 ml were pipetted into appropriate polypropylene tubes, the remaining plasma was refrozen at -70° C for subsequent assays. One 50 mg talc tablet (Goldleaf Pharmacal Co.) was crushed and added to each sample tube (1 tablet/5ml plasma). Tubes were mixed by rotation then agitated end-to-end for 30 minutes. After centrifugation in the Sorvall Centrifuge at 10,000 rpm, the supernatant was discarded and the pellet washed with water and then eluted with 2 ml of Acetone-1MHCl (1:1 v). The Acetone-HCl extracts were evaporated under Nitrogen gas to dryness. Samples were reconstituted in 0.5ml, 0.05 M acetic acid. After centrifugation (5 minutes in Sorvall) 100 μl of the supernatant were pipetted into tubes prepared for radioimmunoassay.

Appendix D

SILICIC ACID TREATMENT

All procedures were carried out at 4° C to minimize possible enzymatic conversions. 300 mg silicic acid were added to 5 ml plasma and mixed by end to end rotation for 30 minutes in a cold room. After centrifugation, the extract was washed 2 times with ice cold deionized water and saved for estimating recovery. The 2 water washes and the adsorbed peptides were desorbed from silicic acid by a mixture containing 2 ml MeOH:1M HCl (8:2). The preparation was mixed followed by centrifugation at 10,000 rpm at 4° C. The supernatants were recovered and dried under a gentle stream of Nitrogen and reconstituted with 0.5 ml of .05 M acetic acid. After 5 min centrifugation, the supernatants were prepared for RIA.

Appendix E

MATERIALS USED IN RADIOIMMUNOASSAY

ANTIBODY

β-endorphin serum labeled "Christine, #2, Bleed #3," is stored at -70° C. It is contained in 1 ml aliquots in 15 ml polypropylene centrifuge tubes. 9 ml of β-endorphin assay buffer was pipetted into the tube to make a total volume of 10 ml of a 1:10 solution of β-endorphin antiserum. From this 1:10 solution, 10 μl were carefully pipetted (with a P-20 Eppendorf pipette) into another 15 ml polypropylene centrifuge tube. To this second tube, 4.0 ml of β-endorphin assay buffer was added to make a final concentration of 1:4000. This was sufficient for 75-80 tubes.

LABEL

Radioactive ^{125}I- β-h-endorphin was purchased from Peninsula Laboratories (Belmont, Ca) in the amount of 10 μ Curies. This was subsequently divided into 10, 1 μ Ci aliquots. Upon dilution to 25 ml, 100 μl contained 5000 - 6000 cpm.

STANDARD

Standards are already prepared (Tejwani et al., 1983) and kept (stored) in 10 μl = 10 ng or 1 aliquots in -70^{0} C freezer. To this amount 990 μl buffer was added to bring volume to 1000 ul. The polypropylene tube became the 1000 pg tube used for the standard curve serial dilution.

BUFFER

To make 200 ml β-endorphin assay buffer add:

1. 20 ml of stock 1.0 M PO4 buffer at pH 6.0
2. 200 mg of gelatin (0.1 %)
3. 585 mg NaCl (0.05 M)
4. 50 mg of Thimersol (0.025 %)
5. 700 mg of EDTA (0.350 %)
6. 200 µl of Triton X-100 90.1 % v/v)

Use 170 ml deionized water to which is added all ingredients except Triton and mix gently to avoid foaming. (Heating the stoppered bottle with hot tap water helps to dissolve the materials quickly). Finally add 200 µl Triton X-100 to the warmed buffer and mix gently. When stored at 2-8^{0} C the buffer is stable for six weeks.

CHARCOAL

To 100 ml of β-endorphin assay buffer the following was added

1. 1.6 g Norit A (charcoal)
2. 160 mg (0.16 gm) Dextran T-70

Appendix F

STANDARD CURVE PREPARATION

Table 21

Preparation of Tubes for Standard Curve

	Buffer *	Ab *	Label *	Charcoal *
Specific Binding	100	100	100	500
Blank (NSB)	200	----	100	500
Total	200	----	100	Buffer
pg/ml (*b*-endorphin)				
1.9	----	100	100	500
3.9	----	100	100	500
7.8	----	100	100	500
15.6	----	100	100	500
31.3	----	100	100	500
62.5	----	100	100	500
125	----	100	100	500
250	----	100	100	500
500	----	100	100	500
1000	----	100	100	500

Note: * = μl volumes

Appendix G

PILOT STUDY DATA

Table 22

Means and SD of Pre and Post-MCAT of TAI Scores (Pilot Study)

	TAI						
		Worry		Emotionality		Total	
	N	M	SD	M	SD	M	SD
Pre-MCAT	5	12.20	1.92	14.80	5.21	6.60	1.34
Post-MCAT	5	13.40	3.20	14.20	4.60	6.60	1.67

Table 23

Mean and SD of Pre and Post-MCAT of b-e (Pilot Study)

		b-endorphin	
	N	M	SD
Pre-MCAT	5	80.12	9.10
Post-MCAT	5	57.76	22.37

Table 24

Subject Profile of Pilot Study

ID	S	R	K	PreW	PreE	PreT	PostW	PostE	PostT	b-e Pre	b-e Post
K	0	0	1	12.00	12.00	6.00	11.00	12.00	5.00	80	96
D	0	1	0	10.00	14.00	6.00	12.00	10.00	5.00	81	59
T	0	1	0	13.00	24.00	9.00	19.00	22.00	9.00	85	44
S	1	1	0	15.00	12.00	6.00	13.00	14.00	7.00	64	40
KE	0	0	1	11.00	12.00	6.00	12.00	13.00	7.00	84	49

Table 25

GPA's and MCAT Scores of Pilot Study

		MCAT Scores					
ID	GPA	BIO	CHEM	PHYS	SCIPROB	READ	QUAN
K	2.54	2	4	7	4	2	6
D	3.07	10	8	10	9	7	8
T	2.96	5	8	8	7	5	8
S	3.33	7	10	7	6	8	9
KE	3.32	9	9	10	8	5	5

Note: S=Sex, 0=Male, 1=Female, R=Repeating MCAT, K=Attended Kaplan preparation program.

Appendix H

MEANS, SD, AND DIFFERENCES BETWEEN MEANS FOR ALL SUBJECTS

Table 26

Mean and SD of Pre and Post-MCAT of B-e (All Subjects)

		b-endorphin	
	N	M	SD
Pre-MCAT	17	86.41	65.32
Post-MCAT	17	64.94	12.50

Note: These data were not used in any of the analyses where serum β-endorphin was considered as a correlate.

Appendix 1

STANDARD CURVE RESULTS

Table 27

Experimental Data

	Assay 1		Assay 2		Assay 3	
	M CPM	%Bind	M CPM	% Bind	M CPM	% Bind
spb	1367.50	100	2358.50	100	1716.75	100
nsb	219.50	0	900.75	0	559.75	0
total	2822.75	245.88	3807.25	261.18	3443.50	297.62
1.9	1077.50	93.85	1428.25	98.01	1042.50	90.10
3.9	1040.00	90.59	1385.25	95.03	1022.50	88.37
7.8	1025.50	89.32	1386.25	95.09	954.00	82.45
15.6	957.00	83.36	1288.50	88.39	933.00	80.68
31.3	794.00	68.16	1164.25	79.87	733.00	66.87
62.5	657.00	57.27	871.25	59.56	628.25	54.29
125	479.25	41.74	601.25	41.24	404.25	34.93
250	305.00	26.56	490.25	33.63	324.25	28.02
500	221.75	19.31	348.00	23.87	206.25	17.82
1000	121.75	10.60	148.00	10.18	172.50	14.90

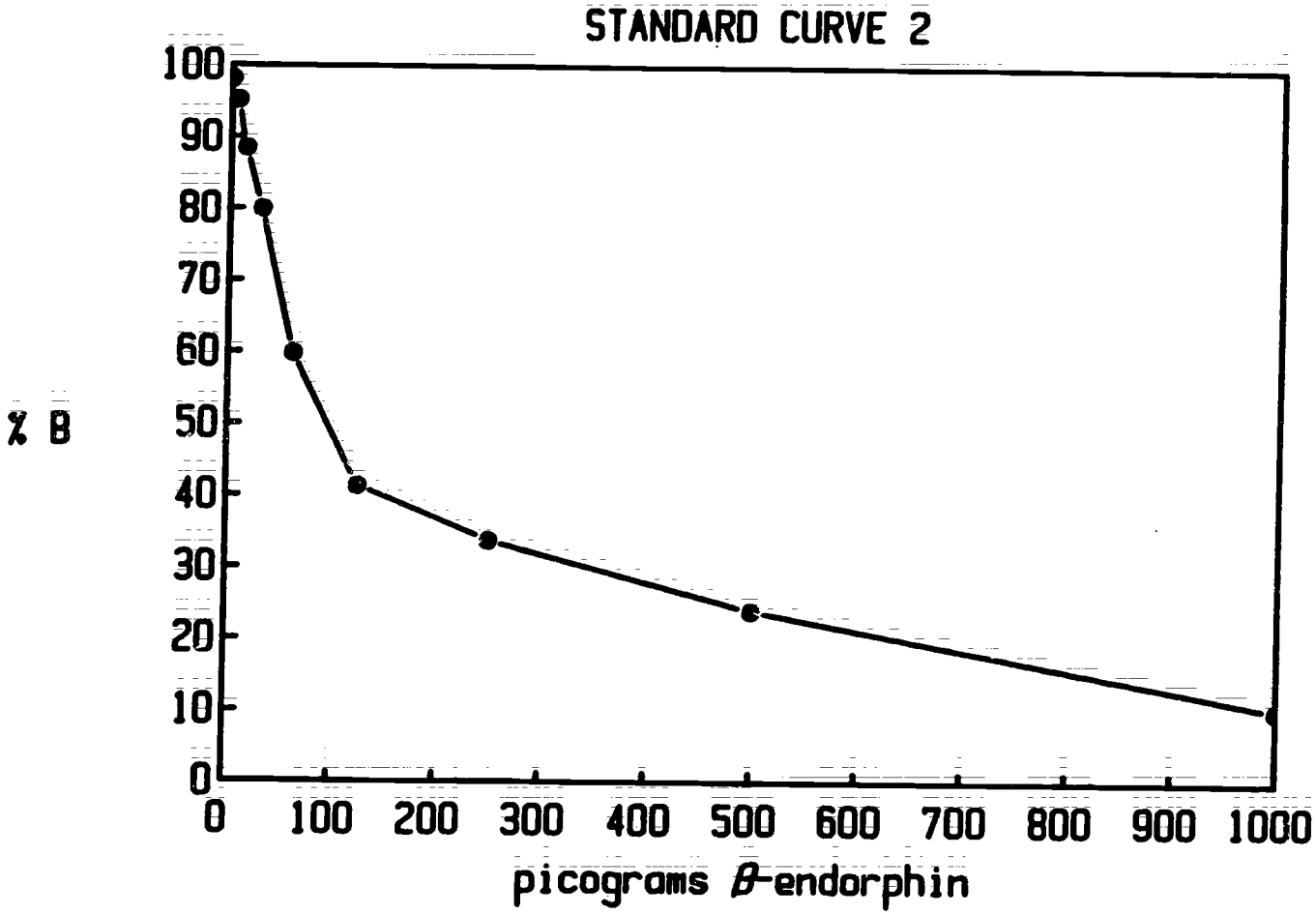


Figure 7: Standard Curve- RIA 2

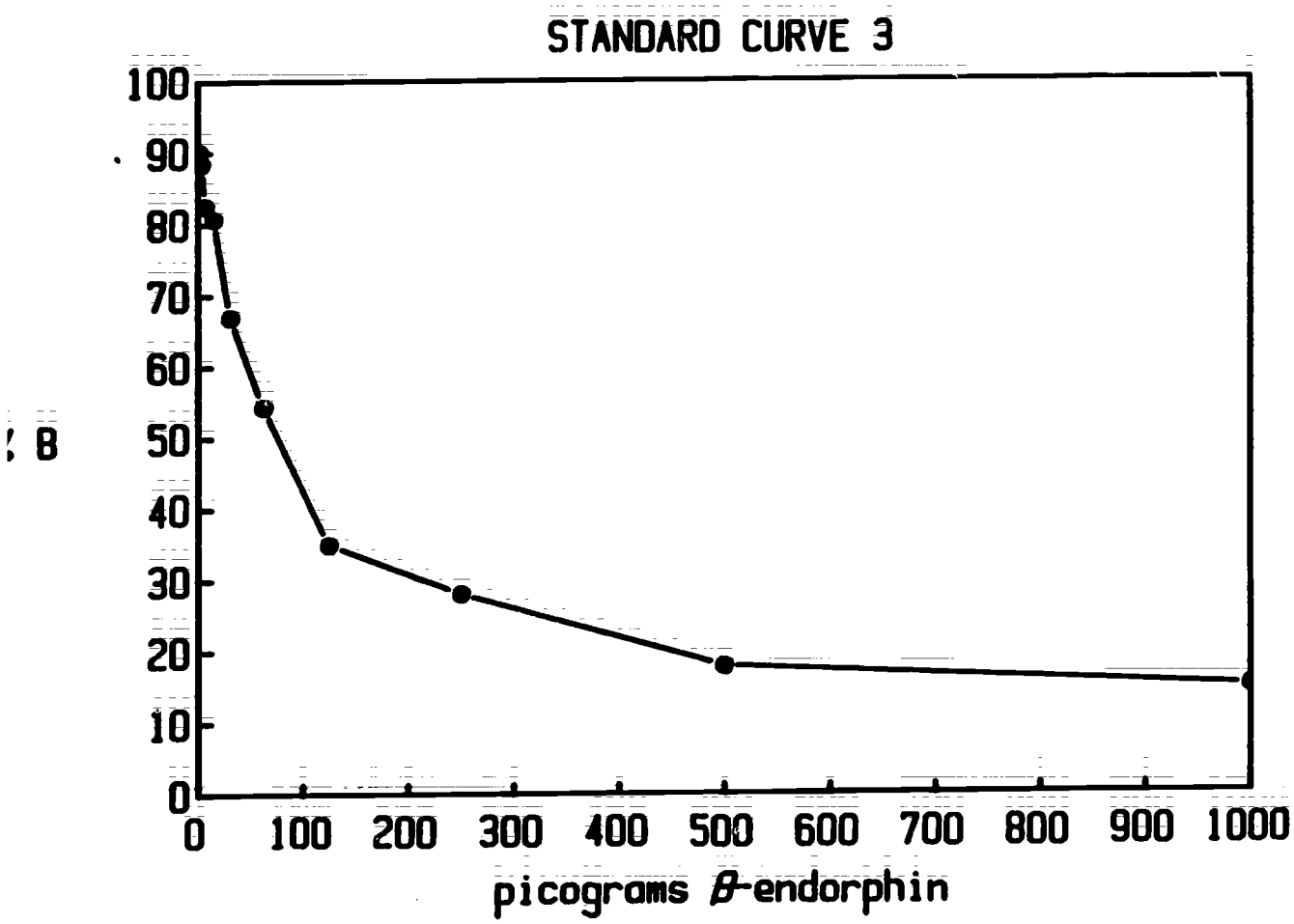


Figure 8: Standard Curve- RIA 3

Appendix J

MEANS OF THREE ASSAYS

Table 28

Beta-endorphin Results- All Subjects from Main Study

	M PRE-MCAT			T	M POST-MCAT			T
A	69.61	48.50	54.16	58	91.20	41.65	15.27	49
B	58.16	46.44	59.95	55	94.82	136.72	77.26	103
C	60.95	58.19	41.94	54	53.81	58.59	47.86	54
D	47.66	46.49	39.27	44	68.65	56.70	37.76	55
E	64.97	58.15	53.07	59	68.41	74.10	82.00	75
F	49.63	50.66	61.68	54	53.72	60.78	61.97	59
G	77.56	469.40	147.57	232	59.99	62.47	73.94	65
H	48.56	51.40	36.30	45	71.72	71.54	63.68	69
I	233.71	290.50	118.52	215	66.86	77.65	55.20	67
J	59.93	367.70	87.08	172	70.06	77.78	82.84	77
K	54.24	48.32	24.32	42	79.97	66.76	65.94	72
L	45.71	46.73	16.61	36	59.29	72.49	54.00	62
M	58.42	78.33	34.74	57	55.06	65.40	53.12	58
N	74.70	62.44	25.86	54	66.48	59.92	56.82	61
O	70.82	362.68	74.64	170	63.94	67.26	55.99	62
P	57.90	58.30	27.98	48	61.01	56.26	41.94	53
Q	70.75	78.82	76.90	74	76.22	67.26	47.20	63
			Mean Total - 86				Mean Total - 65	

Table 29

Mean of Three Assays with Outliers Removed

	M PRE-MCAT				M POST-MCAT			
				T				T
A	69.61	48.50	54.16	58	91.20	41.65	15.27	49
C	60.95	58.19	41.94	54	53.81	58.59	47.86	54
D	47.66	46.49	39.27	44	68.65	56.70	37.76	55
E	64.97	58.15	53.07	59	68.41	74.10	82.00	75
F	49.63	50.66	61.68	54	53.72	60.78	61.97	59
H	48.56	51.40	36.30	45	71.72	71.54	63.68	69
K	54.24	48.32	24.32	42	79.97	68.76	65.94	72
L	45.71	46.73	16.61	36	59.29	72.49	54.00	62
M	58.42	78.33	34.74	57	55.06	65.40	53.12	58
N	74.70	62.44	25.86	54	66.48	59.92	56.82	61
P	57.90	58.30	27.98	48	61.01	56.26	41.94	53
Q	70.75	78.82	76.90	74	76.22	67.26	47.20	63
		Mean Total = 52				Mean Total = 61		

Appendix K

SUBJECT PROFILES

Table 30

Main Study Subject Profiles

ID	S	R	K	PreW	PreE	PreT	PostW	PostE	PostT	b-e Pre	b-e Post
A	0	0	0	9.00	11.00	8.00	8.00	11.00	8.00	58	49
B*	0	0	1	16.00	15.00	7.00	12.00	13.00	9.00	55	103
C	1	0	0	11.00	14.00	6.00	11.00	10.00	6.00	54	54
D	1	0	1	8.00	14.00	9.00	8.00	15.00	7.00	44	55
E	1	0	0	10.00	16.00	9.00	12.00	16.00	9.00	59	75
F	1	0	0	13.00	13.00	7.00	10.00	15.00	6.00	54	59
G*	0	0	0	8.00	17.00	5.00	8.00	14.00	5.00	232	65
H	0	0	0	9.00	23.00	12.00	8.00	20.00	10.00	45	69
I*	0	0	1	10.00	16.00	8.00	10.00	14.00	7.00	215	67
J*	1	0	0	12.00	11.00	4.00	11.00	10.00	4.00	172	77
K	0	0	0	10.00	8.00	5.00	8.00	10.00	4.00	42	72
L	0	0	0	14.00	15.00	5.00	13.00	13.00	6.00	36	62
M	0	0	0	12.00	17.00	10.00	12.00	15.00	7.00	57	58
N	0	0	0	11.00	11.00	7.00	10.00	10.00	7.00	54	61
O*	0	0	1	12.00	19.00	8.00	13.00	18.00	7.00	170	62
P	0	0	1	11.00	17.00	10.00	11.00	16.00	9.00	48	53
Q	1	0	1	16.00	14.00	7.00	16.00	15.00	8.00	74	63

* = Outliers

Note: S=Sex, 0=Male, 1=Female, R=Repeating MCAT, K=Attended Kaplan preparation program.

Table 31

GPA's and MCAT Scores-All Subjects

		MCAT Scores					
ID	GPA	BIO	CHEM	PHYS	SCIPROB	READ	QUAN
A	3.43	11	9	11	9	9	11
B	3.35	7	4	5	4	4	6
C	3.54	9	8	9	8	9	5
D	3.62	11	10	7	11	10	7
E	3.57	8	10	9	8	9	7
F	3.03	10	7	9	7	10	9
G	3.83	12	12	11	13	12	14
H	3.69	7	8	8	9	6	8
I	3.41	7	9	9	8	10	11
J	3.84	11	12	10	10	11	7
K	3.89	11	12	11	11	9	10
L	3.29	10	11	9	10	9	9
M	3.46	8	7	10	9	11	7
N	3.84	11	9	9	10	11	8
O	2.76	4	5	8	5	4	6
P	3.19	5	8	8	7	7	4
Q	3.70	6	8	9	8	7	8

Table 32

Number of Science Courses - All Subjects from Main Study

	Biology				Chemistry		Physics
	Bio	Zoo	Gen	Micro	Biochem	Chem	Physics
A	20	42	01	00	01	70	30
B	10	10	10	00	00	70	30
C	20	10	00	02	01	70	30
D	10	00	00	00	01	60	30
E	20	00	00	05	00	70	30
F	20	61	00	00	00	40	20
G	20	10	00	00	00	70	30
H	20	00	01	01	00	70	30
I	20	30	00	00	00	50	30
J	20	33	00	01	00	60	30
K	20	00	00	00	00	70	30
L	20	00	00	00	00	70	30
M	20	10	01	00	01	70	30
N	20	21	02	01	01	60	30
O	10	10	00	01	00	90	40
P	20	10	00	00	00	70	30
Q	70	00	00	00	00	40	30

Note: The first digit in the double set indicates the number of undergraduate courses and the second number depicts the graduate courses in the subject area.

Appendix L

CORRELATION MATRICES

SRI

AUSTIN

.0246

SCI

1951

SXI

POSTU

.2309

PREV9

SEX

-.0943

PUB79

COST11

.5108

PREV

POSTN

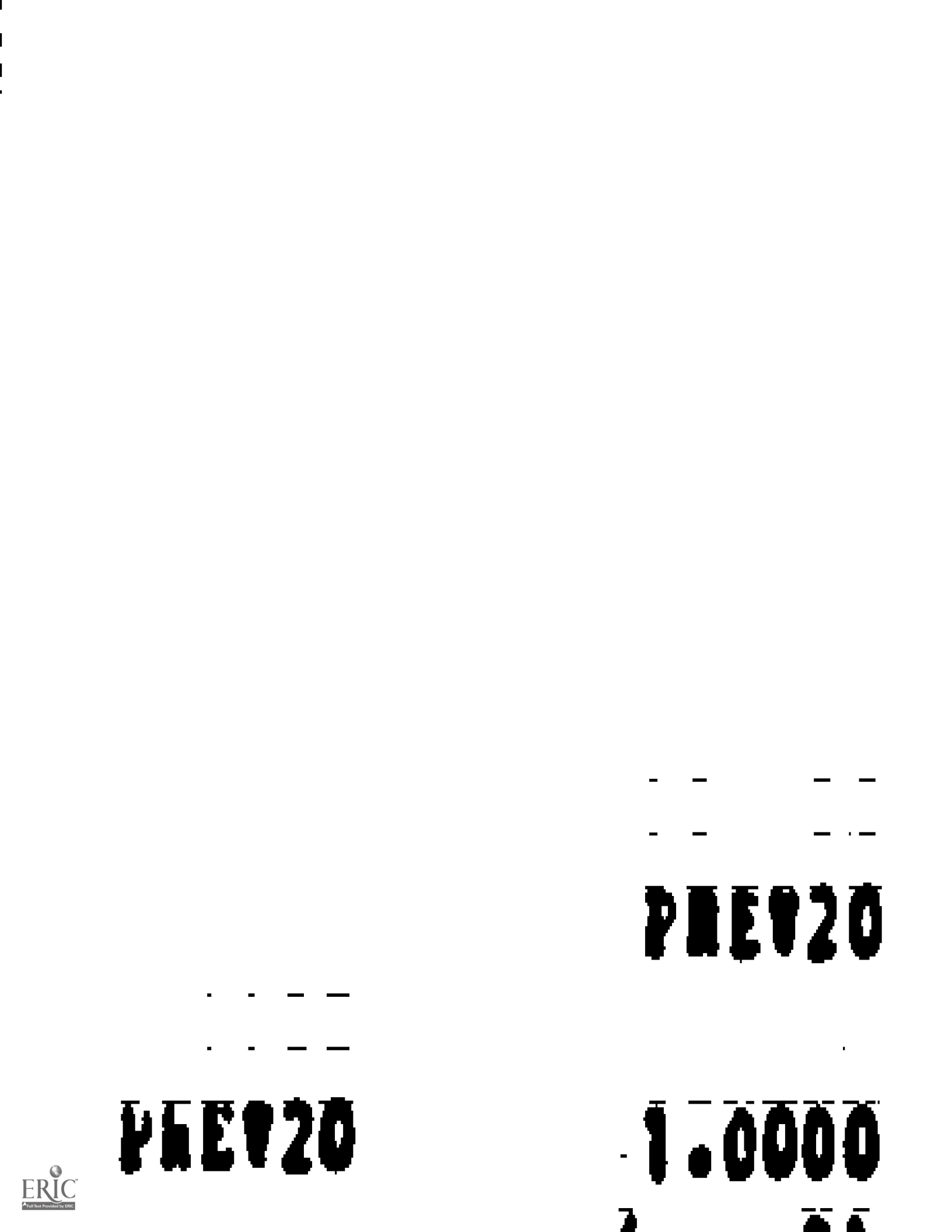
.0593

(17)

P= .021

Correlation Matrix continued

	PREV20	POSTV1	POSTV2	POSTV3	POSTV4	POSTV5	POSTV6	POSTV7	POSTV8	POSTV9	POSTV10
REV9	-.1819	.1498	-.1204	.0502	-.0708	.4818	-.0943	-.3913	.1708	.7536	.1726
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .485	P= .566	P= .645	P= .848	P= .787	P= .050	P= .719	P= .120	P= .512	P= .000	P= .508
REV10	-.2884	.2887	.0913	-.0380	-.0985	-.1650	.0715	.1674	.0398	.2692	.8862
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .262	P= .261	P= .728	P= .885	P= .707	P= .527	P= .785	P= .521	P= .879	P= .296	P= .000
REV11	.2398	.3620	.2207	.3913	-.2715	-.0466	.0838	.1204	.3941	.3841	.3403
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .354	P= .153	P= .395	P= .120	P= .292	P= .859	P= .749	P= .645	P= .118	P= .128	P= .181
REV12	-.1672	.1449	.2029	.2029	-.1061	.1280	-.1413	.0526	.2558	.2985	.2585
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .521	P= .579	P= .435	P= .435	P= .685	P= .624	P= .589	P= .841	P= .322	P= .245	P= .316
REV13	-.2805	.2193	.5470	-.0729	-.2861	-.1883	-.1650	-.1337	.2387	.3073	.3585
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .275	P= .398	P= .023	P= .781	P= .266	P= .469	P= .527	P= .609	P= .356	P= .230	P= .158
REV14	-.1499	-.2263	-.4364	-.1273	.4708	-.1690	.3039	.1273	-.1905	-.3941	-.1341
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .566	P= .382	P= .080	P= .626	P= .056	P= .517	P= .236	P= .626	P= .464	P= .118	P= .609
REV15	.0377	.4202	-.0458	.1488	-.0269	-.0177	-.4424	.0458	.1499	.5209	.3207
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .886	P= .093	P= .861	P= .569	P= .918	P= .946	P= .075	P= .861	P= .566	P= .032	P= .209
REV16	-.0177	.2008	-.4662	.0215	.0848	.2448	.2397	-.0215	-.3099	.6579	.3737
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .946	P= .440	P= .059	P= .935	P= .747	P= .344	P= .354	P= .935	P= .226	P= .004	P= .140
REV17	.6600	.3915	-.1062	.3105	.5096	.0127	-.1195	.2451	.0789	-.0708	.0442
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .004	P= .120	P= .685	P= .225	P= .037	P= .962	P= .648	P= .343	P= .775	P= .787	P= .866
REV18	.0291	.0440	-.2357	.1650	-.1387	-.0913	.2339	.4360	-.0154	-.0681	-.1216
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .912	P= .867	P= .362	P= .527	P= .596	P= .728	P= .366	P= .080	P= .953	P= .795	P= .642
REV19	-.1174	.5259	-.0190	-.1804	.1788	.1177	.0694	.1804	.0622	.3910	.4901
	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)	(17)
	P= .654	P= .030	P= .942	P= .488	P= .492	P= .653	P= .791	P= .488	P= .813	P= .121	P= .046



PREV20

POSTV11 .3594

(17)

PE .157

PREV20

ACATDIO

-.2631

(17)

P= .308

PUB DATE 20

.503

POSTN

17

POSTV11

PREV9

.5108

(17)

P= .036

POSTV11

PREV20

.3594

(17)

P= .157

POSITIVE

POSITIVE

1.0000

(0)

POSTV11

R.CATDIO -.6721

(17)

POSTVII

.4959

POSTV

ACADIO

SEX

.1051

17)

p= .570

	ACATBIO
VALV9	-.5102
	(17)
	P= .036

[redacted]

	NCATBIO
POSTV11	-.6721
	(17)
	p= .003

NCATBIO

NCATBIO

1.0000

(0)

Pr .

ACATDIO

POSTW -.5801

(17)

P= .015

POSTU

SLX

.2309

(17)

Px .372

POSTW

.0593

(17)

P= .021

POST0

PHEV20

.5031

(17)

P= .016

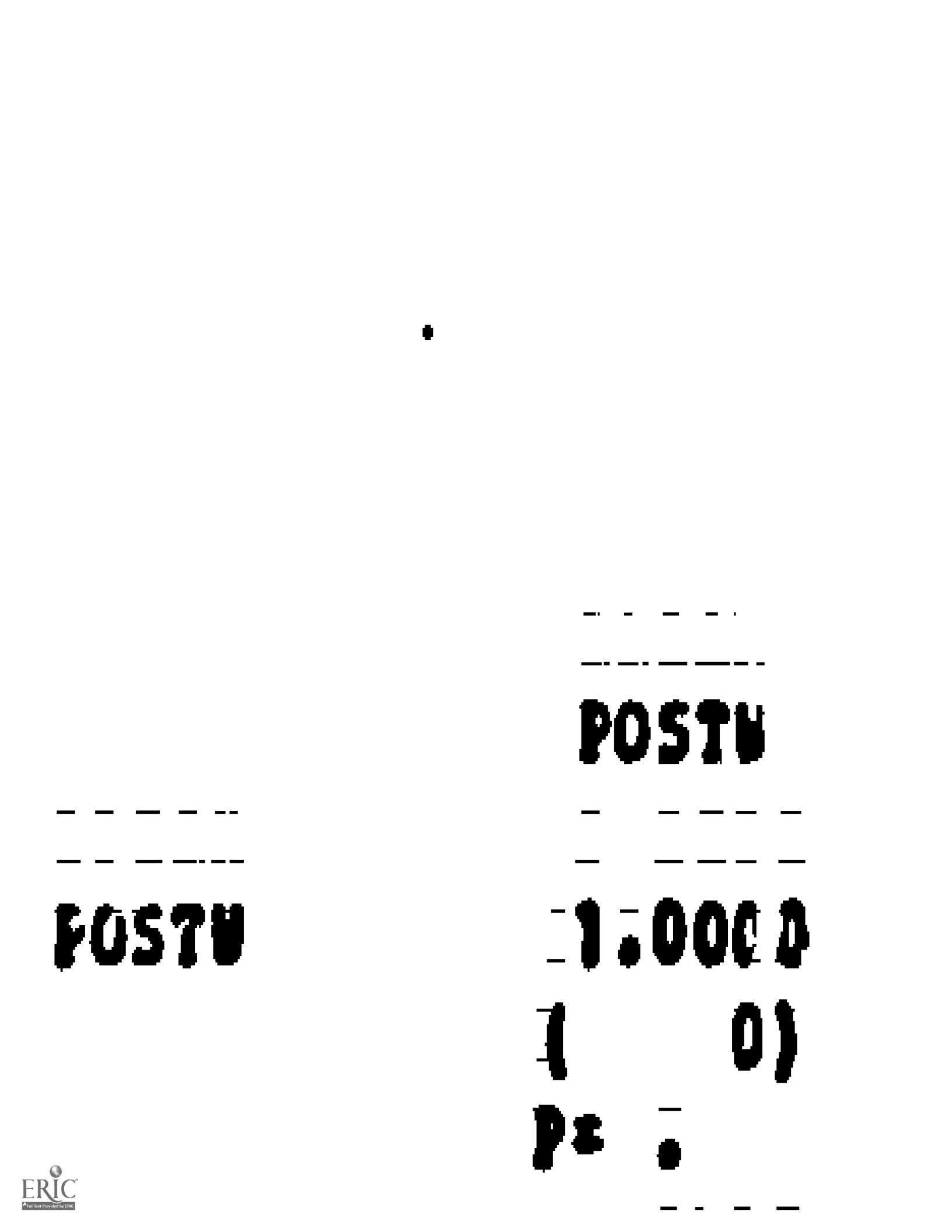
POST01

.1700

POSTV

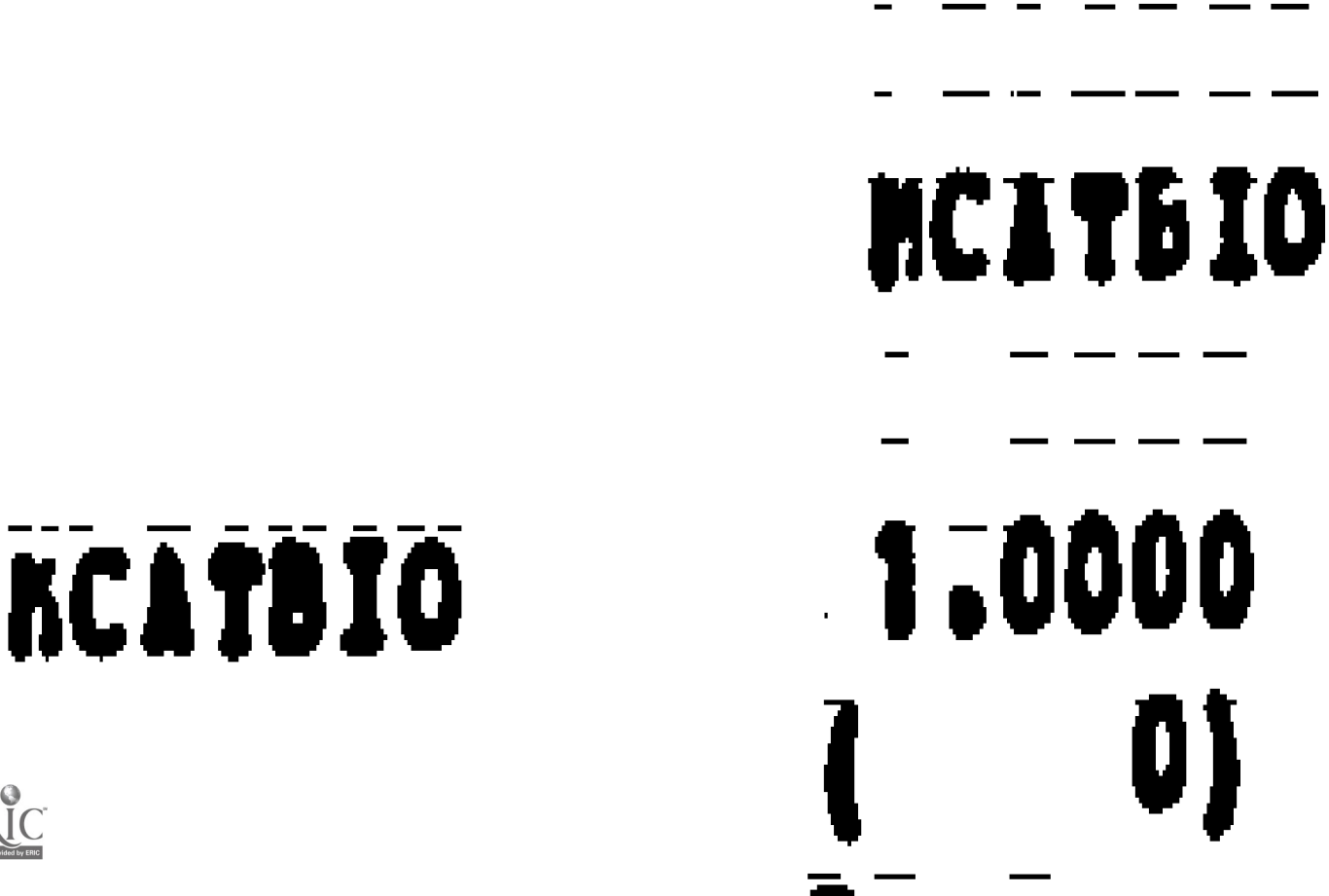
POSTV11 .0959

	POSTV
MCATBIO	-.5801
	(17)
	P= .015




NCATBIO

	MCAT610
PKEV9	-.6072
	(12)
	P= .036



POSTV

ACATBIO

-.5316