A PHARMACOLOGICAL STUDY OF THE SITE AND MECHANISM OF
ACTION OF N-ALLYLNORMORPHINE

DISSERTATION

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

By

FRED THEODORE GALYSH, B.S., M.S.

****

The Ohio State University
1955

Approved by

[Signature]
Adviser
Department of Pharmacy
ACKNOWLEDGMENTS

The author wishes to make grateful acknowledgement to Dr. John W. Nelson, Professor, College of Pharmacy, for his constant and never-failing advice, help and encouragement which have been invaluable in carrying out this investigation. He also wishes to acknowledge a special debt of gratitude to Dr. Arthur Tye, Associate Professor, College of Pharmacy, for his advice and interest which have been both encouraging and helpful, and to Dr. Bernard V. Christensen, Retired Dean of the College of Pharmacy for his many expressions of kindness and aid. To the remainder of the faculty of the College of Pharmacy, I wish to express a humble thanks for their advice and guidance. Last but by no means least, I wish to acknowledge the unrewarding aid of my wife, Jean, whose patience has known no bounds.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION AND HISTORICAL REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>Early History of N-Allylnormorphine</td>
<td>5</td>
</tr>
<tr>
<td>Review of Recent Literature</td>
<td>8</td>
</tr>
<tr>
<td>Established Clinical Uses of Nalorphine</td>
<td>21</td>
</tr>
<tr>
<td>Objectives of this Investigation</td>
<td>26</td>
</tr>
<tr>
<td>LOCALIZATION OF THE SITE OF ACTION OF DRUGS</td>
<td>28</td>
</tr>
<tr>
<td>MECHANISM OF ACTION OF DRUGS</td>
<td>34</td>
</tr>
<tr>
<td>NEURAL AND CHEMICAL CONTROL OF RESPIRATION</td>
<td>37</td>
</tr>
<tr>
<td>The Respiratory Centers</td>
<td>37</td>
</tr>
<tr>
<td>Rhythmicity of the Respiratory Centers</td>
<td>39</td>
</tr>
<tr>
<td>Types of Normal Breathing</td>
<td>43</td>
</tr>
<tr>
<td>Chemical Control of Respiration</td>
<td>44</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>52</td>
</tr>
<tr>
<td>Part I</td>
<td>52</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>54</td>
</tr>
<tr>
<td>Experimental Design and Procedure</td>
<td>54</td>
</tr>
<tr>
<td>Results</td>
<td>56</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>58</td>
</tr>
<tr>
<td>Experimental Design and Procedure</td>
<td>58</td>
</tr>
<tr>
<td>Results</td>
<td>59</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>61</td>
</tr>
<tr>
<td>Experimental Design and Procedure</td>
<td>61</td>
</tr>
<tr>
<td>Results</td>
<td>62</td>
</tr>
</tbody>
</table>
LIST OF TABLES

TABLE I.--Effect of Nalorphine on Respiration in Dogs Anesthetized with Pentobarbital sodium .................. 57

TABLE II.--Respiratory Minute Volume and Rate in a Dog Anesthetized with Pentobarbital Sodium. 58

TABLE III.--Effect of Nalorphine on Respiration in Dogs Anesthetized with Amobarbital Sodium .... 60

TABLE IV.--Respiratory Minute Volume and Rate in Dogs Anesthetized with Amobarbital Sodium. 60

TABLE V.--Effect of Nalorphine on Respiration in Dogs Anesthetized with Urethane. 63

TABLE VI.--Effect of Nalorphine on Respiration in Dogs Anesthetized with Secobarbital Sodium. 65

TABLE VII.--Respiratory Minute Volume and Rate in a Dog Anesthetized with Secobarbital Sodium. 65

TABLE VIII.--Effect of Morphine on the Respiration of a Dog Anesthetized with Secobarbital Sodium. 68

TABLE IX.--Effect of Nalorphine on Respiration Depressed by Morphine in Dogs Anesthetized with Secobarbital Sodium. 70

TABLE X.--Graded Dose-response Data Relative to Nalorphine Antagonism of Morphine-induced Respiratory Depression. 72

TABLE XI.--Effect of Nalorphine on Respiration Depressed by Morphine in a Dog with Denervated Chemoreceptors (Dog No. 15). 75

TABLE XIII.--Adrenal Ascorbic Acid of Rats as Affected by Various Drugs. 96

TABLE XIV.--Adrenal Cholesterol of Rats as Affected by Various Drugs. 106

TABLE XV.--LD50 and LD99 of Morphine Sulfate and PD50 of Nalorphine Hydrochloride in Normal and Adrenalectomized Rats (in mg./Kg.). 109
# LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE 1-A</td>
<td>46</td>
</tr>
<tr>
<td>FIGURE 1-B</td>
<td>47</td>
</tr>
<tr>
<td>FIGURE 2</td>
<td>76</td>
</tr>
<tr>
<td>FIGURE 3</td>
<td>82</td>
</tr>
<tr>
<td>FIGURE 4</td>
<td>83</td>
</tr>
<tr>
<td>FIGURE 5</td>
<td>85</td>
</tr>
<tr>
<td>FIGURE 6</td>
<td>110</td>
</tr>
<tr>
<td>FIGURE 7</td>
<td>112</td>
</tr>
<tr>
<td>FIGURE 8</td>
<td>114</td>
</tr>
<tr>
<td>FIGURE 9</td>
<td>126</td>
</tr>
</tbody>
</table>
INTRODUCTION AND HISTORICAL REVIEW

Although morphine has been one of the most important drugs in the physician's armamentarium for many years, its serious disadvantages have always been recognized. Along with the dangers inherent in overdosage of the drug, serious side effects have limited its usefulness. Among these side effects may be listed depression of respiration, slowing and weakness of the pulse, constipation, nausea and vomiting, excitation in some individuals and the ever present danger of addiction.

Because of these disadvantages and the virtual indispensability of morphine for the relief of severe pain, many attempts have been made to find substitutes possessing the analgesic properties of morphine but devoid of its serious side effects. The complexity of the morphine molecule has given chemists an opportunity to prepare many synthetic derivatives, and chemical alteration of the natural alkaloidal base has resulted in compounds having increased analgesic activity (heroin or diacetylmorphine and Dilaudid or dihydromorphinone), decreased analgesic activity (codeine or methylmorphine and Dionin or ethylmorphine), or entirely different properties (apomorphine). Other useful drugs (methadone,
meperidine, 3-hydroxy-N-methylmorphinan or Dromoran, etc.), while not morphine derivatives, bear some chemical relationship to morphine and exhibit some of its therapeutic as well as toxic and addictive properties.

Respiratory depression following the use of narcotics is commonly observed and is perhaps the most dangerous symptom of opiate intoxication. Opiate poisoning may occur as a result of overdosage, due either to untoward reactions to average doses of opiate or to errors in calculation with the subsequent administration of overdoses of the drugs. Outside the hospital, acute poisoning with potent analgesics, either intentional or accidental, is rare because of the lack of availability of these agents. Among addicts, however, respiratory depression which requires medical treatment is a fairly common occurrence. This is especially true in those individuals addicted to the potent synthetic analgesics which, in general, have a lower margin of safety than do the naturally occurring alkaloids. Most of the synthetics are not excreted by the kidney, as is morphine, but depend on normal active liver function for their detoxication; therefore, individuals with liver disease require smaller doses since normal average doses may result in poisoning (1).

Treatment of such opiate depression in the past has been largely symptomatic and supportive, and in many
instances has been unsuccessful. The older literature contains the antidotal suggestions to "walk the patient" or "keep the patient awake at all costs". These suggestions are almost entirely useless in mild poisoning and totally impossible in overwhelming intoxication. The treatment of acute morphine or opiate poisoning has consisted of gastric lavage to remove any remnants of the drug if it has been taken orally. Emetics are of no value since the vomiting center is depressed. Various analeptic agents and respiratory stimulants, such as caffeine, metrazol, nikethamide and atropine, have been used to combat failure of the respiratory center. If the respiration failed in spite of these measures, artificial respiration was employed and continued as long as there was the least trace of cardiac activity.

When the above-mentioned analeptics are resorted to in the treatment of narcotic intoxication, their use must be attended with extreme caution since even seemingly slight overdosage of these agents may induce convulsions and death from respiratory failure. The efficacy of these drugs in treating opiate narcosis has been variously described (2, 3). Picrotoxin is said to be contraindicated (4). Consequently, the introduction of the narcotic antagonist, N-allylnormorphine, into the therapy of opiate intoxication was of great significance. This substance is now listed in New and Nonofficial Remedies and bears
the generic name Nalorphine.

Chemically, N-allylnormorphine is similar to morphine, from which it is also derived, the only difference being that an allyl group replaces the methyl group on the nitrogen atom. The structures of these two compounds, as well as that of normorphine are illustrated below.

![Morphine](image1)

![Normorphine](image2)
The hydrochloride salt of \( N \)-allylnormorphine, \( \text{C}_{19}\text{H}_{21}\text{NO}_3 \cdot \text{HCl} \), is a white, odorless powder with a fairly high water solubility. When dissolved in water, it forms a clear, colorless solution which turns yellow on standing. The pH of a 1 per cent aqueous solution is 4.5 to 5.5.

**Early history of \( N \)-allylnormorphine.**—Heimann (5) in 1915 reported that simple demethylation of morphine, codeine and other related alkaloids does not change qualitatively the action of these alkaloids, although their analgesic effects are considerably diminished. The substitution of the imino (=NH) group in norcodeine by an \( N \)-allyl group, however, was shown by von Braun and his coworkers to produce a remarkable alteration in the pharmacologic action of this substance (6).
It remained for Pohl (7) to find that N-allylnorcodeine stimulated the respiration and antagonized the depressing action of morphine upon the respiration. He described an experiment in which sleep had been induced by morphine in a dog. Upon administration of N-allylnorcodeine, a temporary arousal from sleep resulted.

It was not until 1941 that McCawley, Hart, and Marsh (8) reported the synthesis of a compound differing from morphine only by the substitution of an allyl for a methyl group on the nitrogen atom of the heterocyclic ring. They acetylated morphine with acetic anhydride to protect the hydroxyl groups. The N-methyl group was removed by the action of cyanogen bromide with decomposition to normorphine which base reacted with allyl bromide to form N-allylnormorphine. In the same publication, these authors pointed out that N-allylnormorphine appeared to have a stronger antagonistic action toward depression of respiration evoked by morphine than did N-allylnorcodeine. Hart (9) made some further observations upon the antagonistic activity of N-allylnormorphine in a subsequent publication.

In 1942, an alternate method for the preparation of N-allylnormorphine was described by Weijlard and Erickson (10). Their development was the subject of U. S. Patent 2,364,833, which was assigned in 1944 to
The first extensive study of the pharmacological effects of N-allylnormorphine in animals was undertaken by Unna (11). He reported in 1943 that N-allylnormorphine is about as toxic, but much less effective than morphine in raising the threshold for pain in mice. Small doses, in contrast to morphine, did not depress respiration; very large doses, however, produced respiratory arrest, and all animals succumbing in the acute toxicity studies to N-allylnormorphine died of respiratory failure. Customarily fatal doses of morphine were harmless to mice that had been protected with the antagonist. Unna found, furthermore, that an injection of N-allylnormorphine prior to that of morphine prevented analgesia (mice), respiratory depression (rabbits), and the general depressing (dogs) and stimulatory (cats) effects as well. When morphine was administered and its effects allowed to develop, a subsequent injection of N-allylnormorphine abolished the analgesic effect of the former drug (mice), restored the depressed respiration to normal levels (rabbits) and abolished other toxic manifestations of morphine in cats and dogs.

Unna was the first individual to postulate that the site of action of N-allylnormorphine is central. He indicated that since the site of action of morphine is
generally assumed to be central, it would appear that N-allylnormorphine by virtue of its chemical relationship to morphine exerts its action upon the same centers as morphine rendering them less sensitive to morphine.

In 1944, Hart and McCawley (12) reported additional studies, confirming Unna's findings. They, too, found that depression of respiration by morphine is prevented if N-allylnormorphine is given previously or is abolished if the latter drug is given after morphine. A significant new contribution was added by these investigators. They utilized the D'Amour and Smith analgesimetric method (13) to show that N-allylnormorphine is at least as potent as morphine in raising the pain threshold of rats. Hart and McCawley, in summary, concluded that replacement of =N-CH₃ by =N-CH₂-CH=CH₂ in morphine: 1) eliminates the respiratory depressant action; 2) does not diminish the analgesic action; 3) alters the action on the gastrointestinal tract; 4) alters the excitant action in cats; and 5) antagonizes various other actions of the parent compound.

Despite these early reports little interest was manifest in the drug for the next five or six years.

**Review of Recent Literature.**--N-allylnormorphine remained clinically untried until about five years ago.
when Huggins, Glass, and Bryan (14) and Smith, Lehman, and Gilfillan (15) reported experimental studies which demonstrated that this drug overcame the respiratory depression caused by various narcotic drugs. (For convenience and brevity, N-allylnormorphone will be referred to hereafter by its generic name, nalorphine). Huggins et al. (14), upon administering nalorphine to dogs anesthetized with barbiturates, found that it was effective in preventing the respiratory depression produced by codeine, Dilaudid, metopon, and methadone as well as by morphine, but was comparatively ineffective against that produced by Demerol. Other workers have studied the drug in greater detail and found it to be equally effective in combatting the depressive effects of Dromoran, Pantopon and Demerol (16, 17, 18, 19, 20, 21, 22).

All early investigations indicated that nalorphine was a specific narcotic antagonist. It was reportedly not active against the depression produced by cyclopropane, ether, nitrous oxide, barbiturates or other depressant drugs of non-narcotic character. These early reports showed that nalorphine promptly reversed the respiratory depression and increased both the minute volume and rate of respiration in patients depressed by large doses of narcotics. It also prevented the occurrence of respiratory depression when administered prior to (about 30
minutes) a large therapeutic dose of morphine. The drug also in many cases reversed the fall in blood pressure, decrease in pulse pressure, cardiac arrhythmia and loss of superficial and deep reflexes produced by narcotic drugs. It altered the electroencephalographic pattern from that of deep sleep to that of the waking state in patients poisoned with morphine and its derivatives.

While it is often desirable to administer narcotics to relieve pain during labor, physicians are always conscious of the possibility of such drugs producing profound respiratory depression in the fetus. The depression caused by barbiturates, scopolamine, and narcotic analgesics during the first stage of labor and frequently intensified by inhalation anesthetics during the later stages of labor increases the risk of severe respiratory depression in the mother and the newborn. With these facts in mind, Eckenhoff and his associates (18, 23) studied the use of intravenous nalorphine in combatting neonatal narcosis produced by parturient sedation. In very extensive studies involving more than a thousand deliveries, they found that infants born to mothers receiving nalorphine and narcotics, without general anesthesia, showed a significant reduction in the need for resuscitation and in the time required to gasp and breathe. The efficacy of nalorphine was substantially
reduced when narcotized mothers received nitrous oxide anesthesia, but there still was a significant shortening of time to gasp and breathe, and a decreasing need of resuscitation. The narcotic antagonistic effects of nalorphine were not apparent in infants born to opiate treated mothers who had been given ether anesthesia. These workers also used nalorphine successfully in the treatment of neonatal apnea due to maternal narcosis by injecting the drug directly into the umbilical cord vein of apneic infants who had received oxygen and artificial respiration because they failed to breathe spontaneously after delivery. Further investigation of this use will no doubt be carried out, particularly with regard to nalorphine's relative ineffectiveness in the treatment of asphyxia of non-narcotic origin in the newborn.

In patients severely depressed and in deep coma as a result of narcotic poisoning, nalorphine was only partially effective in lightening the depth of the coma. Fraser et al. (24) successfully treated two patients who were severely depressed and in deep coma as a result of methadone poisoning. The respiratory depression was relieved promptly and consciousness was regained within a short time after the administration of nalorphine.

Nalorphine has also been used to obtain partial relief of respiratory depression resulting from a narcotic
plus a barbiturate. However, it was stated that nalorphine did not relieve the depressant action of barbiturates; therefore, the degree of improvement varied with the relative depressant effect of the narcotic. In the induction of inhalation anesthesia, where respiratory depression has resulted from preanesthetic medication with opiates, nalorphine has been employed to relieve this and thus facilitate induction with an inhalation anesthetic. (25)

It has been reported that nalorphine will cause symptoms of withdrawal within 15 minutes when given to narcotic addicts and is therefore a valuable agent for the rapid diagnosis of narcotic addiction (26, 27, 28). However, there is danger involved in this procedure, since the patient may experience violent withdrawal symptoms and a fatality might result. Signs of withdrawal may also occur in individuals who have received several doses of a narcotic for therapeutic purposes. The data available at the present time do not justify recommending the use of nalorphine as a diagnostic in narcotic addiction except by those experienced in dealing with addicts and when doctors and patients are fully aware of the risks involved.

Nalorphine does not produce serious toxic effects unless very large doses are given. The lethal dose has
not been established in man, but as much as 75 mg. has been given to non-tolerant former morphine addicts without causing dangerous symptoms (26). However, it has been suggested that no more than 40 mg. of nalorphine be administered in a single dose to adults. High dosage is usually accompanied by dysphoria, miosis, pseudoptosis, lethargy, mild drowsiness and sweating. Occasionally nausea, heaviness in the limbs, hot and cold flashes, pallor and postural hypotension are observed. It has been observed that nalorphine acts synergistically when given to patients after a dose of morphine which does not cause marked depression (27, 28). When, however, a definite depression has been caused by a narcotic analgesic, nalorphine causes a spectacular increase in respiratory rate and minute volume and elevation of blood pressure, if it has been depressed. Improvement of the patient is usually dramatic. A crisis may be reversed, with cyanosis disappearing and a deeply comatose patient becoming arousable.

Nalorphine is quite inactive orally, but acts within seconds when given intravenously and within minutes by subcutaneous injection. Dramatic effects may be obtained with repeated doses of 10 to 20 mg. However, the duration of antidotal action is only 2 to 3 hours or less, and if the patient has been poisoned with a long-acting
drug such as morphine, Dromoran, or methadone, a second dose may be needed.

The majority of investigations of the pharmacology of nalorphine have been published only within the past 2 or 2½ years. These have been concerned with such diversified studies as:

1) Whether various newer analgetic agents are antagonized by nalorphine and other allyl derivatives of potent analgetics.

2) Whether nalorphine has analgesic potency when administered alone and whether it antagonizes analgesia induced by known potent analgetics.

3) Whether nalorphine has intestinal antispasmodic action.

4) Analgetic-antagonist combinations in an attempt to find one in which the analgesic activity would be preserved but the respiratory depressant action obviated.

5) Site and nature of the antagonism between nalorphine and various narcotic and barbiturate drugs. These newer investigations will be briefly reviewed in an attempt to clarify the present status of pharmacological knowledge in reference to nalorphine.

More recently it has been found that nalorphine also antagonizes other potent narcotic analgesic agents, including isomethadone, heptazone, alpha-acetylmethadol
and Misentil (29, 30, 31, 32). As has been pointed out, Huggins et al. (14, 33) found no antagonism of nalorphine to meperidine in animals, but Bickenhoff et al. (17) reported nalorphine to be effective in meperidine narcosis in man. In view of this controversy, Winter et al. (34) undertook an investigation to present additional data on this question. From their extensive experiments in rats, dogs, and rabbits, they concluded that nalorphine is an effective antagonist to the effects of meperidine upon rectal temperature, respiratory rate, heart rate, pupil diameter, pain threshold, and general depression. These investigators, in a different type of experiment, found that nalorphine antagonized a compound unrelated chemically to any of the opiates or synthetic analgetic agents. This was 1, 1-bis-2-thienyl-3-dimethylamino butene-1 a potent synthetic narcotic (35).

Fraser and Isbell reported that the following compounds exhibited pharmacological activity very similar to that of nalorphine (36): N-allyldiacetylnormorphine (I), N-propyl-dihyronormorphine (II), L-3-hydroxy-N-allyl-morphinan (III), L-3-methyl ether of N-allyl-morphinan (IV), L-3-acetoxy-N-allylmorphinan (V), and L-3-hydroxy-N-propargyl-morphinan (VI). III and IV are of interest because of their prolonged effectiveness (as compared with nalorphine) and because they are orally
effective. YI is of interest since it produces significant analgesia in mice.

It has been earlier indicated that Unna (11) and Smith et al. (15) found nalorphine to possess little more than a trace of analgesic action in animals, while Hart and McCawley (12) reported it to be about as potent as morphine. Recent work by Winter et al. (34) in rats and dogs support the conclusions of Unna and Smith. This viewpoint has gained support from work done in man by Lasagna and Beecher (37). Even at the present writing, the question of whether nalorphine has significant analgesic action is unsettled; however, the preponderance of evidence indicates that it does not.

Much work has been done in respect to finding the amount of nalorphine required to antagonize the analgesic effects of morphine. Unna (11) used 50 mg./kg. of nalorphine to counteract the analgesic effect of morphine in mice, and Smith et al. (15) used 10 mg./kg. in rats. Neither of these authors attempted to find a minimal effective dose of nalorphine, or to construct a dose-response curve. There is nothing in their data to indicate that they might not have obtained equally good antagonism to the effect of morphine with much smaller doses. On the other hand, Mckenhorff et al. (17, 18) found that as little as 5 to 20 mg. in patients sufficed
to counteract the respiratory depressant effect of narcotic analgesics. In Fromherz and Pellmont's experiments (30), 0.25 mg./kg. completely antagonized 15 mg./kg. of morphine in rats. Winter (34) also indicated that nalorphine antagonizes the analgesic effect of many times its weight or molecular equivalent of analgesic drug.

A significant amount of work has been done upon the intestinal antispasmodic action of nalorphine. Beal and Schapiro (36) demonstrated that nalorphine diminished spontaneous gastrointestinal motility in man, and inhibited motor activity following stimulation by morphine. Gruber and Gruber (39) found that nalorphine prevented or antagonized the increase in intestinal tone produced by either morphine, Dromoran, Dilaudid, metopon, meperidine, methadone, or Nisentil. Winter et al. (34) tested the antispasmodic activity of nalorphine on the isolated ileum of the guinea pig and of the rabbit. Their data showed that although nalorphine has some atropine-like action on the intestine, it is very weak, amounting dosage-wise to about 1/40,000 the activity of atropine upon acetylcholine or papaverine induced contractions. In spite of this finding, Orahovats et al. (40) have succeeded in using nalorphine to protect dogs from nausea and vomiting due to morphine.

The early investigations of nalorphine were undertaken
in a search for agents with the analgesic potency of the opiates but without their respiratory or circulatory depressant properties (12). Although it was soon apparent that respiratory and circulatory depression produced by opiates could be counteracted by nalorphine, it also became obvious that the antagonist per se would depress (17). Theoretically, if properly selected doses of a depressant and an antagonist are injected simultaneously, depression should not occur. It is possible to combine suitable doses of a barbiturate and an analeptic so that narcosis fails to develop with intravenous injection (4). The attempt to combine opiates and opiate antagonists in order to abolish the depressant effects and preserve the analgesia was inevitable. Several efforts in this direction have been made (41, 42, 43). The most recent suggested that the combination of levo-Dromoran and levallorphan in a ratio of 10 to 1 would result in maximal analgesia without depression of the respiration of circulation (43). Eckenhoff and his coworkers in a very recent publication (44) presented data which did not substantiate the above-mentioned work. They found that combinations of opiates and antagonists appear to produce the effects of the opiate, per se. The ability of opiate antagonists to counteract narcotic depression appears to depend upon the existence of such depression prior to the
injection of the antagonist (28, 45). The antagonists appear to be relatively ineffective for treatment of mild depression. Bearing in mind the above facts and also the fact that these antagonists are depressant per se, it is not surprising that simultaneous injections of opiate and antagonist produce respiratory depression. It would indeed be surprising if the antagonist could antagonize certain effects of the opiates and leave other properties unaffected. Lasagna and Beecher (37) obtained results comparable to those of Lockenhoff (45). However, Orahovats et al. (46) found that morphine/nalorphine mixtures given intravenously in dogs and cats showed no significant effects on blood pressure and respiration in contrast to the depressant effects of morphine, given alone at the same dose levels. Therefore, it is obvious that this whole question of preferential antagonism is highly unsettled at the present writing.

Nalorphine was thought for a long time to be an effective antagonist against only a few specific opiates and closely related synthetic derivatives. Salomon et al. in a recent publication (47) agreed with Unna (11) who previously indicated that the action of nalorphine appeared to be competitive on the basis of its chemical similarity, occupying specific receptors or displacing the narcotic agents from these receptors by chemical competition
(competitive inhibition). Similar competitive action has been reported in a large variety of drugs (sulfanilamide vs. para-aminobenzoic acid and histamine vs. anti-histaminics). This, however, would not explain its action against demerol which has very little similarity to morphine. Employing up to 40 mg. total dose in man, Eckenhoff et al. (17, 18) found nalorphine to be of no value against certain members of the barbiturate series, i.e., thiopental, secobarbital. However, Dulfano et al. in 1953 (48) reported the successful treatment of two patients suffering from thiopental and Surital respiratory depression. In an attempt to resolve this apparent contradiction, experiments were performed by Vivante et al. (49) to determine whether or not nalorphine is able to antagonize the respiratory depression produced in dogs by pentobarbital and certain other drugs. In addition, studies were made of the mechanism of its action in pentobarbital depression. These workers found that nalorphine significantly increased the respiratory rate, the total ventilation and the ventilatory equivalent for oxygen in pentobarbital depression. Nalorphine was also found to be an effective respiratory stimulant in dogs anesthetized with a chloralose-urethane mixture. Further data obtained by these investigators led them to conclude that nalorphine probably acts centrally on the respiratory
centers.

So it is seen that nalorphine is an effective antagonist against a wide variety of potent depressant drugs related pharmacologically to morphine, whether related chemically or not. All of these facts indicate that the antagonism is biological, not chemical.

The above section of this dissertation has been written in an effort to review the highlights in the recent literature on nalorphine. It does not cover all the work which has been published on this increasingly important drug. For instance, many possible clinical uses for nalorphine have been suggested in the literature, but only a few have become well established in therapeutics.

Established Clinical Uses of Nalorphine.—At present nalorphine has three distinct clinical uses, all related to its ability to antagonize undesirable effects of narcotic drugs:

1) The prevention and treatment of respiratory depression in the newborn.

2) The treatment of poisoning with narcotics.

3) The diagnosis of physical dependence (active addiction to narcotic drugs).

The use of narcotic drugs for relief of pain during labor has always been beset with difficulties. While
it is frequently desirable to administer analgesic drugs
during labor, the knowledge that such agents might pro-
duce serious respiratory depression in the fetus has
been a deterrent. Early clinical investigations (18, 23)
of nalorphine showed that this drug would possibly
provide a solution to this problem. The ability of
nalorphine to combat infant narcosis was strikingly
evident in patients given opiates but no general anes-
thetic. Less but still definite respiratory-stimulating
effect was observed in the group receiving opiates plus
nitrous oxide, but not in the group receiving opiates
plus ether. The administration of nalorphine, therefore,
appeared to be a valuable procedure for the prophylaxis
of asphyxia neonatorum when mothers have been deeply or
moderately depressed with morphine or drugs pharmacolo-
gically related to this opiate. When used in this way,
10 mg. of nalorphine is usually given to the mother not
less than 5 and not more than 25 minutes prior to deli-
very. Although many more cases must be studied before
the final place of nalorphine in combatting asphyxia
neonatorum due to morphine, methadone, and other narcotics
can be determined, the results obtained to date are so
promising that the drug is on hand and ready for use in
respiratory depression of the newborn in the obstetric
services of most hospitals and in the bags of many
Severe acute poisoning with opiates is not commonly found in this country, but moderate degrees of poisoning are frequently encountered. Ordinarily, such cases occur because of unusual sensitivity to opiates, because of errors in the calculation of doses, and because of frequent overdosage among narcotic addicts. Since nalorphine is a more or less specific antidote for narcotic depression, it has replaced cautious, symptomatic, nonspecific analeptic therapy utilizing caffeine, nikethamide, and sympathomimetic amines. Sufficient information is not yet at hand for the formulation of any absolute rules concerning the dosage of nalorphine in opiate poisoning. Treatment is usually begun by the administration of 10 mg. of nalorphine either subcutaneously or intravenously. If the desired stimulation of respiration is not obtained, additional doses are given at intervals of 15 minutes. In reality, failure of nalorphine to induce spectacular respiratory stimulation should cast doubt on the accuracy of the diagnosis of narcotic depression. When respiratory stimulation is produced, the patient is observed carefully and the drug not repeated unless respiratory depression recurs. The patient does not invariably become fully awakened; though nalorphine may convert a comatose patient into one easily arousable by manual stimulation, such
patients may remain sedated and drowsy for many hours. This actually causes no harm as long as respiratory function is well maintained. These facts indicate that nalorphine does not antagonize completely all of the pharmacological actions of morphine. Thus the sedative effects are incompletely combatted. Neither does nalorphine abolish morphine convulsions (50). In fact the limited efficacy of nalorphine in antidoting morphine in man is due to its insignificant effect on the important central nervous system stimulating component of morphine toxicity.

Administration of nalorphine to patients addicted to various potent narcotic analgetics precipitates, within minutes, signs identical with those that follow abrupt withdrawal of these agents. Thus, its administration to addicts may be followed by typical abstinence changes such as yawning, rhinorrhea, lacrimation, goose flesh, vomiting and restlessness. Obviously, nalorphine is useful in diagnosing the presence of physical dependence (active addiction) on these drugs. In the past, it was necessary to isolate persons suspected of addiction in an environment so carefully controlled as to make smuggling of drugs impossible, to withhold narcotics, and to observe the patient for withdrawal symptoms over a period of two to five days. With nalorphine, this cumbersome and time
consuming procedure is not necessary. It is possible to make a diagnosis of addiction in an hour. The procedure is contraindicated in persons with advanced organic disease and has serious medico-legal implications. Before using nalorphine in diagnosing addiction, a complete history and physical examination is obtained. The patient's written permission to carry out the test is obtained, and the test is always conducted in the presence of witnesses. Three mg. of nalorphine are given subcutaneously. If signs of abstinence have not appeared after 20 minutes, an additional 5 mg. is given. If a definite abstinence syndrome does not appear after 20 more minutes, a final dose of 8 mg. is administered. This cautious method of using multiple small doses is quite necessary because, in strongly addicted persons, large doses of nalorphine may precipitate such intense abstinence symptoms that a fatality might result. If symptoms of abstinence of sufficient intensity appear to permit a definite diagnosis, small doses of morphine sulfate, repeated at intervals of one-half hour are administered in an attempt to make the patient more comfortable. A negative nalorphine test does not exclude the possibility that the patient has been taking occasional doses of narcotics, nor does it prove that he was not actively addicted a week before the test was done. Furthermore,
a negative test does not exclude the possibility of active addiction to meperidine; nalorphine does not precipitate abstinence symptoms in meperidine addiction, unless the patient has been taking 1600 mg. or more daily. The reason for this has not yet been elucidated.

Objectives of This Investigation.--The literature review previously presented in this dissertation indicates a multitude of avenues for possible research on nalorphine. There occur many opportunities for research either of an applied or of a fundamental nature. A prominent controversy is much in evidence concerning the specificity of action of nalorphine upon the respiratory depression produced by various central nervous system depressant drugs. An insight into the specificity or lack of specificity of action of nalorphine would be much desired. Therefore, the primary objective of the investigation to be reported herein is to conduct an inquiry into the anti-respiratory depressant action of nalorphine with the ultimate goal of contributing to the knowledge relating to the site of action of this drug. A second objective is to elucidate as much as possible the specific neurophysiological mechanisms underlying some of the actions of nalorphine in its antagonism of opiate-induced effects in the experimental animal. The chief uses and value of
information to be derived from such studies will be discussed in the following chapter.
One of the fundamental problems of pharmacology is the determination of the site of action of a drug. A second closely related problem is the determination of the mechanism of its action. These two problems are so closely interrelated that much of the information that is listed under the mechanism of action of drugs in the pharmacological literature is probably some division of the localization of the site of action rather than specific or even unspecific information concerning how a drug acts after it reaches the site of action. It may be desirable to review some of the basic methods for the localization of the site of action of drugs and to discuss the usefulness of the information derived from such studies.

Generalizations are comparatively simple and usually obtained by a process of elimination but a final analysis of the site of action of a specific drug may be quite difficult or impossible.

Drugs may act at the point of application, during transport in the body, by reflex effects through the nervous system, by reaching a threshold concentration in a particular tissue, or by reaching a threshold concentration in a particular cell. Some or all of these
factors may be involved. Drugs that act only at the site of application are said to have local or topical action, while drugs that act only after absorption are said to have systemic or general action.

Various levels of information concerning areas involved in drug action can be reached. The limitations are usually those imposed by the methods employed. From very early times, anatomists, physiologists and pharmacologists have inquired into the site of action of drugs by utilizing essentially physiological or surgical approaches.

It was by such methods that Claude Bernard localized the site of action of curare. He excluded the circulation to one leg of a frog without disturbing the motor nerve to the muscles of this leg. He then injected a solution of curare into the frog. Stimulation of the nerve leading to the leg with the intact circulation produced no effect; stimulation of the nerve leading to the leg which was prevented from coming in contact with the drug still produced the usual muscular contraction. Stimulating the muscle directly with electric current or acid produced an effect. Using these observations, and with confirmation by others only slightly more refined, Bernard concluded that the curare acted at the nerve ending, but that it did not act on the muscle directly nor on the
central nervous system of the frog. A century later we still say that curare acts at the myoneural junction to prevent the transmission of nerve impulses from the somatic nervous system to the muscle and thus paralyzes the muscle by inhibiting its response to all but local stimuli.

More recently several workers have shown that another drug, 3-o-toloxy-1,2-propanediol (Myanesin), which essentially paralyzes skeletal muscle, does so at a much higher level in the central nervous system and involves the spinal tracts. Such localization of action can be accomplished by careful section and stimulation above and below the area suspected and also by recording of action potentials from various tracts in the central nervous system.

The anesthetic barbiturates depress the central nervous system to the point that no somatic impulses are sent out to the skeletal muscle system and paralysis occurs. In this instance it has been demonstrated that these drugs act in the higher centers, since electrical stimulation of the outflowing nerves at any point below the brain will produce contraction of skeletal musculature.

By these physiological and surgical techniques it can be demonstrated that inhibition of skeletal muscular function can be accomplished at three different places.
Similar physiological exclusion procedures involve removing a portion of the responsive system and testing the drug to elucidate whether or not the response is still intact. In addition drugs may be applied to the isolated tissue itself to determine if the drug acts here or elsewhere.

Biochemical procedures are another approach to the general problem of the localization of the site of action of a drug. Various enzyme systems can be isolated in functional condition and definite concentrations of the drug applied. By such a technique some of the barbiturates have been shown to block the reduced flavoprotein-cytochrome b complex in the stepwise oxidation of glucose to provide energy for the brain. The action of drugs upon enzyme systems is very closely allied to their specific mechanism of action.

The pharmacological approach to site of action studies has been primarily based upon the use of blocking agents. The use of this method may be exemplified as follows. The pharmacologist may find that a drug produces a given effect when given parenterally. The next step is to determine if it still produces this effect after the administration of another drug, which may have an antagonistic or blocking action. If no response is elicited, the first drug is classified among other drugs
which are blocked or antagonized by the second drug. Thus this type of reasoning, based on the ability of a drug to produce an effect like another drug, and for both these drugs to be blocked by a third, is of value for the compartmentalization or classification of drugs into groups.

Many physicochemical approaches have been utilized in an attempt to solve the problem of site of action. The procedures that are capable of leading to the most precise information about the site of action of a drug are the modern ones that involve labeling a drug with a radioactive or other than the usual isotope of some atomic constituent. The "tagged" molecules can be followed by determination of their presence with a mass spectrometer in the case of non-radioactive heavy isotopes or with some radiation sensitive device in the case of isotopes that undergo radioactive decay. These procedures are based on the fact that the animal body cannot distinguish between heavy and light isotopes under ordinary circumstances nor between radioactive and stable isotopes as long as the radiation emitted does not destroy too many cells. If the specialized equipment and the trained personnel are available, and the drug can be synthesized from the intermediates available, it is possible to obtain precise information concerning the distribution of a drug in the
tissues of the body. Whether the drug acts in all these tissues may often be questioned; it is often difficult to determine what concentration of a drug is necessary to produce a given action and what amount of it is needed to be non-preferentially taken up by indifferent tissues. The tracer techniques also usually do not give information concerning any change the drug may have to undergo in the body before it becomes active. In spite of these and other disadvantages, these techniques are potentially the most accurate in determining the site of action of drugs.

The primary uses and value of the localization of site of action studies may be summarized as follows:

1) To classify drugs into groups for ease of study.
2) To avoid using a drug for a condition if the area in which the drug is usually active is not functional.
3) To provide information which may be of use in the study of rates of absorption and excretion of drugs.
4) To provide information which may be useful in the preparation of satisfactory and efficient dosage forms.
5) To provide preliminary data for the elucidation of the mechanism of action of drugs.
Pharmacology today suffers from the drawback that there is a lack of universally accepted basic useful theory for the action of drugs. Many relations found between the exposure to drugs and the cellular or tissue response are capable of more than one explanation; furthermore, the various explanations may differ fundamentally.

Drug effects may, in general, be said to occur as a result of:

1) The addition of some specific acting foreign substance.

2) The removal of a chemical substance present in tissue by a drug.

3) The interference with normal metabolic function produced by a drug.

4) The non-specific production of some physico-chemical changes in colloid or osmotic effects, etc., by a drug introduced into the tissues.

A more specific classification of drug effects may be formulated only after much more extensive investigation has been carried out at the biochemical or enzymatic level of drug action.

As has been pointed out already, the general methods of determining the mode of action of drugs yield
information primarily concerning the site of action rather than the mode of action. For example, the physiological approach involves the use of excised or localized tissues and may demonstrate, for example, that a drug acts through the central nervous system or locally in a particular area or division. The biochemical approach classically involves the use of isolated enzyme systems and may indicate, for instance, that anesthetics may depress a specific enzyme system in the central nervous system, but does not yield much information as to how this produces loss of consciousness. The physicochemical approach treats of reaction rates and attempts to conclude from such data as is obtained from kinetic studies how a drug may act. Finally, the pharmacological approach may use drugs as blocking agents, antagonists and so on.

The majority of our present information concerning the action of drugs has been obtained in one of these ways. We know, in general, upon which structures of the body most drugs act and in some instances in which part of a given structure. The question of mechanism of action of a drug on a specific group of cells is difficult if not impossible to answer in many instances.

Most of the current theories regarding the mechanism of action of drugs involve surface phenomena such as surface tension, polarity, template effects, blocking
effects, "active patches" and competitive effects involving essential metabolites in enzyme systems. Gradually evolving from mechanism of action studies is the science of biochemorphology which treats of structure-action relationships.
Inasmuch as a major portion of this investigation is involved with antagonism of morphine-induced respiratory depression by nalorphine, it may be well to review the neurogenic and chemical control of respiration in the light of some of the more recent information upon this subject. By so doing, many phenomena may be more easily and intelligibly discussed in the experimental chapter.

The Respiratory Centers. — As early as 1810, Legallois (51) described a small region in the floor of the fourth ventricle of the brain, the destruction of which caused death due to respiratory arrest. Flourens somewhat later (52) elaborated the concept of a center to denote a compact nucleus of quite small size in the floor of the fourth ventricle near the obex. He named this region the "noeud vital" (vital knot). It was later found that this center was a bilateral structure and that full intercommunication existed across the midline.

By carefully probing the medulla with bipolar stimulating electrodes oriented by a Horsley-Clarke stereotaxic instrument Pitts et al. (53) located in the ventral formation of the medulla of the cat immediately overlying the cephalic four-fifths of the inferior olive.
a region from which could be obtained maximal inspirations involving both thorax and diaphragm. Inspiratory apnea continued as long as the stimulation was maintained and was not interrupted by any natural expiratory movement. This was considered to be the inspiratory center.

Another portion of the dorsal reticular substance responded to electrical stimulation by arresting of natural inspiration and a tetanic contraction of expiratory muscles. This area was taken to be the expiratory center. It was not possible to prolong expiratory apnea because natural inspiration ultimately broke through; this was an indication of the primary importance of the inspiratory center. Thus Pitts and his associates indicated that the expiratory region was located dorsal to the inspiratory region which occupies most of the ventral reticular substance at the level of the inferior olive.

Gesell et al. (54), recording action potentials from the medullary structures by means of bipolar needle electrodes, found inspiratory and expiratory electrical activity present in the bulb, but they were unable to show any regularity in the mutual situation of the structures involved. These authors concluded that "scattered reticular grey matter" is an adequate name on account of the scattered distribution of the inspiratory and expiratory neurons. Brookhart (55) also found a
similar scattered distribution of respiratory structures upon stimulating the bulb with needle electrodes. He has questioned the existence of compact, sharply delineated 'centers' for inspiration and expiration in the medulla. He does agree, however, that the reticular formation is the seat of the primary respiratory neurons. Amoroso et al. (56) favor the view of Brookhart in rats, cats, and dogs.

**Rhythmicity of the Respiratory Centers.**—It was thought until very recently that the inspiratory and expiratory centers could not by themselves maintain a normal respiratory rhythm, because the inspiratory center discharged impulses continuously and provoked sustained inspiration. This activity of the inspiratory center was postulated to be periodically inhibited by impulses from the pneumotaxic center and from the lungs, thus permitting expiration to take place.

These earlier hypotheses of the origin of respiration, in which two extramedullary self-activating circuits are postulated, may be more closely defined as follows. First, there is the Hering-Breuer reflex which is supposed to be set in motion by the act of inspiration which inflates the lungs and stimulates stretch receptors in their substance. These initiate in the afferent fibers
of the vagus nerve impulses which are supposed to inhibit further inspiration, presumably by way of the nucleus solitarius and the expiratory center. The second circuit is supposed to be activated by impulses from the inspiratory center which are sent rostrally to the 'pneumotaxic' center in the upper regions of the pons, which in turn relays impulses back to the expiratory center to inhibit inspiration. Either circuit alone was supposed to be capable of maintaining rhythmic respiration; but, if both were removed, the inspiratory center was hypothetically incapable of cyclic breathing, and could produce only a state of sustained inspiration, continuing until death from anoxia occurred.

Recently, it was found by Hoff and Breckenridge (57) that neither of these circuits are indispensable to the respiratory cycle, but that respiration continues after both vagi and pons are eliminated by section of the vagi and appropriate transection of the brain stem between the pons and medulla the boundary between which is marked by the acoustic tubercles. Since the Hering-Breuer reflexes and the circuit between the medulla and the pneumotaxic center are thus obviated, the primary generation of the respiratory act is therefore confined within the medulla itself. The medullary centers then obviously have an intrinsic periodicity. The concept of
an inherent rhythmicity of the respiratory centers was confirmed by the transection experiments of Hukuhara et al. (58) on vagotomized rabbits, cats, and dogs.

It is not known precisely just how the avalanching rhythm of inspiratory discharge is arrested at its acme, only to start again after a period of quiet. It is thought by some that such periodicity represents the reciprocation between self-exciting and self-inhibiting circuits within the cortex, ending in fluctuating excitation and inhibition of the pyramidal neurons. The interneuronal connections of the medullary reticulum are probably of a complexity sufficient to support activity based on recurrent excitatory and inhibitory circuits. Kaada (59) has made an extensive study on the localization of the cortical areas from which inhibition or acceleration of respiratory movements can be elicited in monkeys, cats, and dogs, as well as in one infant chimpanzee and in eight human patients. Two distinct inhibitory fields were found: (a) A "medial-basal inhibitory field," and (b) a "temporal-inhibitory field." An acceleration of respiratory movements could be produced by stimulation of part of the motor region. It was demonstrated that the respiratory effects obtained by electrical stimulation of these fields are mediated directly "down-stream" by cortico-subcortical fibers, and by section of the pyramids
it was shown that the response is of extrapyramidal nature. The responses obtained were well-integrated acts and represented almost all characteristics of respiratory movements. The author therefore concluded that the effects are probably due to activation of respiratory centers rather than to a direct activation of the spinal motor neurons.

It has been pointed out that vagotomy is almost without influence at the medullary level, however, it may have important consequences at the higher levels. It has been suggested that afferent discharge from the vagus forms an important part of the regulation at a supra-medullary level of the balance between the facilitatory and suppressor systems in which the actual timing of separate impulses is largely lost. Kerr and his associates (60) believe that the principal role of the vagus is as an important source of afferent drive for the suppressor system. Bozler and Burch (51), who studies the effect of vagotomy on respiration in closed and open chest experiments on dogs, concluded that pulmonary vagal fibers have an excitatory effect on inspiration. As the effect of vagal block was independent of the mechanical state of the lungs, it was assumed that the volume receptors initiating the Hering-Breuer reflexes were not involved.
Types of Normal Breathing.—Within the range of normal breathing there are three fundamental patterns. The most significant of these is eupnea, in which rate, depth and the expiratory base line are approximately constant. Variations in metabolic demand may alter these without fundamental change in the basic nature of the pattern. With marked increase in metabolic demand, and especially with increase in temperature, a second type of breathing—panting—may be produced, usually by a more or less abrupt change. This type is distinguished by its lower amplitude and markedly increased rate. In the dog panting usually involves a rate of 150-200 or more per minute. The third type of respiration is the "sighing" type, described particularly by Christie (62) in man, designated as "superimposed" in the dog by Swindle (63), and called "gasping" by Lumsden (64, 65).

Against this background of patterns seen in normal circumstances there develops a variety of abnormal respiratory rhythms which include Cheyne-Stokes respiration, Biot's breathing, and apneusis or breath-holding. It has become apparent in the course of earlier studies on respiratory mechanisms (57, 66, 67, 68) that many of these patterns develop from a combination of basic medullary potentialities as they are variously suppressed or enhanced by supramedullary influences.
Chemical Control of Respiration.—The respiratory movements are so regulated as to control the rates at which O₂ is added to and CO₂ removed from the alveolar air of the lungs. At rest breathing normally is rather slow and shallow because the rates of removal of O₂ from the alveolar air and of addition of CO₂ to it are relatively slow. During exercise, however, the rate and depth of breathing must be stepped up in order to supply the increased demand for O₂ and to excrete the increased quantity of CO₂ formed in tissue oxidations. At high altitudes where the partial pressure of O₂ in the inspired air is decreased, the rate of pulmonary ventilation is increased in compensation. The rate of excretion of CO₂ from the lungs is of importance in regulating the acid-base balance and pH of the blood and tissues. Thus, when the pH of the blood falls below normal, as in the case of diabetic acidosis, respiratory movements are increased to blow off more CO₂, thereby decreasing the dissolved CO₂ and H₂CO₃ and H⁺ ion concentration of blood and tissue fluids. In conditions of alkalosis in which the pH of the blood and tissue fluids rises above normal, respiration is depressed and the rate of removing CO₂ is decreased. This causes the partial pressure of CO₂ in the tissues and blood to build up, thereby increasing the H₂CO₃ and lowering the pH. The rate at which CO₂ is
excreted by the lungs is of primary importance in regulating the acid-base balance of the body, and it is through automatic control of the respiratory movements that this is accomplished.

The chemical control of respiration is mediated directly or indirectly through the respiratory center located in the medulla oblongata, which sends impulses to the respiratory apparatus. The neurons of the respiratory center are stimulated directly by increases in CO₂ pressure and H⁺ ion concentration (decreases in pH). Conversely, the center is depressed by decreases in CO₂ pressure and H⁺ ion concentration (increases in pH).

Chemoreceptors located in the carotid and aortic bodies situated at the bifurcations of the carotid arteries and the arch of the aorta, respectively, also play a role in the chemical regulation of respiration (See Figures 1-A and 1-B, pp. 46, 47).

Also lying at the bifurcation of the common carotid artery and also at the arch of the aorta are another distinct type of receptors, pressoreceptors or baroreceptors. Both have respiratory effects. The receptors in the aortic and carotid bodies which respond to changes in the chemical composition of the blood play a relatively small part in the regulation of circulation, but they are of considerable importance in the control of
Figure 1-A.—Location and innervation of the carotid receptors. 1. Thyroid artery. 2. Lingual artery. 3. Common carotid artery. 4. External carotid artery. 5. Occipital artery. 6. Carotid sinus. 7. Carotid body. 8. Internal carotid artery. 9. Carotid nerve. 10. Glossopharyngeal or ninth cranial nerve.

Respiration. These chemoreceptors are stimulated by a decrease in oxygen tension, by an increase in hydrogen ion concentration or carbonic acid, and by nicotine, cyanide, hydrogen sulfide, and other drugs.

It appears that the chief effect upon the chemoreceptors is exerted by decreased O₂ pressure and that effects produced by changes in CO₂ and H⁺ ion concentration are of little physiological significance.
Sensitiveness of chemoreceptors to small changes in oxygen partial pressure can be demonstrated in animals if care is taken to avoid depression by anesthetics. Activity increases as oxygen saturation of hemoglobin diminishes, almost in linear relation, with a maximum at 15 per cent oxyhemoglobin.

Sensitiveness to an increase in hydrogen ion concentration or CO\textsubscript{2} has been demonstrated in perfusion experiments in which the carotid body and sinus nerves are intact. In physiologic conditions these stimuli are not of much importance, since a change of 0.1 in pH is needed to produce the minimum (threshold) response and variations of this magnitude seldom occur in normal subjects.

The chemoreceptors are not very sensitive to changes in arterial CO\textsubscript{2} partial pressure, which must be of the order of 10 mm. to produce effects. The respiratory centers, on the other hand, respond to changes in arterial CO\textsubscript{2} partial pressure of 0.075 mm. Hg. Therefore, CO\textsubscript{2} cannot be considered as the normal stimulus of the chemoreceptors.

A salient feature of the chemoreceptors is their great resistance compared with that of the respiratory centers. Thus anoxia of a certain intensity and duration depresses the respiratory center, but does not disturb
the chemoreceptors. Resistance of the chemoreceptors to lack of oxygen is perhaps an important factor in maintaining respiration in serious anoxia.

A sharp rise in arterial blood pressure produces apnea, whereas a sudden fall in arterial blood pressure produces hyperpnea. The apnea accompanying a rise in pressure results mainly from stimulation of the pressoreceptors, which lie within the adventitia and media of the carotid sinuses, aortic arch, and the innominate artery adjacent to the arch. Impulses from these receptors reflexly inhibit the inspiratory center. The hyperpnea accompanying a fall in blood pressure results mainly from a withdrawal of these inhibitory impulses which are normally discharged at low frequency when the pressure is at a normal level. Although the pressoreceptor impulses may normally exert a tonic restraining influence on the respiratory center, the reflexes which they elicit are of much less general significance than the chemoreceptor reflexes in the regulation of respiration.

Gray (69) demonstrated that each of the chemical and physical factors which regulate breathing exerts a stimulating or depressing effect independent of all the others, and that the character of the breathing at any given time is the resultant of the arithmetical sum of
all these partial effects. With an individual at rest, the pulmonary ventilation is controlled largely by the action of CO₂ and H⁺ ions upon the respiratory center. Gray has developed an equation by which the combined effects of CO₂ tension and H⁺ ion concentration upon respiration may be calculated. This equation is as follows:

\[ VR = 0.22H^+ / 0.262 pCO₂ - 18.0, \]

in which \( VR \) = number of times alveolar ventilation is increased over the resting value due to H⁺ ion and CO₂ changes, \( H^+ \) = H⁺ ion concentration of arterial blood in billionths of a mol per liter, and \( pCO₂ \) = the alveolar pressure of CO₂ in millimeters of Hg.

In the normal individual at rest with an arterial blood pH of 7.44, \( H^+ = 36.3 \) billionths of a mol per liter and alveolar \( pCO₂ \) is of the order of 38.2 mm. The respiratory response in this individual is zero:

\[ VR = 0.22 \times 36.3 / 0.262 \times 38.2 - 18 = 0 \]

If such an individual breathes 5 per cent CO₂ so that the arterial pH drops to 7.336 (\( H^+ = 46.1 \)) and the alveolar \( pCO₂ \) rises to 46.7 mm, the rate of pulmonary ventilation becomes: \( VR = 0.22 \times 46.1 / 0.262 \times 46.7 - 18 = 4.4 \). Thus, under the stimulus of 5 per cent CO₂ in the inspired air, the respiratory center increases the alveolar ventilation to 4.4 times the normal rate.
From the above calculations it can be seen that both 
H+ ion concentration and pCO₂ in the blood exert marked 
effects upon pulmonary ventilation rate.
EXPERIMENTAL

The individual experiments comprising this investigation differ one from the other in several respects—in specific objectives, basic design, procedures, and types of data obtained. For this reason, the various experiments will be discussed individually. The results obtained and the information supplied thereby will be discussed and correlated in the following chapter of this dissertation.

PART I

Some work has been done in respect to finding the amount of nalorphine required to antagonize the analgesic effects of morphine (17, 18, 30, 34). These efforts have been discussed earlier. However, there has been very little work done in behalf of determining the minimum dose of nalorphine necessary to antagonize morphine-induced respiratory depression. Eckenhoff et al. (17, 18) used 5 to 20 mg. of nalorphine to counteract the respiratory depressant effect of variable doses of narcotic analgesics in human patients. More recently, Costay and Bonnycastle (32) indicated that a ratio of 5:1 morphine/nalorphine was required to produce a return to normal of depressed minute volume in the rat. This
is the only publication (to the author's knowledge) which has reported dosage-effect data of this kind.

Because of the scarcity of information on minimum effective doses of nalorphine required to combat respiratory depression, it was decided to study nalorphine antagonism of morphine-induced depression. The dog was chosen as the experimental animal inasmuch as dogs and monkeys are thought to react to opiate-type drugs similarly to man. This is not deemed to be true of felines and rodents, which exhibit certain atypical effects to these drugs, particularly in the case of the former.

The first problem which presented itself was the selection of a suitable anesthetic which would not be antagonized by nalorphine. It was necessary to anesthetize the animals in order to be able to satisfactorily record minute volume. A logical possibility, of course, might have been the use of volatile anesthetics such as ether or cyclopropane since neither of these two agents are antagonized by nalorphine. However, the difficulty of their administration and control of the depth of anesthesia almost immediately ruled out their use.

Another possibility was the use of one of the intermediate or short-acting barbiturates, such as pentobarbital or amytal. Since pentobarbital is readily
available and produces very satisfactory anesthesia in the dog, it was selected for use in this experiment, which was undertaken before Vivante et al. (49) reported that nalorphine also antagonized this barbiturate. Adrian (25), however, had reported somewhat earlier that nalorphine was useful in obtaining partial relief of respiratory depression resulting from a narcotic plus a barbiturate. However, it was thought that the degree of improvement varied only with the relative depressant effect of the narcotic. Nevertheless, it seemed desirable to verify this assumption by a suitable experiment before undertaking the proposed study of nalorphine antagonism of morphine-induced respiratory depression in dogs anesthetized with pentobarbital sodium.

**EXPERIMENT 1**

**Experimental Design and Procedure.**—This experiment was designed to determine whether or not nalorphine is able to antagonize the respiratory depression produced by pentobarbital sodium in dogs. Male mongrel dogs, weighing from 8 to 11 Kg. were anesthetized with 35 mg./Kg. of pentobarbital sodium given intravenously. An intratracheal rubber catheter equipped with an inflatable French type latex cuff was inserted into the trachea well beyond the level of the larynx. The cuff was blown
up with air by use of a hypodermic syringe and needle suitably attached to the stem of the cuff. This procedure prevented any passage of air between the catheter and the walls of the trachea. The catheter was connected to a one-way flutter valve mechanism which in turn was led into a wet test gas meter\(^1\) which was sensitive to as little as 10 ml. of expired air. By means of the valve mechanism the anesthetized animal was permitted to inhale room air on inspiration. On expiration, however, the valve opening to the outside was closed off by the pressure of the exhaled air which was blown out through the second flutter valve into the gas meter. The rubber tubing serving to join the various pieces was made as short as possible to decrease the dead space to a minimal volume. Minute volume could be obtained by observing the excursion of the indicator on the gas meter during the period of one minute. Similarly respiratory rate could be determined by observing the individual small excursions of the indicator with each expiratory movement.

A time interval of 15 minutes was allowed after administration of the anesthetic for intubation of the trachea and inflation of the cuff and for the respiration to become stabilized. The minute volume and respiratory rate were then determined at five minute intervals for a

\(^1\)Precision Scientific Co., Chicago, Illinois
duration of two hours. At the end of one-half hour, a
dose of 10 mg./Kg. of nalorphine hydrochloride was ad-
ministered intravenously. Each animal served as its own
control inasmuch as the means of the minute volumes and
rates recorded over the first 30 minutes were considered
to be normal or control values for that particular
animal.

Results.—Table I shows the results of such studies
in two dogs. The figures in the third column of the
table represent the means and the respective standard
errors of the values obtained for ventilation minute
volume over 30 minute periods. The column headed P
indicates the probability of the difference from control
being due to chance. Respiratory rate is given in breaths
per minute.

It is immediately apparent from the table (page 57)
that the administration of nalorphine results in a
remarkable improvement in both respiratory minute volume
and rate as compared with the depressed control levels.
Nevertheless, it may be argued that this agent does not
in reality bring about an antagonism of depressed res-
piration; perhaps the observed increase in ventilation and
rate are manifestations of a spontaneous decrease in
anesthetic depth. Therefore, Dog No. 1 was utilized
Table I.—Effect of Nalorphine on Respiration in Dogs Anesthetized with Pentobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time (min.)</th>
<th>Ventilation (l./min.±S.E.)</th>
<th>P Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-30 (Control)</td>
<td>1.37 ± 0.08</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>1.71 ± 0.07</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>1.71 ± 0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.07 ± 0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>0-30 (Control)</td>
<td>1.57 ± 0.12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>2.08 ± 0.07</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2.42 ± 0.14</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.55 ± 0.18</td>
<td>0.02</td>
</tr>
</tbody>
</table>

six days later in a similar experiment in which the administration of nalorphine was omitted. This control test indicated that the dose of pentobarbital sodium used in the experiment was sufficient to maintain a rather uniform state of depressed respiration over the full period of 2 hours and 15 minutes (see Table II).

The preceding experiment, however, did not show that a true depression existed as a result of the administration of pentobarbital. Therefore, Dog No. 2 was given a much smaller dose of the anesthetic in a subsequent
Table II.—Respiratory Minute Volume and Rate in a Dog Anesthetized with Pentobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time (min.)</th>
<th>Ventilation (l./min.±S.E.)</th>
<th>P Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-30 (Control)</td>
<td>1.28 ± 0.15</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>1.35 ± 0.17</td>
<td>0.90 16</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>0.82 ± 0.09</td>
<td>0.20 9</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>1.23 ± 0.18</td>
<td>0.90 11</td>
</tr>
</tbody>
</table>

Experiment (20 mg./Kg.). Minute volume and rate were again recorded over the same time interval. The mean values for ventilation volume and rate were approximately double those shown in Table II. This would indicate that the larger dose of pentobarbital used in the first experiments actually does produce a profound respiratory depression.

**EXPERIMENT 2**

Experimental Design and Procedure.—Experiment 1 indicated that pentobarbital would be an unsuitable anesthetic to be used in a study of nalorphine antagonism of morphine effects. It would be quite difficult to evaluate antagonistic effects against both the anesthetic and the narcotic.
It was approximately at this point of the investigation that Vivante et al. (49) published their report concerning nalorphine antagonism of pentobarbital sodium-induced respiratory depression. There were no additional publications indicating that nalorphine might antagonize other barbiturates. Therefore, this experiment was undertaken to determine whether the depressive effects upon respiration produced by amobarbital sodium are also prevented by nalorphine.

The experimental procedure was essentially the same as that reported for Experiment 1.

Results.—Table III lists the data obtained in the studies with amobarbital sodium. Table IV summarizes the data obtained in a control study to show that an improvement in respiration does not occur as a result of lightening of anesthesia. Dogs 3 and 4 were anesthetized by administering 45 mg./Kg. of amobarbital sodium intravenously and 15 minutes were then allowed for respiratory stabilization. Then one-half hour later these dogs were given 10 mg./Kg. of nalorphine hydrochloride intravenously. Dog No. 5 received only the initial anesthetic dose of amobarbital sodium.
Table III.— Effect of Nalorphine on Respiration in Dogs Anesthetized with Amobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time (min.)</th>
<th>Ventilation (l./min.±S.E.)</th>
<th>P</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0-30 (Control)</td>
<td>2.30±0.02</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>2.62±0.08</td>
<td>0.02</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>3.36±0.22</td>
<td>0.01</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>5.46±0.54</td>
<td>0.01</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>7.06±0.57</td>
<td>0.001</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>0-30 (Control)</td>
<td>2.92±0.04</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>3.04±0.10</td>
<td>0.50</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>3.22±0.06</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>5.34±0.66</td>
<td>0.02</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>5.78±0.33</td>
<td>0.001</td>
<td>12</td>
</tr>
</tbody>
</table>

Table IV.— Respiratory Minute Volume and Rate in a Dog Anesthetized with Amobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time (min.)</th>
<th>Ventilation (l./min.±S.E.)</th>
<th>P</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0-30 (Control)</td>
<td>2.48±0.08</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>2.45±0.16</td>
<td>0.80</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2.18±0.13</td>
<td>0.20</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.07±0.10</td>
<td>0.10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>2.48±0.58</td>
<td>&gt;0.90</td>
<td>13</td>
</tr>
</tbody>
</table>
It became quite apparent that amobarbital sodium would also be unsuitable as an anesthetic agent. It should be mentioned that the probability figures in the tables were determined by application of the "t" test.

**EXPERIMENT 3**

Experimental Design and Procedure—Experiments 1 and 2 indicated that nalorphine may antagonize a great number of the various barbiturate anesthetics. It therefore seemed desirable to study an anesthetic which is chemically unrelated either to the opiates or the barbiturates. This experiment was designed, then, to study the effects of nalorphine upon respiration in dogs anesthetized with urethane.

It should be pointed out that although some of the dogs were utilized several times in these various experiments, at least six days were allowed for recovery to take place to obviate the possibility of obtaining additive effects due to drug accumulation and to prevent the occurrence of tolerance phenomena.

The experimental procedure was essentially the same as that reported in Experiment 1. Dogs 4 and 5 were anesthetized with 1200 mg./Kg. of urethane (10% solution) given intravenously. Because of the necessity
of administering urethane in such a high concentration, 25 minutes instead of 15 minutes were allowed from the time that the anesthetic was administrated until recording of minute volume and rate were begun. The large volume of urethane solution was injected very slowly over a period of about the first ten minutes.

Results.—Table V lists the means of the values obtained for minute volume and rate in the dogs anesthetized with urethane. Once again the respiratory responses were observed at five minute intervals for 30 minutes to obtain control means, after which 10 mg./Kg. of nalorphine hydrochloride were injected by the intravenous route.

It would seem from the data listed in Table V (page 63) that urethane would be a satisfactory anesthetic to use in those animals in which studies upon nalorphine antagonism of morphine-induced respiratory depression were to be conducted. The data indicates that urethane is not antagonized by nalorphine. There is one serious objection to its use, however; namely that this anesthetic has a very long duration of action. To illustrate this fact, it may be pointed out that Dog No. 4 did not regain consciousness for more than 72 hours. Dog No. 5 died apparently from dehydration sometime
Table V.--Effect of Nalorphine on Respiration in Dogs Anesthetized with Urethane.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time (min.)</th>
<th>Ventilation (l./min. ± S.E.)</th>
<th>P</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0-30</td>
<td>4.14 ± 0.27</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>3.73 ± 0.45</td>
<td>0.60</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>3.42 ± 0.38</td>
<td>0.20</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.76 ± 0.27</td>
<td>0.05</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>0-30</td>
<td>3.05 ± 0.10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>3.14 ± 0.27</td>
<td>0.80</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2.91 ± 0.28</td>
<td>0.70</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.88 ± 0.07</td>
<td>0.30</td>
<td>14</td>
</tr>
</tbody>
</table>

between 60 and 72 hours. Since it was desired to use the same animal in a series of studies on nalorphine/morphine effects, urethane, unfortunately, would not be an appropriate anesthetic.

EXPERIMENT 4

Object and Procedure.--A paper, published by Gruber (70), appeared while Experiment 3 was in progress. This
investigator found that no protection was offered by nalorphine against the acute toxic effects of secobarbital sodium in mice. It occurred that this short-acting barbiturate might be one agent of this series which might not be antagonized by nalorphine. Accordingly, the present experiment was carried out to study the effects of nalorphine on respiration of dogs anesthetized with secobarbital sodium. Again the experimental procedure was fundamentally the same as that outlined in Experiment 1.

**Results.**—The results are tabulated in Tables VI and VII. Dogs 6 and 7 were anesthetized with 25 mg./Kg. of secobarbital sodium introduced intravenously. After respiratory data were recorded at five minute intervals for one-half hour, 10 mg./Kg. of nalorphine hydrochloride were given intravenously and respiratory data again obtained at five minute intervals for one and one-half hours. Dog No. 8 received only the anesthetic and thus served as a control in this series.

Upon learning that nalorphine did not improve respiration in Dogs 6 and 7 (see Table VI, page 65), this same experiment was repeated in the same three dogs. This time Dog No. 6 was utilized in the control study. The results obtained were very similar to those reported
Table VI.--Effect of Nalorphine on Respiration in Dogs Anesthetized with Secobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time</th>
<th>Ventilation (l./min. ± S.E.)</th>
<th>P</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0-30 (Control)</td>
<td>3.39 ± 0.07</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>3.44 ± 0.20</td>
<td>0.90</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>3.04 ± 0.08</td>
<td>0.80</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.69 ± 0.13</td>
<td>0.05</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>0-30 (Control)</td>
<td>2.93 ± 0.15</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>2.20 ± 0.31</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2.97 ± 0.01</td>
<td>0.90</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.33 ± 0.23</td>
<td>0.10</td>
<td>8</td>
</tr>
</tbody>
</table>

Table VII.--Respiratory Minute Volume and Rate in a Dog Anesthetized with Secobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time</th>
<th>Ventilation (l./min. ± S.E.)</th>
<th>P</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0-30</td>
<td>2.99 ± 0.05</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>3.13 ± 0.07</td>
<td>&gt;0.20</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>3.24 ± 0.11</td>
<td>0.50</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>3.03 ± 0.03</td>
<td>0.80</td>
<td>6</td>
</tr>
</tbody>
</table>
on the preceding page and, therefore, the data will not be recorded herein. This experiment was interpreted to show that secobarbital would be a suitable anesthetic agent in a study of nalorphine antagonism of morphine-induced depression of respiration. Such a study was undertaken in Experiment 5.

EXPERIMENT 5

The first problem to be solved before nalorphine effects could be studied was the determination of an intravenous dose of morphine which would produce a marked depression of respiratory rate and minute volume for a duration of at least two hours after its administration. Various experiments were performed in which dogs anesthetized with 25 mg./kg. of secobarbital sodium were given graded doses of morphine sulfate intravenously. (All drugs were administered intravenously in all subsequent experiments in parts I and II). The following doses were tried: 0.5, 1, 2, 3, 5, and 10 mg./kg. It was found that the highest mentioned dose produced too great a depression and the animal died of respiratory and circulatory failure. Doses below 5 mg./Kg. did not produce consistent effects on respiration. However, a dose of 5 mg./kg. of morphine sulfate produced a depression of both ventilation volume and rate to a level
of 40 to 60% of normal or control levels time after time. A total of 4 dogs were used at the 5 mg./Kg. dosage level of morphine sulfate.

Procedure.—The dogs were initially anesthetized with 25 mg./Kg. of secobarbital sodium, their tracheae were intubated, and the apparatus arranged for recording of minute volume and rate as described in Experiment 1. Respiratory responses were observed at five minute intervals for thirty minutes, the dose of morphine sulfate was administered, and the responses observed once more at five minute intervals for two additional hours.

Results.—In Table VIII will be found the data obtained from one of the dogs receiving a dose of 5 mg./Kg. of morphine sulfate. These data are representative of that obtained from the four dogs receiving this dosage of the opiate. It is apparent that morphine produces a profound depression of respiration of dogs anesthetized with secobarbital sodium (Table VIII, page 68).
Table VIII.—Effect of Morphine on the Respiration of a Dog Anesthetized with Secobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time (min.)</th>
<th>Ventilation (l./min. ± S.E.)</th>
<th>P</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0-30 (Control)</td>
<td>5.14 ± 0.21</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>2.91 ± 0.02</td>
<td>&lt; 0.02</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2.84 ± 0.11</td>
<td>0.05</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.65 ± 0.07</td>
<td>0.05</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>2.60 ± 0.09</td>
<td>0.05</td>
<td>14</td>
</tr>
</tbody>
</table>

**EXPERIMENT 6**

This experiment was conducted to determine the approximate dose of nalorphine required to just antagonize the effects of morphine on the respiration of dogs anesthetized with secobarbital sodium.

**Procedure.**—The design of this experiment is much the same as that given in the preceding one. The dosage of morphine sulfate used in all the following experiments in this group was standardized at 5 mg./Kg. The dose of secobarbital was also standardized at 25 mg./Kg. The anesthetic was administered, 15 minutes was allowed for arranging the apparatus and for the
respiration to become stabilized. Then the respiratory responses were observed at five minute intervals for 30 minutes. At this point a dose of nalorphine hydrochloride was given followed within 1-2 minutes by the standard dose of morphine sulfate. The responses were again observed at five minute intervals for two additional hours.

Results.—Much experimental work had to be done in order to establish a dose of nalorphine that would just antagonize the standard dose of morphine. It was found that doses of 10, 5 and 1 mg./Kg. of nalorphine hydrochloride would prevent any significant depression of respiration by morphine. Therefore, smaller doses were tried. Dog No. 8 received 0.1 mg./Kg., Dog No. 7, 0.5 mg./Kg. and Dog No. 9, 0.75 mg./Kg. of nalorphine. The respiratory data from these three dogs is recorded in Table IX (page 70).

It is seen from the following table that approximately 0.75 mg./Kg. of nalorphine is required to protect dogs anesthetized with secobarbital against respiratory depression induced by 5 mg./Kg. of morphine sulfate.
Table IX.—Effect of Nalorphine on Respiration Depressed by Morphine in Dogs Anesthetized with Secobarbital Sodium.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Time (min.)</th>
<th>Ventilation (l./min. ± S.E.)</th>
<th>T</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0-30 (Control)</td>
<td>3.94 ± 0.09</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>3.13 ± 0.01</td>
<td>0.05</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>2.61 ± 0.11</td>
<td>0.05</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.04 ± 0.10</td>
<td>0.05</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>2.32 ± 0.03</td>
<td>0.02</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>0-30 (Control)</td>
<td>4.44 ± 0.04</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>1.97 ± 0.00</td>
<td>0.001</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>1.89 ± 0.16</td>
<td>0.05</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>1.55 ± 0.04</td>
<td>0.01</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>1.58 ± 0.08</td>
<td>0.02</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>0-30 (Control)</td>
<td>3.04 ± 0.23</td>
<td>0.90</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30-60</td>
<td>3.16 ± 0.16</td>
<td>0.90</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>60-90</td>
<td>3.09 ± 0.11</td>
<td>0.90</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>90-120</td>
<td>2.94 ± 0.09</td>
<td>0.90</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>120-150</td>
<td>2.89 ± 0.22</td>
<td>0.80</td>
<td>14</td>
</tr>
</tbody>
</table>
The purpose of this experiment is to more closely define the minimum dose of nalorphine which is effective against the chosen standard dose of morphine.

Procedure.--The design and procedures of Experiment 6 were employed in this experiment. In preliminary tests it was found that 0.7 mg./Kg. was a sufficient dose to antagonize the standard dose of morphine. A new series of dogs was obtained (Nos. 10, 11 and 12). A graded dose scheme was selected with 0.7 mg./Kg. as the medium dose. High and low doses, 0.9 and 0.55 mg./Kg. respectively, were chosen in order to obtain a logarithmic spacing between the three doses. These doses were administered at weekly intervals to the individual dogs in the series, until each of the dogs had received the three doses. The order of dosing was randomized according to a modified Latin square design.

Results.--A summary of the results is shown in Table X (page 72). From this table it is seen that the ratio of the doses of opiate to allyl antagonist required to maintain control values of minute volume and respiratory rate is approximately of the order of 7:1.
Table X.— Graded Dose-response Data Relative to Nalorphine Antagonism of Morphine-induced Respiratory Depression

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Dose of Nalorphine (mg./Kg.)</th>
<th>Time (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.55</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>V*</td>
<td>R**</td>
</tr>
<tr>
<td>10</td>
<td>2.81</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3.69</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2.89</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.83</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.63</td>
<td>13</td>
</tr>
<tr>
<td>11</td>
<td>1.88</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1.60</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1.20</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.12</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1.15</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>8.40</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>6.62</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5.69</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>4.59</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3.98</td>
<td>18</td>
</tr>
</tbody>
</table>

V* signifies mean ventilation (l./min.).
R** signifies mean respiratory rate (breaths/min.).
C*** signifies control mean values before the antagonist and opiate are administered.

It is noted that 0.55 mg./Kg. of nalorphine did not completely protect against morphine depression (P = 0.05).
PART II

The experiments in Part II were undertaken to study the significance of the aortic and carotid body chemoreceptors as well as the respiratory center relative to the site of the antagonistic activity of nalorphine against morphine-produced respiratory depression.

EXPERIMENT 8

Experimental Design and Procedure—The basic design of this experiment differed as follows from that described in the preceding experiment. The expired air as it left the gas meter was shunted into a latex membrane tambour which was adjusted to record the expiratory excursions on a smoked drum. By suitably connecting the poles on the gas meter to a signal magnet in series with a dry cell battery, it was possible to record expiratory volume in increments of 3 liters (one excursion through 360° of the indicator on the gas meter). By the use of an automatic electrical timing device, intervals of ten seconds and one minute were also recorded on the smoked drum just below the volume-recording signal magnet. A femoral vein of the dogs was cannulated for injection of drugs and the blood pressure of the animals was recorded by means of a mercury manometer connected to a
femoral artery.

In order to make the surgical manipulation possible, the dose of the anesthetic, secobarbital sodium, was increased to 35 mg./Kg. The dose of morphine sulfate was conversely reduced to 3 mg./Kg., to avoid severe depression of circulation and respiration. The amount of nalorphine administered amounted to 1 mg./Kg.

After the blood vessels had been cannulated and the trachea intubated in this series of dogs, the following procedures were followed in the three dogs:

Dog No. 13—Bilateral carotid chemoreceptor denervation.

Dog No. 14—Bilateral vagotomy to eliminate aortic chemoreceptors.

Dog No. 15—Both of the above procedures.

Two hours was allowed for the dogs to recover from the denervation procedures. Then the effect of nalorphine on the respiratory depression produced by morphine was studied.

Results.—Table XI (page 75) is a resumé of the data obtained and the procedure followed in the remainder of this experiment. Figure 2 (page 76) is a photograph illustrating normal respiration and blood pressure records in the carotid denervated and vagotomized dog, the same
<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Ventilation (l./min.)</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.23</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>3.36</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>3.39</td>
<td>17</td>
</tr>
<tr>
<td>12</td>
<td>3 mg./Kg. Morphine Sulfate I. V.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2.04</td>
<td>13</td>
</tr>
<tr>
<td>20</td>
<td>1.78</td>
<td>13</td>
</tr>
<tr>
<td>25</td>
<td>1.76</td>
<td>13</td>
</tr>
<tr>
<td>27</td>
<td>1 mg./Kg. Nalorphine Hydrochloride I. V.</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3.26</td>
<td>12</td>
</tr>
<tr>
<td>35</td>
<td>3.31</td>
<td>15</td>
</tr>
<tr>
<td>40</td>
<td>3.45</td>
<td>15</td>
</tr>
<tr>
<td>45</td>
<td>3.11</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>2.98</td>
<td>12</td>
</tr>
<tr>
<td>55</td>
<td>2.74</td>
<td>11</td>
</tr>
<tr>
<td>60</td>
<td>3.00</td>
<td>12</td>
</tr>
<tr>
<td>65</td>
<td>2.65</td>
<td>11</td>
</tr>
<tr>
<td>70</td>
<td>2.76</td>
<td>12</td>
</tr>
</tbody>
</table>
Figure 2.—Blood Pressure (A) and respiration (B) in a chemoreceptor denervated dog (Dog No. 15). C: respiratory volume in increments of 3 liters. D—timer, recording intervals of 10 seconds and 1 minute. Unmarked segment: normal respiration and blood pressure. At 1: carotid arteries clamped bilaterally for 45 seconds. At 2: 1 mg. NaCN I.V. At 5: 3 mg./Kg. morphine sulfate I.V. At 6: 1 mg./Kg. nalorphine hydrochloride I.V.

records after the administration of morphine and also after nalorphine.

It has been quantitatively demonstrated in this experiment that neither bilateral carotid chemoreceptor
denervation nor bilateral vagotomy nor both significantly alter stimulation of depressed respiration by nalorphine.

Technique of Chemoreceptor Denervation.--The aortic glomus chemoreceptors may be denervated by simply separating the vagus nerves from their sheaths, doubly ligating them, and cutting between the ligatures.

Denervation of the carotid chemoreceptors is a little more complicated. The carotid artery is traced cephalad until the carotid sinus is located. This general area is cleared of all loose, peripheral connective tissue. One ligature is slipped around all the structures lying between the sinus and the external carotid artery (this should include the occipital artery and the carotid sinus-glomus nerves). A second ligature is slipped around the same structures and moved about 3-4 mm. from the first ligature. A cut is made between the ligatures (see Fig. 1-A, page 46). Completeness of denervation may be determined by injection of sodium cyanide. No change in amplitude of respiratory movements should be noted if the denervation is satisfactory.

It would appear that the mechanism of action of nalorphine in combatting narcotic respiratory depression
is primarily a central one. This inference is supported by the fact that there is no correlation between the increase in ventilation and the drop of blood pressure after intravenous administration of either morphine or nalorphine. Furthermore, the respiratory response was still elevated when the blood pressure had returned to its normal value. This, of course, does not necessarily eliminate the possibility of a partial contribution of a reflex pressure effect on ventilation.

A further indication that the effect of nalorphine is possibly central is demonstrated by the similar responses observed after denervation of the peripheral chemoreceptors.

The present experiment has as its objective the study of the effect of nalorphine on the electrical excitability of the respiratory center when depressed by morphine.

**Experimental Design and Procedure.**—This experiment was undertaken for the purpose of determining the action of nalorphine on the inspiratory center during deep morphine narcosis, making use of a rather new criterion for determining the state of activity of the respiratory center, namely, observation of responses to electrical stimulation, as well as rate and tidal air. This
technique was used by Wells et al. (71) and Robert de Ramirez de Arellano (72) in 1944 and 1947, respectively.

As indicated earlier, the inspiratory portion of the respiratory center has been accurately located in the ventral reticular formation of the medulla of the cat by means of the Horsley-Clarke stereotaxic instrument (53). Electrical stimulation of this region results in a marked inspiratory response involving both thorax and diaphragm with fixation of the chest in inspiration.

Since there is no direct evidence as to the precise effect of nalorphine on the respiratory center itself, it was decided to study the influence of this drug on the electrical excitability of the inspiratory region.

The mongrel dogs used in these experiments weighed 12.5 to 24 Kg. They were anesthetized with secobarbital sodium, 35 mg./Kg. Additional anesthesia was given in increments of 3 mg./Kg. as required during the surgical manipulations. The medulla was exposed from the dorsal aspect by placing the animal in a prone position and ventroflexing the head. After cutting the skin exactly in the midline from the protuberantia occipitalis downwards, the temporal muscles were detached from their dorsal insertions and retracted out of the way. Then the neck muscles were slowly detached from their insertions in the occiput by use of a fine spatula. Kaolin paste was used
liberally to control bleeding. This procedure exposes the atlanto-occipital membrane which was cut in the midline together with the dura. The membrane and the dura were drawn back to the muscles laterally. If necessary, the opening was widened by removing part of the occipital bone.

The inspiratory center was then localized by means of a unipolar electrode oriented either manually or by means of a Chambers micromanipulator. In either case the electrode holder was fixed to the head of the animal so that the electrode could not be displaced by any movement of the animal. The electrode consisted of a fine hypodermic needle stylet (27 gauge) which was varnished except at the very tip. An indifferent electrode consisting of a copper bar was inserted into the rectum to complete the circuit. A Grass¹ physiological stimulator (Model 3B) discharging at a frequency of 100 per second with a pulse delay of 2 milliseconds, delivered square-wave monophasic impulses. The least voltage required to produce and maintain inspiratory apnea was determined. This voltage, varying between 0.15 and 0.75 volts in the different animals, was designated as the threshold stimulus. The inspiratory response produced by the threshold stimulus was in every case much less than that produced by stimuli.

¹Grass Instrument Co., Quincy, Massachusetts.
of higher voltage. The trachea was intubated and the apparatus adjusted for recording on smoked kymograph paper as in the previous experiment. The inspiratory volume response to electrical stimulation of the center, and readings of tidal air, minute volume and rate were taken at intervals before any drugs, after morphine, and after nalorphine antagonized the morphine effects.

**Results.**—A total of 36 experimental dogs were used in obtaining the data reported below. Of this number, only 10 of the animals yielded satisfactory data. The remainder were unsuitable because of accidental damage to vital areas in the brain stem or because the inspiratory center could not be definitely localized as indicated by atypical physiological responses upon electrical stimulation.

The three figures which follow illustrate and summarize the results obtained in this series of studies. Figure 3 shows the results obtained in a single animal. These data are typical of those obtained in three dogs in which the inspiratory response was studied before and after the administration of morphine and after nalorphine antagonism of the effects of morphine on respiration.

Figure 4 (page 83) indicates the results obtained in a dog in which the peripheral chemoreceptors were bilaterally denervated. Here again the inspiratory response to
Figure 3. Intact dog. Stimulus constant at 0.6 V. throughout. At A, 1 mg./Kg. of morphine sulfate I.V. The resultant depression of respiratory activity is indicated in the graphs. At B, 1 mg./Kg. nalorphine HCl I.V. The improvement in the respiratory condition and increased sensitivity of the inspiratory center of the animal is evident. Similar qualitative results were obtained in two other dogs.
Figure 4.—Denervated dog. Stimulus constant at 0.38 V. throughout. At A, 1 mg./Kg. of morphine sulfate I.V. The resultant depression of most of the modalities of respiratory activity is indicated in the graphs. At B, 1 mg./Kg. nalorphine HCl I.V. The improvement in the condition of respiration and the increased sensitivity of the inspiratory center of the animal is evident. Similar qualitative results were obtained in two other animals.
electrical stimulation was studied before and after the administration of morphine and after nalorphine antagonism of the morphine-induced respiratory depression.

It was of interest to determine whether nalorphine might also increase the sensitivity of the inspiratory center to electrical stimulation in the absence of prior depression by morphine. Therefore, a series of experiments were carried out in which nalorphine was administered 30 minutes prior to the injection of morphine. The results of a typical experiment of this type are graphically illustrated in Figure 5 (page 85).

The results of this experiment may be summarized as follows:

1) Morphine decreases the sensitivity of the inspiratory center to electrical stimulation both in intact and chemoreceptor-denervated dogs.

2) Nalorphine increases the sensitivity of the morphine-depressed inspiratory center to electrical stimulation in intact dogs and in dogs in which the peripheral chemoreceptors have been denervated.

3) Nalorphine does not significantly alter the responsiveness of the non-depressed inspiratory center of the intact dog to electrical stimulation.

Morphine alone, 1 mg./Kg., was given to a dog in a control experiment. This animal showed no appreciable
Figure 5.—Intact dog. Stimulus constant at 0.71 V. throughout. At A, 1 mg./Kg. of nalorphine HCl I.V. Note the gradual depression of minute volume and lack of significant effect on response of the inspiratory center to electrical stimulation. At B, 1 mg./Kg. morphine sulfate I.V. Note the further depression of ventilation volume and rate and ineffectiveness of morphine upon the inspiratory response. Qualitatively similar results were observed in two additional dogs.
spontaneous recovery from morphine depression of the inspiratory response and of ventilation volume during the one and one-half hour period of observation.

**PART III**

It was reported by Winter and Flataker (73) in 1951 that cortisone antagonizes hypnosis and catalepsy as seen in rats. ACTH had the same effects.

Morphine can influence the secretory activity of both adrenal medulla and cortex in experimental animals. It can liberate adrenalin in dogs (74) and can deplete adrenal ascorbic acid and cholesterol in rats (75). This may be an indication of pituitary-adrenal activation (76). The rate of liberation of adrenal hormones may influence the depression and other activities of the potent narcotics. Further evidence of this is provided by the fact that adrenalectomy markedly increases the toxicity of morphine (77). MacKay and MacKay (78) found that the adrenal cortex hypertrophied when a rat was treated with increasing doses of morphine. More recently similar results have been observed by Sung, Way, and Scott (79) while studying rats made tolerant to morphine and methadone.

Since numerous other agents have been shown to deplete adrenal ascorbic acid and since there appear to be gaps in the knowledge as to the manner in which such compounds
exert this effect, studies were conducted in rats to investigate the relative degree of specificity of the adrenal response to morphine as well as to nalorphine, to determine whether nalorphine antagonizes or reverses the depletion of cholesterol and ascorbic acid of the adrenal by morphine, and to assess whether adrenocortical activation by morphine is an incidental effect due to stimulation of ACTH production by the anterior pituitary or, indeed, a significant effect from the standpoint of certain pharmacological actions of this narcotic.

**EXPERIMENT 10**

Simultaneous depletion of both ascorbic acid and cholesterol from the adrenal gland has been shown to be a fairly specific index of corticoid output. The association of Vitamin C with the formation and secretion of cortical hormone was first suggested by Zwemer and Lowenstein (80). They have isolated, from the adrenal, a water-soluble steroid in which they believe Vitamin C is attached to Ring D of the steroid nucleus apparently by carbon-to-carbon union. According to these investigators this compound which has a high degree of instability in water solution breaks down in an acid medium to form a steroid without cortical activity and Vitamin C. The latter has been isolated and its antiscorbutic properties
confirmed. If this claim is true, it would clarify the alterations in adrenal ascorbic acid that are observed following the injection of ACTH. It might also account for the rapid and early depletion of ascorbic acid in response to stimulation of the gland since this may represent the discharge of preformed hormone which is followed by a slower change in cholesterol as new quantities of hormone are synthesized.

**Experimental Design and Procedure**—The dinitrophenyl-hydrazine method has been utilized for the determination of adrenal ascorbic acid in rats injected with physiological saline solution (control), with morphine, with nalorphine and with the combination of the opiate and the antagonist. This method \((81, 82, 83)\) is used to determine ascorbic acid, dehydroascorbic acid, and diketogulonic acid. The result obtained may be a measure of only the ascorbic acid, or it may be a composite value for two or three components. In normal animals under ordinary dietary conditions, ascorbic acid occurs entirely, or almost entirely, in its reduced form. This assumption is consistent with the fact that animal tissues contain systems that reduce dehydroascorbic acid to ascorbic acid \((84, 85)\). Damron et al. \((86)\) found either zero values or trace amounts of dehydroascorbic acid and diketogulonic acid in
normal guinea pigs; only when abnormally large amounts of ascorbic acid, dehydroascorbic acid, or diketogulonic acid were injected into the animal was the presence of dehydroascorbic acid and diketogulonic acid observed in the tissues in appreciable amounts. Therefore, when applying this method to the analysis of tissues from normal animals under usual dietary conditions, it may be assumed that the values obtained are entirely ascorbic acid measurements.

**Principle of Reactions Involved.**—Ascorbic acid is oxidized to dehydroascorbic acid by mild oxidizing agents. One of the most useful oxidizing agents is Norit, having the double advantage of serving both as an oxidizing agent and a clarifying agent for the removal of pigment from animal tissue extracts. Dehydroascorbic acid undergoes spontaneous transformation into diketogulonic acid, slowly in mildly acid solution, and very rapidly in solution at a pH below 1.0 and in a neutral or alkaline medium. When treated with 2,4-dinitrophenylhydrazine, dehydroascorbic acid and diketogulonic acid form a derivative, a bis-2,4-dinitrophenylhydrazone, in which the 2,4-dinitrophenylhydrazine is coupled to carbon atoms 2 and 3. The rate of coupling with diketogulonic acid is faster than that with dehydroascorbic acid. There is evidence that coupling with 2,4-dinitrophenylhydrazine occurs only with
diketogulonic acid and that dehydroascorbic acid must undergo transformation to diketogulonic acid before coupling takes place (87).

When the bis-2,4-dinitrophenylhydrazone of dehydroascorbic acid or diketogulonic acid is treated with 85% H₂SO₄, the derivative undergoes a molecular rearrangement and a highly stable, reddish brown product is formed which absorbs maximally at 500 to 550 and 350 to 380 millimicrons. The color obtained in this way may be measured photometrically.

**Specificity of the Method.**—The dinitorphenylhydrazine method has a high degree of specificity. The color used is given by 2,4-dinitrophenylhydrazine derivatives of six-carbon and five-carbon sugarlike compounds. The rate of coupling of sugars with 2,4-dinitrophenylhydrazine is very slow in comparison with that of the oxidation products of ascorbic acid. The osazones of the sugars also decompose in sulfuric acid with the high acid concentrations used; this is one reason for waiting 30 minutes after developing the color before taking readings in the spectrophotometer. The coupling reaction is carried out in a medium containing the reducing agent thiourea—a provision that avoids interference from non-ascorbic acid chromogens. Investigators have found no
interference when this method is applied to animal
tissues. It is well adapted to all types of animal ex­
perimention.

One of the outstanding difficulties in the use of
some methods for the determination of ascorbic acid is
presented by the effect of metal ions, such as Cu++,
Cu+, Fe++, Fe++, and Sn++, which may catalyze the
oxidation of ascorbic acid or may reduce the dye. These
metal ions have no effect in the dinitrophenylhydrazine
method; oxidation is an essential step in the analysis
and the coupling with 2,4-dinitrophenylhydrazine is
carried out under reducing conditions. The advantages
of the dinitrophenylhydrazine method in the analysis of
products containing metal ions are well shown by the
work of Chapman, et al. (88).

Reagents.—The following reagents were required
for determination of adrenal ascorbic acid:

1) 6% trichloroacetic acid solution, used for
extraction of the glands.

2) Dinitrophenylhydrazine-thiourea reagent, made
by dissolving 2 Gm. of 2,4-dinitrophenylhydrazine in
100 ml. of approximately 9 N H₂SO₄ and then adding 4 Gm.
of reagent grade thiourea, shaking until dissolved, and
filtering. This reagent was kept in a refrigerator,
filtered occasionally and freshly prepared biweekly.
It was checked before each use for the presence of active reducing agent by placing 2 ml. of 1% HgCl₂ in a test tube and adding the reagent dropwise. The addition of 3-5 drops produced a copious precipitate of HgCl if adequate thiourea were present.

3) Acid-washed Norit. This was prepared by placing 200 Gm. of Norit in a large flask and adding 1 liter of 10% HCl. This was heated to boiling; then filtered with suction. The cake of Norit was removed to a large beaker, washed thoroughly with 1 liter of distilled water and filtered again. The washing procedure was repeated once and the Norit allowed to dry overnight in an oven at 110-120° C.

4) 85% sulfuric acid.

**Extraction and Processing of Tissues.**—Albino Wistar strain rats, weighing 150-225 Gm., were injected subcutaneously with the respective drugs and exactly 90 minutes later were killed by stunning, decapitated and exsanguinated. Both adrenal glands were removed taking care to preserve the integrity of the connective tissue capsules but removing any adhering fat. The capsule was preserved because its removal is associated with a stripping away of a portion of the layer of the zona glomerulosa cells of the cortex, which is undesirable
in a determination of this kind. The pair of glands was immediately weighed on a direct-weighing analytical balance\(^1\) to the nearest 0.0001 Gm. This procedure occupied approximately the same time in each experiment, so that any loss in weight due to drying could be assumed to be approximately constant. Immediately after weighing, the glands were placed in a test tube containing 5 ml. of 6% trichloroacetic acid.

The tissues were ground and macerated in a mortar with acid-washed sand, using small portions of the 6% trichloroacetic acid solution. An amount of extracting fluid was taken which was estimated to yield 1 to 10 micrograms of ascorbic acid per ml. of fluid. It was found that a total volume of 50 ml. was sufficient in all cases. Trichloroacetic acid must be present in the extracting fluid to precipitate the protein, to prevent adsorption of the vitamin on the Norit and to facilitate oxidation. Ascorbic acid is not oxidized quantitatively by Norit unless the extracting solution contains trichloroacetic acid, which is preferentially adsorbed on the Norit, eluting active oxygen in quantities sufficient for rapid oxidation.

When the extraction was complete, 1 Gm. of acid-washed Norit was added to the extract. The extract was shaken vigorously and filtered.

\(^1\)Christian Becker Projectomatic Balance, Christian Becker Company, Inc.,
Four ml. of the Norit filtrates were pipetted into clean test tubes. To all but one (the blank) of the tubes was added 1 ml. of dinitrophenylhydrazine-thiourea reagent. Then all the tubes were placed in a water bath maintained at 37° C.; the bath was equipped with an efficient thermostat to maintain a constant temperature. The tubes were left immersed in the water bath for 3 hours. They were then removed and placed in a pan of ice water containing generous quantities of ice. To all of the tubes in the ice bath 5 ml. of 85% H₂SO₄ was added very slowly from a buret, one drop at a time over a period of about one minute. Concentrated sulfuric acid was used for lubricating the stopcock of the buret instead of stopcock grease. One ml. of dinitrophenylhydrazine-thiourea reagent was added to the blank tube. All the tubes were shaken thoroughly while in the ice water to obtain complete mixing, and then removed to a rack. After 30 minutes the contents of the tubes were transferred to ½-inch matched test tubes (cuvettes) and the transmittance values read in a photoelectric colorimeter. The blank tube was used to set the colorimeter at 100% transmittance. The diffraction grating monochromator was set to transmit at 540 millimicrons.

¹Bausch & Lomb Spectronic 20 Colorimeter, Bausch & Lomb Optical Co., Rochester 2, N. Y.
Calibration Curve and Calculations.—Since quite extensive work was done with ascorbic acid, it was desirable to prepare a calibration curve or chart. This curve was prepared by using the same colorimeter and the same reagents which were used in the method.

One hundred mg. of ascorbic acid of the highest purity (as determined by melting point data) was dissolved in 100 ml. of the 6% trichloroacetic acid solution. This then was oxidized with Norit as described above. Ten ml. of the dehydroascorbic acid solution obtained was pipetted into a 500 ml. volumetric flask, and made up to the mark with the acid solution.

A series of standard solutions were prepared by pipetting 5, 10, 20, 30, 40, 50, and 60 ml. of the diluted standard solution into 100 ml. volumetric flasks and making up to volume with the trichloroacetic acid solution. Four ml. of each of these standard solutions was pipetted into the colorimeter tubes and treated in the manner described above for the Norit filtrates of the tissue extracts. A calibration chart was prepared by plotting, on semilogarithmic paper, the percentage of transmittance on the ordinate scale and the concentration of ascorbic acid (in micrograms) on the abscissa. A total of three separate calibration curves were constructed; all of these proved to be practically coincidental.
With a standard calibration curve the following equation could be used for calculating the content of ascorbic acid in tissues:

\[
\text{Micrograms from curve } \times \frac{\text{ml. extract}}{1000} \times \frac{100}{\text{Sample wt. in Gm.}} = \text{mg./100 Gm. of tissue.}
\]

Results.—The results of this experiment are summarized in Table XIII. The column headed \( P \) indicates the probability that the respective values differ from the control values by chance.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Ascorbic Acid (mg./100 Gm. ( \pm ) S.E.)</th>
<th>( P )</th>
<th>No. of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline controls— 1 ml./Kg.</td>
<td>315 ( \pm ) 13</td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>Morphine sulfate— 20 mg./Kg.</td>
<td>222 ( \pm ) 11</td>
<td>0.01</td>
<td>18</td>
</tr>
<tr>
<td>Nalorphine HCl— 20 mg./Kg.</td>
<td>314 ( \pm ) 11</td>
<td>&gt;0.90</td>
<td>6</td>
</tr>
<tr>
<td>Morphine sulfate / nalorphine HCl— of each, 20 mg./Kg.</td>
<td>311 ( \pm ) 18</td>
<td>0.90</td>
<td>12</td>
</tr>
</tbody>
</table>

*The drugs were allowed to exert their action for 90 minutes before the animals were sacrificed.
This data indicates that nalorphine antagonizes morphine depletion of adrenal ascorbic acid when the two drugs are administered simultaneously. Nalorphine given alone does not produce any significant depletion of adrenal ascorbic acid.

**EXPERIMENT II**

It may be assumed that an increased output of hormone, particularly in a gland that possesses only a small reserve, is accompanied by characteristic metabolic changes associated with the synthesis and release of the hormone. This is particularly so when a precursor or an essential constituent of the hormone is present in the gland in quantities much greater than those present in the other tissues. One instance of this is the presence in the adrenal cortex of cholesterol which appears to be specifically related to the formation of the hormone and which appears to reflect the changes in its secretion.

The outstanding chemical characteristics of the adrenal cortex are its high content of cholesterol and ascorbic acid. No other organ of the body except the corpus luteum approaches the organ in its high concentrations of both these substances.

Another characteristic of the adrenal cholesterol is that approximately 90% of it is present in the gland
in ester form. This is to be compared with 50% of ester found in the liver and 10% found in the brain. It is generally understood that a high proportion of esterified cholesterol is indicative of a high rate of turnover of both the steroid and its associated fatty acids. The evidence for the ultimate conversion of cholesterol into the cortical hormones by the cells of the gland is even at present quite indirect. The circumstantial evidence, however, appears to be strong, and this view is strengthened even more by the demonstration that cholesterol is converted into progesterone in man (89).

Experimental Procedure.—The method of Sperry and Webb (90) has been used for the determination of adrenal cholesterol in rats injected subcutaneously with physiological saline solution (controls), with morphine, with nalorphine, or with the combination of the narcotic and allyl antagonist.

The special reagents required for this study are outlined below:

1) Solvents: Acetone-absolute ethanol (1:1)
   Acetone-ether (1:2)
   Ether

All of these solvents were redistilled. The ether was tested for peroxide content by shaking with 10% potassium
iodide in the dark for a period of one hour. If any color developed, the ether was treated with an acidified 5% ferrous sulfate solution to purify the ether.

2) 0.5% digitonin solution in 50% ethanol.
3) 50% KOH.
4) Glacial acetic acid and 10% glacial acetic acid.
5) Acetic anhydride, 99-100% pure.
6) Cholesterol solution containing 100 mg./100 ml.
in glacial acetic acid. Standards for preparation of a calibration curve were made by diluting this solution with glacial acetic acid. The cholesterol was purified before use by recrystallizing it from anhydrous alcohol and drying in a vacuum desiccator over phosphorus pentoxide.

The albino Wistar strain rats, weighing 150-240 Gm., were injected subcutaneously with the respective drugs and the adrenal glands were removed much the same as in Experiment 10. The tissues were removed either 1.5 or 3 hours postinjection as indicated in a subsequent table. The tissues were weighed rapidly and placed in conical test tubes to which had been added 2 ml. of the alcohol-acetone mixture. The tissues were minced by grinding with a pinch of sand and closely fitting stirring rod. The extractive mixture was heated to boiling by immersion in a hot water bath and then it was allowed to cool to room temperature. The supernatent solution was decanted into
a 10 ml. volumetric flask. The tissue was re-extracted several times more in the same manner with small volumes of the same solvent. After the contents of the volumetric flask cooled to room temperature, it was made up to volume and mixed. The mixture was filtered through hot-alcohol-washed filter paper into a clean test tube. Rapid filter paper was used to minimize evaporation of the solvent. One ml. aliquots of the clear solution were used for determination of total cholesterol by the above-indicated method.

The one ml. aliquots were placed in dry, graduated, 12 ml. centrifuge tubes containing one drop of 50% KOH. The mixture was then stirred with a glass rod until no droplets of alkali solution were seen at the tip of the tube.

Meanwhile, a large amber glass jar containing a layer of sand 3 cm. in depth was heated until the temperature of the sand was 45°C. The above tubes were then placed in the sand; the jar was tightly covered and placed in an incubation oven at a temperature of 38°C for 30 minutes. This procedure is necessary to hydrolyze the cholesterol esters present in the sample.

When this was complete, the tubes were removed to a rack and cooled to room temperature. The stirring rods were raised and the acetone-ethanol mixture added to the 2 ml. graduation. The excess alkali was neutralized to
the phenolphthalein end-point with 10% acetic acid (from 5 to 7 drops were usually required). An extra drop of acetic acid and 1 ml. of the digitonin solution were then added and the mixture stirred thoroughly with the glass rods, which remained in their respective tubes throughout the procedure except during the centrifugation to be described subsequently. The tubes were again placed in the sand-containing jar, which was covered tightly and left at room temperature. At least 16 hours were allowed for complete precipitation of cholesterol digitonide. Digitonin forms an insoluble complex with cholesterol, but not with its esters. This reaction, in general, is given by those sterols in which the C3 hydroxyl group and the C10 methyl group lie on the same side of the molecule plane (cis-hydroxy sterols). Although the exact nature of the reaction is obscure, the C3 hydroxyl must be free; sterol esters do not react.

At the end of this time the tubes were transferred to a rack. The contents were stirred gently to free the precipitate which adheres to the walls of the tube near the surface of the liquid. The rods were removed without contact with the upper part of the tube and placed on a rack without loss of adhering precipitate. The tubes were now centrifuged for 15 minutes at about 2800 r. p. m. If any particles were still suspended at the end of this
time, the tubes were re-centrifuged at a higher speed. The centrifugate was decanted and the tubes allowed to drain for a few minutes; the last drop was removed by touching the lip of the tube to a clean towel.

The rods were returned to their respective tubes. The walls of the tubes and the rods were washed with 1.5-2.0 ml. of the acetone-ether mixture. The contents were stirred thoroughly, the rods returned to the rack and the centrifugation repeated for a duration of 5 minutes. The centrifugate was again decanted and the precipitate washed once more in the same manner with ether. The tubes were centrifuged a third time and removed to a rack. The rods were returned to their proper tubes which were then placed in a water bath at 40°C for two or three minutes in order to secure complete drying of the precipitate.

The tubes were now placed in the order of development and reading of color in a sand bath at 110-115°C for 30 minutes. The sand bath was removed from the oven. One ml. of glacial acetic acid was added to the first tube while still in the hot sand. The contents were stirred vigorously and the tube was left in the sand while acid was added to the next 2 or 3 tubes (2 or 3 minutes in all). The solution was again stirred and the tube was removed from the sand, cooled, and placed in a
water bath maintained at 25° C. and kept in the dark. This process was continued until all the tubes were in the water bath in the order of reading of the color.

An amount of acetic anhydride sufficient for the number of samples to be read was placed in a glass stoppered flask and chilled in an ice bath. Then concentrated H₂SO₄ was added in the proportion of 1 ml. to 20 ml. of acetic anhydride with agitation during addition. The stopper was inserted and the flask removed from the bath and shaken vigorously for a few moments and returned to the bath.

About 10 minutes later, when the reagent had chilled, the first tube was removed from the 25-degree water bath, wiped dry and 2 ml. of the sulfuric acid-acetic anhydride reagent added. The contents was stirred vigorously, the rod was removed and the tube was returned to the water bath. The above process was repeated with each tube in the series at regular time intervals such that the samples could be read in the colorimeter 30 to 31 minutes after the addition of the sulfuric acid-acetic anhydride reagent. The samples were transferred to ½-inch test tubes (cuvettes) and the transmittance values read in a photoelectric colorimeter¹. A blank sample containing 1 ml. of acetic acid and 2 ml. of the sulfuric acid-acetic anhydride

¹Bausch & Lomb Spectronic 20 Colorimeter, Bausch & Lomb Optical Co., Rochester 2, N. Y.
Calibration Curve and Calculations.—Again it was desirable to prepare a calibration curve for cholesterol much the same as the one prepared for ascorbic acid in the preceding experiment.

One hundred mg. of cholesterol which had been re-crystallized and stored carefully was dissolved in 100 ml. of glacial acetic acid with the aid of gentle heat. From this solution a series of standards were prepared containing 0.04, 0.06, 0.08, 0.10, 0.12 and 0.15 mg. of cholesterol per ml., respectively. One ml. aliquots of each of these standard solutions were pipetted into colorimeter tubes and the color-developing sulfuric acid-acetic anhydride reagent added. A calibration chart was prepared by plotting, on semilogarithmic paper, the percentage of transmittance on the ordinate scale and the concentration of cholesterol (in milligrams) on the abscissa. A total of four separate calibration curves were constructed; all of these curves proved to be practically coincidental.

With a standard calibration curve, the following equation could be used for calculating the content of
cholesterol in the tissues:

\[
\text{Milligrams from curve} \times \frac{\text{ml. extract}}{1000} \times \frac{100}{\text{Sample wt. in Gm.}} = \frac{\text{Gm.}}{100 \text{ Gm. of tissue}}.
\]

Results.—The results of this experiment are summarized in Table XIV (page 106). The column in the table headed \( P \) indicates the probability that the values differ from the respective control values by chance.

It is seen from this table that, although morphine did not cause a significant depletion of cholesterol, the action of nalorphine when administered simultaneously with morphine was in the direction of an increase in the amount of this sterol in the adrenal glands when determined \( 1\frac{1}{2} \) hours postinjection. It was reported by Long (91) that the depletion of cholesterol after ACTH is maximal at 3-6 hours. Therefore, the second series of animals were exposed to the action of the various drugs for 3 hours before removal of the glands. In these animals morphine produced a significant depletion of cholesterol as did also nalorphine. When the animals were injected with both the opiate and the allyl antagonist and the glands analyzed after 3 hours, there was a very marked depletion of cholesterol.
Table XIV.—Adrenal Cholesterol of Rats as Affected by Various Drugs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol (Gm./100 Gm. ± S.E.)</th>
<th>F</th>
<th>No. of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Saline controls— 1 ml./Kg.</td>
<td>3.73 ± 0.15</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>*Morphine sulfate— 20 mg./Kg.</td>
<td>3.71 ± 0.16</td>
<td>&gt;0.90</td>
<td>12</td>
</tr>
<tr>
<td>*Morphine sulfate / nalorphine HCl— of each, 20 mg./Kg.</td>
<td>5.11 ± 0.18</td>
<td>&lt;0.01</td>
<td>11</td>
</tr>
<tr>
<td>**Saline controls— 1 ml./Kg.</td>
<td>4.36 ± 0.08</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>**Morphine sulfate— 20 mg./Kg.</td>
<td>3.86 ± 0.06</td>
<td>0.02</td>
<td>6</td>
</tr>
<tr>
<td>**Nalorphine HCl— 20 mg./Kg.</td>
<td>3.89 ± 0.11</td>
<td>&lt;0.10</td>
<td>5</td>
</tr>
<tr>
<td>**Morphine sulfate / nalorphine HCl— of each, 20 mg./Kg.</td>
<td>2.88 ± 0.07</td>
<td>&lt;0.001</td>
<td>6</td>
</tr>
</tbody>
</table>

*Duration of drug action was 1.5 hours.
**Duration of drug action was 3.0 hours.
In 1951 Winter and Flataker (73) reported that exogenous cortisone markedly reduces the threshold for the effectiveness of nalorphine antagonism of dl-methadone hydrochloride in rats. It seemed desirable to design an experiment which would yield information concerning the significance of the integrity of the adrenal glands and endogenous corticoids to the activity of nalorphine in combatting the acute toxic depressive effects of morphine. For this reason the present study was undertaken.

**Experimental Design and Procedure.**—The essential design of this experiment was that of an acute toxicity study conducted in both normal rats and in rats which were adrenalectomized and maintained only on water and Purina dog pellets. Preliminary studies conducted in several rats indicated that bilaterally adrenalectomized rats will survive at least five days post-adrenalectomy. Of this group the first rat died on the sixth day after extirpation of the glands; the remainder survived at least two weeks.

The LD$_{50}$ of morphine sulfate was determined in both normal rats and in rats three days after adrenalectomy. A 24-hour period of observation was chosen. The doses
administered to the various dose groups versus the per cent mortality were plotted on logarithmic probability paper in order to obtain dose-per cent effect curves. The Litchfield and Wilcoxon (92) graphical method was used for determining whether a straight line fitted the plotted points satisfactorily and for estimating the LD$_{50}$ with confidence limits.

From the same curves, the respective LD$_{99}$s were also determined. The LD$_{99}$s determined for the normal and adrenalectomized rats were administered to other similar groups of normal and adrenalectomized animals. The secondary groups of rats, however, also received, together with the LD$_{99}$s of morphine sulfate, graded doses of nalorphine hydrochloride. The dose of the antagonist necessary to protect 50% of the animals against death produced by the LD$_{99}$ of morphine sulfate was determined. This dose of nalorphine hydrochloride was designated as the PD$_{50}$.

**Results.**—The results of these toxicity studies are recorded in Table XV (page 109). Figures 6, 7, and 8 (pp. 110, 112, 114) illustrate the dose-effect curves from which the LD$_{50}$s and the LD$_{99}$s of morphine sulfate and the PD$_{50}$ of nalorphine hydrochloride were determined.
Table XV.—LD\textsubscript{50} and LD\textsubscript{99} of Morphine Sulfate and PD\textsubscript{50} of Nalorphine Hydrochloride in Normal and Adrenalectomized Rats (in mg./Kg.).

<table>
<thead>
<tr>
<th></th>
<th>Normal Rats</th>
<th>Adrenalectomized Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD\textsubscript{50}</td>
<td>57 (40 to 81)</td>
<td>13 (8.4 to 20)</td>
</tr>
<tr>
<td>LD\textsubscript{99}</td>
<td>140</td>
<td>79</td>
</tr>
<tr>
<td>PD\textsubscript{50}</td>
<td>2.6 (1.6 to 4.1)</td>
<td>Indeterminable</td>
</tr>
</tbody>
</table>

The PD\textsubscript{50} for nalorphine could not be determined in adrenalectomized rats inasmuch as doses below 20 mg./Kg. produced 100% mortality. A dose of 20 mg./Kg. resulted in two survivals out of ten injected animals, while 50 mg./Kg. protected only one animal in ten from acute death from the LD\textsubscript{99} of morphine sulfate.
FIG. 6
MORPHINE DOSE EFFECT LINE
LD₅₀ AND LD₇₀ NORMAL RATS

PER CENT DEAD

DOSE (MG./KG.)
Calculations for Figure 6. (LD$_{50}$ and LD$_{99}$ of Morphine Sulfate in Normal Rats).

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. Dead</th>
<th>Observed Percentage</th>
<th>Expected Percentage</th>
<th>O-E</th>
<th>Diff. to (Chi)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0/6</td>
<td>0.0 (1.05)</td>
<td>0.35</td>
<td>0.7</td>
<td>0.014</td>
</tr>
<tr>
<td>35</td>
<td>1/6</td>
<td>16.7</td>
<td>10.3</td>
<td>6.4</td>
<td>0.045</td>
</tr>
<tr>
<td>60</td>
<td>4/9</td>
<td>44.4</td>
<td>55.0</td>
<td>10.5</td>
<td>0.050</td>
</tr>
<tr>
<td>85</td>
<td>9/10</td>
<td>90.0</td>
<td>84.8</td>
<td>5.2</td>
<td>0.021</td>
</tr>
<tr>
<td>100</td>
<td>10/10</td>
<td>100.0 (97.6)</td>
<td>92.6</td>
<td>5.0</td>
<td>0.038</td>
</tr>
</tbody>
</table>

$$K = 5 \quad N^1 = 9 \quad n = 3 \quad \sqrt{N^1} = 3$$

Animals/dose = 8.2

$$LD_{84} = 84/57 = 1.47$$
$$LD_{50} = 57/39 = 1.46$$
$$LD_{16} = 39/2.93 = 13.3$$

$$S = \frac{2.93}{2} = 1.47$$
$$fLD_{50} = 82.77/3$$

$$LD_{50} \times fLD_{50} = 81$$

$$fLD_{50} = 1.42 \quad LD_{50}/fLD_{50} = 40$$

LD$_{50}$ with 19/20 confidence limits:---

57 (40 to 81) mg./Kg.

LD$_{99}$:--- 140 mg./Kg.
FIG. 7
MORPHINE DOSE EFFECT LINE.
LD₅₀ AND LD₇₉ ADRENALECTOMIZED RATS

PER CENT DEAD

DOSE (MG./KG.)

5 10 20
Calculations for Figure 7. (LD$_{50}$ and LD$_{99}$ of Morphine Sulfate in Adrenalectomized Rats).

<table>
<thead>
<tr>
<th>Dose</th>
<th>No.</th>
<th>O.</th>
<th>E.</th>
<th>O-E</th>
<th>Contrib. to (Chi$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1/10</td>
<td>10</td>
<td>11.4</td>
<td>1.4</td>
<td>0.002</td>
</tr>
<tr>
<td>10</td>
<td>4/10</td>
<td>40</td>
<td>37.5</td>
<td>2.5</td>
<td>0.003</td>
</tr>
<tr>
<td>20</td>
<td>7/10</td>
<td>70</td>
<td>71.5</td>
<td>1.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\[ K = 3 \]
\[ \sqrt{N^1} = 4.47 \]
\[ n = 1 \]

Animals/dose = 10

\[ \text{LD}_{84} = 28/13 = 2.15 \]
\[ \text{LD}_{50} = 13/5.9 = 2.20 \]
\[ \text{LD}_{16} = 4.35/5.9 = 0.35 \]

\[ S = \frac{4.35}{2} = 2.18 \]

\[ f_{LD_{50}} = S^2 \cdot 77/4.47 \]

\[ LD_{50} = 20 \]

\[ f_{LD_{50}} = 1.55 \]

\[ LD_{50}/f_{LD_{50}} = 8.4 \]

LD$_{50}$ with 19/20 confidence limits:

13 (8.4 to 20) mg./Kg.

LD$_{99}$:

79 mg./Kg.
FIG. 8
NALORPHINE DOSE EFFECT LINE
50% NORMAL RATS
Calculations for Figure 8. (PD<sub>50</sub> of Nalorphine Hydrochloride in Normal Rats Injected With an LD<sub>99</sub> of Morphine Sulfate.

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. Dead</th>
<th>O. E.</th>
<th>0-E</th>
<th>Contrib. to (Chi)&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.79</td>
<td>1/10</td>
<td>10</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>1.4</td>
<td>4/10</td>
<td>40</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>2.5</td>
<td>4/10</td>
<td>40</td>
<td>48</td>
<td>8</td>
</tr>
<tr>
<td>4.4</td>
<td>7/10</td>
<td>70</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td>7.9</td>
<td>10/10</td>
<td>100(96.5)</td>
<td>89</td>
<td>11</td>
</tr>
</tbody>
</table>

K = 5  \[ \frac{N^1}{n} = 30 \]
\[ n = 3 \]
\[ \sqrt{\frac{N^1}{n}} = 5.47 \]
Animals/dose = 10

PD<sub>84</sub> = 6.4
\[ \frac{2.46}{2.46} \]
PD<sub>50</sub> = 2.6
\[ \frac{2.41}{2.41} \]
PD<sub>16</sub> = 1.08
\[ \frac{4.87}{4.87} \]

\[ S = \frac{4.87}{2} = 2.44 \]

\[ t_{PD50} = S^2 / \sqrt{5.47} \]
\[ PD_{50} \times t_{PD50} = 4.1 \]
\[ t_{PD50} = 1.59 \]
\[ PD_{50} / t_{PD50} = 1.6 \]

PD<sub>50</sub> with 19/20 confidence limits:

2.6 (1.6 to 4.1) mg./Kg.
DISCUSSION

A study has been made of nalorphine antagonism of the undesirable depressive effects of morphine in an effort to study the possible site(s) and mechanism(s) of action of the former drug.

Part I of this investigation involved screening for an anesthetic agent, the respiratory depressant effects of which were not reversed by nalorphine. It was found that nalorphine successfully antagonizes the depressant effects of certain barbiturates, such as pentobarbital sodium and amobarbital sodium. Additional studies indicated that it did not antagonize the effects of either urethane or secobarbital upon respiration of the dog. Secobarbital was employed as an anesthetic in experiments designed to determine the minimum dose of nalorphine required to prevent respiratory depression produced by morphine in dogs.

Part II describes studies undertaken for the purpose of investigating the importance of the peripheral chemoreceptors in reference to the site and mechanism of the antagonistic action of nalorphine against morphine. Here also the action of nalorphine on the medullary inspiratory center was studied by the use of another criterion for determining the state of activity of the center, namely, the observation of responses to direct electrical stimulation. These responses were compared and correlated.
with data simultaneously obtained on minute volume, tidal air and respiratory rate.

The third section of this investigation (Part III) sought to partially elucidate the relationships between the pituitary-adrenal axis and the respective effects of morphine and nalorphine.

From the data obtained in Part I, it is apparent that nalorphine possesses a stimulatory effect upon the respiration of dogs even when depressed by certain drugs of the barbiturate series. Rather high doses were employed here because it was desired to determine whether or not an antagonistic effect actually existed. The dosages of nalorphine were sufficient to stimulate a deeply depressed animal even during apnea, yet in animals with rather light anesthesia (as in the dogs anesthetized with secobarbital) no harmful effect was observed. Occasionally body movements and a return of reflexes were noticed but no convulsions were ever produced, although not infrequently there was an increase in intestinal peristalsis, resulting in defecation.

The increased respiratory response was primarily an effect of nalorphine itself and not due to the spontaneous lightening of the anesthesia level as evident from observations on animals in which the respiration after pentobarbital or amytal alone remained unchanged.
for as long as five hours.

It is probable that the mechanism of action of nalorphine in combatting barbiturate depression is primarily a central one. This implication is supported by the observations of Vivante et al. (49) who reported that pentobarbital depression of the respiration in dogs is antagonized by nalorphine even after denervation of the peripheral chemoreceptors. That cortical influences are not involved was also evidenced from their observations that decerebration of the animal did not alter the stimulatory response produced by nalorphine.

The observations that nalorphine antagonizes the respiratory depression produced by certain barbiturates as well by morphine would imply that nalorphine does not have a specific action. Since barbiturates and opiates possess different chemical configurations, any hypothesis regarding the specificity of action of nalorphine against depression produced by opiates only seems unnecessary, at least in vivo.

If an analogy exists between the regulation of respiration in the dog and man, it seems justifiable to conclude that nalorphine deserves extensive clinical trials in cases of depression by the barbiturates, pentobarbital and amobarbital.

That a species difference may exist in respect to
nalorphine antagonism of barbiturate effects is indicated by the observations of Boyd et al. (93). They obtained no evidence that single doses of nalorphine hydrochloride significantly affected the respiratory rate or duration of narcosis in albino rats under pentobarbital sodium anesthesia.

Marshall and Kosenfeld (94) observed that in cats and dogs anesthetized with sodium phenobarbital and also injected with morphine, oxygen reduced respiratory minute volume and this effect was abolished by denervation of the sino-aortic mechanism. This was explained on the basis that, after morphine, respiration is largely maintained by sino-aortic chemoreceptor reflex mechanisms for which anoxemia is the adequate stimulus. Schmidt (95) found that morphine reduced the sensitivity of the respiratory center to CO₂ but enhanced the sensitivity of the carotid body to cyanide. Similar effects of morphine on the respiratory center and chemoreceptor activity were reported by Dripps and Dumke (96).

Henderson and Rice (97) found that, in urethanized rabbits, 3 to 5 mg./Kg. of morphine slowed respiratory rate and frequently reduced tidal volume. Concomitantly, all vagal respiratory reflexes were enhanced; i.e., morphine enhanced the respiratory accelerating effects of continuous low frequency (10 to 20 per sec.) stimulation
of the proximal end of the severed vagus nerve, the accelerating effects of high frequency (50 to 75 per sec.) stimulation during inspiration and the slowing effect of high frequency stimulation during expiration. These investigators concluded that, since such effects are also noted under conditions of lowered alveolar CO₂, the similar effects of morphine could be explained on the basis of lowered CO₂ in the respiratory center and reflex arcs even though the drug produces a rise in alveolar CO₂ concentration, or lowered sensitivity of the central mechanism to CO₂.

Enhancement of the carotid sinus pressure reflex by morphine in man has been observed by Rovenstine and Cullen (98). Marri and Hauss (99) have reported that morphine enhances respiratory reflexes in response to stimulation of pressoreceptors in the carotid sinus.

In view of these reports and the similarity of the structural configuration of morphine and nalorphine, it was felt that perhaps the action of the latter drug might be subserved by chemoreceptor, pressoreceptor or vagal stretch reflexes. However, Experiment 9 further indicated that the effect of nalorphine is probably central since stimulation of morphine-depressed respiration was still observed after denervation of the peripheral chemoreceptors. In addition, there was no correlation between the increase
in ventilation and the transient hypotension produced by nalorphine under the experimental conditions (see Figure 2 and Table XI). The respiratory response was still evident when the blood pressure had returned to its normal level. Furthermore, denervation of the pressoreceptors as tested by clamping of the carotid arteries bilaterally and of the chemoreceptors as tested by injection of cyanide would necessarily eliminate the possibility of a partial contribution of a reflex effect on ventilation.

The data reported in Experiment 10 indicates that nalorphine, administered to dogs depressed by morphine, produces a considerable stimulation or increase in sensitivity of the inspiratory portion of the respiratory center to electrical stimulation. This occurs both in intact dogs and in those animals in which the peripheral aortic and carotid sinus chemoreceptors have been denervated.

A significant observation, however, was the fact that nalorphine does not significantly alter the responsiveness of the non-depressed inspiratory center of the intact dog to electrical stimulation. This observation may be the answer to the question of why nalorphine did not antagonize the effects of urethane or secobarbital upon respiration. It is well known that urethane acts quite satisfactorily on animals to produce a rapid and profound narcosis with little change in the circulation
or respiration. Accordingly Dog No. 4 anesthetized with urethane exhibited mean control ventilation volume of 4.14 liters per minute (Table V). The same dog anesthetized with amobarbital sodium had a mean control ventilation volume of only 2.92 liters per minute (Table IV). This may indicate that urethane does not depress respiration to any large extent. Similarly, the dosage of secobarbital sodium (25 mg./Kg.) used may also have very insignificant effect upon respiration. Therefore, perhaps because the respiratory center was not depressed by these two anesthetic agents, nalorphine showed no stimulation.

It is difficult to explain why nalorphine exerts its effects only on the previously depressed respiratory center. With the knowledge presently at hand, it may only be said that physiologically altered functions are restored to their normal or nearly normal states by medicinal agents more readily than normal functions are changed by the same agents. An outstanding example of this is the lack of typical pharmacological activity of digitalis and its preparations on the normal heart. Thus while the output of the normal heart is diminished or unchanged by digitalis preparations.

It would be extremely desirable to carry out further experiments to test the effect of nalorphine on the
inspiratory center under various other conditions:

(1) in the absence of any nalorphine effect on higher centers, as in the decerebrate animal with respiration depressed by morphine; (2) in the absence of any depressant agent whatsoever, as in a dog decerebrated under ether anesthesia and allowed to recover. Respiration in the latter case would be in a state of depression not associated with a drug effect.

It is interesting to note that nalorphine is not alone in having an action only in the presence of a previously depressed center. It has been reported that the greater part of the stimulatory action of metrazol on both the inspiratory and expiratory portions of the respiratory center depends on the presence of an actual depression, particularly that produced by barbituric acid derivatives (72).

There are many examples of drugs in addition to nalorphine which may reduce the response produced by another; such drugs are known as antagonists. Such antagonism may be mutual or unidirectional. Nalorphine-morphine antagonism appears to be of the latter variety. Three types of antagonism have been described. They are termed physiological, chemical, and competitive.

Physiological antagonism is a term used to describe the situation in which two drugs acting on different or
identical systems produce effects which are mutually antagonistic. For example, nitroglycerin causes a fall in blood pressure and epinephrine causes a rise.

Chemical antagonism implies that the active drug combines chemically with another drug, its antagonist to form thereby a compound with either no activity or substantially less than the original activity. Under certain circumstances the body itself may be considered as providing the antagonist. The acetylation of the sulfonamides in the body is an example of this process.

Because of their greater prevalence and usefulness, the competitive antagonists are perhaps of more interest. Ever since pharmacological investigations of nalorphine were first conducted, this drug was reputed to exert its actions against morphine by acting as a competitive antagonist. In this category are those agents which have as their characteristic action the ability to interfere with the eventual combination between the stimulus and the responding system. As the name implies, this interference exists in the form of a competition between the agonist and its antagonist for a particular site of action for which both have an affinity. Most often the antagonist does not elicit a response from the tissue with which it combines. Dose-response relationships within a competitive system may be explained on the basis
Some of the rules which have been worked out for competitive systems may be enumerated as follows:

1) The response of a competitive system increases with increasing concentrations of the active drug in the form of an exponential curve relating response to concentration of active drug.

2) For each concentration of antagonist there is a similar curve for the active drug which always starts at the origin and approaches the same response maximum. Thus, regardless of the amount of antagonist, a sufficiently great concentration of the active drug can overcome the effects of its presence in a truly competitive system (see Figure 9, page 126).

3) Frequently, the relationship between the concentration of active drug required to produce a particular effect and the concentration of antagonist is not linear. The curvature may be in the direction of an effect of the antagonist disproportionately greater than the dose. Among several possible explanations of this phenomenon is one which implies that more than one molecule of antagonist combines with the receptor and that this combination occurs in stages.

It is quite evident that a primary criterion for truly competitive antagonism is the characteristic finding
Figure 9. The progressive alteration of the dose-response curve of an active drug (D) by increasing doses of competitive antagonist (A).

that there is a constant ratio between agonist and antagonist over a wide range of concentrations. If one molecule of antagonist prevents half the effectors from reacting with one molecule of active drug, then 1000
molecules of antagonist must prevent half the effectors from reacting with 1000 molecules of active drug.

Some of the data obtained in this investigation is difficult to fit into such a ratio, and this is a priori evidence that the particular mechanism in the case of nalorphine antagonism of morphine is not a simple competitive inhibition. It was found that a dosage ratio (mg./Kg.) morphine sulfate: nalorphine hydrochloride of 7.1: 1.0 was required to completely prevent respiratory depression by 5 mg. of morphine sulfate given intravenously to dogs. When calculated as the free alkaloidal bases, the ratio is 6.0: 1.0. This ratio was not constant in all the experiments, however. For example, from Table IX it is seen that a ratio of 50:1 produced a maximum depression of respiration of about 50%; while a ratio of 10: 1 produced a maximum depression of respiration of approximately 35% of control values.

Gruber (70) also obtained evidence that nalorphine antagonized morphine by a mechanism other than true competitive inhibition. He observed that nalorphine when given to mice produces observable protection only against certain doses of morphine. As the dose of morphine was increased, the optimum dose of nalorphine decreased. As the dose of nalorphine was increased above this optimum dose, additive toxicity developed.
From all of this data, it is hypothesized that nalorphine is a pharmacodynamic agent in its own right, which stimulates the depressed respiratory center. It probably does not exert its action passively by simple occupation of receptor sites to the exclusion of depressant agents.

The data in Part III clearly indicate that morphine depletes adrenal ascorbic acid. The depletion of adrenal cholesterol by morphine is less pronounced and requires a longer time to develop. The mechanism of this action is undoubtedly via the ability of morphine to stimulate the release of ACTH from the anterior pituitary since it has been shown (100) that the integrity of the pituitary is essential for this effect. Nalorphine reverses these effects of morphine until at least 1½ hours postinjection. The combination of morphine and nalorphine showed a highly significant depletion of cholesterol in the adrenal when determined three hours after injection however. This might be explained by the fact that the effect of nalorphine may last only approximately 2 hours whereas morphine effects may have a duration of 6-8 hours. This explanation ceases to be suitable when it is observed that morphine alone does not produce nearly as profound a depletion. Therefore, it may be postulated that morphine and nalorphine in these high dosage ranges (20 mg./Kg. of
each) may act synergistically in the direction of morphine effects.

Conceivably, morphine activates the anterior pituitary gland to release ACTH by one or more of several independent or interdependent mechanisms:

1) A reflex secretion of endogenous epinephrine which then acts on the pituitary.

2) A release of tissue histamine, which might result in a release of epinephrine from the adrenal medulla, the latter which then might act on the pituitary.

3) A fall in the level of circulating adrenal cortical hormones as a result of increased cellular utilization.

4) A direct action on the anterior pituitary.

5) A neural or neuro-humoral discharge in the hypothalamus.

The first three possibilities have virtually been ruled out by various investigations reported in the literature (75, 100). Results with adrenal demedullated rats have shown conclusively that the adrenal response to morphine occurs in the absence of the release of adrenal medullary epinephrine. It has been shown by Nasmyth (75) that tissue release of histamine following morphine is not an important factor in depleting adrenal ascorbic acid. Finally, an increase in the level of circulating cortisone failed to block the release of ACTH
following the injection of morphine.

In the light of recent evidence that the adrenocortical response to stress may be controlled by the hypothalamus (100), it seems more likely that morphine may act via a neural or neurohumoral mechanism. That morphine acts upon the hypothalamus is evidenced by the fact that cats with lesions in the caudal hypothalamus fail to exhibit the characteristic symptoms of hyperthermia and hyperactivity following morphine administration. Further evidence is indicated by the antidiuretic effect of morphine, which is blocked by pituitary stalk section or hypophysectomy. By inference it may be stated that nalorphine also may act on the hypothalamus in the central nervous system since it effectively blocks the antidiuretic effect of morphine.

Zauder (101) found that when morphine was administered to hypophysectomized rats, the depression produced was much greater than that produced by the same dose in normal animals. Gross et al. (102) reported a greater depression in adrenalectomized than in normal dogs following morphine. Similarly, a severe depression is seen when analgesic drugs are given to patients suffering adrenal insufficiency. One factor is common to all three conditions, inability to secrete adrenal cortical hormones. Since morphine causes a release of adrenal cortical hormones, it is possible that the stimulatory phase of
-131-
morphine effects is due not to a depression but a stimulation by the cortical steroids. This view has been confirmed recently by clinical experiences with ACTH and cortisone. Several workers have reported that patients receiving these two compounds experience a marked euphoria (103, 104, 105). Winter and Flataker (73) reported that exogenous cortisone markedly reduces the threshold for the effectiveness of nalorphine antagonism of dl-methadone hydrochloride in rats. The present investigation has shown that the integrity of the adrenal glands and therefore the presence of endogenous corticoids are necessary for the action of nalorphine in combatting the acute toxic effects of morphine in rats. Could it be possible that nalorphine itself causes a release of massive quantities of corticoids into the blood stream which act in the direction of stimulation of the central nervous system and more specifically the respiratory center? It would seem apparent that this were so from the present studies on adrenal cholesterol studies. On the other hand, the experimental data gathered in the adrenal ascorbic acid studies would tend to refute this hypothesis. This apparent contradiction may be resolved only by further experimentation and study. It may even be further postulated that nalorphine may stimulate the release from the adrenal cortex of some as yet unreported very potent
substance which aids the animal to overcome periods of extremely severe and acute stress as would be produced by an LD99 dose of morphine.

Much is still in need of being done before a final statement may be made concerning the site and mechanism of action of this very interesting narcotic antagonist. However, in summary it may be stated that nalorphine is rather unspecific in its action; it is effective against a wide variety of narcotic and barbiturate depressants. This agent acts directly on the depressed inspiratory center, increasing its sensitivity to electrical and most probably normal physiological stimuli. Its antagonistic effects may be mediated in part by an action upon the adrenal cortex either directly or indirectly.
SUMMARY AND CONCLUSIONS

An investigation has been carried out involving a study of the antagonism by nalorphine of the undesirable depressive effects of morphine in an attempt to partially elucidate the site and mechanism of action of the former drug. Experiments were performed to determine the minimum dose of nalorphine which was just sufficient to prevent respiratory depression by a chosen dose of morphine in dogs. Further studies, utilizing direct electrical stimulation of the medullary inspiratory center, were made in intact and chemoreceptor-denervated animals. Finally, an investigation was made of the importance of the pituitary-adrenal axis as it may be concerned with the mechanism of the antagonistic action of nalorphine. The following conclusions were reached as a result of these studies:

1. Nalorphine antagonizes respiratory depressant effects of pentobarbital and amobarbital in dogs anesthetized with these barbiturates. This is evidenced by a significantly increased respiratory rate and ventilation volume.

2. Nalorphine is not an effective respiratory stimulant in dogs anesthetized with urethane or secobarbital sodium.

3. The optimal dosage ratio (expressed in mg./Kg.)
morphine sulfate: nalorphine hydrochloride required to completely prevent respiratory depression by 5 mg./Kg. of morphine sulfate given intravenously to dogs is 7.1: 1.0.

4. It is most likely that nalorphine antagonizes the depressant effects of morphine by a mechanism other than true competitive inhibition. The allyl compound is a pharmacodynamic agent in its own right, which produces stimulation of the depressed respiratory center.

5. The stimulatory effect of nalorphine against morphine is not altered after complete bilateral carotid body and sinus denervation and bilateral vagotomy.

6. Nalorphine probably acts centrally on the respiratory centers in overcoming morphine depression.

7. Nalorphine does not significantly alter the responsiveness of the non-depressed inspiratory center of the dog to electrical stimulation.

8. Nalorphine produces a pronounced inhibition of the adrenal ascorbic acid depleting effect of morphine.

9. Combinations of morphine and nalorphine in high doses may act synergistically in depleting adrenal cholesterol in albino rats.

10. Nalorphine has no effect upon adrenal ascorbic acid content when administered alone; however, it is equivalent to morphine in depleting adrenal cholesterol when it is given alone.
11. The integrity of the adrenal glands is necessary for the action of nalorphine in combatting the acute toxic effects of morphine in albino rats.

12. Many avenues of future research are suggested by the data reported in this investigation. A study of nalorphine effects upon respiratory depression not associated with a drug effect would be extremely desirable. Another interesting investigation might involve conducting an acute toxicity study (similar to that of Experiment 12) in hypophysectomized rats.
BIBLIOGRAPHY


A U T O B I O G R A P H Y

I, Fred Theodore Galysh, was born in Cleveland, Ohio April 17, 1929. I received my secondary school education in the public schools of Oil City, Pennsylvania. My undergraduate training was obtained at the School of Pharmacy, University of Pittsburgh, from which I received the degree Bachelor of Science in Pharmacy in 1952. From The Ohio State University, I received the degree of Master of Science in 1954. While in residence at The Ohio State University, where I specialized in pharmacy (pharmacology), I was appointed Fellow of the American Foundation for Pharmaceutical Education. I have held this Fellowship for three years while completing requirements for the degree Doctor of Philosophy.