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TITLE Studies on Analgesia: Analysis of Algesimetric Methods and the Evaluation of 1-Methyl-4-(3-Hydroxyphenyl)-4-Piperidyl Ethyl Ketone

AUTHOR John Reed Lewis

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STUDIES ON ANALGESIA

ANALYSIS OF ALGESIMETRIC METHODS AND THE EVALUATION OF 1-METHYL-4-(3-HYDROXYPHENYL)-4-PIPERIDYL ETHYL KETONE

By

John A. Lewis
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Committee in Charge:

Professor M. H. Seevers, Chairman
Professor F. F. Blicke
Professor A. A. Christman
Associate Professor G. M. Roe
Instructor F. E. Shideman

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John R. Lewis

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Introduction

The discovery of the analgesic action of ethyl-1-methyl-4-phenylpiperidine-4-carboxylate (Demerol, Meperidine) by Eisleb and Schaumann (1) in 1939 was probably the greatest advance in the field of analgesia since Sertürner isolated morphine from opium in 1805. The importance of this discovery lies in the fact that this was the first example of high analgesic activity in a relatively simple chemical compound and has led to the synthesis of other synthetic analgesics.

The indispensable nature of opium was emphasized by Sydenham in 1680 when he wrote, "Among the remedies which it has pleased Almighty God to give to man to relieve his sufferings, none is so universal and so efficacious as opium." (2) This statement has remained true until the present decade.

The first use of opium as a medicament is not known; however, according to some Hebrew scholars there is reference to poppy juice in the Bible. The earliest definite and authentic references to the milky juice of the poppy

are found in Greek and Latin literature. Theophrastus, in the third century B.C., referred to the milky juice of the poppy as meconium.

The original home of the poppy was in Asia Minor from which it was carried to Greece. Opium was used extensively by Arabian physicians and was introduced to the natives of the west by them.

By the sixteenth century European physicians were well versed in the use of opium. The only opium preparations in use at that time were crude extracts and many pharmaceutical preparations of opium still in use are of antique origin. Tincture of opium or laudanum dates from Paracelsus (1490-1540). It has been said that much of his success was due to the bold way in which he administered opium to his patients. He called it the "balm of immortality". Paregoric (which comes from the Greek term meaning "soothing") was originated in the eighteenth century as an elixir for asthma by Le Mort, the professor of chemistry at Leyden. Dover's powders was introduced as a diaphoretic agent in 1708.

Further advances in the use of opium depended on progress made in the field of chemistry. It is significant that the first alkaloid to be discovered and isolated was the chief active principle of opium which was named morphine. This discovery by Sertürner led to the isolation of other alkaloids and the use of pure alkaloids rather than crude extracts in therapeutics soon followed.

The outstanding property of morphine and related opiates is their analgesic action. Although their side-effects such as nausea, vomiting, constipation, diarrhea, miosis, respiratory depression and narcosis are undesirable in many cases, the fact that the opiates cause addiction is the most serious disadvantage to their chronic use. For this reason much effort has been devoted to the discovery of compounds possessing analgesic activity comparable to morphine but without the addicting properties.

At the present time no compound has yet been discovered which has satisfactory analgesia and is non-addicting. As stated previously it was not until 1939 that a non-opiate drug was found to have high analgesic activity. The fact that this was over a century from the time of isolation of morphine raises the question as to the possible cause of such slow progress in this field. It certainly was not because the problem was unrecognized but was probably due to several reasons in both the chemical and biological fields.

One factor of importance on which the development of new analgesic drugs depended was the progress in our knowledge of the basic anatomy and physiology of pain.

The present concept that pain is a specific sensation is comparatively new and our knowledge of the anatomical basis of pain is even more recent and still incomplete. Pain was not considered as one of the senses but was classified with pleasure among the passions of the soul (3). The theory that pain was a separate and distinct sense was first

suggested by Avicenna in the eleventh century and although it was intimated several times in the years following, it did not become prominent until after the classical experiments of Blix and Goldscheider in the 1880's. By this time there were three prevailing theories: the pain-pleasure theory, intensive theory and the sensory theory. During the following ten years there was a heated controversy in the literature among the proponents of these various theories. Even such workers as Blix and Goldscheider changed their allegiance from time to time as new results were obtained. The experiments of von Frey lent strength to the sensory theory and by 1900 it began to appear in textbooks. Although there were still many unsolved problems, the other theories were gradually dropped.

In the development of the sensory theory von Frey noted that if the skin were probed with stiff hairs there were certain areas from which pain alone could be elicited. He thought that the free nerve endings were the organs of pain but his direct experimental evidence was meager. The neurohistological basis of cutaneous pain was established in 1940 by Woollard, Weddell and Harpman (4). They demonstrated that cutaneous pain is subserved by the finer medullated and non-medullated nerve fibers bearing free endings. Similar nerve endings have been demonstrated in other tissues, e.g. the center of the cornea, which give

only a sensation of pain (5). In regards to the conduction nerves, Gasser (6) found that painful impulses are carried by both myelinated and unmyelinated fibers of various sizes.

Although there is much to be learned concerning the central representation of pain and particularly the effect of analgesics on it, the pain pathways to the brain have been described. Painful impulses are carried to the cord either directly by somatic nerves or indirectly in sympathetic nerves via the white rami communicantes. The cell bodies are located in the posterior root ganglia or comparable sensory ganglia of the cranial nerves. In entering the cord the posterior root divides into a lateral and medial division. The pain fibers pass through the lateral division and enter Lissauer's tract where they send collaterals up and down the cord for one or two segments. These end in the grey matter of the posterior horn where they synapse with other fibers forming the 2nd neurone in the path to the brain or with short internuncial neurones to anterior horn cells of the same or opposite side. From the posterior horn the 2nd order neurones cross at the same level and ascend in the lateral spinothalamic tract. The spinothalamic tract proceeds without break to end in the ventral portion of the lateral nucleus of the thalamus. From this thalamic nucleus there are projections to the cortex which are predominantly to the post central convolution. At least from an anatomical basis there are other

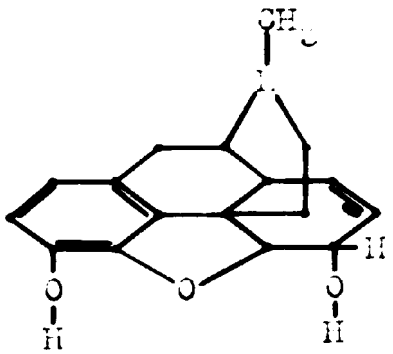
possible pathways by which pain impulses may reach the cortex. In addition to the cortical projections there are numerous other projections from this nucleus of the thalamus to other thalamic nuclei, to the pulvinar and to the hypothalamus. Besides providing additional pathways for pain impulses to the cortex it appears that these association pathways may be important in some of the reactions seen with pain or with some analgesic drugs.

Following proof and acceptance of the sensory theory it became possible to devise suitable methods for the study of pain. Unlike other sensory receptors the pain receptors can be stimulated by any type of stimulus if of adequate intensity. Mechanical, chemical, electrical and thermal stimulation have all been used in the study of pain.

The earliest experiments on humans were done by using the von Frey hairs as the stimulus. Seevers and Pfeiffer (7) in 1936 studied the action of some opiates with the von Frey hair method in normal humans and demonstrated the applicability of the method to the quantitative study of analgesic drugs. The next advance in algesimetry was the thermal radiation method of Hardy, Wolff and Goodell (8) published in 1940. The quantitative evaluation of numerous analgesic drugs were made by Wolff and his co-workers (9). This method is the one most widely used today.

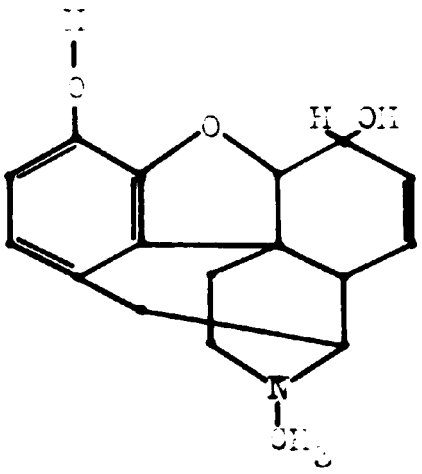
The fact that pain is a subjective phenomenon accounts in part for the difficulty encountered in assaying the effectiveness of analgesic drugs. The measurement of pain responses and analgesia is even more difficult in experimental animals than humans. The large number and variety of methods in the literature is an indication of this fact. A summary of the various animal and human methods was published by Goetzl et al. (10) in 1945. These will be discussed in greater detail below.

The synthesis of new active drugs is usually based on the chemical structure of known compounds. The determination of the exact structural formula of morphine proved to be an extremely difficult chemical problem. The fact that this problem was not solved for many years delayed the synthesis of new analgesic drugs. Knorr (11) in 1889 was the first to suggest a formula and since that time many others have been proposed. The most probable of these is the one advanced by Gulland and Robinson (12) in 1925. It best explains the complicated and exceptional reactions of the morphine group of alkaloids. The accepted structure is:

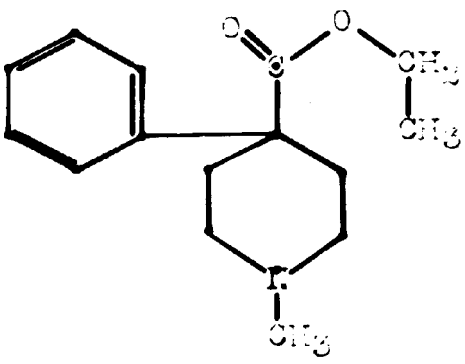


Although morphine has not been synthesized, many of its derivatives have been made and studied. In an attempt to find a potent analgesic drug that did not produce addiction, several hundred compounds were prepared and studied during the ten years from 1929 to 1939. This work was carried out under the auspices of the Committee on Drug Addiction of the National Research Council. Most of these compounds were derivatives of the morphine molecule or closely related compounds. Many of the results of this cooperative study are summarized by Krueger, Eddy and Dunwalt (13). Important contributions relating chemical structure and pharmacological action of the morphine structure resulted from this work but the initial goal was not reached. The most promising compound of those studied was methyldihydromorphinone, metopon, which although addicting, has some advantages over morphine. The pharmacology of this compound has been reviewed by Eddy (14).

The discovery of Demerol greatly stimulated interest in synthetic analgesics since it is not a derivative of morphine although certain structural similarities have been pointed out by Schaumann (15):



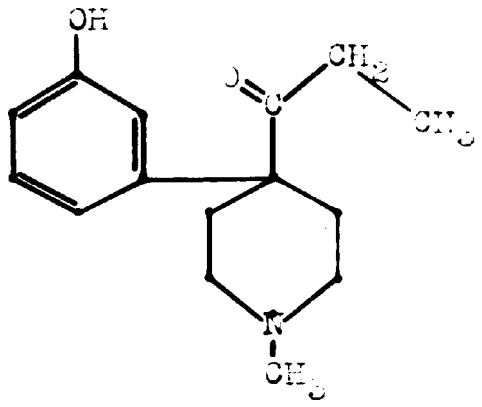
Morphine



Demerol

Since 1940 many compounds based on the Demerol structure have been made and studied (Schaumann (15), MacDonald, Woolfe, Bergel et al. (16), Foster and Carman (17), Randall and Lehmann (18)). Following World War II the U. S. Dept. of Commerce published a report (19) of analgesic compounds prepared by the Germans. The activities of a number of compounds related to Demerol were given in this report. The most active compound in this group was 1-methyl -4-(3-hydroxyphenyl)-4-piperidyl ethyl ketone (also known as German No. 10730, M.H. 1009, Metopemidone). This compound has the same basic phenyl-piperidine nucleus as Demerol, but contains a meta-hydroxy group on the phenyl ring and has an ethyl ketone in place of the ethyl carboxylate of Demerol.

Its formula is given below:



In some respects this compound resembles the morphine structure more than does Demerol. The Germans reported that it was ten times more active than Demerol. The results of a study made of the actions of this compound are reported in Part II of this thesis.

With the synthesis of many new compounds it became necessary to develop suitable methods for screening analgesic activities in experimental animals. Many methods have been proposed in the past as noted in the summary by Doetzl et al. (10). In this summary various algesimetric methods are discussed with reference to (a) the choice of stimulus, (b) selection of the receptive field and (c) application of the stimulus and observation of the response.

Our experiments were directed toward finding a suitable method which could be used routinely to screen large numbers of compounds for analgesic activity in experimental animals.

There are a number of factors to consider in selecting a suitable screening method. The method must give accurate and reproducible results which lend themselves to statistical analysis. It should not be too time-consuming in order that large numbers of animals can be tested in a given time. It should be relatively simple to operate.

The screening methods most commonly used in various laboratories today utilize a radiant thermal stimulus. Although there are certain criticisms to the use of a thermal stimulus, it is a convenient form and its intensity can be measured and maintained constant during the course of an experiment. Thus we selected this form of stimulus for our study.

Albino rats were chosen as the experimental animal for a number of reasons, namely: (a) they are readily available, (b) their cost and maintenance are not too expensive, (c) they are easily handled, and (d) they are small enough not to require large quantities of test material.

Our studies of methods using a radiant thermal stimulus with albino rats are reported in Part I of this thesis.

Part I - Analysis of Algesimetric Methods

The method of Hardy, Wolff and Goodell (8) proved so successful in the measurement of the pain threshold and analgesia in man that it was soon adapted to animal experiments by D'Amour and Smith (20) and Andrews and Workman (21). The latter workers used the Hardy-Wolff apparatus and technique with dogs as the experimental animal. They used a characteristic reflex twitch of the musculature of the back as an indication of the sensation of pain. The intensity of the stimulus was measured by a wattmeter connected in the lamp circuit. They showed that a linear relation exists between the readings of the wattmeter and the radiometer which is used to measure the intensity of stimulus of the standard Hardy-Wolff apparatus. D'Amour and Smith used albino rats in their method. The stimulus was applied to the tip of the rat's tail and the response used as the end-point was a typical twitch of the tail.

We had an apparatus similar to that described by Winder et al. (22) available in our laboratory. It was arranged in such a way that a beam of light could be focused on the tip of a rat's tail as in the D'Amour and Smith method. The duration of the stimulus was set at three seconds and the threshold stimulus as measured by a watt-meter was determined. This was the first method to be considered in our study.

The results of a typical experiment with morphine are shown in figure 1.

This method was used to test several new synthetic compounds and it was found that analgesic activity could be determined from the increase in pain threshold. This method of screening has one major disadvantage and was, therefore, discontinued. The disadvantage arose from the difficulty in manageability of the animals. In order to establish the pain threshold it was necessary to take several readings on each animal. It was found that stimuli should not be repeated more than once per minute. (This interval between stimuli was found to be necessary also by Winder et al. (53).) The difficulty encountered was that of keeping the animal quiet and its tail in position for the stimulus for the length of time required to determine the threshold.

After this technique was discontinued in our laboratory, an article by Thorp (50) describing the same method appeared in the literature. He published results obtained with morphine and heroin and found it was necessary to train the rats before using them for the test.

The method studied next was that proposed by D'Amour and Smith in which the reaction time of the animal is measured when a stimulus of constant intensity is applied to the tip of a rat's tail. The apparatus used was essentially the same as that described by Hart (24). It consisted

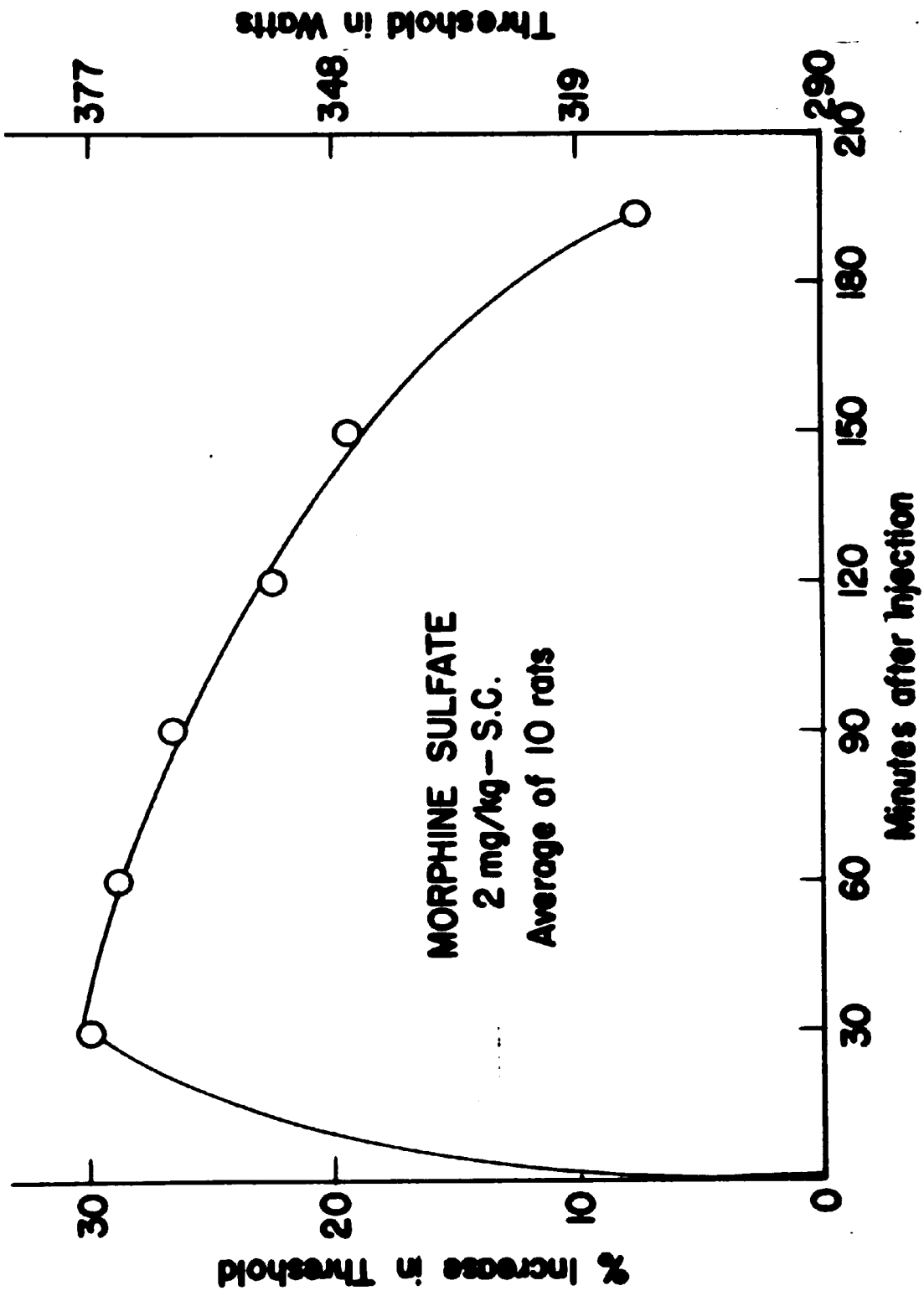

% Increase in Threshold
0
10
20
30
Threshold in Watts
290
319
348
377
Minutes after Injection
30
60
90
120
150
180
210
MORPHINE SULFATE
2 mg/kg – S.C.
Average of 10 rats

Figure 1

of a 50 candle power light in a suitable reflector whose heat was focused by a lens on the tip of a rat's tail. An electric timer was connected through a switch to the light circuit so that it was in operation only during the application of the pain stimulus. This procedure was much more convenient to use than the previous method because the stimulus [illegible] only [illegible] of reading. The intensity of the stimulus was adjusted to give normal responses [illegible] seconds. The results obtained by this method with morphine [illegible] are shown in figures [illegible] and 3. Figure [illegible] gives the time-effect [illegible] of different doses of morphine. [illegible] the dose-response relationship. When the percentage increase in reaction time is plotted against the log dose, all points except that for the lowest dose fall about a straight line.

It is interesting to note that for the one dose level of [illegible] the percentage increase in reaction time agrees favorably with the percentage increase in pain threshold obtained by the first method. A dose-response curve was not determined using the first method so a comparison of the sensitivity of the two methods cannot be made.

During the time these experiments were being conducted a slight modification of this method was published by Davies, Raventos and Walpole ([illegible]). In place of a light as the source of the heat stimulus, they used a small coil of resistance wire heated by an electric current and placed

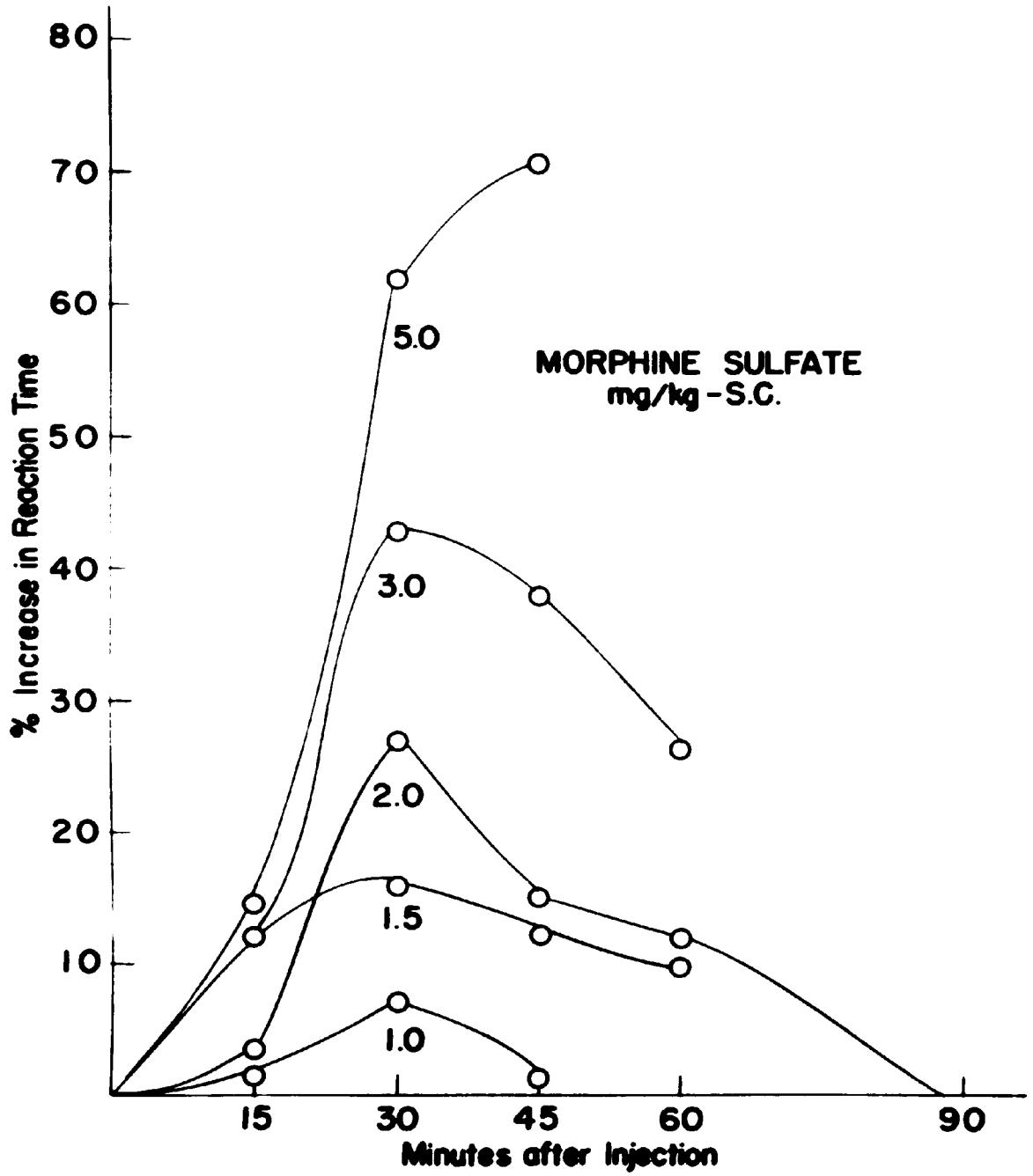

80
70
60
50
40
30
20
10
% Increase in Reaction Time
MORPHINE SULFATE
mg/kg - S.C.
5.0
3.0
2.0
1.5
1.0
15
30
45
60
90
Minutes after Injection

Figure 2

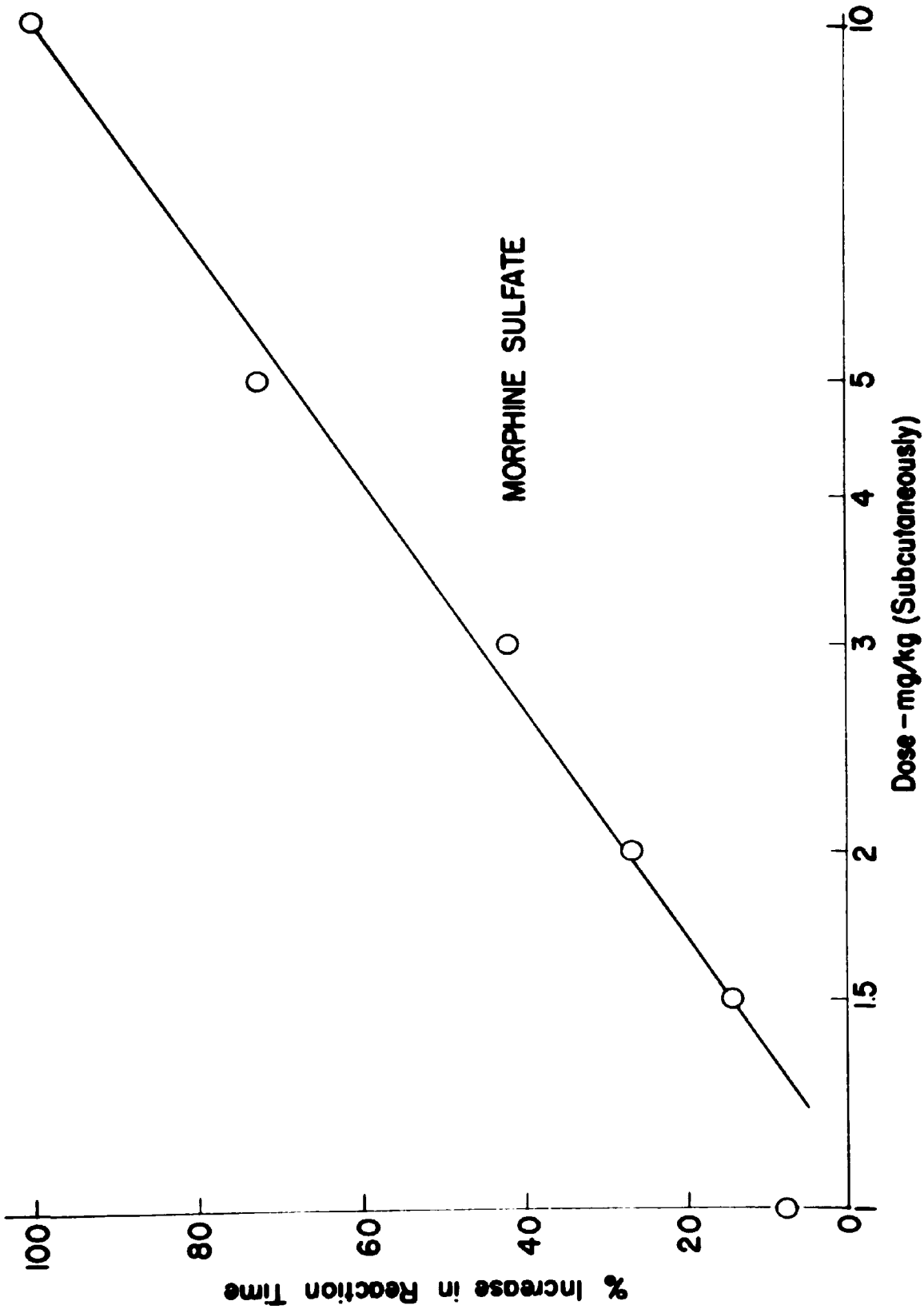

MORPHINE SULFATE
% Increase in Reaction Time
Dose – mg/kg (Subcutaneously)
0
20
40
60
80
100
1
1.5
2
3
4
5
10

Figure 3

beneath the end of a rat's tail. They show dose-response curves obtained with several analgesic drugs but since they used a different route of administration than that employed by us, the results obtained cannot be compared.

The determination of reaction time instead of pain threshold is more convenient and less time consuming. There are, however, two disadvantages to the use of the rat's tail as the site of stimulation. First, if the degree of analgesia is complete the tail may be withdrawn before the reaction occurs. In this case the animal cannot be used again. Second, some difficulty is experienced in keeping the animal's tail in position for the application of the stimulus. This restlessness was not present in all the animals but was observed in some normally and became more apparent after injection of those compounds causing central nervous system stimulation.

The next method investigated was the one proposed by Ercoli and Lewis (4d). This was a modification of the D'Amour and Smith method, the difference being that a shaved area of the rat's back was used as the site of stimulation in place of the terminal portion of the tail.

The apparatus used consisted of a 500 watt projection lamp, the light of which was focused by means of lenses exactly on a 10 mm. aperture in a lucite screen. The intensity of the light was controlled by a variable transformer and determined by a radiometer placed at the

aperture in the lucite screen. A shutter which was automatically synchronized with an electric stop clock was interposed between the light and lens.

In testing a compound the animal was held vertically with a shaved area of its tail against the aperture of the lucite screen. The shutter was opened and the reaction time measured to the first perceptible response, usually twitch in the skin. The normal control reaction time was about 2.5 seconds. After injection of the test compound, the reaction times were determined at various intervals to determine the time of maximum effect and duration of action of the compound.

In order to determine the effect of repeated exposures in the same animal, normal reaction times were determined on three groups of 20 rats each. One reading was taken on Group 1, two readings at a 30 minute interval were taken on Group 2, and three readings at 30 minute intervals were taken on Group 3. The results are given in Table 1. It was found that repeated determinations over the period of an hour did not alter the normal average reaction time. In subsequent tests only one control reading was made prior to injection of the test compound.

Several known drugs were used in order to ascertain the applicability of the method for determining quantitatively analgesic activity. The results obtained with morphine, Demerol and antipyrine are given in Figure 4.

Table 1

Normal Reaction Time of Rats

Group	Average Reaction Time (seconds)		
	0 min.	30 min.	60 min.
A	3.4 ± .20 *		
B	3.4 ± .05	3.4 ± .25	
C	3.6 ± .17	3.4 ± .26	3.3 ± .26

* Standard deviation

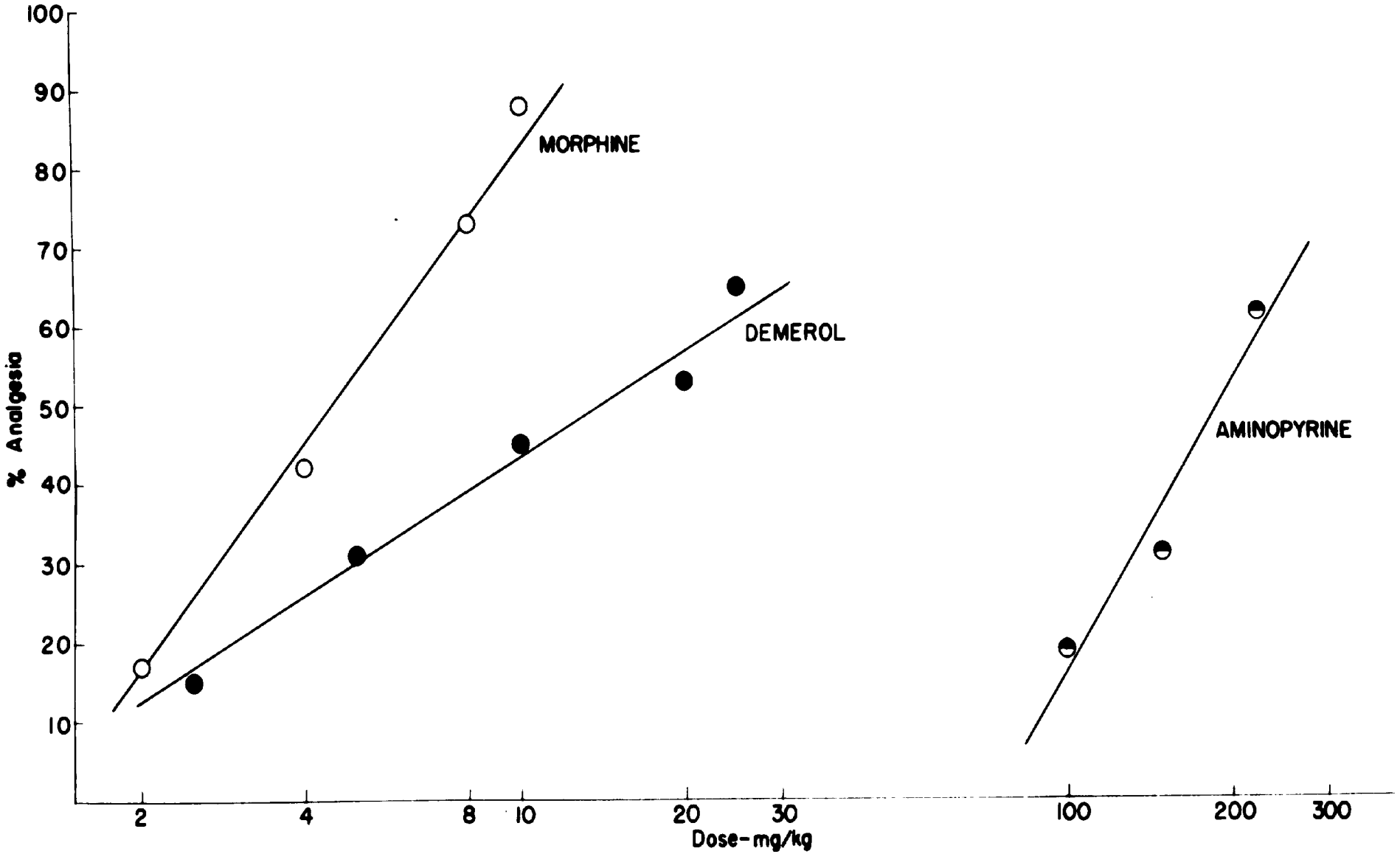

100
90
80
70
60
50
40
30
20
10
% Analgesia
MORPHINE
DEMEROL
AMINOPYRINE
2
4
8
10
20
30
100
200
300
Dose-mg/kg

Figure 4

These three examples represent drugs with widely different degrees of analgesia as noted by the dosage range, nevertheless a straight line dose-response curve can be obtained for each one. The fact that the slopes between morphine and Demerol differ indicates that Demerol becomes relatively less active as the dose is increased. This has also been noted by Davies et al. (21) and by Woolfe and MacDonald (27). However, with sufficiently large doses of Demerol complete analgesia can be obtained in the rat as well as with morphine. This is not possible with aminopyrine. By complete analgesia, we mean a state in which the animal gives no response to the stimulus even though burning of the skin may occur. This phenomenon deserves special consideration in the calculation of results obtained with potent analgesic drugs.

Since this method of evaluation of analgesia is that which uses the reaction time to a standard painful stimulus as the dependent parameter, it becomes necessary for purposes of calculation to assign a finite value to those animals which become completely analgized. It is our opinion that this value should be the time at which the animal fails to react to the stimulus and yet should not be long enough to cause a significant degree of burning. Hardy et al. (8) stated that burning occurs at intensities twice the threshold value for pain perception in man. Winder et al. (28) observed that blisters formed at

intensities two to three times the threshold value with mined pigs. We have observed in our standard procedure that the animals were burned when exposed to the stimulus for 4-6 times their normal values. We also observed that if an animal did not react within a time twice its normal the probability was great that it would not react at a longer time of exposure. On the basis of this we felt that a value of twice the normal reaction time would closely approximate the value we sought to use in our calculations as representing complete analgesia. The question arose, however, whether this would hold true for various reaction times or intensities of stimulus. In order to obtain more information on this subject the following experiment was performed.

The normal reaction times of a group of rats were obtained when exposed to thermal radiation of various intensities. Ten rats were used at each of the various intensities. The animals were then injected subcutaneously with 3 mg./kg. of d-iso-methadone, a potent analgesic drug, which we had found produced complete analgesia in approximately all of the animals at the standard intensity setting. Thirty minutes following the injection each rat was exposed for 2 and 3½ times its normal reaction time. After twenty-four hours the animals were examined for possible

burns. The degree of injury was graded as follows:

1 = small wheal (blister)

2 = large wheal (blister)

3 = ulcer formed

The average degree of injury was calculated by dividing the total injury score of the group by the number of animals in the group which were scored. Some animals gave a muscle twitch in less than the exposure time which prevented tissue injury so could not be used in calculating the degree of injury. It was too difficult to control the animals at the lowest intensity to obtain accurate results for the longer exposure periods so they are omitted from the table.

The results are given in table 2 and shown graphically in figure 6.

The normal reaction time at various intensities is shown by the solid line on the graph. Twice these values are shown by the dotted line and the broken line indicates a possible curve of significant tissue injury. Theoretically there is an intensity below which there would be no reaction even at an infinite time. As the intensity is increased the reaction time decreases, but even at an infinite intensity there would be a definite reaction time due to the time of passage of the impulse through the reflex arc. In regards to tissue injury it is obvious that at very high intensities burning will occur in less time than the reaction.

Table V

Intensity of Stimulus-Reaction Time Relationship

Intensity Scale Setting (micro amperes)	Average Normal Reaction Time (seconds)	Average Degree of Tissue Injury	
		Exposure X Normal	Exposure 3* X Normal
30	10.10	----	----
31	6.54	.125	.500
36	5.52	.400	1.000
43	4.58	.448	.667
47	3.43	.500	1.500
61	2.43	1.250	2.000
75	1.62	2.000	----

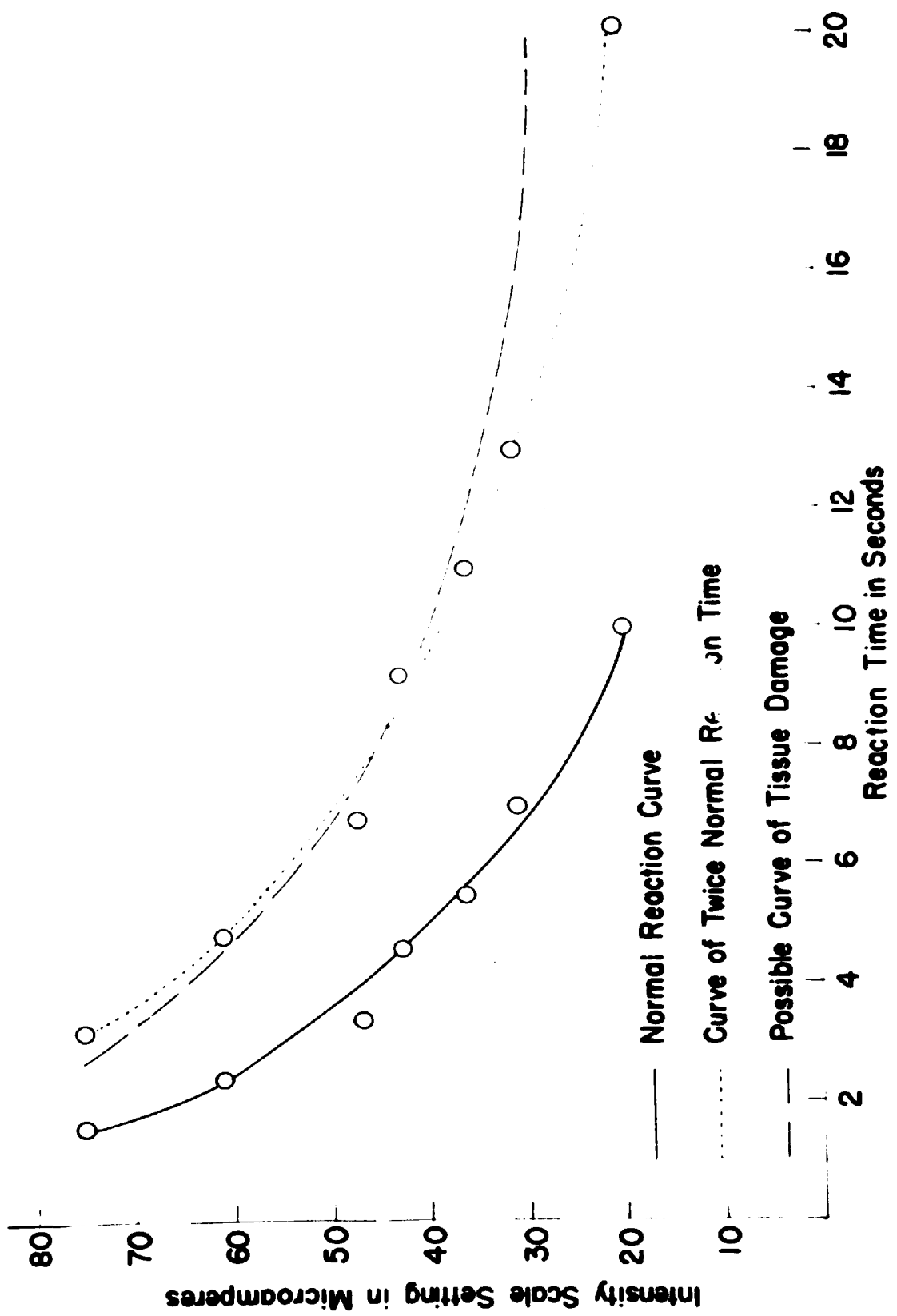

Intensity Scale Setting in Microamperes
80
70
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
Reaction Time in Seconds
Normal Reaction Curve
Curve of Twice Normal Re on Time
Possible Curve of Tissue Damage

Figure 5

Our experiment covers only the middle portion of the complete intensity-reaction time curve. The information obtained indicates that for a constant percentage increase over the normal reaction time more tissue injury occurs at a high intensity than at a low intensity. The degree of injury increases considerably, even at the lower intensities, when the exposure time is increased from twice to two and one-half times the normal.

From the examination of the animals and the results obtained it is our opinion that an average degree of tissue injury of 0.5 or less does not represent an appreciable degree of injury. If this value is taken as the maximum allowable, then twice the normal reaction time at an intensity of 47 micro amps (our standard intensity) would be the maximum time of exposure. At lower intensities a slightly greater increase in time might be allowed but not exceeding 2½ times the normal even at the lowest intensity tried in this experiment. At higher intensities the maximum exposure time would be less than twice the normal.

From the results obtained in this experiment, it appeared that a value of twice the normal reaction time should be the maximum value assigned to those animals with complete analgesia. Since this value is an increase of 100% over the normal, then percentage increase in reaction time represents percent of complete analgesia. The calculations of degree of analgesia reported herein were made on this basis.

The study of this method indicates that it is suitable for the screening of compounds for analgesic activity. Quantitative data can be obtained and comparisons made with known analgesic drugs although these results may not be transferable directly to clinical human use. The method is comparatively easy to perform and the apparatus is simple and easily standardized so that consistent control results are obtained. The speed with which the determinations can be made enables one to test large numbers of animals thus increasing the statistical significance of the results. The disadvantages noted with the previous method have been circumvented by this method. The area of the back which can be used is large enough so that if one spot is burned other spots can be tested without interfering with the accuracy of the results obtained. Since the animal is held in position for stimulation, the difficulty in manageability is not encountered as when the tail was stimulated.

Although certain criticisms of the method have been made, the fact remains that it has proved to be reliable in the evaluation of potent analgesic drugs. (Equivocal results have been obtained with analgesics like aspirin.) There are no known analgesic drugs whose activity has not been demonstrated by this method and it is very unlikely that in screening new synthetic compounds those which possess analgesic action will be overlooked.

PART II - Evaluation of 1-Methyl-4-(3-Hydroxyphenyl)-4-Piperidyl Ethyl Ketone

Following the disclosure of the high analgesic activity of 1-methyl-4-(3-hydroxyphenyl)-4-piperidyl ethyl ketone (19) (referred to as WIN 1539 in this thesis) we undertook a study of some of the actions of this compound. Scott et al. (28) published a report of several compounds in the German series. They also reported high analgesic activity for this compound. Reports of its clinical use by Kirchhof (29) and Lund (30) appeared more recently in the literature.

This compound was of special interest because of its close chemical similarity to Demerol. Schaumann (15) and MacDonald et al. (16) reported on a large series of compounds related to Demerol, but the reports of the Germans and of Scott indicated that WIN 1539 had the highest analgesic activity of any Demerol derivatives previously studied. In our investigation the actions of WIN 1539 were compared with Demerol and/or morphine.

Action on the Central Nervous System

Analgesic Activity

Evaluation in Rats:

In order to determine the analgesic activity of WIN 1539, the method of Ercoli and Lewis was used as described previously. Four groups of ten rats each were

tested at four dose levels at weekly intervals for four weeks. The design of the experiment was such that each group received each dose once during the period. The compound was injected subcutaneously as an aqueous solution. Reaction times were determined at 30 and 60 minutes following administration of the drug. The maximum reaction occurred within 30 minutes which time was used in calculation of activity. A summary of these results is given in table 5 and a graph of average responses is shown in figure 6.

From the graph it is apparent that WIN 1539 is much more active than morphine or Demerol. The slope of the Demerol curve differs from that of WIN 1539 so no direct comparison of activities can be made. However, in general WIN 1539 appears to be about ten times more active than Demerol. This is in agreement with results reported previously (19, 28) and obtained by different methods. Our results obtained in rats indicate that WIN 1539 is about 5 times more active than morphine.

Potentiation of Analgesia:

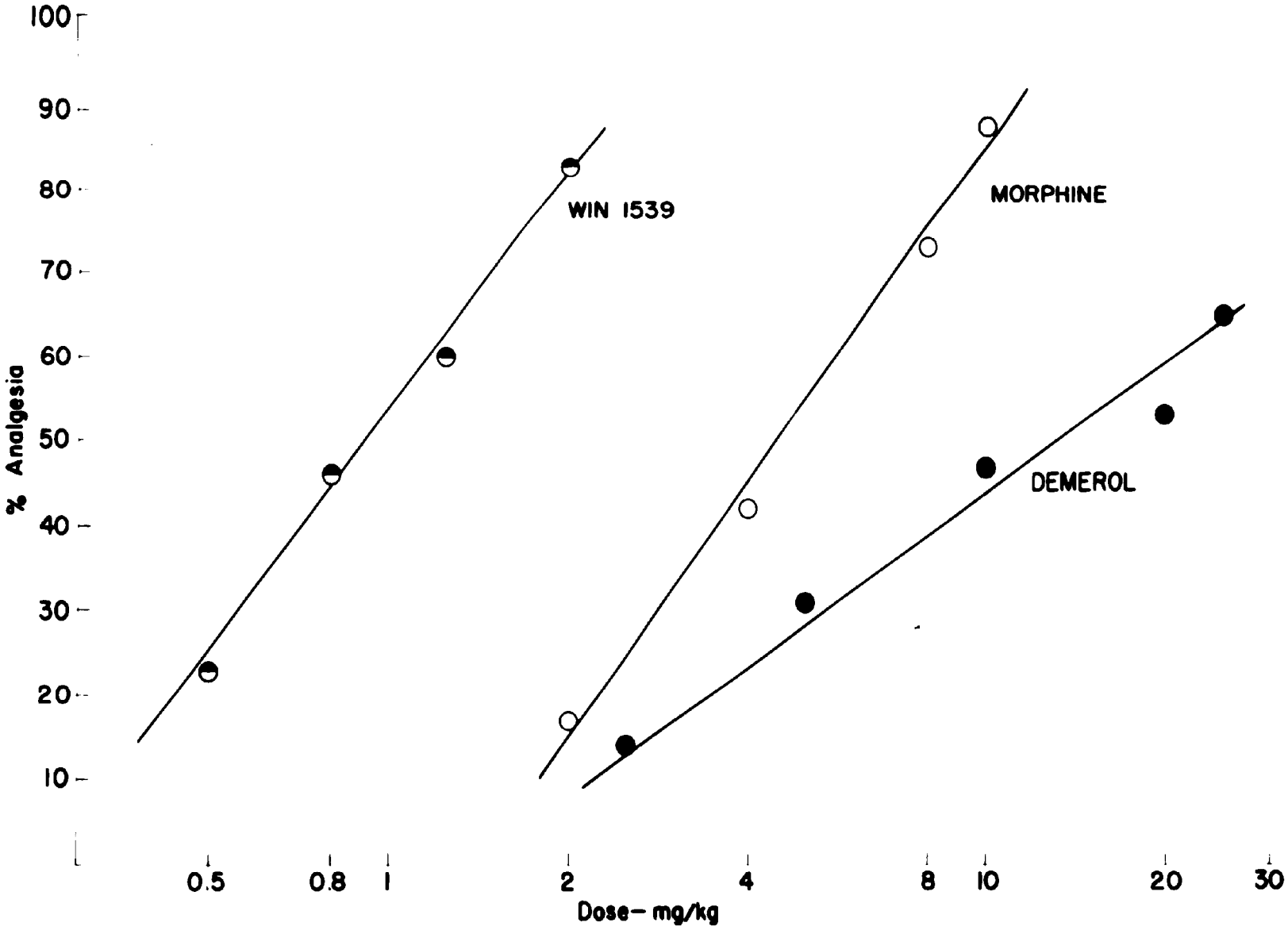
The fact has long been recognized that some of the actions of morphine resemble those of stimulation of the autonomic nervous system. Slaughter and Hunsell (31) suggested that acetylcholine plays a role in morphine analgesia by demonstrating that Prostigmine potentiated the effect of morphine and that atropine antagonized the effect.

Table 5

Analgesic Activity of WIN 1539

Group of Rats	Week 1		2		3		4	
	Dose*	% Analgesia	Dose*	% Analgesia	Dose*	% Analgesia	Dose*	% Analgesia
A	.5	19.2	2.0	82.0	1.25	48.6	0.8	59.0
B	.8	50.5	0.5	21.1	2.0	90.8	1.25	77.0
C	1.25	57.2	0.8	36.2	0.5	29.7	2.0	85.0
D	2.0	75.1	1.25	56.6	0.8	38.9	0.5	20.6

* Dose in mg./kg.


100
90
80
70
60
50
40
30
20
10
% Analgesia
WIN 1539
MORPHINE
DEMEROL
0.5
0.8
1
2
4
8
10
20
30
Dose – mg/kg

Slaughter et al. (32) (33) also reported that good pain relief was experienced by humans with a combination of Prostigmine and morphine. However, Smith et al. (34) observed no potentiation of morphine analgesia by Prostigmine in rats but did find that scopolamine antagonized the effect of morphine. Seevers (7) and Hardy (8) found also that scopolamine reduced the degree of analgesia produced by morphine.

Ivy et al. (35) reported that epinephrine produced analgesia in man and dogs. The earlier literature on this subject is reviewed by Ivy. In later articles (36,-38) Ivy and co-workers demonstrated that dextroamphetamine produced analgesia and potentiated morphine analgesia.

Although a completely adequate explanation of these actions of morphine with autonomic drugs has not been given, we thought a comparison of morphine with WIN 1539 in combination with certain drugs acting on the autonomic nervous system would indicate similarities or differences of analgesic action between the two drugs. The method previously described was used to determine the degree of analgesia in rats. The analgesic drug and the potentiating drug were injected subcutaneously simultaneously. The potentiating drugs used were pilocarpine as an example of a cholinergic drug, eserine, an anticholinesterase, and epinephrine, a sympathomimetic drug. A summary of results obtained is given in table 4. It is interesting to note

Table 4

Potentiation of Analgesic Action

Potentiating Drug	Dose mg./kg.	Percent Analgesia at minutes after injection of drugs: Morphine 2.0 mg./kg. 30'	Morphine 2.0 mg./kg. 60'	Morphine 2.0 mg./kg. 120'	WIN 1539 0.5 mg./kg. 30'	WIN 1539 0.5 mg./kg. 60'	WIN 1539 0.5 mg./kg. 120'
Pilocarpine	5.0	38	41	0	33	20	3
Pilocarpine	10.0	69	67	11	71	49	20
Pilocarpine	15.0	72	94	49	89	60	29
Eserine	0.1	31	25	14	6	3	0
Eserine	0.2	56	56	0	24	15	0
Eserine	0.4	67	44	3	54	9	0
Epinephrine	0.25	14	25	14	17	31	25
Epinephrine	0.50	61	47	58	25	37	37
Epinephrine	1.00	80	88	88	47	65	62

that with pilocarpine and eserine evidence of parasympathetic stimulation was observed in addition to the analgesia.

It is apparent that the analgesia of morphine and WIN 1559 is potentiated by pilocarpine, eserine and epinephrine and the degree of analgesia is increased with an increase in the dose of potentiating drug. The fact that analgesia is potentiated by both cholinergic and adrenergic drugs may seem paradoxical, however, some recent investigations offer a partial explanation of this phenomenon. Puharich and Goetzl (39) and Friend and Harris (40) have reported that the analgesic action of morphine was reduced in adrenalectomized rats indicating that the release of epinephrine from the adrenal medulla plays a role in morphine analgesia. Similar results were obtained in adrenalectomized dogs by Gross et al. (41). These workers and Christensen and Gross (42) have reported on studies made on the relationship between the action of several analgesic drugs and autonomic drugs.

If epinephrine is a mediator in analgesia as suggested, then the effects of pilocarpine and eserine may be explained on a similar basis. Feldberg et al. (43) have shown that acetylcholine stimulates the secretion of epinephrine from the adrenal medulla and eserine enhances this action. They found that pilocarpine also acts similarly. It has been demonstrated (44) (45) that morphine has an anticholinesterase action and causes a secretion of epinephrine. The

anticholinesterase action of WIN 1559 has not been studied but such an action has been reported for Demerol (41) and it may be assumed that WIN 1559 also has this action.

We have made no attempt to determine the mechanism of analgesic action in these experiments but believe that these results and others reported below indicate that the analgesic action of WIN 1559 is mediated through the same mechanism as that of morphine.

Excitatory action in cats:

Some species of animals are stimulated by morphine whereas a depression is more commonly observed in others. The stimulating action of morphine is most easily demonstrated in cats. The effect of WIN 1559 in cats has been studied and the results compared with those of morphine excitation. The drugs were injected subcutaneously and the animals observed for several hours. These observations are given in table 5.

The most constant observation was mydriasis which was seen in all cats. Two out of twelve animals that received morphine vomited, but over half of the animals in each group showed evidence of nausea such as excess salivation and retching.

Taking into account the variation in response between animals, there does not appear to be any appreciable difference between the action of WIN 1559 and morphine in regard to the excitatory action in cats. This action of

Table 5

Excitatory Action in Cats

Cat Number	Dose mg./kg. Morphine	Observations
# 4153	1	Mydriasis, salivation, animal quiet, apparently normal.
# 4175	2	Mydriasis, restless, many senseless random movements, hallucinations, circus movements, clonic convulsions lasting two minutes, very apprehensive.
# 4174	2	Mydriasis, evidence of nausea, very nervous and excitable.
# 4148	3	Mydriasis, emesis, salivation, animal sits in one position and stares at wall, occasionally strikes at an imaginary object.
# 4157	3	Mydriasis, emesis, apprehensive, hallucinations, some ataxia, nervous, rubs against objects and other cats in cage
# 4129	5	Mydriasis, apprehensive, hallucinations, intoxication, rubs against objects and other cats.

Table 5 cont'd

Cat Number	Dose mg./kg. WIN 1559	Observations
# 4173	1	Mydriasis, evidence of nausea, excitable, apprehensive, some senseless backward movements.
# 4171	1	Mydriasis, evidence of nausea, slightly nervous.
# 4549	1	Mydriasis, animal sits quiet crouched in one position, stares at wall, apprehensive, sleeping at 5 hours after injection.
# 4543	1	Mydriasis, emesis, apprehensive, sits in corner, stares at wall, sleeping at 6 hours after injection.
# 4149	2	Mydriasis, evidence of nausea. restless, hallucinations, strikes at imaginary objects on wall.
# 4172	2	Slight mydriasis, quiet no excitement.
# 4168	3	Mydriasis, evidence of nausea, emesis, quiet, sits in crouched position, no excitement.
# 4167	3	Mydriasis, evidence of nausea, apprehensive, very excitable and nervous.

Table 5 cont'd

Cat Number	Dose mg./kg. WIN 1539	Observations
# 4528	3	Mydriasis, evidence of nausea, sits in crouched position, hallucinations, apprehensive, rubs against other animals.
# 4536	3	Mydriasis, apprehensive, hallucinations, runs back and forth in cage, very nervous.
# 4538	5	Mydriasis, sits in corner, stares at wall, hallucinations, some nervousness.
# 4555	5	Mydriasis, evidence of nausea, apprehensive, hallucirations, muscle twitches, rubs against objects and other cats in cage, nervous.

WIN 1539 resembles more closely morphine than Demerol, since Barlow (46) found that the wild or senseless random movements which characterize the action of morphine in the cat are not seen following the administration of Demerol.

Action on Gastro-intestinal tract.

In addition to its action on the central nervous system a second important action of morphine is on the gastro-intestinal tract. Its constipating action was used by the Ancients for relief of diarrheas and dysenteries. The following experiments were performed with WIN 1539 to determine its action on the gastro-intestinal tract.

Effect on excised intestinal segments:

WIN 1539 and Demerol were tested for their activity against barium chloride and acetylcholine induced spasms of the rabbit ileum and against histamine induced spasms of the guinea pig ileum by the procedure described by Miller, Becker and Tainter (47). The results are given in table 6. These data indicate that the spasmolytic activity of WIN 1539 is considerably greater than Demerol against all three spasmogenic agents.

Effect on intestine in situ:

Kymographic recordings were made of a segment of the intact ileum of anesthetized dogs by a modification of the Barbour method as described by Jackson (48). Aqueous solutions of the drugs were injected into the exposed femoral vein. A total of six dogs was used in these experiments. The most marked effect on the intestine following the administration of WIN 1539 was a decrease in tonus. There was no significant change in amplitude or frequency of the contractions. Doses of WIN 1539 as low as 0.01 mg./kg.

Table 6

Relative *in vitro* Spasmolytic Properties

of WIN 1539 and Demerol.

Compound	Barium Chloride Induced Spasms* Effective Dilution	Barium Chloride Induced Spasms* % Papaverine	Acetylcholine Induced Spasms* Effective Dilution	Acetylcholine Induced Spasms* % Atropine SO_4	Histamine Induced Spasms* Effective Dilution	Histamine Induced Spasms* % Papaverine
WIN 1539	1:1,000,000	650	1:3,200,000	2.5	1:1,000,000	475
Demerol	1:500,000	200	1:98,000	0.08	1:205,000	98

* Values obtained with intestinal strips of six animals.

produced a definite decrease in tonus. These small doses had no effect on blood pressure, heart rate or respiration. Larger doses (1-2 mg./kg.) did not cause stimulation of the intestine but only a relaxation of longer duration. The results obtained in a representative experiment are shown in figure 7.

Roentgenographic study of the rate of movement of a barium meal through the gastro-intestinal tract:

The method used was a modification of the one described by Gershon-Cohen and Shay (49). Adult male albino rats were starved for approximately 24 hours. They were then administered a barium meal, consisting of a 50% suspension of barium sulfate in 0.5% gum tragacanth, by intubation in a dose of 0.5 cc/100 gm. of body weight. The analgesic compounds were injected subcutaneously as aqueous solutions immediately preceding the barium meal. The doses used are indicated in table 8. These doses had been found to produce approximately the same degree of analgesia in rats. Roentgengrams were made at 1, 2, 3 and 4 hours following the administration of the barium meal. The results are summarized in table 7. The time for complete evacuation was not determined, but the data obtained indicate that there is a delay in the emptying time of the stomach due to the action of each of the analgesic drugs. Morphine caused the greatest and demerol the least delay. The delay resulting from WIN 1806 was almost as great as from morphine. Morphine

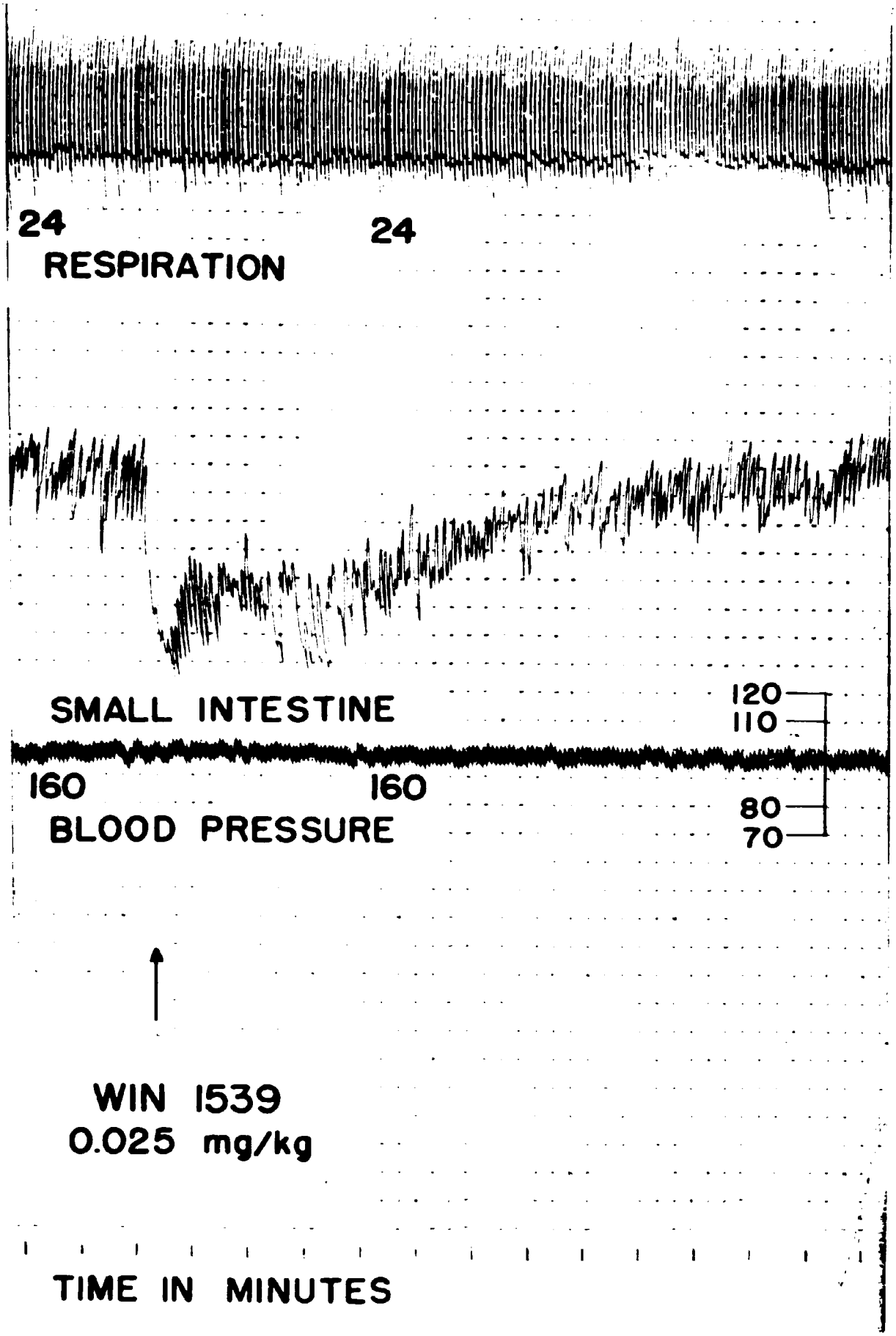

24
24
RESPIRATION
SMALL INTESTINE
120
110
80
70
160
160
BLOOD PRESSURE
WIN 1539
0.025 mg/kg
TIME IN MINUTES

Table 7

Rate of Movement of a Barium Meal through the Gastro-intestinal Tract of Albino Rats.

Drug	No. of Animals	Approximate percent of barium meal remaining in stomach Hours after administration 1	2	3	4	Location of remainder of barium meal 4 hours after administration
Untreated Controls:	7					
Mean		15	9	7	4	Cecum and feces
Range		2-50*	2-25	0-25	0-15	
Morphine (10 mg/kg):	5					
Mean		100	96	86	49	Jejunum and ileum
Range		-	90-100	75-95	25-95	
Demerol (30 mg/kg):	9					
Mean		58	46	34	26	Lower ileum and cecum
Range		20-100	10-95	2-95	2-90	
WIN 1699 (5 mg/kg):	10					
Mean		89	70	46	44	Jejunum, ileum and some
Range		80-100	20-95	10-80	5-80	in cecum of 4 animals

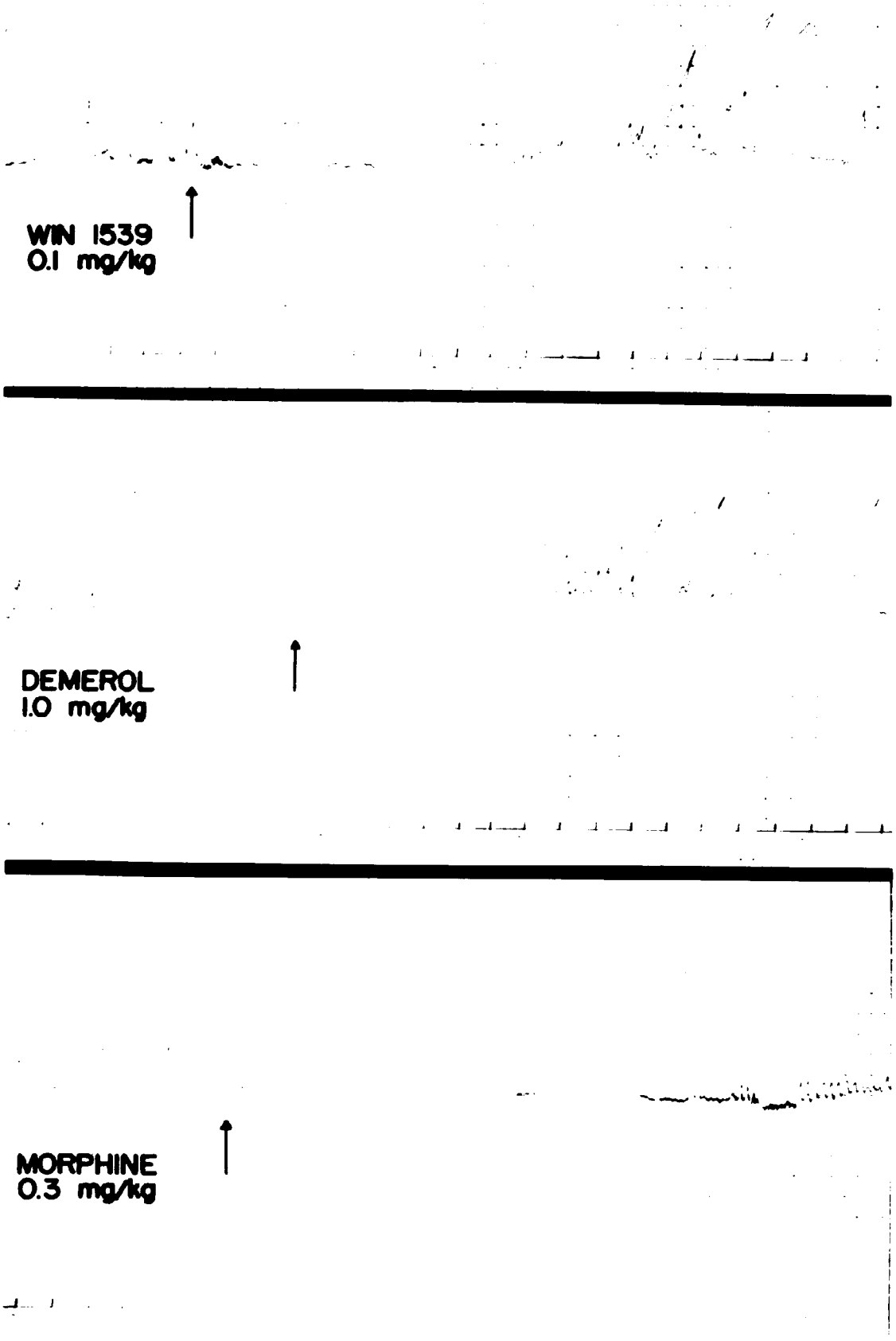
* 2% indicates a visible trace -- other values are estimates of percentage of total volume as determined from area and density of barium meal.

appears to act somewhat longer than WIN 1539 which is also true of its analgesic effect. Harr (50) reported that morphine caused a decrease in distance traversed by a carbon suspension in the intestine of rats and that Demerol was much less active in this respect. This is undoubtedly related to the emptying time of the stomach and our results agree with these.

Effect on stomach in situ:

Since the roentgenographic study showed that the analgesic drugs tested caused a delay in the time of passage of a barium meal from the stomach, the effect of these drugs on the motility and tone of the intact stomach was investigated. Jackson's method (48) of recording contractions of the dog's stomach was modified for use with rats. The rats were anesthetized with sodium pentobarbital and the apparatus attached so that recordings of the pyloric portion of the stomach were obtained. The drugs were injected into the exposed saphenous vein.

A total of twelve animals were used in these experiments. Figure 8 is a record obtained in a representative experiment. It was observed that WIN 1539 in a dose of 0.1 mg./kg. caused a marked decrease in the motility of the stomach. In contrast, 1.0 mg./kg. of Demerol produced an increase in tone and in some experiments also an increase in frequency of contraction. In two experiments morphine caused a response similar to that obtained with WIN 1539 in that the peristaltic waves were abolished.


WIN 1539
0.1 mg/kg
DEMEROL
1.0 mg/kg
MORPHINE
0.3 mg/kg

The marked decrease in the motility of the stomach caused by WIN 1559 or morphine could account for the delay in the emptying time. The delayed emptying time of the stomach is an important factor in the constipating action of morphine. Gruber, Hart and Gruber (51) reported that Demerol produced a stimulation of the stomach and pylorus in unanesthetized dogs. We obtained similar results in rats. In clinical use Demerol does not have a constipating effect. Even though morphine has an inhibitory action on the stomach of some species of animals (Krueger, et al. (15)), Veach (52) found that it is predominantly motor to the human stomach. Because of differences in the digestive processes from species to species it cannot be stated that the actions obtained with WIN 1559 in these experiments would be the same for man.

Development of Tolerance

The development of tolerance to analgesic action is one of the disadvantages of the repeated use of opiates in the treatment of chronic pain. This makes it necessary to increase the dose which in turn increases the degree of tolerance, thereby setting up a vicious cycle. Tolerance has been defined by Himmelsbach (53) as "The gradual decrease in the effect produced by repeated administration of a drug necessary to produce the same effect as did the initial dose." Most investigators believe that tolerance is intimately associated with the addiction liability of a drug. The development of tolerance to morphine has been investigated in several species of animals and this subject has been comprehensively reviewed by Krueger, Eddy and Sumwalt (13). The criterion of the development of tolerance to morphine in most animal experiments has been the disappearance of the narcotic effect whereas in clinical practice the decrease in analgesic effect is more important. It has been demonstrated that tolerance to the various actions of morphine develops at different rates and to different degrees. It is of considerable importance that the development of tolerance to the analgesic action of WIN 1539 be determined.

Studies in rats.

A number of other new synthetic analgesics which are listed below were included in this study for purposes of comparison. Andrews (54) has reported that in patients,

previously addicted to morphine, tolerance develops to the pain threshold raising effects of Demerol. By measuring the duration of analgesia, Scott et al. (55) found that tolerance develops to this action of methadone in rats. Isbell, et al. (56) reported that tolerance develops to methadone in mice, dogs and man.

Analgesic activity in male albino rats was determined by the Ercoli and Lewis (26) modification of the radiant thermal stimulus method of D'Amour and Smith (20) as described previously.

The compounds investigated were morphine sulfate (10 mg./kg./day), WIN 642-4, l-methadone hydrochloride (2 mg./kg./day), WIN 1783-2, l-isomethadone hydrochloride (3 mg./kg./day), WIN 1559, 1-methyl-4-(3-hydroxyphenyl)-4-piperidylethyl ketone hydrochloride (3 mg./kg./day) and WIN 1161-2, 1-ethyl 1,1-diphenyl-3-dimethylaminobutyl sulfone hydrochloride (3 mg./kg./day). The dose of each compound was selected so that the value for the average initial analgesic reaction was between 90 and 100 per cent. Data from a group of twenty rats were obtained for each compound. These rats were given daily subcutaneous injections and their analgesic reactions and body weights determined at weekly intervals.

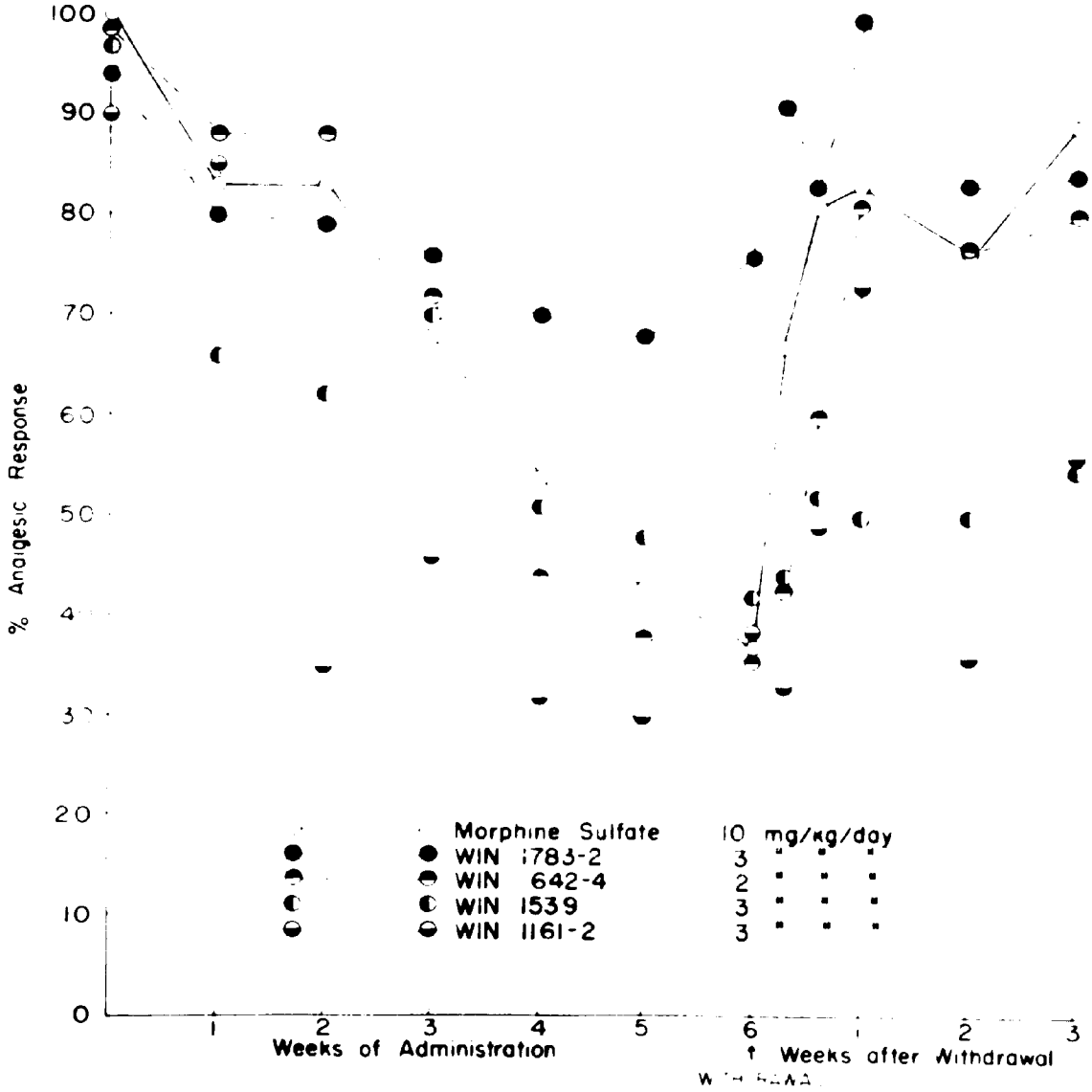
The administration of the drugs was discontinued at the end of six weeks. At this time, each group was subdivided into three smaller groups. On the second, fourth

and seventh days following withdrawal one of these smaller groups was again tested for the analgesic response. On the second and third week after withdrawal the whole group on each compound was tested for analgesia. The groups were divided in this manner so that the time interval between the test doses of the compound for any one animal would be large enough to have as little influence as possible on tolerance. We had previously determined that no tolerance develops when the doses are given at weekly intervals.

The results of the analgesic determinations are given in figures 9 and 10. Daily administration of these compounds leads to a progressive decrease in the degree of analgesia produced. There is some difference among the compounds, however, with regard to the degree and rate of development of tolerance. Tolerance developed most rapidly to WIN 1161-2. The analgesic response to this compound was 40 per cent of its initial value within two weeks and it remained at this low level until the sixth week when administration was discontinued. The least amount of tolerance developed to WIN 1785-2. At the end of the first week, the response was 85 per cent of its initial value and changed little after this time so that it was still approximately 80 per cent at the end of six weeks. The development of tolerance to all the other compounds including WIN 1609 was quite similar and at the end of

EFFECT OF DAILY ADMINISTRATION

ON THE ACTION OF SOME ANALGESIC COMPOUNDS

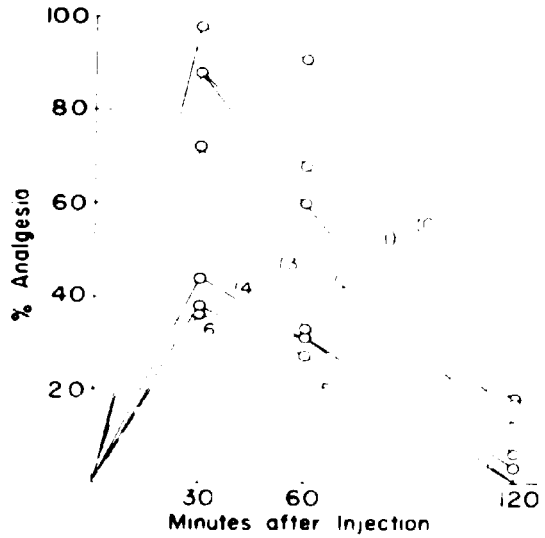


TIME-ACTION CURVES OF ANALGESICS DURING DEVELOPMENT OF TOLERANCE

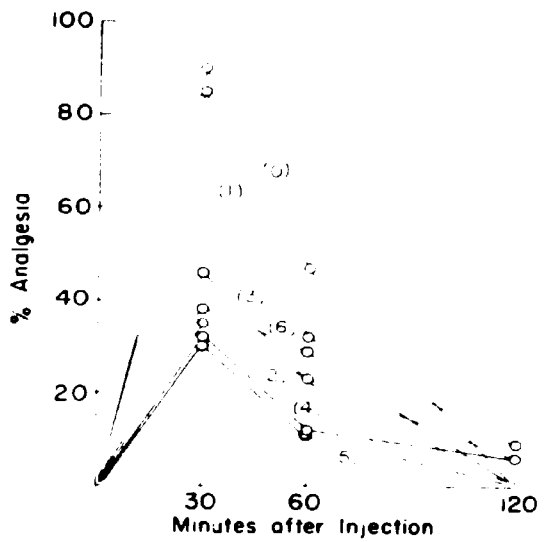
Numbers in parentheses indicate week of determination · (0) = Initial

TIME-ACTION CURVES OF ANALGESICS DURING DEVELOPMENT OF TOLERANCE

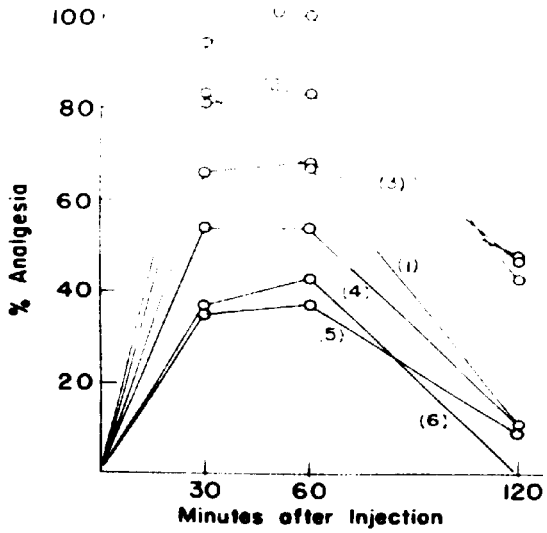
Numbers in parentheses indicate week of determination: (0) = Initial



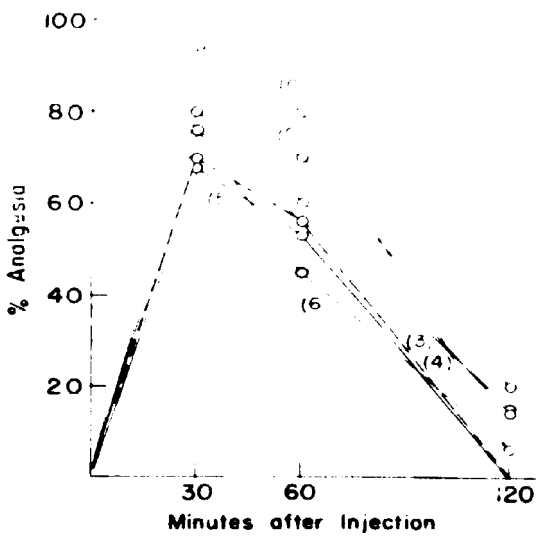
WIN 642-4 (2.0 mg/kg)



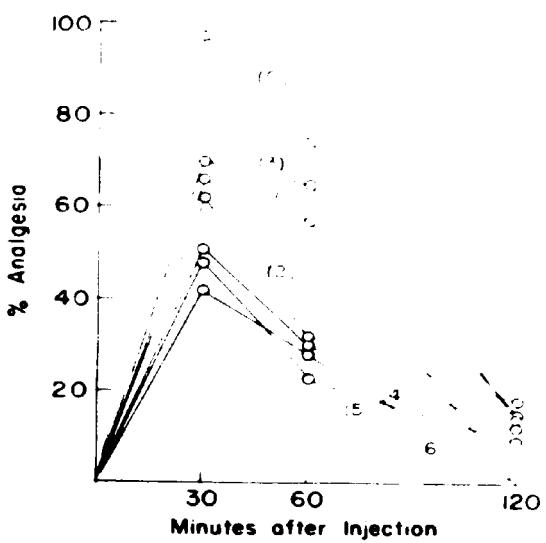
WIN 1161-2 (3.0 mg/kg)



Morphine (10 mg/kg)



WIN 1783-2 (3.0 mg/kg)



WIN 1539 (3.0 mg/kg)

six weeks of daily administration the analgesic response was found to be only about 40 per cent of the initial value.

It is interesting to note that there was no significant change in the preinjection reaction time of the animals during the course of the experiment.

Figure 10 shows the time-action curves for the compounds. These indicate that morphine has a greater duration of action than the other compounds. The maximum effect of morphine is reached at 60 minutes following injection whereas the peak effect of the other compounds is obtained at 30 minutes. As tolerance develops there is a decrease in duration of action that is probably related to the decrease in maximum effect.

In addition to the decrease in the analgesic response that results from daily administration of these drugs, there were definite changes in the side-effects. The characteristic side-effects observed with all the compounds, except morphine, with the doses used here are: slight catalepsy, absence of corneal and wink reflexes and exophthalmos. The rats receiving morphine showed a slight depression. After the animals had been medicated for one week, the most characteristic side-effects were nervousness and excitability which made their handling more difficult. The depression caused by morphine disappeared after three weeks of administration and was replaced by

excitation. These effects were observed after each daily injection and continued for the duration of the experiment. The excitation occurred within about 30 minutes after injection and lasted for about two hours.

Joel and Ettinger (57) also observed that during the development of tolerance to morphine in rats the initial narcotic phase disappeared and excitation became prominent. Tatum, Seevers and Collins (58) reported that the excitation phase of morphine action masked the depressant phase in the tolerant state. The compounds tested in this experiment did not differ essentially from morphine in this respect.

We did not see the increase in preinjection hyperirritability during the daily administration of the compounds that Himmelsbach, Gerlach and Stanton (53) found in rats receiving morphine and which they interpreted as evidence of addiction.

The rates of recovery from the tolerance after withdrawal of these compounds are given in figure 9. The fact that smaller numbers of animals were used for the first three tests after withdrawal probably accounts for part of the variations observed in some of these responses. At the end of three weeks after withdrawal, none of the groups had recovered their original analgesic response, although differences were observed in the degree of recovery with the various compounds. WIN 1783-2, which showed the least amount of tolerance, gave 90 per cent of the initial response

at the end of three weeks after withdrawal. Of the compounds developing the greatest degree of tolerance, the most rapid recovery was observed with morphine and WIN 642-2, these giving responses of 90 and 82 per cent of the normals respectively at the end of three weeks after discontinuance of medication. Compounds WIN 1559 and WIN 1161-2 showed the least recovery for three weeks after withdrawal. WIN 1161-2 gave a response of about 62 per cent of normal and WIN 1559 about 57 per cent.

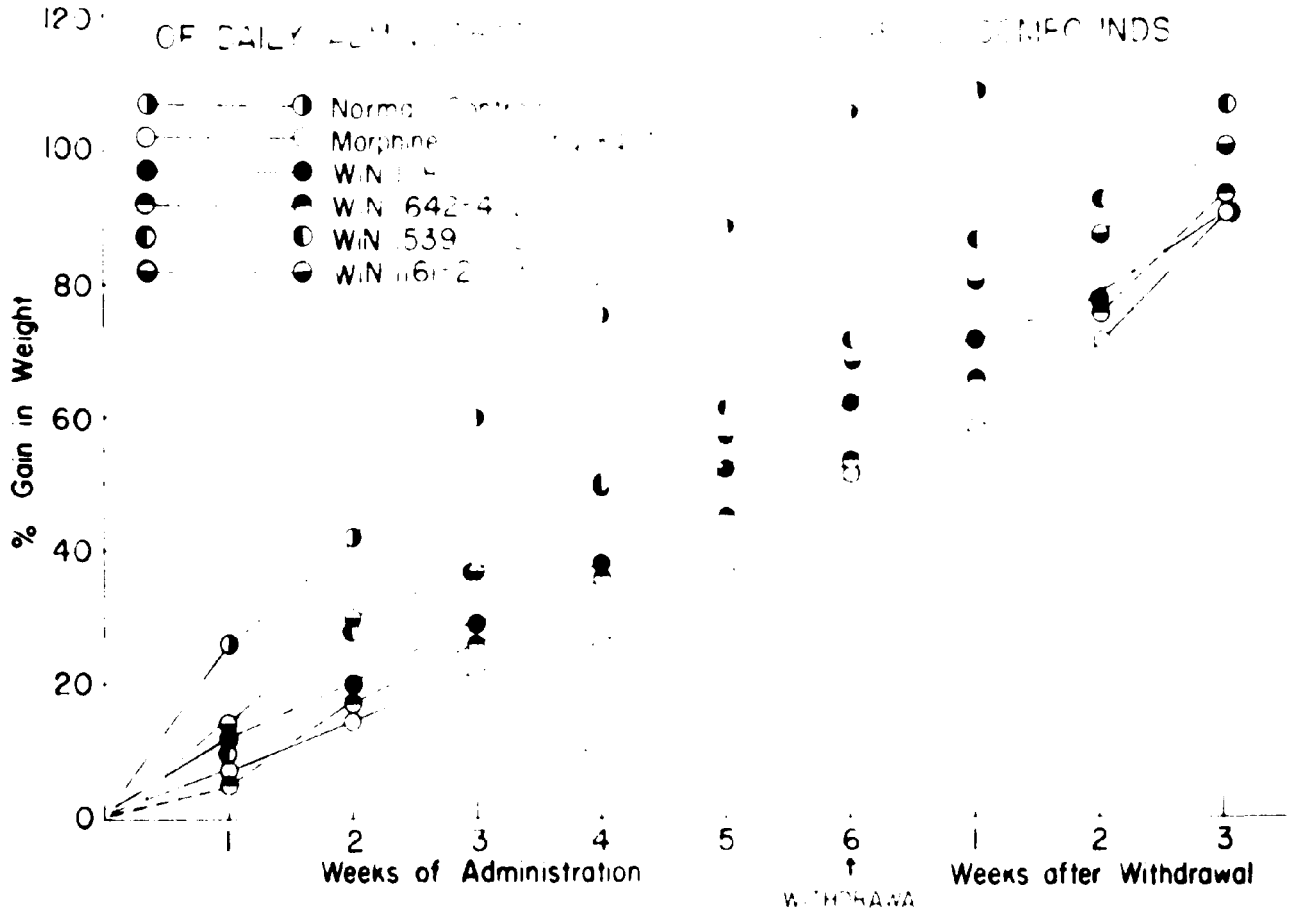
After withdrawal of the drugs, the animals were observed for possible abstinence symptoms. No symptoms were observed at 24 hours, but at 48 hours after withdrawal the animals exhibited a marked degree of excitation as evidenced by increased activity, fighting and biting one another and by chewing on the wires of the cages. This excitation corresponded to that which was observed following the daily injection of the drugs during the test period. At 96 hours there was still some excitation present but it was less than that observed at 48 hours. This excitation was increased after the injection of the drugs during the withdrawal period. Two weeks after withdrawal, the group receiving morphine still developed a marked degree of excitation following injection. A slight excitation was observed in animals receiving WIN 1559 and WIN 1161-2. With WIN 642-4 and WIN 1783-2, slight catalepsy, absence of corneal reflex,

exophthalmos and only a very slight excitability was observed. Essentially the same effects were observed three weeks following withdrawal.

In order to determine whether cross tolerance had developed between WIN 1559 and morphine, ten of the animals which were tolerant to the action WIN 1559 28 days following withdrawal were given 10 mg./kg. of morphine. There was an indication that little or no cross tolerance had developed from WIN 1559 to morphine. The cross tolerance from morphine to WIN 1559 was not determined.

The average weights along with the percentage gains in weight are given in figure 11. There was a decreased rate of growth in all groups receiving daily drug injections. Although it was not measured in this experiment, we believe this is due in part to a decreased consumption of food. Even after withdrawal of the drug there was no appreciable change in the rate of growth. Morphine caused the greatest and WIN 1559 the least depression of growth.

These experiments indicate that tolerance develops to the analgesic action of WIN 1559 at a rate and to a degree comparable to that of morphine. The recovery from the tolerance was not as rapid with WIN 1559 as with morphine. The symptoms observed following withdrawal of the drugs were similar with all compounds.

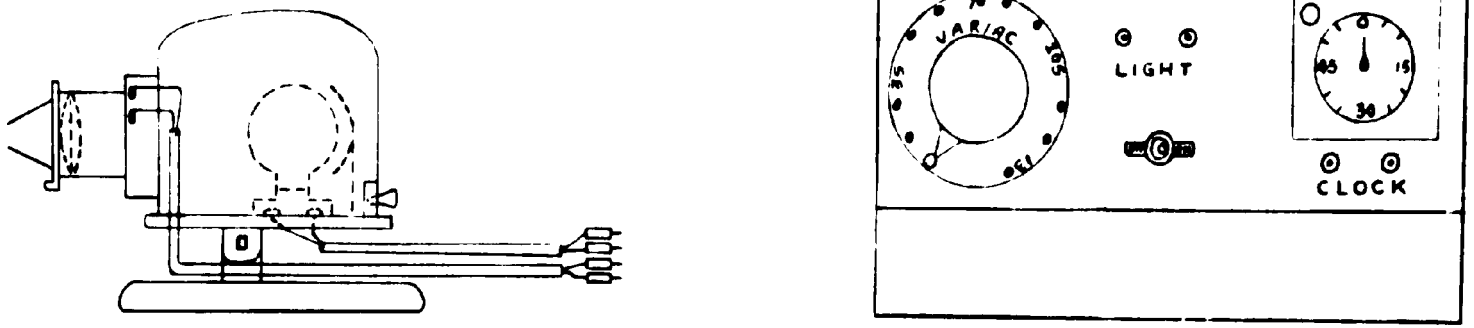

120
100
80
60
40
20
0
% Gain in Weight
Morphine
WIN
WIN 539
1
2
3
4
5
6
1
2
3
Weeks of Administration
Weeks after Withdrawal

Studies in Dogs

In view of the results obtained in rats and the importance of tolerance in the treatment of chronic pain it seemed advisable to determine the development of tolerance to the actions of WIN 1559 in another species.

Dogs were selected as the experimental animals inasmuch as several actions of the compound could be conveniently studied simultaneously with the analgesic actions. Six dogs were given WIN 1559 and three were given morphine sulfate. The drugs were injected subcutaneously daily in aqueous solutions. The initial dose of both drugs was 1 mg./kg. and as tolerance developed this was increased.

Analgesic action of the drugs was determined at weekly intervals by a modification of the Andrews and Workman method (21). The time in seconds necessary to elicit a muscle twitch was determined when a thermal stimulus of constant intensity was applied to a shaved, blackened area of the dog's back. The apparatus consisted of a portable light containing a shutter which was synchronized with an electric stop clock so that when the shutter was opened the clock started and at the time the muscle twitch was noted the shutter was released which stopped the clock. The intensity of the stimulus was adjusted by means of a variable voltage transformer and standardized so that the normal reaction time was about 3.0-3.5 seconds. A schematic drawing of the apparatus is shown in figure 12. We observed that there was a large variation in reaction



SCHEMATIC DRAWING OF ANALGESIC APPARATUS

time with different areas of the back; however for a given small area the reaction time was constant; so for any one determination, the same area of the back was stimulated. After the injection of an analgesic drug, an increase in the time of stimulation greater than twice the normal would produce a burn of the skin. For this reason the maximum time of exposure was limited to 8 seconds, this being designated as complete analgesia. Even though no response could be elicited with the thermal stimulus a strong pinch of the skin would elicit a muscle twitch in some cases; so the degree of analgesia was not complete to all methods of stimulation.

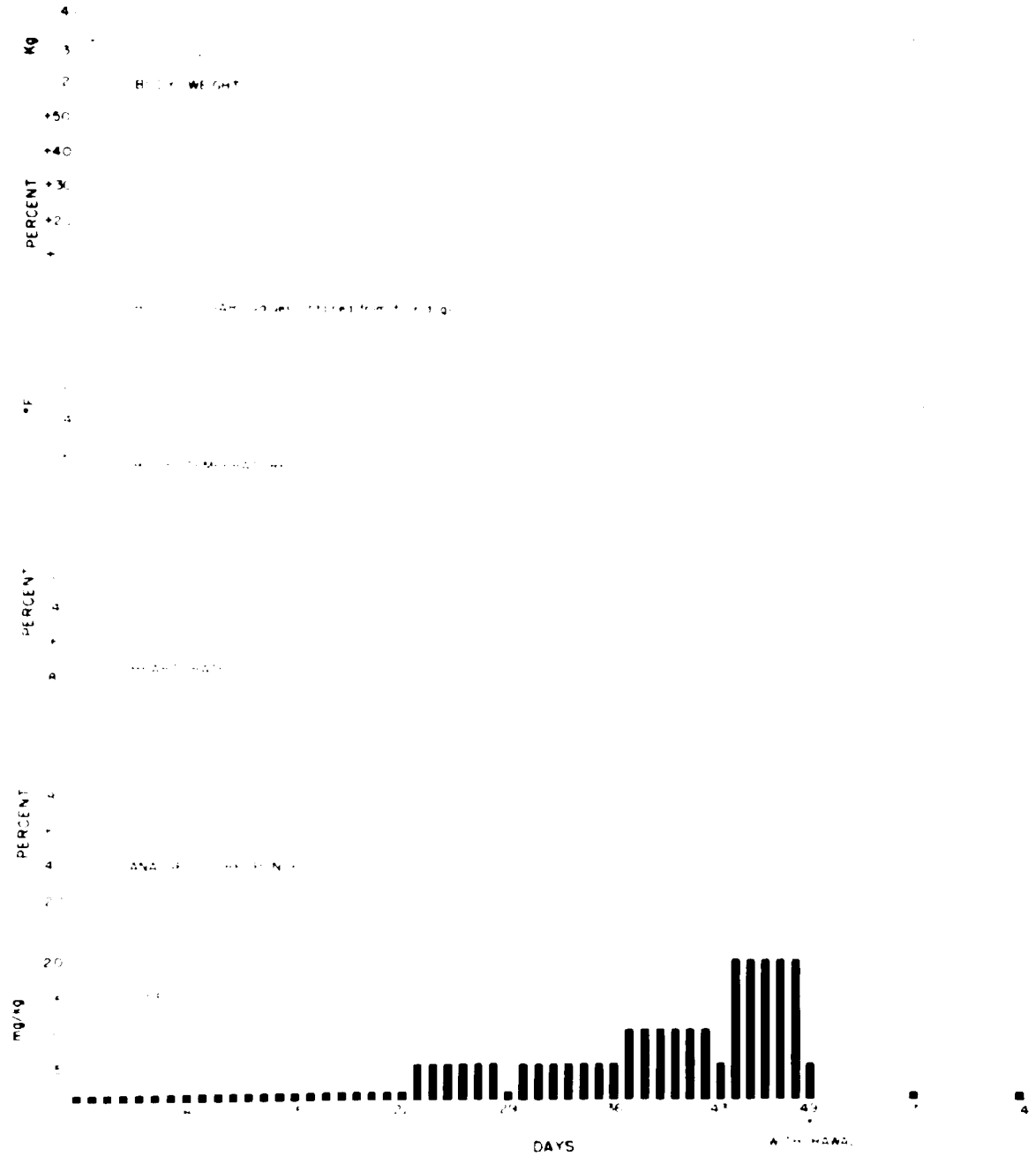
The hyperglycemic response was determined according to the method of Schales and Schales (55) on 4 dogs receiving WIN 1539 and 2 dogs receiving morphine. Blood sugar levels were determined on samples taken just prior to injection of the drug and at 1, 2, 3 and 4 hours following the injection.

Other observations made at weekly intervals following the administration of the drugs included heart rate, body temperature, respiration, side-effects and hematology.

At the end of 7 weeks the administration of the drugs was discontinued and the animals observed for possible abstinence symptoms. At periods of 1 and 2 weeks after withdrawal of the drugs the animals were again given test doses of the drugs to determine the recovery from the tolerance. The results obtained with WIN 1539 are shown graphically in figure 13.

RESPONSES TO WIN 1539

values are averages obtained from six dogs



Analgesia:

After 22 daily doses of WIN 1539, at 1 mg./kg. only one animal gave a lower analgesic response than initially but the duration of analgesia was somewhat shorter. When the daily dose was increased to 5 mg./kg. for 6 days, a test dose of 1 mg./kg. produced a smaller response than initially in all but one animal. There was no evidence of a decreased response to test doses of 5 mg./kg. until the daily dose had been increased to 20 mg./kg. After this dose had been given for 5 days the analgesic response to a 5 mg./kg. test dose was decreased in two animals but in the other four there was complete analgesia.

The development of tolerance to the analgesic action of morphine was more rapid than with WIN 1539. On the 14th day of the 1 mg./kg. dose level there was definite evidence of a decreased analgesic response but a 5 mg./kg. dose on the 15th day produced complete analgesia. Tolerance developed to this dose in one week in two dogs and two weeks in the other. As the dose was increased tolerance developed rapidly so that on the 49th day no analgesia was produced by doses of 40 mg./kg. in one dog and 80 mg./kg. in the other. One animal receiving morphine died on the 55th day of the experiment as a result of a laboratory accident.

One week after withdrawal four dogs showed complete analgesia with a dose of 1 mg./kg. of WIN 1539 and on the second week all but one had recovered from the tolerance. The two dogs in the morphine group had recovered from tolerance to the analgesic action by the second week but at doses of 5 mg./kg. and 10 mg./kg. respectively.

Heart Rate:

The average pre-injection heart rate of the WIN 1539 group was slightly greater at the end of drug administration than at the beginning. As the dose of the drug was increased the degree of bradycardia following drug injection became greater. This indicates that no tolerance developed to this action of the drug. When the animals were tested with a 1 mg./kg. dose on the first and second week following withdrawal of the drug the pre-injection heart rate and the per cent decrease following the injection of the drug were essentially the same as at the beginning of the experiment.

The pre-injection heart rate of one dog receiving morphine had increased at the end of 4 weeks but it was no greater in the other two animals at the end of 7 weeks of morphine administration than at the beginning. There was no significant change in the bradycardia following the injection of the drug during daily administration.

Body Temperature:

There was no appreciable change in the pre-injection rectal temperatures during the course of the experiment. The hypothermia produced by WIN 1539 increased slightly with the increase in dosage. We interpret this as an indication that no tolerance developed to this action of the drug.

There was an indication that tolerance developed to this action of morphine in one dog inasmuch as the decrease in body temperature became less as the daily administration was continued and the dose increased.

Hyperglycemia:

There was no significant change in the average pre-injection blood sugar level during the course of the experiment. There was a development of tolerance to the hyperglycemic action of WIN 1539. A slight decrease in the hyperglycemia following the injection of the drug was observed after 3 weeks of daily administration of 1 mg./kg. When the dose was increased to 5 mg./kg./day for 6 days, a test dose of 1 mg./kg. on the seventh produced no increase in blood sugar. There was evidence of development of tolerance to the 5 mg./kg. dose by the end of 7 weeks during which time the daily dose had been increased.

One dog receiving morphine had developed a tolerance to the hyperglycemic action of this drug by the third week. The other animal showed no hyperglycemic response to morphine at

any time during the experiment. It is assumed that this animal had developed a tolerance since a hyperglycemia would be expected from the higher doses used.

Hematology:

There was no significant changes in hemoglobin concentration, hematocrit, red cell count or white cell count during the administration of the drugs or following withdrawal.

Respiration:

The effect of the drugs on respiration was not determined quantitatively because of the many factors which influence this action. One consistent observation with both drugs, however, was an increase in respiratory rate which appeared 5-15 minutes following injection of the drugs. This increase, which was so great that the dogs panted for several minutes, was followed by a marked decrease in rate which appeared to be associated with the degree of sedation produced by the drug. We observed no marked change in these responses during the daily administration of the drugs.

Side Effects:

Both drugs produced a marked sedation in the dogs. However, it was not of such a degree to be classed as narcosis. The animals were drowsy and quiet but could be aroused easily by a sharp noise. This action appeared

about 30 minutes following the injection of the drugs. Evidence of the development of tolerance to this effect was a decrease in duration of the action with continued daily administration of the drugs.

Vomiting occurred infrequently and was not observed in any of the dogs receiving WIN 1539.

A conditioned salivary response appeared prior to the injection of the drugs during the second week of the experiment. The flow of saliva became greater following the injection of the drugs but usually ceased about an hour afterwards. Occasionally a slight rhinorrhea was observed following the injection.

Body Weight:

Of the dogs receiving WIN 1539 three gained weight and the other three lost whereas all of the dogs on morphine lost weight. This loss in weight was apparently due to a decreased intake of food since the animals which lost weight refused to eat their regular rations. For this reason they were given supplemental feedings of milk and raw meat. Two of the dogs on morphine became constipated but there was no evidence of constipation in any of the animals receiving WIN 1539.

Withdrawal Symptoms:

After the administration of the drugs was discontinued, the animals were observed for abstinence symptoms. The average heart rate of the WIN 1539 group was only slightly

higher 24 hours after withdrawal than on the day of withdrawal. The dogs receiving morphine had an increased heart rate which reached its maximum 48 hours following withdrawal. Twenty-four hours following withdrawal of WIN 1539 there was a slight increase in the average blood sugar level, but at 48 hours it was practically normal. We observed no pyrexia following withdrawal of the drugs. The most noticeable reaction observed was that of excitability. The animals appeared quite restless and whined and barked. They also evidenced a desire for attention. These reactions were most marked the first day after withdrawal but by the end of a week following withdrawal the animals acted normally.

From a practical standpoint, the most important factor in the chronic administration of analgesic drugs is the development of tolerance to the pain-relieving action. Tolerance to the other actions of analgesic drugs is variable both in degree and rate of development. Morphine tolerance in the dog has been studied by many investigators and there are a few reports on tolerance to some of the newer synthetic drugs. Scott et al. (55) observed that tolerance developed to the analgesic action of methadon and Wikler and Frank (60) reported that dogs became tolerant to the sedative, analgesic and hypothermic effects of methadon.

We have found that tolerance develops to the analgesic action of WIN 1539 in dogs but not as rapidly as with mor-

phine. This difference between the two drugs was not observed in our previous experiments with rats. However, the dosage schedule was different and there may be a species difference in this regard.

We noted that tolerance developed to the sedative actions of WIN 1539 but there was apparently no tolerance to the cardiac slowing effect. Similar results have been reported for methadon (56, 60). Unlike the results with methadon, we observed no tolerance to the hypothermic action of WIN 1539. Finnegan et al. (61) reported the development of tolerance to the hyperglycemic action of morphine and methadon. We have observed that tolerance also developed to the hyperglycemic action of WIN 1539. The development of tolerance to the analgesic action appears to parallel that of the hyperglycemic action more closely than any other effect studied.

The withdrawal symptoms observed were not marked. There was only a slight increase in heart rate of our animals in contrast to the tachycardia reported by Scott (55) in dogs receiving methadon. We observed no pyrexia following withdrawal of the drugs as was reported for methadon (56). If the excitability and restlessness which we observed are indications of physical dependence, there was no difference between those dogs receiving WIN 1539 and morphine. There are many factors which influence the development of tolerance and addiction to drugs in experimental animals. Of

these, frequency of administration and rate of increase of the dose are important as well as the duration of the administration. Our animals received daily injections for 7 weeks. Although we were able to demonstrate the development of tolerance with this regimen, the withdrawal symptoms were not marked. Isbell (56) administered methadon to dogs 4 times a day for 10 weeks and obtained abstinence symptoms more severe than those reported here.

Summary

Several algesimetric methods were studied for their applicability as screening methods for testing compounds for analgesic activity. The one found to be most suitable in our hands consisted of stimulating a shaved area of a rat's back with a radiant thermal stimulus and determining the time of reaction for a twitch of muscle of the back.

1-Methyl-4-(3-hydroxyphenyl)-4-piperidyl ethyl ketone hydrochloride (WIN 1539) was found to have analgesic activity about five times that of morphine and ten times that of Demerol.

The analgesia produced by WIN 1539 was potentiated by pilocarpine, eserine and epinephrine. This action was similar to that of morphine.

WIN 1539 produces excitation in cats which is indistinguishable from this action of morphine.

WIN 1539 was found to have a stronger spasmolytic action than Demerol on the stimulated isolated intestinal strip. A decrease in tone of the intact small intestine of the dog was observed. WIN 1539 caused a greater delay than Demerol in the emptying time of the rat's stomach but morphine retarded emptying more than either of these drugs. Demerol caused a stimulation of the intact rat's stomach whereas WIN 1539 and morphine decreased the motility of this organ.

Tolerance developed to the analgesic action of WIN 1539, when administered daily to rats, at a rate and to a degree comparable to that of morphine. The recovery from the tolerance was not as rapid with WIN 1539 as with morphine. The withdrawal symptoms observed with WIN 1539 and morphine were similar.

The daily administration of WIN 1539 to dogs for seven weeks produced tolerance to the analgesic, sedative and hyperglycemic actions of the drug. No tolerance developed to the bradycardia or hypothermia caused by the drug. Recovery from the tolerance occurred in 1-2 weeks following withdrawal. Tolerance did not develop as rapidly to WIN 1539 as to morphine in contrast to results obtained in rats. Anorexia and loss of weight was not as marked in the animals receiving WIN 1539 as in those receiving morphine. A slight increase in heart rate, slight hyperglycemia, excitability and restlessness were the only symptoms noted following withdrawal of the drugs.

The pharmacological actions of WIN 1539 are very similar to those of morphine.

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