ENDOCRINE CHANGES

ASSOCIATED WITH MORPHINE TOLERANCE

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INTRODUCTION

The reasons for undertaking the researches described herein are presented first to acquaint the reader with the nature of the problem selected by the candidate.

The main objectives and purposes of this investigation may be stated. They are to further explore and correlate the various systemic and hormonal changes occurring in the animal body during the development of tolerance and addiction to analgesics of the morphine group. A better understanding of the mechanisms of drug action concerned in the development of the addictive state is desired. Through study of the inter-related activities of the autonomic nervous system in association with changes in the anterior pituitary and the adrenal cortex, it is hoped that some rational medical procedure might be suggested for delaying or preventing the development of drug addiction.

For clarification several of the terms mentioned frequently throughout this discourse should be defined at this time. The name morphine is often used alone, for convenience to describe a condition, state or action caused also by the other opiate analgesics and the synthetic analgesics since those actions are common to this group of drugs. The term addiction, as herein used, refers to that condition produced only by repeated use of morphine and the allied synthetic analgesics. Only this group of analgesic drugs causes true addiction with its characteristic features of tolerance, mental and physical dependence and the appearance of the withdrawal syndrome on cessation of the drug. Other habit forming drugs such as the barbiturates, cocaine, chloral hydrate,

nicotine or alcohol do not fully meet the requirements of an addictive drug nor develop true addiction on repeated use. Also, a distinction should be made between the terms tolerance and addiction used herein, although it is realised that these two words are often used interchangeably in referring to morphine addiction since the presence of one condition implies, and occurs along with, the other effect. Tolerance develops to many drugs besides the morphine group of analgesics. In morphine addiction tolerance, while important, is but one feature and this condition does not develop to all of the many effects of morphine on the body.

In order to appreciate the problems involved in carrying on an experimental study dealing with addiction, some background information of the history, chemistry and pharmacology of morphine and the synthetic analgesics is offered. Previous experimental approaches to studying the problem of morphine addiction are considered also.

PHARMACOLOGY

A. Historical: Present Clinical Status of Morphine.

Morphine stands out as the first alkaloid isolated from crude plant sources and used for medicinal purposes; this significant medical advance was accomplished by the French pharmacist Sertuerner about 1807(1). Previous to this, opium, obtained as the dried juice of the incised capsule of the oriental poppy, had been used since antiquity as a sleep-producing narcotic and as an analgesic for the relief of pain.

The superiority and usefulness of morphine as a pain relieving drug and for other therapeutic purposes, such as cough suppression, allaying of apprehension, stopping of diarrhea, and compelling sleep remains unchallenged even today. No other drug has proven as reliable or as effective for these various clinical conditions. But, with morphine--as is true for other worthwhile and beneficial contributions in this world--there are compensatory drawbacks in its use. These are the many undesirable actions that are an integral part of the general systemic effects of morphine on the animal body and which appear in high incidence even with therapeutic doses. These undesirable actions, such as nausea and vomiting, dizziness, constipation, respiratory depression, sweating, and the like seem inseparably bound together with the beneficial actions of morphine. However, in some disorders these untoward actions are therapeutically useful, such as employing the respiratory depressant effects for relief of the dyspnea and air-hunger of heart failure, the constipating action for diarrhea or the diaphoretic action for producing sweating in upper respiratory infections. Nevertheless, too many divergent

actions, both beneficial and undesirable, are resident in the molecule of morphine. The ideal preparation sought would be one retaining the therapeutically useful features of morphine without the undesirable ones; this seems impossible for a single agent so that the search must be extended, at present, to the development of several preparations to satisfy this need.

Undoubtedly, the one untoward action of morphine and the synthetic analgesics which is most objectionable and certainly of no value therapeutically is that of tolerance and addiction. Through an elucidation of some of the underlying factors and mechanisms concerned in the development of the addictive state, ways and means might be found to lessen the degree and rapidity of its progress.

B. An Experimental Approach to Studying the Problem of Morphine Addiction and Undesirable Actions.

Two general approaches to the problem of finding methods to reduce the addiction liability of morphine and the frequency of its undesirable actions are available. Both have been explored previously but the possibilities they offer in providing some solution to this problem are far from exhausted. The attack may be carried out through a systematic biochemorphologic appraisal of morphine and the synthetic analgesics. A complete evaluation of the relationship of the chemical structure to that of pharmacological activity of the many analgesics now available is needed. More experimental information is required as to their relative analgesic potency, the incidence of untoward actions they cause and, particularly, their propensity to produce addiction. The monumental

work carried on from 1928 to 1940 by the pharmacologists Nathan B. Eddy, Hugo Kruger and their co-workers and the chemist Lyndon Small summarized all the known biochemorphologic knowledge of morphine and newly discovered related compounds up to that time(2). But, since then, many new synthetic analgesics have been made available with only incomplete evaluations of them having been made thus far. While there has been a never-ending search for drugs that possess marked analgesic properties, yet lack the undesirable actions, it has been impossible thus far to find an analgesic which allows a separation of the analgesic components of the morphine molecule from the other components. Nevertheless, the approach to this problem through a study of the relationship of chemical constitution to activity with the purposeful synthesis of new compounds has proven fruitful in the past and appears promising for the future.

Another approach to the problem of finding less objectionable and addictive drugs than morphine lies in developing methods to counteract or antagonize those properties. Other drugs, used in conjunction with morphine, have been found to not only modify some of the untoward actions but to intensify the analgesic effect. Such drugs as magnesium sulfate(3), prostigmin(4), d-amphetamine(5) and certain vasoconstrictors such as epinephrine(6) injected previous to the use of morphine intensify the resultant analgesia but do not appreciably lessen the untoward effects. Recently, as an extension of the observations to be reported here, other workers in the Department have shown that preliminary administration of certain sympathoadrenolytic compounds potentiates morphine and l-isomethadone analgesia in the rat(7).

In this present study both methods of approaching the problem of decreasing the undesirable actions of morphine have been followed.

New and recently synthesized analgesics which, from a biochemorphologic standpoint, appear to offer advantages over morphine in some respects have been studied. The major interest has been in studying the effects of repeated use of certain dihydrogenated ergot compounds over a period of time on the development of hyperglycemia in the rabbit as an index of the degree and extent of addiction.

C. Chemistry and Structural Formula of Morphine and Related Analgesics.

The more important aspects of the chemistry and the pharmacology of the morphine analgesics are reviewed here. A basic knowledge of the diverse actions of morphine on the animal body with an explanation of the pharmacological mechanisms concerned provides a background or starting point from which may be launched an attack on the problem of tolerance in morphine addiction.

1. Structural Formulae. Structurally, most organic chemists agree that the morphine molecule consists of the phenanthrene nucleus, an oxide bridge, a tertiary amine attached to the phenanthrene ring, and two hydroxyl groups each attached to opposite poles of the phenanthrene ring. The commonly accepted structural formula for morphine is shown in Figure 1. The formulae of some of the newer synthetic analgesics, such as meperidine, methadone and l-isomethadone is presented in Figure 2.

2. Some Biochemorphologic Aspects of The Morphine Molecule. The structural formula for morphine (Figure 1) shows two hydroxyl groups attached to either end of the phenanthrene ring. The hydroxyl on the left is known as the phenolic hydroxyl and that to the right as the alcoholic.

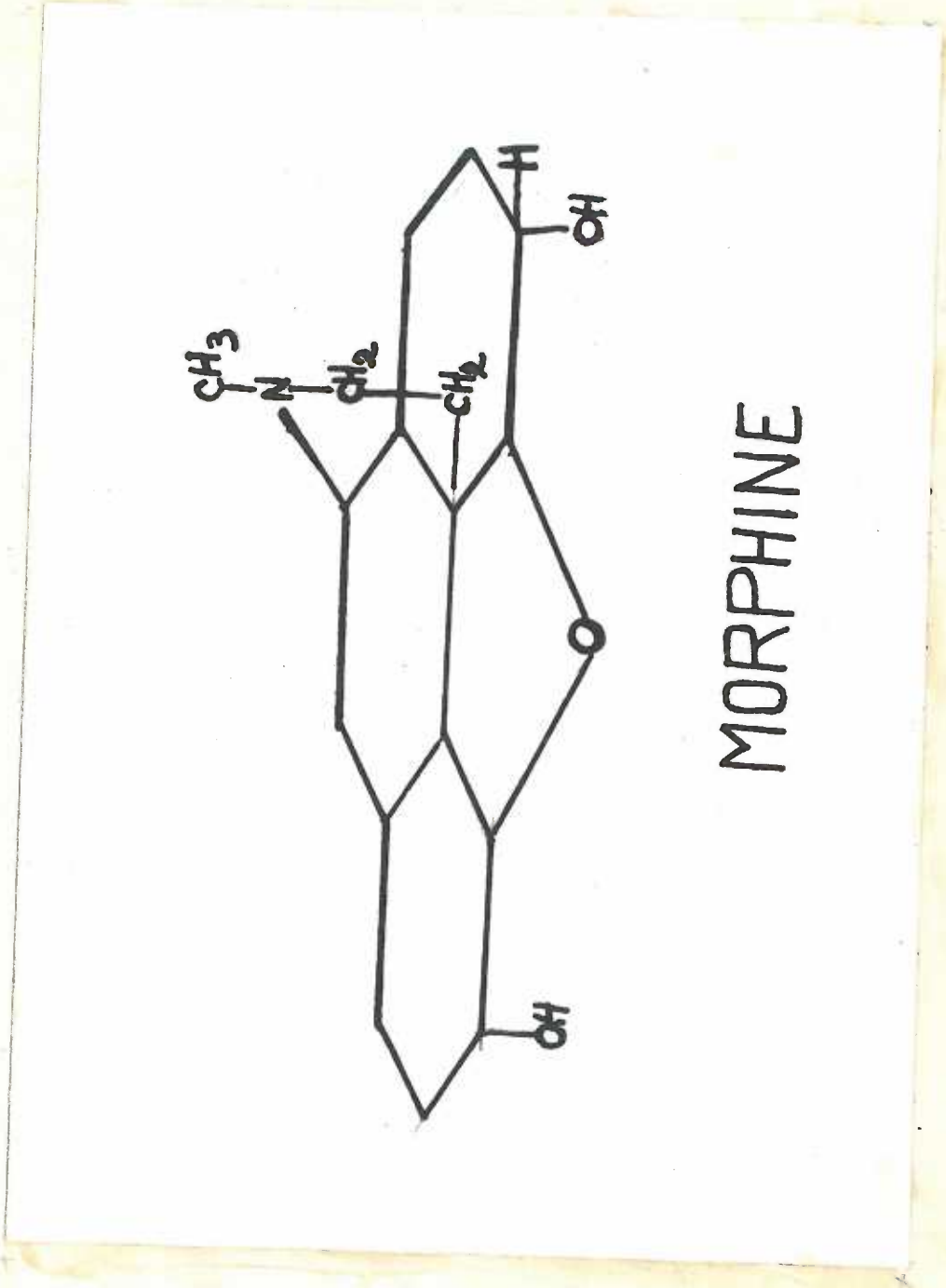


Figure 1. Structural Formula of Morphine.

Through the pioneer investigations of Eddy, Krueger and their co-workers [2], it has been shown that definite and very important properties characteristic of morphine may be assigned to each of these hydroxyls. For example, masking of the phenolic hydroxyl by substitution with a methyl group changes the morphine molecule to codeine. By this rather simple change many of the active properties of morphine have been quantitatively reduced with codeine still retaining considerable analgesic activity. Compared to morphine, codeine shows a lower incidence of untoward actions, is less depressant to respiration, less constipative, and is less addictive. When the alcoholic hydroxyl in morphine is masked (along with saturation of its adjacent double bond in the phenanthrene ring) by replacement with a ketonic oxygen to form dihydromorphinone (dilaudid), both the analgesic and the respiratory depressant properties of morphine are exaggerated. When both the phenolic and alcoholic hydroxyls are masked by replacement with acetyl groups to form heroin, not only is the compound more analgesic and depressant to respiration than morphine but it rapidly causes addiction. Some general principles dealing with the biochemorphologic relationships between morphine and other related analgesics may be formulated. Unfortunately, they do not permit an immediate prediction of what properties or actions may be expected from the synthesis of morphine-like compounds which retain some of the structural features but are altered to differ slightly from the morphine molecule. It is only by experimental screening and pharmacologic study that these properties can be discovered[8].

With the discovery and introduction of meperidine by Eisleb and Schaumann[9] in 1939, it became evident that potent analgesic activity

could be resident in compounds of different structure than that assigned morphine (Figure 2). From preliminary clinical study and early experience, along with insufficient experimental investigation (due to interuption by the War), it was first thought that meperidine did not cause addiction. For the first time it appeared that it might be possible to separate the property of potent analgesia of the type provided by morphine from that of addiction. But, within a year or so after its introduction and wide-spread use in the United States, it soon became evident that meperidine was nearly as vicious as heroin in its propensity to cause addiction[10].

Stemming from the original chemical and pharmacologic studies which led to the discovery of meperidine was the introduction of the amidone (methadone) series of analgesics by the German workers. Methadone, itself, was not developed or used very extensively by the Germans during the War but, when brought back to this country in 1946, it was subjected to intensive experimental investigation[11] and soon found wide-spread clinical trial[12][13]. Since methadone rather closely resembled meperidine in chemical structure and some of its other pharmacologic actions besides that of analgesia, it was expected that it, too, would prove addictive. Recently, this has been shown to be the case clinically[14]. Several analogues or isomers of methadone are now being studied experimentally and clinically; so far, the most promising of these is l-isomethadone which has been used in the studies reported on in this discourse.

A closer examination of the structural formulae of morphine, meperidine, and methadone (Figure 2) reveals several structural arrangements

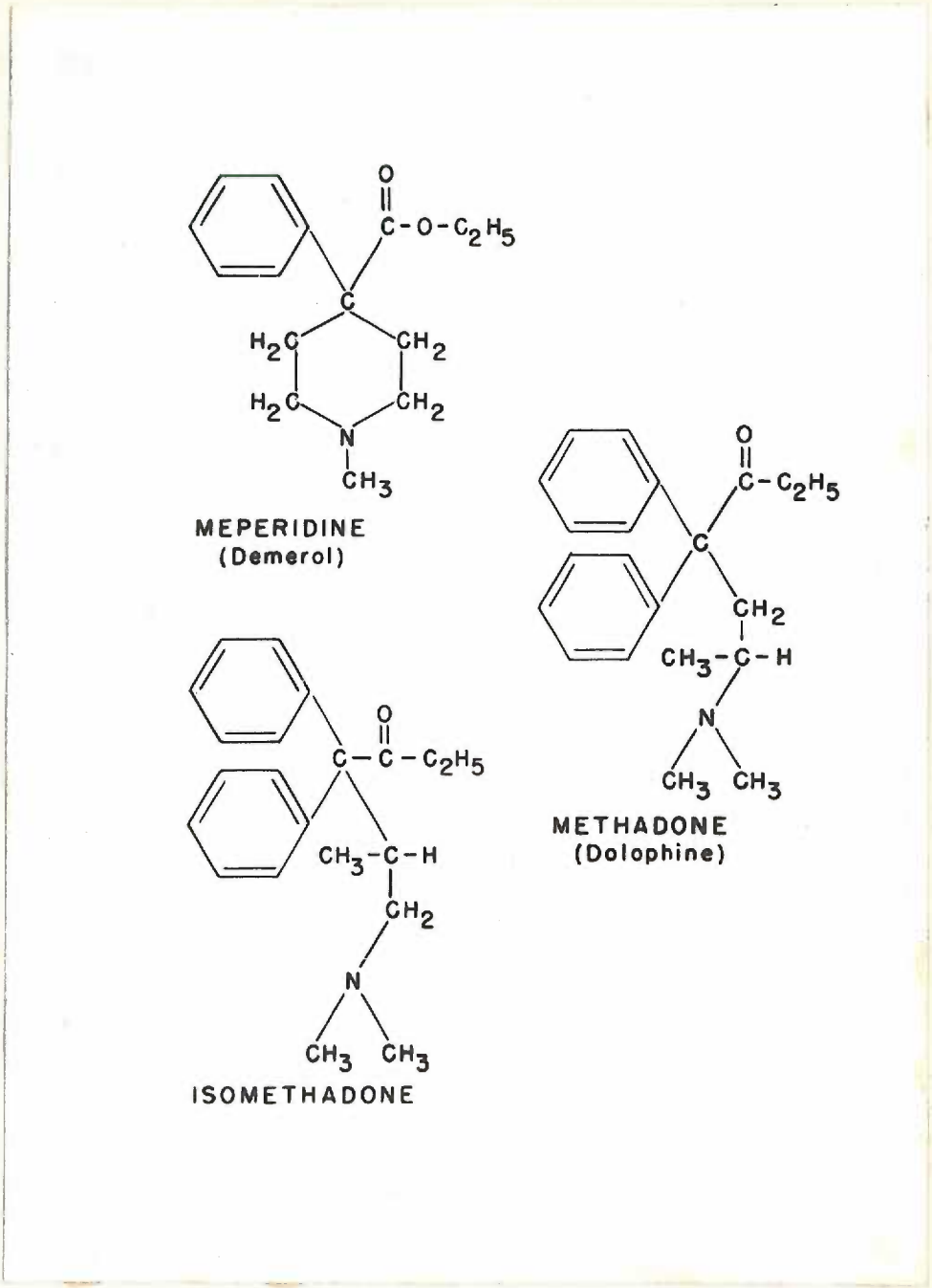


Figure 2. Structural formulas for meperidine, methadone, and isomethadone.

common to each. All compounds show a tri-alkyl nitrogen and all show a ketone or other oxygen. Chen and his associates[15] have studied more than two hundred synthetic compounds related to methadone. It has been found that the elimination of the amino-N atom destroys completely the activity of the compound; and, the ketonic and amino groups must be on the opposite sides of the diphenyl C-atom.

D. Pharmacologic Actions of Morphine.

Only the most prominent actions and properties of morphine having a direct bearing on the problem of tolerance and addiction require mention here. Emphasis will be placed on the actions on the autonomic nervous system since it is in this manner that the influence of morphine is extended to activate certain endocrine organs during the development of addiction.

1. The C.N.S. Actions of Morphine and The Synthetic Analgesics. The most prominent actions of these compounds is analgesia which is thought to occur by depression of the thalamic region of the central nervous system, possibly aided by the release of epinephrine. Small doses are effective in relieving some types of pain. The severer the pain, the larger the dose required up to the maximum tolerated single dose of 30 milligrams given intravenously for the excruciating pain of coronary thrombosis. Beyond this dose, no greater analgesia can be produced as the toxic effects limit the amount of drug which can be safely given. Thus, the increase in analgesic potency with dosage obtains in linear fashion only up to a certain point. But, of all the drugs known to man--with the exception of complete anesthesia--morphine is the sole

agent which comes most nearly to being effective in complete obtundation of pain without causing unconsciousness.

The narcotic effect of morphine compelling sleep when only therapeutic amounts are administered is less prominent than its analgesic action. It is this action, resident in opium, which impressed the ancients more than its pain-relieving qualities. In fact, Sertuerner named the alkaloid he extracted from opium, morphine, after the god of sleep, Morpheus. The newer synthetic analgesics such as meperidine and methadone differ quantitatively from morphine in that they cause sleep only when used in fairly large doses and some cumulation of the drug occurs.

Euphoria, or the production of pleasant thoughts and a sense of superior well-being, or if sleep ensues of happy optimistic or sometimes erotic dreams, frequently occurs following the administration of therapeutic doses of morphine. It is more likely to occur if morphine has been taken by a person not suffering pain. There is a dictum prevalent in the minds of medical men that pain is the "antidote" for the toxic and untoward effects of morphine. This seems true with respect to the occurrence of euphoria in those suffering pain,--they hardly ever experience this action. Euphoria is claimed by some to be one of the underlying causes of the development of addiction brought on by the desire of the individual to repeat the pleasant experiences again and again. Meperidine and methadone, however, readily produces euphoria in addicts (14).

Mention should be made of the fact that morphine depresses the heat-regulatory center in the hypothalamus which leads to a loss of heat from the body through vasodilatation and sweating as well as decreased

metabolic activity[16]. This vasomotor action with vasodilatation may be of some importance in the mechanism of morphine analgesia[7].

On the medullary centers the actions of morphine and its congeners consist in a confusing mixture of stimulation and depression. Foremost is the depression of the respiratory center evident even with very small doses. The newer synthetic analgesics are less depressant to the respiratory center than the opium analgesics. The vomiting center is stimulated by morphine and to a lesser degree by the synthetic analgesics. The cough center is depressed by morphine and were it not for its addictive properties, it probably would be the most useful drug available in the treatment of the dry non-productive cough.

2. Actions on The Autonomic Nervous System. In addition to the prominent sedative and analgesic actions of morphine, the autonomic nervous system centers and peripheral ramifications are influenced by morphine. Morphine has been found to produce a definite anti-cholinesterase effect in the ganglia and at the peripheral ends of the parasympathetic nerves which leads to cholinergic stimulation of the innervated organs. At the same time there is liberation of epinephrine due to both central stimulation and from cholinesterase inhibition with acetylcholine release in the adrenal medulla. Generally, for a given dose of morphine, the parasympathetic effects outweigh those of adrenergic stimulation. While it is claimed that the autonomic effects of morphine appear less prominent than its C.N.S. actions, it is the former which are responsible for many of the untoward actions.

a. Actions on Parasympathetic Division. The following symptoms noted from the administration of morphine are suggestive of parasympa-

thetic stimulation. Following ordinary doses of morphine, the heart rate is slowed. This action is central since sectioning of the vagi or administration of sufficient atropine will prevent this slowing of the heart rate. The constipative action of morphine is due largely to stimulation of the parasympathetic system, both centrally on the vagus center and peripherally through cholinesterase inhibition with acetylcholine release. Added to this effect which causes segmental peristalsis and prevents the downward progression of intestinal contents through segmental constriction as well as clamping of the sphincters, is the direct stimulatory actions of morphine on intestinal smooth muscle. Compounds such as meperidine and methadone are spasmolytic to the isolated intestinal strip but in the intact animal their parasympathetic actions outweigh any local depressant effect so that these drugs are constipative also.

On arterial smooth muscle morphine has some direct tonic action. However, any tendency to cause a rise in blood pressure is counteracted through central vasomotor action leading to peripheral vasodilatation. Since the synthetic analgesics tend to depress smooth muscle and still retain most of the parasympathetic actions and effects on the vasomotor centers, they are more likely to cause hypotonia when given in large doses.

b. Actions on The Sympathetic Division. The actions of morphine on the sympathetic nervous system are difficult to evaluate objectively as distinct activities since, as previously mentioned, the parasympathetic system also is stimulated simultaneously. Through direct stimulation of the sympathetic centers centrally and by cholinesterase

inhibition in the adrenal medulla, morphine leads to epinephrine release. The effects of this release of epinephrine are manifold. Our interest in these sympathetic actions of morphine lies in the fact that the liberated epinephrine causes hyperglycemia(17), probably activates the pituitary-adrenocortical system(18) and plays a role, through either causing vasoconstriction(6) or vasodilation(7) in the production of analgesia.

3. The Undesirable Actions of Morphine.

a. Clinical Aspects. With the exception of the analgesic actions of morphine, any of its other effects may in certain conditions be considered undesirable. Conversely, many of the side effects may be utilized for therapeutic purposes. Already suggested have been the employment of the respiratory depressant, the diaphoretic and the constipative actions therapeutically. In other situations such as in the control of chronic pain in patients who desire to be up and around, the hypnotic effects of morphine are undesirable; here, recourse is had to less narcotic agents such as metopon or demerol. In heart conditions, the stimulation of the vagus by morphine may be desirable in slowing the heart rate. Oftentimes the nausea and vomiting provoked by the morphine derivative apomorphine may be employed in the treatment of poisoning.

b. Addiction and Tolerance. The development of addiction and physical dependence to the analgesics greatly limit their usefulness both from a therapeutic as well as an economic standpoint. Were it not for this outstanding undesirable action, the far-reaching benefits of morphine, or other analgesics, could be made available for a longer time to countless thousands of patients who rapidly become addicted and

tolerant. The use of large and frequent doses no longer controls pain and the toxic actions resulting preclude continuation of the drug. And, were it not for this abhorrent property of morphine, thousands of addicts now dependent upon morphine would no longer be subject to the untold misery this affliction causes and the millions of dollars now spent to care for them and control the extent of addiction would be saved.

A pharmacologic definition and explanation of addiction may now be offered. Reference has been made already to the addictive state (page 2) so that the following applies to the requirements of a drug for causing addiction.

Definition of Addiction: True addiction is caused only by the opium or synthetic analgesics. Other drugs, including the antipyretic analgesics such as the salicylates and acetanilid do not meet the requirements stated below. A clear-cut distinction is to be made between the term habit-forming and that of addiction.

The four requirements for a drug to cause addiction are:

(1) Ability to develop tolerance to its effects; this means that with subsequent use, larger and larger doses of the drug must be administered to produce the original desirable analgesic or sedative effect. With this necessary administration of larger and larger doses, the factors which develop addiction are given more opportunity to exert their actions and so lead to addiction.

(2) Ability to develop dependence to the drug so that repeated administrations are sought. By this is meant that repeated exhibition of the drug effects in the body lead to both a mental and a physical desire for the drug. Simply stated, this effect may be looked upon

as a "substitution" effect with the morphine molecule, through its repeated actions, replacing and taking over certain functions ordinarily served by other agents or nutritive substances essential to the welfare of the body. Without the presence of morphine, these cells undergo certain changes which are abnormal and lead to the production of the withdrawal symptoms.

(3) Ability to exhibit withdrawal symptoms upon removal of the drug. The symptoms of withdrawal from morphine follow a characteristic pattern typical for this drug or the synthetic analgesics.

(4) Ability of a drug to alleviate the symptoms of withdrawal. Only morphine or certain allied synthetic analgesics have the ability to immediately stop the symptom complex of withdrawal. This may be explained as due to the fact that the administration of such drugs fulfills the need of the cell for their presence; hence, the cell is now returned to the state or condition existing during the addiction state.

It is evident from the above that the features of tolerance, dependence and the exhibition of withdrawal symptoms on drug withdrawal are closely interwoven and dependent one on the other. Thus, the presence of any one feature following repeated administration of morphine implies the presence of the other; this fact is not generally appreciated by the clinician.

B. Factors Concerned in the Development of Addiction.

The development of tolerance is a characteristic property of the phenanthrene alkaloids of opium and the newer synthetic analgesics. Tolerance does not develop uniformly to all the actions of morphine.

The depressant action on respiration and the analgesic action decrease with continued use of the drug unless the dose of the drug is increased. But, tolerance to the excitatory effects such as pupillo-constriction and to constipation, another parasympathetic stimulatory effect, does not develop. As a rule, after two weeks of continued use of the same dose of morphine so that its effects are maintained, the usual depressant effects disappear. After withdrawal of the drug and allowing time for the re-establishment of the normal milieu of the cells, tolerance disappears. But, if the drug is again resumed, tolerance develops even more rapidly than before.

The underlying factors or mechanisms concerned in the development of tolerance and addiction are not well understood. That addiction is a true abnormal physical condition, rather than a psychic imbalance, has been demonstrated many times by the experimental production of addiction in animals. In man, it is true, that both mental and physical factors are concerned but even here, addiction can be readily produced in the perfectly normal, well-adjusted psychologically, individual. Thus, our attention should be more directed to the physical changes caused by morphine in addiction.

Theories of Addiction. Various theories have been offered to explain the underlying mechanisms concerned in addiction. Transformation of morphine to oxymorphine has been proposed with this compound supposedly being responsible for the stimulatory effects, such as those witnessed during withdrawal. However, such actual transformation of morphine to oxymorphine has been disproved(19). Another theory which may now be dismissed is that of antitoxin formation due to repeated

exhibition of the effects of morphine. The claim that the serum of addicted animals is protective against the action of morphine has been discarded[20]. Increased destruction of morphine has been reported, but later studies found that the destruction rate of both addicted animals and in normal animals was the same[21]. Still another theory advanced was the decreased rate of absorption. However, addicts tolerant to large oral doses are also tolerant to the same dose given intravenously, a route which allows for the full concentration of morphine to reach the brain.

The theory of the causation of addiction which appeals most to the pharmacologist is that of Tatum and Seevers[22]. Briefly, this theory is based on the fact that morphine has a bivalent action, one depressant and the other stimulant, so that tolerance rapidly forms to many of the depressant effects such as analgesia and narcosis, but not to the stimulant actions. Thus, with a certain dose of morphine, the depressant or sedative and analgesic effects rapidly wear off after it has been given over a period of time, but the stimulant actions persist. The unequal and sharply differentiated tolerance existing between these two effects of morphine also explains the appearance of the withdrawal symptoms. In fact, the early symptoms of withdrawal are of excitatory origin and following each dose of morphine some manifestations of abstinence appear. One might even go so far as to claim that some of the after-effects, such as nausea, dizziness, nervousness, constipation, and other untoward symptoms are actually expressions of withdrawal. Eddy[6] has pointed out that in transferring the addicted patient from one analgesic to another that the symptoms seen, such as nausea and vomiting, sweating,

apprehension and the like, during this process are often misinterpreted as being due to the new drug when actually they are expressions of withdrawal from the previously used analgesic. As tolerance to the depressant effects of morphine develops, the individual becomes disturbed and uneasy earlier and earlier following administration of the drug. Thus he seeks to have morphine more frequently and in larger amounts since, through experience, he knows this will temporarily allay withdrawal manifestations. The dose and the rhythm of frequency of morphine administration is increased up to where a point is reached where his tolerance to the toxic effects is broken. He either succumbs to the effects of morphine or it becomes necessary to stop further administration of the drug.

F. Methods for Studying the Development of Addiction.

1. Clinical Observations. In the human receiving opiate analgesics for the relief of chronic protracted pain addiction ensues even more rapidly than the patient's physician thinks. Many patients demand more and more frequent drug not so much because of the severity of their pain as because they actually show withdrawal symptoms soon following administration of the drug and become wakeful, apprehensive and uncomfortable at shorter and shorter intervals.

2. Experimental Methods Available for Studying Addiction. The development of tolerance and addiction in the experimental animal is usually controlled by limiting drug administrations to once daily at a specified time and by compensating for the effects of tolerance by allowing gradual increments in the dosage each week.

Several methods may be employed to measure the degree and extent of addiction in the experimental animal. In such studies it is essential that control or base levels be first established so that the changes resulting during addiction can be compared with the original, normal values.

Among the tests used for measuring the development of tolerance and addiction to analgesic drugs are:

a. Tests for Decreasing Analgesic Potency. Various techniques may be employed for measuring the changes in the threshold to painful stimuli over a period of time and when the same dose is continued. The D'Amour-Smith[23] rat tail method, th Brecli-Lewis[24] or the Andrew's method[25] all employ radiant heat as the painful stimulus.

b. Tests for Changes in Hypnotic Effects. Hafner's method[26] of the ability of the animal to "right" itself or stand up, Barlow's[27] tranquilising method measuring changes in the jumping or twitching activity of the supine, tied down rat, or Abreu's[28] suspended cage method for measuring activity of the rat have all been used for addiction studies.

c. Measurement of Respiratory Depressant Effects. Various modifications of Dresser's[29] method to measure the respiratory rate and tidal air exchange have been proposed as a method for measuring the changes in this effect occurring during addiction. Phatak and Samay[30] have used the oxygen consumption chamber method to measure the effects of long-continued administration of barbiturates to guinea pigs and this method could be employed to study the effects of continued administration of analgesics.

d. Changes in the Hyperglycemic Response to Morphine. Single injections of morphine cause a prompt rise in the blood sugar level in man and other animals[17]. As later discussed when morphine is given over a period of time, this hyperglycemic response becomes less and less and gradually disappears.

Because workers in this laboratory have been concerned for some time with studies dealing with the hyperglycemic responses of both the morphine-like analgesics and the barbiturates, this method is looked upon as a practicable experimental technique for studying addiction and tolerance. Since the research reported here has dealt largely with the hyperglycemic response following administration of morphine, the importance of this subject demands that it be discussed in a separate section.

MORPHINE HYPERGLYCEMIA

A presentation of the knowledge gained from previous studies dealing with morphine hyperglycemia is offered. This is best done in orderly fashion by separately considering the effects and mechanisms concerned when only single doses have been given, or when repeated administration over a period of time has been practiced.

A. Effects of Single Doses of Analgesics on the Blood Sugar Levels.

A number of workers in the past have been interested in the hyperglycemia caused by single doses of morphine and related opium derivatives(2). Based on the earlier observations of Asche fe(31) and De Dodo (32), Emerson and Phatak(33) in 1937 showed that single doses of morphine, dihydromorphinone (dilaudid) and dinitrophenylmorphine produced hyperglycemia in the fasting rabbit. Studies on the newer synthetic analgesics of the methadone series by Phatak, Maloney and David(34) in 1948 have shown that these substances cause hyperglycemia in the same fashion but to less degree than morphine. More recently Kimura and De Beers(35) have employed the hyperglycemic method to compare several of the newer synthetic analgesics as to addiction potentialities.

From these studies it has been found that single doses of morphine (15 milligrams per kilogram), meperidine (70 - 100 milligrams per kilogram), methadone (2 - 3 milligrams per kilogram) and other synthetic analgesics cause a rise in the blood sugar of the fasting rabbit from the normal of around 80 milligrams per cent to 120 milligrams per cent. The peak of the response is reached about one hour after subcutaneous injection and the hyperglycemic response lasts for several hours.

larger doses, up to a certain point limited by the toxic actions of the drug, provoke greater rises in the blood sugar so that some linear relationship exists between the dose and response.

Use of the hyperglycemic response in animals allows observations to be made on other effects produced by morphine. This permits a correlation between the degree of the effects exhibited and the extent of hyperglycemia; it also permits observation as to whether or not respiratory depression can be ruled out as a factor in causing hyperglycemia.

B. Mechanism of Morphine Hyperglycemia.

The mechanism concerned in morphine hyperglycemia appears to be a stimulation of the autonomic centers which involve areas of the posterior hypothalamus. The pathways concerned here are the sympathetic centers in the hypothalamus (diencephalon), thence via sympathetic pathways in the spinal cord to the splanchnic nerves and to the adrenal medulla. The hyperglycemia is due to the sympathetic stimulation of the adrenal medulla leading to a liberation of epinephrine secondarily inducing glycogenolysis in the liver. The actual liberation of epinephrine by the adrenal medulla is effected by the release of acetylcholine at the sympathetic endings of the splanchnic.

While only indirect proof has been offered to support the hypothesis that morphine hyperglycemia results from direct stimulation of the sympathetic centers to indirectly cause epinephrine release, several experiments would seem to support this contention. Brooks, Goodwin and Willard (36) showed that decerebration through a midcollicular area, performed twelve to twenty four hours previously, prevents morphine hyperglycemia

in cats. Also, Kobayashi[37] showed that splanchnicotomy or adrenal demedullation would prevent this hyperglycemia as would double adrenalectomy. Kato[38] found that removal of the adrenal cortices, without demedullation, decreased morphine hyperglycemia but not to the same extent as did adrenalectomy. Watanabe[39] found that the larger the dose of morphine sulfate given, the greater were the increases in blood sugar and lactic acid and that both these responses were suppressed by splanchnicotomy. Thus did de Bodo et al[32] from these studies and those of their own performed on spinal animals conclude that "the morphine hyperglycemia is due to stimulation of supraspinal centers with the subsequent release of epinephrine which causes glycogenolysis in the liver."

Since morphine causes respiratory depression, the factor of asphyxia must be considered in explaining the mechanism of morphine hyperglycemia. Conditions such as asphyxia which limit the supply of oxygen to the tissues leads to rapid loss of liver and muscle glycogen. Anoxia produces tissue acidosis leading to an increased rate of glycogen breakdown.[40] Elias[41] has shown that the intravenous injection of acid into dogs causes rapid breakdown of liver glycogen with resulting hyperglycemia. Stewart and Rogoff[42] found, as did others, that denervation or demedullation of the adrenals will prevent morphine hyperglycemia but does not prevent asphyxial hyperglycemia. Also at a time when the hyperglycemia induced by morphine was disappearing, and the blood sugar might have returned to normal, the respiration was apt to be even more depressed than earlier, at a time when distinct hyperglycemia was present. Stewart and Rogoff further concluded that morphine hyperglycemia was essentially different from asphyxial. It has

been shown by Anton[43] that hyperventilation does not suppress morphine hyperglycemia.

Langley and Clarke[44] observed that the increase in blood sugar and in the liver glycogen which is characteristic of hypoxia requires the presence of the adrenal cortex. However, when a dose of adrenal cortical extract, which in itself does not affect the carbohydrate levels is administered as maintenance therapy to the adrenalectomized rat, it permits the full development of the carbohydrate changes characteristic of anoxia[44].

Since hyperglycemia is but one of the features of morphine action, interest has been shown in studying whether or not morphine hyperglycemia could be prevented. Successful attempts along this line have been the observation[45] that insulin lowers blood sugar levels in morphine hyperglycemia as much as in other hyperglycemias; that morphine hyperglycemia is much greater after partial destruction of the pancreas[46]; that phenobarbital prevents this hyperglycemia by antagonising the morphine effects centrally[47]; and that tetraethylammonium chloride (etamon), a ganglionic blocking agent, similarly affects morphine hyperglycemia.

Thus, from the previous experimental evidence it may be concluded that the hyperglycemia following single injections of morphine is provoked due to epinephrine release and the resultant glycogenolysis which takes place in the liver. Other effects due to morphine such as respiratory depression play a minor role in producing this distinctive type of hyperglycemia. Not only does this release of epinephrine caused by morphine lead to hyperglycemia, but other measurable effects such as ascorbic acid depletion and eosinopenia may be observed when morphine

provoke epinephrine as their instigating factor, will be considered in the next section of this thesis.

C. Effects of Repeated Administration of Analgesics on the Hyperglycemic Response.

When large doses of morphine or other analgesic drugs of this type are given daily to rabbits over a period of several weeks, the usual hyperglycemic responses become progressively less. This phenomenon was first noted by Ascho Ho[31], and later by Emerson and Phatak[33], Phatak, Maloney and David[34], Kimura and DeBoer[35], and, recently, by Kimura, DeBoer, Walts and Keith[48]. When the same initial dose of morphine is used daily and blood sugar determinations made weekly, at the end of four or five weeks there is practically no hyperglycemic response shown. However, if one allows increments in dosage at the beginning of each week, the hyperglycemic response, although lessened, occurs due to the increased stimulus from the added amount of drug given.

Another phenomenon may be observed during the development of addiction when the blood sugar responses are studied. Emerson and Phatak pointed out that after a period of twenty-four to forty-eight hours of withdrawal, the fasting blood sugar tends to increase compared to the original fasting level. Or, if the experiment is stopped at the end of seven or eight weeks with the abrupt withdrawal of morphine, the hyperglycemic response reoccurs. The height of this hyperglycemic response coincides with the height of the withdrawal symptoms, both lasting for several days. With codeine, methadone and meperidine, the withdrawal hyperglycemic response is never as great as that for morphine[34].

Similarly, the hyperglycemic response occurring during the development of tolerance to these compounds is not as great as with the morphine type of analgesics.

D. Effects of Repeated Administration of Analgesics on the Adrenal Cortex.

An orderly consideration of the indirect actions of morphine would call for discussion of this subject later. However, early studies revealed that rabbits given daily administration of morphine with weekly increments in dosage for periods of six to eight weeks showed cortical hypertrophy at autopsy (Emerson and Phatak)(33). With this cortical hypertrophy compression atrophy of the adrenal medulla occurred. This important observation, made over fifteen years ago, pointed out the close relationship between the action of repeated administration of morphine and the activity of the adrenal cortex.

Similarly, in 1927 MacKay and MacKay(49) found adrenal cortex hypertrophy in rats given repeated injections of morphine. The cortical hypertrophy was greater the larger the dose used and the tolerance attained and, especially, in those rats which resisted large doses of morphine. This led these workers to believe that the reason uremic individuals often tolerated very large doses of morphine was because of adrenal cortical hypertrophy. They inferred that in some way the activity of the hypertrophied cortex allowed an increased resistance to morphine, as they had noted in their rats.

Phatak and his co-workers(33),(34) studied the addiction liability of several synthetic analgesics by comparing the phenomena of decreased

hyperglycemic response during repeated administration of the drugs, the reappearance of the hyperglycemic response upon withdrawal and the changes in the adrenal cortex with similar changes effected by morphine.

INTERRELATED CHANGES DURING TOLERANCE

The interrelated changes occurring in the functional activity of various organs from repeated administration of morphine and other analgesics will now be considered. Since the provocative agent responsible for these changes is epinephrine, the first part of this discussion deals with the various actions of this substance during the development of morphine tolerance. It has been pointed out above that the hyperglycemic response following morphine administration is due to liberation of epinephrine which, in turn, causes glycogenolysis. The hyperglycemic response then may be used as an index of epinephrine released, and indirectly as a measure of epinephrine action on other organs when administration of morphine is repeated. Thus, during development of tolerance to morphine, the hyperglycemic effect of epinephrine continues along with its effects on production of other hormonal secretions.

It is our purpose here to consider those interrelated changes that occur in the body due to continued epinephrine release and subsequent stimulation by it of various end organs such as the liver, the anterior pituitary, and the adrenal cortex. When correlated, these activities, from an insight into their mechanisms, may reveal some of the features of morphine tolerance.

A. General Effects of Epinephrine Release.

Excitation of the sympathetic nervous system by various stress stimuli will alarm and activate many parts of the body. The liberation of epinephrine from the adrenal medulla results in a marked amplification

of the initial nervous impulse through stimulation of adrenergic nerve endings. One might consider that the autonomic nervous system serves as the key to the activation of the organism as a whole in response to acute stresses. The liberation of epinephrine makes an individual or animal more responsive mentally, results in dilation of the pupils for better vision, mobilizes liver glycogen to provide glucose as a source of ready energy for muscle and brain, and stimulates the anterior pituitary to secrete adrenotrophic hormones to build up a stress resistance through increased adrenal cortical activity. Thus, as a result of stress activity, the sympathetic nervous system produces readily available energy at the expense of carbohydrate reserves. Although many of the sympathetic manifestations due to morphine injection are masked by the simultaneous activation of the parasympathetic nervous system, nevertheless the role of the sympathetic nervous system in alarming the body to cope with stressful conditions cannot be minimized.

B. Physiologic Purpose of Epinephrine Release: Stress.

Selye[50] has shown that the release of epinephrine occurs following certain "stress" situations such as shock, cold, hunger, poisoning by toxic substances and tissue damage. Hence the administration of morphine, which provokes epinephrine release, may likewise be looked upon as a "stress" situation. Especially is this true when repeated injections of morphine are given and there is repeated release of epinephrine resulting from this stress. It is the released epinephrine which continually stimulates target organs and tissues.

C. Epinephrine Release and Carbohydrate Metabolism.

During stress, whether due to repeated morphine injections or other conditions, the utilization of the readily available carbohydrate reserves is counterbalanced by the stimulation of the adrenal cortex. For example, Engel[51] states..."Since all types of stress are followed by evidence of increased protein catabolism and activation of the adrenal cortex in normal, but not in adrenalectomized or hypophysectomized animals, whereas stress regularly results in hypoglycemia and death in the latter, it may be that an increased need for carbohydrate is the first effect of stress in general". It has been shown that the initial response to injury is an elevation in blood glucose with smaller increases in lactate and pyruvate, due presumably, to reflex stimulation by epinephrine. However, at a later stage the adrenal cortical hormones are involved in the formation of new carbohydrate through the mobilization and facilitation of protein catabolism. Also, in stress conditions it has been found that the adrenalectomized animal's blood sugar falls progressively, showing "an inadequate ability to call into play those mechanisms by which the normal animal protects itself against carbohydrate deprivation. Carbohydrate utilization appears to be proceeding at a rate which is excessive in terms of availability of carbohydrate from preformed sources and by gluconeogenesis[53]". But, in the absence of stress, Russell[54] showed that normal and adrenalectomized rats required the same rate of glucose infusion to maintain their blood sugar levels after evisceration.

D. Relationship Between Epinephrine Induced Release of Adrenocorticoids and Carbohydrate Metabolism During Morphine Tolerance.

The rate at which the liver releases glucose depends primarily on the level of blood glucose to begin with and is inversely related to it(55). However, the level at which the "glucostat" threshold is set is determined by a balance of certain hormones from the anterior pituitary, the adrenal cortex, and the insulin. The role played by insulin may be demonstrated by the depancreatised animal which fails to deposit glycogen(56) but shows a restoration of this function when exogenous insulin is injected(57). When the anterior pituitary hormone is in excess, the liver threshold for glycogen is maintained at high level; conversely, lack or absence of this hormone reduces this threshold and hypoglycemia results(58). This effect of the anterior pituitary hormone is a direct one and is also brought about by stimulation of the adrenal cortex. Insulin favors the utilization of glucose while, on the other hand, certain hormones from the adrenal cortex and the pituitary facilitate gluconeogenesis from protein, and also inhibit the utilization of glucose(40).

With respect to the part by Thyroid played in blood sugar regulation, its role is characterised by its tendency toward elevations and depression of blood glucose levels, and are manifested in the respective states of hyperthyroidism and hypothyroidism(59). In a depancreatised, hypophysectomised animal, the blood sugar approaches normal(60). The role of epinephrine is an emergency mechanism which prevents sudden changes of the blood sugar.

Increased release of corticosteroids also affects the blood glucose

levels. An explanation of the phenomenon of the progressive decrease in the hyperglycemic response during repeated morphine administration may be the stabilizing effect of corticosteroids on the blood sugar. With the increased blood titer of corticosteroids due to repeated morphine or epinephrine (stress) injections, there would be a tendency to limit or inhibit the usual hyperglycemic response or prevent the hyperglycemia from being as marked as it otherwise would be. Selye[63] has found that cortisone would inhibit, although not completely suppress, epinephrine hyperglycemia when cortisone was administered prior to epinephrine injection. Chiu and Needham[62] have shown that certain adrenal extracts and steroids when added in vitro to liver slices increased the glycogen production and inhibited glycogen breakdown showing that the point of action of the released corticosteroids is on the liver insofar as reduction of hyperglycemia is concerned. Also, desoxycorticosterone[63] has been shown to inhibit hyperglycemia due to epinephrine.

Other evidence may be cited to show that epinephrine, or its release induced by morphine, activates the adrenal cortex. The depletion of adrenal cortex as cortic acid, which results after administration of epinephrine, does not occur in hypophysectomized animals[64]. Furthermore, adrenotrophic hormone, not epinephrine directly, is the stimulating factor acting on the adrenal cortex to cause the elaboration and liberation of its hormones[65]. Thus, in order for epinephrine to influence the adrenal cortex, a preliminary release of adrenotrophic hormone is necessary so that it appears that the integrity of the pituitary-adrenal cortical system is essential.

B. Anterior Pituitary Activity During Repeated Administration of Morphine: Adrenotrophic Activity.

The part played by the anterior pituitary due to epinephrine stimulation and the subsequent variegated effects produced have been investigated. Hume and Wittenstein(66), finding that the destruction of nuclei in the anterior hypothalamus abolishes the ability of epinephrine to cause adrenocortical activity, reported that there must be a humoral agent released from these anterior hypothalamic nuclei which stimulates the anterior pituitary to secrete adrenocorticotrophic hormone. However, adrenocorticotrophic secretion is not prevented after the severance of neural connections between the pituitary and the hypothalamus, nor is the discharge of ACTH prevented from pituitary transplant in the anterior chamber of the eye(67). Therefore, it is evident that neither direct neural nor neuromuscular connections with the hypothalamus are necessary for the release of adrenotrophic hormone. McDermott et al(68) found that a transplanted pituitary will respond to the direct application of epinephrine. On the basis of this evidence, and other work, Long and his associates(69) believe epinephrine to be the normal stimulus for the release of adrenotrophic hormone.

The decrease noted in cholesterol content and ascorbic acid content of the adrenal gland serves as a measurable index of adrenotrophic activity(70). It has been shown also that morphine will cause adrenal ascorbic acid depletion(71) although it does not act directly on the adrenal cortex(72). An index of the secretory activity of the adrenal cortex, itself, is the increase in circulating neutrophiles together with a decrease in the eosinophiles and lymphocytes(73). This also

holds true in morphine addiction(74).

From the evidence presented, it should be clear that morphine stimulates the release of epinephrine which, in turn, acts directly on the anterior pituitary to liberate adrenotrophic hormone causing adrenal ascorbic acid depletion, as well as the other peripheral actions attributed to epinephrine release. The adrenotrophic hormone, through stimulating the adrenal cortex, causes a release of oxycorticosteroids which influence the bone marrow and lymphatic tissues resulting in neutrophile leucocytosis, lymphopenia and eosinopenia.

F. Epinephrine Effects Influencing Analgesia.

The effects of morphine on the autonomic nervous system may exert an influencing factor on the degree of analgesia produced. While the major locus of action of morphine in producing analgesia is by depression of the thalamus centrally, recent studies indicate a modifying effect on analgesia by either acetylcholine or epinephrine, or both. It has been shown by Slaughter(4) that opiate analgesia is potentiated by prostigmine. The mechanism concerned here, according to Cross(6), is possibly cholinesterase inhibition at the splanchnic terminations in the adrenal medulla leading to increased supplies of acetylcholine. Acetylcholine serves as the activator of the adrenal medulla and thus morphine indirectly leads to an outpouring of epinephrine. Cross and his coworkers(6), as well as others such as Ivy and Gootzl(5), and Zauder(75) maintain that epinephrine plays an important role in analgesia and that epinephrine and other vasoconstrictor drugs alone can produce analgesia. Although Cross(6) found that morphine, methadone and meperidine no

longer produced analgesia in adrenalectomized dogs, Harris and Friend[76] showed experimental evidence that the adrenal medullary portion, but not the cortex, is essential in opiate analgesia.

G. Morphine as a "Stress" Stimulus in Addiction.

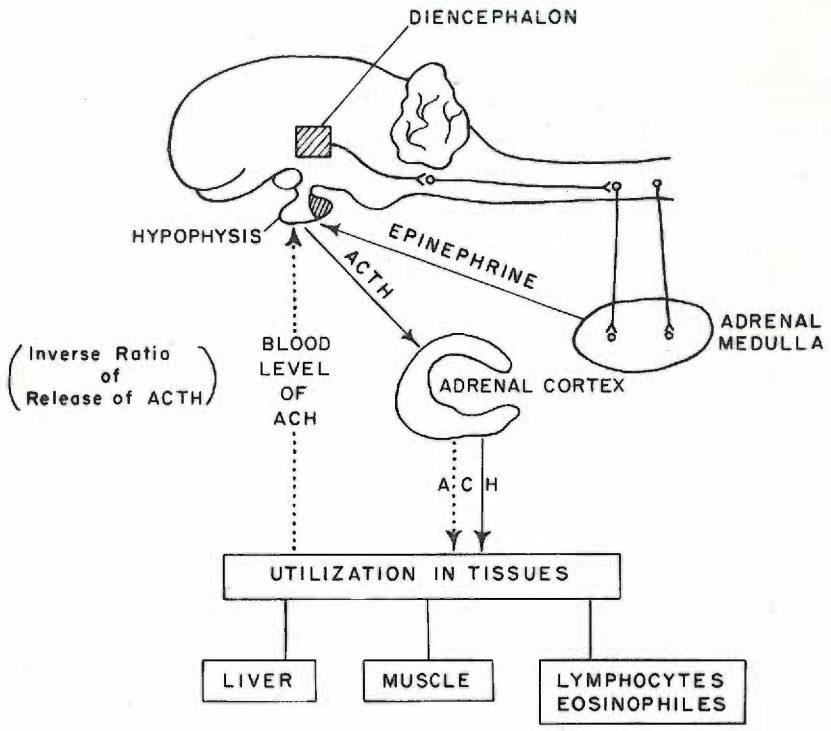
Having presented evidence to show that a close relationship exists between the administration of morphine and epinephrine release, and that many of the pharmacologic effects of morphine are actually due to epinephrine, we may go a step further and consider these effects as a response to stress. Based on Selye's[50] efforts, it is seen that repeated exposure to any stress results in a sequence of events Selye calls the "adaptation syndrome". This is an attempt on the part of the body tissues and structures to compensate or adjust to the stress. The adaptation syndrome may be divided into three distinct stages, as follows: (a) The "alarm reaction" in which resistance to the stress has not, as yet, been acquired or developed. This stage is accompanied by a marked liberation of epinephrine. (b) The "stage of resistance" during which the adaptation is optimal, with the interrelated activities of the anterior pituitary and adrenal cortex responding adequately to maintain adjustment of the body to the stress situation. (c) The "stage of exhaustion" when, through overactivity, the acquired resistance is lost. It should be pointed out that during the stage of resistance, the animal may still respond to new or different stresses.

Since morphine provokes epinephrine release, and the chain of pharmacologic events resulting are in many ways identical with the changes occurring in stress situations, morphine may be considered as a stress

stimulus. Similarly, the repeated administration of morphine may be considered as calling forth the adaptation syndrome in the animal with the three stages elicited depending on how long the stress is continued. As tolerance to a certain dose is acquired, hyperglycemia does not occur. However, if the dose of morphine is increased during the development of tolerance, hyperglycemia reappears as a result of the increased stress. The hypertrophy of the adrenal cortex is but another phase of this stage of resistance due to repeated or continued injections of morphine serving as the stressor. Withdrawal of morphine, likewise, represents a new stress and evokes a hyperglycemia as indicated by Fhatak et al[33][34]. That withdrawal of morphine serves as a new stress is also likely, as seen in morphine addicts who exhibit severe and dramatic withdrawal symptoms, though quite dissimilar from those attributed to morphine during establishment of tolerance and addiction.

From considering Selye's adaptation syndrome, one gains the impression that while activation of the pituitary-adrenocortical system evolves slowly, once this system has been called into play its activities are not easily stopped or interfered with. During the alarm reaction the sympathetic nervous system is excited with liberation of epinephrine which serves to call forth certain protective reactions. For example, epinephrine serves as the "trigger" mechanism to set the pituitary-adrenocortical system into operation. Once this system has been activated, it gains momentum. However, one of the regulatory limiting factors controlling these secretory activities is the ability of the peripheral tissues to utilize released cortical hormones[77]. Sayer's concept of the rate of utilization as a governing factor controlling

pituitary-adrenocortical activity comes from the observation that cortical hormone will prevent the hypertrophy which occurs following prolonged exposure to stress[78]. Thus, administration of exogenous corticosteroids obviates the necessity for the adrenal cortex to hypertrophy to increase its secretion to meet the demands for the hormones induced by stress. On this basis McDermott[79] believes that two factors govern the regulation of the adrenotrophic secretion: (1) epinephrine and (2) the rate of utilization of cortical hormones. A graphic presentation of this concept of McDermott is presented in Figure 3. Two approaches to modification of tolerance seem evident when governing factors are understood: (1) To check the action of epinephrine on the anterior pituitary so that the adrenocortical system is not activated. Possibly this may be done by the use of adrenergic blocking agents which also may prevent hyperglycemia or protect analgesia. This possibility is what we intend to examine in our experiment. (2) To raise the blood titer of corticosteroids exogenously so that a state of functional hypophysectomy is created, thus producing an atrophy of the adrenal cortex[80]. An expression of this state of functional hypophysectomy is shown by hypersensitivity to insulin due to a deficiency of cortisone when overdosage of desoxycorticosterone is given[81]. It has been claimed that desoxycorticosterone will enhance morphine analgesia and that cortisone reduces it[82]. Since it appears that these two corticosteroids are antagonist with respect to morphine analgesia, one might consider the use of desoxycorticosterone along with morphine when it is necessary to use the latter over a period of time. Unfortunately, with the production of a functional hypophysectomy and



PROPOSED MECHANISM OF CONTROL OF THE SECRETION OF ADRENAL CORTEX STEROIDS

(McDermott et al; Yale J. Biol. Med: 23, 1950)

Figure 3. Proposed mechanism of control of the secretion of adrenal cortex steroids.

insufficient secretion of corticoids, the use of desoxycorticosterone continuously may be required since withdrawal of desoxycorticosterone is followed by signs and symptoms of Addison's Disease[83]. This, however, would not be an interdicting factor in the patient dying of malignant disease who, at best, has only a few months to live.

With respect to repeated administration of morphine and adrenal cortex activity, it has been already mentioned that repeated injections of morphine lead to adrenal cortex hypertrophy, as seen at autopsy of tolerant rabbits[33] and that, as found by MacKay and MacKay[49] that adrenal hypertrophy was greater, the greater the tolerance exhibited to the effects of morphine in both man and animals. More recently, Winter and Flataker[82] showed that cortisone and ACTH reduce morphine analgesia and antagonise the narcotic and toxic effects of morphine. Conversely, it has been shown that adrenalectomy renders the animal more susceptible to morphine intoxication[84]. Thus, it appears that the activity of the adrenocorticoids limits the extent of the desirable actions of morphine, namely that of analgesia and narcosis, and, through this action, as a response to stress during the period of increased resistance, is responsible, in part for tolerance. If adrenal cortex hypertrophy could be limited, with limitation of the secretion of the corticoids, an approach might be made to the problem of forestalling morphine tolerance and thus the need for rapidly increasing the dose of morphine to effect satisfactory pain relief in the suffering patient.

H. Reasons For Use of the Morphine Hyperglycemic Response as a Test of Tolerance.

Hyperglycemic responses become less and less at about the time tolerance to analgesia and narcosis is established. The underlying mechanism producing tolerance to hyperglycemia and, also, tolerance to analgesia are either identical or very closely interdependent. As morphine is repeatedly administered the adrenal cortex hypertrophies which, in effect, would raise the blood titre of corticosteroids. The excess corticosteroids then are produced as a normal response to morphine stress and would be expected to nullify both the hyperglycemia and analgesia resulting primarily from the released epinephrine. Cortisone appears to inhibit or retard glycogenolysis (62)(85). It also reduces morphine analgesia (82). Thus an appraisal of the developed tolerance to hyperglycemia may be taken as a measure of compensatory changes occurring in the functional activity of the adrenal cortex in response to repeated stress of morphine administration.

EXPERIMENTAL

The research work reported in this thesis involved three separate experiments. The procedures followed and materials used are described in the discussion of each experiment. Since each of these experiments were carried out on rabbits, and part of the study dealt with the hyperglycemic and other responses, the general method followed and the drugs used may be mentioned first.

Animals. Albino male rabbits, weighing from 2 to 3 kilograms at the start of the experiment, and about four months of age when procured, were used. A few females were inadvertently used although all animals were kept in separate cages during the experiment. Purina chow was fed, with greens given once or twice a week. For obtaining blood samples, the animals were brought from their quarters to the laboratory; otherwise, the animals were kept and given their daily injections in quarters maintained at a constant, even temperature of 24° C. The animals were maintained in good condition throughout the experiments although several of those injected with l-isomethadone showed some irritation at the site of injection. All animals were weighed daily and the analgesic drugs were injected on the basis of mg. per kilogram body weight. Saline and CCK #179 were injected at a constant total dose of 0.5 cc. for the former and 0.1 mg./kg. for the latter. This same dose for CCK #179 was injected half an hour previous to morphine and l-isomethadone in the chronic blockade of hyperglycemia produced by these drugs.

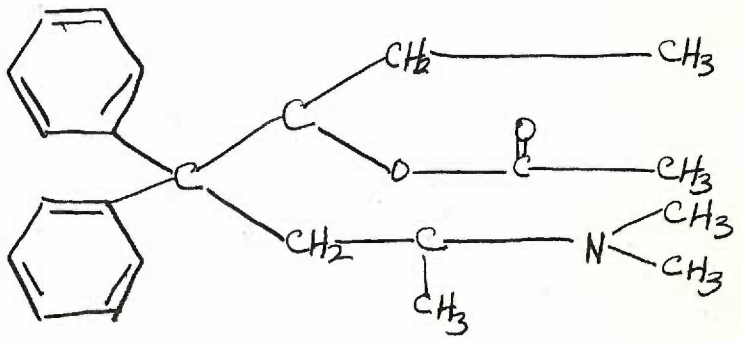
Drugs. Morphine Sulfate served as the control drug in these experiments to establish the base values for making comparisons. Its formula

(Fig. 1) has been shown and its actions have been previously described. Similarly, the formulae (Fig. 2) for methadone and l-isomethadone have been presented. Since the other synthetic analgesic used in this study, alpha acetyl methadol, has not been previously described a brief description of this drug is offered here. The formula is shown in Figure 4. Alpha acetyl Methadol is a new drug recently released for experimental trial. Little published work on this drug has appeared so far. Fraser and Isbell[86] used the three forms of alpha acetyl methadol available, the dextro rotatory (l-antimer), the laevo rotatory (d-antimer) and the racemic dextro-laevo rotatory form, experimentally in human addicts. They found that the dl-form, which we used, induced intense morphine-like euphoria when given in a dose of 30 mg. Euphoria was evident within thirty minutes after the injection and persisted for thirty to forty-eight hours. Cumulative effects were observed when the d-antimer (laevo rotatory form) was given in a dosage of 15 mgs. twice daily for three days. All forms relieved abstinence from morphine and when given orally to addicts that were tolerant to 160 to 400 mgs. of morphine subcutaneously per day, all prevented appearance of abstinence from morphine. In other patients given the drug for two weeks with abrupt removal of the drugs, mild withdrawal symptoms were noted.

In the mimeographed pamphlet[87] describing the preliminary pharmacologic study on alpha acetyl methadol, presumably done by Charles A. Winter at the Merck Institute for Therapeutic Research, it is pointed out that the LD/50 of all three forms for mice by subcutaneous injection is roughly 40 mg./kg., that the levorotatory (d-antimer) form produces delayed toxicity as does the racemic form containing the

levorotatory fraction. Also, the racemic form is more active than methadone with respect to analgesic activity. Winter says..."By comparison, methadone has been calculated by various investigators to have an LD/50 ranging from 20 to 50 mg./kg." Thus alpha acetyl methadol compares favorably in this respect with methadone. Beecher and Keats[88] mention the curious properties described by Fraser and Isbell[86] "who found that the levoisomer (30 mg.) on subcutaneous administration did not produce a morphine-like effect, which they described as 'euphoria', until after some nine hours, whereas after oral administration the chain of morphine-like reactions appeared in 1 or 2 hours". In clinical studies, Beecher and Keats found that subcutaneous doses of 20 mg. given once satisfactorily relieved the pain of 56% of the patients which, as they point out, is well below the effectiveness of 10 mg. of morphine. However, David and Seuler[89] in a study of the use of dl alpha acetyl methadol over a period of time find this compound excellent for the relief of chronic pain at an oral dose of 5 to 10 mg. three or four times daily.

The dihydrogenated ergot alkaloids and ergotamine used for adrenergic blocking action in these experiments may be described very briefly. It has long been known that ergotamine, in small doses, causes arteriolar vasoconstriction and a stimulation of smooth muscle, particularly the uterus. This action appears to be direct on the smooth muscle concerned. When ergotamine is given in several repeated doses, the response to both pressor and inhibitory adrenergic stimulation is blocked (Rothlin[90]), and this effect produced on the blood vessels negating the effects of an injection of epinephrine, is known as adrenalin reversal. The

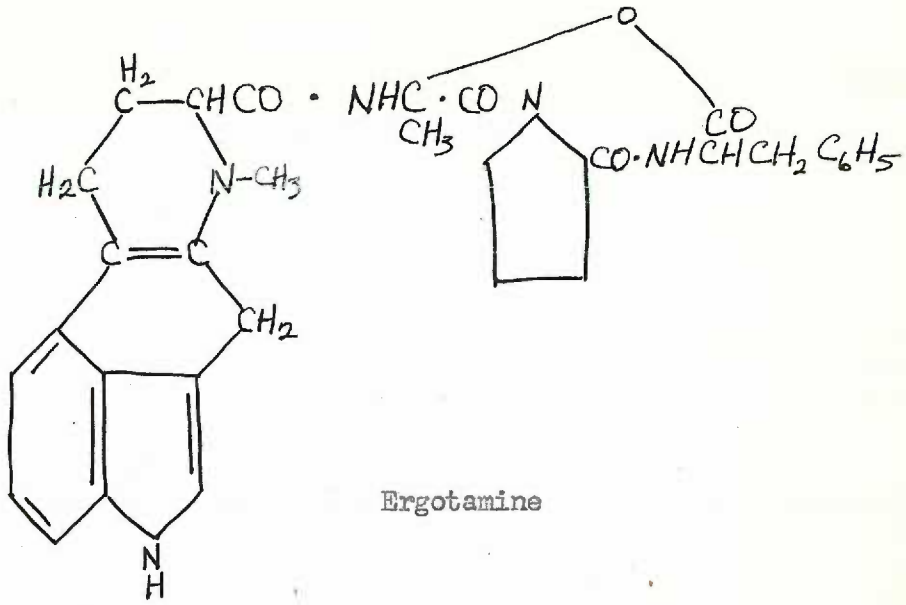


Alpha-acetylmethadol

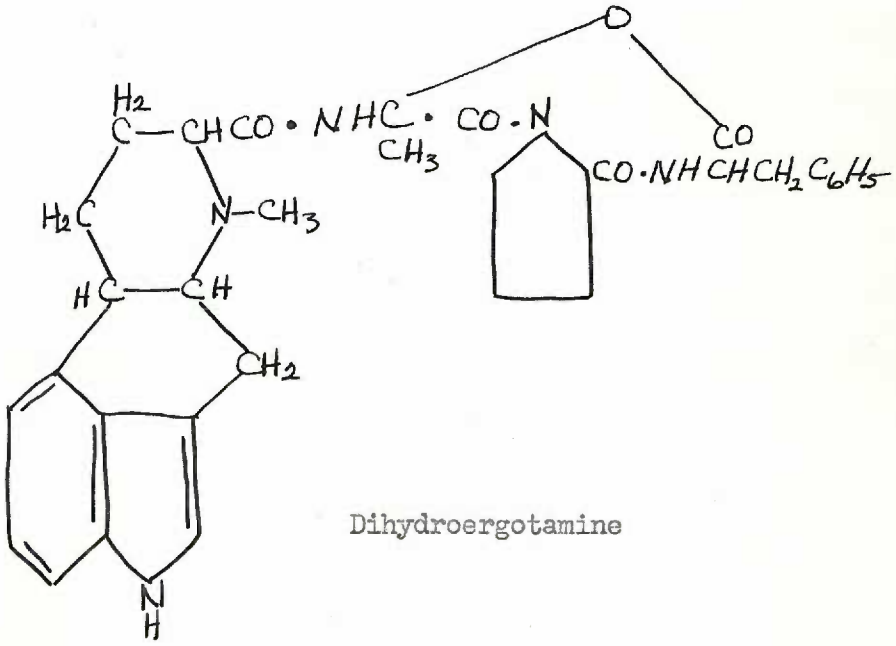
Figure 4. Structural Formula of Alpha-acetylmethadol.

dihydrogenated ergot compounds such as Dihydroergotamine (DHE #45), Dihydroergocornine (DHO #180), and CCK #179 ("Hydergine", an equal mixture of dihydroergocornine, dihydroergocristine and dihydroergokryptine derived from the alkaloids of ergotoxin) lack some of the prominent actions of ergotamine and ergotoxin. For example, in small doses these drugs do not cause smooth muscle stimulation nor vasoconstriction; (91) the uterus is not stimulated; and, their main action is adrenolysis. (92) They are much more potent than ergotamine in causing adrenalin reversal. (93)

Although both DHE #45 and DHO #180 are dihydrogenated compounds and therefore claimed to act mainly as "sympathicolytic" agents, a distinction is to be made pharmacologically between dihydroergotamine and the two newer compounds dihydroergocornine (DHO #180) and its combination called CCK #179 (Hydergine(R)). Dihydroergotamine retains a certain amount of direct vasoconstrictor action in small dose but not to the degree that ergotamine does. Usually, with ordinary therapeutic doses, as in man, the adrenosympathicolytic effects are preponderant although it is difficult to predict exactly in which way the organs innervated by the sympathetic system will respond to a given dose of DHE #45 in the therapeutic, or small dose, range. Dependence on the vasoconstrictor actions of DHE #45 is the basis for the use of small doses of this drug in migraine. On the other hand, dihydroergocornine (DHO #180) and CCK #179 show practically no direct constrictor action on smooth muscle and the effect they generally produce is vasorelaxation in small doses. Speaking of both the "constrictor" type of ergot preparations, ergotamine and dihydroergotamine, and the relaxant type, dihydroergocornine and



Ergotamine



Dihydroergotamine

Figure 5. Structural Formulas for Ergotamine and Dihydroergotamine.

CCK #179. Mickerson[94] says, "Although the ergot alkaloids are true adrenergic blocking agents, they unfortunately also have very potent effects upon the CNS. They all act on the CNS to depress reflexes in concentrations lower than those required to produce true adrenergic blockade". While Mickerson rightfully objects to the use of such terms as adrenolytic, sympatholytic and adrenosympathicolytic, whatever the terms that may be used to designate the mode of action of these drugs, it still holds true that, in small doses, the newer dihydrogenated ergot alkaloids such as dihydroergocornine and CCK #179 are more likely to effect depression of the tone of smooth muscle, particularly arterial. Hence, the value of these newer dihydrogenated ergot compounds in the treatment of peripheral vascular diseases[95] and in hypertension[96].

Determination of Blood Glucose. Animals were divided into groups of four to six. An initial fasting glucose determination following one-half cubic centimeter of physiological saline, given intravenously, was made on each rabbit. Following this, to observe the response of the animal, further determinations were made at one-half hour, one hour and two hours. In the second and third experiment, the blood glucose was determined similarly but at one hour, two hour and four hour periods. Blood specimens were obtained by cutting and bleeding the left marginal ear vein. Determinations were made in duplicate using the Hagedorn-Jensen micro-method[97]. All animals were fasted from twelve to fourteen hours. Drugs to be tested were injected intravenously into the right marginal ear vein or subcutaneously in the back.

Eosinophile Counts. Previous reference[73] has been made to the observation that epinephrine, through its anterior pituitary-adrenocortico

trophic mechanism, causes a depression of the eosinophile count. Morphine has been found to produce this effect also[74]. Accordingly, we undertook to study the eosinophile responses following injections of morphine to rabbits but found that the responses varied erratically and that little consistent effect was noticeable on the 4 hour eosinophile depression with the dose of morphine (15 mg./kg.) we used. Consequently, rats which have shown a reliable response in the assay of ACTH activity were used for this study. Male Sprague-Dawley rats, weighing from 100 to 300 grams were injected subcutaneously with one-half cc. of saline subcutaneously, with [redacted] mg./kg. of morphine sulfate, 0.1 mg./kg. of CCK #179 and a combination of [redacted] mg./kg. of morphine sulfate one-half hour following an injection of 0.1 mg./kg. of CCK #179. Direct counts of blood eosinophiles were made on freshly flowing samples of tail blood previous to injection and four hours after the injections using the method described in Wan's "A Syllabus of Laboratory Examination in Clinical Diagnosis",[98]. Diluting fluid (5 ml. 2% eosin, 5 ml. acetone, 90 ml. of distilled water) was stored in the refrigerator and filtered before using. Using two standard pipettes, a 1:20 dilution of the blood sample was then placed on the shaker and shaken for thirty seconds. Drops from the middle portion of each pipette were placed on each side of 0.1 mm. depth of the Levy special counting chambers. After the count was made, the calculation was done as follows:

$$\frac{\text{Number of eosinophiles} \times 20 \times 10}{\text{Number of one millimeter squares}} = \text{number of eosinophiles/cmm.}$$

Adrenal Cortex Changes. At the conclusion of the chronic experiments the animals were administered intravenous sodium pentobarbital,

infused with formalin fixative and sacrificed. Both adrenal glands were removed in their entirety, weighed, and immediately sectioned for histological examination and placed in 10 per cent formalin fixative. Other tissues such as the liver and kidney were also removed for study of tissue changes. Sections of the liver and kidney were made, but are not reported on in this study since the changes observed were not significant to this study.

Absorbic Acid Content of Adrenal Cortex. An attempt was made to make determinations of the ascorbic acid content of the adrenal cortices obtained from the rabbits at autopsy both by tissue staining and chemical methods, but due to technical difficulties, it was impossible to make such observations in a reliable manner. Such studies have to be made under the rather exacting conditions and careful laboratory facilities which we did not have at the time. Furthermore, the tissues were removed from formalin infused animals.

RESULTS

The results are presented in tabular form for the three experiments performed in this research. The first experiment, called Experiment 1, deals with the study to determine the blocking effects of the ergot alkaloids on morphine hyperglycemia, the second study, Experiment 2, serves as a control study for the subsequent experiment. In experiment 2, the hyperglycemic responses from increased doses of morphine and l-isomethadone given over a period of nine weeks, were studied. The effects of a constant dose of saline given to one group of rabbits and a constant dose of CCK #179 given to another group were also studied as controls. Experiment 3 is the crucial experiment in this study. To two groups of rabbits increments of dosage of morphine and of alpha acetyl methadol were given for a prolonged period of thirteen weeks. The purpose of this was to compare the effects on tolerance to hyperglycemia from alpha acetyl methadol with morphine. The group given increments of dosage of morphine served as a control for comparison with two other groups given combined treatment with CCK #179, administered one-half hour before the analgesic. One of these was given the combination of CCK #179 and morphine and the other CCK #179 and l-isomethadone. In this experiment, we wished to find out whether or not continuous daily use of CCK #179 with its blocking action on hyperglycemia at the minimal effective dose of 0.1 mg./kg. would have any effect in modifying the development of tolerance as measured by variations from normal blood glucose concentration.

The results of these three experiments are best considered by an

analysis of the tables made for each experiment, which follows:

Experiment 1

Evaluation of Results Shown in Tables I, II, III, IV, V and VI.

Tables I to IV show the normal variations found in the blood glucose level of rabbits when saline 0.5 cc. is injected; for morphine sulfate 15 mg./kg.; for DHO #180 0.5 mg./kg. alone, DHO #180 0.6 mg./kg. given one-half hour previous to morphine sulfate 15 mg./kg.; DHE #45 0.6 mg./kg. alone, DHE #45 0.6 mg./kg. administered one-half hour previous to morphine 15 mg./kg.; Ergotamine 0.6 mg./kg. and CCK #179 0.2 mg./kg. or 0.5 mg./kg. given one-half hour previous to morphine sulfate 15 mg./kg. The results are compiled in Table V which shows the group averages for blood glucose after intravenous saline, morphine, DHE #45 and DHO #180. It shows that morphine intravenously in a dose of 15 mg./kg. produces an elevation of blood glucose which persists or steadily rises even two hours after such an injection. Both DHO #180 and DHE #45 produce an upward variation in the blood glucose level from the fasting controls which may or may not be equivalent to "hyperglycemia".

Table VI presents the group averages for blood glucose changes after preliminary injections of Ergotamine, DHE #45, DHO #180 (0.6 mg./kg.) and CCK #179 (0.2 and 0.5 mg./kg.) followed by intravenous morphine sulfate 15 mg./kg. All the ergot compounds, at the specified dose levels, were quite successful in the complete blockade of morphine hyperglycemia. This occurred and is evident in spite of the possible, mild upward variation in the blood glucose level produced by DHE #45 and DHO #180, as seen in Table V. (See also Figure 6)

TABLE I

CHANGES IN THE BLOOD GLUCOSE LEVELS FOLLOWING SALINE AND MORPHINE SULFATE IN THE FASTING RABBIT

DRUGS USED	RABBIT NO.	BLOOD GLUCOSE (mg./%) FASTING	½ hour	1 hour	2 hours	REMARKS
Normal	16	86	80	88	73	Note slight variations in normal values
Control	17	83	90	73	73	
Saline	18	91	77	89	93	
Group	19	90	93	88	89	
	20	99	85	77	86	
Morphine	16	98	132	162	171	Note the considerable increase in glucose level in all rabbits
Sulfate I.V.	17	109	142	153	160	
15 mg./kg.	18	107	162	177	178	
	19	81	128	150	166	
	20	85	137	146	164	

TABLE II

BLOCKADE OF MORPHINE HYPERGLYCEMIA BY DIHYDROMORPHINONE (DHO #130) IN THE RABBIT

DRUGS USED	RABBIT NO.	BLOOD GLUCOSE (mg./100 cc.) FAST-ING	DHO ½ HR.	AFTER MORPHINE ½ hr.	1 hr.	2 hrs.	REMARKS
Normal Control Group	16	86		80	88	73	Note slight variations in normal values
	17	88		90	73	73	
	18	91		77	89	93	
	19	90		93	88	89	
	20	99		88	77	86	
DHO #130 I.V. 0.6 mg./kg.; Morph. Sulf. 15 mg./kg. I.V.	16	94	80	82	80	76	Complete block of hyperglycemic response
	17	105	99	95	95	90	
	18	91	79	82	84	80	
	19	81	75	97	81	79	
	20	93	97	91	91	86	
Normal Control Group	6	85		75	103	89	
	7	93		95	89	89	
	8	98		98	84	93	
	9	89		96	102	105	
	10	96		93	89	98	
				(- after DHO -)			
DHO #130 I.V. ONLY 0.5 mg./kg. (Used old "weak preparation")	6	75		84	90	99	Sl. hyperglycemic effect noted in all animals; considered in range of normal variation.
	7	80		88	99	88	
	8	86		102	113	98	
	9	90		91	108	99	
	10	91		91	94	110	

TABLE XII

BLOCKADE OF MORPHINE HYPERGLYCEMIA BY DIHYDROERGOTAMINE (DHE #45) AND ERGOTAMINE IN THE FASTING RABBIT

DRUGS USED	RAB-BIT NO.	FAST-ING	BLOOD GLUCOSE (mg./100 cc.) TIME AFTER INJECTIONS ½ HOUR	1 HOUR	2 HOURS	REMARKS
Normal	11	98	104	106	79	
Saline	12	115	92	115	102	
Control	13	111	102	106	102	
Group	14	97	102	106	101	
	15	102	95	101	106	
			(DHE #45)	(after morphine)		
DHE #45 I.V.	11	120	122	116	124	117	
0.6 mg./kg.;	12	97	94	91	87	98	Note complete
Morph. Sulf.	13	99	94	88	87	91	blockade of
15 mg./kg.	14	101	94	91	102	131	morphine hyper-
	15	86	96	98	85	102	glycemia.
			(Ergotamine)	(after morphine)		
Ergotamine	11	(died previous to experiment)				
0.6 mg./kg.;	12	78	70	75	70	72	
Morph. Sulf.	13	77	75	(died 10 mins. after)		
15 mg./kg.	14	75	79	84	92	83	Complete block-
	15	68	70	70	77	74	ing noted.
Normal	1	70		77	66	77	
Saline	2	75		92	89	92	
Control	3	77		86	65	-	
Group	4	70		68	81	70	
	5	74		66	72	88	
DHE #45	1	64		78	60	75	No alteration in
ALONE	2	66		75	85	96	blood glucose lev-
0.6 mg./kg.	3	69		60	78	76	els caused by
I.V.	4	60		89	82	85	DHE #45 alone
	5	75		73	77	82	

TABLE IV

BLOCKADE OF MORPHINE HYPERGLYCEMIA BY CCK #179 (Hydergine (R)) IN THE FASTING RABBIT

DRUGS USED.	RAB-BIT NO.	BLOOD GLUCOSE (mg./%) FAST-ING	CCK #179 ½ hr.	AFTER MORPHINE ½ hr.	1 hr.	2 hrs.	REMARKS
Normal Control Saline Group	1	70		77	66	81	
	2	75		92	83	92	
	3	77		86	65	-	
	4	70		68	81	70	
	5	74		66	72	88	
CCK #179 0.5 mg./kg.; Morph. Sulf. 15 mg./kg. I.V.	1	78	74	81	77	74	Morphine hyperglycemia blocked; note recurrence hyperglycemia in Nos. 4 and 5 after 1 hr.
	2	83	67	63	77	71	
	3	78	69	74	84	72	
	4	71	63	67	88	67	
	5	72	72	89	97	76	
CCK #179 0.2 mg./kg.; Morph. Sulf. 15 mg./kg.	6	74	83	65	63	70	Complete blockade with added hypoglycemic effect after M.S. given.
	77	81	79	65	66	68	
	8	83	52	65	65	63	
	9	84	75	81	63	68	
	10	85	74	63	70	65	
Normal Control Saline Group	6	85		75	103	89	
	7	93		95	89	89	
	8	98		98	84	93	
	9	89		96	102	105	
	10	96		93	89	98	

TABLE V

GROUPS AVERAGES FOR BLOOD GLUCOSE DETERMINATIONS AFTER INTRAVENOUS ADMINISTRATION OF SALINE, MORPHINE AND ERGOT DRUGS TO FASTING RABBITS

DRUGS USED	AVERAGE BLOOD GLUCOSE (mg./100cc.) FASTING CONTROL	½ HOUR	1 HOUR	2 HOURS	REMARKS
NO DRUG OR SALINE USED	75	78	75	65	Normal variations noted.
MORPHINE SULFATE 15 mg./kg. I.V.	96	140	160	170	Marked hyperglycemia
DHE #100 0.5 mg./kg. I.V.	84	91	100	97	Slight hyperglycemia?
DHE #45 0.6 mg./kg. I.V.	67	75	76	83	Normal range ?

TABLE VI

GROUP AVERAGES FOR BLOOD GLUCOSE DETERMINATIONS AFTER A PRELIMINARY INJECTION OF AN ERGOT COMPOUND FOLLOWED BY MORPHINE SULF- INTRAVENOUSLY IN THE RABBIT

COMBINATIONS OF DRUGS USED	BLOOD GLUCOSE LEVELS (mg./ 100 cc.) FASTING CONTROL	AFTER ERGOT ½ hr.	AFTER MORPHINE SULFATE ½ hr.	1 hr.	2 hrs.
ERGOTAMINE 0.6 mg./kg.; Morph. Sulf. 15.0 mg./kg.	75	74	76	80	76
CCK #179 0.6 mg./kg.; Morph. Sulf. 15.0 mg./kg.	76	69	76	85	72
CCK #179 0.2 mg./kg.; Morph. Sulf. 15.0 mg./kg.	61	73	68	65	67
MIO #190 0.6 mg./kg.; Morph. Sulf. 15.0 mg./kg.	95	86	89	86	82
DHE #45 0.6 mg./kg.; Morph. Sulf. 15.0 mg./kg.	101	100	97	97	109

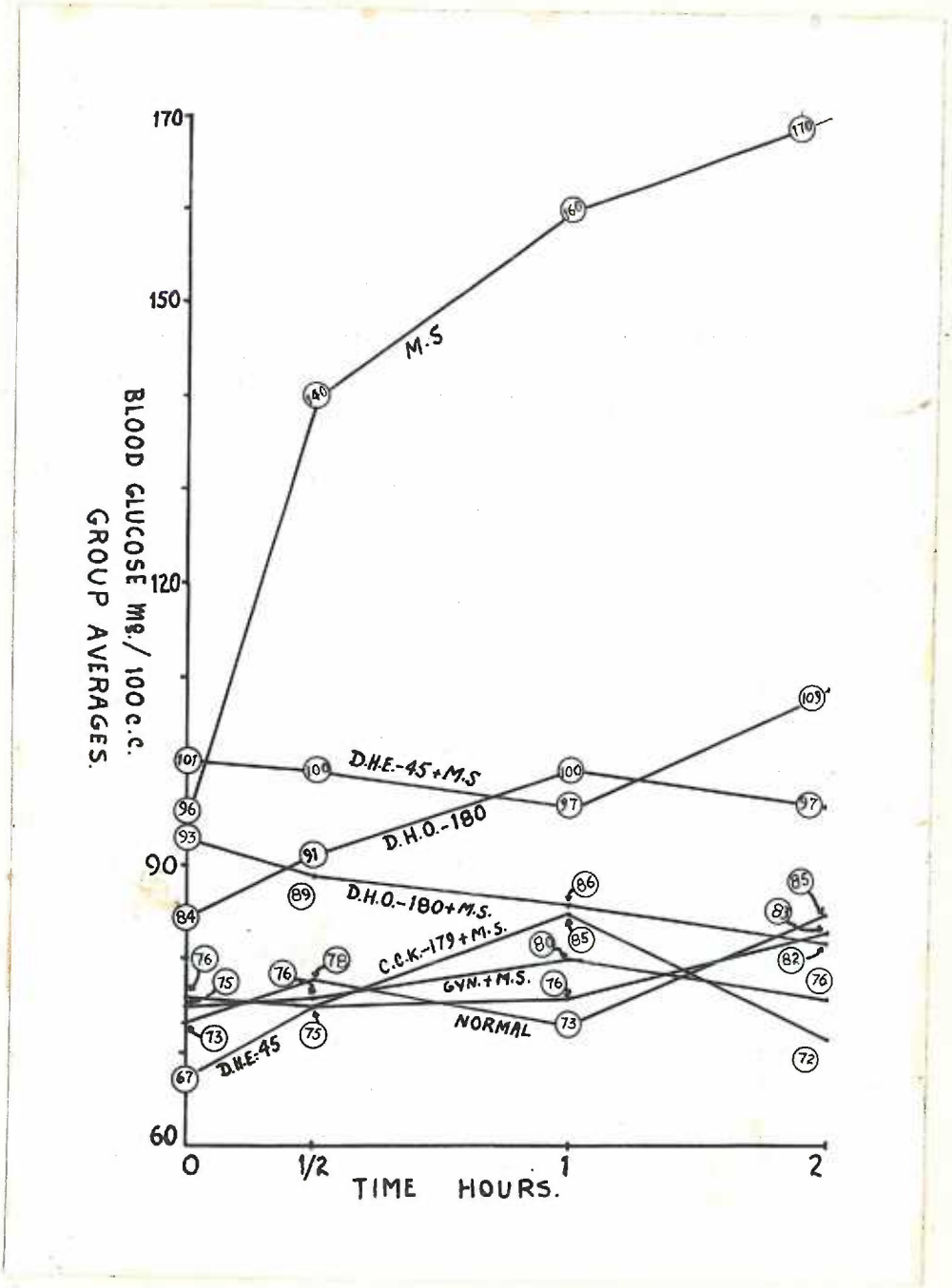


Figure 6. Blockade of morphine hyperglycemia with various ergot alkaloids.

Experiment No. 2

Evaluation of Results Shown in Tables VII, VIII, IX, and X.

Tables VII to X show the results of control studies for Experiment No. 3. Tables VII and VIII show the natural variation in blood glucose response of rabbits to prolonged daily injections of saline and CCK #179 under the experimental conditions of the handling and housing of the animals. Tables IX and X show similar observations with prolonged treatment with morphine and l-isomethadone.

Table IX shows the expected hyperglycemic response to initial stress of injecting 15 mg./kg. morphine sulfate and its modification and disappearance during development of tolerance which follows the continued, prolongation of the same stress by gradually increasing its intensity. When tolerance is acquired to a particular dose, a sufficient increase in the dose still provokes the hyperglycemia as indicated by blood glucose rise at the beginning of the seventh week. Thus, the animals still retain their ability to respond to such an intensified stimulus by the same mechanism of resistance or defense. Instead of the intensification of the specific stimulus, a different one such as withdrawal of the drug can also serve as an adequate stimulus provided its intensity or strength exceeds the threshold for the newly established compensatory mechanisms of altered homeostasis. It is not necessary for the animals to resort to the identical mechanisms of resistance as those utilized during development of tolerance, such as epinephrine-ACTH-adrenal cortex hyperactivity by morphine. They now respond with the stimulated activity of the adrenal cortex to mobilize carbohydrate reserves as suggested by the delayed rise of blood glucose during the withdrawal phase[33].

Epinephrine hyperglycemia is prompt when non-tolerated doses of morphine are injected in animals otherwise tolerant to smaller doses of morphine (e.g., the blood glucose rise shown in Table IX after the seventh week dose).

The withdrawal of saline or CCK #179 in the doses injected (Tables VII and VIII) can act only as a non-specific stress and its effects are easily overcome within the physiological limits of homeostasis. They are not sufficiently noxious to produce any noticeable changes in the metabolic activity of the animals.

TABLE XXI

THE AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY INJECTIONS OF SALINE FOR NINE WEEKS AND DURING WITHDRAWAL

WEEK OF OBSERVATION	TREATMENT AND PROCEDURE	AVERAGE BLOOD SUGAR (mg./%) FAST-ING	HOURS AFTER INJECTION 1 hr.	2 hrs.	4 hrs.	MAXIMUM VARIATION PLUS	MINUS
CONTROL	0.9% SALINE S.Q.	100	95	90	94	-	10
START OF 1st. Week	"	100	91	89	76	-	23
1st. Week	"	103	108	97	95	5	8
2nd. Week	"	(no observations made)					
3rd. Week	"	83	80	81	77	-	6
4th. Week	"	97	91	91	92	-	5
5th. Week	"	105	94	85	79	-	26
6th. Week	"	79	76	78	76	-	3
7th. Week	"	99	93	89	84	-	15
8th. Week	"	92	81	80	84	-	8
9th. Week	Withdrawal	81	76	71	75	-	5
2nd. day	Withdrawal	87					
3rd. day	Withdrawal	77					
4th. day	Withdrawal	68					
5th. day	Withdrawal	89					

TABLE XXXI

THE AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY INJECTIONS OF CCK #179 FOR NINE WEEKS AND DURING WITHDRAWAL

WEEK OF OBSERVATION	TREATMENT AND PROCEDURE	AVERAGE BLOOD SUGAR (mg./%) FAST-ING	HOURS AFTER INJECTION 1 Hr.	2 Hrs.	4 Hrs.	MAXIMUM VARIATION FROM MEANS	
CONTROL	" "	84	77	88	84	4	7
1st. Wk.	CCK #179 0.1 mg./kg	102	96	101	102	-	6
2nd. Wk.	"	90	85	89	83	-	7
3rd. Wk.	"	72	74	71	70	2	2
4th. Wk.	"	90	80	80	80	-	10
5th. Wk.	"	91	84	77	72	-	19
6th. Wk.	"	90	83	87	82	-	8
7th. Wk.	"	92	90	83	83	-	9
8th. Wk.	"	77	77	78	80	3	-
9th. Wk.	Withdrawal	75	72	75	70	-	5
2nd. Day	Withdrawal	78					
3rd. Day	Withdrawal	77					
4th. Day	Withdrawal	77					
5th. Day	Withdrawal	73					

TABLE IX

THE AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY INJECTIONS OF MORPHINE SULFATE WITH WEEKLY INCREASES IN THE DOSE FOR NINE WEEKS AND DURING WITHDRAWAL

WEEK OF OBSERVATION	TREATMENT AND PROCEDURE	AVERAGE BLOOD SUGAR (mg./%) FAST-ING	HOURS AFTER INJECTION 1 hr.	2 hrs.	4 hrs.	MAXIMUM VARIATIONS PLUS	MINUS
CONTROL	- -	93	84	83	85	-	10
1st. Wk.	H_2S_4 15 mg. per kg. S.Q.	120	162	178	148	68	-
2nd. Wk.	15 mg./kg.	90	116	134	106	44	-
3rd. Wk.	25 mg./kg.	83	80	76	77	-	5
4th. Wk.	30 mg./kg.	99	91	96	93	-	8
5th. Wk.	40 mg./kg.	92	89	81	77	-	15
6th. Wk.	50 mg./kg.	89	90	88	85	-	4
7th. Wk.	60 mg./kg.	97	108	117	105	20	-
8th. Wk.	70 mg./kg.	76	86	77	76	10	-
9th. Wk.	WITHDRAWAL	75	75	80	79	5	-
2nd. Day	Withdrawal	80					
3rd. Day	Withdrawal	83					
4th. Day	Withdrawal	81					
5th. Day	Withdrawal	103					

TABLE X.

THE AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY INJECTIONS OF L-DESOMETHADONE WITH WEEKLY INCREMENTS IN THE DOSE FOR NINE WEEKS AND DURING THE WITHDRAWAL.

WEEK OF OBSERVATION	TREATMENT AND PROCEDURE	AVERAGE BLOOD SUGAR (mg./l) FAST-ING	HOURS AFTER INJECTION ½ hr.	1 hrs.	4 hrs.	MAXIMUM VARIATION RISE	FALL
CONTROL	- -	98	92	92	98	-	6
1st. Wk.	5.0 mg./kg. L-Isometh.	92	103	130	113	38	-
2nd. Wk.	5.0 mg./kg.	94	159	122	106	65	-
3rd. Wk.	5.0 mg./kg.	100	115	105	95	15	5
4th. Wk.	5.0 mg./kg.	91	95	91	82	4	9
5th. Wk.	6.0 mg./kg.	87	103	147	93	60	-
6th. Wk.	6.0 mg./kg.	75	98	105	76	30	-
7th. Wk.	6.0 mg./kg.	91	108	99	82	17	9
8th. Wk.	7.0 mg./kg.	78	92	108	-	30	-
9th. Wk.	7.0 mg./kg.	95	90	86	-	-	9
10th. Wk.	Withdrawal	86	80	76	-	-	9
2nd. day	Withdrawal	96					
3rd. day	Withdrawal	86					
4th. day	Withdrawal	93					
5th. day	Withdrawal	77					

Experiment No. 3 - Results

The results of this experiment are shown in Tables XI, XII, XIII and XIV. The average blood sugar levels are shown for the group of rabbits tested for initial control hyperglycemic responses at the beginning of the experiment and for each week that these determinations were made. Tables XI show the results obtained when morphine alone was used and Table XII for the new synthetic analgesic, alpha-acetyl methadol. These two experiments were performed simultaneously in order to provide a morphine control so that, under identical conditions of our experiment, the usual hyperglycemic responses observed with continued administration of morphine could serve as points for comparison for alpha-acetyl methadol. Also, the experiment using morphine alone served as a control experiment, in its entirety, for comparison with the hyperglycemic responses and other effects resulting from the combination of CCK #179 and l-isomethadone (Table XIV) and CCK #179 given prior to morphine over a period of time. A similar experiment for l-isomethadone alone, given over a period of nine weeks discussed previously (Table X) serves, in part, as the control for the experiment studying the combined effects of CCK #179 and l-isomethadone (Table XIV).

A detailed evaluation of the results observed in each experiment, as shown in the respective tables, follows:

Table XI. The initial subcutaneous dose of 15 mg./kg. of morphine sulfate produced a mild rise of the blood sugar level not, however, sufficient to produce glycosuria as, this rise, is still below the kidney threshold for glucose excretion. This dose did not significantly depress respiration. The gradual increments of dosage given each week,

from 15 to 60 mg./kg. showed the following results: (a) By the end of the sixth week, a gradually decreasing blood sugar level, revealed when the blood sugar determinations were made at the beginning of the sixth week and a plus variation of only 3 mg./% was observed. This indicates the establishment of a good tolerance to the hyperglycemic effects of morphine sulfate injections by the absence of any but normal variations in the fasting blood sugar levels during the four hours of the test; (b) The drop in fasting blood sugar level to 67 mg./% at the beginning of the seventh week (after the dose of 60 mg./kg. had been continued during the preceding week) indicates developing tolerance; (c) When next the dose of morphine was raised to 70 mg./kg., a mild hyperglycemic response was noted with the blood sugar level showing a plus variation of 30 mg./%. From the sixth to the thirteenth week with a daily dose of 70 mg./kg., which was not raised until the fourteenth week, the determinations show definite tolerance to the hyperglycemic response since no rise in blood glucose was noted. With the dosage raised to 80 mg./kg., on the fourteenth week, there is still no added hyperglycemic response, the blood glucose levels remaining within the normal range. Either the increment of 10 mg./% in the dose of morphine sulfate was insufficient to provoke added release of epinephrine or, by this time, it can be assumed that the animals were in a state of an anterior pituitary-adrenal cortex homeostasis adjusted at an accelerated stage.

The withdrawal period, started at the end of fourteen weeks treatment with morphine and studied for five days, show no changes in the fasting blood sugar responses which can be considered as other than in the range of normal variations. In fact, the average blood glucose for

five days of the withdrawal period was 83 mg.% which is lower than the initial blood glucose response of ~~80~~ 93 mg.%. This response does not differ from that seen during the five day withdrawal period for the group of rabbits given morphine sulfate over a period of nine weeks (Table IX). The failure of the hyperglycemic response to appear during the withdrawal period might be considered as due to the animals reaching a stage of high resistance after a prolonged treatment with morphine. Sufficient time was allowed for full development of tolerance to hyperglycemia before doses were increased. And prior to withdrawal, the animals were maintained on a well tolerated dose which neither materially handicapped them nor sapped their resistance to the exhaustion stage.

Table XII. The subcutaneous injections of 3 mg./kg. of alpha-acetyl methadol produces a significant rise of the blood sugar level in rabbits when given as the initial dose. When compared to morphine (Table XI) it is seen that tolerance to this dose develops very slowly and gradually as indicated by the slow and gradual subsidence of the hyperglycemic response. Tolerance to 3 mg./kg. was fully evident by the beginning of the seventh week, when the glucose determinations were made and, instead of a plus variation a minus variation of 19 mg.% was observed. The dose of 3 mg./kg. was continued to complete the seventh week of treatment and at the start of the eighth week, as shown in the table, an increment in the dose to 4 mg./kg. was found to produce a mild rise in the blood glucose level. With continuance of the daily dose of 4 mg./kg. and weekly observations of the blood glucose response, it is found that a good hyperglycemic response is noted for the beginning of the ninth week, and then for the subsequent four weeks, up to the start of the

fourteenth week. On withdrawal, there is a continued tolerance shown by the failure of the blood glucose levels to vary more than a few milligrams per cent.

Compared to morphine sulfate administered in the dose range of 15 to 80 mg./kg., alpha acetyl methadol permits only a very restricted limit for raising the dosage for testing the rapid development of tolerance because of its narrow range of permissible dosage. While the initial dose of 3 mg./kg. produced satisfactory hyperglycemic responses and maintained these response for a period of six weeks, an increase to 4 mg./kg. appeared the largest the rabbits could withstand without severe toxic effects. Doses above 4 mg./kg. produce marked muscular convulsions with death due to respiratory paralysis, even when the animal is tolerant to the hyperglycemia of 3 mg./kg.

That the animals do develop tolerance to hyperglycemia produced by alpha acetyl methadol by injections of this drug is evident from the results shown for the dose of 3 mg./kg. given over a period of the first seven weeks and, again, with the increased dose of 4 mg./kg. given from the eighth week to the end of the experiment, a period of six weeks.

Table XXXX. At the beginning of the first week, the initial blood glucose response to 15 mg./kg. of morphine sulfate, without modification by previous injection of CCK #179, indicates established expected hyperglycemia. At the beginning of the second week when the dose is raised to 25 mg./kg., but now preceded by 0.1 mg./kg. of CCK #179 half an hour previously, the hyperglycemic response is blocked whereas in the control unprotected group (compare with Table XX) morphine sulfate at the same dose still produces a hyperglycemia. At the beginning of the sixth week,

where tolerance to 50 mg./kg. of morphine sulfate is evident in the morphine control group (Table XX), a similar lack of hyperglycemia is observed here even though the animals were not protected by the blocking effects of CCK #179 on the day of the test. The same is true at the eighth week and the twelfth week.

The use of CCK #179 previous to morphine injection in tested animals does modify the initial hyperglycemia or subsequent hyperglycemia due to non-tolerant doses. However, once tolerance to the hyperglycemia for a particular dose is established, withholding of CCK #179 injections previous to the same test doses of morphine sulfate fails to show a rise of blood sugar. The challenging dose increment was not sufficient to test the efficacy of CCK #179 in protecting hyperglycemia in our experiments.

Table XIV. From this table it is seen that weekly increments in dosage were not allowed due to the fact that the dose of 5 mg./kg. seemed high and would occasionally cause toxic reactions, such as convulsions, in the animals. When an attempt was made to increase the daily dose to 6 mg./kg. one animal of this group died soon after the injection in convulsions and respiratory failure. Consequently, the dose for the group was lowered to 5 mg./kg. after a week's cautious trial of the 6 mg./kg. dose. At the beginning of the fifth week, the four remaining animals were able to withstand a daily dose of 6 mg./kg. and, at the beginning of the eighth week the dose was raised to 7 mg./kg. Again, this higher dose was not well tolerated so that at the beginning of the eleventh week, the dose was reduced to 6 mg./kg. and continued at this level until the conclusion of the experiment.

Since we were interested in studying the effects of maintaining a continuous blockade of epinephrine hyperglycemia resulting from injections of l-isomethadone over a period of time, it was necessary to assay the initial hyperglycemic response to the use of l-isomethadone. A dose of 5 mg./kg. of l-isomethadone injected at the beginning of the first week's test, without the previous administration of CCK #179, indicates a substantial blood sugar raising effect. Thereafter, CCK #179 was always injected daily one-half hour prior to the dose of l-isomethadone, except on test days, the beginning of the sixth week, the eleventh week and the thirteenth week. The results of the sixth week show that when tolerance to 6 mg./kg. of l-isomethadone was still undeveloped, blood sugar level still rises, unaided by the blocking action of CCK #179.

There was no significant upward change in the fasting blood sugar values during development of tolerance or during the withdrawal period, from the control one at the beginning of the experiment.

The range of doses used to develop tolerance to l-isomethadone is very much limited, from 5 to 7 mg./kg., owing to the toxic convulsive effects of the drug to which tolerance does not appear to develop even with or without CCK #179 intervention.

TABLE XI

THE AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY SUBCUTANEOUS INJECTIONS OF MORPHINE SULFATE WITH WEEKLY INCREMENTS IN DOSAGE FOR FIFTEEN WEEKS AND DURING WITHDRAWAL

Week of Observation	Dose mg/kg	Average Blood Glucose (mg./%) FAST-ING	Hours After Injection 1 hr.	2 hrs.	4 hrs.	Maximum Variation Plus	Minus
Control.	0	103	109	82	99	6	21
1st.	15	97	128	101	97	31	—
2nd.	25	76	103	113	88	36	—
3rd.	30	89	102	102	93	13	—
4th.	40	94	94	92	90	—	4
5th.	50	82	83	84	76	2	6
6th.	60	91	94	89	86	3	5
7th.	70	67	97	84	69	30	—
8th.	70	85	83	85	77	—	8
9th.	70	79	81	82	73	3	6
10th.	70	—	—	—	—	—	—
11th.	70	92	88	84	78	—	14
12th.	70	91	91	86	—	—	5
13th.	70	97	93	95	—	—	4
14th.	80	105	103	104	—	—	1
15th.	With drawal.	90	88	91	—	1	2
2nd. Day.	With drawal.	88					
3rd. Day.	With drawal.	89					
4th. Day.	With drawal.	86					
5th. Day.	With drawal.	91					

TABLE XII

THE AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY SUBCUTANEOUS INJECTIONS OF ALPHA-ACETYL METHADOL FOR FOURTEEN WEEKS AND DURING WITHDRAWAL

WEEK OF OBSERVATION	DOSE mg/kg.	AVERAGE BLOOD GLUCOSE(mg./%) FASTING	HOURS AFTER INJECTION 1 hr.	2 hrs.	4 hrs.	MAXIMUM VARIATION PLUS	MINUS
Control	0	95	87	88	88	—	0
1st.	3	88	135	133	—	47	—
2nd.	3	90	136	128	93	46	—
3rd.	3	89	112	125	81	36	—
4th.	3	88	100	88	76	12	12
5th.	3	92	105	95	88	13	4
6th.	3	80	81	97	82	17	—
7th.	3	93	91	74	—	—	19
8th.	4	111	127	128	—	17	—
9th.	4	98	109	116	98	18	—
10th.	4	88	96	89	—	8	—
11th.	4	97	100	95	—	3	2
12th.	4	97	102	100	—	5	—
13th.	4	98	103	96	—	5	2
14th.	with drawal.	86	88	90	—	4	—
2nd. Day.	with drawal.	77					
3rd. Day.	with drawal.	84					
4th. Day.	with drawal.	85					
5th. Day.	with drawal.	85					

TABLE XIII

THE AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY SUBCUTANEOUS INJECTIONS OF 0.1 MILLIGRAM PER KILOGRAM OF CCK #179 PRIOR TO WEEKLY INCREMENTS IN DOSAGE OF MORPHINE AND DURING WITHDRAWAL

WEEK OF OBSERVATION	MORPHINE DOSE mg/kg.	BLOOD GLUCOSE (mg./%) FAST- ING	AFTER INJECTION ½ hr.	2 hrs.	4 hrs.	MAXIMUM VARIATION PLUS MINUS		REMARKS
CONTROL	0	95	87	73	91	-	22	
1st. Wk.	10	91	138	151	99	60	-	CCK not given when tested for hyperglye.
2nd. Wk.	25	77	86	87	77	10	-	
3rd. Wk.	30	93	84	89	78	-	15	
4th. Wk.	40	87	97	96	88	10	-	
5th. Wk.	50	82	82	87	78	5	-	
6th. Wk.	60	98	95	90	86	-	12	CCK not given prior to test Only 50 mg/kg M.S. given
7th. Wk.	70	76	82	78	68	6	8	
8th. Wk.	70	84	81	82	77	-	7	CCK not given prior to test
9th. Wk.	70	82	81	88	80	6	-	
10th. Wk.	70	-	-	-	-	-	-	Not tested
11th. Wk.	70	93	85	75	69	-	27	
12th. Wk.	70	92	94	85	-	2	7	CCK not given prior to test
13th. Wk.	70	94	95	91	-	1	3	
14th. Wk.	60	96	94	89	-	-	7	
15th. Wk.	Withdrawal	90	91	94	-	4	-	
2nd. Dy.	"	94						
3rd. Dy.	"	91						
4th. Dy.	"	84						
5th. Dy.		95						

TABLE XIV

AVERAGE BLOOD SUGAR VALUES FOR A GROUP OF RABBITS GIVEN DAILY SUBCUTANEOUS INJECTIONS OF 0.1 MILLIGRAM PER KILOGRAM OF CCK #179 PRIOR TO INCREMENTS IN DOSAGE OF L-ISOMETHADONE AND DURING PERIOD OF WITHDRAWAL.

OBSERVATION	DOSE mg/kg.	BLOOD GLUCOSE (mg./%) FASTING	AFTER INJECTION 1 hr.	2 hrs.	4 hrs.	MAXIMUM VARIATION PLUS	MINUS	REMARKS
CONTROL	0	95	86	83	84	-	12	
1st. Wk.	5	78	132	140	-	62	-	CCK not given dy. of test.
2nd. Wk.	5	94	99	86	73	5	21	
3rd. Wk.	6	96	105	98	79	9	17	One animal died in convulsions
4th. Wk.	5	87	89	84	72	2	15	
5th. Wk.	6	94	100	89	88	6	6	
6th. Wk.	6	71	83	104	79	33	-	CCK not given
7th. Wk.	6	92	92	74	-	-	18	
8th. Wk.	7	103	101	92	-	-	11	
9th. Wk.	7	108	108	111	103	3	5	
10th.Wk.	7	78	84	83	-	6	-	
11th.Wk.	6	92	91	90	-	-	2	CCK not given
12th.Wk.	6	90	89	91	-	1	1	CCK not given
13th.Wk.	6	94	94	92	-	-	2	No CCK given
14th.Wk.	Withdrawal Period	94	92	92	-	-	2	
2nd. Dy.	"	86						
3rd. Dy.	"	96						
4th. Dy.	"	96						
5th. Dy.	"	73						

INFLUENCE OF SINGLE INJECTIONS OF MORPHINE SULFATE ON THE EOSINOPHIL COUNT

The results obtained from eosinophil counts made on rat's blood before and four hours after injections of saline, CCK #179, Morphine Sulfate and a combination of CCK #179 and morphine are shown in Table XV. The following conclusions may be made:

1. Morphine sulfate, in a single dose of 8 mg./kg. caused a significant reduction of eosinophils at the four hour period. The average percent reduction for the nine rats studied was 49.2 per cent.

2. CCK #179, in a single dose of 0.1 mg./kg. given to six rats caused a significant reduction of the eosinophils of 42.0 per cent.

3. When CCK #179 was administered one-half hour before morphine, in these same dosages, there was a reduction of 53.3 per cent.

4. Under the conditions in which this study was performed, and with the number of animals used, no statement can be made whether or not previous administration of CCK #179 before morphine had any effect in blocking the action of epinephrine on the anterior pituitary. Both CCK #179 and morphine, in the doses used, caused approximately the same reduction in the number of eosinophils as did the combination of CCK #179 and morphine.

TABLE XV

Eosinophil Counts in Rats Following Administration of Saline, CCK #179, Morphine Sulfate and A Combination of CCK #179 and Morphine

Drug Used	Dose Used	Eosinophil Counts: Normal Control	Eosinophil Counts: After Injection 4 hours		Percentage Reduction	"p" Significance
	mg/kg				%	
Saline	½ cc. total	218	185		15.1	
		194	152		21.5	
		200	164		18.0	
		169	130		23.0	
		181	159		12.1	
		184	144		21.6	
				Average	18.5	< 0.05
CCK #179	0.1 mg/kg	188	136		27.7	
		200	113		43.5	
		224	74		66.9	
		120	67		44.1	
		231	162		29.8	
		220	132		40.0	
				Average	42.0	< 0.05
Morphine Sulfate	8 mg/kg	255	117		54.1	
		200	55		72.5	
		166	115		30.6	
		266	100		62.4	
		228	100		56.1	
		266	222		16.5	
		212	71		66.4	
		180	50		72.2	
		156	137		12.1	
				Average	49.2	< 0.01
Morphine Sulfate; CCK #179	8 mg/kg 0.1 mg/kg	248	112		54.8	
		169	88		47.8	
		228	100		56.1	
		159	82		48.4	
		180	88		51.1	
		194	74		61.8	
				Average	53.3	< 0.05

HISTOLOGICAL EXAMINATION OF ADRENAL CORTICES

The adrenal glands obtained from the rabbits used in Experiment No. 2 and in Experiment No. 3 were sectioned and the adrenal cortices examined by a pathologist. Comparisons were made by examining sections of the adrenal gland obtained from normal rabbits. The degree of cellular changes observed in the treated animals was graded on the basis of the hydropic changes noted with definite cellular atrophy designated as 5 plus, marked hydropic degeneration as 4 plus, definite hydropic degeneration as 3 plus, moderate hydropic degeneration as 2 plus and no apparent hydropic change as 0. These changes, as graded, are shown in Table XVI. The results shown in Table XVI reveal that morphine and alpha acetyl methadol, when given over a prolonged period to rabbits, definitely show more cellular changes than hydergine (CCK #179) or saline controls. The combination of CCK #179 with either morphine or l-isomethadone did not reduce the degree of the cellular changes noted in the adrenal cortices, compared to morphine or l-isomethadone when administered alone. The cellular changes of l-isomethadone were variable.

TABLE XVI

REPORT ON HISTOLOGICAL EXAMINATION OF ADRENAL CORTICES OF RABBITS GIVEN PROLONGED TREATMENT WITH SALINE, OCK #179, MORPHINE SULFATE, L-ISOMETHADONE, MORPHINE SULFATE AND OCK #179, AND L-ISOMETHADONE AND OCK #179

I. Designations Used To Grade Cellular Changes According To The Degree of Hydropic Changes Noted In Adrenal Cortex:

Atrophy	5 plus
Marked hydropic degeneration	4 plus
Hydropic degeneration	3 plus
Moderate hydropic degeneration	2 plus
No or minimal change	0
Mild hydropic degeneration	1 plus

II. Degree of Cellular Changes Noted in Cortices of Rabbits Given Various Analgesics and OCK #179 With Control Drugs Over A Period of Time.

MEDINOXINE (OCK #179) 0.1 mg/kg 8 weeks	SALINE (0.5 cc.) 8 weeks	MORPHINE SULFATE 15 mg/kg* 8 weeks	MORPHINE SULFATE 15 mg/kg* 13 weeks
2 plus	1 plus	2 plus	3 plus
1 plus	0	3 plus	4 plus
1 plus	1 plus	3 plus	3 plus
0	1 plus	3 plus	4 plus
2 plus	1 plus	4 plus	
0	1 plus		
Aver. 1 plus	1 plus	3 plus	3.5 plus

L-ISOMETHADONE* PLUS OCK #179 0.1 mg/kg 13 weeks	L-ISOMETHADONE* 3 mg/kg* 8 weeks	MORPHINE* PLUS OCK #179 0.1 13 weeks	ALPHA ACETYL METHADOL* 3 mg/kg 13 weeks
3 plus	1 plus	4 plus	4 plus
2 plus	2 plus	4 plus	4 plus
4 plus	5 plus	3 plus	4 plus
4 plus	3 plus	2 plus	2 plus
	4 plus	4 plus	
Aver. 3.2 plus	3 plus	3.4 plus	3.5 plus

SUMMARY OF RESULTS

The following summary is offered as to the conclusions to be drawn from the results of this research:

1. Ergot alkaloids, such as ergotamine, dihydroergotamine (DHE #45), dihydroergocornine (DHO #180) and Hydergine(R) (CCK #179) which is a mixture of dihydroergocornine, dihydroergocristine and dihydroergokryptine, are found effective in blocking the adrenergic hyperglycemia produced by morphine in rabbits.

2. Morphine sulfate 15 mg./kg., l-isomethadone 5 mg./kg., and alpha acetyl methadol 3 mg./kg. produce a measurable hyperglycemia in rabbits on initial injection of these analgesics.

3. When these analgesics are administered daily to rabbits in increasing doses or at the same dose level for successive weeks, the initial hyperglycemia to injections of these drugs diminishes or disappears, indicating developing or established tolerance.

4. If such drugs are given in doses higher than those to which tolerance is established, they again produce hyperglycemia, indicating that the mechanism producing adrenergic hyperglycemia is still capable of responding to the increased stress.

5. Saline 0.5 cc., or CCK #179 0.1 mg./kg. administered daily to rabbits shows no significant variations in the blood glucose levels to such a non-specific stress under the conditions of handling or housing, during the acute or chronic experimental procedures described.

6. When tolerance to hyperglycemia by the analgesics studied is allowed to develop by utilizing slow increases in their dosage or using

the same dose over a few weeks before the next increase, tolerance develops slowly. This is noted by observing the variations in the fasting blood glucose values from week to week or during withdrawal which are minimal. On the other hand, as noted by Emerson and Pfeiffer[33] in previous work, when rapid build up of tolerance is attempted by rapid increments of the dosage, greater variations between the initial fasting blood levels and those from week to week or during withdrawal are seen. The results of our studies utilizing slow increases in dosages indicate that the interrelated endocrine mechanisms involved in morphine hyperglycemia are capable of shifting to maintain higher levels of glucostatic equilibrium.

7. Daily injections of CCK #179 (adrenergic blocking agent) previous to administration of morphine sulfate and l-isomethadone over a period of 12 weeks blocks the rise of blood glucose resulting from such injections.

8. Since tolerant doses of the analgesic drugs are not hyperglycemic, the blocking action of CCK #179 on their hyperglycemic effects could not be tested in rabbits receiving both CCK #179 and the analgesic drugs by withholding CCK #179 on the days the blood glucose determinations were made. Our results fail to reveal whether CCK #179 did or did not modify development of tolerance.

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