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BETA ENDORPHIN LEVELS IN BURNED PATIENTS

by

Geraldine May Goosen

A Dissertation Submitted to the Faculty of the

COLLEGE OF NURSING

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1985
THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read
the dissertation prepared by Geraldine M. Goosen
entitled Beta Endorphin Levels in Burned Patients

and recommend that it be accepted as fulfilling the dissertation requirement
for the Degree of Doctor of Philosophy.

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DEDICATION

This research is dedicated to Mr. David C. Hale for his constant understanding, confidence, availability, and assistance, especially during times of discouragement.
ACKNOWLEDGMENTS

As this goal nears completion, I would like to acknowledge those individuals who served as stimuli, assistants, resource people, and encouragement. From the outset, committee members in both major and minor disciplines, namely, Drs. Beverly McCord, Arlene Putt, Suzanne VanOrt, George Hohmann, Mary Wetzel, and William Ittleson, served as mentors and available resources for course work, research and continued professional growth. A very special credit is extended to Dr. Ada Sue Hinshaw, Major Advisor, for her untiring and extremely capable assistance during completion of the course work and especially during research activities. Her guidance and availability while conducting this dissertation research facilitated completion in a very positive manner. Requisite activities for completion of the doctoral degree were changed from stressful to meaningful and educational via the qualities of the above individuals as well as other faculty at the University of Arizona.

Laboratory research knowledge, skill, and equipment required to complete the biochemical assay utilized in this beginning research effort would have been impossible without the support and guidance from Drs. Milos Chvapil and Clemond Eskelson.

A very special "thank you" to the staff at the George David Peak Burn Unit and the Barnes General Hospital Burn Unit. Drs. Boyd Terry and William Monafo, Directors of the Units, were extremely
helpful in accessing the facilities. This clinical research would have been impossible without the assistance and enthusiasm of Mrs. Cindy Boyd, Patient Care Manager, and Mrs. Beverly Weber, Head Nurse, and their respective staff.

The confidence and assistance provided by Dr. Phyllis Drennan, Dean of the School of Nursing, University of Missouri, Columbia, Missouri, during data collection activities served as continuous stimuli to complete clinical research. She opened doors to clinical agencies, resources, and staff that facilitated completion of the project. Mrs. Juanita Black's patience and perseverance during the writing and completion of this document will always be appreciated. Last, but certainly not least, I would like to acknowledge the confidence and encouragement provided by my son, Michael, and my mother, Clara.

This project was funded in part by a Biomedical Research Development Grant, University of Missouri, and Predoctoral Funding from the Division of Nursing.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>x</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xi</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Statement of the Problem</td>
<td>7</td>
</tr>
<tr>
<td>Statement of Purpose</td>
<td>8</td>
</tr>
<tr>
<td>Significance of the Problem</td>
<td>8</td>
</tr>
<tr>
<td>2. CONCEPTUAL MODEL AND REVIEW OF LITERATURE</td>
<td>12</td>
</tr>
<tr>
<td>Construct Level</td>
<td>14</td>
</tr>
<tr>
<td>Stimulus</td>
<td>14</td>
</tr>
<tr>
<td>Biopsychosociocultural Events</td>
<td>16</td>
</tr>
<tr>
<td>Concept Level</td>
<td>19</td>
</tr>
<tr>
<td>Burn Injury</td>
<td>19</td>
</tr>
<tr>
<td>Pain</td>
<td>26</td>
</tr>
<tr>
<td>Referential Level</td>
<td>44</td>
</tr>
<tr>
<td>Index of Injury Severity</td>
<td>44</td>
</tr>
<tr>
<td>β-Endorphins</td>
<td>47</td>
</tr>
<tr>
<td>Analgesia</td>
<td>52</td>
</tr>
<tr>
<td>Summary</td>
<td>55</td>
</tr>
<tr>
<td>3. DESIGN AND METHODOLOGY</td>
<td>57</td>
</tr>
<tr>
<td>Design</td>
<td>57</td>
</tr>
<tr>
<td>Settings and Sample Selection for Research</td>
<td>57</td>
</tr>
<tr>
<td>Operationalization of Variables</td>
<td>60</td>
</tr>
<tr>
<td>Burn Severity Index</td>
<td>62</td>
</tr>
<tr>
<td>β-Endorphins</td>
<td>69</td>
</tr>
<tr>
<td>Analgesia Equivalency Score</td>
<td>79</td>
</tr>
<tr>
<td>Data Collection Protocol</td>
<td>84</td>
</tr>
<tr>
<td>Protection of Human Subjects</td>
<td>87</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>87</td>
</tr>
<tr>
<td>Summary</td>
<td>88</td>
</tr>
<tr>
<td>4. DATA ANALYSIS</td>
<td>90</td>
</tr>
<tr>
<td>Burn Severity Index</td>
<td>90</td>
</tr>
<tr>
<td>β-Endorphins</td>
<td>93</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS--continued

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesia Equivalency Scores</td>
<td>96</td>
</tr>
<tr>
<td>Pattern of β-Endorphin Levels</td>
<td>102</td>
</tr>
<tr>
<td>Relationship Between β-Endorphins and BSI</td>
<td>105</td>
</tr>
<tr>
<td>Relationship Between AES and BSI</td>
<td>108</td>
</tr>
<tr>
<td>Relationship Between β-Endorphins and AES</td>
<td>108</td>
</tr>
<tr>
<td>Summary</td>
<td>111</td>
</tr>
</tbody>
</table>

5. DISCUSSION, CONCLUSIONS, IMPLICATIONS, AND RECOMMENDATIONS. 112

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Findings, Discussion, and Conclusions</td>
<td>112</td>
</tr>
<tr>
<td>Burn Severity Index</td>
<td>112</td>
</tr>
<tr>
<td>β-Endorphin Levels</td>
<td>113</td>
</tr>
<tr>
<td>Analgesia Equivalency Score</td>
<td>117</td>
</tr>
<tr>
<td>Conclusions</td>
<td>118</td>
</tr>
<tr>
<td>Design Issues</td>
<td>118</td>
</tr>
<tr>
<td>Conceptual Model Issues</td>
<td>119</td>
</tr>
<tr>
<td>Implications for Nursing Practice</td>
<td>120</td>
</tr>
<tr>
<td>Recommendations for Further Study</td>
<td>123</td>
</tr>
</tbody>
</table>

APPENDIX A: THEORETICAL FRAMEWORK. 127

APPENDIX B: REPORT OF PILOT STUDY. 139

APPENDIX C: HUMAN SUBJECTS FORMS. 146

APPENDIX D: BURN CHARTS. 151

APPENDIX E: β-Endorphin (125I) RIA KIT. 153

APPENDIX F: LINEAR-LOGARITHMIC REGRESSION AND STANDARD CURVES. 179

APPENDIX G: TABLES FOR VALIDITY ISSUES--EQUIANALGESIA. 184

APPENDIX H: INDIVIDUAL PATIENT PLOTS. 187

REFERENCES. 209
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Distribution of Patients by Sample Size, Age, Sex, and Agency.</td>
<td>61</td>
</tr>
<tr>
<td>2 Burn Severity Index</td>
<td>63</td>
</tr>
<tr>
<td>3 Interrater Agreement Between the Researcher and Observer from Each Clinical Agency for the Burn Severity Index</td>
<td>70</td>
</tr>
<tr>
<td>4 Distribution of β-Endorphin Levels from One Non-Burned Subject: Mean, Standard Deviation, and Standard Error with all Samples and with Removal of Outlyers</td>
<td>77</td>
</tr>
<tr>
<td>5 Equianalgesia Table</td>
<td>80</td>
</tr>
<tr>
<td>6 Calculation Sheet for Analgesia Equivalency Score</td>
<td>81</td>
</tr>
<tr>
<td>7 Data Collection Sheet</td>
<td>85</td>
</tr>
<tr>
<td>8 Distribution of Total Body Surface Area Burned by Sex and Agency</td>
<td>92</td>
</tr>
<tr>
<td>9 Distribution of Burn Severity Index Scores by Sex and Agency</td>
<td>94</td>
</tr>
<tr>
<td>10 Distribution of the Number of Samples Obtained for β-Endorphin Levels From Each of the 28 Patients by Number of Days Samples Were Collected for Each Agency</td>
<td>95</td>
</tr>
<tr>
<td>11 Central Tendency and Dispersion for β-Endorphin Levels Grouped Together by Days Post Burn</td>
<td>97</td>
</tr>
<tr>
<td>12 Central Tendency and Dispersion for Analgesia Equivalency Scores Grouped Together by Days Post Burn</td>
<td>99</td>
</tr>
<tr>
<td>13 Distribution of Mean Analgesia Equivalency Scores for 28 Patients by Days Post Burn</td>
<td>100</td>
</tr>
<tr>
<td>14 Spearman Correlation Coefficients for Analgesia Equivalency Scores Grouped Together by Days Post Burn</td>
<td>101</td>
</tr>
</tbody>
</table>
**LIST OF TABLES--continued**

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Distribution of β-Endorphin Levels Over Days Post Burn: Mean and Median</td>
<td>103</td>
</tr>
<tr>
<td>16</td>
<td>Spearman Correlation Coefficients for β-Endorphin Levels Grouped Together by Days Post Burn</td>
<td>104</td>
</tr>
<tr>
<td>17</td>
<td>Spearman Correlation Coefficients Between β-Endorphin Levels and the Burn Severity Index Grouped Together by Days Post Burn</td>
<td>107</td>
</tr>
<tr>
<td>18</td>
<td>Spearman Correlation Coefficients Between Burn Severity Index and Analgesia Equivalency Scores Grouped Together by Days Post Burn</td>
<td>109</td>
</tr>
<tr>
<td>19</td>
<td>Spearman Correlation Coefficients Between β-Endorphin Levels and the Analgesia Equivalency Scores Grouped Together by Days Post Burn</td>
<td>110</td>
</tr>
</tbody>
</table>
# LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Conceptual Model</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>Chemical Structure of Morphine</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>Subset of Individual Patient Plots</td>
<td>106</td>
</tr>
</tbody>
</table>
ABSTRACT

Nursing activities directed at maintaining patient comfort incorporates time and energy. Nurses and researchers continue to search for adequate methods and information to quantify pain. The common mode of therapy is the administration of narcotics, which do not consistently relieve the pain described by traumatically injured patients.

Discovery of endogenous opiates, such as $\beta$-endorphins, provided the potential for acquiring additional physiologic information regarding neuro-endocrine activities associated with pain. Consistent findings of concentrated $\beta$-endorphins in areas of the central nervous system previously identified as pain pathways prompted clinical researchers to determine $\beta$-endorphin levels in patients experiencing pain.

The purposes of this investigation were to study $\beta$-endorphin levels in burn injured patients by describing: (1) the pattern of $\beta$-endorphin levels in burn injured patients during the first two weeks following injury, (2) the relationship between $\beta$-endorphin levels and the severity of the burn injury, (3) the relationship between analgesia taken by patients and the severity of the burn, and (4) the relationship between $\beta$-endorphin levels and the amount of analgesia given to the burn patient.
Plasma samples for \( \beta \)-endorphin levels were obtained from 28 burned patients over a two-week interval. New England Nuclear \( ^{125} \text{I} \) \( \beta \)-Endorphin Kits were used to assay the plasma samples. In addition, information was tabulated from the patient's chart to complete the Burn Severity Index. Narcotic analgesia taken 24 hours before obtaining the blood sample were summarized and categorized according to the Equianalgesia Table.

Descriptive and correlational statistics showed no significant relationships between \( \beta \)-endorphins over time, \( \beta \)-endorphins with burn severity, \( \beta \)-endorphins with the analgesia equivalency score, or burn severity with the analgesia equivalency scores. \( \beta \)-endorphin levels were elevated above normal in all 28 patients. Five patients displayed the anticipated declining pattern over the two-week interval post burn. Many erratic peaks and troughs in \( \beta \)-endorphin levels were observed with some peaks associated with clinical events. The findings of elevated \( \beta \)-endorphin levels have implications for nursing practice and provide stimulus for continued nursing research.
CHAPTER I

INTRODUCTION

It was a saying of the ancients, "Truth lies in a well," and to carry on this metaphor, we may justly say that logic does supply us with steps, whereby we may go down to reach the water.--Isaac Watts

Pain is an example of a universal condition which has as many different expressions as the cultures and societies which exist in our world. Studies which review pain with childbirth are support for the above statement. The dichotomy for the impact of childbirth extends from women being incapacitated for weeks to women returning immediately after delivery to the labor fields (Sternbach, 1978). This is simple evidence that pain is perceived differently and since the physiologic process of childbirth is similar in female humans, it would appear that cultural influences impact heavily on this process. The family unit which reflects cultural mores may be the most immediate impact on pain behavior.

Sternbach & Tursky, (1965), Sternbach (1968), Fordyce (1973), and Swanson et al. (1976) supported the thesis that pain behaviors may be influenced by environmental contingencies. The process whereby children acquire the attitudes, values, and behavioral skills needed to function as adults in society is largely one of observational learning. Baron and Byrne (1977) stated, "It now appears that children can acquire everything from language to moral
values, and from sexual identity to a knowledge of the social norms of their society through observational learning. . . " (p. 315).

There is reason to believe that the full range of pain and illness behavior is also strongly affected by social modeling.

Evidence of the impact of modeling during the socialization of pain behavior comes from contrasts of a number of naturally occurring groups. Consistencies within groups and differences between groups indicate that parents and significant others serve as models and exert sanctions for what would be recognized as either adaptive cognitive, emotional or social coping strategies in response to actual or anticipated noxious stimulation, or for maladaptive behavior inconsistent with organic pathology (Sternbach, 1978).

Displays of pain and suffering and other persons' reactions to these displays provide powerful examples of interpersonal behavior with immediate and long-term effects.

Culture is viewed as the whole fabric of ways for living which distinguishes a human society. This includes modes of communication, the pattern of organization or structures, the complexities of role and status and the technologies which seem to manipulate the physical world. Man's physiological orientation is largely a cultural phenomenon. The understanding of the significance and role of social and cultural patterns in human physiology is necessary to clarify those aspects of human experience which remain puzzling if studied only within the physiological frame of reference.
Pain is basically a physiological phenomenon with many aspects of perception and reaction. The biological function of pain is to provoke special patterns directed toward avoidance of the noxious stimulus which presents a threat to the individual (Sherrington, 1900). Reference is frequently made to the pain experience which includes pain sensation and feeling states. In human society, pain, like so many other physiological phenomena, acquires specific social and cultural significance and certain reactions to pain can be understood in the light of this significance. The process of investigating cultural attitudes toward pain includes differentiation between apprehension and anxiety. Pain apprehension reflects the tendency to avoid the pain sensation as such. Pain anxiety is a state of anxiety provoked by the pain experience (Wolff, Langley & Langley, 1968).

Cultural traditions dictate to members of a given society not only whether they should expect and tolerate pain but also the correct response to the pain experience. The rules may vary with sex, age, and social status. Since people in a society usually comply to the rules or mores it is important to have a working knowledge of these rules when caring for the patient. The best sources from which to elicit the kind of information that will supply the needed data to treat the symptomatology are the patient and the family (Zborowski, 1969).

Zborowski (1952) drew the conclusions that similar reactions to pain demonstrated by members of different ethnocultural groups do not necessarily reflect similar attitudes of pain. He further
concluded that reactive patterns similar in terms of their manifestations may have different functions in various cultures. Varying attitudes of pain have prompted studies which resulted in some controversy regarding variables such as age, sex, and culture per se. In general, the results showed that pain tolerance decreases with age; men tolerate more pain than women; whites tolerate more pain than orientals, while blacks occupy an intermediate position (Woodrow et al., 1972; Weisenberg et al., 1975).

Cognitive activities such as cultural values, anxiety, attention, and suggestion all have an effect on pain experience. There is evidence that the sensory input is localized, identified in terms of its physical properties, and evaluated in terms of past experience (Sternbach & Tursky, 1965).

Livingston (1943) and Melzack (1973) suggested that prolonged intense pain and low level input may produce neural activity that subserves memory-like processes related to pain. This speculation is important and may serve to explain the presence of pain in the absence of a detectable lesion. The concept of memory-like mechanisms in pain is supported by convincing experimental evidence in men and animals, but the neural mechanisms associated with long-term memory of a sensory experience are not clearly understood (Melzack, Konrad, & Dubrowsky, 1969).

Frequently, cognitive processes critically determine the nature of the pain experience. The individual in pain tends to interpret its source and personal meaning in terms of the immediate
environment, his or her past history, and the future implications of any injury or disease (Sternbach, 1978).

Pain management has evolved into a multi-million dollar business. In addition, the cost factor related to energy expenditure, human suffering and inability to meet role expectations is overwhelming. Numerous research studies are directed toward some component of pain. Clinicians continue to search for a method to quantify pain, subsequently, providing a more objective basis for planning and intervening with the body's response to painful stimuli. Even though there is a wealth of existing literature which relates to some aspect of the concept of pain, researchers continue to seek additional insight and clearer understanding of individual response to painful stimuli.

Concern is especially great with patients that experience tremendous levels of pain and discomfort during conditions such as burn injury. The number of burn injuries requiring hospitalization continues to approach 100,000 annually in this country (Artz, Moncrief, & Pruitt, 1979). Thus, the need for concern is justified for persons admitted to an emergency room blackened, painful, and shocked. Only minutes before the burn injury, this same person was a normal, healthy, functioning member of society with role responsibilities. Lying before this individual is a pathway of strange environment, strange persons, loss of autonomy, separation from family, loss of employment, removal of skin, painful procedures, hospital expenses, and a myriad of other phenomena which may end in permanent body changes, disability, and/or death. The total recovery
period may take weeks or even years. Throughout the entire treatment and rehabilitation course, degrees of pain and discomfort are present. The person entered this state with a unique personality, individual characteristics, individual lifetime goals, a personal level of stress tolerance, and a repertoire of learned behaviors. The role of nursing includes collaboration with the patient, burn team members, and the family to return this individual to society in the best possible condition. More specifically, the nurse is responsible for planning and delivering care which will contribute to comfort, recovery, and prevent complications (Fagerhaugh, 1974; West & Shuck, 1978). Clinicians continue to share concern regarding the management of pain from the burn injury per se, as well as pain from manipulation and treatment of the wounds (Perry, Heidrick, & Ramos, 1981; Walkenstein, 1982).

The discovery and research of morphine-like properties in neuropeptides have stimulated new thoughts regarding pain perception and management (Goldstein, 1976; Almay et al., 1978). These endogenous opioids have now been found in the brain, pituitary gland, adrenal glands, blood, urine, and milk (Goldstein, 1976; Leong Way, 1980). One opioid identified, β-endorphin, is a large peptide containing 31 amino acids and is believed to be one of the neuromodulatory substances involved in the control of pain perception. There are well-described neuronal pathways in which β-endorphin has been identified (Bloom et al., 1978). This opioid has produced analgesia to experimental noxious stimuli when injected into the ventricle of rats (Bradbury et al., 1976) and recently has
been injected intrathecally to produce analgesia in intractable pain syndromes (Oyama et al., 1980).

β-endorphin is present within the pituitary (Li & Chung, 1976) and is thought to be released into the circulation during periods of stress (Fraioli et al., 1980). Pain and stress are congruent factors in the burn injured patients; therefore, the use of these individuals as research subjects appears to be a logical choice. To date, empirical data regarding β-endorphin levels in trauma and/or burn patients have not appeared in the literature. This research then is the beginning of many projects that will contribute to knowledge regarding β-endorphin levels following burn injury.

Statement of the Problem

Pain management of burn patients remains one of the greatest challenges to clinicians, primarily nurses. Chemical agents such as parenteral analgesics and anesthesia inhalants have been utilized for years with relative success (Sternbach, 1978). Use of relaxation techniques has also resulted in varying success (Mersky, 1976; Wickramasekera, 1979; Wernick, Jaremko, & Taylor, 1981).

To achieve maximum benefits in pain management, all parameters of pain, physiological, psychological, and sociocultural, must be assessed and quantified. Qualitative data obtained through verbal query and written questionnaires have been helpful, but this information especially assists in evaluating the psychological and sociocultural components. To date, physiological evaluation has been
made from individual patient response, pain scales, and an estimation of discomfort from the depth and extent of injury. The nurse is faced with taking in available information related to pain, analyzing and synthesizing the data and providing treatment based on an individual or group consensus (West & Schuck, 1978).

β-endorphins as endogenous opiates may be the key to increased understanding of pain perception from a physiological viewpoint. Quantitative information regarding the level of endogenous opiates in burned victims may be potential data for nurses and other clinicians to utilize in formulating clinical judgements for pain management. The problem of this study was to describe β-endorphin levels following burn injury.

Statement of Purpose

The purposes of this study were to: (1) describe the pattern of β-endorphin levels in burn injured patients during the first two weeks following injury, (2) describe the relationship between β-endorphin levels and the severity of the burn injury, (3) describe the relationship between analgesia taken by patients and the severity of the burn, and (4) describe the relationship between β-endorphin levels and the amount of analgesia given to the burn patient.

Significance of the Problem

A major role of nurses is to maintain comfort for assigned patients. Comfort is frequently achieved through diminution of painful stimuli perception. Even though chemical agents (drugs) are
ordered by the physician, choices regarding the frequency and quantity of dosage administered rest with the nurse. In addition, the use of other comfort modalities such as repositioning, relaxation techniques, and educating the patient are activities usually carried out by the nurse. Clinical judgments made by the nurse are precluded by knowledge regarding theories of pain, physiological, sociocultural, and psychological parameters of pain and individual characteristics related to pain. Understanding the potential effectiveness of chosen methods for pain management is paramount.

Recent studies concerning endogenous opiates have stimulated new thoughts and interest for nurses. Since the concept of endogenous opiates is still in infancy, the relationship of endorphins to clinical practice is speculative. Endogenous opiates are believed to mediate pain relief; furthermore, they can be measured. The goal of each clinician and researcher associated with and interested in pain is to find and utilize some objective method to determine the amount of pain and to evaluate when clinical methods are effective in decreasing pain. Therefore, the concept of endorphins holds much promise regarding clinical management of pain response.

Research efforts to look at pain tolerance, pain threshold, and the declared source of pain (organic or psychogenic) began with chronic pain patients. β-endorphin levels were found to have a much broader range with chronic pain patients than those of normal patients. Patients classified as having mainly organic pain syndromes were found to have significantly lower endorphin levels
than patients with predominantly psychogenic pain syndromes. A treatment variable of relaxation (biofeedback) was believed to be instrumental in reducing β-endorphin levels in chronic low back pain patients. Simultaneously, clinical follow-up provided information for increase in ability and scope of physical activities in those patients with decreased β-endorphin levels (Van Knorring et al., 1978; Hossobuchi et al., 1979; Putt et al., 1981).

Elevated β-endorphin levels were demonstrated in patients during labor and abdominal surgery. These studies were especially meaningful for indexing pain with altered β-endorphin levels since it was possible to compare pre-pain, intra-pain, and post-pain in the same individual (Csontos et al., 1979; Dubois et al., 1981; Cahil & Akil, 1982).

To date, a study has not been reported that reflects the relationship between β-endorphin levels and verbal or written reports of pain. A correlation between reports of pain and physiologic variables associated with pain would be an asset in the clinical management of pain.

To effectively manage pain, nurses as clinicians must incorporate all available information. Thus, the nurse must be knowledgeable regarding the value of endogenous opiates in pain management. If endogenous opiates such as endorphins and enkephalins are available in the body to decrease perception of painful stimuli, then what is the value of chemical agents commonly given to patients? Clear understanding of the relationship between endogenous and exogenous opiates will hopefully be gained from
continued clinical research whereby information can be obtained regarding endogenous opiate levels following adverse stimuli. This research effort will be followed by studies to determine the relationship of β-endorphin levels to perceived pain and experimental studies to determine methods for enhancing the body’s capabilities.

In summary, Chapter I, an introduction to the study, includes the biopsychosociocultural parameters of pain, the problem statement, statement of purpose, and significance of the study. Each topic relates information of β-endorphin levels in burned patients to the role and function of nursing and implies the importance of knowledge regarding β-endorphin levels in burn patients to clinical nursing judgments. The following chapter includes a conceptual model and background information for implementing the study.
Chapter II contains a description of the conceptual model with supportive data from literature to clarify definitions and relationships specified. A design depicting selected variables and their relationship is presented in Figure 1. The conceptual model is derived from a broader theoretical framework related to the stress response and management of trauma victims (Appendix A). The conceptual model is related to pain reactions experienced by burn victims. Pain is viewed as one important stressor which impacts heavily on burned patients and requires careful nursing management. Intrinsic and extrinsic components of the model are consistent with the Gibb's theoretical framework (Gibbs, 1972). The extrinsic terms, construct, concept, and referential, will be defined according to Gibbs (1972). These components will provide the foundation for statements which comprise the intrinsic components of the model, appearing as axioms, postulates, propositions, transformational statements, and theorems. A slight modification from the temporal sequencing proposed by Gibbs (1972) is evident in the conceptual model. Two stages are identified with Stage 2 preceded by Stage 1. The time interval has not been specified. The unit term for this theory is adults. Selected statements from review of the literature provide support for definitions and relational statements.
Figure 1. Conceptual Model
Construct Level

The two substantive terms, stimulus, and biopsychosocio-cultural reaction are defined and comprise the construct level for the conceptual model. Construct is the term applied when a theorist regards the definition of a term as neither complete nor empirically applicable (Gibbs, 1972, p. 125).

Stimulus

In 1970, Toffler predicted future events and factors which could influence the life of everyone in the existent environment. Toffler stated:

We have in our time released a totally new social force—a stream of change so accelerated that it influences our sense of time, revolutionizes the tempo of daily life, and affects the very way we "feel" about the world around us (Toffler, 1970, p. 17).

Murphy (1971, p. 46) further explained that "change is constant and universal, and man is continuously faced with the unchanging law that change will occur within man's environment, between man and his environment, among man's relationships with other men, and with man himself."

As society marched forward into this era of change it became clear that some understanding regarding the impact of change on the individual was necessary. Vast amounts of change soon became identified as the source of stimulus, stimuli, and/or stressors to which the individual must respond or react. The response has been described since the early 1900's by Selye (1973, p. 692) as the
"nonspecific response of the body to any demand made upon it."

The individual, faced with societal as well as personal everyday stimuli, must adapt. Mechanic (1976) referred to the importance of viewing adaptation as a transactive process between people and their life situations. The process of adaptation depends on the degree of fitness between the skills and capacities of individuals and the types of challenges with which they are confronted. To the extent that capacities are fitted well to the challenges, the flow of events is routine and ordinary.

Lazarus (1977) stated that every instance of adaptive activity between an individual and his environment is appraised cognitively as to its significance for the person's well-being. Appraisals underlie the quality and intensity of the emotional stress state. Coping methods, or self-regulatory processes, as well as cognitive appraisals, are key mediators of the individual's stress reaction. Psychological and concomitant physiological response to threat by humans is neither uniform nor simple. The stimulus must first be perceived, then interpreted in the context of prior experience, and finally, if read as a threat, the stimulus will be confronted by the psychological barriers of coping methods. Lazarus (1977) suggested that the key mediator of Selye's General Adaptation Syndrome may be psychological. He further implied that the individual first recognizes the threat and cognitively appraises the potential impact.

Stimulus arising from the internal and external environment is perceived by the body through the central nervous system.
Chemoreceptors, neurotransmitters, and efferent nerve routes provide the pathway for stimuli to the cerebral cortex or spinal cord (Cotman & McGaugh, 1980). The elements of a behavioral interaction between organism and environment are conventionally represented in terms of stimulus and response events. The environment can be divided into several stimulus classes based upon the functional role of such events in behavioral interactions. One class, eliciting stimuli, regularly precedes and elicits reflexive or relatively fixed and stereotypic responses. A second class of reinforcing stimuli consists of environmental events that follow as the consequence of responses and influence the frequency with which those responses will recur in future behavioral interactions. The third major class of behaviorally relevant environmental events, discriminative stimuli, function as antecedent or concurrent occasioning events or circumstances. In contrast to eliciting stimuli, discriminative stimuli do not elicit responses in the reflexive sense, but rather they influence the frequency of those responses which have previously been followed by reinforcers in their presence (Skinner, 1965). Stimuli precipitate body reactions or responses which can be observed behaviorally and/or physiologically.

Biopsychosociocultural Events

The term, biopsychosociocultural, reflects recent acknowledgment in literature that the body functions as a total entity. Physical, psychological, social, and cultural influences are concomitantly active and together create the behavior, reactions, or
responses of the body (Pelletier, 1978).

Within the last decade, professional nurses have become cognizant of the need to understand and manage body responses as a unified concept, holistic. The holistic approach replaces the belief that a body reaction or response is initiated from essentially one parameter and that the behavior observed is only reflective of that parameter. Certainly there are concepts with a heavier connotation to one parameter. An example is pain. Even though health professionals now are aware of psychosociocultural influences, pain is essentially and frequently viewed as a physiological entity.

To assess and manage concepts with attention to biological, psychological, social, and cultural parameters requires a broad and integrative knowledge base. Management outcomes which incorporate aspects of the total body, reflecting uniqueness, will ultimately result in greater satisfaction for the individual and subsequently for the total society (Chaska, 1978).

Biopsychosociocultural responses are influenced by age, sex, religion, ethnic or cultural group, education occupation, relational patterns, and health level. These variables are further influenced by heredity, environment, general health habits, behavioral varieties, and past illnesses (Goosen & Bush, 1979).

Definitions for the constructs, stimulus, and biopsychosociocultural which comprise the construct level of the Conceptual Model include:
Stimulus. The definition for a stimulus can include a factor, event, or variable which constitutes a change from the usual (Toffler, 1970). An external stimulus may be physical, environmental, social, or cultural in nature. An internal stimulus may arise from personal structural characteristics, psychological processes, physical growth and development, body repair development, body repair mechanisms, behavioral characteristics, and physiological alterations which occur with a disease process or when usual health patterns are not followed (Dubos, 1965; Goosen & Bush, 1979).

Biopsychosociocultural reaction. The definition for a biopsychosociocultural reaction is immediate activity of the body to internal and/or external stimuli (Selye, 1978). Even though the body is viewed as a total unit and interrelatedness is acknowledged, ongoing clinical observations reflect activities which can be categorized in the biological, physiological, psychological, and/or sociocultural realm.

The first set of relational statements for the Conceptual Model are Axioms (A) which are derived from the previously defined substantive terms and provide support for the following statement (Gibbs, 1972, p. 167):

A1: Among adults, the greater the stimulus at Stage 1, the greater the biopsychosociocultural reaction at Stage 2.
Concept Level

The terms burn injury and pain comprise the concept level of the model. Concepts are substantive terms defined in such a way that the definition is regarded as complete but not empirically applicable (Gibbs, 1972, p. 128).

Burn Injury

To comprehend the impact of burn injury, it is requisite to review the anatomy and physiology of the skin, physical agents which impose thermal damage to the body, burn wound classification and relevant systemic response to thermal injury.

Anatomy and Physiology of the Skin. The skin is composed of two layers: the outer layer, called the epidermis, and the inner layer, known as the dermis, or corium. The epidermis consists of stratified squamous epithelial tissue; the dermis is composed of fibrous connective tissue. Underlying the dermis is the loose subcutaneous tissue, which is comprised of areolar, and in some cases, adipose tissue which overlays the subcutaneous fat pad.

The terms "thick skin" and "thin skin" are frequently used. In the areas of thick skin, the palms of the hands and the soles of the feet, the epidermis has five layers. In the areas of thin skin, all other body surfaces, the epidermis has only four layers. The five epidermal layers, from the surface inward, include the stratum corneum, stratum lucidum (missing in thin skin), stratum granulosum, stratum spinosum, and stratum germinativum (abuts on the dermis), (Jacoby, 1979).
The stratum corneum is composed of keratin fibers surrounded by a lipid monolayer. Since lipids are water repellant, the stratum corneum layer acts as the vapor barrier for the body. When large areas of the stratum corneum are damaged, as in a burn, (even one of a very superficial type), extensive fluid loss occurs, adding greatly to the fluids that must be replaced (Sauer, 1980).

The innermost layer, the stratum germinativum, is also of great importance since it constantly produces new cells that move toward the surface and so renew the other epidermal layers. It is the presence of the stratum germinativum layer that determines whether or not a burned area will require grafting. If all vestiges of it are destroyed, regeneration cannot take place (Sauer, 1980).

The dermis, or corium, serves as a supporting and nutritional bed for the epidermis. The dermis is composed of two layers. The outermost layer (that next to the epidermis) is called the papillary layer; the innermost layer is called the reticular layer. The predominant fiber in the dermis is collagen. Scattered throughout the collagen are connective tissue cells (the mast cells) performing the functions of secretion, phagocytoses (the histiocytes), and repair (the fibroblasts), (Peacock, 1984).

The epidermal appendages found in the dermis include the hair follicles, the sebaceous glands, and the sweat glands. When a burn destroys the epidermal layer, the epithelial cells of the external sheaths of the hair follicles, sebaceous glands, and sweat glands can grow to form new epithelium.
The nerve fibers in the skin come from a nerve plexus deep in the dermis. Naked nerve endings between the layers of the stratum germinativum receive pain sensation. The pin prick test, often used to ascertain the depth of burn injury, is not a particularly reliable test for initial evaluation (Langley, Telford, & Christensen, 1980).

The sensation of touch is provided by Meissner's corpuscles, which are located just below the epidermis. The corpuscles of Vater Pacini (for pressure), of Ruffini (for heat), and Krause's end bulbs (for cold) are all located in the dermis and subcutaneous fascia. A deeply burned patient, therefore, complains of less pain, whereas a patient with partial-thickness burn has much pain. Sensory function returns to the skin about two months after grafting, but the nerve regeneration process may continue for many years (Langley et al., 1980).

Burn Wound Classification. The trend today is toward the use of the terms "partial thickness", "deep dermal", and full thickness" in describing burn wounds. The term partial thickness is used to describe a wound that has the ability to heal without grafting. This wound can resurface itself from the stratum germinativum of the epidermis. The deepest epithelial cells capable of regeneration are present in the sheaths of the epidermal appendages, deep in the dermis and subcutaneous tissue. The epithelium also regenerates from residual basilar cells in the rete pegs (Artz et al., 1979).

The superficial partial thickness wound characteristically takes two forms. The surface may be covered with blisters or bullae of varying sizes and when these are removed the epidermis beneath it
is weeping, glistening, bright pink or bright red, and is exquisitely sensitive to temperature changes, exposure to air, and light touch. Deep partial thickness wounds are waxy white but still soft and elastic, and, although they are sensitive to pressure, they are insensitive to light touch or soft pin prick. These wounds were previously considered to be full thickness wounds but with the preservation of the remaining epithelial elements with effective topical therapy these wounds heal spontaneously, albeit with extensive hypertrophic scarring.

A deep dermal burn is a partial-thickness wound that frequently has the gross appearance of a third-degree, or full thickness, burn. This type of injury has the potential to heal without grafting. However, infection, mechanical trauma, or obliteration of the blood supply to the affected part can destroy the deep epithelial cells and convert a partial-thickness deep dermal burn wound to a full-thickness wound.

In a full-thickness burn wound, all the epithelializing elements are destroyed, both in the epidermis and in the epidermal appendages necessitating autografting in order to be closed properly (Jacoby, 1979). The full thickness thermal injury is hard and dry, tan or fawn colored, and with exposure to air in a matter of a few hours becomes desiccated, parchment-like and translucent. The thrombosed dermal vessels beneath the surface can readily be discerned. Full-thickness injury results in a very inelastic eschar which leads to compression of deeper tissues when edema forms.

Full-thickness wounds are the result of exposure to flame for
more than very brief periods of time, immersion scalds, or intense radiant energy. They can be caused by less significant thermal transfers in the very elderly or the very young in whom thickness of tissue is less than that of persons in the prime of life.

Because of the significant variations in skin thickness throughout the body, exposure to the same temperature for the same duration in different parts of the body will result in burns of different depths. For example, to have a full-thickness burn of the upper portion of the back without prolonged exposure to intense heat would be unusual, whereas a relatively short duration of exposure is required for full-thickness injury of such areas as the abdomen, the anterior neck, or the medial portion of the upper extremities.

Another type of burn which is clinically confusing is that seen characteristically in spill scalds or immersion scalds, particularly in infants, young children, and the very elderly. The tissue is red but not wet and has a dry appearance. The red discoloration under these circumstances is due to hemoglobin fixed in the tissues which gives it the red appearance. This is a deep wound, not a partial thickness wound (Artz et al., 1979).

The extent and depth of the burn injury is utilized as a guide to the treatment methodology. The Rule of Nines (division of the body surface in multiples of nine) is an accepted guide for determining the extent of total body surface area (TBSA) involved. Several modifications have been made with the original Rule of Nines to accommodate the difference in body proportions during developmental years (Artz et al., 1979). In recent years computer
programs have been developed to assist in estimating the extent and depth of injury.

Systemic Response to Burn Injury. Continued research with burn patients reveals new knowledge regarding the body's general response to the impact of heat. The overwhelming influence which the injury has is reflected in burn mortality. A statement by Moncrief summarized the effects: "Systemic changes occurring subsequent to thermal injury are rapid in onset, prolonged in duration, dramatic in intensity, and fatal in outcome if not treated or corrected" (Artz et al., 1979, p. 28).

A major systemic change addressed in the independent variable relates to the respiratory system; however, changes in the cardiovascular, renal, and gastrointestinal systems are prevalent and contribute to the total understanding of burn injury. Damage to the skin influences the extent of systemic change and currently are viewed together by burn clinicians as they consider the impact of calories/time relationship to the body. Changes in the cardiovascular system resulting in poor tissue perfusion may directly alter individual pain perception. The concept of hypermetabolism is important to incorporate with the issue of pain. Increase in body metabolism results from increases in epinephrine and norepinephrine following burn injury (Wilmore, 1974). Since adrenocorticotropin hormone (ACTH) and β-endorphins result from the same precursor molecule, β-lipotropic hormone (BLPH), some thought must be given to total systemic reactions as well as hypermetabolism for comprehensive understanding of the total effects from burn injury.
The term, pulmonary burn, is not a true description of the injury sustained by the respiratory tract. Sufficient heat to traumatize the pulmonary parenchyma rarely goes beyond the posterior pharynx (Achaver et al., 1973). Damage to the bronchial tree may occur from inhalation of smoke resulting from incomplete combustion of certain substances. Plastic materials or polymers release a fume and smoke which is extremely irritating, causing an inflammatory reaction, congestion of submucosal veins and bronchial arteries, and marked edema of the mucosa.

Respiratory involvement can occur by upper airway obstruction, lower respiratory tract injury, and restriction of chest excursion by constricting burns of the chest. Upper airway obstruction may occur from edema of the pharynx which results in obstruction at the larynx. As fluid resuscitation progresses, inflamed areas will become edematous, thus contributing to upper airway obstruction. Deep circumferential burns of the neck will also cause obstruction of the airway.

Lower respiratory tract injury is due to smoke inhalation which may cause injury to the central airways and/or peripheral airways. Early assessment of the patient with potential respiratory injury reveals a history of the injury occurring in an enclosed space and/or loss of consciousness. Clinical assessment would reveal burns of face and neck, carbon in the sputum, dyspnea, hoarseness, inflammation of the face and larynx, singed nasal hairs, dry lips, dryness of the nasal openings, and a clear chest x-ray. Laboratory tests reveal a decreased arterial paO₂ and elevated carboxyhemoglobin (Artz et al., 1979).
Respiratory complications have become a leading cause of death since the improvement of fluid and wound management. Therefore, constant monitoring for respiratory changes such as increasing dyspnea, labored breathing, and hypoxemia is vital.

Pain

Perception and Response to Pain. The word "pain" means different things to each individual. Even though anatomical structures are similar, the psychological, cultural and societal variables that are unique to each person have much influence on the perception of pain and the subsequent responses to pain. As mentioned previously, there is a wealth of information available in literature relevant to pain and the response to pain. In an attempt to summarize information related to pain into meaningful groundwork for the specified conceptual model, the following outline will be followed: (1) definitions related to pain, (2) theories of pain, (3) physiological parameters of pain, and (4) burn pain.

Definitions Related to Pain. Since there are numerous pain descriptors and different terms or phrases used to classify pain, definition of these words is requisite for clear understanding between persons communicating about pain. Pain is a highly personal, variable experience which is influenced by cultural learning, the meaning of the situation, attention and other cognitive activities (Sternbach & Tursky, 1965). Melzack (1973) considered pain to be a complex, perceptual and affective experience determined by the unique past history of the individual, by the meaning of the stimulus to
him, by his "state of mind" at the moment, as well as by the sensory
nerve patterns evoked by physical stimulation (p. 134).

Liebeskind (1977), stated that "pain means many different
things; and the variables which correlate with, inhibit, or enhance
one kind of pain, and the neural mechanisms which underlie it, may
not be associated with or influence other kinds" (p. 41). Mersky
(1975) defined pain as "an unpleasant experience which we primarily
associate with tissue damage or describe in terms of tissue damage or
both" (p. 319).

Research studies involving the relationship between pain
perception and culture have prompted the need for differentiating
between sensation threshold, pain perception threshold, and pain
tolerance level. Sternbach and Tursky (1965) defined sensation
threshold as the lowest stimulus value at which sensation is first
reported. They further defined pain tolerance level as the point at
which the subject refuses to tolerate further pain. Hardy, Wolff,
and Goodell (1952) defined pain perception threshold as the lowest
stimulus level at which a person reports feeling pain.

Most clinicians differentiate between acute pain and chronic
pain. Acute pain is related to a known or well defined cause and
follows a predictable course. When healing is completed, the pain
usually disappears. Acute pain has a rapid onset, the phasic
component, followed by a tonic component which varies in length.
Chronic pain may begin as acute pain with the tonic component lasting
far beyond the healing time. Chronic pain may also begin as a low
level input and continue for extended periods of time despite
intervention. Chronic pain may involve neural mechanisms which are more complex than acute pain. Involvement of adjacent body areas may also occur (Melzack & Dennis, 1978). The previous definitions are included to clarify the researcher's conceptualization of pain and differentiate between terms which are commonly used by clinicians when discussing pain.

Lazarus (1977) stated, "Of course, nearly everyone will agree that the only way we can know anything about another person is through his behavior" (p. 553). Through pain behaviors, pain is recognized and interpreted by clinicians. Behaviors may include verbal descriptors, increase in heart rate, limping, rubbing a body part, sweaty palms, grimacing, or other overt expressions. Behaviors have meaning to the person demonstrating the characteristics as well as to the observer.

Theories of Pain. The concept of theory based practice is not foreign to the area of pain management. Even though there fails to be common agreement, theories exist which have meaning and value for clinical practice. Pain theory development has contributed to the current overall understanding of pain. The usual pattern of theory development is evident in that affect, physiology, and psychology were each advocated by different individuals or groups. Currently, there is evidence for each which reflects the holistic construct. Pain theories are grouped into the following major orientations: affect, specificity, pattern and gate control. The first three are traditional and fail to provide a comprehensive account of pain phenomenon. The gate control theory attempts to
integrate understandings derived from the three traditional theories on the basis of new clinical evidence and assumptions.

After reviewing all the pain theories which are briefly summarized, the Gate Control Theory may be perceived as a concerted effort to incorporate and explain neurological systems and activity associated with pain perception and response. Assumptions pertinent to the gate control theory incorporate psychological and cognitive aspects related with pain. Since this researcher views pain as a complex phenomena it is comfortable to view this theory as the most promising. Research to test this theory continues and recent studies suggest the possibility that endogenous opiates, primarily enkephalins, play a role in the gating mechanism (Watkins & Mayer, 1982; Terman et. al., 1984). Thus, the possibility exists that endogenous opiates may further clarify this theory in the future.

The affect theory considers pain to be an emotion rather than a sensation. Dating back to Aristotle, the theory views pain as emotional quality that colors all sensory events. Emotion was viewed as the opposite of pleasure. As early as 1894, Marshall supported this belief when he suggested that pain is an emotional quality, or guide, that colors all sensory events. He used a very broad description of pain. For example, he described listening to badly played music as painful. Bereavement was also viewed as pain. In the early 1900's, Sherrington proposed that pain had a sensory and affective dimension. His view was that affective tone is an attribute of all sensation, and among the attribute tones of skin sensation is skin pain. Titchener (1909) viewed pain and
unpleasantness on a continuum. His assertion was that there is a continuum of feeling in conscious experience which differs from sensation. A description of how affect and unpleasantness is linked with pain was not addressed. Nevertheless, the recognition of affect or a pleasant, unpleasant feeling is considered an important aspect when currently evaluating pain (Melzack, 1973).

Specificity theory is the traditional theory which is still being utilized in medical and graduate schools and many times presented as factual information. In general, the specificity theory proposed that pain is a specific sensation, proportional to the extent of tissue damage. The theory implied a fixed, straight through transmission system from somatic pain receptors to a pain center in the brain (Sternbach, 1978).

Descartes in 1644 (as quoted in Melzack, 1973) provided classical description of the theory. He conceived of the pain as a straight through channel from skin to brain. The person feels and responds to pain. Descartes' view remained until the nineteenth century when physiology developed as a science. Anatomists and physiologists looked more closely at sensory nerves and their ability to link the brain with the outside world.

In 1842, Muller contributed the five classical senses with the sense of touch being the sensation related to pain. Specific energy was believed to relay messages to specific brain areas where the quality of the sensation was interpreted; thus, each sensory nerve had a distinct brain center (Melzack, 1973).
Specificity theory continued to expand as new neurophysiological information was made available. Currently, specificity theory proposes that a mosaic of specific pain receptors in body tissue projects to a pain center in the brain. The theory further maintains that free nerve endings are pain receptors and generate pain impulses that are carried by A delta and C fibers in peripheral nerves and by the lateral spinothalamic tract in the spinal cord to a pain center in the thalamus (Sternbach, 1978).

Von Frey's theory extended the physiological component of specificity theory by looking at skin receptors. He utilized existing information from Muller, Helmholtz, and Volkmann to identify the four types of sensory spots: touch, cold, warmth, and pain. Anatomists were identifying tissue types via the microscope and Von Frey linked these types with the four sensations. Consequently, Meissner corpuscles were identified as the touch receptors, Krause end-bulbs the cold receptors, Ruffini end-organs the warmth receptors, and Pacinian corpuscles detect pressure (Melzack, 1973). The sensorial end-organ is an apparatus by which an afferent nerve fiber is rendered amenable to some particular physical agent and at the same time rendered less amenable to other excitants. The value of one particular kind of stimulus is lowered while the value of the threshold of other kinds of stimuli may be heightened.

As opposition arose to the specificity theory, several new theories presented by Goldscheider, Nafe, Livingston, & Noordenbos were grouped into a pattern theory. Through studies of patients with syphilis, Goldscheider proposed that stimulus intensity and central
summation are the critical determinants of pain. Pain sensation became cumulative and the response increased as time increased. He believed that the large cutaneous fibers comprise a specific touch system, while the smaller fibers converge on dorsal horn cells which summate their input and transmit the pattern to the brain. Pain then results when the total output goes beyond a certain level. Pain can occur from excessive stimulation or pathological conditions. The long delays and persistent pain observed in pathological pain states are due to abnormally long time periods of summation (Melzack, 1973).

The peripheral pattern component of pattern theory simply implied that excessive peripheral stimulation produces nerve impulses which are interpreted centrally as pain. Nafe (1934) suggested that all cutaneous qualities are produced by spatial and temporal patterns of nerve impulses. Since all nerve fiber endings are viewed as alike, the pattern from pain is formed by the intensity of the stimulation.

Livingston (1943) was the first to suggest specific neural mechanisms to account for the remarkable summation phenomena in clinical pain syndromes. He proposed that intense, pathological stimulation of the body sets up reverberating circuits in spinal pools that can be triggered by normally non noxious inputs and generate abnormal volleys that are interpreted centrally as pain. Goldscheider (1964) proposed that the spinal summation path that transmits the pain signals to the brain consist of slowly conducting, multi-synaptic fiber chains. Goldscheider's approach may possibly explain the phantom limb pain problem.
The sensory interaction theory is derived from Goldscheider's original beliefs and further proposes that a rapidly conducting firing system inhibits synaptic transmission in a more slowly conducting system. Noordenbos' theory (1959) was an important contribution to sensory interaction concepts. He viewed the small fibers as carriers of the nerve impulse pattern that produces pain and the large fibers as inhibitors to transmission. A shift in ratio of large to small fibers with an increase in small fibers would result in excessive pain.

The Gate Control Theory was proposed by Melzack and Wall (1965) and reflected an effort to integrate the previous theories as well as to clarify the role of the central nervous system in pain response. The theory was named because of a gate control system which modulates sensory input from the skin before it evokes pain perception and response. Impulses are transmitted to three spinal cord systems: the cells of the substantia gelatinosa in the dorsal horn, the dorsal column fibers that project toward the brain, and the central transmission (T) cells in the dorsal horn.

The Gate Control Theory proposed that neural mechanisms in the dorsal horn of the spinal cord act like a gate which can increase or decrease the flow of nerve impulses from peripheral fibers to the central nervous system. Somatic input is therefore subjected to the modulating influence of the gate before it evokes pain perception and response. The degree to which the gate increases or decreases sensory transmission is determined by the relative activity in large diameter (A beta) and small diameter (A delta and C) fibers and by
descending influences from the brain. When the amount of information that passes through the gate exceeds a critical level, the neural areas responsible for pain experience and response are activated. Like all theories, the gate control theory has two facets: a conceptual model which is the basis of the theory, and explanatory mechanisms which show how the model functions (Melzack & Wall, 1965).

The small (A delta and C) fibers play a highly specialized and important role in pain processes. They activate the T cells directly and contribute to their output. The substantia gelatinosa appears to be the most likely site of the spinal gating mechanism (Wall, 1964; Melzack & Wall, 1965). The substantia gelatinosa receives axon terminals from many of the large and small diameter fibers. The dendrites of cells in deeper laminae project into the gelatinosa. The substantia gelatinosa consists of a highly specialized, closed system of cells throughout the length of the spinal cord on both sides. The area receives afferent input from large and small fibers and is able to influence the activity of cells that project to the brain. Melzack and Wall (1965) proposed that these substantia gelatinosa cells act as a spinal gating mechanism by modulating the conduction of nerve impulses from peripheral fibers to spinal cord transmission cells.

Melzack and Wall (1965 and 1970) proposed that sensory fibers transmit patterned information, depending on the specialized properties of each receptor fiber unit about pressure, temperature, and chemical changes at the skin. These temporal and spatial patterns of nerve impulses have two effects at the dorsal horns: they
excite the spinal cord T cells that project the information to the brain, and they activate the substantia gelatinosa which modulates or gates the amount of information projected to the brain by the T cells.

There are two ways in which the cells of the substantia gelatinosa can act as a gating mechanism that influences the transmission of impulses from afferent fiber terminals to spinal cord cells (Melzack & Wall, 1970; Cotman & McGaugh, 1980; Pinsker & Willis, 1980). They can act directly on the presynaptic axon terminals and thereby block the impulses in the terminals or decrease the amount of transmitter substance which they release. They can also act post synaptically on the spinal transmission cells by increasing or decreasing their level of excitability to arriving nerve impulses. Melzack and Wall (1965) proposed that the effect is primarily presynaptic. Hongo, Jankowska, and Lundberg, 1969, supported that modulating effects are exerted post synaptically on the spinal transmission cells.

The Gate Control Theory also proposed that a mechanism called the central control trigger activates two subsystems in the brain: the brainstem reticular formation and the cortex. The reticular formation exerts a powerful inhibitory control over information projected by the gate control system. The central control system acts very rapidly in identifying, evaluating, and selectively modifying the sensory inputs and also clearly interacts with the action system (Melzack, 1973). The central control system is activated only when the output of the T cells exceed a certain
critical level and is controlled by the central control system. When the output reaches or exceeds a critical level, the T cells are transmitted to the reticular and cortical projection subsystems mentioned above. Activation of reticular structure underlies the motivational drive and unpleasant effect that trigger the organism into action toward escape or attack. The selection and modulation of the sensory input through the cortical projection subsystem provide sensory discriminative information as to the location, magnitude, and spatiotemporal characteristics of the noxious stimulus. The theory assumes that activities in the action system plus cognitive information processed at the central control system interact with one another to influence the motor mechanisms responsible for the complex pattern of overt behavior in response to noxious stimulus (Melzack, 1973).

Physiological Parameters of Pain. During the past decade much has been published regarding the physiological parameters of pain. As neurophysiologists explore deeper into the anatomy and physiology of the central nervous system, knowledge is acquired regarding sensory perception, neuron pathways, and motor response.

The essential function for the neuron system is communication. Communication is important within the body from cell to cell, as well as communication with the external environment. The endocrine system shares the function of communication. Communication messages are converted to electrical impulses which are transmitted from the receptor to a central nuclei where they synapse, evoke the same message in other cells, and eventually result in appropriate
responses (Williams & Warwick, 1975). Responses recognized by clinicians may include perspiration, increased heart rate, pallor, increased blood pressure, increased oxygen consumption, increased rate and depth of respiration, muscle tension, dilated pupils, limping, nausea, and vomiting. Other behavioral responses might include an increase in gross motor activity, verbal expressions, flexion and withdrawal reflexes, spasms of smooth or skeletal muscles, and increased body activity (Bruegel, 1971; Melzack, 1973; Jacox, 1977).

Neurophysiologists classically designate the synapse as the area where control of information occurs. When a message (action potential) arrives at a synapse, several possibilities exist. The impulse may terminate or be inhibited from passing to the next neuron. The impulse may pass on to the next neuron in a facilitated manner. Facilitation may occur by summation or be influenced by convergence or divergence. Lastly, the stimuli may be blocked or facilitated by therapeutic drugs or chemicals. Nevertheless, the sum of all influences impinging upon the synapse finally determines if transmission of the message will occur (Noback & Demarst, 1977).

Synapses are inhibitory Type I or excitatory Type II, depending on the type of neurosecretory substance released from their respective presynaptic membranes. Two major classes of transmitter substance have been identified which include acetylcholine and the catecholamines. Acetylcholine is known to be present in the initial part of the Renshaw Loop and catecholamines are important in the reticular system of the brain and spinal cord, in the hypothalmus,
corpus striatum, and substantia nigra (Williams & Warwick, 1975).

The identified neurotransmitters probably represent only a fraction of the actual existing numbers. Many more may be unknown than are known and many of the suspected transmitters may be precursors of, or breakdown products of, the real transmitters yet to be identified. Early literature on endogenous opiates refer to these neuropeptides as neuroreceptors and neuromodulators (Goldstein, 1978). More recent information reflects reference to the endogenous opiates as neurotransmitters. Enkephalins especially have been linked with neurotransmission in the spinal cord (Watkins & Mayer, 1982; Dupont et al., 1981).

The probability that Substance P, an identified transmitter, is interrelated with endogenous opiates has support from research findings. Substance P distribution has been identified in areas of the central nervous system associated with pain and analgesia such as the substantia gelatinosa of the spinal cord dorsal horn and the spinal trigeminal nucleus (Hokfelt, Ljungdahl, & Terenius, 1977). Since Substance P has been implicated as a sensory transmitter of the afferent small diameter fibers in the dorsal horn of the spinal cord, it is possible that endogenous opiates such as enkephalins and endorphins inhibit the neurotransmission by inhibiting the release of Substance P (Jessell & Iverson, 1977). Like endogenous opiates, the role of Substance P remains controversial since it appears to have a dual nociceptive action. At low doses, Substance P is believed to promote the release of β-endorphins which concomitantly inhibit pain transmission. High doses of Substance P tend to excite
nociceptor neurons (Frederickson, Burgis, & Harrell, 1978).

In the skin there are large numbers of pain receptors; each area receives the branches of several pain neurons and these branches overlap extensively; therefore, a single stimulus affects the receptors of several sensory units. The number of sensory units and their associated pain receptors in a peripheral structure is correlated with the degree of representation of that structure in the central pain pathways of the spinal cord, brainstem, and brain (Stephens, 1980). Cellular or tissue injury, from whatever cause, activates specific pain receptors either by direct damage or by release of chemical mediators of pain.

Transmission of pain information occurs via Class A and Class C fibers. The Class A alpha fibers are thought to be important in modification and inhibition of pain transmission in substantia gelatinosa. Delta fibers of Class A are usually associated with clear localized first pain of the specific pain pathway. Class C fibers are usually associated with vague, dull, generalized pain related to the nonspecific second pain pathway (Bishop, 1959; Burgess, 1974). Many investigators have studied the Class A and Class C fibers in relation to pain from a peripheral as well as from a central consideration (Bishop, 1964; Collins et al., 1960).

All sensory nerve fibers enter the spinal cord via the dorsal roots. The nerve cell bodies of these sensory neurons lie in the dorsal root ganglia. The central processes of the neurons enter the cord and synapse with neurons in the dorsal horn of the cord. The
dorsal roots contain the nerve cell bodies of all afferent neurons and include many different types and sizes of cells, including some autonomic ganglion cells. The impulse is created and communicated to the spinal cord gray laminar cells via specialized systems (Kuffler & Nicholls, 1976).

The first of these specialized systems is the spinothalamic system (Wall, 1964). Touch, pain, and temperature will activate this system. Pain and temperature information become the lateral spinothalamic tract, while touch impulses comprise the anterior spinothalamic tract. Two subpathways (neospinothalamic and paleospinothalamic systems) have been described. The neospinothalamic system terminates in the posterior and ventrobasal nuclei of the thalamus. The paleospinothalamic system extends into the reticular formation of the medulla and midbrain, also to the dorsolateral nuclei of central gray of the midbrain, and terminates in the intralaminar nuclei of the thalamus (Rodman & Smith, 1979). Spinothalamic subpathways terminate in the areas of the brain where large concentrations of β-endorphins have been identified.

The second projection system of importance in pain is the lemniscal system. This system originates from large Class A alpha fibers of the medial root division which enter the dorsal funiculi (dorsal columns) and ascend to the medulla to synapse with the second-order neurons of the nucleus gracilis or cuneatus. After synapsing in these nuclei, the message is carried by these second order neurons to the opposite side to ascend in the medial lemniscus projection system. Axons terminate by synapsing with the third order
neurons of the ventral and medial thalamus, again an area of high concentration for β-endorphins (Rodman & Smith, 1979).

Burn Pain. In partial-thickness injury, a great deal of pain exists due to the injury of epidermal layers which act as a protective covering for the nerve endings. Patients complain of intolerable stinging and burning reminiscent of the pain experienced at the time of the initial injury (Andreason et al., 1972).

Theoretically, full-thickness injuries may be less painful since the depth of injury includes nerve fibers and destruction of nerve endings would not allow for transmission of stimuli. Clinically, patients do complain of discomfort with full-thickness injury. Several factors may contribute to this phenomenon. First, burn injury is usually mixed in depth. The depth of burn is related to the number of heat calories exposed to the skin over a period of time. The calorie/temporal ratio varies, resulting in varied depth of injury. Therefore, on an area of skin which appears to be completely full-thickness injury there may be areas of partial-thickness injury where nerve endings remain functional (Artz et al., 1979).

A second factor to consider in full-thickness injury includes the parameters of pain associated with burn injury. Emotional factors play a tremendous role in pain expression and response. If the individual has been apprised of the extent of injury, the anxiety associated with outcome, surgical procedures, and future implications may offset the physiological response to partial-thickness injury (Fagerhaugh, 1974).
The pain of burn injury is often conceptualized as continuous, with no periods of comfort. Clinically, patients are able to experience periods of rest and sleep. Several patterns of pain have been observed and supported by research. The greatest amount of discomfort is experienced when the wounds are being treated and during active motion such as ambulation and position change. Critical treatments which cause extreme discomfort include dressing changes, hydro-therapy or tanking, debridement, soaking of dressings with 0.5% silver nitrate solution, and replacement of dressings (Wagner, 1977; Perry et al., 1981).

The pain experience varies with the stages of burn management. During the acute phase, active therapy is continuous, but as the wounds begin to heal, pain begins to subside. The acute phase is followed with the grafting phase. Once the wounds are covered with new skin, pain is minimal; however, the burn wound pain is now replaced with pain in the donor site areas. Even after the wounds are covered and healed, patients experience the discomfort of rehabilitation. Pain during rehabilitation is due to contracture formation, stiff joints, and regeneration of nerve tissue in healing wounds (Fagerhaugh, 1974; Wagner, 1977; Perry, et al., 1981).

Refinement in describing the alterations of burn injury to the usual pain pathways remain a requisite. Hopefully, as neurophysiologists become more knowledgeable with the normal pain pathways, the interest will shift to studying patients with altered perception at the nerve ending sites.
The following definitions of the concepts, burn injury and pain, are derived from the previous review of literature:

**Burn injury.** Burn injury is defined as a type of stimulus resulting from energy, usually in the form of heat, imposed upon the body which results in local and systemic damage to the body (Artz et al., 1979).

**Pain.** Pain is defined as a complex, perceptual, and affective experience determined by the unique past history of the individual by meaning of the stimulus to him, by his state of mind at the moment, as well as by the sensory nerve patterns evoked by physical stimulation (Melzack, 1973, p. 134).

These concepts, burn injury and pain plus the previously identified constructs, stimulus and biopsychosocial reaction, form the basis for postulates (P) in the conceptual model. The two substantive terms, concepts, alone form the propositional (PR) statements (Gibbs, 1972, p. 178). The relational statements, postulate, and propositions are as follows:

P1: Among adults, the greater the stimulus at Stage 1, the greater the burn injury at Stage 1.

P2: Among adults, the greater the biopsychosociocultural reaction at Stage 2, the greater the pain at Stage 2.
PR1. Among adults, the greater the burn injury at Stage 1, the greater the pain at Stage 2.

Referential Level

Referentials are intrinsic terms that designate a formula in the extrinsic part of a theory. A referential appears as a capitalized acronym, the purpose being to signify that the meaning of the term is technical and relating to a particular theory (Gibbs, 1972, p. 129). Referentials include BSI (Burn Severity Index), BE (β-endorphin Levels), and AES (Analgesia Equivalency Score).

Index of Injury Severity

In recent years effort has been exerted to establish some format for identifying the extent of injury for multiple trauma victims. Historically, the patient was classified according to the system affected, such as bone fractures or internal injuries. As emergency medical systems became more prevalent, the individuals with more than one system injury were kept alive and transported to hospitals with service and equipment to manage severe trauma (Baker et al., 1974). As trauma became a leading cause of death in middle aged adults, the need to have some type of system that viewed the body holistically became evident. Trauma indices were developed that could be calculated and documented on the patient's medical record. Several of these indices are presented and discussed in literature which include: The Abbreviated Injury Scale and The Comprehensive
Injury Scale (Committee on Medical Aspects of Automotive Safety, 1971); The Injury Severity Score (Baker et al., 1974); The Trauma Index (Kirkpatrick & Youmans, 1971); and The Multi Attribute Severity Scale (Gustafson & Holloway, 1975). More recently a severity index for 11 different physiologic parameters chosen to reflect the patient's physiologic status is used initially and periodically following injury. The index consists of a circle diagram of physiologic status showing variables of cardiac index, heart rate, mean arterial blood pressure, venous pH, venous pCO₂ cardiac mixing time, cardiac ejection fraction, pulmonary mean transit time, systolic ejection time, right atrial pressure (CVP), arteriovenous oxygen content difference, and mixed venous pO₂ (Trunkey, 1983).

The need for a systemized index to categorize burn severity was also recognized. For a number of years it has been evident that severity of injury and extremes of age are categories to observe when predicting recovery ability. The first effort to accumulate data was evidenced by Fellar (1970). He developed a National Burn Information Exchange Center at the University of Michigan, Ann Arbor, Michigan. Prior to the development, the Rule of Nines had been utilized to identify the total body surface area injured. However, the Rule of Nines did not incorporate other physiological and demographic aspects that appeared to impact the patient's recovery. Fisher et al. (1976) raised concern about the need for a burn severity grading system. At the Seventh Annual Meeting of the American Burn Association (1975), advantages were proposed for using a burn severity grading which included:
(1) Providing a means for defining segments of the burn population and identifying variation in a burn unit's patient profile.

(2) Permitting all burn units, large and small, to compare their clinical performance with themselves and others.

(3) Providing a basis for establishing treatment standards along broad categories as required by PSRO and other auditing agencies.

(4) Encouraging identification of treatment goals other than survival.

(5) Obligating innovators, advocates, and critics among us to specifically define which grades of burn will benefit or suffer from a given innovation.

(Fisher et al., 1975, pp. 254)

In 1979, Zawacki et al. developed a multifactorial Probit Analysis utilizing the factors of age, TBSA, full thickness area, prior bronchopulmonary disease, abnormal $\text{paO}_2$, and airway edema to predict survivors. The data were reported from one facility and, even though they found the index to be a reasonable predictor of death, there was concern about outlying cases not reflected in the mathematical model. There was also concern about the use of data from one facility being utilized to predict outcome in another.

Tobiasen et al. (1980 and 1982) reported data from a Practical Burn Severity Index which incorporated sex, age, inhalation injury, presence of full-thickness burn, and percent body surface area injured. The Burn Severity Index was proposed as a predictor of injury outcomes, an indicator as to the type of hospital in which the patient should be treated, and a way of facilitating audit of burn care (Tobiasen et al., 1980). Due to the large number of patients and the varied settings used to develop the index, the decision was
made to use the Burn Severity Index to quantify severity of the burn injury in the present study.

**β-Endorphins**

Due to current literature information which relates β-endorphins with pain, β-endorphin levels (BE) were chosen as an index for pain. Several paragraphs will be devoted to describing β-endorphins and differentiating them from other known endogenous opiates.

The term endorphin is a combination of the words "endogenous" and "morphine". This generic name was proposed by Eric Simon for substances which combine with opiate receptors in the brains of mammals. Endogenous peptides appeared to occupy the same receptor sites as morphine and elicit the same effects but were believed to have a three to four times greater affinity for the receptors than morphine (Loh & Law, 1977).

In 1964, Li discovered a polypeptide substance in sheep pituitary gland with properties different from most pituitary hormones. The new substance was named β lipotropin (β LPH). The amino acid sequence was then elaborated as a 91 amino acid peptide which was believed to be a potential precursor for other hormones and biologically active peptides. The specific amino acid sequence identified for human β-endorphin is: H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH (Li, Liang, & Loh, 1976). The pentapeptide, Tyr-Gly-Gly-Phe-Met (Met enkephalin) was identical to the
61 - 65 amino acid residue of B LPH (Hughes et al., 1975). The primary structure of alpha endorphin is H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH. The primary structure of delta endorphin has the same primary structure as alpha endorphin with one additional Leu as the COOH terminal residue in position 17 (Guillemin, 1978). Two pentapeptides, Met Enkephalin and Leu enkephalin, have been isolated with less activity when compared to endorphins and morphine (Loh & Law, 1977).

Endorphins were found in varying amounts in all vertebrates. Most studies reflect the use of extracts from pigs, beef, sheep, rats, and human pituitaries (Goldstein, 1976). The peptides were found in the brain, spinal fluid, adrenal medulla, and adrenal cortex. Receptor sites were also identified in the vas deferens of rats (Schulz et al., 1979) and guinea pig ileum (Puig et al., 1977). When looking carefully at the adrenals the greatest amount of endorphins were concentrated in the medulla (Hexum, Yang, & Costa, 1980).

Matsukura et al. (1979) conducted a study with four Japanese monkeys to determine the concentration of β-endorphin in the brain. The immunoreactive β-endorphin was reported in pg/mg net weight. Areas of the brain that contained concentrations beyond 400 pg/mg included the corpus callosum, optic chiasma, olfactory tract, lateral, dorsomedial, and ventromedial hypothalamus, pallidum, Habenula, interpenduncular nucleus of the midbrain, pyramis, dorsal root of spinal cord, and the thoracic ganglia of the sympathetic nervous system.
Krieger et al., (1977) assayed fresh adult bovine brain for its content of immunoreactive β lipotropin, ACTH, and β-endorphin. Highest concentrations of β lipotropin were present in the hypothalamus, hippocampus, central grey rostral mesencephalic level, pons, striatum, and spinal cord. Lesser concentrations of β lipotropin were present in other parts of the limbic system, brain stem, cortex, and thalamus. Immunoreactive ACTH concentrations were highest in the hypothalamus and hippocampus with markedly lesser concentrations being present in all other aforementioned areas of the brain. Immunoreactive β-endorphin concentrations were also high in the hypothalamus and hippocampus with lesser amounts in other areas of the brain.

Goldstein (1976) found that concentration of endorphins was about eight times higher in the posterior pituitary than in the anterior pituitary. Most of the concentration was in the region of the cleft, suggesting its presence primarily in the pars intermedia.

Endorphins were believed to originate in the pituitary and then liberated to other areas of the brain, CNS, and the adrenal medulla. However, only minimal changes in the level of serum endorphins were detected following hypophysectomy. At one month post hypophysectomy, the level of endorphins was found within twenty percent of the preoperative serum level (Goldstein, 1976; Ogawa et al., 1979).

In 1978, Suda, Liotta, and Krieger were unable to detect endorphins from the plasma of normal human subjects. However, Csontos et al. (1979) and Hollt et al. (1979) readily measured the
endorphin levels in normal human subjects. Each of these studies reflected other vital information for clinically evaluating and understanding the activities of endorphins. The peptide β-endorphin and ACTH levels were both elevated in patients suffering from Addison's disease, Cushing's disease, and Nelson's syndrome. This information is consistent with rat studies conducted by Guillemin et al. (1977) and Hollt, Prezowlovk, and Herz (1978) when they reported that β-endorphin and ACTH are released simultaneously from the anterior pituitary into the blood stream in response to various stress treatments.

In a study conducted by Blasig et al. (1978), rats were confronted with the experience of a new environment and stressful handling procedures. The body temperature increased within minutes and the plasma β-endorphin level increased dramatically.

The studies which have been conducted to identify and clarify the properties of endorphins have reflected the use of naloxone, a morphine antagonist, which also has an affinity for the opiate receptor sites present in the body (Goldstein, 1978). The use of this agent has been helpful to identify behavioral change following administration of the drug.

Continued research has led to further delineation and characterization of β-endorphins. Akil et al. (1981) and Gubler et al. (1982) describe three families of opiates: pro-opiomelanocorticotropin, pro-enkephalin, and pro-dynorphin. β-endorphin is believed to be contained in the pro-opiomelanocorticotropin precursor molecule which has its own gene. There is accumulating support for the
location of $\beta$-endorphin in centers especially concerned with pain and stress. Furthermore, $\beta$-endorphin measured in the plasma are probably generated in the anterior and intermediary pituitary, outside the blood/brain barrier (Akil et al., 1981). There appears to be no interaction between the $\beta$-endorphins secreted within and outside the blood/brain barrier. Therefore, clear differentiation of their actions is hopefully forthcoming.

Studies continue which demonstrate simultaneous production of $\beta$-endorphin and chemical stress mediators such as ACTH, cortisol, and epinephrine (Guillemin et al., 1977; Akil et al., 1981; Holaday et al., 1979; Colt, Wardlaw, & Frants, 1981; Pert, 1982). Clinical studies which implicate $\beta$-endorphins in hypotension, decrease in respiration rate, decrease in temperature, decrease in cardiac output, decrease in pulmonary wedge pressure, and change in glycogen, insulin, cyclic adenosine monophosphate, and other hormones continue to make knowledge of human $\beta$-endorphin levels necessary for management of the acutely ill individual (Barden et al., 1981; Lang et al., 1982).

An example of $\beta$-endorphin influence in acute illness is reflected in an animal study conducted by Gahhos et al., 1982. Three groups of pigs with induced septic shock were studied. The group given naloxone provided evidence of decrease in the hemodynamic and hormone parameters persistent with septic shock. The group of pigs given continued doses of morphine manifested ongoing low blood pressure, increased pulmonary wedge pressure, increase in substance P, and no change in cardiac output. Thus, clinical relevance of
β-endorphins becomes more and more exciting as time allows researchers to pursue areas of interest, concern, and clinical expertise.

Analgesia

The type of analgesia utilized for pain management in burn injured patients varies according to the personal preference of the attending physicians, staff evaluations as to success of certain agents in decreasing pain, and the tolerance of the patient. Although morphine has been identified as the analgesia most frequently used, other agents such as codeine and meperidine are given (Perry et al., 1981).

The term opioid refers to any natural or synthetic drug that has morphine-like pharmacological actions. The term is used interchangeably with narcotic analgesia. The opioids are employed primarily for the relief of pain, but their use entails the risk of producing physical and sometimes psychological dependence. There are as yet no agents effective against severe pain that are entirely free of this risk (Jaffee & Martin, 1975).

Morphine, the alkaloid that gives opium its analgesic actions, remains the standard against which new analgesics are measured. Morphine and its surrogates produce their major effects on the central nervous system (CNS) and the bowel. At present, the mechanisms by which the opioids exert their effects remain uncertain. Stereospecific saturable receptors for opioids and opioid antagonists have been studied in vertebrate neural tissues by several
Ample evidence exists that opioids interact with more than one neurotransmitter, either directly or indirectly. Opioids decrease the release of acetylcholine (ACH) from some peripheral and central cholinergic neurons, and elevate ACH levels (Harris & Dewey, 1973). Opioids seem to inhibit the release of catecholamines from certain peripheral neurons, but increase the release, synthesis, and turnover of catecholamines in the CNS (Smith & Sheldon, 1973). Attempts to understand the role of various transmitters in any one specific opioid action, such as analgesia, have not yielded clear results.

In man, morphine produces analgesia, drowsiness, changes in mood, and mental clouding. A significant feature of the analgesia is that it occurs without loss of consciousness. The extremities feel heavy and the body warm, the face, and especially the nose, may itch, and the mouth becomes dry. In addition to relief of distress, some patients experience euphoria. If the external situation is favorable, sleep may ensue.

Since morphine is the standard by which to measure all strong analgesia and structurally related drugs, comparisons of the onset, peak duration, and the equianalgesic dose to 10 milligrams (mgs) of morphine have been made. Information then allows the clinician to view narcotic analgesia in light of the drug's comparison to morphine 10 mgms.

A study conducted by Beaver, 1980, utilized the equianalgesia table with cancer patients. By prescribing the equivalency dose of
other narcotics and comparing the effects, Beaver found that except for distinctions based on relative potency, time-effect considerations, and oral-parenteral potency ratio, there are few clinically important differences among strong narcotics. At equianalgesia doses, their dependence liabilities are the same, and there is little evidence from controlled studies that adverse effects differ qualitatively or quantitatively. However, for unknown reasons, an individual patient may experience an adverse effect with one of these drugs but not with an equianalgesic dose of another (Beaver, 1980).

The following definitions constitute the Referential Level for the Conceptual Model:

**Burn severity index (BSI).** A simple and clinically useful system for measuring severity of burn injury. Multivariate logistic regression is used to predict death from sex, age, presence of inhalation injury, presence of full-thickness burn, and percent of total body surface area burned (Tobiasen et al., 1982).

**β-Endorphins (BE).** An endogenous opiate, used to index pain, with morphine-like properties produced within the CNS and some endocrine tissues. Endorphins are located in areas of the CNS concerned with pain perception and in endocrine tissues associated with stress response (Cahill, 1983).

**Analgesic equivalency score (AES).** A classification system used to index pain which has an assigned equivalency
The above referentials are used along with concepts to formulate additional relational statements for the Conceptual Model. A transformational statement (TFS) includes a concept and a referential. A theorem (TH) contains constituent terms which are referentials and represent the final step in theory construction (Gibbs, 1972, p. 190). Transformational statements and theorems for this study are as follows:

TFS1: Among adults, the greater the burn injury at Stage 1, the greater the BSI at Stage 1.

TFS2A: Among adults, the greater the pain at Stage 2, the greater the BE level at Stage 2.

TFS2B: Among adults, the greater the pain at Stage 2, the greater the AES at Stage 2.

TH1A: Among adults, the greater the BSI at Stage 1, the greater the BE at Stage 2.

TH1B: Among adults, the greater the BSI at Stage 1, the greater the AES at Stage 2.

Summary

A Gibbs framework is utilized for the conceptual model with review of literature organized and presented relevant to specified variables. Definitions of each construct, concept and referential are presented following the review of literature. Both the extrinsic and intrinsic theory for the conceptual model are delineated. The
theorems of the intrinsic theory are consistent with the purposes for this research study. Chapter II provides the substantive groundwork for Chapter III which addresses the design and methodology for implementing the conceptual model.
Chapter III
DESIGN AND METHODOLOGY

The study design, description of subjects and settings, data collection procedures, techniques for measurement and analysis are presented in this chapter. Decisions for methodology were influenced by a pilot project conducted on four burned patients. A summary of the information obtained from the pilot study may be found in Appendix B.

Design

This study is a descriptive, causal modeling/correlational design (Sellitz et al., 1976; Blalock, 1972). Developing a theoretical model required thought, knowledge, and creativity in the chosen field. The previous chapter reflected each of these issues as applied to physiological variables and provided the substantive information for this data collection effort.

Settings and Sample Selection for Research

Two settings were utilized for data collection which are located in close geographic proximity. These settings include the George David Peak Burn Unit at the University of Missouri Hospital and Clinics, Columbia, Missouri, and the Barnes General Hospital Burn Unit at St. Louis, Missouri. The medical directors for each unit
have managed burn patients for a number of years and are nationally and internationally recognized for their expertise. The nursing staff also have expertise in burn management and actively participate in regional and national burn organizations. Agency approval was obtained according to guidelines established by the Health Science Institutional Review Boards for each setting. Permission was also obtained from the Human Subjects Committee of the Arizona Health Sciences Center (See Appendix C).

The George David Peak Burn Unit has the capacity for six patients and admits an average of 125-150 burn patients annually. The Barnes Hospital Burn Unit is a seven bed unit that admits an average of 150-175 burn patients annually. Each of these units admit children which are included in the above numbers. The combination of the George David Peak and the Barnes Hospital burn units was reasonable because of similarities in burn management. The medical directors and the nursing staff for the two units work collaboratively with each other. Similarities in management include fluid resuscitation, types of analgesia given (Morphine and Tylenol #3), conservative surgical procedures, and partial occlusive dressings. The major topical antimicrobial agent for the George David Peak Burn Unit is silvadene. Cerium nitrate is the agent more frequently used at the Barnes Hospital Burn Unit. At this time no clear differentiation has been made regarding pain response between these two antimicrobial agents.

The sample included patients admitted to the burn unit that met the following criteria: (1) thermal injury diagnosed as partial-
or full-thickness injury, (2) male or female above the age of 21 years, (3) burn injury within 24 hours of admission to the unit, (4) literate in English, and (5) agreed to participate in the study by signing the Human Subjects Research Approval Form designated for the particular institution.

Only thermally injured adults were selected for patients due to the uncertainties of estimating extent of injury from electrical burn injury (Baxter, 1972; Hunt, 1976). Also, issues related to the independent and dependent variables studied are not clearly specified in the literature regarding children.

Obtaining pre-burn information from the patient is impossible; therefore collecting baseline data is imperative as soon as possible following injury. Patients admitted and retained by a general hospital for longer than 24 hours post injury were excluded from the study. To assure clear understanding of the patients role in this study, all participants were literate in English.

Considering the three variables in the study, a total of 30 patients were used as a minimal number for data collection. No attempt was made to randomize the subjects for this descriptive study or to obtain equal numbers of patients from each of the burn units. Prior to data analysis, two patients were removed from the study due to the inability to retrieve their medical records. Thus, a total of 28 patients were used for analysis. Of these 28 patients, 19 (68%) were hospitalized at the George David Peak Burn Center, University of Missouri-Columbia, and 9 (32%) were patients at Barnes General Hospital Burn Unit, St. Louis, Missouri. Only two females were
included in the study, one in age group 21-30 and the other in age group 61-70. A total of 15 patients (54%) fell between the age group of 21-30. An additional 5 (18%) fell in the age group 31-40. Thus, a total of 20 (72%) of the total group of patients that contributed data ranged in age from 21 to 40. Three patients were in the 41-50 age group, 1 in the 51-60 age group, 3 in the 61-70 age group, and 1 in the 71-80 age group. The range of age for the total group of patients from both clinical sites was from 21-80. The mean age for the total group was 36.57 and the standard deviation 15.2. A frequency distribution for the data is included in Table 1.

Operationalization of Variables

The independent variable, Burn Severity Index, was described in the previous chapter and identified in the model as a referential for the concept, burn injury, in Stage 1. The two dependent variables chosen for study included β-endorphin levels and an analgesia equivalency score. Each of these variables was collected over a time interval of two weeks. The dependent variables were identified as referentials for the concept, pain, in Stage II for the conceptual model.

The following paragraphs include description of two indices and the biochemical assay which was utilized for organizing and analyzing the data for this descriptive study. Validity and reliability issues pertinent to each index and the radioimmunoassay are also addressed.
Table 1. Distribution of Patients by Sample Size, Age, Sex, and Agency.

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>St. Louis</th>
<th>Columbia</th>
<th>Total N</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>5 (17.9%)</td>
<td>*10 (35.7%)</td>
<td>*15 (53.67%)</td>
</tr>
<tr>
<td>31-40</td>
<td>2 (7.1%)</td>
<td>3 (10.7%)</td>
<td>5 (17.9%)</td>
</tr>
<tr>
<td>41-50</td>
<td>1 (3.6%)</td>
<td>2 (7.1%)</td>
<td>3 (10.7%)</td>
</tr>
<tr>
<td>51-60</td>
<td>0</td>
<td>1 (3.6%)</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>61-70</td>
<td>1 (3.6%)</td>
<td>*2 (7.1%)</td>
<td>*3 (10.7%)</td>
</tr>
<tr>
<td>71-80</td>
<td>0</td>
<td>1 (3.6%)</td>
<td>1 (3.6%)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>9 (32%)</td>
<td>19 (68%)</td>
<td>28 (100%)</td>
</tr>
</tbody>
</table>

* = 1 Female in group

Range of Age: 21-80

Mean of Age: 36.57

Standard Deviation for Age Group: 15.2
Burn Severity Index

A Burn Severity Index utilizing the variables of sex, age, inhalation injury, presence of full-thickness burn, and percent of body surface area burned was used to index the burn injury (see Table 2). Age and sex were readily available from the patient or the chart. The presence of full-thickness burn and the percent of total body surface area injured were criteria provided by the physicians. Standard forms on which the extent of injury was tabulated for each of the clinical settings are included in Appendix D. The presence of inhalation injury was inferred when two or more of the following criteria were met: (1) history of a closed space fire, (2) facial burns with singed nasal hair, (3) carbonaceous-stained sputum or oral mucous membranes, and (4) stridor or labored breathing. Information pertinent to the Burn Severity Index was accumulated from the medical record by the researcher at the end of the two-week interval.

Validity and Reliability Issues. The initial work for the Burn Severity Index was conducted on 969 patients from two major burn centers and 65 community hospitals (Tobiasen et al., 1980). The purpose of the study was to develop an objective and quantitative index to measure the severity of burn injury. The index was assessed for predictive validity to outcome mortality and successfully cross-validated to different burn population samples. Predictive variables utilized in the study were available prior to treatment without the use of laboratory tests or special instrumentation. Only
Table 2. Burn Severity Index.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Female</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

| Age (Yr) | 0-20 | 1     |
|          | 21-40| 2     |
|          | 41-60| 3     |
|          | 61-80| 4     |
|          | 81-100| 5    |

<table>
<thead>
<tr>
<th>Inhalation Injury</th>
<th>Full-thickness burn</th>
<th>Total BSA burn (%)</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-10</td>
<td>11-20</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>21-30</td>
<td>31-40</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>41-50</td>
<td>51-60</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>61-70</td>
<td>71-80</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>81-90</td>
<td>91-100</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total burn score</th>
<th>Threat to life</th>
<th>Probability of Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>Very low</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>4-5</td>
<td>Moderate</td>
<td>.98</td>
</tr>
<tr>
<td>6-7</td>
<td>Moderately Severe</td>
<td>.8-.9</td>
</tr>
<tr>
<td>8-9</td>
<td>Serious</td>
<td>.5-.7</td>
</tr>
<tr>
<td>10-11</td>
<td>Severe</td>
<td>.2-.4</td>
</tr>
<tr>
<td>&gt;12</td>
<td>Maximum</td>
<td>&lt;=.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhalation Injury</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of Closed Space</td>
<td>Date of Onset</td>
</tr>
<tr>
<td>Facial Burns with Singed Nasal Hair</td>
<td></td>
</tr>
<tr>
<td>Carbonaceous-Stained Sputum or Oral Mucous Membranes</td>
<td></td>
</tr>
<tr>
<td>Stridor or Labored Breathing</td>
<td></td>
</tr>
<tr>
<td>Total =</td>
<td></td>
</tr>
<tr>
<td>Total =</td>
<td></td>
</tr>
</tbody>
</table>
admission variables available to specialized as well as more general health care delivery systems were included in the 1980 study. A subset of 590 patients with complete data was treated in specialty burn treatment facilities. One half of this subset was used to develop the statistical model for a burn severity injury scoring system. The remaining 295 patients comprised the validation sample for burn centers, with 317 community hospital patients accounting for the second validation sample. The statistical mode developed on the estimation sample was assessed for its goodness of fit to both validation samples.

To assess the extent to which coders agreed on any given variable, an intercoder reliability study was conducted. Each of four coders abstracted ten identical cases selected from the medical records library. The selected cases were chosen to represent a wide spectrum of severity of burn injury. Coders agreed, on the average, on 94% of the coded responses. Forty pretreatment variables were abstracted from patient records, including age, sex, race, burn locations, percentage second-degree burns, percentage third-degree burn, percentage combined second- and third-degree burn, total percentage body surface burned, presence of inhalation injury, pre-existing medical conditions, time in days from injury to hospital admission, and burn etiology.

Exploratory analysis of these 40 variables was completed using multiple regression on the estimation sample. Eleven of the 40 variables in the regression equation were statistically significant at P < 0.05. Variables included were age, sex, presence of
inhalation injury, percentage second-degree burn, percentage third-degree burn, percentage total body surface burned, as well as burns to the foot, arm, cranium, abdomen, and back. The estimation of the probability of death for each patient, based on the total outcomes for all cases in the estimation sample, utilized the multivariate logistic function. The maximum-likelihood computation procedure was used to select the best subset of variables and corresponding weights. An $R^2$ of 1 indicated that the model is discriminating perfectly among those patients who died and those who survived, while an $R^2$ of 0 indicated that the model did not differentiate between the two groups. The $R^2$ for the model presented was equal to 0.67 ($\chi^2 [7] = 178.669; P < 0.001$). Overall, the logistic regression equation correctly classified 93.2% of the estimation sample and correctly placed 78% of the nonsurvivors and 96.3% of the survivors. These percentages represent clear improvements over the base-rate probabilities (17% for death and 83% for survival).

The complete statistical model was validated on the remaining subset of burn center patients. The model generalized to the new sample and correctly classified 92.6% of the cases including 79% of the deaths and 94% of the survivors. Finally, the statistical model was validated on the sample of cases from the community hospitals. Greater than 99% of survivors were correctly classified by the statistical model. The results for nonsurvivors, however, were reported as inconclusive because only six (1.7%) of the community hospitals.
hospital sample died. The statistical model correctly classified three (50%) of the patients in the community hospital (Tobiasen et al., 1980).

A second study was conducted (Tobiasen et al., 1982) to develop a simple and clinically useful system for measuring severity of burn injury. An estimation sample of 590 case records of burn victims from two burn centers was audited to provide data for the initial development. An additional 762 case records from 65 community hospitals and three burn centers provided data for the cross validation of the index. The 762 case subset was referred to as the validation sample. The variables for the scale presented earlier were used. The relative proportion of the estimated coefficients for each variable compared to every other variable were identified and a new statistical model was developed. The new statistical model was based on coded values of the variables, which were constructed to reflect the relative size of the estimated parameters based on the raw values of the variables.

The multivariate logistic model consisting of sex, presence of inhalation injury, presence of full-thickness burn, and the recoded values for age and percent of the total BSA burned presented an $R^2$ of 0.57 ($x^2 [5] = 283.5; p .001$). The coefficients for each variable were nearly equal (the range is 0.86 to 1.2) so that any one variable may be eliminated from the model without loss of predictive power. A third logistic regression was calculated using the sum of each patient's coded value for all five variables as a single predictor
variable in the analysis. The $R^2$ for the model, based on the score variable was $0.569 \ (x^2 [1] = 282.6; \ p = 0.001)$ and represents a negligible decrease in predictive power from the five variable models. Therefore, the abbreviated model of burn severity as presented appears to be both feasible and appropriate.

The abbreviated model, based on each patient's composite score of the five severity variables, was used to calculate probability of survival. The number of patients who lived and died are grouped in two probability classes of survival: (1) probability of survival of less than or equal to 0.5, and (2) probability of survival of greater than 0.5. The statistical model classified 75% of those who died into high risk categories. Conversely, it classified more than 95% of the survivors into low risk categories. The model, therefore, is an accurate tool for classifying the burn patient who is at risk and the patient who is not at risk. The new model then is viewed as an appropriate tool for use in both the triage of patients and the evaluation of outcomes of treatment.

To be utilized as a referential for this study a determination was made regarding the consistency or equivalence of the Burn Severity Index in yielding measurements of the same traits in the same subjects. A potential weakness of this data collection approach is the fallibility of the observer/recorder. The greater the interpretive burden upon the observer, the higher the risk of observer error or bias. The accuracy of observer ratings and classifications can be enhanced by careful training. Interrater (or interobserver) reliability is estimated by having two or more trained
observers watching and/or recording some event simultaneously and independently recording the relevant variables according to a predetermined plan or coding system. The extent to which the raters agree or disagree is then determined. Dichotomous data with two raters is evaluated for reliability. Interrater agreement "represents the extent to which different judges tend to make exactly the same judgments about the rated subject (Tinsley & Weiss, 1975, p. 359)." On a numerical scale, agreement means that raters assign exactly the same values on the same person. Interrater reliability "represents the degree to which the ratings of different judges are proportioned when expressed as deviations from their means (Tinsley & Weiss, 1975, p. 359)." Since this researcher was interested in the ability of two judges (dichotomous data) to rate the same group of data rather than the degree to which the ratings of two judges are proportional, only interrater agreement was pursued (Tinsley & Weiss, 1975).

The Lawlis and Lu (1972) measure of interrater agreement was utilized due to the flexibility in selecting a criterion for agreement. The Lawlis and Lu (1972) index allows for total agreement with identical ratings, ratings that differ by no more than one point or ratings that differ by no more than two points. For the Burn Severity Index, a rating of no more than one point is viewed as in agreement. All disagreements that exceed the criterion are of equal seriousness. The non-parametric chi-square test of the significance of interrater agreement was used. A significant chi-square indicated that the observed agreement was greater than the agreement that could
be expected on the basis of chance (Lawlis & Lu, 1972).

Five charts were randomly selected and rated by the researcher and by the individual identified in each institution that assisted with data collection. Interrater agreement of the BSI between the observer/rater at Barnes Hospital and the researcher was 100%. There were no discrepancies with the final number identified, nor with the information obtained from the subject's chart from which the judgments were made.

One disagreement was identified between the observer/rater at Missouri University and the researcher resulted in 83% interrater agreement. When the data were examined more closely, it was found that the proper information had been retrieved from the chart and accurately recorded on the information sheet. The error arose when the information was transferred from the recorder information sheet to the Burn Severity Index. In summary, only one disagreement arose in the total interrater activity for the BSI. Thus, the goal of no greater than one disagreement was met. Table 3 presents the interrater data from both clinical agencies for the BSI. The total percent agreement between the researcher and the observer for both agencies was 98.3%.

β-Endorphins

To measure β-endorphin levels, blood samples (7 ml.) were drawn from each patient selected on admission to the burn unit every 24 hours for 7 days and every 48 hours for an additional 7 days. The proposed sequencing allowed for a potential of 11 samples to be
Table 3. Interrater Agreement Between the Researcher and Observer From Each Clinical Agency for the Burn Severity Index.

<table>
<thead>
<tr>
<th>Agency/Patient No.</th>
<th>BSI By Researcher</th>
<th>BSI By Observer</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Louis/No. 1</td>
<td>8</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>St. Louis/No. 2</td>
<td>7</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>St. Louis/No. 3</td>
<td>8</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>St. Louis/No. 4</td>
<td>8</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>St. Louis/No. 5</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>Columbia/No. 1</td>
<td>6</td>
<td>5</td>
<td>83%</td>
</tr>
<tr>
<td>Columbia/No. 2</td>
<td>9</td>
<td>9</td>
<td>100%</td>
</tr>
<tr>
<td>Columbia/No. 3</td>
<td>8</td>
<td>8</td>
<td>100%</td>
</tr>
<tr>
<td>Columbia/No. 4</td>
<td>5</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>Columbia/No. 5</td>
<td>5</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>10</strong></td>
<td><strong>70</strong></td>
<td><strong>98%</strong></td>
</tr>
</tbody>
</table>
obtained from each subject.

Acquisition of blood samples ended when the patient was discharged, refused to continue participation in the study, or expired. Reasons for the inability to acquire samples at any given time were tabulated on the data collection sheet.

Blood Collection. The following protocol was completed by all individuals obtaining blood samples:

1. All supplies for obtaining blood samples were chilled in ice water, i.e., syringes and EDTA Vacutainers (BD Vacutainers Brand).
2. The sample was obtained prior to wound manipulation and/or prior to a surgical procedure.
3. A sample of 7-8 ml of whole blood was drawn from the patient at the same time that samples were obtained for other analyses.
4. Sample was immediately chilled on ice and transported to the laboratory for processing and freezing within 15 minutes of withdrawal from the patient.
5. All samples were Centrifuged at 2000 RPM's for 10 minutes at 4°C.
6. Plasma was pipetted into a chilled polypropylene tube for measurement.
7. 100 µl 1N HCl/ml plasma were added to each specimen.
8. Each sample was marked with hospital number, date, and time.
9. Plasma was stored at -70° C until extraction and analysis. (Cahill, Matthews, & Akil, 1983)

The samples were retained in -70° C until all samples were collected. Dry ice was utilized to transport the samples with the researcher to the University of Arizona Health Sciences Center, Tucson, Arizona, for analysis. The Surgical Biology Laboratory, directed by Dr. Milos Chvapil, University of Arizona College of Medicine, was the setting for sample analysis. Dr. Clemond Eskelson, a biochemist in the Surgical Biology Laboratory, has been analyzing β-endorphins for the past four years. This researcher has worked closely with Eskelson and his laboratory staff to develop knowledge and skill in performing the biochemical assay for β-endorphins.

Plasma Extraction. The Sep-Pak C18 Cartridge (Waters Associates, Inc., Milford, MA) was utilized for plasma extraction. The Sep-Pak C18 cartridge is a reverse phase liquid chromatographic system which retains hydrophobic species. The following steps were followed for plasma extraction:

1. Plasma was thawed and acidified to pH 2 with 1N HCl;
2. Sample was centrifuged at high speed (2400 RPMs) for 10 minutes to remove fibrin;
3. Sep-Pak C18 was activated in the following manner:
   Washed with 5 ml methanol;
   Washed with 5 ml 8 M urea;
   Washed with 10 ml double distilled water
4. Plasma sample was slowly applied to Sep-Pak C18;
5. Washed with 10 ml double distilled water;
6. Washed with 10 ml 4% glacial acetic acid;
7. Sep-Pak C18 was eluted with 5 ml glacial acetic acid and 90% ethanol (1:24);
8. Eluate was dried and stored at -70°C until assayed.

(Cahill et al., 1983)

The samples were assayed according to instructions specific for the New England Nuclear Corporation β-endorphin (125) RIA kit, Catalog No. NEK-003 (Appendix E). β-endorphin levels are reported in picograms per milliliter (pg/ml) of plasma.

Three assays (A, B, and C) utilizing two kits at two different time intervals were necessary to analyze acquired plasma samples for β-endorphin levels. All procedures were followed as specified in the instructions. After the samples were counted, the standard curve for each assay was utilized to determine the concentration of β-endorphins in the samples. Standard curves for assays A, B, and C closely approximate the curve derived by researchers at the New England Nuclear Corporation. Both curves are plotted on graph paper and presented in Appendix F. As delineated in Catalog No. NEK-003 (Appendix E) the normalized percent bound (%B/Bo) was calculated from the counts obtained from the standards and samples. The %B/Bo was used to acquire the picograms of β-endorphins per milliliter (pg/ml) of plasma. The standard curves for each assay performed and the formula used to derive β-endorphin levels from the %B/Bo are presented in Appendix F.

It is imperative to note that the reported picograms reflect all opiate activity and cross-reactivity that contributed to binding
activity from associated or precursor molecules such as βLPH. The sensitivity for the New England Nuclear β-endorphin Kit is low for this activity as previously reported.

Validity and Reliability Issues. The word "precision" is used to describe the reproducibility (reliability) of results. Precision is defined as the "agreement between numerical values of two or more measurements that have been made in an identical fashion" and can be expressed in absolute or relative terms (Skoog & West, 1978, p. 44). A numerical determination of precision can be achieved through deviation from the mean without regard to size, standard deviation, variance, correlation, and the spread or range of numerical data (Skoog & West, 1978, p. 44).

Chemical analysis is replicated in an effort to decrease determinate and indeterminate error. Determinate error includes personal errors of the experimenter, instrumental errors which include tools and environmental factors upon the tools, and method error which refers to problems within the framework of the analysis which addresses problems with chemicals, reactions, contaminants, etc. Determinate error may be constant or proportional. Constant error will become more serious as the size or quantity decreases. Proportional error also changes with the size of the agent (Skoog & West, 1978, p. 46).

An experienced, competent chemist will have less determinate error than a neophyte. Differences can be determined by having two persons conduct the same procedure at the same time, place, reagents, etc., and comparing the results. Much determinate error has
previously been eliminated from this assay through repetition. Following the completion of each assay discussion with a colleague will assist in determining and correcting possible errors.

Personal error which existed for this assay included: lack of knowledge, miscalculations, failure to recheck instruments, inaccurate weights, improper pipetting, skipping test tubes, inadequate stirring, and inadequate incubation. Instrumental errors include pH meter malfunction, effects of air when weighing, environment, temperature, freezing, improper supplies (glass vs. plastic), and improper calibration of machines. Method errors include outdated chemicals, untoward reactions of chemicals and improper filtration process. Most determinate error has been eliminated through self discipline, astuteness, rechecking, and recording. Also, total concentration is imperative while calibrating, preparing, and pipetting chemicals.

Indeterminate error arises from uncertainties in a measurement that are unknown and cannot be controlled by the scientist. As reflected by the word, indeterminate error cannot be determined, therefore it cannot be eliminated. If measurement error is great and all possible determinate error is eliminated it may be necessary to plan for rechecking and comparison to see if insights can be gained by working with another scientist. Since indeterminate errors tend to distribute themselves they do not greatly affect the precision (Skoog & West, 1978, p. 46).

Duplication of samples is standard procedure for a biochemical assay. Calculation of the reliability coefficients between samples will provide insight into determinate error. Correlations in the
.80's and .90's are desirable (Anastasi, 1976, pp. 108-109); however, chemists are determined to have correlation above .90 when a standard curve is being established for calculation of samples (Eskelsen, 1983). The reliability can be interpreted as the true variance which remains stable overtime. True variance is referred to as stability of measurement and is requisite in a biochemical assay.

One non-burned individual consented to the withdrawal of 60 ml whole blood to be used for analysis of β-endorphins and ultimately determination of precision for the β-endorphin assay. Plasma was processed according to the protocol specified for patient samples. A total of 13 aliquots of 2 ml plasma were assayed with patient samples. Resulting data included a range of counts per minute (CPM) from 1534 to 2238. The calculated normalized %Bo/Bo was used to determine the error rate. With data from all 13 samples in the equation, the mean was 73.71, standard deviation was 8.69. Standard error was 2.41; thus, a total error rate of 11%. It was determined that three low outlyers could be discarded and still retain significance at the .05 level. Removal of three outlyers allowed a range of 1910 to 2237 AV CPM. Again, the %Bo/Bo were recalculated, resulting in a mean of 77.42, standard deviation of 5.097, and standard error of 1.611. This error rate of 6.5% is within the guidelines followed by Eskelson (1984) of not greater than 10% for biochemists. Table 4 delineates the data from each sample obtained from the volunteer along with the findings.

"The term accuracy denotes the nearness of a measurement to its accepted value and is expressed in terms of error" (Skoog & West,
Table 4. Distribution of β-Endorphin Levels from One Non-Burned Subject: Mean, Standard Deviation, and Standard Error with All Samples and with Removal of Outlyers.

<table>
<thead>
<tr>
<th>CPM</th>
<th>Av. CPM</th>
<th>Av. NCPM</th>
<th>% Bo</th>
<th>% B/Bo All</th>
<th>% B/Bo Outlyers</th>
<th>β-Endorphins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1902</td>
<td>1918</td>
<td>1661</td>
<td>46.49</td>
<td>70.67</td>
<td>70.67</td>
<td>5</td>
</tr>
<tr>
<td>2136</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. 2340</td>
<td>2238</td>
<td>1989</td>
<td>55.68</td>
<td>84.63</td>
<td>84.63</td>
<td>0</td>
</tr>
<tr>
<td>1960</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. 1990</td>
<td>1975</td>
<td>1726</td>
<td>48.3</td>
<td>73.44</td>
<td>73.44</td>
<td>4</td>
</tr>
<tr>
<td>1885</td>
<td></td>
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<td></td>
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<td></td>
</tr>
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<td>4. 1993</td>
<td>1939</td>
<td>1690</td>
<td>47.3</td>
<td>71.9</td>
<td>71.9</td>
<td>5</td>
</tr>
<tr>
<td>1710</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. 1734</td>
<td>1722</td>
<td>1473</td>
<td>41.23</td>
<td>62.67</td>
<td>*</td>
<td>8.5</td>
</tr>
<tr>
<td>1890</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. 2036</td>
<td>1963</td>
<td>1714</td>
<td>47.97</td>
<td>72.97</td>
<td>72.97</td>
<td>4.5</td>
</tr>
<tr>
<td>1566</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1534</td>
<td>1285</td>
<td>35.97</td>
<td>54.67</td>
<td>*</td>
<td>13</td>
</tr>
<tr>
<td>1590</td>
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</tr>
<tr>
<td>8. 2060</td>
<td>2075</td>
<td>1826</td>
<td>51.11</td>
<td>77.69</td>
<td>77.69</td>
<td>2.5</td>
</tr>
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<td>1998</td>
<td></td>
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</tr>
<tr>
<td>9. 2110</td>
<td>2054</td>
<td>1805</td>
<td>50.52</td>
<td>76.79</td>
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<td>3</td>
</tr>
<tr>
<td>1766</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. 1864</td>
<td>1815</td>
<td>1566</td>
<td>43.83</td>
<td>66.63</td>
<td>*</td>
<td>7</td>
</tr>
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<td>1989</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. 2327</td>
<td>2158</td>
<td>1909</td>
<td>53.43</td>
<td>81.23</td>
<td>81.23</td>
<td>1</td>
</tr>
<tr>
<td>2320</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. 2116</td>
<td>2218</td>
<td>1969</td>
<td>55.11</td>
<td>83.77</td>
<td>83.77</td>
<td>.5</td>
</tr>
<tr>
<td>2114</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. 2202</td>
<td>2158</td>
<td>1909</td>
<td>53.44</td>
<td>81.22</td>
<td>81.22</td>
<td>1</td>
</tr>
</tbody>
</table>

Mean: 73.71 77.42
Standard Deviation: 8.69 5.1
Standard Error: 11.00 6.49
According to biochemists, once precision of the assay is established and a standard curve derived, the results from samples are then calculated. The basic principle of radioimmunoassays addresses the issue of accuracy. The principle is competitive protein binding where a radioactive and a nonradioactive antigen compete for a fixed number of antibody binding sites. When unlabeled antigen from standards or samples and a fixed amount of the labeled antigen are allowed to react with a constant and limiting amount of antibody, decreasing amounts of the labeled antigen are bound to the antibody as the amount of unlabeled antigen is increased. As earlier addressed, accuracy is synonymous with validity and is the proportion of variance that the observed scores share with the true scores and the degree to which a set of indicators measure the concept it is intended to measure (Zeller & Carnine, 1980, p. 73).

Collection and processing of blood samples were described earlier in this discussion. The specified procedure was followed as precisely as possible to minimize the destruction or loss of ability to recover β-endorphin from the plasma. One person, designated as a research assistant, at each setting was responsible for carrying out the specified steps. Research assistants completed a minimum of four practice sessions with the researcher. At the practice sessions, blood samples were obtained from a volunteer and each step was completed until the individual was comfortable and clearly understood the importance of the procedure and the elements of time. Training and retraining the individuals in the use of equipment such as the balance scale and centrifuge was repeated as often as necessary to
assure comfort and ease with utilization. Index cards containing each step procedural were available with supplies needed to process the blood sample. Supplies included polypropylene tubes, labeling tape, and pipettes, 1N HCl, and syringes for measurement.

Due to the tremendous expense of acquiring and assaying plasma for β-endorphin levels, it was not feasible to compare results between blood samples obtained by the researcher and blood samples obtained by the assistants prior to implementation of the study. The assistants were selected based on their interest, availability, and commitment to research. They were viewed as accountable individuals and it was believed that the training sessions synchronized activities. Due to the many uncertainties which exist in the clinical setting, the researcher and assistants recorded any procedure deviation on the Data Collection Sheet.

Analgesia Equivalency Score

Since no effort was made in this study to control the amount and type of analgesia given to the burn patients, the equianalgesia table presented in Goodman and Gillman (1978) was employed to tabulate and equate narcotic analgesia administered to the patient in a 24-hour interval prior to obtaining the blood sample for Beta endorphin analysis. The analgesia administered was equated to Morphine 10 mgm. as designated in Table 5. Only the narcotics given to patients in this study are included in the table. Table 6 is the form used to tabulate the information for the narcotic analgesia to the specified increments of Morphine.
Table 5. Equianalgesia Table

<table>
<thead>
<tr>
<th>Drug</th>
<th>Onset (minutes)</th>
<th>Peak (hours)</th>
<th>Duration (1 hour)</th>
<th>1½ (hours)</th>
<th>Equianalgesic Doses (mg) IM:Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>within 20</td>
<td>½ to 1½</td>
<td>up to 7</td>
<td>2 to 3</td>
<td>10:60</td>
</tr>
<tr>
<td>Methadone</td>
<td>10 to 15</td>
<td>1 to 2</td>
<td>4 to 6</td>
<td>22 to 25</td>
<td>10:20</td>
</tr>
<tr>
<td>Meperidine</td>
<td>10 to 15</td>
<td>½ to 1</td>
<td>2 to 4</td>
<td>3 to 8</td>
<td>75:300</td>
</tr>
<tr>
<td>Codeine</td>
<td>15 to 30</td>
<td>1 to 1½</td>
<td>4 to 6</td>
<td>3 to 4</td>
<td>130:200</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>---</td>
<td>---</td>
<td>4 to 5</td>
<td>---</td>
<td>15:30</td>
</tr>
</tbody>
</table>

1 After IV administration, peak effects may be more pronounced, but duration is shorter. Duration of action may be longer with the oral route.
2 Data are based on acute, short-term use. Chronic administration may significantly affect pharmacokinetics and decrease the oral-parenteral dose ratio. The morphine oral-parenteral ratio decreases to approximately 1.5-2.5:1 upon chronic dosing.
3 Duration and half-life increase with repeated use due to cumulative effects.
Table 6. Calculation Sheet for Analgesia Equivalency Score.

<table>
<thead>
<tr>
<th>DATE</th>
<th>HOSPITAL NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent</td>
<td>Amount</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Summary Form

<table>
<thead>
<tr>
<th>Agent</th>
<th>Total Amount Given in 24 Hours</th>
<th>Equivalency to Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Amount Given in 24 Hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Amount Given in 24 Hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total Amount Given in 24 Hours</td>
<td></td>
</tr>
</tbody>
</table>

1-10 mg. Morphine = 1
10-20 mg. Morphine = 2
20-30 mg. Morphine = 3
30-40 mg. Morphine = 4
40-50 mg. Morphine = 5
50-60 mg. Morphine = 6
60-70 mg. Morphine = 7
70-80 mg. Morphine = 8
80-90 mg. Morphine = 9

The Morphine Equivalency Score—Analgesia Equivalency Score will be recorded on the Data Collection Sheet.
Validity and Reliability Issues. Information available to support the value of the equianalgesia dose includes comparison of chemical structures and comparison of similar desired effects and untoward effects. The structure of morphine originally proposed by Gulland and Robinson in 1925 has been tested and remains the accepted chemical configuration (See Figure 2).

Many synthetic narcotics have been derived from the morphine molecule. For this study it is important to know that narcotics commonly used in the burn unit, such as codeine, are structurally similar to morphine. Viewed as a validity issue, Table A in Appendix G depicts the comparison of selected positions of chemical radicals in the morphine molecule and compares these with other narcotics. Morphine and codeine have comparable radicals with the exception of position 3 (Goodman & Gilman, 1975, p. 246).

Opiate receptors in the CNS mediate analgesic activity. Narcotic agonists occupy the same receptors as endogenous opioid peptides and both may alter the central release of neurotransmitters from afferent nerves sensitive to noxious stimuli. In addition to the analgesia effect, narcotics have a variety of secondary pharmacological effects. A table comparing the effects for various narcotic analgesia agents is also included in Appendix G. Blank space indicates that no such activity has been reported. Information regarding chemical radicals and comparison of effects from narcotic analgesia agents are presented to support the validity of the equianalgesia scoring system.
Figure 2. Chemical Structure of Morphine

(Goodman and Gilman, 1975, p. 246)
An interrater test was conducted to determine the accuracy and consistency with which the researcher retrieved information from the patient's chart and the accuracy of using the equianalgesia scoring table. The same charts and raters used for the Burn Severity Index were used for the Analgesia Equivalency Score. However, accumulation of information for the two scales was conducted consecutively instead of simultaneously. An interrater agreement consistent with the Burn Severity Index was sought and obtained. There was 100% agreement in the daily Equianalgesia Score between the raters/observers at both Barnes Hospital and the University of Missouri. When the information was carefully observed, some discrepancies were found in the data transferred from the patient chart to the observer/rater forms. However, none of the differences was of sufficient magnitude to alter the range of equivalent.

**Data Collection Protocol**

Subjects entering the clinical settings that met the delimitations for the study as determined by the researcher and/or research assistant were approached to participate in the study. Data for the three variables were collected according to the methodological guidelines. Pertinent information was recorded on the Data Collection Sheet (See Table 7). It is important to note that only one BSI is obtained from each patient compared to a series of information for the β-endorphin levels and equianalgesia scores. The sequence listed below was followed for each subject:
Table 7. Data Collection Sheet

<table>
<thead>
<tr>
<th>Admission Date</th>
<th>Total Body Surface Area</th>
<th>BSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Partial-Thickness</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Full-Thickness</td>
<td></td>
</tr>
<tr>
<td>Hospital Number</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dates</th>
<th>Days Post Burn</th>
<th>Adm. Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endorphin Levels</td>
<td>pg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Time Drawn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Special Information about Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AES</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A. Admission - Blood sample was drawn, processed, and stored. Permission was obtained from individuals with minor burns. If patient was incoherent, blood samples were drawn and saved until patient was physically able to give permission or refused to participate in the study. In some instances, permission was obtained from a family member.

B. First DPB - Blood sample was drawn with routine a.m. blood analysis prior to wound manipulation. Sample was processed, stored, and specific information recorded on Data Collection Sheet.

C. Second DPB - Blood sample drawn, processed, stored and recorded.

D. Third DPB - Blood sample drawn, processed, stored and recorded.

E. Fourth DPB - Blood sample drawn, processed, stored and recorded.

F. Fifth DPB - Blood sample drawn, processed, stored and recorded.

G. Sixth DPB - Blood sample drawn, processed, stored and recorded.

H. Seventh DPB - Blood sample drawn, processed, stored and recorded.

I. Ninth DPB - Blood sample drawn, processed, stored and recorded.
J. Eleventh DPB - Blood sample drawn, processed, stored and recorded.

K. Thirteenth DPB - Blood sample drawn, processed, stored and recorded.

L. Fourteenth DPB - The researcher obtained information from the chart necessary to complete the Burn Severity Index and the Analgesia Equivalency Scores. All samples and information were stored at the University of Missouri until completion of data collection.

Protection of Human Subjects

The purposes and anticipated outcome of the study were discussed with the patients and family members that met the delimitations. A consent form was prepared and signed according to the guidelines of the educational and clinical institutions involved. Patients were informed that they could withdraw from the study at any time without untoward effects on continued therapy. Risk factors and measures taken to alleviate these possibilities were discussed. Approval notification forms from the University of Arizona, University of Missouri Hospitals and Clinics, and Barnes Hospital are included in Appendix C.

Statistical Analysis

Descriptive, quantitative analysis of data from the 28 patients previously presented began with a univariate procedure performed on data from all three variables to determine the amount of
skewness, extreme values, and to check for normality. Descriptive statistics including the mean, standard deviation, median, and range for each of the variables were obtained. The BSI yielded only one score from each patient. The β-endorphin levels and AES's were obtained over a two-week time interval with the possibility of 11 data sets. Plots of AES with BSI for each time period, β-endorphin levels on admission and β-endorphin levels on days 1 through 13 with BSI, and AES with β-endorphin levels for each time period were constructed to determine if any relationship among the variables might be apparent.

Spearman Correlation Coefficients were determined for the BSI with β-endorphin levels, BSI with AES's, and β-endorphin levels AES's all over the two-week time interval. The Spearman Correlation was chosen as the appropriate measure because of randomization of subjects and the small sample size.

Due to an interest in visualizing the β-endorphin levels over time, individual plots were constructed for all patients with six or more data samples available. Data analysis includes a description for each of the three referentials presented in the conceptual model. The description will be followed by a presentation of the comparative findings which are organized according to the purposes of the study.

**Summary**

Chapter III outlines the design and methodology which was followed for data collection. The sample is delineated that was selected from the burn settings. Use of the Burn Severity Index,
β-endorphin levels, and Analgesia Equivalency Scores as referentials is clearly specified. Tables are utilized to exemplify the referentials. Validity and reliability issues for each variable are addressed. Both information from literature and interrater reliability performed by the researchers comprise the support for validity and reliability of the referentials.

A step by step protocol as to the data collection activities is included. A summary statement regarding human subjects and a plan for statistical analysis of the data were presented.
CHAPTER IV
DATA ANALYSIS

This chapter contains a description of the data obtained for each referential and descriptive analysis of the data for each purpose. Data were derived from 28 patients obtained as a convenience sample from two clinical settings as described in the previous chapter. Findings of the investigation will be addressed, for each of the following purposes, to: (1) describe the pattern of \( \beta \)-endorphin levels in burn injured patients during the first two weeks following injury, (2) describe the relationship between \( \beta \)-endorphin levels and the severity of the burn injury, (3) describe the relationship between analgesia taken by patients and the severity of the burn, and (4) describe the relationship between \( \beta \)-endorphin levels and the amount of analgesia given to the burned patients.

**Burn Severity Index**

Data contributed from the 28 patients for the concept, burn injury, included age, sex, total body surface area (TBSA) burned, full-thickness injury and presence of inhalation injury. The data were utilized to obtain the Burn Scverity Index (BSI) score which was a one time score for each patient.

As delineated in Table 1, the study is inadvertently biased for men and early adult age. A total of 26 men and 2 women comprised
the sample with 53.5% of the patients falling between the ages of 21 to 30. Of these 53.5%, 50.5% were men. An additional 5 male patients were between the ages of 31 to 40. Thus, 71.4% of the patients selected for study ranged between ages 20 to 41. Gender and age were not analyzed specifically as variables; however, both factors were utilized for computing the BSI score. None of the variables considered in the study was assumed to be gender or age specific.

The range for TBSA injured in the sample was from 5.75% to 86.5%. Specific TBSA injuries were placed in categories as designated in the BSI, Table 2. Of the 28 patients in the sample, 11 (39.3%) fell in category 1 (1-10% TBSA). This group included 1 female. An additional 6 patients (21.4%) were included in category 2 (11-20% TBSA). Thus 17 patients (60.7%) had less than 20% TBSA injury. The remainder of the group ranged from a total of 1 to 4 patients in categories 3 through 9 except for category 7 (61-70%) which contained no patients. The above data are delineated in Table 8.

The range of full-thickness injury was from .7 to 77% of the TBSA injuries described. This range of full-thickness injury was manifested in 18 (64.3%) of the total patients in the sample.

Four of the 28 patients (14.3%) had simultaneous inhalation injury. Two of these patients had information in their chart which met all four of the criteria specified on the BSI, including history of closed space, facial burns with singed nasal hair, carbon stain on oral mucous membranes or in the sputum and stridor or labored
Table 8. Distribution of Total Body Surface Area Burned by Sex and Agency.

<table>
<thead>
<tr>
<th>TBSA</th>
<th>St. Louis</th>
<th>Columbia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>1-10%</td>
<td>3 (10.71%)</td>
<td>*8 (28.57%)</td>
<td>*11 (39.29%)</td>
</tr>
<tr>
<td>11-20%</td>
<td>2 (7.14%)</td>
<td>4 (14.29%)</td>
<td>6 (21.43%)</td>
</tr>
<tr>
<td>21-30%</td>
<td>2 (7.14%)</td>
<td>2 (7.14%)</td>
<td>4 (14.29%)</td>
</tr>
<tr>
<td>31-40%</td>
<td>1 (3.57%)</td>
<td>0</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>41-50%</td>
<td>1 (3.57%)</td>
<td>2 (7.14%)</td>
<td>3 (10.71%)</td>
</tr>
<tr>
<td>51-60%</td>
<td>0</td>
<td>*1 (3.57%)</td>
<td>*1 (3.57%)</td>
</tr>
<tr>
<td>71-80%</td>
<td>0</td>
<td>1 (3.57%)</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>81-90%</td>
<td>0</td>
<td>1 (3.57%)</td>
<td>1 (3.57%)</td>
</tr>
</tbody>
</table>

Total N = 9 (32.14%) N = 19 (67.86%) N = 28 (100%)

Range of TBSA: 5.75% - 86.5%

* = 1 Female in group
breathing. Two patients had the first three criteria but did not manifest stridor or labored breathing. Only three criteria were necessary to identify the presence of inhalation injury.

The BSI was obtained on each of the 28 patients and ranged from a minimum of 3 to maximum of 15 (See Table 9). The lowest score attainable with the index is 2 with 18 the highest number possible. The mean for the total group was 6.18 with a standard deviation (S.D.) of 3.03. The median for the group was 5. The issue of non-variance is reflected in the range, mean, and S.D.

**β-Endorphins**

β-endorphin levels for each plasma sample obtained from the 28 patients in the study were reported in pg/ml of plasma and comprise one index for the concept, pain, in the conceptual model. Blood samples were obtained on admission, daily for 7 days, and every 48 hours for an additional 7 days. A maximum of 11 samples were possible from each patient. All admission samples were drawn within 24 hours of the injury. Even the one patient that did not have an admission sample designated, the first blood sample drawn and identified as the sample for day 1 was drawn before the end of the 24 hours following injury.

Table 10 presents the distribution of samples obtained from the 28 patients. There were admission samples only drawn from 6 patients. Two patients provided 2 samples, 2 patients provided 6 samples each, 1 patient provided 7 samples, 3 patients provided 9 samples each, and 2 patients provided 10 samples each. A total of 12
Table 9. Distribution of Burn Severity Index by Sex and Agency.

<table>
<thead>
<tr>
<th>BSI</th>
<th>St. Louis</th>
<th>Columbia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td></td>
<td>3 (10.71%)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>(10.71%)</td>
<td>*5</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>(7.14%)</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>(7.14%)</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>(3.57%)</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>(3.57%)</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td></td>
<td>*1</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Total  N = 9 (32.14%)  N = 19 (67.86%)  N = 28 (100%)

Range of BSI : 3 - 15

* = 1 Female in group
Table 10. Distribution of the Number of Samples Obtained for β-Endorphin Levels from each of the 28 Patients by Number of Days Samples were Collected for Each Agency.

<table>
<thead>
<tr>
<th>No. of Possible Samples</th>
<th>Total of Samples Collected (%)</th>
<th>Missouri University No./%</th>
<th>Barnes General Hospital No./%</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Adm</td>
<td>6 (21.43%)</td>
<td>6 (21.43%)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2 (7.14%)</td>
<td>2 (7.14%)</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>2 (7.14%)</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>1 (3.57%)</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>3 (10.71%)</td>
<td>3 (10.71%)</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>2 (7.14%)</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>11</td>
<td>13</td>
<td>12 (42.86%)</td>
<td>5 (17.86%)</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>28 (100%)</td>
<td>19 (67.86%)</td>
</tr>
</tbody>
</table>
patients (42.9%) were accessible for the full set of samples planned for the study.

Of the 16 patients that did not yield complete sets of data, 3 patients refused to participate in the study but gave permission for the samples to be obtained on admission to be analyzed and reported. A total of 11 patients were discharged and 2 patients expired before all data could be collected. Isolated missing samples which occurred when the centrifuge did not work, when the patients were in the operating room for extended periods of time, or when it was impossible to draw enough whole blood to retrieve the adequate amounts of plasma are not delineated in the table.

The range of β-endorphin levels for all samples during the two-week interval was from 4 to 500+ pico grams per milliliter (pg/ml) of plasma (Table 11). This reflects an obvious increase above the normal range of 0-15 pg/ml reported in literature (Csontos et al., 1979; Wardlaw & Frantz, 1979; Colt, Wardlaw, & Frantz, 1981; Thomas, Fletcher, & Hill, 1982).

**Analgesia Equivalency Score**

The Analgesia Equivalency Score (AES) was obtained from data recorded on the patient's chart and represented the second referential for the concept, pain, identified in the conceptual model. These data were not retrieved from the chart until all possible blood samples had been drawn. A concerted effort was made to include all narcotic analgesics taken by the patients 24 hours before the blood sample was drawn for β-endorphin analysis. Therefore, an
Table 11. Central Tendency and Dispersion for \( \beta \)-Endorphin Levels
Grouped Together by Days Post Burn.

<table>
<thead>
<tr>
<th>( \beta )-Endorphin Levels</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adm.</td>
<td>27</td>
<td>116.59</td>
<td>125.13</td>
<td>84</td>
<td>4</td>
<td>500</td>
</tr>
<tr>
<td>Day 1</td>
<td>22</td>
<td>75.73</td>
<td>45.29</td>
<td>66</td>
<td>6</td>
<td>163</td>
</tr>
<tr>
<td>Day 2</td>
<td>20</td>
<td>77.25</td>
<td>59.99</td>
<td>59</td>
<td>7</td>
<td>214</td>
</tr>
<tr>
<td>Day 3</td>
<td>20</td>
<td>69.45</td>
<td>53.34</td>
<td>55</td>
<td>5</td>
<td>181</td>
</tr>
<tr>
<td>Day 4</td>
<td>20</td>
<td>86.95</td>
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admission AES was not available. The anticipated pattern for the AES was an increased amount for the first 2 to 3 days post burn followed with a gradual decline over the remaining two-week interval.

Table 12 presents the numerical information for the mean, standard deviation (S.D.), median, and range for the AES's obtained over days post burn (DPB). The range of AES for the 28 patients was from 2 to 7. The mean for the total scores on the first DPB was 2.27 with a S.D. of 1.78. A slight rise occurred on the second DPB (mean = 2.35, S.D. = 1.31). A decline occurred on the third DPB (mean = 1.55, S.D. = 0.89). A mean range of 1.42 to 1.80 was maintained until the thirteenth DPB when there was another decline (mean = 1.00, S.D. = 0.74). A bar graph depicting the distribution of the mean AES's over the two-week interval for all 28 patients is presented in Table 13.

The Spearman correlation was used to determine the relationship between days for the AES's. Significant relationships (p = .05) were identified in the matrix (See Table 14). Some meaningful patterns have emerged from this variable. Day 1 correlated with day 4 (r = .49, P = .05) and day 5 (r = .83, P = .001). Day 3 correlated with day 6 (r = .72, P = .003). Day 4 correlated with day 5 (r = .73, P = .002), day 6 (r = .52, P = .05), and day 9 (r = .69, P = .009). Day 5 correlated with day 6 (r = .54, P = .05) and day 7 (r = .83, P = .001). Day 6 correlated with day 9 (r = .58, P = .03) and day 11 (r = .66, P = .03). Day 7 correlated with day 11 (r = .71, P = .02). Day 9 correlated with day 11 (r = .97, P = .0001) and day 13 (r = .71, P = .05), and day 11 correlated with day 13 (r = .76, P = .03).
Table 12. Central Tendency and Dispersion for Analgesia Equivalency Scores Grouped Together by Days Post Burn.

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Table 13. Distribution of Mean Analgesia Equivalency Scores for 28 Patients by Days Post Burn.
Table 14. Spearman Correlation Coefficients for Analgesia Equivalency Scores Grouped Together by Days Post Burn.

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</table>
In summary, the AES's followed to some degree the anticipated pattern of progression over the two-week interval post burn injury. Also, moderate correlations are reflected between consecutive days which indicate that the amount of analgesia taken on one given day post burn had some relationship with the amount of analgesia taken on the immediately following days post burn.

**Pattern of β-Endorphin Levels**

β-endorphin levels for each patient were aggregated and analyzed for central tendency and dispersion from admission through the thirteenth DPB (See Table 11). The mean β-endorphin levels on admission were 116.13 with a S.D. of 125.13 followed with a reasonably gradual decline until the ninth DPB (mean = 57.50, S.D. = 39.04). The change reflects a 44% decline over 8 days. The observed incline on days 11 (mean = 64.46, S.D. = 52.33) and 13 (mean = 99.18, S.D. = 69.03) was not anticipated. Two bar graphs are presented in Table 15 to picture more clearly the compared pattern of the mean and median distribution of β-endorphin levels over the two-week interval.

Spearman correlation was used to determine the relationship of β-endorphin levels between days for all patients over the two-week interval. A matrix presenting this data is available in Table 16. One significant correlation was found between β-endorphin levels on admission and day 11. Since no pattern is evident with surrounding correlations, this is viewed as a random, isolated event.

The non-occurrence of significant correlations and subsequent
Table 15. Distribution of β-Endorphin Levels over Days Post Burn: Mean and Median

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Table 16. Spearman Correlation Coefficients for β-Endorphin Levels Grouped Together by Days Post Burn.

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<th>Day 6</th>
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lack of pattern emergence prompted the plotting of β-endorphin levels individually for those patients with six or more sets of data. This yielded a total of 18 plots which are presented individually in Appendix H. These plots have a common axis of 0 to 300. Outliers above 300 are identified. The goal for this exercise was to determine subsets which could possibly have some visual/common pattern development.

Only one subset was identified which included those patients admitted with high β-endorphin levels that decline over time (patients 1, 6, 12, 19, and 21). This subset is presented in Figure 3 with the plots of all five patients to demonstrate the occurrence of the pattern over time. The remaining plots contained such erratic peaks and troughs that no pattern could be discerned.

In summary, the most meaningful information derived from this purpose is the finding that β-endorphin levels are elevated above normal following burn injury. Another finding is that β-endorphin levels are extremely variable over time with this group of burned patients.

Relationship Between β-Endorphins and BSI

Correlation coefficients were estimated between β-endorphin levels over the two-week interval post burn and the BSI (See Table 17). It was anticipated that these two variables would have high correlations, especially on admission and day 1 and 2 DPB because the BSI is taken only on admission. Instead, the correlations obtained did not support the possibility of any significant relationships between these two referentials.
Figure 3. This subset includes individual plots for those patients that were admitted to the burn unit with higher \( \beta \)-endorphin levels that declined over the two weeks post burn injury. Patients in this group include 1, 6, 12, 19, and 21.
Figure 3. Subset of Individual Patient Plots

- = Patient 1
○ = Patient 6
■ = Patient 12
□ = Patient 19
▲ = Patient 21
Table 17. Spearman Correlation Coefficients Between β-Endorphin Levels and the Burn Severity Index Grouped Together by Days Post Burn.

<table>
<thead>
<tr>
<th>BSI</th>
<th>Adm.</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 9</th>
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<tr>
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<td>-0.06</td>
<td>0.07</td>
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</table>
Relationship Between AES's and BSI

The correlation coefficients between the AES's and BSI over time are presented in Table 18. Again, it was anticipated that these two referentials would have high correlations on the first 2 days. It is clear from the matrix that there are no significant correlations. Therefore, it is assumed that a meaningful relationship between these two referentials is absent. It is probable that the nonvariability for the AES's affected the correlational results.

Relationship Between β-Endorphin Levels and AES

Correlational coefficients were determined between β-endorphin levels and the AES's from all 28 patients over the two-week interval. This matrix is presented in Table 19. Several significant correlations are isolated, i.e., β-endorphin level 7 with AES day 2 (r = .51, p = 0.04), β-endorphin level 13 with AES day 3 (r = .71, p = 0.01), β-endorphin level 10 with AES day 4 (r = .71, p = .01), and β-endorphin level 5 with AES day 9 (r = .60, p = .03). These correlations are scattered and appear to be reflective of chance since a particular pattern does not emerge. In summary, there is no evidence of meaningful relationships between these two referentials, β-endorphin levels, and the AES by DPB.
Table 18. Spearman Correlation Coefficients Between Burn Severity Index and Analgesia Equivalency Scores Grouped Together by Days Post Burn.

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Table 19. Spearman Correlation Coefficients Between β-Endorphin Levels and the Analgesia Equivalency Scores Grouped Together by Days Post Burn.

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Summary

This chapter described the data obtained from 28 burned patients that address the three referentials selected for research. Descriptive statistical analysis of the data is included in narrative and table form. Even though few of the relationships for each purpose studied were significant, the data are presented as estimated.

The data obtained from this group of patients (N = 28) failed to demonstrate a consistent pattern of β-endorphin levels across all subjects over time. One subset with the anticipated pattern was identified. Overall, β-endorphin levels were elevated above normal which is clear from the statistics derived from central tendency analysis as well as from individual graphic (plot) presentations.

Data analysis described will be presented as findings and compared to clinical information in the following chapter. The comparative findings will provide the basis for conclusions, implications, and recommendations.
Summary and discussion of the findings for the research effort will be presented for each referential with inclusion of relevant clinical events. Discussion of the findings related to the design and conceptual model follows. Conclusions are presented based on analysis of the data obtained. Implications for nursing and recommendations for further study provide closure for the chapter.

Findings, Discussion, and Conclusions

Burn Severity Index

Discussion of the demographic factors which provide some basis of understanding for the findings of the study are incorporated in the BSI. The major problem associated with age, sex, and burn injury is that the sample was skewed to young, male patients (71.4% between ages 21-40) with minor burn injuries (60.7% < 20% TBSA). To provide an even distribution for each of these factors, an alternative would have been to identify and include only those numbers of patients desired in each category of age range, sex, and TBSA. Since the picture obtained is reflective of national statistics (Artz et al., 1979), an extended study would be requisite
with criteria placed on the number of patients needed in each category.

The BSI score was acquired as a one-time reading for each patient and correlated with the β-endorphin levels and AES, which provided data over a two-week time interval. It was anticipated that the higher BSI scores would correlate positively with high β-endorphin levels and increased AES's, especially for the admission data. Correlations between the BSI and β-endorphin levels as well as AES were not found to be significant at any time. These correlations may have been affected by the nonvariance of the BSI scores for this particular sample of burn patients. The BSI scores, too, could have benefited from predetermined criteria factors.

Another issue worth consideration is whether the use of an index that incorporates age, sex, TBSA, full-thickness injury, and inhalation injury (all excellent predictors of survival) is of greater or lesser value than viewing each of these variables individually for correlates with other variables. Complete reorganization of the data obtained and reanalysis may provide some insight related to this concern.

β-Endorphin Levels

The anticipated pattern of change for the β-endorphin levels in burned patients over the two-week interval post injury included a marked elevation on admission and possibly through day 2 post burn, followed by a gradual decline over the remaining two-week interval. The major finding for the variable, β-endorphin levels, is that the
levels were elevated above normal limits during the two-week time interval following burn injury. These elevations are difficult to explain in relation to pain alone since the correlations with AES's, another pain indicator, were not significant. Also, the issue that pain is greater with the magnitude of injury as reflected by the BSI, thus stimulating increase in \( \beta \)-endorphin levels, did not hold since the correlations between \( \beta \)-endorphin levels and BSI were not significant at any time. Documentation of perceived pain was not included in this research, so it is possible that the evident elevations in \( \beta \)-endorphin levels may still relate to some aspect of pain, other stressful events, and/or a combination of stressful events with pain. \( \beta \)-endorphins may not be an appropriate index for pain. Examples of additional stressful, simultaneous events may be tissue injury, surgical procedures, treatment modalities, and hospitalization.

Another finding is the extreme variability in \( \beta \)-endorphin levels both within and between patients. The variability is evident in the range (4 to 500+pg/ml), the high standard deviations reported (Table 10), and visual observations of individual plots. Since the study design is unique in regard to the sample and the data collection over time, it is not possible to obtain clearly delineated support from the literature for these findings. Support is present for elevations in \( \beta \)-endorphin levels following stressful events (Akil et al., 1976; Lewis et al., 1981; Dubois et al., 1981). There is also some indication in literature that change in \( \beta \)-endorphin levels, following a stressful event, can occur rapidly (Stewart, 1980). Due
to the published research that sudden peaks in β-endorphins are precipitated by stressful events in addition to pain, the sudden peaks in β-endorphin levels noted on individual patient plots were compared to tabulated clinical events on the data collection sheets prepared for each patient. There were some clinical happenings that coincided with peaks in β-endorphin levels. Some examples of peaks visible in individual patient plots, Appendix H, include: Patient 1 was admitted with severe burns following a suicide attempt (admission β-endorphin level = 500+ pg/ml); patient 6 was admitted following a homicide attempt by a brother (admission β-endorphin level = 500+ pg/ml); patient 11 died within 15 minutes of the last sample drawn (β-endorphin level, day 13, = 175 pg/ml); patient 15 had a psychiatric consult on day 6 post burn (β-endorphin level, day 6, = 270 pg/ml); patient 19 acquired two fractures in combination with the burn injury (admission β-endorphin level = 275 pg/ml); and patient 25 had an elevated β-endorphin level preoperatively (β-endorphin level on day 6 = 309 pg/ml). While these are isolated events and not generalizable, the identification of concomitant clinical situations may have future relevance when planning additional research to relate biochemical findings to clinical situations.

The excitement of some positive clinical findings, even though devoid of statistical significance, is tempered with concerns regarding methodological issues. A fairly large number of sudden declines, troughs, in β-endorphin levels to the normal range (0-15 pg/ml) required consideration. Examples of these are clear in the individual plots for patients 10, 11, 15, 18, 19, 25, 26, and 28 (See
Appendix H.). Conversely, one extreme elevation (500+ pg/ml) of β-endorphin in patient 24 was keyed back to the data collection sheet on a day that the research assistant identified a 1.5-hour delay in getting the plasma sample to the freezer. The incident reflects concerns in literature that delay in the freezing of plasma samples will allow breakdown of precursor β-endorphin molecules present (Pert & Snyder, 1974).

Due to the lack of funding for this expensive assay and the extreme distance between data collection sites and the biochemical laboratory, methodological studies which could have provided insight into the magnitude of the problem were not conducted. For example, a comparison of procedures for acquiring and processing samples between the research assistants at the two clinical agencies was not performed.

The New England Nuclear β-Endorphin Kit is readily accessible for purchase by researchers that are utilizing β-endorphin analysis as a variable in research. Cross-reactivity and sensitivity is a problem with this assay as described earlier in this paper (Thomas et al., 1981). A possible contribution to this low sensitivity may be the extraction process. Developers of the kit have not designated an explicit extraction procedure that is successful with this product (Appendix E). The extraction protocol utilized for samples drawn from the burned patients was developed and tested by Cahill et al. (1983). This protocol was used two times by the researcher, prior to extraction of patient samples, as well as by Dr. Eskelson, and was found to be satisfactory for β-endorphin recovery. Nevertheless,
refinement and comparison of the extraction procedure with other processes, as well as more careful specification of techniques such as time intervals for filtration, would yield greater confidence in obtained results as recovery patterns are improved for β-endorphins.

Sensitive assays with reported low cross-reactivity have been developed by biochemists that have devoted their research efforts to β-endorphins (Csontos et al., 1979; Akil et al., 1982; Pert & Snyder, 1984). Animal models, such as rabbits, have been used to produce sensitive antibodies. These antibodies are retained by researchers for future personal use and not available for purchase.

Analgesia Equivalency Score

The AES was chosen as a referential for the concept pain after completion of the pilot study. The scale was proposed for use as designed by Goodman and Gilman (1975) and Beaver (1980). The major concern with this scoring system is the issue of sensitivity. It was noted while using the scale to tabulate the narcotic analgesia taken by the burned patients that a clear ending and beginning in range overlaps for two categories. For example, 10-20 mgs. = 2, 20-30 mgs. = 3. The problem arises as to which score to use, 2 or 3, for 20 mgs. of narcotic analgesia. Also, discrimination is weak since the patient taking 20.5 mgs. of analgesia would receive a score of 3 as would the patient that took 29.5 mgs. over a 24-hour period.

Despite the above concerns, some correlations between AES's over days were significant and patterns were beginning to emerge which indicated that amounts of analgesia taken on a given day did
influence the amounts taken on subsequent days. Correlational findings between the AES and BSI, as well as AES with β-endorphin levels, were not significant. Continued use of this score as an index for pain in burn patients is questionable until comparative studies have been made between the AES and a valid, reliable pain scale. Also, clarifying the categories for the scale is requisite. Increasing the categories by reducing the span would be one method of increasing the sensitivity for the scoring system.

Conclusions

Two issues can be concluded from the research activities and analysis of the data collected from 28 burned patients. Firstly, it can be concluded that burn injury precipitated an elevation in β-endorphin levels over the two-week interval post burn. Secondly, the β-endorphin levels were variable over the two-week interval.

Design Issues

A descriptive, causal modeling/correlational design was appropriate for this study based on the current status of information regarding clinical relevance of β-endorphins. The sample was a purposive convenience sample which resulted in weighting of patients toward minor burn injury and young age. This skewness reduced variability in two referentials, AES and BSI. The protocol was readily implemented in both clinical agencies with adequate flexibility to accommodate clinical problems.
Conceptual Model Issues

The conceptual model (Figure 1) which directed the research activities was derived from literature and knowledge from clinical practice. Previous testing had not been performed. The referential, BSI, was selected to index the concept, burn injury. Even though the index incorporated major variables which are known predictors of survival from burn injury, the index did not correlate with the two indices for pain, β-endorphin levels, and AES. Thus, the two relational statements, theorems, are not substantiated with this sample of patients.

β-endorphin levels as an index, referential, for pain were elevated, which suggests that β-endorphin levels may reflect pain which is identified as a biopsychosociocultural reaction. However, correlations between the AES, a second referential for pain, were mostly nonsignificant, which may indicate that additional indices need to be tested for pain in combination with β-endorphin levels or that β-endorphins are indexing other biopsychosociocultural reactions in addition to, or in combination with, pain. The possibility also exists that β-endorphin levels are not a valid index for pain.

Before utilizing this conceptual model to guide future research, a careful review of substantive bases must be conducted and the relational statements reconsidered. It is difficult to determine if the problem rests with the instruments used in this research or if the relational statements are not appropriate. Consideration will be given to development of the identified constructs, concepts and referentials into a feedback model. β-endorphin activity is
progressively linked to neuro-endocrine activity which functions from a feedback system (Krieger, Yamaguchi, & Liotta, 1981). Therefore, with such background information, plus the findings from this investigation which indicate that β-endorphin levels change rapidly from a probable wide range of stimuli, use of a feedback system for a conceptual framework will also be explored.

Implications for Nursing Practice

Identification and explanation of the role of β-endorphins in the human body have occurred during the last decade. Only within the last two to three years has information regarding the relevance of β-endorphins to clinical practice been addressed (Thomas et al., 1982; Dubois et al., 1981; Matthews et al., 1982; Cahill & Akil, 1982). Information regarding the role of β-endorphins has appeared in nursing literature with emphasis on opiate activity (Huhman, 1982; Zaloga et al., 1984). This emphasis is reflective of literature in general. However, the question is now being raised regarding other stressful events which may precipitate elevation of β-endorphins. It must be remembered that pain is a stressful event, but elevated β-endorphin levels have been found in patients before pain has been experienced by the patients (Cahill, 1983). Since nurses are constantly in touch with individuals experiencing varied stress-producing events, they should be apprised of the associated research.

In addition to searching for the trigger mechanism for β-endorphins, researchers are looking more closely at biopsychosociocultural reactions which may be associated with
elevated β-endorphins. Some examples include perpetuating shock syndromes (Gahhos et al., 1982), increasing depression (Matthews et al., 1982), and decreasing hemodynamic parameters (Lang et al., 1982). This global view would initiate speculation that elevated β-endorphin levels may be both therapeutic and nontherapeutic.

An example of untoward or nontherapeutic physiological activities associated with elevated β-endorphin levels has been studied in the shock patient. Shock syndromes precipitated by infection, fluid imbalance, and neurological or cardiovascular disorders are frequently irreversible. Recognition that elevated β-endorphin levels decrease blood pressure, decrease heart rate, decrease respiratory rate, and are associated with high cortisol levels (Gahhos et al., 1981; Dubois et al., 1981) led to speculation that β-endorphins perpetuate the shock syndrome by not allowing the body to respond normally due to decreased hemodynamics. This research is especially vital to nurses since they daily assess the physiological hemodynamic parameters identified. The associated implication then is that nurses be knowledgeable regarding these recent findings, especially when they are caring for patients with documented elevations in β-endorphins. The overall concern for nursing would be, "How can nursing action alter nontherapeutic β-endorphin levels or enhance therapeutic activities of β-endorphins?" Certainly, to arrive at answers, such a multifaceted question requires continued clinical nursing research and astute nursing observations.
Since common nursing responsibility includes pain management, especially in patients that have tremendous tissue injury such as burns, application of knowledge regarding β-endorphin levels to pain management is a key issue. Even though this research did not clearly link β-endorphins to pain, the findings did support the presence of elevated β-endorphins in burn-injured people. If β-endorphins modulate pain and the levels are elevated, what role does exogenous morphine play in diminishing painful stimuli? The question is speculative and currently unanswered.

To further extend the issue of pain management, it is important to consider the information that the body contains receptors for narcotic analgesia and non-narcotic analgesia (Mayer & Watkins, 1981). If this information is accurate, would patients that have elevated β-endorphin levels receive greater benefit from non-narcotic analgesia during the interval that β-endorphins are above the normal limits? Again, continued research and careful observation by nurses will provide insight into this concern.

Findings from this investigation support the biopsychosociocultural (holistic) construct for nursing practice. Since significant relationships were not established between the physiological referentials, it is requisite to speculate regarding other factors which influence change in β-endorphin levels in the burned patient. Some individual, incidental clinical events were linked with elevated β-endorphin levels in patients which comprised the sample for this study. These events could be categorized as biological and as psychosociocultural. Therefore, the nurse
practitioner and nurse researcher must continue to identify holistic events which may influence β-endorphin levels in individuals experiencing severe injury.

The issue could be raised regarding the psychosociocultural concept of control and the relationship to pain. Current, research supports the premise that patient control of the amount and time analgesia is administered via a computerized intravenous system results in less analgesia administered and greater relief from pain (Bennett & Griffin, 1983). Given the opportunity to control management of painful stimuli, will patients be able to critically analyze and evaluate biopsychosociocultural response?

There are many and varied ways that nurses can perceive the overall implications for β-endorphins, the issue of biopsychosociocultural reaction, and the relationship to management. β-endorphins are only one minute segment of a complex neuro-endocrine system. Nevertheless, the role which β-endorphins play in overall body function and response may be of great magnitude.

**Recommendations for Further Study**

Even though findings related to correlation of β-endorphin levels over the two-week interval post burn with BSI and AES were not significant, research should continue regarding the relevance and implications of β-endorphin levels to clinical practice. Certainly, the findings of increased β-endorphin levels in burned patients over the two-week interval post burn and the frequent erratic patterns
demonstrated which corresponded to some clinical events, plus current literature, stimulate interest for continued clinical investigation.

The two major issues which arise when reviewing the many events associated with the completed research findings center around methodological issues and control of clinical variables. As mentioned previously in this chapter, the use of the New England Nuclear β-Endorphin Kit is reasonable for isolated research studies; however, if long-term clinical research with β-endorphins is anticipated, careful consideration should be given to acquiring antibodies that would provide higher sensitivity and lower cross-reactivity (Hollt et al., 1978; Nader et al., 1980; Cahil et al., 1983). The use of these antibodies for developing assays greatly reduces the cost when compared to the commercial kits. If developing antibodies is not a feasible function, combining research efforts with individuals who have developed satisfactory assays would be a reasonable alternative.

Regardless of the direction taken for assaying samples, refinement of the many facets associated with sample acquisition and processing, plus the extraction process, is in order. Comparing techniques for sample retrieval, time intervals, and use of specific equipment and supplies are requisite. Results from such comparative studies would provide insight into changes that can be tolerated in the protocol when samples are acquired in a clinical setting. For example, it would be helpful to know the length of time a sample can be stored on ice before breakdown of the β-endorphin precursor molecule occurs. Also, the difference in β-endorphin recovery needs
to be determined when a refrigerated or a nonrefrigerated centrifuge is used for processing the sample.

Comparing times, methods, and chemicals for extraction would also be invaluable. Even though the protocol for this research had been tested before publication (Cahil & Akil, 1982) and previously tested by this researcher, discrepancies regarding minimal filtration time for plasma, procedure for lyophylization of the eluate, and the amount of solution for reconstitution prior to assaying were issues that required discussion. These issues may have been instrumental in altering the β-endorphin recovery which was reflected in decreased β-endorphin levels.

The issue of specifying criteria and controlling variables has previously been raised in this chapter. Since speculation remains regarding many issues related to β-endorphins, the most desirable way to control variables is via animal research (Cunningham & Mitchell, 1982). Even though information retrieved from animal studies is not directly transferable to human clinical situations, the data would provide guidelines for additional clinical studies.

An alternative to animal research is controlling clinical variables to the most possible extent and greatly increasing the number of patients planned for study. For example, instead of limiting future clinical studies to burn patients, multiple trauma victims could be studied.

A number of possible clinical studies could be pursued that would clarify or exemplify the reported findings. Several key issues
include the determination of β-endorphin levels at more frequent intervals, and comparison or differentiation of pain with other stressful events. Careful tabulation of events with several patients in conjunction with obtaining frequent blood samples (every 1 to 2 hours) would assist in explaining the varied β-endorphin levels obtained with the burn patients over the proposed intervals in this research. Careful documentation of events observed or declared by the patient would provide insight into simultaneous changes in β-endorphin levels.

Certainly, comparison between β-endorphin levels and subjective pain scales would provide insight into patient perception of pain. This type of comparison could be conducted during intervals of wound manipulation such as dressing changes and debridement. To link pain perception and β-endorphin levels with stress response, comparison could be made between cortisol levels and β-endorphins (Fraioli et al., 1980; Holaday et al., 1979), as well as comparisons between subjective indices for pain and stress.

Even though more questions were raised than answered regarding the relevance of β-endorphin information to nursing, the underlying message remains that nurses be knowledgeable regarding current and ongoing research. In addition, since nurses are at the bedside to observe patient behavior, active involvement in clinical research regarding β-endorphin levels would be appropriate and extremely challenging, thus promoting the science of nursing and providing basis for nursing for actions.
APPENDIX A
THEORETICAL FRAMEWORK

The theoretical framework proposed for this study, as well as future research, is a Gibbs (1972) format. The underlying basis for statements in the model have been derived from literature concerning stress, reaction, response, adaptation, and body alterations resulting from trauma. Many and varied resources were utilized to derive the definitions and other componential statements. The review of literature reflects empirical observations and testing; however, the stated design has not been tested. As a beginning researcher, it is personally important to identify a framework that is consistent with information derived from literature and years of clinical practice and education. If the theory as proposed requires major alteration after testing, then it would follow that the rationale for clinical practice and teaching may also require rethinking and renovation. Therein lies the excitement and challenge of clinical research and clinical practice.

This theory is congruent with Gibb's definition of theory which includes "a set of logically interrelated statements in the form of empirical assertions about properties of infinite classes of events or things (Gibbs, 1972, p. 5)." Components of the model and statements which comprise the intrinsic and extrinsic theory are also
Stress Adaptation Model for Trauma Victims

Legend:
- **A**: Axiom
- **P**: Postulate
- **PB**: Proposition
- **TFS**: Transformational Statement
- **Th**: Theorem
- **TSl**: Trauma Severity Index
- **WCS**: Wound Culture & Sensitivity
- **BE**: Beta Endorphins
- **AES**: Analgesic Equivalency Score
- **ACTH**: Adrenocorticotropic Hormone
- **BW**: Body Weight
- **CBC**: Complete Blood Count
- **SSPEFR**: Signs and Symptoms for Physical, Emotional, and Psychological Response
consistent with Gibbs. A Stress-Adaptation Model for Trauma Victims provides a diagrammatic view of this framework. Statements of assumptions and values, along with the extrinsic theory, will provide some clarification for the empirical assertions and relational statements which comprise the intrinsic theory.

Stress adaptation model for trauma victims

Intrinsic theory includes the unit terms, temporal elements, substantive terms, and statements in the form of empirical assertions about properties of infinite classes of events or things (Gibbs, 1972). The extrinsic part of the theory includes the definitions of the terms used in the intrinsic theory and also includes procedural instructions and specifications to follow when testing the theory. The following paragraphs include assumptions and values, substantive terms, definitions, and brief information to support the statements.

The following assumptions and value statements are relevant to this theoretical framework:

1. Man is a biopsychosocial being in constant interaction with his environment. (Roy, 1976)

2. Adaptation is necessary at all times, to varying degrees. (Selye, 1976)

3. Adaptation is essential to personal development, progress, and simply adjusting to the business of daily life. (Mechanic, 1976)
4. The perception of the extent to which a particular stimuli or life event is stressful is idiosyncratic, differing from individual to individual. (Dohrenwend, 1974)

5. Qualitatively different stimuli of equal toxicity do not necessarily elicit exactly the same syndrome in different people.

6. Adaptive responses are observable.

7. The same degree of stress, induced by the same stimulus, may produce different lesions in different individuals. (Selye, 1956)

8. Each adult possesses unique variables which influence the adaptive response.

9. Health is synonymous with adaptation.

10. Endorphins are chemical indicators of pain.

11. Pain, hypermetabolism, and sepsis are common reactions to traumatic stimuli.

12. Trauma produces body reactions to which the body must adapt.

The unit term for this theory is "adults". They and their family members must be able to speak English and comprehend communication necessary to conduct the study.

The three substantive terms, stimulus, biopsychosociocultural reaction, and adaptation, will be defined and comprise the construct level of this theory. Construct is the term applied when a theorist regards the definition of a term as neither complete nor empirically applicable (Gibbs, 1972, p. 125).
A **stimulus** can include a factor, event, or variable which constitutes a change from the usual (Toffler, 1970). An external stimulus may be physical, environmental, social, or cultural in nature. An internal stimulus may arise from personal structural characteristics, psychological processes, physical growth and development, body repair mechanisms, behavioral characteristics, and physiological alterations which occur with a disease process or when usual health patterns are not followed (Dubos, 1965; Goosen & Bush, 1979).

**Biopsychosociocultural reaction** is immediate activity of the body to internal and/or external stimuli (Selye, 1977). Even though the body is viewed as a total unit and interrelatedness is acknowledged, ongoing clinical observations reflect activities which can be categorized in the biological (physiological, psychological, and/or sociocultural reaction).

**Adaptation** is the constant alteration which individuals make in their patterns of interaction within the environment. These alterations perpetuate the survival of the individual and increase the individual's utility, performance, and pleasure within the chosen environment (Mechanic, 1976).

The five terms, trauma, pain, hypermetabolism, sepsis, and adaptational level, will be defined and compromise the concept level of this theory. Concepts are substantive terms defined in such a way that the definition is regarded as complete but not empirically applicable (Gibbs, 1972, p. 128).
Trauma: An injury to living tissue caused by an extrinsic factor such as an agent, force, or mechanism (Walt & Wilson, 1975).

Pain is a complex, perceptual, and affective experience determined by the unique past history of the individual by meaning of the stimulus to him, by his "state of mind" at the moment, as well as by the sensory nerve patterns evoked by physical stimulation (Melzack, 1973, p. 134).

Hypermetabolism is a graded physiologic response to a stressful stimulus. The metabolic response depends upon the magnitude of the injury and increases in a linear manner up to 40-50% total body surface area burned (Wilmore, 1974).

Sepsis - The major description for this term is a wound that contains more than 100,000 (10±) organisms in each gram of tissue involved. Additional signs may include a rise or sudden fall in temperature, chills and rigor, leukocytosis or leukopenia, glycosuria, tachycardia, or tachypnea. Later manifestations may be confusion, nausea, vomiting, ileus, peripheral vasoconstriction, hypotension, oliguria, obtundation, and death (Artz et al., 1979).

Adaptation levels are five phases at which adaptation may occur and include cognitive appraisal (Lazarus, 1977; Mechanic, 1976), coping mechanisms (Vaillant, 1977), neurophysiological arousal, emotional response, physiological response, and intensification of both neurophysiologic and psychological responses (Selye, 1978).

Referentials are intrinsic terms that designate a formula in the extrinsic part of a theory. A referential appears as a capitalized
acronym, the purpose being to signify that the meaning of the term is technical and relating to a particular theory (Gibbs, 1972, p. 129). Referentials include TSI (Trauma Severity Index), BE (β-endorphin levels), AES (Analgesic Equivalency Score), ACTH (Adrenocorticotropic Hormone), BW (Body Weight), WCS (Wound Culture and Sensitivity), and CBC (Complete Blood Count). These referentials are derived from the biological and physiological activity concept. Additional referentials identified in the model include SSPEPR (Signs and Symptoms for Physical, Emotional, and Psychological Response). This referential will not be developed further until data from the first segment of the model are evaluated. Definitions for the referentials pertinent to this study are as follow:

**Trauma Severity Index:** A numerical classification which summarizes the magnitude of injury imposed on the entire body. This approach incorporates the concept of multi-organ involvement from the external impact.

**β-Endorphin (Endogenous Opiate):** A 31-peptide chain with a molecular weight of approximately 3,400. β-endorphin is found in the brain with concentration in the pituitary gland. This amino acid chain is a fragment of a 91-peptide chain identified as β-lipotrophic Hormone (BLPH), (Hughes et al., 1975). The levels of β-endorphin have been altered in individuals reporting pain (Guillemin et al., 1977).

**Analgesic Equivalency Score:** A classification and assigned equivalency score for narcotic agents.
Adrenocorticotropic Hormone: A hormone secreted by the anterior lobe of the pituitary gland which is one regulation of cortisol secretion directly related to stressful stimuli (Henry, 1979, p. 442).

Body Weight: A measure in kilograms which reflect the total body mass.

Wound Culture and Sensitivity: Examination of morphology, staining characteristics, biochemical characteristics, and serologic reactions which identify bacteria. Differentiation is made between gram negative and gram positive characteristics followed by microscopic examination to identify specific bacteria. Sensitivity studies identify the antibiotic which will inhibit growth of the bacteria (French, 1971).

Complete Blood Count: A hematologic examination which provides the hemoglobin, red blood cell count, hematocrit, reticulocytes, white blood cell count and, most importantly, a differential count which specifies the number of granulocytes and lymphocytes (French, 1971).

Referents are the values computed after applying the referential formula. Referents by themselves do not constitute evidence for or against any theory. Referents become evidence only when a prediction about them is derived from the theory. Epistemic statements which link referentials with sets of referents are theorems and are imperative when testing a theory (Gibbs, 1972, pp. 290, 292).
Hypotheses are derived assertions which are formal predictions about referents. Once a hypothesis has been derived, the next step for the investigator is to describe the actual relationship between the two sets of referents. A true hypothesis only indicates congruence, the correct prediction of the direction of a relationship (Gibbs, 1972, p. 296).

As stated earlier, intrinsic theory includes those statements in the form of empirical assertions about properties of infinite classes of events or things. These are categorized as axioms, postulates, propositions, transformational statements, and theorems. Axioms are direct intrinsic statements in which the substantive terms are constructs (Gibbs, 1972, p. 167). Both a construct and a concept comprise a postulate (Gibbs, 1972, p. 178). When two substantive terms are concepts, a proposition is formed (Gibbs, 1972, p. 178). A transformational statement includes a concept and a referential. A theory is not testable without transformational statements (Gibbs, 1972, p. 180). A theorem contains constituent terms which are referentials. They present the final step in theory construction and are derived from the "sign rule" (Gibbs, 1972, p. 190).

Referents alone do not constitute evidence for or against any theory. When referents are declared as consistent or inconsistent with a theory, a conclusion is made. Epistemic statements link referentials in a theorem with sets of referents. Each epistemic statement refers to a particular set of referents (Gibbs, 1972, p. 294). A hypothesis is derived by the application of the sign rule to
a theorem and two epistemic statements (Gibbs, 1972, p. 295).

Temporal elements described by Gibbs will not be followed precisely for this framework. A staging sequence will be utilized which reflects occurrence of the referentials. Stage 1 is the initial impact of the traumatic stimulus on the body. This stage temporally precedes Stage 2 which is manifestation of biopsychosociocultural reaction. Stage 3 follows with identification of adaptational behavior.

The following statements comprise the intrinsic theory:

AXIOMS:

A1: Among adults, the greater the stimulus during Stage 1, the greater the biopsychosociocultural reaction at Stage 2.

A2: Among adults, the greater the biopsychosociocultural reaction at Stage 2, the lesser the adaptation at Stage 3.

POSTULATES:

P1: Among adults, the greater the stimulus at Stage 1, the greater the traumatic injury at Stage 1.

P2: Among adults, the greater the biopsychosociocultural reaction at Stage 2, the greater the pain at Stage 2.

P3: Among adults, the greater the biopsychosociocultural reaction at Stage 2, the greater the hypermetabolism at Stage 2.
Among adults, the greater the biopsychosocial-cultural reaction at Stage 2, the greater the sepsis at Stage 2.

Among adults, the greater the adaptation at Stage 3, the greater the adaptational level at Stage 3.

PROPOSITIONS:

Among adults, the greater the traumatic injury at Stage 1, the greater the pain at Stage 2.

Among adults, the greater the traumatic injury at Stage 1, the greater the hypermetabolism at Stage 2.

Among adults, the greater the traumatic injury at Stage 1, the greater the sepsis at Stage 3.

Among adults, the greater the pain, hypermetabolism, and sepsis at Stage 2, the greater the adaptational level at Stage 3.

TRANSFORMATIONAL STATEMENTS:

Among adults, the greater the traumatic injury at Stage 1, the greater the TSI at Stage 1.

Among adults, the greater the pain at Stage 2, the greater the BE at Stage 2.

Among adults, the greater the pain at Stage 2, the greater the AES at Stage 2.

Among adults, the greater the hypermetabolism at Stage 2, the greater the ACTH level at Stage 2.
TFS3B: Among adults, the greater the hypermetabolism at Stage 2, the greater the loss of BW at Stage 2.

TFS4A: Among adults, the greater the sepsis at Stage 2, the greater the WCS at Stage 2.

TFS4B: Among adults, the greater the sepsis at Stage 2, the greater the CBC at Stage 2.

THEOREMS:

TH1: Among adults, the greater the TS1 at Stage 1, the greater the BE at Stage 2.

TH2: Among adults, the greater the TS1 at Stage 1, the greater the AES at Stage 2.

TH3: Among adults, the greater the TS1 at Stage 1, the greater the ACTH at Stage 2.

TH4: Among adults, the greater the TS1 at Stage 1, the greater the BW loss at Stage 2.

TH5: Among adults, the greater the TS1 at Stage 1, the greater the WCS at Stage 2.

TH6: Among adults, the greater the TS1 at Stage 1, the greater the CBC at Stage 2.

TH7: Among adults, the greater the TS1 at Stage 1, the greater the SSPER at Stage 3.
APPENDIX B
REPORT OF PILOT STUDY

Purposes

This pilot study was conducted as a preliminary to the dissertation research. The purposes of the study were to:

1. Evaluate the protocol as specified in a clinical setting.
2. Evaluate the feasibility of acquiring the samples, processing the samples, and placing the samples in the subzero freezer within 15-20 minutes of acquisition.
3. Evaluate the extraction process on patient samples.
4. Evaluate the assay procedure.

Methodology

Setting

The setting for data collection was the George David Peak Burn Unit, University of Missouri Hospital and Clinics, University of Missouri, Columbia. This is a 7-bed unit that admits children and adults with burn injury. Nursing and medical staff have expertise in burn management and participate in on-going inservice education regarding the burn injured patient.
Sample

The sample consisted of 4 patients admitted to the burn unit that met the following delimitations: (1) thermal burns diagnosed as partial- or full-thickness between 20% and 60% total body surface area, (2) male or female between the ages of 21 to 60 years of age, (3) burn injury within 24 hours of admission to the unit, (4) literate in English, and (5) agree to participate in the study by signing the Human Subjects Research Approval Form.

The sample was comprised of all male patients, ages 54, 31, 21, and 62. In order according to age, the following percent of total body surface area was burned: 20%, 40%, 15%, and 51%. All were thermally injured and all patients agreed to participate in the study. The 5% total body surface area burned patient was admitted to the study due to lack of patients that met the delimitations.

The pilot project began March 31, 1984. By August 1, 1984, only two patients had been admitted--both during the month of April. Since plans were to terminate the project on September 1, 1984, it was decided to admit any patient to the study that agreed to participate, regardless of extent of injury or age.

Data Collection

Baseline data were collected on all patients upon admission to the burn unit including estimated extent and depth of injury and a blood sample for β-endorphins. Following admission, blood samples for β-endorphins were obtained every 24 hours for 7 days, then every
other day (every 48 hours) for the remaining 7 days or until discharge from the hospital. Thus, a total of 11 samples were possible from each patient.

Blood samples (10-12 ml.) were obtained by the researcher or the research assistant that had been trained in the procedure for processing samples. The samples were obtained at the same time blood was drawn for other laboratory analyses to eliminate the possibility of complications in the patient from additional venipuncture. The blood samples were transferred immediately to a polypropylene tube containing 0.5 ml. of bacitracin (2 mgm/ml) and EDTA (920 Mn). All syringes and tubes were chilled and the sample secured in ice during transfer to the biochemical laboratory. Samples were then placed in a centrifuge at 2000 RPM's for 10 minutes. The temperature of the centrifuge was maintained at 0° centigrade. The plasma was pipetted (polypropylene material) to the test tube and frozen in a marked container. The label displayed the hospital number of the patient, date, time, and number of the sample in sequence. The samples were retained in -20° centigrade freezer until all samples were collected. Dry ice was utilized to transport the samples with the researcher to the University of Arizona, Tucson, Arizona, for analysis. The Surgical Biology Laboratory, under the direction of Dr. Milos Chvapil, University of Arizona, College of Medicine, was the site for assay analysis. The researcher has worked closely with the laboratory staff, especially Dr. Clemond Eskelson, to develop skill in implementing the assay.
Analysis of the samples were conducted according to instructions specified for the New England Nuclear Corporation β-Endorphin (¹²⁵I) RIA Kit, Catalog No. NEK-003 (See Appendix E). β-endorphin levels were reported in pico grams per milliliter of plasma (pg/ml).

Protection of Human Subjects

The purposes and anticipated outcomes of the study were discussed with the patients and family members that met the delimitations. A consent form was prepared and signed according to the guidelines for the University of Missouri Hospital and Clinics Institutional Review Board. Patients were informed that they may withdraw from the study at any time without untoward effects on continued therapy. Risk factors and measures taken to alleviate those possibilities were discussed.

Analysis of the Data

β-endorphin levels were plotted in line graph form to visualize the levels over days post burn injury. Due to the small number of patients obtained for the pilot study, no further analyses were implemented.
Results

Briefly, this study was conducted to determine the feasibility of the protocol and to evaluate the methods of procedure. The following results were obtained:

After several orientation sessions with burn unit nursing staff for each shift, the protocol was implemented. Major concerns were whether the routine times designated for sample collection were feasible. It was gratifying to determine early in the pilot study that the staff perceived the research as important and communication was prompt.

Maintaining supplies and solutions became a problem when personnel from the pharmacy destroyed solutions that were stored in the medication sample refrigerator. On two occasions, the entire supply of EDTA and bacitracin was destroyed because of the dates on the bottles. This resulted in lost blood samples since only one person in the pharmacy was familiar with the research and knew the procedure for preparing the solution. Communication and posted signs did not solve the problem due to numbers of pharmacy personnel involved.

A problem was identified when the amount of EDTA and bacitracin placed in test tubes in advance was found to decrease due to evaporation. Also, if less than 10-12 ml. of whole blood was obtained and placed in the tube with 0.5 ml EDTA and bacitracin, the proportion was inaccurate. It was unknown as to the effects of bacitracin on later binding potential of \( \beta \)-endorphin activity.
These issues were discussed with Dr. Eskelson, and following a brief review of literature, the decision was made to use B-D vacutainer tubes for blood collection. These contain EDTA 0.7 ml. and are readily accessible in the burn unit. The use of vacutainers had been reported several times in literature even though these are glass tubes as opposed to polypropylene.

The time interval between obtaining the sample and placing the plasma in subzero freezing did not exceed 20 minutes. This was achievable even though the biochemistry laboratory was on the 6th floor and the burn unit is on the 3rd floor.

A recurring difficulty was the acquisition of blood samples from patients with bilateral burns of the upper extremities. Peripheral veins in the feet were attempted for venipuncture sites, but frequently only 1-2 ml. of blood could be drawn at one time. This resulted in missed samples. The use of arterial lines in relatively small to medium injuries were not realistic and presented the potential for too great a risk for the patient. Therefore, it was decided that these problems were congruent with clinical research and would need to be evaluated on an individual basis.

Samples were transported without difficulty to the University of Arizona for assaying. The NEN kit was implemented for plasma from volunteers before the patient samples were assayed. It was decided to utilize the Sep-Pak C-18 filter with the extraction protocol that had been published by Cahil and Akil (1982). The
procedure was implemented with minimal difficulty. It was found that plasma samples filter at varying times, apparently due to the amount of fibrin remaining in the plasma. The Sep-Pak filtration system accommodated 8 samples simultaneously. The total preparation, washing, filtration, and eluting process required from 2-3 hours of time, depending on the speed of sample filtration.

A standard curve was established by the researcher and, following extraction, the samples were assayed according to the instructions provided. Results of the β-endorphin levels determined from the 4 patients in the sample are presented in Figure 1. As noted in the figure, all β-endorphin levels were elevated above normal (0-15pg/ml). The peak to 360 pg/ml which occurred on day 9 for patient 2 occurred after surgical excision. The patient experienced blood loss with a replacement need for multiple units of packed red cells following surgery.

Even though the numbers of samples obtained for the pilot research were minimal, it was decided to implement the protocol for the dissertation research with change in the type of container in which the blood sample was obtained.
APPENDIX C

HUMAN SUBJECTS FORMS

UNIVERSITY OF MISSOURI-COLUMBIA

INTER-DEPARTMENT CORRESPONDENCE

TO
Ms. Geraldine M. Goosen
Nursing
5421 Nursing School

FROM
Dr. C. W. Woodruff, Chairman
Institutional Review Board
H764 HSC

RE: Project #1154--"Beta Endorphin Levels in Burned Patients"

April 1, 1983

Dear Ms. Goosen:

The above research project has been given an expedited review, and as a result of that review, has been found to impose no more than minimal risk on the research subjects. You will, therefore, be allowed to obtain the oral consent of the research subject as a means of obtaining informed consent. If a patient is unable to give informed consent, you must obtain the consent of the subject's legally authorized representative. This will ordinarily be a spouse and if there is no spouse, the next-of-kin or legally appointed guardian.

The IRB will call for an interim report on this project in six months and every six months thereafter. Please report any untoward or unexpected developments during the investigation to the IRB. Use the assigned number (#1154) to identify your project in any future correspondence with this office.

Sincerely,

Dr. C. W. Woodruff, Chairman
Institutional Review Board

cc Dr. Boyd Terry

P.S. I understand you talked to Mr. Hodges about the necessity of making a note in the chart stating that the patient is participating in a research project. I am attaching a copy of our guidelines for obtaining oral consent. If you have further questions, please do not hesitate to call me.
STATEMENT TO HUMAN STUDIES COMMITTEE

Principal Investigator: William H. Hoang, M.D.
Present at: Surgery
Examination to: William H. Hoang, M.D.

Date: August 1, 1983

Title of Project: Beta Endorphins in Burn Patients: A Descriptive Study
Grant Agency: University of Missouri, Biomedical Research Development Grant
Grant Number: New X Renewal X (R1) Not Funded

1. Does this project involve human subjects? No X Yes

2. What animal(s) will be used? None

3. Does this project involve Recombinant DNA experiments? No X Yes

4. Does this project involve the administration of radioactive material to human subjects? No X Yes

If yes: a. List radioactive material to be used

b. Submit two (2) copies of letter of approval from the Chairman of the Radioactive Drug Research Committee.

Research involves an investigational new drug or an investigational medical device, the Committee must be assured of clearance by the Food and Drug Administration. State the name of the drug or device involved

If the IND Number or IDE Number, also submit two (2) copies of FDA clearance and of pertinent literature on the use of the drug or device in animals and humans, including all information on anticipated hazards, side effects, or toxicity. If research is privately funded, include two (2) copies of indemnification agreement from sponsor.

Submit two (2) copies of the Statement to Human Studies Committee, a summary of the investigative work and the plan of investigation as it appears in the grant. Budget data is not required.

Signature of Department Chairman

Signature of Principal Investigator

Committee Approval Number: 831109

ACTION OF COMMITTEE

Signature of Committee Chairman

HUMAN SUBJECTS

Exempt Review Expedited Review Low Risk Medium Risk High Risk Duration of Approval

AUG 24 1983 12 months
Geraldine M. Goosen, R.N., M.S.
Route 3
Cole Camp, Missouri 65425

Dear Ms. Goosen,

We are in receipt of your project, "Beta Endorphin Levels in Burn Patients", which was submitted to this Committee for review. The procedures to be followed in this study pose no more than minimal risk to the participating subjects. Regulations issued by the U.S. Department of Health and Human Services (45 CFR Part 46.110(b)) authorize approval of this type project through the expedited review procedures, with the condition that subjects' anonymity be maintained. Although full Committee review is not required, a brief summary of the project procedures is submitted to the Committee for their information and comment, if any, after administrative approval is granted. This project is approved effective 2 August 1984.

Approval is granted with the understanding that no changes or additions will be made to either the procedures followed or the consent form(s) used (copies of which we have on file) without the knowledge and approval of the Human Subjects Committee and your College or Departmental Review Committee. Any physical or psychological harm to any subject must also be reported to each committee.

Please be advised that the signed consent forms generated by this study must be surrendered to the authorities at the institutions where the individual subjects are enrolled. This will ensure their accessibility in the event that institution officials require the information in your absence. These forms need not be retained by the University of Arizona.

Sincerely yours,

Milan Novak, M.D., Ph.D.
Chairman
Human Subjects Committee

KN/jm

cc: Ada Sue Hinsaw, R.N., Ph.D.
College Review Committee
SUBJECT'S CONSENT FORM

Project Title: Beta Endorphin Levels in Burn Patients

My name is Geraldine M. Goosen, RN, MS, a doctoral candidate from the College of Nursing, University of Arizona, Tucson, Arizona. You are being asked to participate in a study conducted at the Burn Unit to determine the Beta endorphin levels in burn patients. Beta endorphins are morphine-like substances that are secreted in your own body. You have been selected for the study because you have been admitted to the burn unit with a burn injury and you are beyond the age of 21. A minimum of 30 burn patients will be approached to enroll in the study.

The major goals of the study are to determine the changes in Beta endorphin levels over time following burn injury and to determine the relationship between Beta endorphin levels, depth and extent of burn injury, and the amount of medicine you take for painful discomfort.

If you agree to participate, we will obtain 8 ml. (less than 1 tablespoon) of blood on admission to the burn unit. An additional 8 ml. of blood will be drawn daily for 7 days and every other day until a total of 11 samples are obtained. This will be at the end of 2 weeks. These samples will be taken at the same time blood is being drawn for laboratory tests requested by the physician. It may be necessary to perform a separate "needle stick" to obtain the blood sample, but this is not anticipated. If this situation does occur, you will be informed. In addition, the following information will be taken from your chart: age, sex, evidence of the presence of inhalation injury and depth and extent of burn injury. This study has been discussed with your physician and she/he does not object to your participation.

Of possible benefit to you will be new information about what is available in your own body which may change your perception and response to pain. Also, you will be contributing information which will be used to manage pain for future burn patients. Since the incidence of pain is high in our society, new knowledge which could decrease suffering, time, and money expended in pain management would be a tremendous service to society and to the community. All information found in this study will be available to your and your physician. Results from the study will be shared with burn team members at state and national meetings and in reputable professional journals. The cost to you for participating in this study will be less than 10 tablespoons of blood which is less than one-half cup.
Beta endorphin analysis will be performed at no expense to you.

Since the procedure for performing this study is done routinely for management of your injuries, no unusual reactions, responses, or discomforts are anticipated aside from the usual discomfort from the venipuncture. The risk of infection from entering the vein with a needle is present. Nurses (including myself) and physicians will constantly observe for any evidence suggesting infection. Although the chances of a serious side effect occurring to you are extremely small, in the event of physical injury resulting from the research procedure, financial compensation for wages or time lost is not available.

All records will be kept strictly confidential. Your name will be known only to the physician and the nursing staff. Initially your hospital number will be placed on the blood sample and the data collection sheet. After all data is analyzed, the information will be reported by the number of the sequence in which you entered the study.

"The nature, demands, risks, and benefits of the project have been explained to me as well as the type of treatment as known and available. I understand what my participation involves. Furthermore, I understand that I am free to ask questions and withdraw from the project at any time without affecting my medical care. I also understand that this consent form will be filed in an area designated by the Human Subjects Committee with access restricted to the principal investigator or authorized representatives of the particular department. A copy of this consent form will be given to me."

Subject's Signature Date

Signature of Parent or Legally Authorized Representative Date

I have carefully explained to the subject the nature of the above project. I hereby certify that to the best of my knowledge the subject signing this consent form understands clearly the nature, demands, benefits, and risks involved in participating in this study. A medical problem or language or educational barrier has not precluded a clear understanding of his/her involvement in this project.

Investigator's Signature (optional) Date
APPENDIX D
BURN CHARTS

UNIVERSITY OF MISSOURI
MEDICAL CENTER
ESTIMATION: % BODY BURNS

ANTERIOR POSTERIOR

HEAD A1 A2
NECK
RT. ARM
RT. FOREARM
RT. HAND
LT. ARM
LT. FOREARM
LT. HAND
TRUNK
BLITTOCK
PERINEUM
RT. THIGH
RT. LEG
RT. FOOT
LT. THIGH
LT. LEG
LT. FOOT

0 1 5 10 15 ADULT

A-1/2 HEAD 9 1/2 8 1/2 6 1/2 5 1/2 4 1/2 3 1/2
B-1/2 ONE THIGH 2 3/4 3 1/4 4 1/4 4 1/2 4 3/4
C-1/2 ONE LEG 2 1/2 2 1/2 2 3/4 3 3 1/4 3 1/2

EXTENT OF BURNS

% PARTIAL THICKNESS (1st & 2nd)

% FULL THICKNESS (3rd & 4th)

TOTAL

151
I. INTRODUCTION

Endorphin (from the words "endogenous morphine") is the generic name which is applied to any opioid peptide without designating a particular chemical structure. (1) The major opiate-like peptide found in mammalian pituitary is the 31-amino acid peptide, β-endorphin. (2) It has also been reported to be found in brain. (3) The specific amino acid sequence identified for human β-endorphin is: (4)


In 1964, C. H. Li discovered a polypeptide substance in sheep pituitary gland with properties different from most pituitary hormones. (5) This substance was named β-lipotropin (β-LPH). The amino acid sequence was elaborated in 1965 as a 91-amino acid peptide which was believed to be a potential precursor for other hormones and biologically active peptides. (6) With the discovery and identification of the enkephalins, pentapeptides found in brain, (7) there was much interest in the
pituitary peptide described by Li. The pentapeptide, Tyr-Gly-Gly-Phe-Met (Met-enkephalin), was identical to the 61-65 amino acid residue of β-LPH.\(^{(7)}\)

Goldstein,\(^{(8)}\) in collaboration with Li, showed that whereas the β-LPH itself was not an active opioid peptide, the fragment β-LPH (61-91) was a potent opioid in the guinea pig ileum bioassay. At the same time, Guillemin\(^{(9)}\) showed that β-LPH (61-91) had morphine-like activity \textit{in vitro}. The name β-endorphin was proposed by Li for the fragment β-LPH (61-91).\(^{(10)}\)

Loh\(^{(11)}\) investigated some of the biological properties of β-endorphin. In 1976, he reported the finding that intracerebral injection of β-endorphin caused analgesia and other behavior modifying effects. Similar results were observed when the β-endorphin was administered intravenously.\(^{(12)}\) Several other investigators have demonstrated the opioid behavior of β-endorphin.\(^{(13-15)}\)

There are a number of biologically active peptides which have some structural relationships to the 91-amino acid sequence β-LPH, or fragments thereof. For example, the complete sequence of β-melanotropin (β-MSH) is between residues 41 and 58 of β-LPH; the seven residue segment, Met-Glu-His-Phe-Arg-Trp-Gly, is common to melanotropins and adrenocorticotropins.\(^{(16-17)}\) Two other endorphins, χ-endorphin and γ-endorphin, correspond to the sequences β-LPH (61-76) and β-LPH
(61-77), respectively, whereas β-endorphin is β-LPH (61-91). (18)

Since the discovery of these neurohypophysial peptides, and the reports of structural correlations, there has been much research into the localization and quantitation of these substances. (19,20) Further identification and quantitation of these substances may lead to a better understanding of how the brain functions. It is known that β-endorphin can induce behavioral changes. However, determining the normal function of β-endorphin is one of the puzzles still confronting opiate researchers.

II. EXPLANATION OF THE TEST

The methods for determining levels of endorphin in various tissues have included chromatographic procedures such as HPLC, (21) and radioimmunoassay. (19) Samples are derived from a wide variety of sources including tissue extracts, CSF and serum. Since the levels expected for these samples are quite low, the most sensitive procedure possible is required for the accurate determination of endorphin levels. Radioimmunoassay is the procedure which offers the best sensitivity. The development of adequate antisera for use in the radioimmunoassay system has resulted in the development of competitive protein binding methods with sensitivity at a point where low levels of endorphin can be determined even in the presence of some other substances.

The New England Nuclear β-Endorphin Radioimmunoassay Kit is
based on the use of an iodine-125 labeled β-endorphin as the tracer, and a rabbit anti-β-endorphin serum as the antiserum (specific antibody).

III. PRINCIPLE OF THE METHOD

The basic principle of this radioimmunoassay is that of competitive protein binding where a radioactive and a non-radioactive antigen compete for a fixed number of antibody binding sites. This interaction is illustrated schematically in Figure 1.

When unlabeled antigen from standards or samples and a fixed amount of the labeled antigen are allowed to react with a constant and limiting amount of antibody, decreasing amounts of the labeled antigen are bound to the antibody as the amount of unlabeled antigen is increased.

In the New England Nuclear β-Endorphin \(^{125}\)I Kit, separation of the bound from free antigen is achieved by absorption of the free onto activated charcoal. After centrifugation, the supernatant containing the bound antigen is decanted into a counting tube and the radioactivity is measured. The results obtained for the standards are used to construct a dose-response (or standard) curve, from which the values of the unknown samples may be obtained by interpolation.
Figure 1

\[
\text{Labeled Antigen (Ag\textsuperscript{\star})} + \text{Specific Antibody (Ab)} \rightarrow \text{Labeled Antigen Antibody Complex (Ag\textsuperscript{\star} Ab)}
\]

\[
+ \text{Unlabeled Antigen (Ag)} \rightarrow \text{Unlabeled Antigen-Antibody Complex (AgAb)}
\]

(In standard solutions or unknowns samples)
IV. REAGENT DESCRIPTION AND PREPARATION

The β-endorphin kit is shipped at ambient temperature and should be stored at 2-8°C upon receipt. The following reagents are supplied and are sufficient for 250 assay tubes following the assay protocol.

1 vial - β-endorphin Antibody, lyophilized
2 vials - β-endorphin $^{125}$I Tracer, lyophilized
1 vial - β-endorphin Standard Concentrate, lyophilized
1 vial - β-endorphin Assay Buffer Concentrate, 25 ml
1 vial - β-endorphin Charcoal Suspension concentrate, 25 ml

A. β-Endorphin $^{125}$I Tracer

The kit contains two vials of lyophilized tracer, each containing approximately 1μCi of β-endorphin $^{125}$I on the calibration date. Store the lyophilized tracer at 2-8°C or in the freezer (-20°C). Under these conditions, the tracer is stable for at least six weeks.

To prepare the tracer concentrate, reconstitute one vial by adding 1.5ml of distilled water (the second vial may be reconstituted as needed). The resulting solution is referred to as concentrated tracer solution and contains a total of approximately 1.01μCi of β-endorphin $^{125}$I on the calibration date in 0.13M glycine buffer, pH 3.0, with inert ingredients and a preservative. Store the concentrated tracer solution at -20°C until immediately before use. Under these storage conditions, the stability
of the concentrated solution is at least two weeks.

For use in the assay system, dilute one volume of concentrated tracer with nine volumes of assay buffer. For example, mix 0.5ml concentrated tracer with 4.5ml assay buffer. To ensure that the mass of the tracer being added to each assay tube remains constant throughout the lifetime of the kit, always dilute the concentrated solution one volume to nine volumes disregarding the final counts per minute per aliquot. DO NOT dilute to constant counts. Pipets and/or pipet tips used to transfer tracer solution should be made of polypropylene or siliconized glass. Do not use those made of unsiliconized glass. Once diluted to assay concentration, the tracer should be used within twelve hours. Discard any used diluted tracer. There is sufficient tracer for 250 assay tubes with a 10% excess to allow for unused tracer which is discarded.

INSTRUCTIONS RELATING TO THE HANDLING, USE, STORAGE, AND DISPOSAL OF THIS RADIOACTIVE MATERIAL

This radioactive material may be received, acquired, possessed, and used only by research laboratories and only for in vitro laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use, and transfer are subject to
the regulations and a general license of the U. S. Nuclear
Regulatory Commission or of a State with which the
Commission has entered into an agreement for the exercise
of regulatory authority.

NEW ENGLAND NUCLEAR CORPORATION

1. All radioactive materials should be stored in
   specifically designated posted areas.

2. All work with these materials should be carried out
   only in authorized areas.

3. No pipetting should be done by mouth.

4. There should be no smoking or eating within the work
   area.

5. Hands should be washed after handling radioactive
   materials.

6. Any spilled material should be wiped up quickly and
   thoroughly and the contaminated substances transferred
   to a suitable receptacle. The surfaces involved
   should be washed thoroughly with an appropriate
   decontaminant.

7. When use of the Tracer reagent has been completed,
   empty and decontaminant the vial. This radioactive
   material can be discarded into the sanitary sewerage
   system, provided the discharge concentration does not
   exceed $2 \times 10^{-7} \mu\text{Ci}/\text{ml}$. 
B. Prior to disposal of the empty, uncontaminated Kit and Tracer containers to unrestricted areas, remove or deface the radioactive material labels or otherwise clearly indicate that the containers no longer contain radioactive material.

B. β-Endorphin Antibody

One vial of lyophilized anti-β-endorphin serum is supplied. The lyophilized antiserum is stable for at least four months when stored at 2-8°C. To prepare reagent, reconstitute the antiserum by adding 25ml of distilled water. When reconstituted, the solution contains rabbit antiserum to β-endorphin in a 0.1M phosphate buffer, pH 6.0, with 0.05M NaCl, 5mM EDTA, inert ingredients and a preservative. Store the reconstituted solution at 2-8°C. Under these conditions the solution is stable for at least two months.

C. β-Endorphin Standard Concentrate

One vial of lyophilized, synthetic β-endorphin standard is supplied. To reconstitute, add exactly .5ml of distilled water. The resulting concentrated solution contains 1.01µg of β-endorphin per ml in a 0.2M glycine buffer, pH 3.0, with inert ingredients and a preservative. Immediately before use in the assay, dilute an aliquot of
the stock solution to prepare standards. These standards are used to prepare a dose response curve. A suggested procedure for preparing diluted standards appears in Section VI. Pipets and/or pipet tips used to transfer diluted standards should be made of polypropylene or siliconized glass. Do not use those made of unsiliconized glass. Store the remaining concentrated standard at -20°C. Under these conditions the solution is stable for at least four weeks.

D. β-Endorphin Assay Buffer Concentrate

One vial of assay buffer concentrate is supplied. This concentrated solution is stable for at least two months when stored at 2-8°C. To prepare assay buffer for use in the assay, transfer contents of the vial to a container of appropriate size. Add 100ml of distilled water and mix thoroughly. The diluted assay buffer contains 0.1M phosphate, pH 6.0, 0.05M NaCl, 0.1% gelatin, an inert ingredient and a preservative. When stored at 2-8°C, the diluted buffer is stable for at least two months.

E. β-Endorphin Charcoal Suspension Concentrate

One vial of concentrated charcoal suspension is supplied. Before opening the vial, puncture the septum with a needle to release any pressure that might have built up during shipping. Then carefully open the vial and
transfer the suspension to a beaker of appropriate size. Add 100ml of distilled water and stir using a magnetic stir bar. The resulting suspension contains 0.6% charcoal Norit A in 0.1M phosphate buffer, pH 6.0, with 0.1% gelatin, 0.16% dextran T-70, 0.05M NaCl, 5mM EDTA, an inert ingredient and a preservative. Store the diluted charcoal suspension at 2-8°C. Under these conditions the reagent is stable for at least two months.

V. SAMPLE PREPARATION

Body fluids containing β-endorphin should be handled carefully in order to minimize degradation of the immunoreactive protein. Experiments will soon be performed to study the effect of temperature on the lifetime of β-endorphin in serum and plasma. Until this new data suggests otherwise, it is recommended that serum be stored on ice immediately after collection and assayed as soon as possible, or frozen to be assayed at a later date. The investigator should run necessary controls to determine the effect of sample matrix and sample storage on assay results.

There are many methods for extracting β-endorphin from body fluid and tissues. A variety of these extraction procedures are reported in the literature.(22) We have studied the effect on assay performance of a few of the extraction solvents commonly used for peptide purification. The final assay concentrations of the following solvents have been demonstrated to have no
significant effect on the zero binding and blank values.

0.5M Urea <10% Acetonitrile <5% Ethanol, methanol

The effects of other extraction solvents or sample matrices on assay performance must be determined by each investigator for his own particular system.

VI. PROCEDURE

A. Materials Required

In addition to the reagents supplied with the kit, the following materials are required:

1. Pipettors and/or pipets that can accurately and precisely deliver the required volumes. Pipets and/or pipet tips used to transfer diluted standards, tracer, or samples should be made of polypropylene or siliconized glass. Do not use those made of unsiliconized glass.

2. Siliconized glass test tubes (these can be prepared by treating borosilicate glass tubes with Prosil\textsuperscript{*}), or polypropylene test tubes.

3. Test tube rack.

4. Ice bath.

5. Distilled water.

6. Beakers or flasks.

7. Vortex mixer.

8. Magnetic stirring base and stir bars.

9. Centrifuge, refrigerated, with swinging bucket rotor.

10. Gamma scintillation counter.
B. Preparation of β-Endorphin Standards

An aliquot of the β-endorphin standard concentrate should be diluted with assay buffer to prepare a series of standards used to construct a dose response curve. The suitable range of concentrations appropriate for this assay is between 5pg per 0.1ml and 500pg per 0.1ml. A suggested dilution scheme is shown below. Other dilution schemes which cover the assay range can be used. However, be sure to use the assay buffer provided with the kit for dilution since it contains additives to minimize non-specific adsorption of β-endorphin to the wall of the test tubes.

Note: Use only siliconized glass test tubes, or polypropylene tubes. Use polypropylene or siliconized glass pipets or pipet tips for transferring standard solutions. Do not use pipets made of unsiliconized glass.

A suggested dilution scheme:

<table>
<thead>
<tr>
<th>TUBE</th>
<th>ADD</th>
<th>CONCENTRATION (pg/0.1ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. 0.01ml(101µl) standard</td>
<td>+ 0.99ml assay buffer</td>
<td>1,000</td>
</tr>
<tr>
<td>b. 0.50ml of dilution a</td>
<td>+ 0.50ml assay buffer</td>
<td>500</td>
</tr>
<tr>
<td>c. 0.20ml of dilution b</td>
<td>+ 0.60ml assay buffer</td>
<td>125</td>
</tr>
<tr>
<td>d. 0.40ml of dilution c</td>
<td>+ 0.60ml assay buffer</td>
<td>50</td>
</tr>
<tr>
<td>e. 0.50ml of dilution d</td>
<td>+ 0.50ml assay buffer</td>
<td>25</td>
</tr>
<tr>
<td>f. 0.40ml of dilution e</td>
<td>+ 0.60ml assay buffer</td>
<td>10</td>
</tr>
<tr>
<td>g. 0.50ml of dilution f</td>
<td>+ 0.50ml assay buffer</td>
<td>5</td>
</tr>
</tbody>
</table>

Dilutions b through g should be used for the standard curve.
C. Radioimmunoassay Protocol (Standard Protocol)

A complete understanding of the Protocol and Precautions is necessary to the successful completion of the assay. Read these sections carefully before proceeding with the assay. Pipets and/or pipet tips used to transfer diluted standards, tracer and samples should be made of polypropylene or siliconized glass. Do not use those made of unsiliconized glass.

1. Prepare all reagents according to directions in Section IV. Freshly diluted standards and tracer should be prepared just prior to performing the assay.

2. Equilibrate all reagents to ice bath temperature, and swirl before use.

3. Label 18 siliconized glass tubes or polypropylene tubes for the total counts, blanks and standards, and additional tubes for unknown samples.

4. Place tubes in a test tube rack in an ice water bath.

5. Pipet 200μl of assay buffer into tubes 1-4 (total count tubes and the blanks). Refer to the protocol schematic in Table 1 for steps 5-9.

6. Pipet 100μl of each diluted standard (b through g) into the appropriate tubes.

7. Pipet 100μl of each sample in duplicate into the appropriate tubes.

8. Pipet 100μl of the freshly diluted tracer solution into each tube.
Table 1. \(\beta\)-Endorphin Assay Protocol Schematic

(All volumes are in microfilters)

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Buffer</th>
<th>Standards</th>
<th>Samples</th>
<th>Tracer</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Counts</td>
<td>1,2</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Blank</td>
<td>3,4</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>&quot;0&quot; Standard</td>
<td>5,6</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>100 100</td>
</tr>
<tr>
<td>Standards</td>
<td>7-18</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>100 100</td>
</tr>
<tr>
<td>Samples</td>
<td>19,20,etc.</td>
<td>-</td>
<td>100</td>
<td>100 100</td>
<td></td>
</tr>
</tbody>
</table>

Incubate at 4°C overnight. Add 500\(\mu\)l assay buffer to tubes 1 and 2 and 500\(\mu\)l of the charcoal suspension to the remaining tubes. Immediately mix and centrifuge at refrigerated temperature for 10 minutes at 2,400 rpm. Decant all tubes including the total count tubes.

*If glass tubes are used, lower counting efficiency will be obtained.
9. Pipet 100μl of antiserum solution into the remaining tubes, beginning with tube 5.
10. Vortex all tubes thoroughly for 2-5 seconds.
11. Incubate at 4°C for at least 16 to 24 hours.
12. With the beaker of diluted charcoal suspension in an ice bath, thoroughly mix the suspension with the magnetic stirring control set to moderate.
13. While maintaining stirring, pipet 0.5ml of the charcoal suspension to all tubes except tubes 1 and 2 (total count).
14. Pipet 0.5ml of assay buffer to tubes 1 and 2.
15. Vortex all tubes thoroughly for 2-5 seconds.
16. Immediately centrifuge the tubes in a refrigerated centrifuge at 2,400 rpm for 10 minutes.
17. Decant the total count tubes and the supernatants from all the other tubes into corresponding glass* or polypropylene tubes which are numbered in the same manner as the original series. Rim each tube gently to assure maximum transfer without disturbing the charcoal residue.
18. Count the supernatants in a gamma counter. At the usual counting efficiencies of 50-70%, a counting time of 1 minute per tube is adequate.
19. Calculate results as described in Section VII.
D. Alternate Protocol - Delayed Addition of Tracer

Additional sensitivity can be achieved by using the method of delayed addition of tracer. A preliminary incubation of the system without the labeled endorphin, followed by the addition of tracer leads to a standard curve with characteristics which allow for greater sensitivity than in the usual assay system. A typical curve generated in this way is illustrated in Figure 2. The 50% displacement is at approximately 25pg added, and the usable range is between 5 and 125pg.

In order to use this adapted procedure, it is recommended that you prepare an additional standard dilution at a concentration of 7.5pg/0.1ml. To perform the alternate protocol, follow the standard assay protocol, steps 1-10, omitting step 8 (tracer addition). Then incubate the tubes at 4°C for 24 hours. After this first incubation, pipet 100µl of freshly diluted tracer solution into each tube. Vortex the tubes and incubate them again for 24 hours at 4°C. Then follow the charcoal separation and counting procedures described in steps 12-19 of the standard protocol.

E. Precautions

1. Since the assay is not quite at equilibrium overnight, incubation conditions should be standardized for day-to-day assay consistency. For example,
standardize on an 18 hour incubation time.

2. Pipetting must be reproducible and accurate. Like many other basic proteins, β-endorphin has the tendency to adhere to many surfaces. To minimize assay interference from this "sticking" problem, use only polypropylene or siliconized glass pipets or pipet tips when transferring diluted standard, tracer or samples. Do no use pipets made of unsiliconized glass.

3. In order to minimize any non-specific adsorption of β-endorphin to the walls of the test tubes, use only siliconized glass test tubes. Polypropylene tubes are acceptable.

4. Each vial of tracer contains a resin strip to decrease the amount of free iodide in the solutions after reconstitution. As a result, the counts may decrease at a rate slightly greater than that expected from radioactive decay. The assay remains reproducible as long as the same amount of tracer mass is added to each assay tube. To avoid changes in the curve position, add constant tracer mass to each tube for the lifetime of the kit. This is easily accomplished by standardizing the method of dilution of tracer from the concentrate. Do not adjust the tracer to constant counts for each new dilution.
5. Appropriate controls must be run for all samples derived from extraction procedures. The effect of the solvents used, or the protein content of the extracts on the standard radioimmunoassay procedure must be determined.

VII. PROCEDURE FOR CALCULATING UNKNOWNS

After counting has been completed, the concentration of β-endorphin in the samples is determined by preparing a standard curve. The following method is suggested (see Table 2 for sample calculations).

A. If all tubes have been counted for the same period of time, use the total accumulated counts; otherwise, correct all counts to counts per minute.

B. Average the counts for each set of duplicates.

C. Calculate the average NET counts for all standards and samples by subtracting from each the average blank counts.

D. Determine the percent bound (%B) for each standard and sample by dividing the average net CPM by the average net CPM of the total counts tubes.

E. Calculate the normalized percent bound (% B/B₀) as follows:
<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Avg. CPM</th>
<th>Avg. Net CPM</th>
<th>% Bound</th>
<th>% B/Bo Value</th>
<th>Sample Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Counts</td>
<td>6,235</td>
<td>6,078</td>
<td>5,904</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blank</td>
<td>158</td>
<td>174</td>
<td>-</td>
<td>2.85</td>
<td>-</td>
</tr>
<tr>
<td>&quot;0&quot; Standard</td>
<td>2,547</td>
<td>2,653</td>
<td>2,479</td>
<td>41.98</td>
<td>100</td>
</tr>
<tr>
<td>5pg</td>
<td>2,516</td>
<td>2,521</td>
<td>2,347</td>
<td>39.75</td>
<td>94.7</td>
</tr>
<tr>
<td>10pg</td>
<td>2,283</td>
<td>2,342</td>
<td>2,168</td>
<td>36.72</td>
<td>87.2</td>
</tr>
<tr>
<td>25pg</td>
<td>1,787</td>
<td>1,854</td>
<td>1,680</td>
<td>28.46</td>
<td>67.8</td>
</tr>
<tr>
<td>50pg</td>
<td>1,222</td>
<td>1,250</td>
<td>1,076</td>
<td>18.23</td>
<td>43.4</td>
</tr>
<tr>
<td>125pg</td>
<td>778</td>
<td>794</td>
<td>620</td>
<td>10.50</td>
<td>25.0</td>
</tr>
<tr>
<td>500pg</td>
<td>455</td>
<td>443</td>
<td>269</td>
<td>4.56</td>
<td>10.9</td>
</tr>
<tr>
<td>Sample</td>
<td>1,998</td>
<td>1,976</td>
<td>1,802</td>
<td>30.52</td>
<td>72.7</td>
</tr>
</tbody>
</table>

Table 2. Sample Calculations
\[
\frac{\%B}{B_0} = \frac{\%B}{\%B \text{ of } "0" \text{ standard}} \times 100\%
\]

F. Using semi-logarithmic graph paper, plot \( \frac{\%B}{B_0} \) for each standard versus the corresponding pg \( \beta \)-endorphin added (see Figure 2 for typical standard curves using the standard protocol or the alternative protocol).

G. Determine the pg of \( \beta \)-endorphin in each sample by interpolation from the standard curve. Since identical volumes are used for standards and samples and the standard curve is expressed as pg \( \beta \)-endorphin added, samples may be read directly as pg \( \beta \)-endorphin added.

Caution: Any samples with concentrations which are above the range of the standard curve must be diluted with assay buffer and re-assayed. The values obtained are then multiplied by the appropriate dilution factor.

VIII. LIMITATIONS

A. The following compounds from human systems have been checked for cross-reactivity. The percentages indicate cross-reactivity at 50% displacement compared to \( \beta \)-endorphin.
B. The β-endorphin standards are prepared in the assay phosphate buffer. The effect of other sample matrices upon the assay system must be determined by the investigator.

C. Any exogenous radioactivity in the sample may lead to erroneous results. Proper controls should be assayed to determine the effect on your assay system.

D. The β-endorphin Assay has been designed to assay for human β-endorphin. If assaying β-endorphin from other mammalian systems, the investigator must determine the specific performance characteristics and cross-reactivities for his assay system.

E. The alternate protocol (delayed addition of tracer) yields a more sensitive assay, but may be less precise than the standard assay protocol.

IX. PERFORMANCE CHARACTERISTICS

Reproducibility of the Standard Curve

Day-to-Day reproducibility was determined by constructing standard curves from triplicate points on twelve separate days over a two month period. The values obtained (percent bound) were as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Percent Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-endorphin</td>
<td>100%</td>
</tr>
<tr>
<td>β-lipotropin</td>
<td>50%</td>
</tr>
<tr>
<td>χ-endorphin</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Leucine enkephalin</td>
<td>&lt;0.004%</td>
</tr>
<tr>
<td>Methionine enkephalin</td>
<td>&lt;0.004%</td>
</tr>
<tr>
<td>χ-MSH</td>
<td>&lt;0.01%</td>
</tr>
</tbody>
</table>
To demonstrate the accuracy of the method, known amounts of \( \beta \)-endorphin were added to serum and buffer containing 0.8ng/ml \( \beta \)-endorphin. The assay was performed immediately after this addition and produced the following results:

<table>
<thead>
<tr>
<th>( \beta )-Endorphin Added (pg)</th>
<th>Matrix</th>
<th>( \beta )-Endorphin Recovered (pg/0.1ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Buffer</td>
<td>24</td>
<td>96%</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>19</td>
<td>76%</td>
</tr>
<tr>
<td>50</td>
<td>Buffer</td>
<td>52</td>
<td>104%</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>51</td>
<td>102%</td>
</tr>
<tr>
<td>100</td>
<td>Buffer</td>
<td>95</td>
<td>95%</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

The matrix effect on the above recovery study is further described by the following zero binding and blank values.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Matrix</th>
<th>% Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>Buffer</td>
<td>2.3%</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>3.1%</td>
</tr>
<tr>
<td>&quot;O&quot; Standard</td>
<td>Buffer</td>
<td>41.2%</td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>38.6%</td>
</tr>
</tbody>
</table>
X. REFERENCES


XI. ORDERING INFORMATION

In vitro laboratory testing with the New England Nuclear β-Endorphin [125I] Radiomunoassay Kit requires either a general license or a specific license from the U. S. Nuclear Regulatory Commission or from a State with which the NRC has entered into an agreement for the exercise of regulatory authority.

A general license is issued pursuant to part 31.11 of the U. S. NRC regulations (10CFR31.11) to any physician, clinical laboratory or hospital which obtains a validated, registered NRC Form 483. Holders of this license cannot possess at any one time more than 200 microcuries of I-125.

A specific license authorizing the possession of more than 200 microcuries of I-125 is issued pursuant to part 35.14 of the U. S. NRC regulations (10CFR35.14). Any holder of this specific license is authorized to perform in vitro testing with this Kit without filing NRC Form 483, provided that the licensee is subject to the other provisions of part 31.11.

NEK-003 β-Endorphin [125I] Radioimmunoassay Kit, 250 Tubes.
APPENDIX F
LINEAR-LOGARITHMIC REGRESSION AND
STANDARD CURVES

The normalized percent bound (\(\%B/Bo\)) was determined for each sample and \(\%B/Bo\) for each standard curve was plotted on semi-logarithmic graph paper. The decision was made to utilize a linear-logarithmic regression routine on the Deck 10 computer as programmed by the Department of Surgical Biology to calculate \(X\) (\(\beta\)-endorphin concentrates) from \(Y\) (\(\%B/Bo\)). To lessen the curvature of the standard curve both end points, 5 and 500 picograms, were deleted from the group. To obtain the formula for \(Y\), the following \(X\) and \(Y\) points were given to the computer for each assay:

Assay A:  
10 picograms \(\beta\)-endorphins = 75.99 \(\%B/Bo\)  
25 picograms \(\beta\)-endorphins = 53.19 \(\%B/Bo\)  
50 picograms \(\beta\)-endorphins = 35.76 \(\%B/Bo\)  
125 picograms \(\beta\)-endorphins = 10.86 \(\%B/Bo\)  
Correlation Coefficient = -0.998304  
Estimate of Variance = 6.82283  
\(F = 591.15\) with 1 and 2 degrees of freedom  
\(Y = 7.17941E-3X+1.92593\)
Assay B:  

- 10 picograms β-endorphins = 87.48 %B/Bo  
- 25 picograms β-endorphins = 68.59 %B/Bo  
- 50 picograms β-endorphins = 50.06 %B/Bo  
- 125 picograms β-endorphins = 25.32 %B/Bo  

Correlation Coefficient = 0.993594  
Estimate of Variance = 1.04324E-3  
F = 154.605 with 1 and 2 degrees of freedom  
Y = 4.54005E-3X + 1.95863

Assay C:  

- 10 picograms β-endorphins = 91.45 %B/Bo  
- 25 picograms β-endorphins = 78.12 %B/Bo  
- 50 picograms β-endorphins = 53.87 %B/Bo  
- 125 picograms β-endorphins = 28.39 %B/Bo  

Correlation Coefficient = -0.993252  
Estimate of Variance = 1.02944E-3  
F = 146.691 with 1 and 2 degrees of freedom  
Y = 4.39299E-3X + 1.99025

The concentrations of picogram per milliliter of plasma were made from this formula and the input of Y, %B/Bo. A copy of the standard curve developed originally and the standard curve derived from data output are compared on one sheet marked for each assay.
APPENDIX G

TABLES FOR VALIDITY ISSUES—EQUIANALGESIA

This appendix contains Tables 1 and 2 which are presented to (1) support the concept that narcotic analgesics are similar in chemical structure, and (2) depict that narcotic analgesia produces similar effects in the human body. Information in the tables support the process of equating narcotic analgesia. Therefore, summarizing narcotics administered to burned patients via the equianalgesia table is viewed as a valid approach.
Table 1. Structures of Opioids and Narcotic Antagonists Chemically Related to Morphine.

<table>
<thead>
<tr>
<th>NONPROPRIETARY NAME</th>
<th>CHEMICAL RADICALS AND POSITIONS</th>
<th>OTHER CHANGES**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3*</td>
<td>6*</td>
</tr>
<tr>
<td>Morphine</td>
<td>-OH</td>
<td>-OH</td>
</tr>
<tr>
<td>Codeine</td>
<td>-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>-OH</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>-O</td>
</tr>
<tr>
<td>Naloxone</td>
<td>-OH</td>
<td>-O</td>
</tr>
</tbody>
</table>

* The numbers 3, 6, and 17 refer to positions in the morphine molecule, as shown above.

** Other changes in the morphine molecule are as follow:

1) Single instead of double bond between C7 and C8.
2) CH<sub>3</sub> added at C5.
3) OH added to C14.
4) No oxygen between C4 and C5.

(Ref. adopted from Goodman & Gillman, 1978, p.246.)
Table 2. Comparative Pharmacology

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Analgesic</th>
<th>Anti-tussive</th>
<th>Constipation</th>
<th>Respiratory Depression</th>
<th>Sedation</th>
<th>Emesis</th>
<th>Physical Dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Meperidine</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Codeine</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxycodone</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

APPENDIX H
INDIVIDUAL PATIENT PLOTS

To depict the pattern of $\beta$-endorphin levels over a 2-week interval post burn, individual plots were prepared for those patients with 6 or more samples. The following pages contain the plots for patients 1, 2, 3, 6, 10, 11, 12, 15, 18, 19, 21, 22, 23, 24, 25, 26, 27, and 28.

An attempt was then made to visually group patients with similar patterns on one figure to demonstrate similarities. The last pages of this appendix contain figures of patient plots that have similarities. The erratic peaks and troughs evident in the intervening days between admission and discharge tend to obscure the basic premise for each figure; nevertheless, the patterns are viewed according to the following: Figure 1 depicts those patients with higher $\beta$-endorphin levels at the end of 2 weeks than on admission; Figures 2 and 3 depict those patients that have similar $\beta$-endorphin levels on admission and at the end of the 2-week interval.
Patient #10

β-Endorphin Levels

Days Post Burn
Patient #19

β - Endorphin Levels

Days Post Burn
Patient #22

Days Post Burn

- Endorphin Levels

0 1 2 3 4 5 6 7 9 11 13
Patient #23

Days Post Burn

β - Endorphin Levels

0 1 2 3 4 5 6 7 8 9 10 11 12 13
Patient #24

$\beta$-Endorphin Levels vs. Days Post Burn

Days Post Burn:
- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13

$\beta$-Endorphin Levels:
- 50
- 100
- 150
- 200
- 250
- 300
- 350
- 400
- 450
- 500
Patient #25

Days Post Burn

- Endorphin Levels

Days: 0 1 2 3 4 5 6 7 9 11 13

Levels: 0 50 100 150 200 250 300 350 400 450 500
Patient #27

Days Post Burn

β-Endorphin Levels

0 50 100 150 200 250 300 350 400 450 500

0 1 2 3 4 5 6 7 8 9 10 11 12 13
Figure 1. This subset of plots includes those patients that were admitted to the burn unit with lower β-endorphin levels on admission but had relatively high β-endorphin levels on the last day samples were drawn (Patients 2, 11, 15, and 28).
Figure 1. Subset of Patients with Higher \( \beta \)-Endorphin Levels at the End of the Two-Week Interval Than on Admission
Figures 2 and 3. These two subsets of plots include those patients that had admission and terminal β-endorphin levels that were low and similar. Since patients in this category were numerous, two figures were prepared with the following patients:

Figure 2. Patients 3, 10, 18, 22, and 23.

Figure 3. Patients 24, 25, 26, and 27.
Figure 2. Subset of Patients with Similar $\beta$-Endorphin Levels on Admission and at the End of the Two-Week Interval.
Figure 3. Subset of Patients with Similar β-Endorphin Levels on Admission and at the End of the Two-Week Interval.
LIST OF REFERENCES


