SEPTIC SHOCK AND ENDORPHINS

by

Fivos N. Gahhos, M.D.

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ABSTRACT

There are two patterns of progression in septic shock, the hyperdynamic and the hypodynamic circulatory response. There is evidence to suggest that β-endorphin, an endogenous opiate released at times of stress, plays a role in the pathophysiology of shock. However, the exact mechanism of action is not known. In this study, two experimental models of septic shock in piglets were developed, one closely approximating the hypodynamic clinical setting and another the hyperdynamic. Subsequently, the hypodynamic septic shock model was chosen to investigate the effects of β-endorphin in shock, because of its highly reproducible nature, and close proximity to the 'terminal clinical shock'.

Intravenous boluses of naloxone (group I), morphine (group II) and normal saline (group III) were given to three groups of piglets after two hours of sepsis, which was induced by intravenous infusion of live E. coli.

In group I, blood pressure, cardiac output, cortisol and cyclic AMP levels increased after naloxone, while heart rate and liver glycogen levels decreased. The hemodynamic changes lasted for about thirty minutes. In the second group of animals, morphine resulted in a significant drop in blood pressure and cortisol levels while PWP, substance-P and growth hormone increased. Prolactin levels did not change. No hemodynamic changes were noted in response to normal saline.

From these results it was concluded that during septic shock in piglets, opiate receptor blockade results in transient hemodynamic improvement, and therefore, endogenous opiates are partly responsible for the hypotension and low cardiac output of shock. Morphine decreases pulmonary vascular resistance by both depressing the myocardium and causing pulmonary vasodilatation. Endogenous opiates seem to play a role in the homeostasis of shock; and also appear to work through the adenyl cyclase-cyclic AMP system.

RESUME

Le choc septique peut se développer de deux façons: La réponse du système circulatoire hypodynamique et hyperdynamique. L'évidence suggère que le β-endorphine, un opiat endogène sécrété dans les situations de stress, joue un rôle dans la pathophysiologie de choc. Cependant le mode exact d'action de cet endorphine n'est pas connu. Dans cette étude avec les cochons de lait, deux modèles expérimentaux sur le choc septique ont été développés. Un modèle se rapproche de la situation clinique hypodynamique et l'autre hyperdynamique. Subséquement le modèle hypodynamique de choc septique était choisi pour investiguer les effets de β-endorphine en état de choc à cause de sa nature facilement reproducible et sa proximité au choc clinique terminal.

Des bolus intraveineux de naloxone, groupe I, la morphine, groupe II, et la saline physiologique normale, groupe III étaient donnés à trois groupes de cochons de lait deux heures après le début de l'état septique, cet état provenant d'une infusion intraveineuse de E.coli vivant.

Le groupe I: La pression sanguine, débit cardiaque, le niveau de cortisol, et de AMP cyclique augmentent après la naloxone après que le rythme cardiaque et le niveau de glycogène du foie diminue. Les changements hémodynamiques ont été présents pendant environ trente minutes. Dans le second groupe d'animaux la morphine a causé une chute significative dans le niveau de cortisol, alors que PWP, la substance-P et l'hormone de croissance a augmenté. Le niveau de prolactine n'a pas changé. On n'a pas remarqué de changement hémodynamique dans la réponse au saline physiologique.

On a conclut de ces résultats que pendant un état de choc septique sur les cochons de lait un blocage de récepteur d'opiat a comme résultat une amélioration transitoire hémodynamique et, en conséquence, les opiats endogènes sont, en partie, responsables pour l'hypotension et le faible débit de choc. La morphine entrainait une détérioration de résistance vasculaire pulmonaire en baissant le myocarde et en causant une vasodilatation pulmonaire. Les opiats endogènes semblent jouer un rôle dans l'homéostase de choc, et aussi semblent influencer le système adényl cyclase/AMP cyclique.

PREFACE

The investigations for this thesis were performed in the University Surgical Clinic at the Montreal General Hospital. Results from these investigations were presented at three international scientific meetings. The results described in Chapter II were presented at the Third Annual Meeting of the Shock Society in Lake Ozark, Missouri, and the abstract published in Circulatory Shock, Volume 7, Number 2, 1980, entitled 'Hyperdynamic Versus Hypodynamic Septic Shock: The Role of Focus of Infection'. Results described in Chapter IV were presented at the Sixty-Sixth Annual Clinical Congress of the American College of Surgeons in Atlanta, Georgia, and the abstract published in the Surgical Forum, Volume XXXI. Both Chapters have been submitted for publication as full length papers. Furthermore, the findings in GI hormone changes as described in Chapter IV were presented at the 42nd Annual Meeting of the Society of University Surgeons in Hershey, Pennsylvania in February 1981 and will be published in Current Surgery.

This thesis has been mostly rewritten since its first submission in August 1981. A new section has been added to Chapter I - Introduction - titled 'Endocrinology of Sepsis' while the rest of the chapter is mostly rewritten, updated and a figure added to clarify the structure of endorphins. Chapter II of the original manuscript has been re-edited and titled 'Development of a Model'. Chapters III (methods), IV (results), V (discussion) and VI (conclusions) have also been rewritten and contain the information summarized in Chapter III of the original thesis. Furthermore, results and discussion of GI hormone findings appear which were not present in the first manuscript. In summary, the overall form of the thesis has been changed from one of self-contained and independent chapters of published work to a more traditional format with multiple additions, clarifications and updating of the introduction.

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ABBREVIATIONS

MDF	Myocardial depressant factor
CVP	Central venous pressure
PAP	Pulmonary artery pressure
PWP	Pulmonary wedge pressure
CO	Cardiac output
RV-EDP	Right ventricular end-diastolic pressure
LV-EDP	Left ventricular end-diastolic pressure
LV	Left ventricle
TPR	Total peripheral vascular resistance
PVR	Pulmonary vascular resistance
ADH	Anti-diuretic hormone
ACTH	Adrenocorticotropic hormone
MW	Molecular weight
cAMP	Cyclic adenosine monophosphate
CBG	Corticosteroid binding globulin
CRF	Corticotropin releasing factor
PIF	Prolactin inhibitory factor
PRF	Prolactin releasing factor
TRH	Thyrotropin releasing factor
TSH	Thyroid stimulating hormone
SRF	Somatotropin releasing factor
ATP	Adenosine triphosphate
PDE	Phosphodiesterase
cGMP	Cyclic guanosine monophosphate
GTP	Guanosine triphosphate
CNS	Central nervous system
mRNA	Messenger-ribonucleic-acid
α-MSH	α-melanotropin
CLIP	Corticotropin-like intermediate-lobe peptide
γ-LPH	γ-lipotropin
β-MSH	β-melanotropin
β-LPH	β-lipotropin
α-LPH	α-lipotropin

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INTRODUCTION

Chapter I

DEFINITION OF SEPTIC SHOCK

Shock is a state of circulation in which a functional deficit of tissue perfusion in one or more vital organs is so severe that organ function is impaired and an unstable state develops in which blood flow becomes progressively more deficient. Precise criteria for the diagnosis of shock applicable to all situations are not available, primarily because the term shock itself has not been strictly defined. In experimental animals, diagnosis of the shock stage usually is based upon specific hemodynamic abnormalities, including a reduction in arterial pressure and in cardiac output. In the clinical setting, however, criteria for the diagnosis of shock have usually depended more upon evidence of impaired organ function relating to impaired flow.

The term septic shock, as distinct from other types of shock, implies that a wide spectrum of microbial species can induce shock. In this syndrome, invasion of blood by micro-organisms results in inadequate tissue perfusion by a mechanism not completely understood. Septic shock has been recognized in association with a wide variety of bacterial, fungal, viral, rickettsial and parasitic infections. However, bacterial infection is easily the commonest underlying problem. In about two-thirds of patients, septic shock results from bacteremia with endotoxin-containing Gram-negative bacilli so that the terms 'Gram-negative shock', 'endotoxin shock' and 'septic shock' are often used interchangeably. The commonest Gram-negative bacteria implicated in septic shock are: E. coli, Pseudomonas aeruginosa and Bacteroides fragilis. Sepsis and septic shock is now one of the most important causes of morbidity and mortality following surgery and trauma.

The pathophysiology of shock is complex, but most patients begin with hypovolemia, heart failure, or improper distribution of flow in small vessels. This distribution of flow in peripheral vessels is one of the most confusing aspects of the circulatory system disruption in shock. The defect

consists of maldistribution of flow, with inadequate transport of oxygen at the microcirculatory and cellular level, which leads to the seeming paradox of high cardiac output, while generalized underperfusion kills the patient. The mechanism of the neurohumoral factors that lead to this altered microvascular rheology is not well understood.

PATTERNS OF RESPONSE TO SEPSIS

There seem to be two clinical patterns of progression in septic shock in patients evaluated by Maclean et al (1), one being patients that are warm and vasodilated with high cardiac output and a low peripheral resistance (2). The second group of patients are vasoconstricted with cold and clammy skin, low cardiac output, high peripheral vascular resistance and a high mortality (3,4,5,6). Experiments in dogs have shown that vasoactive kinins can produce the features which we have associated with hyperdynamic septic shock (7). Clinical studies suggest that substances with kinin activity may be released in human sepsis (8,9) and Attar et al (10) have shown decreased kininogen activity in fatal septic shock in man.

In a study by Siegel (11), it was noted that the patients with hypodynamic (low-output) septic shock had poorer ventricular function relationships than those with hyperdynamic (high-output) septic shock. In 1966, Brand and Lefer (12), and Baxter et al (13) independently reported the presence of a cardioinhibitory factor in the plasma of cats and dogs in shock. Both groups termed the substance myocardial depressant factor (MDF). Since that time, extensive work has been done in this field and several MDFs identified. Some scientists believe that MDFs play a significant role in the pathogenesis of circulatory shock (14,15,16), while others deny its existence altogether (17,18), and claim that flow states represent inadequate volume replacement. Advocates of the MDF theory feel that the primary event in any type

of shock is splachnic hypoperfusion which then releases lysosomal hydrolases, primarily from the pancreas, giving rise to MDF, which in turn exerts strong negative inotropic effect on the heart.

Another concept is that of increased arteriovenous shunting in tissue (19,20,21). However, two recent studies, one in patients using radioactive xenon washout for skeletal muscle capillary blood flow (22) and the other in animals using microspheres (23), failed to demonstrate either an increased arteriovenous shunt or a decreased capillary blood flow. From the data presented above several conclusions can be made. Firstly, septic shock can present either as a high or low output state; and secondly, the pathophysiology for the two types of presentations remains obscure despite the numerous hypotheses that have been put forward. However, there is general agreement that septic shock induces initially a hyperdynamic state with a high cardiac output and a low peripheral resistance, unless the patient has previously had a reduced blood volume. After a period of time, the hyperdynamic state gives way to deterioration of cardiac function and ultimate cardiac failure and death.

MONITORING IN SEPTIC SHOCK

Parameters like central venous pressure (CVP), pulmonary artery pressure (PAP), pulmonary wedge pressure (PWP), and cardiac output (CO) can be used to monitor the hemodynamic status of the patient in shock.

The adequacy of the heart as a pump can best be evaluated clinically by relating the cardiac filling pressure to its stroke volume or cardiac output. When the heart or pulmonary vascular bed is not directly involved in the etiology of the low flow state, the adequacy of cardiac filling can be assessed by measurement of CVP (24). This pressure approximates the right ventricular end-diastolic pressure (RV-EDP)

or the mean right atrial pressure. It is a measure of the filling pressure of the right heart and provides a useful assessment of left ventricular filling pressure only when the pulmonary vascular bed is normal and the left ventricle is not primarily involved by a disease process (25,26,27). The normal right ventricular filling pressure may range from a mean of 5 to 10 mm Hg. The importance, however, of the CVP is not that of a single measurement to make an assessment of normality, but rather the response of this pressure as a volume challenge is given to the patient in shock. Response to a fluid load provides the most precise guide as to whether the low flow state is related to an inadequate venous return to the heart or to an abnormality of heart function. A rise in venous pressure without any associated improvement in the signs of reduced tissue perfusion may be taken as evidence that the shock is not volume-responsive. In contrast, an improvement in signs of regional perfusion associated with little or no increase in venous pressure is evidence of a volume-responsive state and an indication for further infusion of volume until the abnormal circulatory state is corrected.

When acute volume expansion is ineffective in restoring the circulation, it must be assumed that cardiac dysfunction is playing an important role in the low flow state. Under these circumstances, it is usually mandatory to identify whether the disturbance resides predominantly in the right ventricle, the pulmonary vascular bed, or the left ventricle, and some measure of the left ventricular filling pressure usually is necessary.

The ingenious Swan-Ganz catheter (28) is a simple means of estimating left ventricular end-diastolic pressure (LV-EDP) without direct left heart catheterization. In most patients without intrinsic lung disease, pulmonary artery diastolic pressure and especially PWP are useful estimates of LV-EDP. Normal left ventricular filling pressure (PWP or PAP diastolic) is taken as up to a mean of 12 mm Hg, and is usually

from 3 to 7 mm Hg higher than right ventricular filling pressure (CVP). In the presence of disease confined predominantely to the left ventricle, the left ventricular filling pressure surpasses right ventricular filling pressure by more than the normal gradient, whereas when right ventricular filling pressure surpasses left ventricular filling pressure, a disease confined predominantly to the right ventricle or to the pulmonary vascular bed should be suspected.

There are several advantages for the shock patient when the Swan-Ganz catheter is used rather than a central venous catheter. First, it permits measurement of PAP diastolic and PWP that estimate LV filling pressures. Second, continuous monitoring of PAP systolic and mean reflect changes in pulmonary vascular resistance secondary to hypoxemia, pulmonary edema, and pulmonary emboli. Third, it permits estimation by thermodilution of cardiac output from the right heart alone. This is obtained by injecting cool saline through the proximal orifice of the Swan-Ganz catheter and detecting the temperature changes at the tip of the catheter. In most patients with shock, the actual measurement of cardiac output is not necessary, for adequacy of cardiac output is reflected in tissue perfusion and organ function. Actual measurements of output become useful only in situations where a therapeutic decision may be based upon the absolute level of cardiac index or where it seems important to monitor the patient's output in order to determine whether therapy is producing a salutary effect.

Although shock is often recognized clinically by a fall in auscultatory blood pressure, it is now recognized that this reduction in cuff pressure does not necessarily correspond to a reduction in intraarterial pressure. The advent of intraarterial pressure monitoring has made it clear that the clinical syndrome of shock may exist in the absence of hypotension. Therefore, a low arterial pressure no longer should be considered a prerequisite for the diagnosis of shock.

When cardiac output falls, a reflex increase in systemic vascular resistance usually is observed. The rise in resistance is not homogeneous throughout the vascular tree and is dependent on the intensity of neural, humoral, and local factors affecting vascular tone. An increase in peripheral vascular resistance (TPR) supports the arterial pressure, alters the regional distribution of cardiac output, and may further depress cardiac output. Since cardiac output is inversely related to outflow resistance, particularly when cardiac function is impaired, a constriction of large and small arteries and arterioles will result in a further reduction of what may already be a reduced cardiac output. Some increase in vascular resistance may be necessary to support life when cardiac output falls, since a very low aortic pressure may result in a critical reduction of cerebral and coronary perfusion. The total peripheral vascular resistance may be calculated using the following formula:

$$TPR = \frac{\text{Mean arterial pressure - Central venous pressure}}{\text{Cardiac output}} \times 80$$

(dynes-sec-cm $^{-5}$)

Another useful parameter in assessing septic shock is the pulmonary vascular resistance (PVR) which is defined as follows:

$$PVR = \frac{\text{Mean pulmonary artery pressure}}{\text{Cardiac output}} \times 80$$

(dynes-sec-cm $^{-5}$)

Regional perfusion is critically dependent upon the state of microvasculature. The large conduit arteries and veins play a relatively minor role in controlling circulation to regional vascular beds, while the smaller vessels and capillaries are critical in the control of regional flow and of microcirculatory

pressures that determine capillary filtration roles. Activation of the sympathetic nervous system with release of norepinephrine is a well understood factor leading to constriction of small arteries, arterioles, and venules (29). Other factors causing an increased pulmonary vascular resistance are hypoxia, respiratory acidosis, and the degree of inflation of the lung. With normal inflation of the lung, the vessel size is increased, whereas at low lung volumes, alveolar collapse will lead to reduced caliber of vessels and increased vascular resistance. Hyperinflation at high pressure will also increase pulmonary resistance by compressing the capillary bed. Pathologic studies in patients dying from the shock syndrome reveal marked capillary dilatation, pulmonary edema, alveolar hemorrhage and atelectasis.

By following these parameters closely during sepsis, we can assess the degree of function of the heart, the intravascular volume of the patient or animal, and the response of the peripheral vasculature. These parameters are available at the bedside and are the main criteria for the evaluation and management of the patient in septic shock.

ENDOCRINOLOGY OF SEPSIS

The importance of hormonal homeostasis in the pathophysiology of sepsis cannot be overemphasized. Furthermore, there is increasing evidence that endogenous opiates, as discussed later in this chapter, play a role in the release of many hormones associated with the pathophysiology of septic shock.

When, as a consequence of severe hypovolemia, bacterial toxins, myocardial infarction, or for any other reason, tissue perfusion drops to shock levels, profound metabolic disturbances occur (30). Bacterial endotoxin is capable of providing direct hypothalamic stimulation with attendant release of adrenocorticotropic hormone (ACTH) (31), vasopressin (ADH) (32) and growth hormone (33). Furthermore, sympathetic neural activity

increases and there is thus enhanced secretion of epinephrine and norepinephrine (34) from the adrenal medulla, of renin (35,36) from the kidney, and of glucagon (37) from the pancreas. The same sympathetic stimulation of circulating cathecolamines may inhibit release of insulin (38,39,40,41) from the pancreas.

Sepsis leads to increased secretion of ACTH which stimulates the adrenals to produce more cortisol (42,33). Elevated plasma levels of cortisol lead in turn to inhibition of further release of ACTH. However, increased plasma levels of cortisol also lead to inhibition of release of vasopressin, growth hormone and prolactin (43). Thus, the feedback effect of cortisol may be general and may extend far beyond the control of ACTH release per se. The response to some stimuli like large hemorrhage or overwhelming sepsis, cannot be suppressed by cortisol, and ACTH release will persist until the stimulus is removed (44). Most patients who die following sepsis die with very high blood levels of corticosteroids (45). As mentioned above, endotoxin can act directly upon the hypothalamic sympathetic control centers to produce epinephrine and norepinephrine release (42). Epinephrine and norepinephrine both increase cardiac output and elevate blood pressure. Denervation of the adrenal stops the secretion of cathecolamines in the adrenal venous blood.

Glucagon produces inotropic cardiovascular effects when given to patients (37) or animals (46) in shock. These effects consist of an increase in cardiac output and stroke volume and a decrease in peripheral vascular resistance despite β-receptor blockade. The main results of increased glucagon secretion in trauma are to produce increases in blood glucose through stimulation of glycogenolysis, gluconeogenesis and lypolysis (47).

Another group of polypeptides has recently been identified (48), which seem to play a part in the pathophysiology of low cardiac output and hypotension of sepsis. These substances are grouped under the name of endogenous opiates and their role in sepsis is discussed in the following sections.

A concise description of the properties and function of each of the hormones investigated in this study will now be given.

a) Insulin and Glucagon

Insulin and glucagon are peptide hormones which are secreted by pancreatic islet cells and exert important regulatory effects on carbohydrate, fat, and protein metabolism. Insulin was isolated in 1922 (49) and its amino acid sequence established by Sanger in the 1950's (50). In 1964, it was synthesized in the lab as reported by Katsoyannis et al (51), while glucagon's structure was confirmed by total chemical synthesis in 1967 (52).

Insulin biosynthesis is the exclusive property of the B cell of pancreatic islet tissue (53), while glucagon is synthesized and secreted mainly by the A cells of the pancreatic islets (54). Small amounts of glucagon have also been extracted from gastric and intestinal mucosa of a number of animal species (55).

Insulin has a molecular weight (MW) of about 6,000 and consists of two polypeptide chains, A and B, joined by disulfide bridges. The only differences noted among the five species of mammalian insulin - beef, pork, sheep, horse, and whale - reside in the sequence of three amino acids within the A-chain (56). Glucagon, on the other hand, is a smaller molecule with a MW of about 3,500 and 29 amino acid residues.

In a classic study with the perfused rat pancreas, Anderson and Long (57), clearly demonstrated the primary role of glucose in stimulating insulin secretion. The mechanism of this stimulation is not known, but it appears to be closely related in some way to the utilization of glucose (58). In addition to glucose, amino acids have also been shown to evoke insulin release, the most potent being arginine and lysine (59). Plasma glucose is the prime regulating agent of glucagon secretion (54,55), but acting to suppress rather than stimulate as it

does in the B cell. By contrast, amino acids, particularly arginine and alanine, are effective stimulators of glycogen secretion, an action which resembles that in the B cell. While glucose is the primary stimulus in both hormones, several lines of evidence (60) strongly implicate cyclic AMP (cAMP) and possibly calcium in the secreting mechanism. This is discussed in detail in the following section entitled 'The Second Messenger System'.

Secretion of insulin and glucagon by the pancreatic islets serves to maintain a constant level of plasma glucose. When plasma glucose alone is altered, as following a high carbohydrate meal, levels of plasma insulin and glucagon often change in a reciprocal manner, reflecting positive and negative influences of glucose on B and A cell secretion, respectively. On the other hand, if a meal is high in protein, plasma glucose will not change appreciably while both insulin and glucagon levels will increase (61).

Insulin is rapidly removed from plasma by the body tissues (62). Comparable rates of removal also have been shown for glucagon, and both hormones are probably handled by systemic tissues in a similar manner. The half-life of plasma insulin is 10 minutes or less. The initial reaction of these two hormones with tissues involves binding of the hormone to specific sites on the cell membrane. Those receptor sites, which are specific for each hormone, have been nearly purified in the past few years (63,64). From those receptor sites the hormone exerts its metabolic effect on that cell and is inactivated and broken down to its constituent amino acids. The question of the nature of the pathway that links the hormone receptor to the intracellular site of degradation remains unanswered.

The vital role of the liver as a source of glucose was shown many years ago by Mann and Magath (65) who studied the effects of hepatectomy in the dog. Although glucose is stored in the form of glycogen in muscle, the absence of glucose-6-phosphatase

prevents its release as free glucose into the blood. The solution to this impasse is conversion of glucose to lactate by glycolysis in muscle, but in the absence of the liver, there is little reconversion of the lactate to glucose by gluconeogenesis. While the kidney possesses the enzymes that are required for gluconeogenesis, the quantitative capacity is not sufficient to maintain normal glucose levels in the absence of the liver. The two major pathways of glucose formation in liver, glycogenolysis and gluconeogenesis, are regulated by insulin. When rates of glucose formation are increased by agents, such as glucagon or epinephrine, the addition of insulin exerts a strong inhibitory effect on glucose formation and release from the liver. Since the effect of insulin to lower blood glucose can be attributed to stimulation of glucose uptake by muscle and adipose tissue, the role of the liver in glucose regulation was not readily recognized. In addition to these hormonal factors, blood glucose itself strongly inhibits hepatic glucose release and stimulates its uptake by effects which are exerted predominantly on glycogen metabolism.

Considerable glucose is also derived by gluconeogenesis. Approximately 20% of the glucose which is produced in this way is synthesized from lactate while amino acids from the breakdown of tissue protein contributes the largest fraction of carbon for gluconeogenesis. The extent to which glycogen deposition is the result of diminished rates of glycogenolysis as opposed to the stimulation of glycogen synthesis is not established with certainty, but is likely that both mechanisms operate. Insulin has been shown to stimulate glycogen synthesis in liver. Although glucagon has no direct effect on protein breakdown in skeletal muscle (66), the hormone does stimulate steps in gluconeogenic pathways in the liver and also directly enhances protein degradation in the liver. It seems possible that the strong stimulatory effect of amino acids on glucagon secretion would aid in these aspects of amino acid utilization.

Finally, it is important to know that in addition to glucagon, there are a number of agents whose actions, in general, are opposite to those of insulin. Such agents include epinephrine, glucocorticoids, growth hormone, and fatty acids. When one or more of these antagonists is greatly increased or decreased in amount, then the effectiveness of insulin will be decreased or increased accordingly, giving rise to significant alterations in metabolic regulation.

b) Cortisol

The adrenal cortex synthesizes three classes of hormones; glucocorticoid, mineralocorticoid, and sex hormone. The most important steroids secreted by the normal adult human adrenal cortex are the glucocorticoid, cortisol, and the mineralocorticoid, aldosterone. The hormonal steroids are all synthesized from acetyl coenzyme A with cholesterol as their common intermediate (67), and retain the basic four-ring sterol structure. Cortisol is reversibly oxidized to cortisone in the tissues, predominantly the liver. Cortisone is biologically inert and has a half-life in plasma less than half of cortisol. In plasma, about 2% of cortisol normally circulates in the free state, a small fraction is nonspecifically bound to serum albumin, and greater than 90% is specifically bound to corticosteroid-binding α_2-globulin (CBG). The cortisol bound to CBG or albumin is metabolically inactive, but is in rapid equilibrium with free cortisol (68). Cortisol is cleared from plasma with a half-life of 70 to 80 minutes mainly by degradation in the liver. The metabolites of cortisol and cortisone are excreted in the urine as conjugates of glucuronic acid.

The synthesis and secretion of cortisol are dependent upon stimulation by ACTH with cAMP as a mediator. Three factors are of major importance in regulating ACTH secretion. First, there is homeostatic negative feedback inhibition of ACTH secretion by circulating cortisol. This inhibition may act at the hypothalamic level by inhibiting the release of

corticotropin-releasing factor (CRF) into the hypothalamic-hypophyseal portal blood vessels. Second, plasma cortisol concentrations show a circadian rhythm, with high levels in the early morning and low levels late in the evening. This rhythm is the result of a circadian rhythm in ACTH secretion and appears to be synchronized by the daily environmental shift from darkness to light. Whether or not this is mediated by CRF secretion is not known. The third important factor governing ACTH secretion is 'stress' (43). While some of the influence of stress may be mediated via hypothalamic CRF release, it is of interest that section of the pituitary stalk not always, and seldom permanently, abolishes stress-induced ACTH release.

The overall pattern of cortisol action might be viewed as promoting the conversion of protein to carbohydrate and the storage of carbohydrate in the form of glycogen (69). It is easier to define the effects of cortisol deficiency or excess, which are manifested in every organ system, than to define the role of glucocorticoids in normal physiology. Some of the effects of cortisol on various organ systems are listed here (69):

1) Central Nervous System. Cortisol seems to have a stimulatory effect on brain excitability, independent of electrolyte effects the mechanism of which is unknown.

2) Musculoskeletal System. Cortisol has catabolic effects on skeletal muscle, and a marked influence on connective tissue integrity. In conditions of cortisol excess, peripheral subcutaneous tissue is decreased and skin becomes thin and friable. Wound healing is delayed and bone formation inhibited.

3) Cardiovascular System. Cortisol appears to potentiate the peripheral arteriolar response to vasoconstrictors. It increases cardiac output when given in pharmacological doses.

4) Gastrointestinal System. Cortisol antagonizes the action of vitamin D in promoting calcium absorption from the gastrointestinal tract. There is no conclusive evidence to support the belief that high levels of glucocorticoids can produce ulceration of normal gastric mucosa.

5) Urinary System. The net effect of cortisol on the kidney is maintenance of the ability to excrete a water load (70).

6) Immune Response. Cortisol has a dramatic catabolic effect on lymphoid tissue manifested by lymphoid and thymic atrophy and lymphopenia in hypercortisolism. It is involved in almost every phase of the inflammatory response by depressing the ability of the body to fight infection.

The importance of the adrenal glands in maintaining life was first recognized clinically by Addison in 1855 and confirmed experimentally by Brown-Seguard in the following year. Much has been learned about the function of the adrenal glands and the hormones they produce since then, but much more remains to be learned.

c) Gastrin

Gastric Secretion

Gastric secretion is controlled by both neural and humoral mechanisms. The parasympathetic (vagus) innervation provides the pathways for secretory stimuli to the gastric mucosa (71). Sympathetic pathways control gastric secretion indirectly, due to their control over vasomotor mechanisms of blood flow to the mucosa.

A number of hormonal mechanisms also are involved in the control of gastric secretion. Examples include the release of gastrin from the gastric antrum, which stimulates gastric secretion, and the release of enterogastrone (72) from the mucosa of the upper small bowel, which inhibits gastric

secretions. It is convenient, when discussing the gastric secretory response to a meal, to divide the total response into different phases: cephalic, gastric and intestinal phases, named according to the area where the stimuli arise. In man, gastric secretion tends to be continuous. For this reason, a fourth phase of gastric secretion, the inter-digestive phase is recognized. During this phase, gastric secretion is continuous at rather low levels of maximal capacity, independent of circulating gastrin levels, and reduced but not obliterated by vagotomy or removal of the antrum. The sight, smell and taste of food which, if agreeable (73), will initiate the psychic or cephalic phase of gastric secretion. This stage is mediated by the vagus nerves by two mechanisms: (i) direct cholinergic stimulation of the oxyntic (parietal) cells; and (ii) cholinergic release of gastrim from the pyloric gland area. In man, the direct effect of vagal stimulation on the parietal cell is probably the more important. The entry of food into the stomach (gastric phase) initiates gastric secretion by distention from the bulk of the meal, and in response to peptides and amino acids, especially glycine, β-alanine, serine, and lysine. Distending the stomach or bathing the gastric mucosa with partially digested proteins or amino acids leads to acid secretion by G-cells. The chemical mediators of acid secretion are clearly acetyl-choline release at postganglion synaptic junctions of the vagus, gastrin released from the G-cell, and the mysterious histamine. Acetylcholine is released not only by vagal stimulation, but also by gastric distention. This may be blocked by atropine. As soon as the stomach is empty and the buffering substances present during digestion are no longer available, the pH of the antrum or the duodenum falls to a point at which the inhibitory effect of acid becomes evident and that period of gastric secretion is brought to an end. During the gastric phase of secretion, some chyme enters the small intestine where after a variable latent period of 1 to 2 hours, it

initiates the intestinal phase (74). This phase probably accounts for less than 20% of the total acid secreted by the stomach. In duration, however, the intestinal phase exceeds the other phases. Following the completion of the intestinal phase, the interdigestive phase occurs. During this phase, a minimal amount of gastric secretion occurs intermittently.

Gastrin

The recent increase in our knowledge of gastrin stems from the chemical definition of gastrin (75) and the use of radioimmunoassay in the detection of minute quantities of this hormone in body fluids (76). Gastrin is produced by the G-cell of the antrum of the stomach and first part of the duodenum. There are two main forms of gastrin. In addition to the unsulfated tyrosine (form I, MW 2,098) and the sulfated tyrosine (form II, MW 2,176) forms, gastrin exists in three size forms: big gastrin, little gastrin, and minigastrin. Big gastrin (G 34; MW 3,839) has 34 amino acid residues; little gastrin (G 17; MW 2,098) is a decaheptapeptide consisting of residues 18 to 34 of G 34; and minigastrin (G 13; MW 1,647) (77) consists of residues 22 to 34 of G 34 or residues 5 to 17 of G 17. G 34 is predominant in the blood after a meal and G 17 in the antrum, the latter being cleared five times faster from the blood than G 37 and being five times more active. Trypsin can convert G 37 to G 17. The carboxy terminal portion of the gastrin molecule is all that is required to activate gastrin receptors on the parietal cell.

The role of the vagus in the modulation of gastric acid and gastrin secretion in mammalian species is not fully understood. It has been shown both to inhibit and stimulate (78) gastrin release under different conditions, and a cholinergic inhibitory pathway for gastrin release appears to exist (79). Cutting the vagus nerves greatly decreases the secretion of acid in response to gastrin (80). Local distention, peptide

or amino acid solutions bathing the G cell area (81), calcium, either in the luminal fluid or in the form of hypercalcemia, stimulate gastrin release. The release of gastrin from the pyloric gland area is markedly inhibited by acid bathing this area of the stomach. A pH of 2 or less is required and can counteract the stimulatory effects both of vagal stimuli and of local distention and chemical factors (81). Thus, as the stomach contents become more acidic during the course of a meal, gastrin release is correspondingly suppressed.

Glucagon is found to inhibit gastrin and pancreatic secretions and also inhibit stomach, small bowel, and gallbladder mobility, while substance-P stimulates mobility of the fundus, antrum and small intestine.

According to Grossman (82), gastrin stimulates gastric secretion of hydrogen ion (83) and pepsin; stimulates contraction of the gastroesophageal sphincter and relaxes the ileocecal sphincter (84). It also stimulates gastric antral motility, and the secretion of bicarbonate, water and pancreozymin by the pancreas (85).

So far no relationship between serum gastrin levels and sepsis or septic shock has been reported. In this study we attempt to establish the changes in serum gastrin levels occurring during septic shock in the pig.

d) Prolactin

Prolactin, a polypeptide hormone, was identified in 1928 as a substance in anterior pituitary extracts capable of causing lactation in the rabbit. Most of the amino acids constituting prolactin are found in growth hormone making their physical and chemical properties very similar. The development of sensitive radioimmunoassays for each hormone has greatly facilitated our understanding of their separate biological roles (86). It is produced by the chromophobe cells in the anterior pituitary. Two types of prolactin have been isolated: a normal sized one with a molecular weight of 22,000 daltons

and a larger one with a molecular weight of 40,000-50,000 daltons (87). The large molecule, probably representing a prohormone, constitutes 10% to 20% of the radioimmunoactivity in normal persons. Freezing and thawing and prolonged storage may cause considerable conversion in vitro of 'big' to 'little' prolactin.

The serum level of prolactin appears to be regulated by stimulation of the hypothalamus and pituitary. No peripheral target feedback humoral response has yet been documented.

The hormone is secreted in a diurnal rhythm described by Parker et al (88) as briefly episodic, and sleep enhanced. Anesthesia and surgical stress significantly raise prolactin levels (89) from preoperative levels to 20 µg/l to greater than two-fold in women undergoing general surgery.

Prolactin is under tonic inhibition from the hypothalamus, via the secretion of a neurohormone termed prolactin inhibitory factor (PIF). The hypothalamic content of catecholamines (dopamine) can account for all the PIF activity of the hypothalamus (90). Evidence is accumulating that dopamine is the only PIF secreted by the hypothalamus. Apomorphine is a potent blocker of the dopamine receptor site; opiates hence promote prolactin secretion. Oral methadone, for example, results in a 70 to 80 ng/ml rise in prolactin (91). Besides PIF, hypothalamic extracts have also been shown to contain at lease two substances that stimulate prolactin release, thyrotropin releasing factor (TRH) and prolactin releasing factor (PRF). There is no doubt that the predominant mode of prolactin regulation is inhibitory.

The role of cAMP in the secretion of prolactin appears to be complex. It had previously been assumed that cAMP was an essential second messenger mediating the action of TRH; recent studies, however, suggest that such a role has not definitely been established.

Prolactin is readily detectable in the plasma of almost all human beings by assays having a sensitivity of 1 to 2 ng/ml. Its half-life is approximately 20 minutes, and normal serum levels in adult males is 4.7±2.8 and women 8.0±4.9 (92). Mean serum prolactin concentrations in boys and girls from one year of age until puberty are in the range of 5 to 10 ng/ml and there is no difference between the sexes. Stress of all kinds tends to release prolactin in humans, in somewhat the same fashion as it does growth hormone, though the two hormones do not necessarily rise in parallel (93). Pregnancy and the act of nursing are powerful stimuli for prolactin release, as is hypoglycemia to a lesser extent (93).

The most important action of prolactin is in preparing the female breast for lactation. In both sexes prolactin may have a direct effect on the gonads, suppressing the secretion of sex steroids and contributing to amenorrhea and impotency. In males, prolactin increases the contents of the seminal vesicles. The presence of liver receptors suggests that prolactin has important hepatic actions. Prolactin might also have a role in maintaining serum osmolality in humans (94).

Recently, a possible role of prolactin in breast cancer has been suggested (95). However, it may be said that though prolactin certainly influences the development of breast cancer in some animals and may do so in man, a clear role of prolactin in human breast cancer has not as yet been defined.

The effects of shock, naloxone and morphine on serum prolactin levels is discussed under the heading β-endorphins and sepsis.

e) Growth Hormone

Growth hormone is produced almost solely by the acidophilic cells of the anterior pituitary. The pharyngeal hypophysis

produces unknown amounts and occasionally may provide growth hormone when the pituitary is ablated.

The 191 amino acids of growth hormone constitute a single chain that is looped back on itself in two places by sulfhydryl bridges. Elution curves on Sephadex columns reveal three molecules with growth hormone immunoreactivity labelled, little, big and large. More than half of big growth hormone may be converted to little during cold storage (96). The mode of action of this hormone has not yet been proven. With a molecular weight of 21,800, it most likely operates on the cell membrane, effecting intracellular changes secondarily. The liver is accountable for most of the clearance in man (97). The serum level is controlled by a negative feedback system with the hypothalamus and pituitary involving releasing somatotropin releasing factor (SRF) and inhibiting somatostatin. The result is a diurnal rhythm of irregular and intermittent spurts with a relationship to age, sex and sleep.

There are a number of stimuli whose locus of action is quite nuclear. These include stress (98), exercise (99) and administration of morphine derivatives (164,166). Martin found that (164) when morphine was administered to unanesthetized rats, growth hormone secretion was stimulated, although it could be blocked by prior administration of somatostatin. Morphine has not been shown to act directly on the pituitary, and its stimulating effect must be dependent on different, yet undetected, neural sites.

As with stimulation, there are conditions that inhibit growth hormone release, such as obesity, elevation of free fatty acid levels and glucocorticoids (100) whose site of action is likewise unknown. Other than the direct use of somatostatin, perhaps the most potent agent accomplishing this is the ingestion of glucose. In normal persons, the ingestion of a glucose load is followed by a fall in serum growth hormone, usually to undetectable levels (101). There

is also increasing awareness that rises in serum growth hormone levels that can occur without a known stimulus: the phenomenon of 'spontaneous' increases in serum growth hormone (102).

Growth hormone has both an insulin-like and an anti-insulin effect on the metabolism of glucose. The insulin-like effect is early and is observed both in vivo and in vitro. However, the effect is clearly attained only with very large local concentrations of the hormone or after injection of unphysiologic amounts of growth hormone. The contra-insulin-like action can be demonstrated by perfusing the forearm with insulin and growth hormone, when the effect of insulin is abolished by growth hormone. Precisely in what manner this contra-insulin effect is mediated is not clear.

Growth hormone causes an increase in the size of the skeleton, the muscle and connective tissue mass, and induces general splanchnomegaly by enhancing incorporation of amino acids into protein. In summary, growth hormone has a powerful anabolic effect.

THE SECOND MESSENGER SYSTEM

Hormones (first messengers) affect target tissue by regulating the enzymatic activity within that tissue. According to our present knowledge, enzymatic regulation can occur in one of two ways. Many hormones act by activating or deactivating enzymes that already exist within the target tissue. This response is mediated through a second messenger system located in the target tissue, the adenyl cyclase-cyclic adenosine monophosphate system (103). The second general method for regulation of target tissue enzymatic activity involves induction of enzyme synthesis via transcription. Transcription is followed by de novo synthesis of the enzyme, which is a much slower process than the activation of preexisting enzyme protein by the adenyl cyclase system.

In the first system, adenyl cyclase, an enzyme contained in the target cells can be stimulated by hormones such as epinephrine, glucagon, thyroid-stimulating hormone (TSH), ACTH, vasopressin, insulin, growth hormone, leuteinizing hormones, serotonin, angiotensin and prostaglandins (104). This enzyme which is located in the cell membrane, probably adjacent to the hormone-binding sites, converts adenosine triphosphate (ATP) to 3', 5'-cyclic adenosine monophosphate (cAMP). cAMP is inactivated by conversion of 5'-adenosine monophosphate (5'-AMP) through the action of a phosphodiesterase (PDE). Recent evidence indicates that at least two different phosphodiesterases exist in mammalian cells. These enzymes probably have separate functions in metabolic regulation involving intracellular cyclic nucleotide content.

Cyclic AMP was discovered by Rall, Sutherland and Bartlet in 1957 (106). The effect of cAMP within the target cells is to activate protein kinases, enzymes that utilize ATP to phosphorylate a protein, often another enzyme, within the cell. Protein kinases contain two subunits. One subunit is catalytic and the other is regulatory. When the two are combined, the kinase is inactivated. The regulatory subunit combines with the catalytic subunit in a way that prevents the latter from functioning. cAMP binds to the regulatory subunit and causes it to dissociate from the catalytic subunit. Once the regulatory subunit no longer is attached, the catalytic subunit is free to act.

Lypolysis and glycogenolysis are two of the most important metabolic processes activated by cAMP-mediated phosphorylation. In the case of lipolysis, triglyceride lipase is the enzyme converted from the inactive to the active form through phosphorylation. In the case of glycogenolysis, cAMP stimulates hepatic glycogen breakdown in response to epinephrine and glucagon. Epinephrine and glucagon increase the role of hepatic cAMP production, which in turn promotes the conversion of inactive phosphorylase b to active phosphorylase a (103)

which then catalyzes glycogen breakdown (106) and glucose production and release by the liver.

Another cyclic nucleotide, the 3', 5'-phosphodiester of guanosine monophosphate, also is present in mammalian tissues. This compound is commonly abbreviated cGMP and is produced from guanosine triphosphate (GTP) by guanyl cyclase, an enzyme that is similar to adenyl cyclase. Like cAMP, cGMP is degraded by phosphodiesterase and is an intracellular modulator for signals delivered from outside the cell by hormones or neurotransmitters. However, the cAMP concentration is ten to fifty times higher than that of cGMP. The concept of alpha and beta receptors initiated by Ahlquist (107) has since been expanded to include the adenyl-cyclase-cAMP system. It is postulated that (108) binding of a hormone to its particular receptor changes the permeability of that cell to calcium. Calcium in turn changes the adenyl cyclase activity. Alpha stimulation increases calcium entry into the cell to reduce adenyl cyclase activity, while beta stimulation reduces calcium entry to provide an increase in adenyl cyclase activity.

The catecholamines exert their effects on target tissues by binding to receptor sites on the cell membrane. The receptors have been divided into two categories, α and β on the basis of the relative potency of various agonists, which activate the receptors, and of various antagonists, which block them. β receptors are most sensitive to stimulation by isoproterenol (isopropylnorepinephrine) and least to norepinephrine, whereas with α receptors, the reverse is the case. Although there is no compelling evidence for the existance of two structurally distinct receptors, it has remained a useful concept since synthetic agents which activate or block only one class of receptors have been developed. Thus, α receptors are selectively blocked by phenoxybenzamine and phentolamine, whereas β receptors are blocked by propranolol. More recently β receptors have been divided into two classes based on the strong inhibition of some β receptors (called β_1) by drugs

like practolol, and of others (called β_2) by drugs such as butoxamine.

In the case of the β receptor mediated actions of catecholamines, there is evidence, as described previously, that catecholamine binding causes activation of the adenyl cyclase-cAMP system with a resultant mediation of the effects of the catecholamines. Recently however, some doubts have been expressed that this mechanism provides a complete explanation of these actions of the catecholamines (109). The exact mechanism by which α-adrenergic agents act is even less clear. Earlier hypotheses proposing that α-adrenergic agents act by decreasing cyclic AMP levels have not been supported.

A list of some of the effects of catecholamines and their apparent receptor types is given in Table 1-1. Epinephrine is more potent than norepinephrine in producing these effects. There are such extreme changes in epinephrine, norepinephrine, glucagon and cortisol levels in shock, coupled with severe ischemic, cellular, structural and functional damage, that changes in the cAMP system might be expected. Few experimental and even fewer clinical studies have been done in this specific area. However, of the experimental studies that have been initiated in this area, all indicate a depression in the normal function of the second messenger system in low flow states (110,111, 112). Studies done on dogs and pigs following hemorrhagic shock (110,113), in humans following major surgical trauma (114), myocardial infarction (115,116), and sepsis (117) have shown increases in circulating plasma cAMP levels when compared to levels in normal patients. The reports on shock induced alterations on tissue PDE are inconclusive and controversial (118). Stress activated hormonal and associated metabolic changes appear to be directly related to significant changes in the second messenger system. Inadequate or excessive response of the second messenger system could result in the decreased ability of the tissues to respond to homeostatic or therapeutic controls and therefore may contribute to irreversibility in shock.

TABLE 1-1

Effector Organ	Receptor Type	Response
Eye		
Radial muscle, iris	α	Contraction (mydriasis)
Ciliary muscle	β	Relaxation for far vision
Heart		
Sinoatrial node	β_1	Increase in heart rate
Atrioventricular node	β_1	Increase in conduction velocity and shortening of functional refractory period
Atria	β_1	Increase in contractility
Ventricles	β	Increase in contractility & irritability
Blood vessels	α	Constriction
	β_2	Dilatation (predominates in skeletal muscle)
Bronchial muscle	β_2	Relaxation (bronchodilatation)
Gastrointestinal tract		
Motility		
Stomach	β	Decrease
Intestine	α, β	Decrease
Sphincters	α	Contraction
Urinary bladder		
Detrusor	β	Relaxation
Trigone and sphincter	α	Contraction
Skin		
Pilomotor muscles	α	Piloerection
Sweat glands	α	Selective stimulation (adrenergic sweating)

ENDOGENOUS OPIATES

> 'Telemachus, grieving for his missing father, is given a drug by Helen. The drug has the power to banish all care, and to make a person so insensitive to painful experiences that one can regard the slaying of one's closest relatives before one's very eyes with indifference'.
>
> from 'Odyssey' by 'Homer'

The remedy offered by Helen to relieve the suffering is none other than opium. Twenty-four centuries later, in 1971, those receptors responsible for Telemachus' euphoria were identified (119). They were found to be located in the central nervous system being most abundant in the caudate nucleus, and closely associated with the known pain pathways. However, they also occur in parts of the nervous system not related to the processing of pain information.

Investigators began looking for an opiate-like substance in the body with the same shape and pattern as the reactive chemicals in opium plants. The generic name endorphins, from endogenous and morphine, was proposed for these then hypothetical substances by Eric Simon at NYU. In the summer of 1975 Roger Guillemin of the Salk Institute in California became interested in these early observations. The isolation of these endogenous ligands of the opiate receptors proved to be relatively simple for him, and was achieved in less than three months from extracts of porcine hypothalamus-neurohypophysis. Since that time, several substances fitting the description of endogenous opiates have been discovered (120,121,122,123).

These polypeptides can be classified in two systems: the enkephalin and the endorphin system. In the enkephalin system there are two pentapeptides which seem to coexist in the same cell. These are methionine-enkephalin and leucine-enkephalin. Methionine-enkephanin is the (NH_2) terminal pentapeptide of β-endorphin which will be discussed later. Enkephalins are found in CNS, ganglia of the GI tract, peripheral nerves such as vagus, adrenal medulla,

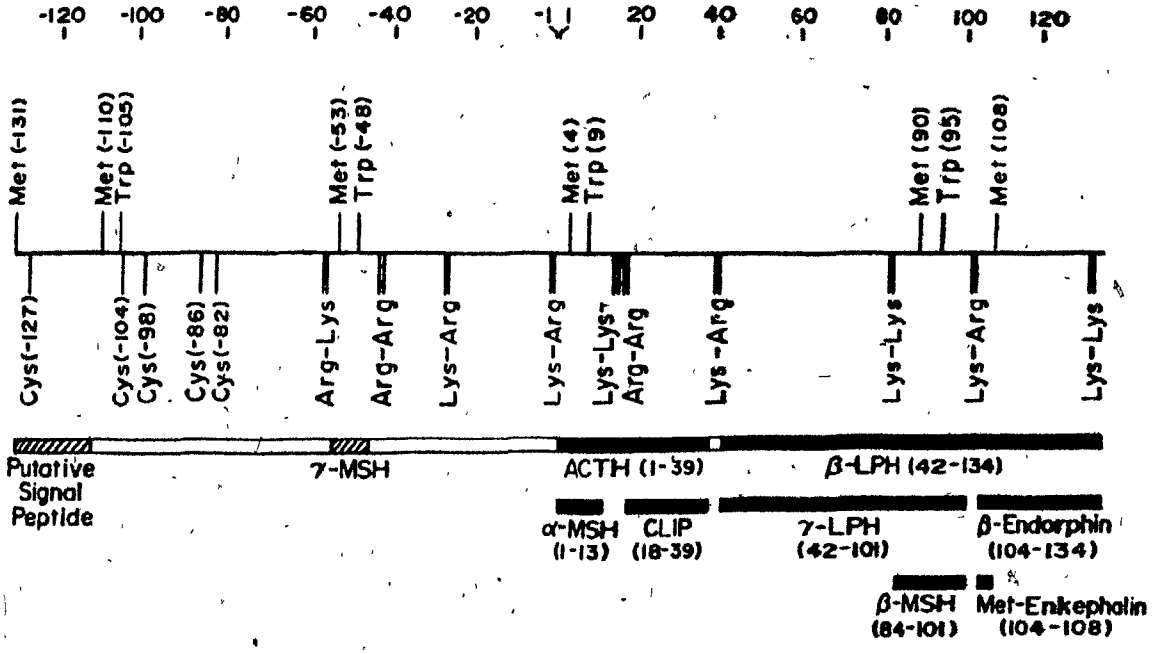
salivary glands and paracrine cells of the GI tract (124,125). The first compound identified in the endorphin system was α-endorphin and its primary structure established in 1976 (126, 127) is H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH.

The primary structure of γ-endorphin was similarly established by mass spectrometry and by Edman degradation and was found to have the same primary structure as α-endorphin with one additional Leu as the COOH-terminal residue in position 17. β-endorphin, the active compound of this system, is a peptide with 31 amino acids. Despite the clinical similarities, there is evidence that the two systems have different biosynthetic origin. β-endorphin appears to be formed from a macromolecule which is also the precursor of β-lipotropin and ACTH.

In 1979, Nakanishi and Inoue (128) published the nucleotide sequence of DNA encoding bovine ACTH and β-lipotropin (β-LPH) precursor messenger-ribonucleic-acid (mRNA). These two hormones which are formed from a large common precursor protein containing small component peptides with biological activity; α-melanotropin (α-MSH) and corticotropin-like intermediate-lobe peptide (CLIP) are derived from ACTH, whereas γ-lipotropin (γ-LPH), β-melanotropin (β-MSH), endorphins and methionine-enkephalin are elaborated from β-LPH. Their results defined the precise locations of ACTH and β-LPH in the precursor protein and predicted the amino acid sequence of its remaining portion. Their findings appear in a more comprehensive form in Figure I-1.

The distribution of β-endorphin is markedly different from that of the enkephalins. It has been identified in the CNS and anterior pituitary (129) in the same cells as ACTH. Stimuli which release ACTH will also release β-endorphin (130). Levels of endogenous opiates have been found to be increased in patients who have high levels of circulating ACTH or normal subjects given metyrapone (131,132,133). Similarly, administration of an endogenous opiate analogue leads to decreased levels of serum ACTH and cortisol (134).

FIGURE I-1



Schematic representation of the structure of bovine ACTH-β-LPH precursor (Nakanishi and Inoue (128)).

γ-MSH	-	γ-melanotropin
β-LPH	-	β-lipotropin
α-MSH	-	α-melanotropin
CLIP	-	corticotropin-like intermediate-lobe peptide
γ-LPH	-	γ-lipotropin
β-MSH	-	β-melanotropin
met-enkephalin	-	methionine enkephalin

The half-life of β-endorphin in blood is of the order of 10 minutes. Pituitary endorphins are released into the blood by various kinds of stress. Their function remains obscure; it is not even clear what target tissue they act upon. β-endorphin is a potent releaser of growth hormone (135) and prolactin (136,137) by an action in the hypothalamus, and vasopressin. Recently an endogenous opiate antagonist contained in efferent neurons has been identified. This peptide has been called substance-P and is implicated in primary sensory neurons as being involved in processing of pain and in axon reflexes causing vasodilation in skin (138) and thus a neurotransmitter. It may be expected that the more stressful and anxiety producing the clinical situation is, the more extensive the endorphin release would be.

Basically two approaches have been followed in investigation of endorphins; 1. the use of the narcotic antagonist naloxone and, 2. the measurement of endorphins in body fluids or tissue. Both approaches may be criticized in that naloxone may not behave ideally, as a pure antagonist, and in that the measurement of endorphin levels may not reflect the actual activity in an endorphin system, which would be better done with turnover studies. Unfortunately, methods are still not available for such studies.

Without going into the potential role of endorphins in the regulation of the motility and function of the gastrointestinal tract, the proper function of autonomic ganglia and other peripheral systems, this short chapter illustrates the complexity of endorphin systems and also the subtle nature of their actions. It may be useful to stay with the dichotomy outlined by Terenium (139), of a neurohormonal system in CNS and periphery with β-endorphin and a neurotransmitter-like system or short-range acting hormonal system with enkephalins. These two systems may be complementary, the former giving a tonic and general activity, the latter a localized amplification. The mechanisms for activating endorphin release is an important goal for future research.

β-ENDORPHIN AND SHOCK

Endogenous opiates are released as a response to various stresses (126,130). The cardiovascular system is an exquisitely sensitive target in stressful situations and also in its response to opiates; both exogenous (140,141, 142,143) and endogenous (144). This led investigators to believe that the stress of shock is infinitely linked to the release of endogenous opiates which depress the cardiovascular system and thus may play an important role in the pathogenesis of shock. In a study (144) designed to determine the effects of met-enkephalin on the ventral surface of the brainstem of cats, where the cardiorespiratory centre lies, it was found that it depressed both blood pressure and heart rate. This effect was reversible by intravenous naloxone. Similar responses were found by administering β-endorphin and Leu-enkephalin into the cisterna magna or subcutaneously (145).

The fact that high levels of ACTH were present during shock led Holaday and Faden to believe that endogenous opiates may be involved in the pathophysiology of various shock states. By using naloxone given intravenously, they were able to reverse the hypotension of endotoxin (146) and hypovolemic (147) shock states in rats. They later showed that the effects of naloxone in endotoxin shock was stereospecific for the (-)-naloxone isomer (148).

Gurll et al applied the same hypothesis to their more sophisticated dog model. By giving naloxone to dogs with endotoxin induced shock, they claimed improved survival and cardiac performance (149), noting that improvement in BP, LV, dp/dt and cardiac output was dose related (150). This improvement applied to canine hypovolemic shock (151,152) as well.

Recently it has also been demonstrated that naloxone given intravenously improves the depressed cardiovascular response after experimental spinal shock in rats (153). As to the

mechanism of action, a CNS mediated cardiovascular effect exists for β-endorphin (154,155) as well as for morphine (140,156). A direct peripheral effect for enkephalins has been proposed. β-endorphin administration to patients resulted in increased serum prolactin levels, but it did not affect plasma growth hormone (157).

Morphine is an opium alkaloid that, in man, produces analgesia (158), drowsiness, euphoria, respiratory depression (159), histamine mediated peripheral vasodilatation (159,160) with resulting hypotension, decreased propulsive contractions (161) and hydrochloric acid secretion in GI tract as well as spasm of the sphincters. In patients with coronary artery disease (162) and in anesthetized dogs (163,141), morphine produced a decrease in oxygen consumption, cardiac index, LV-EDP and cardiac work, and an increase in pulmonary resistance. Morphine stimulates growth hormone and prolactin secretion in rats (164, 165) and goats (166) and monkeys (167) while naloxone causes a reduction in prolactin levels in monkeys (167). Naloxone competes with morphine-like drugs for stereospecific opioid receptor sites and occupies these sites. It has been hypothesized that combination of the receptor with the agonist, but not with the antagonist, produces a conformational change that initiates certain pharmacological responses (168,169). Naloxone of up to 1 gm has been administered to normal subjects without producing any major subjective or hemodynamic effect (170). Recently, naloxone has been recommended for treatment of shock states in man (171).

In an attempt to determine the role of endorphins in the pathophysiology of septic shock and their role in hormonal homeostasis, it was decided to investigate the effects of opiate receptor blockade with naloxone, and stimulation with morphine in our hypodynamic porcine septic shock model. This model is described in detail in the following chapter. The effects of opiate receptor blockade and stimulation were assessed by monitoring changes in hemodynamic, hormonal and biochemical parameters.

DEVELOPMENT OF A MODEL

Chapter II

BACKGROUND

The early hemodynamic pattern in the septic patient usually consists of hypotension, tachycardia, normal or high cardiac output, and a decrease in peripheral resistance and arteriovenous oxygen content difference. The patient appears to be vasodilated and warm. Eventually the patient becomes vasoconstricted with cold clammy skin, the cardiac output and blood pressure drop and a high peripheral vascular resistance becomes established (1,3,4,5).

A wide variety of experimental models have been used and continue to be devised in order to study sepsis. During the last 20 years, endotoxin shock has been the primary method of studying the pathophysiology and treatment of septic shock. However, due to numerous pathophysiologic differences between human septic shock and experimental endotoxin shock, the latter has frequently been criticized as being an inadequate reproduction of the clinical situation. As a consequence, many of the treatment modalities which have had demonstrable effectiveness in endotoxin shock have not been used by clinicians. The reasons for the pathophysiological discrepancies are not known, but it is possible that the clinical septic shock and the experimental endotoxin shock are but two points on the spectrum of the septic state. Shoemaker et al (2) have made numerous attempts to document and outline a pathophysiologic continuum in the various forms of shock in man. Their data, as well as those of others, strongly suggest a progression in human septic shock from a hyperdynamic to a hypodynamic circulatory state. The latter appears to be an essentially preterminal phenomenon and is characterized by profound hypotension, low cardiac output, high total peripheral resistance - clinical signs and symptoms similar to endotoxin shock. Thus, the study of endotoxin shock in animals may, in fact, be applicable to late septic shock in man. To provide a better basis for the development of new therapeutic modalities in clinical

septic shock, an attempt was made to develop an experimental model of septic shock which closely approximated the clinical setting, was highly reproducible and relatively economical. We started by injecting a catalogue strain of E-coli intravenously into anesthetized piglets and monitoring hemodynamic changes.

The idea of using some means other than endotoxin to produce septic shock experimentally is not new. Albrecht and Clowes (180) injected a calcium chloride solution into the hind legs of the dogs recovering from thoracotomy to produce a septic focus. More than half of these dogs survived, and in this group, the circulatory requirements were slightly increased. Among the dogs that died, the characteristic hemodynamic finding was a steady deterioration of the measured indices. In a later study, Clowes and co-workers (181) produced peritonitis in dogs by either ligation of the cecum or the intraperitoneal injection of E-coli and bile. Neither method proved to be lethal, nor were the hemodynamic changes sufficient to warrant the general use of these techniques in the study of septic shock. Blair (182) used the intraperitoneal injection of fecal material to produce peritonitis in dogs and, thereafter, noted the typical acid base and blood gas abnormalities of septic shock. No hemodynamic or survival data were recorded, however, Hermreck and Thal (183) injected fecal material into the hind limbs of dogs and observed a hyperdynamic systemic circulatory response. All of his dogs survived. Perbelini et al (184) injected E-coli into the gallbladder of dogs, following division of the cystic artery and duct. Based upon the circulatory response and mortality two distinct groups were noted. In one, the cardiac index decreased and the total peripheral resistance was elevated. In the other, the cardiac index increased and the peripheral resistance was significantly lower. The average survival time in the former group was three days and, in the latter, five days. Shatney and Read (185) by using the same infected gallbladder model in

Rhesus monkeys, were not able to duplicate the above results. All the animals developed hypodynamic shock and died in periods varying from 12 hours to 13 days. At no time did those monkeys show a hyperdynamic state and there was no evidence of an abscess in the gallbladder area on autopsy.

The above observation suggested that the presence of a 'focus of infection' was primarily responsible for the hyperdynamic circulatory response seen in sepsis. Sepsis induced by the direct intravascular injection of bacteria or endotoxin has no associated focus of infection and produces a low cardiac output type of shock.

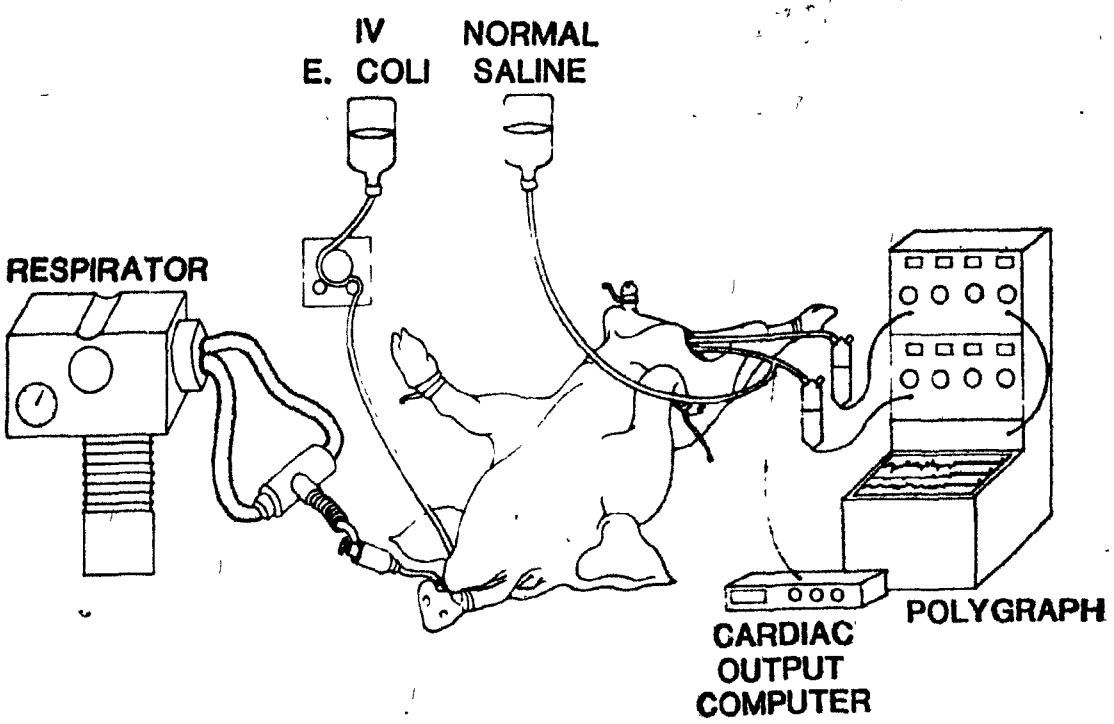
In our attempt to develop an experimental model it was felt that pigs were probably the best species to use next to the primates because of the similarities in their cardiopulmonary and gastrointestinal responses in shock to humans. Their size, in contrast to rats and other small animals, allows for detailed hemodynamic studies. We chose live bacteria instead of endotoxin (186) since pure endotoxemia without bacteremia does not occur in clinical situations (187). We used E coli strain U9-41, which has the pathogenic marker K-1 surface antigen (188). E coli is also one of the most common bacteria recovered from the patients with gram negative sepsis (180). The advantage of using a catalogue strain of E coli is that it is stable, and experiments can be highly reproducible.

A HYPODYNAMIC MODEL

Our investigation started by testing the effects of intravenous infusion of E coli to anesthetized, fasting, young, mixed breed pigs of both sexes, weighing 11-19 kg. A diagram of the experimental set-up appears in Figure II-1.

The six pigs in this group were anesthetized with 30 mg/kg sodium pentothal administered intramuscularly and 20 mg/kg pentobarbital administered intravenously. An endotracheal

FIGURE II-1



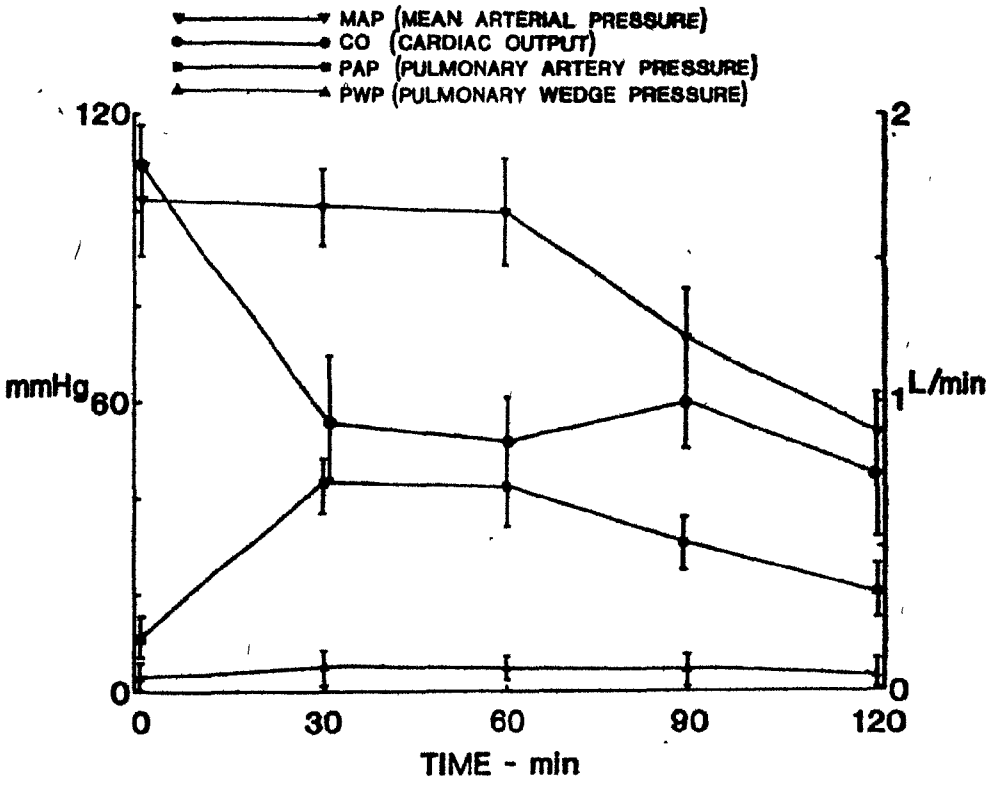
HYPODYNAMIC MODEL: Intravenous administration of E coli to anesthetized piglets with Swan-Ganz catheter and arterial lines connected to polygraph and cardiac output computer.

tube was inserted and connected to a respirator. Light narcosis throughout the experiment was maintained with nitrous oxide 3.5 1/min. Through an inguinal incision, a cannula was introduced into the femoral artery, connected to a pressure transducer and a Grass Model 7D polygraph for continuous blood pressure monitoring. A Swan-Ganz catheter was positioned in the pulmonary artery for continuous PAP recordings, and cardiac output was obtained by using the thermodilution method with an Edwards 9520 CO computer. Animals in this group were given live E coli (strain U9-41) as a continuous intravenous infusion at a concentration of 1.5×10^8 bacteria/kg/minute. They received an average of 20 ml/kg of intravenous saline during the course of the experiment. Blood samples were obtained prior to bacterial administration (controls), one and two hours after beginning bacterial infusion for complete blood count, lactate, pyruvate, cyclic AMP, and cyclic GMP (190).

The following results were obtained. The control mean arterial blood pressure (Figure II-2) was 100±7 mm Hg. This was 101±6 at 30 minutes, 97±7 in one hour and significantly lower 51±7 mm Hg at two hours postbacterial infusion. The cardiac output fell significantly from 2.03±.15 l/min to .93±.11 within 30 minutes ($p<.001$) and remained low throughout. The mean pulmonary artery pressure of 9.8±1 mm Hg increased dramatically to 44±2.4 mm Hg within 30 minutes ($p<.001$) and remained markedly elevated. Pulmonary wedge pressure increased from 2.5±.34 to 4.5±.72 mm Hg within 30 minutes ($p<.05$). Calculated pulmonary vascular resistance increased tremendously with sepsis as seen in Table 2-1 from a control of 386±24 dynes/sec/cm^{-5} to 4,202±309 after one hour of IV E coli infusion and 2,356±470 at two hours. The increase in heart rate from 118 to 140 beats per minute was not statistically significant. The temperature decreased slightly from 35.9±.66°C to 34.7±.84°C in two hours ($p>.1$).

The white blood cell count which was initially 14,100±525 decreased significantly to 2,800±620 ($p<.001$) at two hours.

FIGURE II-2



Hemodynamic changes in animals that received intravenous E coli. All values expressed as mean ±SEM.

TABLE 2-1

	CONTROL	1 HOUR	2 HOURS
Heart Rate beats/min	118±12	140±9	146±10
Stroke volume ml/beat	17.9±2.1	6.07±.55*	7.05±1.2*
Pulmonary vascular resistance dynes/sec/cm^{-5}	386±24	4,202±309*	2,356±470*
Body (°c) temperature	35.9±.66	35.3±.7	34.7±.84
WBC per mm^3	14,100±300	3,870±570*	2,800±620*
Hct %	27.2±.63	29.3±.93	31.4±1.34
Platelets $x10^3$ per mm^3	448±33	232±37*	150±10*
Lactate mg/dl	16.2±1.24	26.2±2.67*	30.2±3.91*
Pyruvate mg %	.27±.13	.36±.19	.36±.17
cAMP nmol/l	34±.71	106±29*	251±67*
cGMP pmol/ml	10.96±3.97	18.0±2.77	16.8±1.23

Changes in some parameters with two hours of intravenous E coli infusion to piglets. All results expressed as mean ±SEM.

* Student t-test $p<.05$.

Platelets also decreased significantly from 448,000±33,000 to 150,000±10,000 ($p<.001$). The hematocrit, however, did not change.

Control value for lactate was 16.2±1.24 mg/dl. Within two hours, lactate had increased significantly to 30.2±3.91 ($p<02$). Cyclic GMP increased significantly from 10.96±3.97 pmoles/ml to 16.8±1.23 ($p<.01$) and cyclic AMP increased from 34±.71 nmoles/l to 251±67 ($p<.01$) at two hours.

In this model where bacteria were introduced intravenously, progressive hypotension, low cardiac output, hypothermia and leukopenia developed. The marked increases in PAP and pulmonary resistance as well as cellular and biochemical changes were consistent with previously reported hypodynamic septic shock models (193,194,195,196). All the piglets died within three to four hours from initiation of IV E coli. This hypodynamic model of septic shock closely approximated the clinical septic shock at its end stage in patients and was highly reproducible. At autopsy the animals were found to be in frank pulmonary edema with both pleural and pericardial effusions. Similarly, other organs were found to be congested as well. At this point it was decided to develop a model with a 'focus of infection' to see whether this actually did result in a hyperdynamic response.

A HYPERDYNAMIC MODEL

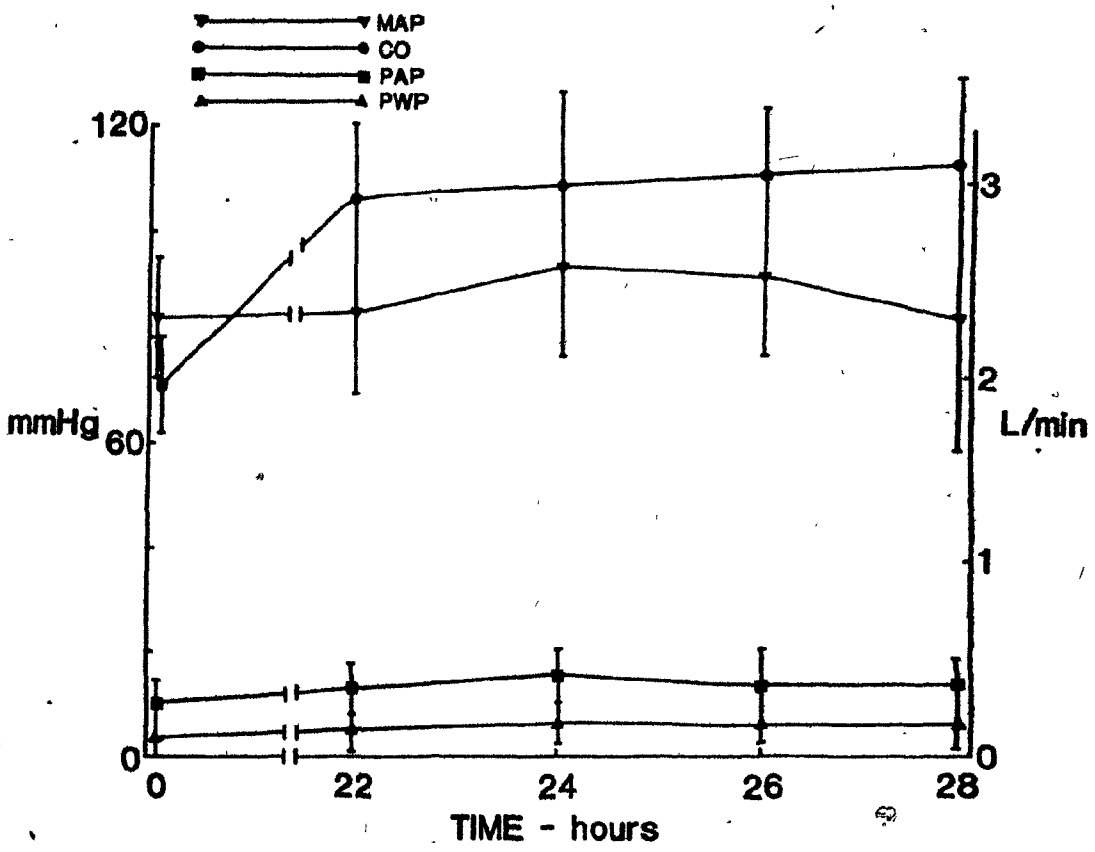
This group consisted of six pigs that were injected with 15 ml of 10^9 E coli/ml (strain Ug-41) intramuscularly into the thigh. Twenty-four hours following this injection, the pigs were anesthetized and an arterial cannula and Swan-Ganz catheter inserted as described in the previous model. Hemodynamic measurements and blood samples were carried out and data was analyzed using the Student t-test. Results are expressed as mean ±SEM.

The control values of the previously described model were used in analyzing the results from these animals since they were in the same weight category. Furthermore, obtaining baseline values prior to injection of E coli would have meant sacrificing a femoral artery and vein, the effects of which would have been another variable.

Tachycardia developed in this group. The heart rate of 184±6 per minute was noted 24 hours after the injection of bacteria intramuscularly. This was significantly higher than the control heart rate ($p<.05$) as well as the rate in corresponding pigs injected intravenously with E coli ($p<.01$). The mean arterial blood pressure as seen in Figure II-3 did not change significantly, however, cardiac output increased to 3.1±.31 l/min at 24 hours. This is significantly higher than the cardiac output in the controls ($p<.05$) and in the intravenous E coli model ($p<.001$). In contrast, mean PAP and PWP did not differ significantly from control values, nor did the calculated pulmonary vascular resistance of 238±49 dynes/sec/cm^{-5}. The body temperature rose to 38.7±.55°C at 24 hours. This was significantly higher than that of the controls ($p<.005$) and the intravenous E coli model ($p<.01$). The WBC of 18,175±1,000 was significantly higher than both in the controls and those receiving intravenous E coli. The platelet count was 245,000±38,000. This was lower than the controls, but was still significantly higher than the animals in hypodynamic shock. The lactate value of 16.9±2.1 mg% was markedly lower than that of hypodynamic piglets ($p<.01$), while the pyruvate value of .45±.21 mg% remained unchanged. On autopsy the thigh where E coli was injected showed a localized, severe, inflammatory reaction. These animals were not followed for mortality rates.

Tissue inflammation, by releasing various kinins, pyrogens and other mediators, can produce a hyperdynamic picture including increased cardiac output, vasodilation, fever and leukocytosis. Sterile irritants, such as castor oil or

FIGURE II-3



Hemodynamic changes in animals that received intramuscular E coli (Hyperdynamic model). The control values at time zero are the control values of the hypodynamic model animals. They are used in this figure since the animals in this group were not instrumented at time zero for the reasons given in the text. All values expressed as mean ±SEM.

calcium chloride can also induce such reactions (180). In practically all clinical sepsis, there is a focus of infection, and the tissue inflammation in response to bacterial invasion may lead to the familiar picture of hyperdynamic sepsis. This particular model satisfied those criteria and confirmed the importance of the elimination of the focus of infection, such as by surgical drainage or excision, which is often associated with a dramatic improvement in the patient's hemodynamic condition.

MISCELLANEOUS TRIALS

Another concept is that in sepsis, patients develop a hyperdynamic state initially, and later lapse into low output shock. Whether septic shock is always preceded by a high output phase was also examined. Gradually increasing doses of E coli, starting with 10^3 bacteria/minute and reaching to 10^9 bacteria/minute, was infused intravenously in a parallel series of porcine experiments. No significant hemodynamic change was observed until the infusion reached the dose of greater than 10^6 bacteria/minute. The hemodynamic response was identical to that observed in the hypodynamic model, namely, a low flow state associated with myocardial depression. Cardiac output and other hemodynamic parameters were observed continuously from the time E coli was administered intravenously. Our observations lead us to conclude that when the bacteria was injected directly into the vein, a hyperdynamic state did not occur before the onset of the low flow state.

To determine if hypovolemia, either as the result of vasodilatation or third space fluid loss, was in part or whole a contributing factor to the hypodynamic state of septic shock large amounts of intravenous saline (135 ml/kg) was infused to a group of five pigs. These five pigs received intravenous E coli in a manner described in the previous section of 'a hypodynamic model', however their PWP was

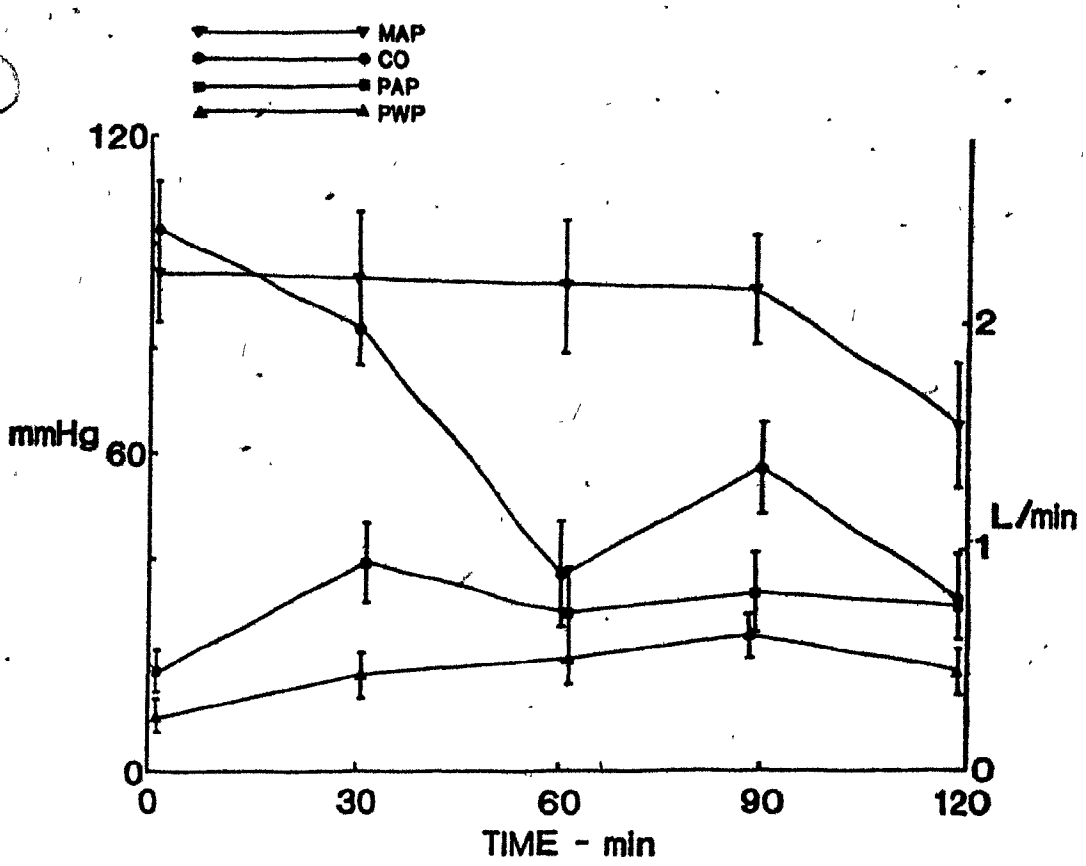
maintained at a range of 10-15 mm Hg. It was noted that the mean arterial blood pressure in this group as noted in Figure II-4 also fell steadily from 91±8 to 60±10 mm Hg within two hours of bacterial infusion ($p<.05$) in spite of volume expansion. Cardiac output decreased from 2.49±.21 l/min to 1.1±.3 within two hours ($p<0.2$). When data from this group was compared to the corresponding ones of the non-volume expanded group they were found not be statistically different. The pulmonary artery pressure again increased significantly ($p<.01$) from 7.6±2.2 to 28±4.6 mm Hg within 30 minutes. Pulmonary wedge pressure of 3.3±1 increased as the result of fluid infusion of 11.3±2.29 mm Hg within 30 minutes ($p<.05$), and was kept high throughout the experiment. The calculated pulmonary vascular resistance increased significantly from 237±55 dynes/sec/cm^{-5} to 1,974±537 at one hour, to 1,548±391 at two hours while temperature changes from a control of 32.7±.94°C to 31.7±2.18 at two hours were not significant ($p>.5$).

At autopsy these animals were found to be in gross pulmonary edema and obvious anasarca with congestion of the GI tract on histological sections. Interestingly, mortality was not effected by the infusion of large amounts of fluid remaining at three and a half to four hours.

THE FINAL CHOICE

During these investigations of an attempt to develop a good model for the study of septic shock the variables were kept to a minimum by using the same species of experimental animal as well as the identical strain and dosage of bacteria. Only the route of bacterial invasion differed, one directly into the blood stream without a local site of tissue inflammation resulting in a hypodynamic state and the other into the muscular mass with abscess formation resulting in a hyperdynamic state. Although the importance of the 'focus of infection' has been recognized or implied by other studies

FIGURE II-4



Hemodynamic changes in the animals that received intravenous E coli with volume expansion. Note PWP which is maintained at 10-15 mm Hg by fluid infusion. All values given as mean ±SEM.

(184,197) notably those of Thal et al (183), this study clearly identified the different roles of local septic inflammation as opposed to that of generalized bacteremia. It is the balance of these two responses that determines the hemodynamic and metabolic manifestations of sepsis.

The following factors influenced the choice of the 'hypodynamic septic shock model' as best suited for the investigation of the role of endorphins in shock:

1. Close approximation to the clinical end stage of septic shock which is the critical period.
2. Highly reproducible results.
3. Reasonable length of time required for completion of experiment: six hours versus several days for the hyperdynamic model.
4. And therefore a more economical model.
5. Repeated daily instrumentation of the animals is impractical if not impossible.
6. Uncertainty in the mortality rates in the hyperdynamic model.

It was decided not to overload the animals with fluids since it had no effect in overall mortality and internal organs were visibly more congested with fluid overload. Similarly, bacterial infusion was kept at a steady concentration throughout the experiment since varying the concentration did not effect the outcome.

METHODS

Chapter III

STUDY OBJECTIVE

The purpose of this experiment was to determine whether endorphins play a role in the pathophysiology of septic shock and to clarify the effects of opiate receptor blockade on various hormones involved in the homeostasis of septic shock. Furthermore, the role of the 'second messenger' system in relation to the mechanism of the action of endorphins was studied.

PREPARATION OF ANIMALS

Fasting young mixed breed pigs of both sexes weighing 13-19 kg were anesthetized as described in Chapter II. After positioning a Swan-Ganz catheter in the pulmonary artery, and inserting an arterial line through the femoral artery a continuous intravenous infusion of E coli (strain U9-41) at a concentration of 1.5×10^8 bacteria/kg/minute was started and continued until the animal expired (about 3 to 4 hours). The animals were divided into three groups on the basis of the drug they received after two hours of bacterial infusion.

Naloxone Group

The six animals in this group that received an average of 20 ml/kg of intravenous saline during the course of the experiment were given naloxone as a 2 mg/kg IV bolus two hours after the commencement of IV E coli. Hemodynamic parameters were recorded at 0, 60, 120, 125, 130, 135 and 150 minutes from the time of E coli infusion was begun. Blood and liver biopsy samples were obtained prior to bacterial administration, 120, 135 and 150 minutes for glucose, liver, glycogen, insulin, gastrin, cortisol, prolactin, lactate, pyruvate cAMP and cGMP determinations.

Morphine Group

In this group of five pigs, the same procedures described in

the Naloxone Group were followed, with the exception that instead of naloxone, morphine as a 4 mg/kg IV bolus was given. Blood samples were obtained for cortisol, prolactin, growth hormone, substance-P, and β-endorphin levels in the times mentioned for the previous group. The radioassay for β-endorphin levels was not successful.

Control Group

In this group of five pigs, similar procedures described in the Naloxone Group were followed. After two hours of IV bacterial infusion, normal saline as a 5 ml/kg IV bolus (equivalent in volume to the naloxone carrier) was given and hemodynamic changes recorded. No morphine or naloxone was given to these animals.

BLOOD ANALYSIS

Glucose

Whole blood for glucose determination was collected in tubes containing fluoride and glucose concentration was determined using the Boehringer Mannheim Test Kit for glucose (Hexokinase Method).

Hematocrit, white blood cell, and platelet counts

Those were obtained with the use of an automated Colder Counter.

Cortisol

Cortisol levels were determined using the RSL (I^{125}) Cortisol Kit made by Radioassay Systems Laboratories of Carson, California.

Radioimmunoassays depend on the ability of an antibody to bind its antigen. To quantitate the antigen, the radioactive and non-radioactive form of the antigen compete for binding sites on its specific antibody. As more non-radioactive antigen is added, less radioactive antigen remains bound until equilibrium

between the free and antibody-bound antigen occurs. Separation of the free from the antibody-bound antigen may be accomplished by several methods.

The RSL Cortisol Kit utilizes a second antibody-PEG technique. This is accomplished by adding a mixture of second antibody-PEG, vortexing vigorously and immediately centrifuging. The radioactivity level of the precipitate is then determined with a gamma counter. The amount of bound cortisol I^{125} found in the precipitate will decrease with increasing amounts of non-radioactive cortisol. Levels of cortisol in samples are determined graphically from a standard curve constructed with results obtained from the cortisol standards.

Specificity of the antiserum: The following materials have been checked for cross-reactivity by the 'kit' manufacturers. The percentages indicate cross-reactivity at 50% displacement compared to cortisol.

Cortisol	100
Prednisolone	58
11-Desoxycortisol	17.5*
Prednisone	1.2
Cortisone	<0.01
Corticosterone	35*
Spironolactone	<0.01
Dexamethasone	<0.01
Progesterone	2.9

* The normal concentrations of these cross-reacting steroids are sufficiently less than that of cortisol so that their overall contributions to the cortisol assay are negligible.

Normal values in man as determined by RSL are:

8:30 AM	7-21 ug/dl or 70-210 ng/ml
4:30 PM	3-11 ug/dl or 30-110 ng/ml

Insulin

The principle of competitive binding analysis was initiated with the development of a radioimmunoassay for insulin by

Berson and Yalow (76).

The RSL Insulin Kit produced by Radioassay Systems Laboratories of California was used for plasma insulin level determinations. The principles and basic procedure of the technique is the same as with cortisol and will not be repeated. The RSL Insulin Kit utilizes the second antibody technique. This is accomplished by adding an antibody specific for immunoglobulin. Following a second period of incubation the precipitate is packed by centrifugation and the supernatant is decanted or aspirated and discarded. The radioactive level of the precipitate is then determined with a gamma counter. The amount of bound insulin-I^{125} found in the precipitate will decrease with increasing amounts of non-radioactive insulin. Levels of insulin in the samples are determined graphically from a standard curve constructed with results obtained from insulin standards.

Specificity and sensitivity (cross-reactivity):

Human insulin	100%
Dog insulin	100
Porcine insulin	100
Pro insulin	19
C-peptide	0.003
Glucagon	0.09

Serum levels of glucagon, prolactin, gastrin and growth hormone were determined using simular RSL Kits for each specific hormone. They all employ the radioimmunoassay principle and technique which is basically similar for each hormone. Therefore, only the cross reactivity data and normal values will be given for these hormones. The detailed description of each method can be found in the booklet included with each kit.

Glucagon

Specificity and sensitivity (cross-reactivity):

Pancreatic-glucagon	100%
Gut glucagon	0.0014

Porcine insulin	0.0005
Porcine gastrin 1 & 2	<0.0003
Human synthetic gastrin 1	<0.0003
T_3	<0.0003
T_4	<0.0003

Normal values as determined by RSL: 50-125 pg/ml.

Gastrin

Human gastrin appears in two almost identical forms, each containing seventeen amino acid residues. The molecular weight of human gastrin I is 2,096 and of gastrin II is 2,176.

Specificity and sensitivity (cross-reactivity):

h Gastrin I	100%
h Gastrin II	100
Cholecystokinin	<0.1
Pancreatozymin	<0.1

Normal values as determined by RSL for gastrin: up to 200 pg/ml.

Prolactin

Specificity and sensitivity (cross-reactivity):

h Prolactin	100%
h Growth hormone	<0.1
h Placental lactogen	<0.1
Anterior pituitary peptide hormones	<0.1

Normal values as determined by RSL:

Males:	7-18 ng/ml
Females:	6-24 ng/ml
Pregnancy:	6 times mean normal value
Lactation:	15 times mean normal value

Growth Hormone

Specificity and sensitivity (cross-reactivity):

h Growth hormone	100%

h ACTH	<0.02%
h FSH	<0.02
h LH	<0.02
h Prolactin	0.3
h TSH	<0.02

Normal values as determined by RSL:

Adult male:	up to 8 ng/ml
Adult female:	up to 30 ng/ml
Children:	up to 10 ng/ml

Substance-P

A radioimmunoassay kit by Immuno Nuclear Corporation (INC) of Minnesota was used (200) which employs simultaneous addition of sample, rabbit anti-substance-P antibody and I^{125} substance-P, followed by an overnight incubation at 4°C. Phase separation is accomplished by the addition of an equal volume of saturated ammonium sulfate in the presence of carrier gamma globulin. The assay is greatly affected by differences in protein and salt concentration; therefore, all re-agents and samples were kept on ice while performing the assay and extracted EDTA plasma frozen immediately after centrifugation at 4°C, to minimize any degradation of substance-P by the presence of enzymes in the samples. The sensitivity of the assay as employed is 8 picograms and the 50% displacement is 44 picograms. The antibody has been tested by INC for cross reactivity with the following neurotransmitters:

Substance-P	100%
Methionine enkephalin	<0.002
Leucine enkephalin	<0.002
β-endorphin	0.008
Eledoisin	<0.002
Physalaemin	<0.002

Plasma can be analyzed if an extraction step is performed. Serum or plasma analyzed without extraction will yield values which do not reflect true substance-P levels. Substance-P was extracted from EDTA plasma with cold analytic grade acetone.

The clarified extract was further purified with ether extraction, followed by air drying of aliquots of the extract and reconstitution in assay buffer. Using this procedure, recovery of substance-P ranges from 68 to 134 percent at 125-500 pg/ml levels as verified by the kit producers.

cAMP

Cyclic AMP determinations in this experiment were done with the use of the Amersham's cAMP Assay Kit. The competitive binding protein assay for cAMP is similar to radioimmunoassay where the reaction between an antigen and antibody provides an assay for the antigen. The method is based on the competition between unlabeled cAMP and a fixed quantity of tritium labeled cAMP for binding to a protein which has a high specificity for cAMP (202). The amount of labeled cAMP-protein complex formed is inversely related to the amount of unlabeled cAMP present in the assay. The concentration of cAMP in the unknown is determined by comparison with a linear standard curve. Separation of the protein bound cAMP from the unbound nucleotides is achieved by absorption of the free nucleotide on charcoal, followed by centrifugation. A sample of the supernatant is removed for liquid scintillation counting. The detailed procedure of the method can be found in the information booklet supplied with the Amersham cAMP Assay Kit.

cGMP

Because of the structural similarity of cAMP and cGMP and the fact that levels of cGMP in most tissues are very much lower than those of cyclic AMP, this work requires a highly sensitive and very specific assay method. Although both CPB and RIA methods have proved satisfactory for cAMP measurements, only RIA methods for cGMP are sufficiently specific to be used without preliminary separation of the cyclic nucleotides. Cyclic GMP is present in most tissues at concentrations 10-fold and occasionally 100-fold lower than those of cAMP. Cyclic GMP levels in urine (203) (0.12-1.09 μmol/24 hours)

and plasma (203) (1.8-6.0 pmol/ml) are approximately half those of cAMP.

In this experiment the Amersham/Searle Cyclic GMP RIA Kit was used. This assay is based on the competition between unlabeled cGMP and a fixed quantity of tritium labeled compound for binding to an antiserum which has a high specificity and affinity for cGMP. The details of the method can be found in the booklet supplied with the kit. Cross-reactivity of various nucleotides with cGMP antiserum causing 50% inhibition is as follows:

cyclic GMP	0.8
cyclic AMP	120,000
AMP, ADP, ATP	$>10^6$
GMP, GDP, GTP	25,000

Lactate

Blood lactate levels were determined with the use of a kit supplied by Boehringer Mannheim GmbH employing the UV-method which was described by Gutmann and Wahlefeld in 1974 (204).

Pyruvate

Blood pyruvate levels were determined using a kit supplied by Boehringer Mannheim GmbH employing the UV-method. This was described by Czok and Lamprecht in 1974 (205).

TISSUE ANALYSIS

Liver Glycogen

Liver glycogen levels were determined using a method described by Somogyi (206) in 1933. This involves the hydrolysis of the frozen liver sample in 30% KOH at 100°C and precipitation with 95% ethanol. The precipitate is dried and analyzed for glucose. The amount is expressed as gm% of glycogen.

RESULTS

Chapter IV

HEMODYNAMICS

A. Naloxone Group

The control mean arterial pressure (Figure IV-1) was 100±7 mm Hg. This went down to 49±8 after two hours of bacterial infusion. After the intravenous injection of naloxone, blood pressure increased to 54±10 at 5, 53±10 at 10 minutes and then it continued to 48±10 at 15 minutes and 40±5 mm Hg at 30 minutes. Systolic and diastolic pressures (Figure IV-2) similarly increased after naloxone injection with the changes being significant at five ($p<0.001$) and ten ($p<0.01$) minutes.

Heart rate increased significantly ($p<.05$) during two hours of sepsis, from 122±11 to 142±10. However, it decreased significantly to 128±9 ($p<.001$) 5 minutes after naloxone injection and then gradually went back to 151±10 at 30 minutes.

Mean pulmonary artery pressure (Figure IV-1) increased from a control of 10±.7 to 26±3.1 after 2 hours of sepsis ($p<.001$). Naloxone produced no change in PAP (Figure IV-1).

Pulmonary wedge pressure did not show any significant change either to naloxone or sepsis.

Cardiac output (Figure IV-4) dropped immediately, from a control of 2.26±.33 L/min. to .96±.19 after 2 hours of sepsis and then increased with naloxone to 1.19±.22 at 5 minutes, 1.13±.25 at 10 minutes, 1.1±.26 at 15 minutes. All these changes were significant ($p<0.05$).

Body temperature remained unchanged throughout the experiment

B. Morphine Group

The mean arterial pressure (Figure IV-3) in this group decreased similarly with sepsis from 98±10 mm Hg to 48±8 at 2 hours. After injection of morphine it decreased significantly to 26±2 at 5 minutes ($p<.05$), 22±4 at 15 minutes and 17±6 at 30 minutes.

Heart rate again increased significantly ($p<.05$) during two hours of sepsis from 125±12 to 160±3, but did not change after the injection of morphine.

Mean pulmonary artery pressure (Figure IV-3), as with the other group, increased significantly ($p<.001$) with sepsis from 11±1 to 30±3, but did not change after morphine.

In this group of animals it was the pulmonary wedge pressure (Figure IV-3) that showed the most dramatic change. After remaining stable at 3±.6 during two hours of sepsis, PWP went to 32±9 mm Hg five minutes after morphine injection and remained high during the subsequent one half hour, coming down to 21±4 after 30 minutes. All these changes were statistically significant ($p<.001$).

Cardiac output (Figure IV-4), which significantly ($p<.01$) decreased with sepsis from 2.05±.24 to .57±.09 L/min did not change after morphine injection. Again, no change in body temperature was observed.

C. Control Group

When the five septic animals were given equivalent amounts of normal saline injections, no significant changes in blood pressure, PAP, PWP, heart rate and body temperature were observed.

HORMONES

A. Naloxone Group (Table 4-1)

Cortisol (Figure IV-5) increased in response to sepsis, 73±15 pg/ml to 109±21 ($p<0.01$), and also thirty minutes after opiate receptor blockade ($p<0.01$). On the other hand, insulin (Table 4-1) decreased 30 minutes after naloxone injection from 37±11 to 23±3.9 μU/ml ($p<0.1$). Glucagon (Figure IV-6) and prolactin (Figure IV-7) levels did not show a response either to sepsis

or opiate receptor blockade, while gastrin (Figure IV-6) increased significantly in response to sepsis from 161±22 pg/ml to 854±140, but did not respond to naloxone.

B. Morphine Group (Table 4-2)

Cortisol, prolactin, growth hormone, substance-P and β-endorphin were measured in this group. Cortisol (Figure IV-5) decreased from 101±18 ng/ml to 68±3 thirty minutes after morphine ($p<0.1$), while substance-P (Figure IV-8) significantly decreased in response to sepsis ($p<0.05$) 91±5 pg/ml to 76±2, and increased in response to morphine ($p<0.05$) at 30 minutes, 76±2 pg/ml to 93±3. Growth hormone increased from 0.82±0.07 ng/ml to 0.9±0.01 after 15 minutes ($p<0.1$), to 0.94±0.08 at 30 minutes ($p<0.1$). Prolactin levels (Figure IV-7) did not change significantly either in response to sepsis or naloxone. The radioimmunoassay for β-endorphin was unsuccessful and no serum β-endorphin levels were obtained.

BIOCHEMISTRY

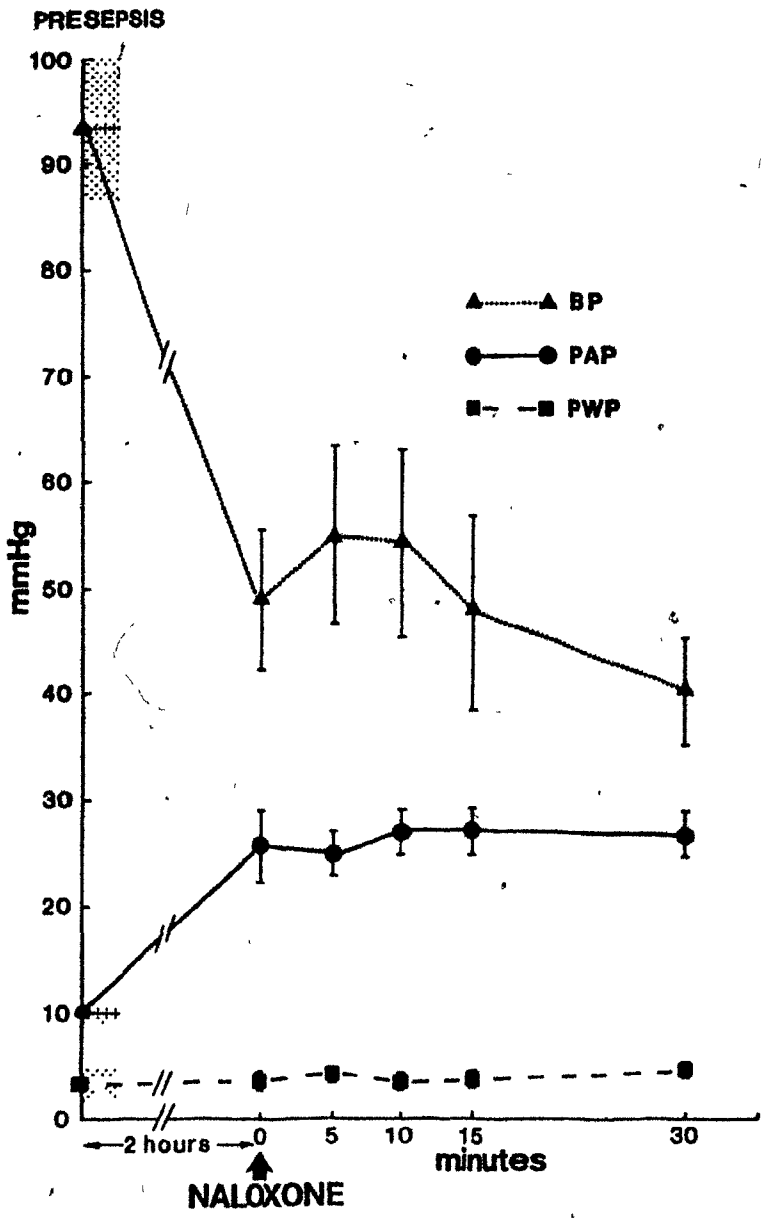
Glucose levels (Figure IV-9) in response to sepsis and naloxone, were kept stable at the expense of liver glycogen (Figure IV-9) which, from a control of 5.2±.64 gm % dropped to 2.3±.2 ($p<.01$) after two hours of bacterial infusion and then further to 1.3±.45 fifteen minutes after naloxone blockade. This was a significant drop ($p<.01$).

Cyclic AMP, cyclic GMP, lactate and pyruvate levels were measured only in the naloxone group. Lactate (Table 4-1) significantly increased in response to sepsis ($p<.01$) from 16.2±1.2 to 26.2±2.7, but, no change was noted with naloxone. Cyclic AMP (Figure IV-10) levels went up in response to sepsis from 28±5.6 μmol/l to 88±29 ($p<.05$) and also 15 minutes 142±35 ($p<.02$) and, 30 minutes after naloxone injection, 253±67 ($p<.001$).

Cyclic GMP (Figure IV-10) went up in response to sepsis ($p<.01$) from 7±.9 pmol/ml to 17.6±3, but did not respond to opiate receptor blockade.

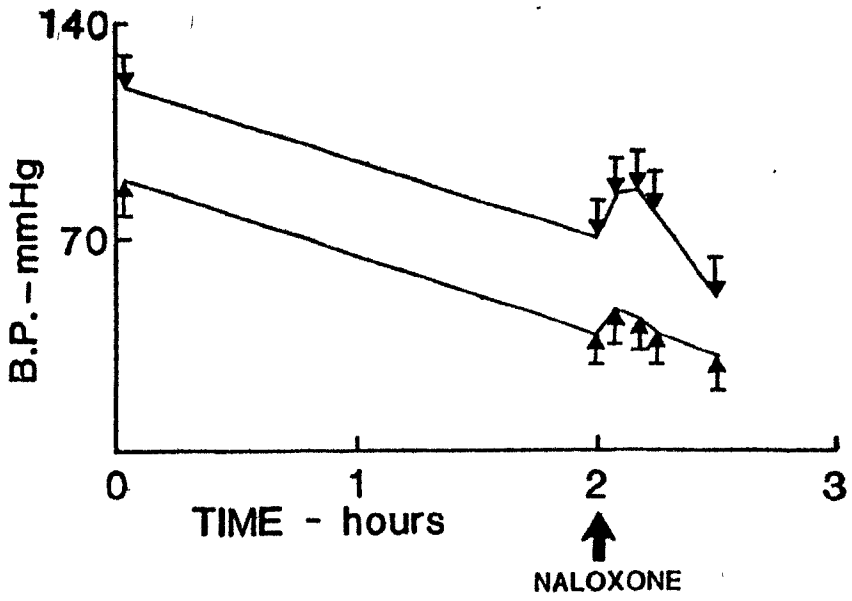
Pyruvate levels (Table 4-1) did not show a response either to sepsis or naloxone.

FIGURE IV-1



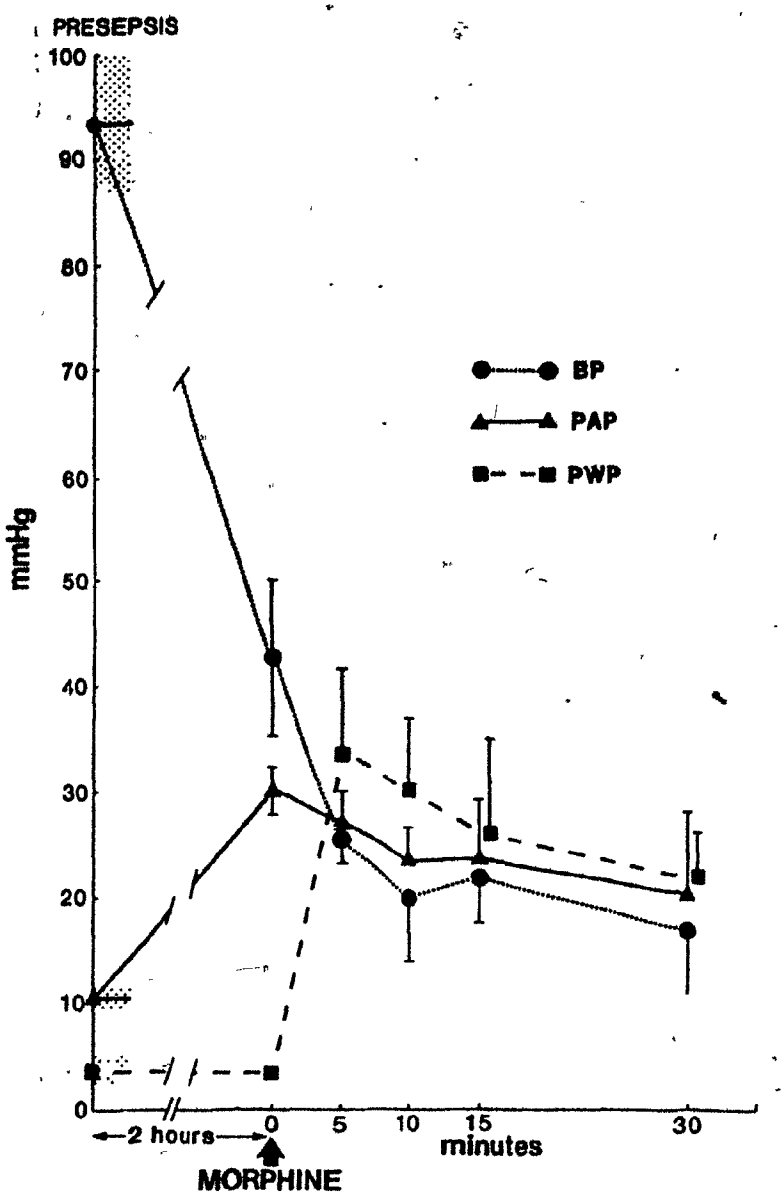
Changes in mean arterial pressure, mean pulmonary artery pressure and pulmonary wedge pressure in response to naloxone after two hours of intravenous E coli infusion. All values represent mean ±SEM.

FIGURE IV-2



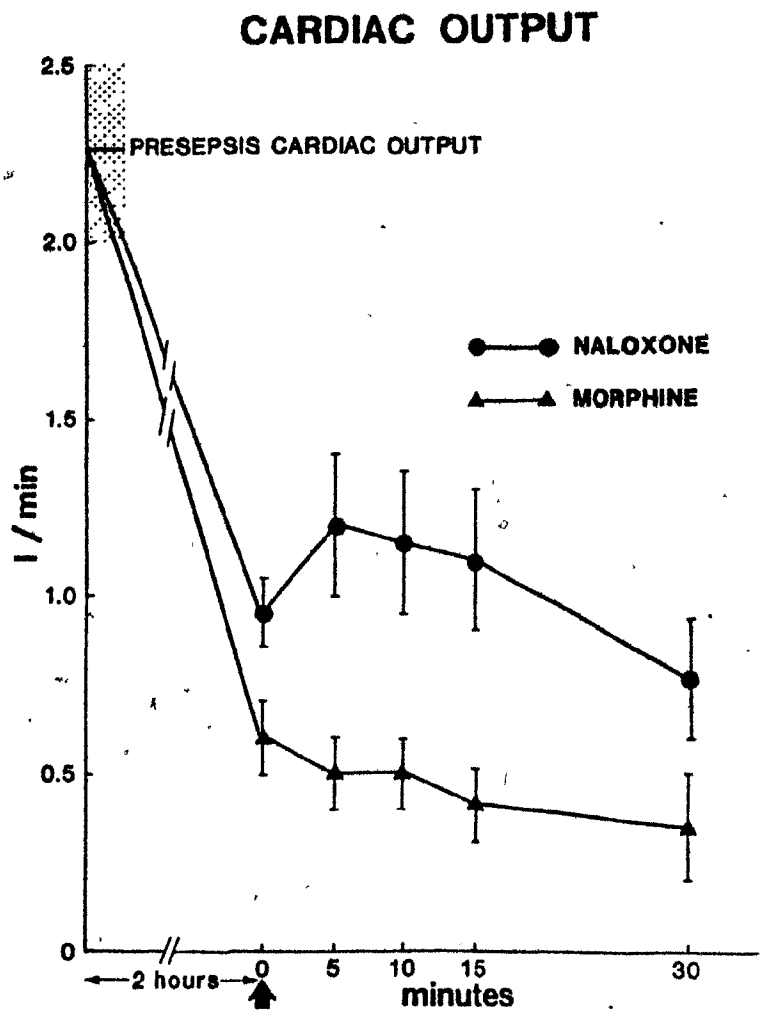
Changes in systolic and diastolic blood pressure in response to naloxone after two hours of intravenous E coli infusion. All values represent mean ± SEM.

FIGURE IV-3



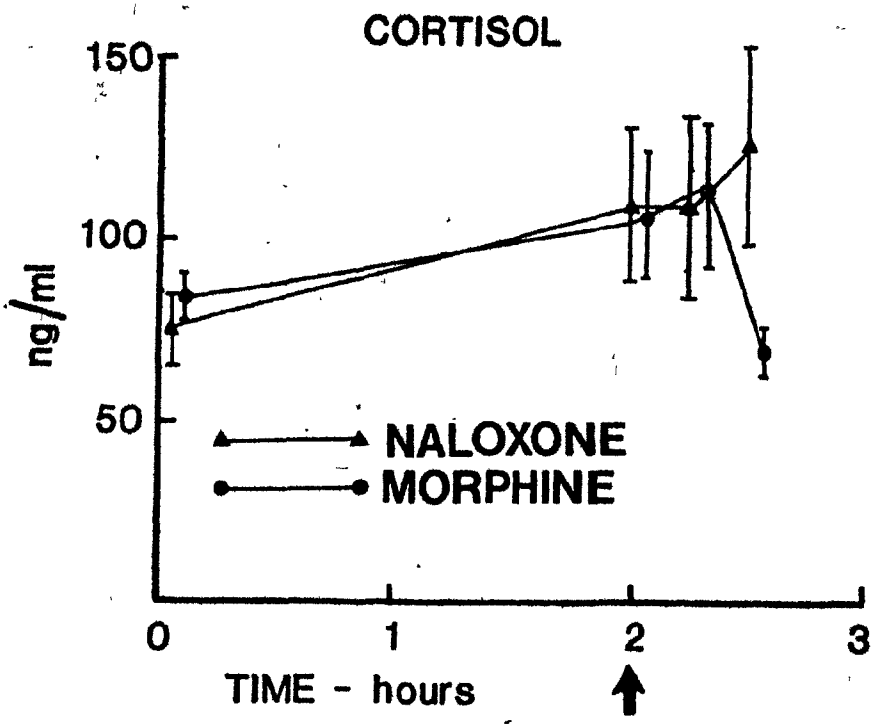
Changes in mean arterial pressure, mean pulmonary artery pressure and pulmonary wedge pressure in response to morphine after two hours of intravenous E coli infusion. All values represent mean ± SEM.

FIGURE IV-4



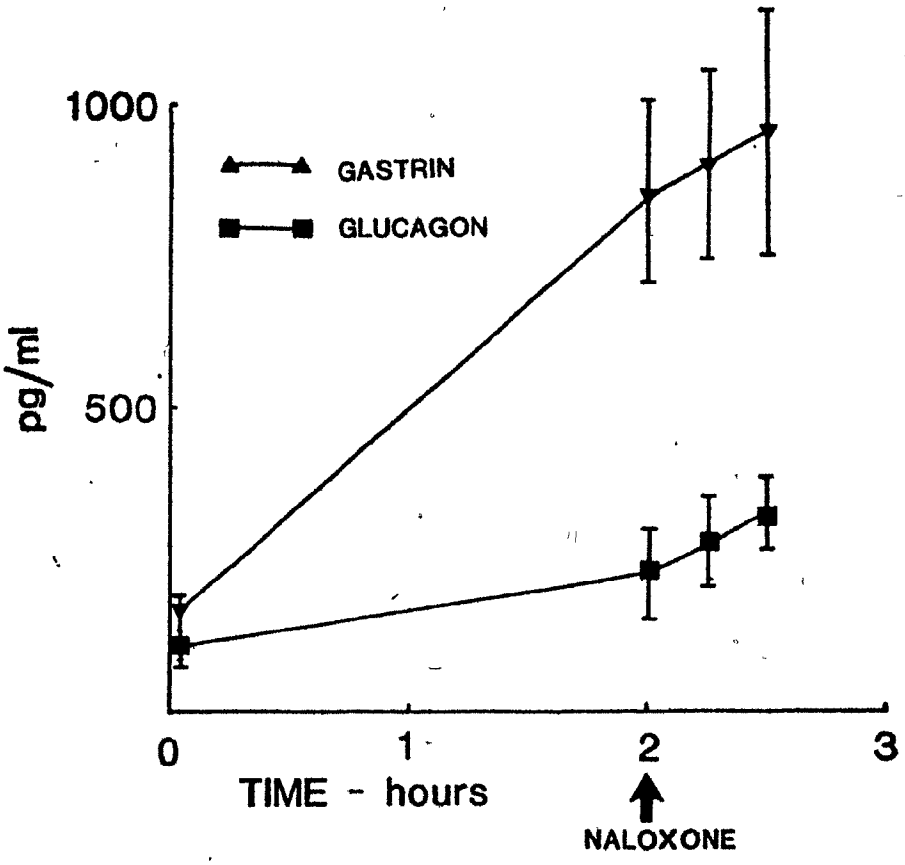
Comparison of the changes in cardiac output in response to naloxone and morphine after two hours of intravenous E coli infusion. Arrow indicates time of drug administration. All values represent mean ± SEM.

FIGURE IV-5



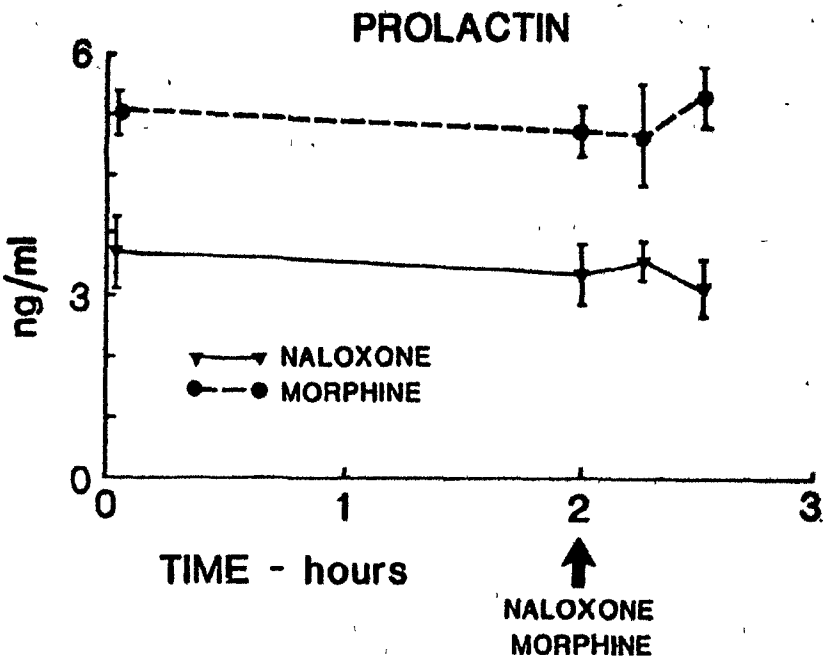
Comparison of the changes in serum cortisol levels in response to naloxone and morphine after two hours of intravenous E coli infusion. Arrow indicates time of drug administration. All values represent mean ± SEM.

FIGURE IV-6



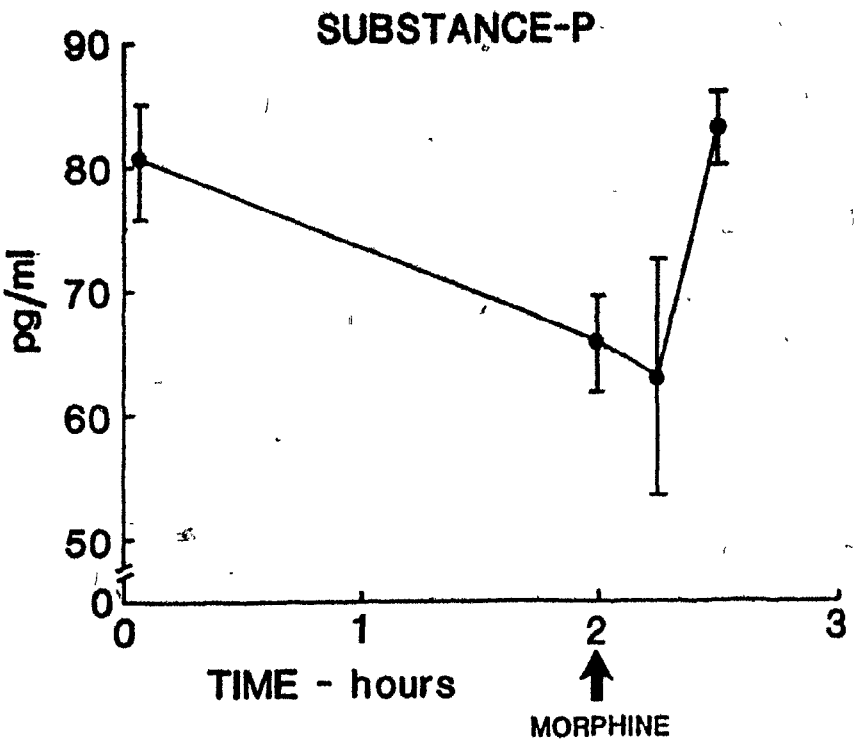
Changes in serum gastrin and glucagon levels in response to naloxone after two hours of sepsis. All values represent mean ± SEM.

FIGURE IV-7



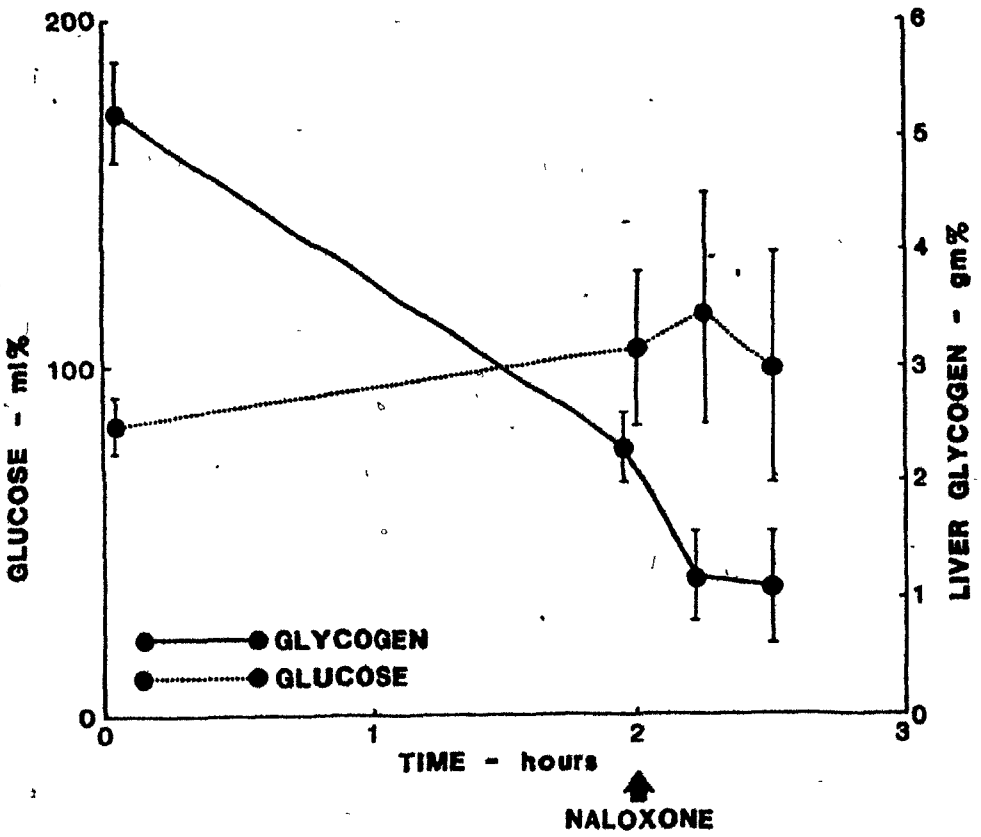
Comparison of the changes in serum prolactin levels in response to naloxone and morphine after two hours of intravenous E coli infusion. Arrow indicates time of drug administration. All values represent mean ± SEM.

FIGURE IV-8



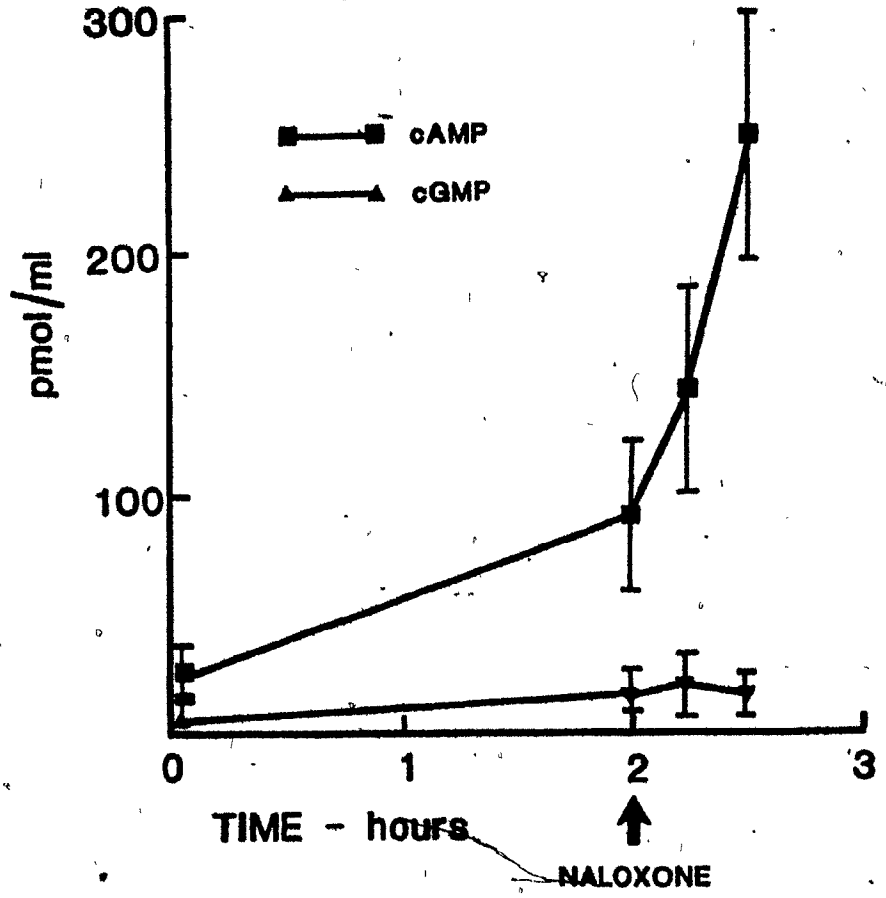
Changes in serum substance-P levels in response to morphine after two hours of sepsis. All values represent mean ± SEM.

FIGURE IV-9



Maintenance of serum glucose levels within normal limits at the expense of liver glycogen. Naloxone administration after two hours of sepsis further depletes liver glycogen levels. All values represent mean ± SEM.

FIGURE IV-10



Changes in cyclic AMP and cyclic GMP levels in response to naloxone after two hours of intravenous E coli infusion. All values represent mean ± SEM.

TABLE 4-1

			Post-naloxone	
	Control	2 hours of sepsis	Fifteen minutes	Thirty minutes
Insulin (μU/ml)	25±2.3	37±11	33±15	23±3.9
Glucagon (pg/ml)	123±16	222±74	278±70	315±65*
Gastrin (pg/ml)	161±22	854±140*	892±148*	850±204
Cortisol (ng/ml)	73±17	109±20*	108±24	125±27
Prolactin (ng/ml)	3.81±.26	3.42±.44	3.61±.3	3.12±.3
Glucose (ml%)	84±7	105±20	116±30	100±30
Glycogen (gm%)	5.2±0.64	2.3±0.29*	1.3±0.45*	1.1±0.47
Cyclic AMP (μmol/l)	28±5	88±29*	142±35*	253±67*
Cyclic GMP (μmol/l)	7±0.9	17.6±3*	20±2	17±1.4
Lactate (ml%)	16.2±1.2	26.2±2.7*	27.2±2.5	30.2±3.9
Pyruvate (ml%)	.27±.06	.36±.07	.28±.08	.36±.07

* $p<0.05$ paired t-test

All values represent mean ± SEM

n=6

TABLE 4-1: Hormonal and biochemical changes in response to naloxone 2 mg/kg IV after 2 hours of IV E coli infusion.

TABLE 4-2

			Post-morphine	
	Control	2 hours of sepsis	Fifteen minutes	Thirty minutes
Cortisol (ng/ml)	80±12	101±18*	113±18	68±3*
Growth hormone (ng/ml)	0.97±0.03	0.82±0.07	0.91±0.01	0.94±0.08
Prolactin (ng/ml)	6.13±.24	5.85±.4	5.56±.71	6.34±.44
Substance-P (pg/ml)	91±5.3	76±2.3*	73±9.4	93±2.6*

* $p<0.05$ paired t-test

All values represent mean ± SEM

n=5

TABLE 4-2: Hormonal changes in response to morphine 4 mg/kg IV after two hours of sepsis.

DISCUSSION

HEMODYNAMICS

The concommitant release of endorphins with ACTH and the presence of high levels of ACTH during shock led Holaday and Faden to suspect that endogenous opiates were involved in the pathophysiology of various shock states. By using naloxone, a pure opiate antagonist, given intravenously, they were able to reverse the hypotension of endotoxin (146) and hypovolemic shock (147) in rats. Gurl and Reynolds (207) implicated opiate receptors and endorphins in the cardiovascular pathophysiology of hemorrhagic shock and endotoxin shock (149) after noting that naloxone produced dose-dependent increases in arterial pressure, cardiac output, stroke volume, and left ventricular contractility. Survival was related to the dose of naloxone used.

These hemodynamic improvements in arterial pressure, and cardiac output were confirmed in our septic pig model. However, they were found to be transient, lasting about thirty minutes, and no further response was noted with repeated administration of naloxone.

It is proposed that the transient nature of the hemodynamic improvement in these animals might be due to the administration of naloxone late in the 'preterminal' stage of the shock state. Others have reported that naloxone, when given in the early stages of shock, returned the blood pressure to preshock levels (146,149).

Morphine administration, on the other hand, resulted in a drop in blood pressure and PAP while (Figure V-1) cardiac output remained unchanged and PWP increased dramatically. This cardiodepressant effect is in agreement with the work of Eckenhoff and Oech (159) in man, and of Lind and Reynolds in anesthetized dogs (141). The hypotension caused by morphine has been attributed partly to low peripheral resistance secondary to histamine mediated peripheral vasodilatation (159,160). Reynolds (141) noted that both low

(.25 mg/kg) and high (8 mg/kg) doses of morphines caused a significant drop in blood pressure and LV-dp/dt in anesthetized dogs while vagotomy and stellectomy abolished the low dose response and caused only a milder hypotension with high doses of morphine. He concluded that morphine decreases arterial pressure and LV dp/dt at low doses through an autonomic mechanism, while the depressor effects in high doses are either on peripheral resistance, myocardial contractibility or both. Further work with anesthetized dogs by Schrank et al (208) revealed as well that morphine (2 mg/kg) given intravenously resulted in significant decreases in CO, MAP, LV dp/dt and myocardial blood flow in spite of a reduction in coronary vascular resistance. However, Leaman et al (209) noted a slight coronary vasodilation in humans after intravenous morphine administration (.2 mg/kg). Schwig et al (163) showed that morphine .3 mg/kg increased pulmonary resistance, and venous plasma histamine levels in anesthetized dogs. Samuel et al (160) noted an increase in forearm blood flow with a decrease in forearm vascular resistance even after brachial plexus block in men that were given 10 mg/ 70 kg of morphine intravenously. Based on work done previously by the same group (142) where CO remained stable following morphine, they concluded that the predominant effect is local.

Zelis et al (144) demonstrated that the dose response curve to continuously infused intra-arterial noradrenaline was unaffected after IV morphine administration. Therefore, they concluded that morphine induced venodilatation was unlikely to be secondary to peripheral α-adrenergic blockade but may be related to attenuation of sympathetic afferent discharge at CNS level (central sympatholysis). Interestingly, Farsang et al (210) recently proposed that the cardiovascular effects of central alpha-adrenoceptor stimulation (clonidine) in rats is mediated by the release of an endogenous opiate.

Our findings of decreased blood pressure in response to intravenous morphine are in agreement with previous reported data in dogs (141,208) and humans (159), while the decrease in pul-

monary vascular resistance with intravenous morphine in septic shock has not been reported previously. This appears to be due to a marked elevation of the PWP and concommitant pulmonary vasodilatation. Therefore, we conclude that morphine both depresses the myocardium and causes vasodilatation during septic shock in pigs. The mechanism of these actions, as discussed earlier, remains controversial. However, there is strong evidence that CNS mediated mechanisms predominate.

It is also noted that naloxone and morphine result in opposite effects on hemodynamic parameters such as mean arterial blood pressure (Figure V-1) and cardiac output (Figure IV-4). This indicated that morphine and β-endorphins produce their effects through the same receptors while the mechanism is not known. Furthermore, the presence of high levels of endogenous opiates during septic shock as reported previously (146,147,149,151, 152) is also confirmed indirectly and so is the fact that endorphins are partly responsible for the hypotension and myocardial depression of septic shock (146,149).

A role of endogenous opioids in thermoregulation has been suggested (148) on the basis of naloxone reversal of thermal effects of exogenously administered opiate agonists. We have found no evidence to support this hypothesis.

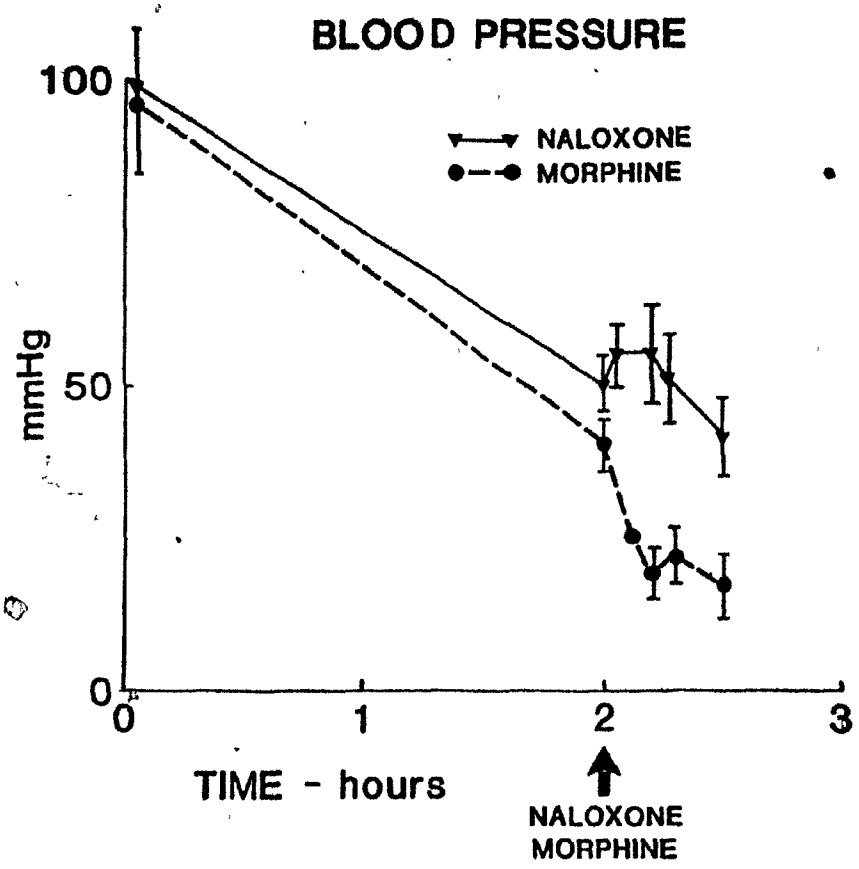
HORMONES

Cortisol

Cortisol is the principle glucocorticoid secreted by the adrenal cortex. Adrenal secretion of cortisol is modulated by a complex negative feedback mechanism involving the CNS, hypothalamus, pituitary and adrenals as described in Chapter I.

In this experiment it was shown that cortisol levels increase in response to sepsis. Opiate receptor blockade in the animal

FIGURE V-1



Comparison of the effects of opiate receptor blockade with naloxone and stimulation with morphine on the blood pressure of the animal in septic shock. All points expressed as mean ± SEM.

with septic shock resulted in an increase in cortisol while morphine caused a significant drop in the serum level of this hormone (Figure IV-5). This is indicative of β-endorphin involvement in the mechanism of cortisol secretion, and it can be postulated that opiates, whether endogenous or exogenous result in a lowering of serum cortisol either through production inhibition or increased clearance from plasma. Since ACTH and cortisol levels are known to be high at times of stress (43), the depressive effect of endogenous opiates may interfere with the defense mechanisms of the body to septic shock. On this basis opiate receptor blockade may have a beneficial effect during septic shock directly by improving hemodynamic parameters, and indirectly by increasing serum cortisol levels.

Prolactin and Growth Hormone

When normal rodents (211), baboons (137), goats (166), and humans (82) are given naloxone intravenously there is a persistent decrease in prolactin levels, while intravenous morphine administration to baboons (157), humans (212), rats (213), and goats (166) results in persistently high levels of prolactin and growth hormone. That increase seems to be dose related in rats (213) and no changes are observed in human growth hormone levels (212). Furthermore, intravenous β-endorphin administration to humans (157) and rodents (213) result in high prolactin levels, and also high growth hormone levels in rodents (213).

In this study it was noted that after one half hour naloxone resulted in a slight decrease in the serum prolactin levels while morphine caused an increase (Figure IV-7). These changes were not statistically significant, and a longer follow-up of these hormones is indicated. Growth hormone levels did not change in response to sepsis or morphine in contrary to previous reports (212,213,166) in nonshocked animals and humans.

Glick et al (211) pointed out that stress might provoke hormone release. This clearly injects difficulties into interpretation of results obtained after using provocative agents for the release of growth hormone. This problem has been appraised in greater detail (212). When repeated injections of normal saline were given intermittently to 12 normal men, it was noted in one study that although initial growth hormone values were low, in about half the patients a substantial rise was observed - generally within 60 minutes of needle placement. Some subjects also showed a later increase of plasma growth hormone three hours after needle placement. There was no correlation of either the early or the late rise in growth hormone with any of the metabolic parameters that were followed. A spontaneous secretory incidence rate of 50 percent is unusually high but must be considered in judging all alleged stimulus of growth hormone secretion.

There is conclusive evidence that endogenous opiates play a role in stimulating hormonal secretions. These neuroendocrine effects seem to be species dependent. As with hemodynamic effects, endogenous opiates and morphine seem to work through the same receptors (213,137,166).

While the mechanism for hemodynamic effects of β-endorphin remains intact during sepsis, that for growth hormone and prolactin regulation seems to be depressed.

This is in agreement with the hypothesis that opiate agonists might increase serum prolactin by reducing hypothalamic norepinephrine release which inhibits prolactin release (137) and since norepinephrine levels are high in shock states, this mechanism may override that of direct prolactin stimulation by β-endorphin.

Substance-P

Substance-P is an eleven amino acid peptide with a structure.

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Gly-Leu-Met-NH_2. It is thought

to be a neurotransmitter active primarily in primary afferent nerve endings (198). It is found in highest concentrations in the dorsal horn grey matter of the spinal cord and also in the dorsal root ganglia. Substance-P has also been found in gut. In physiological experiments, substance-P causes hypotension and secretion of saliva (199).

In this experiment morphine resulted in a significant increase in serum levels of substance-P which has also been shown to increase after a meat meal in dogs (214). Presently very little is known about this substance and its relation to the endogenous opiate systems is just becoming apparent.

Insulin, Glucagon, Gastrin

With respect to hormonal responses to naloxone, no significant changes were noted in glucagon and gastrin while insulin decreased. Once again endogenous opiate blockade seems to be involved in the regulation of a hormone whose function in shock is important, while it has no effect on others. However, thirty minutes may not have been sufficient time for any significant changes in the levels of some hormones. A slower mechanism of response for their secretion or inhibition may be responsible for this, as shown with growth hormone (212).

BIOCHEMISTRY

It is now well established that cAMP has a critical role as a regulator of hormones, enzymes, and other biologically active substances and has been identified as a 'second messenger' in the concept of hormone action proposed by Sutherland (201). Cyclic AMP has been found in most tissues including the mammalian body, fluids and in bacteria. The concentration of cyclic AMP in plasma is usually low, being of the order of 10^{-8}M (8-20 pmol/gm). However, its concentration in urine is relatively high, being in the range of 10^{-6}M (1000^{10} pmol/gm).

Research has suggested that cyclic GMP acts in a similar manner to cyclic AMP as a second messenger in the concept of hormone action. More recently it has become apparent that the roles of cyclic GMP and cyclic AMP may be linked, and that some cellular functions controlled bidirectionally are of two types: those which are stimulated by cAMP and suppressed by cAMP and vice versa. Because cGMP and cAMP can act in opposition to one another in many systems, the relative proportions of one to the other may, under some circumstances, be more important than the absolute concentration of either cyclic nucleotide. Current hypotheses do not, however, completely explain regulation of all systems and much experimental work still remains to be done before the role of cGMP as a biological regulator can be fully understood.

It has been shown that the second messenger response to epinephrine is abolished in hemorrhagic (110,211) and septic (190) shock, with an increase in plasma cAMP and the failure of tissue adenyl cyclase activation by epinephrine (190). The finding of a significant increase in cAMP levels after opiate receptor blockade with naloxone is interesting in view of the increasing evidence of endogenous opiate affecting the second messenger system (215,216). For example, morphine and endogenous opiates have been shown to selectively inhibit catecholamine-induced cAMP accumulation in various brain areas (217). It is possible that endogenous opiates exert a depressant effect by inhibiting cAMP production, on the multihormonal systems which strive to achieve homeostasis in shock and thus, indirectly, at the cellular level, contributing to the chain of homeostatic failures in shock states. The exact significance of the altered intracellular second messenger system has yet to be fully clarified and further efforts to elucidate the effects of endogenous opiates on cellular metabolism are warranted.

CONCLUSION

Chapter VI

CONCLUSIONS

1. Tissue inflammation, by releasing various kinins, pyrogens and other mediators produces a hyperdynamic picture including increased cardiac output, vasodilatation, fever and leukocytosis.

2. Intravenous administration of pathogens in the absence of a focus of infection produces a hypodynamic state with low cardiac output, vasoconstriction, leukopenia and a high pulmonary vascular resistance.

3. A focus of infection with tissue inflammation is necessary for a hyperdynamic response.

4. The low output state in hypodynamic sepsis is not necessarily due to hypovolemia.

5. Opiate receptor blockade with naloxone in hypodynamic sepsis leads to a transient hemodynamic improvement, with hormonal and biochemical changes.

6. Opiate receptors and perhaps endorphins are implicated in the hormonal homeostasis of septic shock.

7. The effects of opiates on receptors seem to be mediated by the adenyl-cyclase-cAMP 'second messenger' system.

8. Naloxone may be an important temporizing measure or a useful adjunct to shock therapy.

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