EVALUATION OF FIVE ANALGESIMETRIC METHODS USING SMALL LABORATORY ANIMALS

by

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PREFACE

Ever since the dawn of history man's constant effort has been to fight against the sufferings of his fellow-beings. Among these, pain has attracted his maximum attention. Thus, from time immemorial, alleviation of pain has been the primary aim of physicians and many methods have been tried to achieve this. Most of the prescriptions in the early scrolls are towards the treatment of such conditions. From the cave man era when people offered sacrifices to spirits to get relief from suffering down to the present day, great advances have been made in the field of medicine. Much interest is still being shown in the search for agents that will kill pain. Although numerous pain remedies or analgesics are now available, present-day scientists are constantly trying to develop safer and more effective agents to relieve pain.

One of the earliest analgesics used was opium which the early Greek and Roman military surgeons mention. In 1803, Sertürner separated morphine from opium. This was the first time that an active principle - in this case an alkaloid in the form of a white crystalline powder - had been separated from a crude plant substance. Today, morphine stands as the most potent and most widely used analgesic. Unfortunately, the many side effects accompanying the use of morphine and its derivatives and, above all, the development of tolerance and addiction, have made necessary the continued search for other

analgesics lacking these unwanted effects. Consequently, in the past half-century many new pain remedies have been discovered and tried and still more are currently under investigation.

Today, the pharmacologist who seeks to develop new remedies must be able to say how effective a drug is, how it affects the body, how long its action lasts, and, whether or not its side effects or toxicity are minimal or excessive thus either warranting or precluding its use in man. Each of these aspects has to be tested on isolated tissues or laboratory animals and thoroughly evaluated before a drug can be given safely to man. In developing new analgesic drugs and testing them on animals for potency, many laboratory techniques have been devised. From a review of these various methods in order to provide background information for this thesis, it would seem that each investigator attempts to modify or improve existing procedures or to develop entirely new techniques. An evaluation of these different methods would be of great benefit.

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INTRODUCTION

In the study of the analgesic potency of a drug several techniques have been used; however, in all the methods, the first step taken is the production of pain in the experimental animal. Various types of stimuli such as heat, chemical, electrical or mechanical are employed for this purpose. Those techniques employing these stimuli to produce pain and devised to measure the prolongation of the reaction time after drug administration have proven valuable and statistically reliable in screening the narcotic analgesics such as morphine and codeine. But, as far as the non-narcotic analgesics such as the salicylates are concerned, no one particular testing method has been very satisfactory for obtaining precise and measureable data so far. Some investigators, however, claim that the production of pain in small animals by using graded electrical stimuli does provide a reliable method for studying and comparing the analgesic potency of these non-narcotic analgesics.

Several different methods are claimed to be useful and reliable laboratory procedures for determining the analgesic potency of the non-narcotic drugs. The purpose of this research was to study and determine the practicability of using two or three of these methods simultaneously in the same animal in order to evaluate the analgesic potency of both narcotic and non-narcotic drugs. To do this and to obtain simultaneous recordings, it was necessary to slightly modify some of these techniques. Most of these testing procedures have been in use for many years while other newer methods are still in the

experimental stage. In addition to providing information relative to the utility of using combined techniques for studying analgesic potency, this research presents the results of the analgesic potency of several new non-narcotic agents.

Goetzl, Burrill and Ivy (46) in 1934 wrote a critical analysis of the then existing laboratory analgesic testing methods "for the purpose of designing an acceptable standard procedure for measuring and comparing 'pain thresholds'." They point out that most of the early studies on pain were made on human subjects and that the use of animals was only recent. This, they say, may have been because early workers were mainly interested in investigating the pain receptive field in man. Recently, however, attempts have been made to clarify the normal and pathological physiology of the pain receptive organs, of the manner of conduction of pain impulses, and of the nature of the responses to painful stimuli. Further, Goetzl et al (46) designate the ideal criteria for analgesimetric methods:

"1. Algesimetric methods should permit the quantitative determination of threshold values of stimuli.

"2. Algesimetric methods should yield information in quantitative terms as to the least discernible difference between the intensities of two stimuli at every point within the range of useful intensities.

"3. Algesimetric methods should be applicable in both man and animals.

"4. If the different qualities of pain exist, the algesimetric methods should be applicable for the quantitative determination of each quality."

These authors then discuss the different analgesimetric methods employed up to that time in an attempt to formulate an ideal technique.

In 1945, Miller (81) wrote another critical analysis of the analgesimetric methods then available. Following this, much work continued to be done in this field but it was not until 1957 that Beecher (4) again surveyed the subject and, from his extensive work in this field, presented an exhaustive and fascinating review as to the nature of pain as well as the various analgesimetric testing methods then in use. It is amazing to note in Beecher's (4) article that even such crude techniques as used by Haffner (52) in 1929 and others later on have given very good results when compared to some of the more modern methods.

A PHYSIOLOGICAL CONSIDERATION OF THE NATURE OF PAIN

Even today, with all the great development and advancement in science and modern medicine, pain is still one of the least understood subjects. There are too few known and definite facts upon which we can base our thinking. Berger (5) quotes Sherrington, 1947, who described physical pain as "the physical adjunct of an imperative protective reflex." But it is obvious that protective reflexes do not necessarily precede painful sensations. As with most other sensations, there must be an integration of peripheral stimuli by the central nervous system in pain, too. It would be a great achievement when it will be possible to discover how stimuli are translated into perceptions or sensations. So far, neurophysiologists have offered very little in answer to this important question.

Berger (5) has attempted to provide a pharmacological description

of pain by his classification of the analgesic drugs into three large groups:

1. Those that modify the perception of the pain-stimulus at the receptor site.
2. Those that modify the central perception of pain.
3. Those that change the reaction of the individual to the pain experience.

To the first group belong drugs such as the local anesthetics which paralyze peripheral sensory nerve endings to obtund pain, or atropine which relieves smooth muscle spasm by blocking the transmission of cholinergic impulses at the peripheral site. Previously, Beecher (4) concluded that analgesic drugs seem to act mainly on the "reaction component" rather than on the "original sensation". Berger adds to this conclusion that while morphine and its chemically and pharmacologically related synthetic allies and, also, alcohol and anesthetics, no doubt affect the "reaction component" to a great extent, it is not clear as to what extent these drugs affect the perception of pain. In this respect the recent work of O'Dell (35) may be helpful since he used both of the above mentioned components as experimental parameters in the evaluation of analgesics. O'Dell studied codeine, meperidine, dextro propoxyphene and other analgesics employing different methods to evaluate not only the analgesic property but, also, to study voluntary motor activity and interneuronal blockade or stimulation. The effect of each drug on interneuronal pathways was determined by utilizing two reflexes which required polysynaptic transmission of impulses. Thus, by this latter method, he could determine whether

or not the drug possessed skeletal muscle relaxant or stimulant properties. Codeine appeared to show moderately potent analgesic activity over a fairly wide dosage range, but it showed no indication of muscle relaxant properties despite its central depressant effect. Since the depressant action of codeine was found to be in the same dosage range as that where the analgesic activity was evident, the two actions may be said to be inseparable. With meperidine O'Dell found that analgesic activity ranged from moderate to strong over a relatively short dosage span. Some central nervous system dulling or depression occurred but O'Dell felt that this should not play as significant a role in producing analgesia as was the case with codeine. Thus, by using different parameters, it does seem possible to evaluate and compare the effects of analgesics by measuring the central effects of the "reaction component" and the peripheral changes in the "original sensation" at the site of the stimulus.

Berger (5) also points out in his paper that "a substance that would change perception of pain without affecting the reaction to it would clearly be of great scientific and practical interest. Such a substance would raise the threshold to pain reaction. Such a substance might not be addicting as it would not necessarily affect the mind and would not produce euphoria."

AN ANATOMICAL CONSIDERATION OF THE NATURE OF PAIN

The neuroanatomist's work concerns what is probably the most complex and least known area of the body. Gradually, an understanding is being developed of the complicated mechanisms by which the sensory stimuli are changed to nerve impulses, become modified, and finally arrive at various levels of the central nervous system.

The sensory mechanism for pain is, in many ways, unique as Ruch and Fulton (90) point out. These writers call attention to the fact that the sensory end-organs for pain are present throughout virtually all tissues of the body and, on this basis, three kinds of pain may be recognized and designated: (1) superficial or cutaneous; (2) deep pain from muscles, tendons, joints and fascia; and, (3) visceral pain. Together the first two comprise somatic pain. These workers state, also, that the pain endings are unique in that they exhibit only to a limited degree the phenomenon of the adequate stimulus (by definition, adequate stimulus is the form of energy to which the receptor is most sensitive rather than whether or not the stimulus elicits a response). Many types of energy, such as electrical, mechanical, extremes of heat and cold, as well as a wide variety of chemical stimuli are adequate to elicit pain. Thus, it would seem that the pain endings are not specialized to react to any one single form of energy but to a variety of them and, as well, to extreme degrees of different kinds of stimulation.

Ruch and Fulton (90) describe the dendritic processes from the sensory nerve cells, the latter situated mainly in the spinal ganglia

and sensory ganglia of the cranial nerves, as innervating the various tissues of the body. For example, the terminal fibers conducting pain which are located in the superficial layers of the skin are found to branch freely and to form a fine overlapping network of naked fibrils. Because of this overlapping, each area of the skin is innervated by two or more nerves. These investigators offer the following classification for the different types of nerve fibers:

(1) The A fibers - myelinated, somatic, afferent and efferent fibers.

(2) The B fibers - myelinated, efferent, preganglionic axons found in the autonomic nerves.

(3) The C fibers - (a) unmyelinated, the s.C class being the efferent postganglionic sympathetic axons, and, (b) the d.r.C class which are the small unmyelinated afferent axons found in peripheral nerves and dorsal roots.

Using Lloyd's (76) classification, Ruch and Fulton describe the large rapidly conducting A fibers as comprising three distinguishable groups:

Group I fibers which are found only in muscle nerves and, apparently, are not sensory. Impulses carried by these fibers have not been traced to the thalamus and cortex.

Group II fibers found very infrequently in muscle nerves, but which make up a large part of the branches to the cutaneous nerves.

Group III fibers, also termed A fibers (A delta), found in both muscle and cutaneous nerves.

Of these above mentioned fibers, there are at least two types which have been recently identified as the ones conducting impulses initiated by a painful stimulus (44). These are the myelinated A delta (A group III fibers according to Lloyd's terminology) and the d.r.C unmyelinated fibers. The myelinated A delta fibers are mostly 2 - 4μ in diameter and conduct at a speed of 10 - 20 meters per second (m/sec.). They form one third of the total number of myelinated fibers in peripheral mixed nerves and carry a certain type of pain impulse. For example, when a single stimulus is applied to the ending of one such fiber, the subject experiences a sensation similar to a tap. But, on applying a tetanizing stimulus to the same spot, there is a definite experience of pricking pain.

The second group of pain afferents, the unmyelinated C fibers, are very thin afferent axons measuring only about 1μ in diameter and conducting at the slow rate of 1 - 2 m/sec. The number of C fibers in the somatic nerve trunk may be four times as many as the total of myelinated A fibers and comprise as much as twenty per cent of the fibers in a sympathetic trunk. Also, the threshold for C fibers is one hundred times as great as that of the A fibers. As early as 1935 Clark et al (18) showed evidence of the existence of pain reflexes (and, therefore, presumably pain) being elicited by C impulses when the saphenous nerve of the cat was used as the test object. These investigators stimulated this nerve with graded stimuli, and observed the resulting nerve impulses using a cathode-ray oscillograph. Changes in respiration were recorded pneumographically. When the strength of current was increased

to a point sufficient to stimulate the C fibers, the rate and depth of breathing were found to be increased. When the A group fibers were blocked by applying pressure, only the C wave activity was detectable in the electroneurogram. Then, in 1939, Zotterman (107) confirmed the relation of pain to the C fibers. Furthermore, he found that the fine myelinated fibers of Group III (A delta fibers) also conduct pain impulses. The recent work by Brookhart et al (10) demonstrated that fibers from the tooth pulp, which may be considered as a classic pure pain source, have the conduction characteristics of Group III fibers. This finding is consistent with their being myelinated. But they could find no conduction at C fiber rates. Ruch and Fulton (90) also point out that there are fewer unmyelinated fibers in the cranial than in the spinal nerves.

With this background, an approach may be made to understanding the phenomenon of double pain. By double pain is meant the quick, sharp, unpleasant sensation upon which can be related to conduction in the relatively fast A delta fibers together with the slow dull, aching or burning pain which is carried in the C fibers. With respect to the latter, it is known that drugs such as cocaine block the slow C fiber components first. On the other hand, the C fibers appear to be blocked last in the agony of asphyxia. In disease conditions such as neuro-syphilis, the Groups II and III fibers probably are damaged to a greater extent than the C fibers. Thus, when the posterior columns of the spinal cord and the nerve roots have been damaged by syphilis, there is loss of touch and proprioception but no change in the sensation of

pain. However, there is a delay of 1 or 2 seconds in experiencing a painful stimulus. This latent period for the pain response in most tabetic patients and, in the normal individual for the awareness of second pain, are approximately the same. In both tabes dorsalis and nutritional neuropathy there is early loss of light touch, position sense, and vibratory sensibility. These neurological findings can be explained on the basis that the impulses mediating these sensory functions travel in the fast-conducting fibers of the A group. Another observation to be mentioned here is that made by Landau and Bishop (74) who produced asphyxia in man in order to block out the Group III pain impulses or, in other words, the activity of the A delta fibers. When the block was effective and an adequate stimulus applied, the Group III pain impulses could not precede the slow pain impulses along C fibers so that the subject experienced only dull, burning, disagreeable pain.

From the preceding discussion it would appear that there are two distinct afferent pain conducting systems. Two terms introduced by Head and Holmes, which Gerard (44) quotes, fit into these two systems. One is called protopathic sensation which is diffuse and vaguely discriminated; the other the epicritic type of sensation which is discrete and localized. These pain-recording systems function in one sense as antagonistic to one another and, in the other, as complementary (44). Thus, when the epicritic A delta system is stimulated, a clear sharp pain is felt. It does not, however, cause continued suffering. Yet, at the same time, it might be acting to inhibit that kind of pain

caused by stimulation of the protopathic C system. As an example, it may be mentioned that aching pain experienced in the lower portions of the body or itching, such as pruritus ani, may be relieved by section of the ventrolateral columns. On the other hand, this variety of pain is exacerbated by section of the dorsal columns. Also, it has been observed that itching - due to activation of C fibers - may be relieved by scratching, the latter stimulus serving to activate the A delta fibers.

The pathways of the afferent pain fibers may be traced upwards in the spinal cord. Gerard (44) further mentions that those messages ascending in the A delta fibers are carried in the ipsilateral dorsal column. The impulses travel primarily in relatively large fibers and speed along at 50 - 80 m./sec. The impulses coming via the C fibers cross and ascend in the crossed anterolateral columns or the spinothalamic tract. Here they are carried by thin fibers which are perhaps 2μ in diameter and are conducted at a lower speed. The different systems can be traced upwards in these columns to the midbrain at which locale they can be sectioned separately. Then they pass through the thalamus and, finally, on to the somesthetic areas in the cortex. Some of these fibers go to the reticular formation from which other fibers originate to alert the cortex.

Apart from pain, sensations of touch, pressure, warmth, cold, sexual sensations, tickle, itch, and feelings of muscular fatigue travel along the spinothalamic tract. However, some of the fibers carrying touch and pressure sensations also travel along the dorsal column tracts along with the kinesthetic sensations.

TECHNIQUES IN ANALGESIMETRY

A review of the literature over the past fifty years reveals the trial of an amazing variety of laboratory and clinical methods for testing the analgesic potency of drugs. Many kinds of stimuli have been used such as chemical, thermal, mechanical, electrical and even audiogenic to produce experimental pain (4). The animals employed in these studies have ranged from mice, rats, guinea pigs, rabbits, dogs, cats, to even horses and monkeys (71). While this review was concerned mainly with analgesic testing methods used in the laboratory, mention will be made of some of the clinical techniques now in vogue.

In the following description of various analgesic testing techniques abstracted from the literature, the methods are categorized according to the type of pain stimulus employed.

CHEMICAL STIMULATION METHOD

Chemical stimuli for causing pain have been used only in man. Most commonly employed has been the vesicant agent cantharidin to cause a blister. The separated epidermis is then removed from the area and about 0.2 ml. of the test solution applied at intervals of 5 to 10 minutes. The pain threshold was determined by having the patient squeeze a pressure bulb which recorded a tracing on a moving drum indicating the intensity of pain. This was found to be quite consistent for a given individual (4).

THERMAL METHODS

Barbour and Maurer (3) reported in 1920 that the heat of a lighted match applied to the tip of the rat's tail could be used to test the analgesic effect of morphine. Later, in 1934, Hildebrandt (60) produced pain in the guinea pig by applying a metal cylinder containing water heated to 40°C. to a shaved area over the lumbar vertebrae. The duration of the stimulus was 20 seconds. If no defensive reaction developed, the temperature was increased to 45°C. Hildebrandt found that most guinea pigs reacted at 45°C. while at 50°C. the reaction was immediate. Failure to show a response was taken as indicative of analgesia. While this crude thermal technique evoked fairly constant responses in some animals, in others considerable irregularity was noted.

Another thermal method for producing experimental pain in man was introduced by Wolff, Hardy and Goodell (53) in 1940. Since then many modifications of their technique have been made. Also, this procedure has been adapted for use in animals. In the Wolff, Hardy and Goodell experiment, the light from a 1000 watt bulb is focused on the blackened foreheads of the subject. A shutter arrangement allows exposure to the light radiation for exactly 3 seconds. The intensity of the radiation is regulated by a rheostat to a point where the subject just feels pain. This intensity of radiation causing pain is measured radiometrically in gm. cals./sec./cm.^{2} and may be defined as the pain threshold. These workers observed that the threshold for pain was constant and independent of the emotional and physical state of the subject. Also, the

intensity of the stimulus required to produce pain remained the same regardless of the size of the skin area stimulated. After drug administration, changes in pain threshold may be followed until all threshold-raising action has ceased.

Andrews and Workman (2) employed the Wolff-Hardy-Goodell apparatus for measuring the pain threshold in dogs. The mid-dorsum of the thoracolumbar region of the dog was depilated and then blackened with India ink. The light beam was applied to this spot by placing the apparatus against the skin of the animal in the same manner as when applied to the forehead in the human. These experimenters found very close similarity in the pain threshold of dogs to that of man. The response in the dog was a characteristic reflex twitch of the musculature of the back.

In 1941, D'Amour and Smith (19) inspired by the work of Wolff, Hardy and Goodell (53), adapted this method to rats. They used the rays from a Mazda 1184, 6 - 8 volt bulb placed in a reflector. A voltage regulator, transformer and rheostat were used to regulate the intensity of the light beam. A stop watch was connected to the circuit so that it could be operated by the same switch which turned the electric current on or off. A grooved board for holding the rat's tail was placed about 6 inches below the light source. For testing the pain response, the tip of the tail was placed in the groove, the light and stop watch switched on and then, when the response occurred, switched off. The pain response was "a sudden, typical twitch of the tail." A light intensity which evoked the response in about 5 seconds was

found most convenient. Using this technique, D'Amour and Smith (19) carried out a comparative assay of the analgesic potency of five opiate drugs obtaining good results. Individual variation in the animal's response was found to be small when studied under a variety of conditions such as in the adrenalectomized versus the hyper-cortin treated rat, the thyroidectomized versus the hyperthyroid rat, the castrate (male and female) versus the "hyper-estrin", the "hyperprogestrin" and the "hypertestosterone" treated animal, and whether the rat was tested in the day time or night time, in the cold room versus hot room, or when the animal was starved.

Several modifications of the D'Amour-Smith principle have been introduced. Davies et al (21) used a small coil of resistance wire operated from a 6 volt battery as the heat source. The wire was of such gauge and length that it would produce a bright red heat when the circuit was closed. An asbestos plate was placed just above the heat source with asbestos strips glued to the upper surface of the plate so as to form a channel 4 inches long and 1/4 inch wide. At the center of the channel a 1/4 inch hole was bored through the asbestos to allow passage of the heat from the wire coil. With the rat contained in a cylindrical holder of perforated zinc, its tail was placed in the channel so as to have the hole within 1½ inches from the tip. The reaction time was determined similar to D'Amour and Smith's technique but the analgesic effect was taken as the difference between the mean control and the post-drug reaction time. Chen (16) and his associates used this technique in a slightly modified manner.

Ercoli and Lewis (36) used another modification of the Wolff-Hardy-Goodell method in the rat. Instead of the tail, they used a shaved area on the back of the rat which was exposed to a constant heat stimulus provided by a 1000 Masda lamp. The lamp was covered with a metal box containing a small window. Light from this window passed through a biconvex lens of 10.2 cm. diameter and was concentrated at a focal distance of 13.5 cm . A lucite screen in which a small hole was cut for allowing passage of the light was placed against the shaved area of the rat. The animal was then placed so that the exposed area of the skin would be at the focal point. A transformer and a voltmeter were used to vary the light intensity. When this method was used it was found that normal rats showed two distinct sets of reactions: (1) twitching of the skin, particularly over the irradiated zone; and (2) retraction of the entire body in an attempt to escape, or, only escape movements. In the majority of animals the two responses occurred simultaneously or in close sequence after 4 to 5 seconds. The smallest range of error was found when 85 volts were used with the average reaction time being 4 seconds. To express the analgesic activity of various drugs, Ercoli and Lewis grouped the rats according to their different reaction times which varied from 2 to 8 seconds. They chose only those which had a reaction time from 3.8 to 4.46 seconds, again grouping them accordingly. The average analgesic dose of a drug was taken to be that which increased the reaction time to anywhere between 6 and 14 seconds in approximately 40 to 60 per cent of the group. The remaining animals were uniformly distributed between "zero and complete

analgesia". Stimulus was stopped at 15 seconds to prevent burning. They found, also, that hamsters, guinea pigs and cats could be studied by this method. In man when a strength of 85 volts was used, pain occurred suddenly in the form of a pricking sensation. Lengthening the time of exposure by twenty-five per cent caused, instead, a burning sensation. Pain sensation became very pronounced on doubling the exposure time and blisters appeared when it was increased to 14 seconds.

Foster and Carman (40) modified the Ercoli-Lewis method by using higher wattage (1500 watts) with lower voltage (110 V.) producing, thereby, a greater proportion of heat to light They proposed a method for determining analgesic activity quantitatively using the formula $\sqrt{\frac{t_2}{t_1}}$. In this formula t_1 is the normal reaction time in seconds and t_2 the reaction time after the drug.

At about the same time, Thorp (96) tried several methods in animals starting with the original Wolff-Hardy-Goodell method applying the light on a shaved and blackened area of the flanks of guinea pigs and then on rats, and later to the rat's tail. The last procedure was found to be the most sensitive. In their method the time exposure was kept constant while the light intensity was varied. The analgesic effect was measured as per cent increase from control threshold.

Winder et al (103) devised a somewhat complicated apparatus combining features of both the Wolff, Hardy and Goodell (53) and the Ercoli and Lewis (36) methods. It was hoped that this modification would overcome some of the short-comings of the other methods. The main feature of their method was the application of the thermal stimulus at

mechanically regulated periods of time with exposure of the thermal stimulus for a set duration of time. A 500 watt projector lamp was used with its rays reflected by a concave mirror in such a way as to pass through a metal tube of about 3 inches in diameter and to focus on the skin of the animal. The intensity of the light was regulated by a voltmeter. By means of a shutter placed in this tube it was possible to interrupt the light beam. This was done by connecting the shutter to an electric motor which was equipped with a reducing head of gears and an adjustable notched wheel, the latter making one complete revolution every 37.3 seconds and opening the shutter. A duplicate notched disc was fastened by means of a set screw on the inner surface of the first wheel so that the gap of the notch could be widened or narrowed. The length of this gap determined the interval of light exposure through the shutter. The electric motor also operated a fan which drove air from a side shaft into the metal tube to cool the lamp. Guinea pigs, loosely confined in a box, were used and the stimulus was applied to the depilated skin of the dorsum. The response sought was a skin twitch.

Winder and his group chose to use a stimulus of fixed duration and variable intensity. They undertook three different approaches which they described as (a) ascending, (b) descending, and (c) interval splitting. In "ascending" approach the intensity was increased up to a strength at which the reaction was elicited on two successive occasions. In "descending" approach the intensity was decreased until there was failure in eliciting a response on two successive tests. In the

"interval splitting" approach, one response had to be positive and the other negative in two adjacent trials. The last approach was chosen since these workers felt that it would allow systematization of the tests. Furthermore, the results would not be influenced even when large changes in threshold occurred during the time required in this test. They also studied the minimum suitable recovery period in guinea pigs and found that by allowing 70 seconds between stimuli which lasted for 4 seconds, the possibility of accumulation of the effects of stimulation was remote. Employment of dark skinned guinea pigs instead of blackening the fair skinned, lowered the variability of data but further blackening of the skin did not show more improvement. Winder and his group also noted that changes in the room temperature had a remarkable influence on the radiant heat threshold in guinea pigs.

Comparing the D'Amour and Smith and Ercoli and Lewis techniques, Winder et al (103) mention that they chose fixed duration of exposure to stimulus in contrast to fixed intensity of stimulus because the former yielded more uniform results. Besides, they claim that the low intensity radiant heat used by their predecessors may have been one of the factors responsible for the frequent non-finite duration values obtained and which made them treat their data in a semiquantitative manner or on biased averages.

The so-called hot plate method for testing the analgesic potency of drugs and using the mouse was first described by Woolfe and MacDonald (77) (106). The apparatus was a zinc plate which could be

maintained at temperatures of 45°C. or upwards. A hollow glass cylinder 15 cms. in diameter and about 8 inches high was placed on the plate to encase the mouse when it was dropped on the plate. The end-point was taken as raising, kicking or dancing of the hind limbs or if the mouse tried to jump out of the cylinder. After trying various plate temperatures, it was found that at 55°C. all the tested animals reacted within 30 seconds and at 60°C. all reacted within 20 seconds. Since mice, normally, often sit up and lick their front paws, the movement of hind limbs was used as the criterion for acute discomfort. Thirty seconds was taken as the standard exposure time, groups of 10 mice were tested, and the number of mice showing analgesia (those not responding in 30 seconds) was noted. The temperature of the plate was increased in increments of 5°C. from 55°C. to 70°C. Higher temperatures caused damage to the feet.

Eddy (32) (34) modified Woolfe and MacDonald's hot plate technique in order to get a better control of the temperature of the plate. He first used a wooden box frame to support the copper plate and a 40 watt bulb was placed in the box as a heat source. In order to get a uniform temperature, he further modified this by placing the copper plate over a solution of equal parts of ethyl formate and acetone contained in a pan. When this mixture was kept boiling, the covering plate remained at a constant temperature of 55.8°C.

Another device employing a thermal stimulus and used for testing pain responses in mice is the "conduction dolorimeter". It consists of a small wooden box measuring 12 by 6 by 6 inches. Attached to it

are a rheostat, the off-on switch, a heating coil, and a ring thermometer, the latter serving to indicate the temperature. The manufacturers recommend not to exceed the 60 point on the rheostat dial. The mouse's tail is placed in the groove above the coil and the pain reaction noted by a stop watch when the animal flips its tail away. O'Dell (84) (85) used the "conduction dolorimeter" to apply the stimulus to the hind foot of the mouse. This procedure gave consistent results when groups of 20 mice were tested following administration of each dosage of a drug and compared with control groups tested concurrently. The analgesic activity was based on the percentage increase of the reaction time in the treated group from that of the control group.

MECHANICAL METHODS

In 1929, Haffner (52) produced a measureable pain stimulus in the mouse by placing a screw clamp near the base of its tail. When the screw was tightened a certain amount, the pain reflex occurred. While only a rough estimation of the potency of a drug could be made with this procedure, it is to be noted that it was this simple test which led Haffner to the discovery of the analgesic properties of meperidine.

In 1936, Kniazuk (67) tested analgesia by squeezing a mouse's tail with a dissecting forceps which had a wedge-shaped disc mounted between its blades to limit the degree of closure. The smallest degree of compression making the animal cry when the forceps were suddenly closed was taken as the pain threshold. A group of 12 mice was used each time. Later, Molitor and Latven (82) used this technique for comparing a number of analgesics.

Amsler (1) tried to produce pain on the skin of a rat or a dog by pinching it with forceps, by pricking it with a needle, or by burning it with an electric cautery. If no response was elicited after administration of a drug, it was considered to have an analgesic response.

Hesse, Roesler and Böhler (59) used guinea pigs whose depilated skin had been previously injected subcutaneously with 0.05 ml. of croton oil to produce an inflammed area which became very sensitive to pressure. Absence of reaction on pressure was taken to be the criterion for analgesia.

Weiss (98) studied the local anesthetic effect of procaine by using a mechanical device to stimulate the rabbit's cornea with a 100 mg. weighted hair at the rate of 4 times a second; complete anesthesia was obtained when stimuli applied 100 times in 25 seconds failed to cause a response. Keil and Pöhls (66) modified this technique when they investigated the potentiating action of morphine on local anesthetics.

In 1932, Eddy (26) used a cat in order to apply pressure to the tip of its tail by means of a specially calibrated device which made the animal cry or struggle as a response to pain. When 4 of the 5 animals tested showed an increase in the threshold of the required pressure to cause pain, analgesia was considered to be present. Eddy was able to differentiate between decreased sensitivity and complete analgesia. Slaughter and Munsell (94) later used this method to study some other aspects of the action of morphine.

Krueger et al (71) (72) mention that Amadon and Craige used the horse. The technique was to pinch the skin over the shoulder using a long heavy hemostat with rubber covered jaws. Analgesic potency of a drug was determined by the degree to which the jaws of the hemostat could be closed before eliciting a response.

Friend and Harris (41) (58) used Eddy's (26) technique in rats and observed that if the tail became irritated and tender from earlier tests, subsequent tests would not be successful. (This holds true for most pressure methods where a certain amount of trauma is unavoidable.) In order to overcome this objectionable feature, Green and Young (49) introduced a new pressure method for studying analgesic drugs which had many advantages over the earlier pressure techniques. A carefully graded amount of pressure was applied to the tip of a rat's tail by a squeezing device consisting of the head of the plunger of a hypodermic syringe below which the tail tip was placed. As the plunger was lowered vertically the tail was pressed against a fixed wooden board. The plunger was operated by connecting the outlet of the syringe to a T-tube, one arm of which was connected to a U-shaped mercury manometer and the other to another syringe which was placed horizontally. The plunger of this second syringe was driven by a "constant injection" type of device ("Synclock" electric motor) for the sake of convenience and standardization. A mixture of equal volumes of kerosene and liquid paraffin was used to displace air and for transmitting pressure changes in the system. When the test was to be done, the distance between the head of the plunger and the board was adjusted to admit only the tail tip and the manometer scale on the U-tube re-set to give a zero reading.

With the pressure being slowly increased and measured by the mercury manometer, the rat squeaked when the pain threshold was reached. Green and Young (49) reported that this technique caused no tissue damage even when the pressure was increased to four times the control value.

Brodie, Way and Smith (9) modified the pressure method for eliciting a pain stimulus and found their procedure of some value in evaluating the salicylate-type of analgesic drugs. The method consisted in applying a sharp pressure stimulus on the rat's tail about 1 to 2 cm. from its base. The amount of pressure given was calibrated in grams. An aluminum welding rod tapered at one end to a sharp point and manipulated in the barrel of a modified microscope to give the desired grams-pressure produced the stimulus. A full description of this technique is presented in the next chapter under Materials and Methods.

ELECTRICAL STIMULATION

Von Helmholtz (97) used electrical current as early as 1851 for stimulating nerve paths in animals and observed the time values which impulses take to travel. Fleisch and Dolivo (38) mention that Ruckstuhl and Gordonoff introduced the modern use of electricity as a pain stimulus in rabbits almost fifty years ago.

Sivadjian (93) stimulated the paws of rats electrically by putting them in a box with its floor made of bare cross wires. The pain threshold was the smallest stimulus necessary to make the animal cry or try to get its feet off the wires.

Hirschfelder and Ridges (61) applied platinum electrodes connected

to a Harvard inductorium, to a shaved area on the skin of a rabbit. Changes in the respiratory activity of the animal following stimulation indicated a response to the painful stimulus. Macht and Macht (78) applied graded electrical shocks to the scrotum of adult rats and studied their response to pain (a characteristic squeal) before and after the administration of various drugs. Knowlton and Gross (68) measured the widening of the palpebral fissure in dogs as a response to electrical stimulation of the skin. These workers felt that this electric shock method did not elicit the muscle reflex or skin twitch which could be utilised for determining the pain threshold. However, when Andrews and Workman (2) used a radiant heat method, they noted a characteristic reflex twitch of the musculature of the back in the dog after a definite level of stimulation had been reached. Andrews and Workman (2) claim great constancy in this response regardless of the site or size of the area stimulated. Dodds et al (22) placed rats on a wire grid and used the jump reflex as the threshold when varying intensities of electric current were applied.

The tooth pulp of the canine tooth in the dog was used by Koll and Reffert (69) (70) as the site for applying an electric shock to produce a painful stimulus. The electrode was cemented in the drilled tooth and could be left for many months. A typical response to the stimulus was a marked defensive movement of the whole body. After administration of an analgesic drug it was necessary to increase the strength of the current in order to produce the same response. Consistent results were reported with this technique. Goetzl et al (46)

applied electrical stimulation to amalgam fillings in man and in dogs in order to compare the effects of analgesic drugs. Unfortunately, they did not give adequate details of their procedure so that when Miller (81) tried to reproduce their work he was unsuccessful. In summarizing his paper, Miller (81) comments that the tooth pulp method is one which "had scarcely been studied enough to evaluate it fairly." However, Goetzl and his coworkers (45) (46) (48) used electrical stimulation of amalgam fillings to evaluate many analgesic drugs and believed this technique very useful. Goetzl does not consider skin as a good site to use for stimulation since the stimulus is diffuse and other afferent systems will be stimulated along with the pain pathway.

From the findings of Björn, who did a systematic study of the tooth pulp methods, Beecher (4) mentions that the true measure of stimulation is the current applied and not the electromotive force, that it is best to use a single rectangular pulse of 10 milliseconds for stimulating and, also, that it is important to use the same point on a carefully dried tooth to get uniform results.

Fleish and Dolivo (38) stimulated the exposed tooth pulp in the rabbit as a test for analgesia and O'Dell (84) (85) used this technique for studying some non-narcotic analgesics. The tooth pulp was stimulated through a hole drilled in each of the two upper teeth into which were inserted shielded spring electrodes. Square wave shocks were given with each shock lasting not longer than 5 seconds. Intervals of 30 seconds were allowed between shocks. The strength of the shock was varied by changing the voltage. The pain threshold was taken when the

rabbit wiggled its nose and upper lip.

Frommel, Fleury and Schmidt-Ginzkey (43) used the guinea pig's tooth pulp to demonstrate the analgesic property of the drug carisoprodol. However, Berger et al (6) who originally had discovered carisoprodol, had failed to detect analgesia with it when tested by the techniques of Woolfe and MacDonald (106) or with the silver nitrate method of LaBelle and Tislow (73). Frommel et al (42) (87) had used the tooth pulp technique with success in other studies. Their method was somewhat similar to O'Dell's in that the perforation was made in the upper incisor tooth with a 0.5 mm. dentist's drill. The tooth pulp was stimulated by placing the active electrode in the perforation with the neutral electrode being lingual. A Grass stimulator was used with a frequency of 40 cycles per second, square wave, and the time of stimulation was for 0.5 second. The pain threshold was determined when the guinea pig raised its head in a particular manner. Only those animals reacting to a voltage between 2.5 and 3.8 were tested. The analgesic potency of the drug was determined by noting the increase in voltage necessary to cause the same reponse as previously and expressing this in percentage.

An electrical method devised by Reinard and E. M. deBeer for testing analgesics in mice was used by Grewal (50) and found to give good results. Burn (11), with the permission of Reinard and de Beer who did not publish their method, described their technique which involved applying electric shock to the tail of a mouse held in a plastic holder with its tail protruding. In this method electrodes

made of two pieces of clock spring 1 cm. wide were placed 1.5 cms. apart on the tail. A small weight attached to the end of each spring held the electrode against the tail. Good contact was ensured by cleaning the tail with ether and using electrode jelly well rubbed on it and not just smeared. Shocks were produced by a variac and a transformer, with the variac so adjusted to give 8.5 volts at the electrodes as indicated by a voltmeter. With a special rotating device shocks were applied once a second for a duration of 1/26th second. The response sought was a squeak and the stimulus stopped when this was evoked. With this apparatus, 85 per cent of the mice squeaked within five shocks and only these were selected for the experiment. Groups of 10 mice were used and the same dose of the analgesic drug was tested in not less than six different groups. Burn (11) suggested that instead of determining the degree of analgesia in each mouse, the percentage of mice showing some analgesia should be taken into consideration. For example, if any score greater than five or six is taken as evidence of analgesia, to relate the dose of the drug to the percentage of mice showing analgesia and, thus, to find the dose causing analgesia in 50 per cent of mice. The author's claim that using 'squeak' as a response to a pain stimulus appears to be a better way of measuring analgesia since the higher centers are involved in this type of response.

Carroll and Lim (13) evaluated these vocal response methods and reported that they noted tail and hind-limb movements in their rats when voltages lower than those necessary to produce the squeak response were used.

McKenzie and Beechey (79) used a modification of Grewal's (50) method for causing pain in mice by applying electric shock to the tail. The electrodes were a pair of modified small crocodile clips, the teeth of which had been filed off and the shape adjusted to fit the tail. Shocks were produced by using pulses of 50 c.p.s. current for 3 cycles duration and at intensities between 10 and 35 volts which could be varied in steps of 1 volt. Instead of finding the percentage of mice showing analgesia, these investigators calculated the analgesic increment by noting the difference between pre- and post-drug threshold. Pre-drug threshold was determined by stimulating each animal by three shocks at each voltage level and at the rate of 1 per second beginning, and then ascending with a 4 volts increment each time. The lowest stimulus intensity at which the animal responded by a squeak to at least two of three shocks was considered as the "voltage threshold". "Each mouse was next stimulated with an intensity of 2 volts above its voltage threshold by a succession of a series of 1 per second pulses. When two such successive pulses evoked the squeak response, the serial number of the first of these two was noted and designated as the 'repetition threshold'. If it exceeded 7, then the stimulus intensity was increased by 2 volts and the estimation repeated." Six or seven mice were used for each experiment and the difference between a 'repetition threshold' after the drug and the one observed in the same animal before the drug was called an analgesic increment. If no response occurred with 60 shocks after the drug, it was designated as 'greater than 60', and the analgesic increment as 60 regardless of the

pre-drug repetition threshold. Thus the range was between -6 and +60. "The difference between the mean analgesic increment of a treated group and that of the control group was taken as the analgesic effect." The authors also point out that the control group mean was usually zero. Apparently, these workers used the number of shocks required to elicit a response at a particular intensity as their experimental parameter.

MISCELLANEOUS METHODS

The Production of Experimental Arthritis Method

To simulate clinical conditions for which analgesic drugs are used, LaBelle and Tislov (73) produced a markedly painful and inflammed joint by injecting 0.2 ml. of a 1% silver nitrate solution into the ankle joint of one hind leg of the rat. In 24 hours time, the joint showed all the signs of acute inflammation and the animal reacted to the pain caused by flexing the inflammed joint. Only those animals which developed an acutely inflammed joint were used for testing analgesic drugs. Twenty-four hours after the injection of silver nitrate, the drug to be tested was given by stomach tube in a starch suspension. Other similarly prepared animals served as controls. The animals were tested at hourly intervals for five hours with grading of the analgesic compounds done according to the degree of pain relief. The authors found this method quite successful as adequate dosages of the effective compounds afforded marked protection from pain.

The Phenylquinone Test

This test was introduced by Seigmund et al (92) as a valuable and also specific test for comparing and evaluating the non-narcotic analgesics. They found that intraperitoneal injection of 0.25 ml. of a 0.02% aqueous solution of 2-phenyl-1-4-benzoquinone (commonly called phenylquinone) produced a syndrome in mice consisting of "intermittent contractions of the abdomen, twisting and turning of trunk and extension of hind legs, beginning 3 to 10 minutes after injection and persisting for more than 1 hour." Only those mice showing the syndrome within 10 minutes after the injection of phenylquinone were used. After the syndrome appeared, the analgesic drug was given and the animal watched for 5 minutes at 15 minute intervals. All untreated animals showed the 'syndrome' at least once in 5 minutes. When the mouse did not show this syndrome after the drug, analgesia had been produced and this determination was made on an all or none response basis. Other depressant drugs such as the barbiturates (phenobarbital sodium, pentobarbital sodium or butobarbital sodium) did not prevent the syndrome even at doses producing loss of body righting reflexes. Tranquilizers (meprobamate or chlorpromazine hydrochloride) and ethyl alcohol did prevent the syndrome, but only at doses causing marked depression. Also, muscle relaxants such as mephenesin prevented the 'syndrome' but, again, only at paralyzing doses. Intestinal antispasmodics such as atropine sulfate and some of the synthetic atropine substitutes (adiphenine hydrochloride) also failed to prevent it. Antihistaminics (diphenhydramine hydrochloride and tripelennamine hydrochloride), however, did

prevent the 'syndrome', thereby possibly favoring the view that histamine could be a possible chemical mediator in cutaneous pain. Intraperitoneal injection of 0.5% procaine, too, immediately suppressed the syndrome for about 10 minutes suggesting that the centripetal impulses arising after the injection of phenylquinone intraperitoneally are involved in this 'syndrome'. After carrying out the phenylquinone test using many different kinds of depressant drugs as mentioned above, the authors feel that it is specific for testing and evaluating the analgesic potency of both narcotic and non-narcotic analgesics. O'Dell (84) (85) has used this as a second method in mice to study the analgesic property of drugs in his experiments.

Combined Foot Inflammation and Pressure Method

Randall and Selitto (88) introduced a combined foot inflammation and pressure method in the rat which they found satisfactory for studying the analgesic effect of even the salicylates which, so far, has not been done with any degree of accuracy. They first produced inflammation by injecting 0.1 ml. of a 20% suspension of brewer's yeast into the plantar surface of the rat's foot. Pressure was then applied to the foot by means of an apparatus somewhat similar to the one used by Green and Young (49). This consisted of a 10 ml. syringe held vertically with a bullet-shaped wooden peg attached to the head of its plunger. Through the tip of the peg, pressure was applied to the plantar surface of the foot of the rat at the rate of 20 mm. of Hg using a pressure gauge connected by a T-tube to the syringe and an air line. The response was the struggling of the rat.

The authors found that the analgesic effect of the salicylate drugs and phenylbutazone could be measured only on the inflammed foot while that of aminopyrine and the narcotic analgesics could be noted on the inflammed as well as the control foot.

Several people have worked in this laboratory in the past years using some of the methods mentioned above. Everett (37) in 1949 used the technique of stimulating the tooth pulp of dogs by applying electric shocks to an amalgam filled tooth and with this procedure compared the effects of various strength solutions of procaine.

Samler (91) in 1953 studied the enhancement of analgesic activity of morphine sulfate and levo-isomethadone when given with various sympatholytic drugs using a modified D'Amour-Smith apparatus. In this method, the rat was placed on a wooden box with the tip of its tail kept on an asbestos platform in a groove, the center of which had a hole 1.5 cm. in diameter. A microswitch operated a camera shutter, connected to an electric timer which opened and closed the exposure through the opening. The length of exposure was measured in seconds. The heat source was placed below, using a prefocused lamp of 82 candle power and of 6 to 8 voltage (G.E. #2530) with the beam aimed at the opening by means of a biconvex lens 10.2 cm. in diameter and a focal distance of 15.5 cm. To avoid unnecessary stimulation by handling, the rats were restrained in small wire cages. Carter (14) made a further modification of this apparatus when he studied the modification of tolerance to narcotic analgesics by phenothiazine derivatives. He abandoned using the electric timer and regulated the shutter and stop watch by hand.

Also, he used a rheostat to control the voltage. The source of radiation was a 300 watt lamp operated by 120 volts. The reason he discarded the electrically operated shutter connected with the timer was because the heat caused significant sticking and slowing in opening and closing the shutter.

MATERIALS AND METHODS

Five different analgesimetric methods were tried in order to study their effectiveness in evaluating analgesic drugs and to compare these methods with one another. The three species of animals used were rats, mice and guinea pigs with the drugs being given subcutaneously to rats and guinea pigs and intraperitoneally to mice.

The pain stimulus was applied to the tail of the rat by three methods: radiant heat (19) (14), electrical stimulation (50) (79) and traumatic pressure (9). The mice were subjected to the first two testing methods used for the rats as well as by a third test consisting of applying conducted heat to the paws (34) (106). In guinea pigs, only stimulation of the tooth pulp was done (43). A feature of this research was the application of multiple testing stimuli to each animal given in close sequence and at approximately the same time during the experiment. Suitable rest period intervals were allowed between these battery of tests. It was hoped that in this way comparisons could be made of the accuracy and utility of three different analgesic testing methods when the animals were given a certain dose of a drug.

RADIANT HEAT TECHNIQUE
(Figures 1 and 2)

Carter's (14) modification of the D'Amour and Smith method was used. The apparatus consisted of a wooden box in which a 300 watt 130 V. G.E. lamp was housed with its beam focused on a hole in the top of the box by means of a biconvex lens of 10.2 cm. diameter and a focal distance of 15.5 cm. The current flow was regulated by a Powerstat-type of rheostat set at 120. The tip of the rat's tail was placed in a channel over the opening (1.5 cm. in diameter). By means of an asbestos shutter operated manually, the light beam was allowed to either pass through the hole or to be interrupted. The time from the opening to the closure of the shutter was noted in seconds by a stop watch.

Male Sprague-Dawley rats weighing 150 to 200 grams at the start of the study and white male mice weighing 25 to 30 grams were used. Each group consisted of 10 animals. All tests were done in a quiet room kept at uniform temperature of 24°C. to 26°C. Studies using drug injections were done only once a week.

Before the beginning of the experiment each rat was put into a wire mesh cage made to fit its body with the tail hanging out. Before testing, the rat was kept in the cage for 30 minutes in order to accustom him to it. The tests were performed with the animal still in the cage; this prevented any further excitement from handling. Similarly, mice were encased in wire mesh screens when tested.

Figure 1

EXPERIMENTAL SETUP FOR ANALGESIMETRY IN RATS

A. Modified D'Amour-Smith Apparatus
B. Brodie-Way-Smith Apparatus
C. Type 502, Dual Beam, Oscilloscope
D. Powerstat
E. Output Indicator

(The arrow points to the beam of light coming from the heat source, 300 watts, 130 V. G.E. lamp, concentrated on the 1.5 cm. circular opening in the center of the asbestos platform by means of a Biconvex lens with a diameter of 10.2 cms. suspended from the roof of the box by three adjustable metal rods.)

Figure 2

CLOSE-UP OF SETUP FOR ANALGESIMETRY IN RATS

1. Rat's tail in groove with shutter closed before thermal stimulation.
2. Electrodes attached to rat's tail for electrical stimulation.
3. The rod in position to give pressure stimulus.

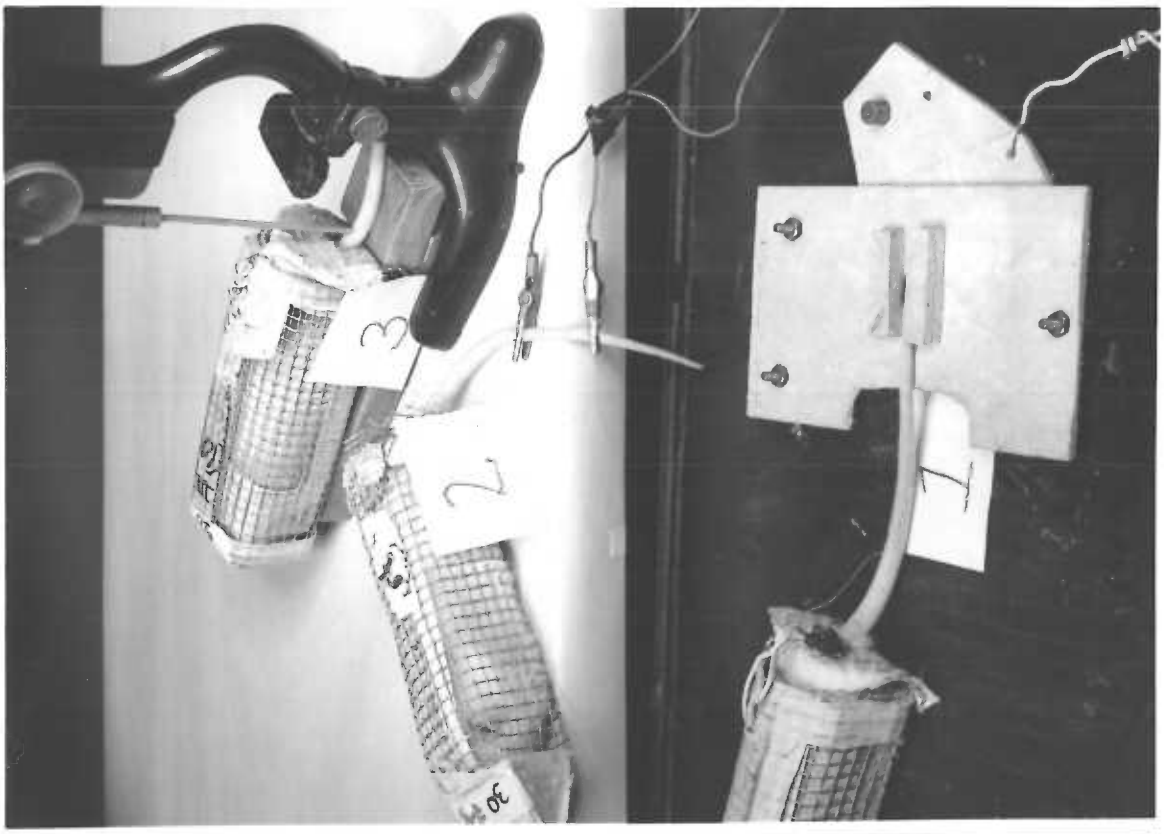


Figure 2

Figure 1

The response to pain was the tail flick. The time from the beginning of exposure to the heat of the light beam when the asbestos shutter was opened to the time the tail flicked was the reaction time. Two control readings were made and the drug then injected. The reaction time was determined again at 15 or 20 minute intervals for the rat and at 30 minute intervals for mice. The peak response was noted. The averages of the mean of the two control responses and of the peak responses after drug injection of the 10 animals in the group were calculated separately. The increase from the control threshold was calculated as follows and noted as the per cent analgesic response. The per cent of analgesic response = $\frac{B-A}{A} \times 100$, where A = the group average control mean value and B = the group average post-injection peak value. To avoid burning the tail, a cut-off time limit was set at 15 seconds for rats and at 12 seconds for mice with the reaction time in such cases taken as 15 and 12 seconds, respectively.

ELECTRICAL STIMULATION
(Figures 1 and 2)

A modification of McKenzie and Beechey's (79) apparatus was used. This consisted of a pair of electrodes made of alligator clips with the teeth filed off and the deep channel filled in with welding material in order to get an increase in the area of contact. The stimulation was applied through the electrodes at about the middle of the animal's tail after electrode jelly had been thoroughly rubbed on to the area

as suggested by Grewal (50) and MacKenzie (79) to ensure good contact. Current was obtained from an output indicator which modified the intensity of the stimulus, the pulses per second, and the pulse width. The last two were kept constant while the intensity, read in volts, was the variable parameter. Since the increase in voltage could not be measured accurately beyond 10 volts by the output indicator, a type 502 Dual-Beam Oscilloscope was used to read the intensity correctly. This showed that the pulses/second as registered on the output indicator was also inaccurate. Finally, the whole apparatus was adjusted so that the animal received current with variable intensity, but with the pulses/second fixed at 55, the pulse width at 1 millisecond, and using repetitive shocks lasting for 1 second each time. The minimum voltage at which the animal squeaked on three successive shocks at 1 sec. intervals was taken as the control threshold. This was determined twice, and if the two readings differed by more than 5 volts, the stimulus was given a third time. After drug injection the threshold was again determined, at intervals, and the percentage increase in response calculated for groups of 10 animals.

TRAUMATIC PRESSURE METHOD
(Figures 1 and 2)

The apparatus described by Brodie, Way and Smith (9) was used to produce a painful stimulus by pressure on the rat's tail. Only rats were used since the tails of mice would be too small for testing by this device. Pressure was applied by an aluminum welding rod 13¼ inches

long by ¼ inch in diameter and tapered at the lower end to a sharp point. This rod was placed in a brass tubing 7¼ inches long with an inside diameter slightly greater than ¼ inch. A spring attached to the rod was arranged to produce graduated pressure when the rod was pressed on the rat's tail. The spring and rod attachment had been calibrated to exert pressures from 10 to 170 grams using 10 gram increments as shown by marks on the upper end of the rod. For convenience, the adjustable barrel of a microscope - from which the eyepiece, revolving nose piece, the stage and the mirror were removed - was used to mount this device. The screw mechanisms of the microscope were used to set the rod at the desired grams-pressure calibration mark. Although the authors suggested the use of young rats weighing from 40 to 60 grams for best results, heavier rats were used in these studies in order to compare their reaction with that of radiant heat and electrical stimulation. When the rat squeaked or showed extreme discomfort on application of a certain grams-pressure, the weight used was read from the calibration. This was the control threshold reading which was determined twice. After drug injection similar determinations were made at regular intervals with the percentage of analgesic response calculated.

CONDUCTED HEAT TECHNIQUE
(Figure 3)

The method used for applying heat to the paws of a mouse was somewhat similar to the Woolfe and MacDonald technique (53). A copper plate measuring 12 by 24 inches was heated by means of copper wires distributed underneath it and uniformly spread. In addition to small screws holding the wires against the plate with a thin insulation in between, an asbestos sheet helped to maintain close contact between the wires and underside of the copper plate. The temperature could be decreased or increased by tightening or loosening a regulating screw. A thermometer with its mercury bulb covered by copper wire in order to give accurate readings by removing the dead space between the bulb and the plate was placed on the top of the plate. The plate was kept at a temperature of 56°C. The mouse was dropped on the heated plate and restrained by a glass cylinder of 6 inches diameter. The time in seconds was noted from the time the mouse was dropped on the plate to when it started licking its hind paws or tried to jump out of the bottle. Two control readings were taken before giving the drug and following injection and two more were taken at 30 and 60 minute intervals. The peak response, measured in seconds, was noted and the per cent of analgesic response determined.

Figure 3

MODIFIED WOOLFE-MACDONALD APPARATUS

(The modified hot-plate for giving conducted heat stimulus to the paws of the mouse, showing thermometer in position with its mercury bulb covered with copper wire thus removing dead space between the bulb and the plate to ensure exact temperature of the plate.)

Figure 4

EXPERIMENTAL SETUP FOR ELECTRICAL STIMULATION OF THE TOOTH PULP IN THE GUINEA PIG

(The figure shows the characteristic manner in which the guinea pig raises its head during stimulation.)

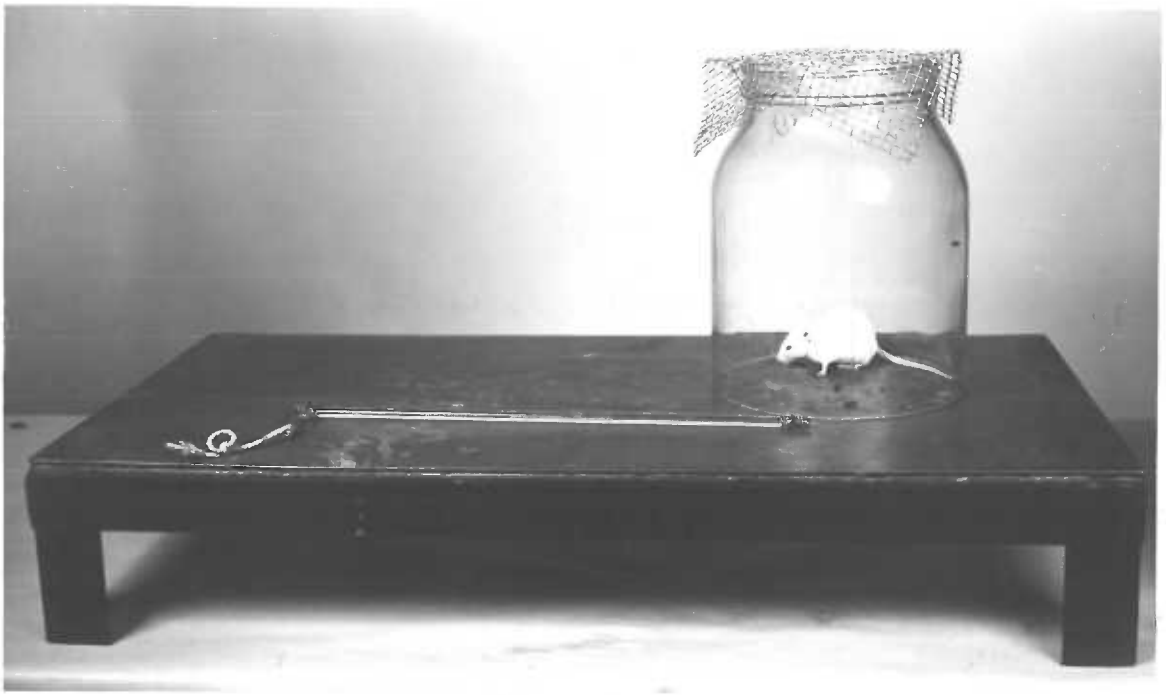


Figure 3

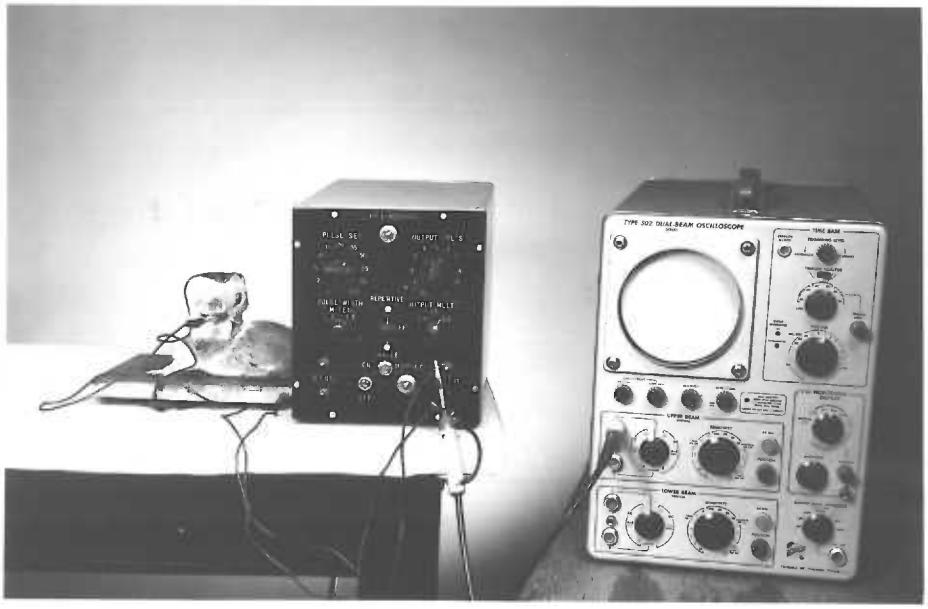


Figure 4

ELECTRICAL STIMULATION OF THE TOOTH PULP IN GUINEA PIGS (Figure 4)

Male guinea pigs weighing from 250 to 300 grams were used although their weight soon exceeded this amount after a few weeks. At first silver amalgam fillings were made in cavities drilled into one of the upper incisors of guinea pigs after anesthetization with 30 mg./kg. of sodium pentobarbital given intraperitoneally. Later, instead of using amalgam-filled cavities, just two notches were made on the lateral side of each of the upper incisor teeth in order to ensure conduction through the electrode to the tooth pulp. As it was found necessary to repeat these notches about every three weeks as the teeth erupted, the procedure was changed to stimulation of the pulp by attaching the electrodes directly to an unprepared tooth, making certain that the electrodes were well shielded and that only the tooth was in contact with them.

Two alligator clips were used as electrodes, one of which was shielded for the tooth and the other, which had been flattened, applied as the neutral electrode to one ear which was rubbed well with electrode jelly.

Otherwise, the same apparatus was used as mentioned above for electrical stimulation of the tail of rats and mice. The pain threshold voltage was noted if the animal reacted in a characteristic manner by squealing and raising its head to three successive stimuli of the same intensity and given for 1 second duration at 1 second

intervals. The rest of the procedure and calculation was exactly as performed for the electrical stimulation of rats and mice.

ANALGESIC DRUGS STUDIED

The drugs tested in this research were the narcotic analgesics morphine sulfate and codeine phosphate, the salicylate antipyretic analgesics sodium salicylate and salicylamide, the non-narcotic analgesics dextro propoxyphene hydrochloride and ethoheptazine citrate, and the investigational drugs EXP-104, SU-8629 and SKF #1340.

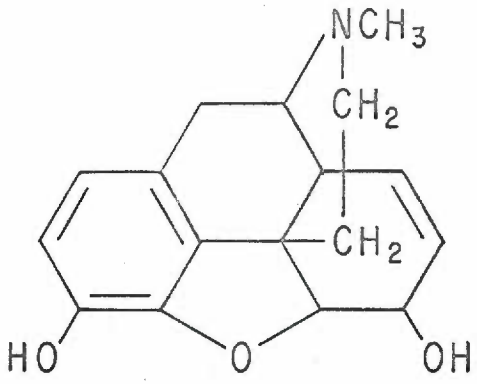
Morphine sulfate, because of its general acceptance as a potent analgesic drug, was chosen as a model drug of the narcotic group along with codeine which is the methylated form of morphine and, hence, closely related to it in action (51). The formulas of these two compounds with the phenanthrene nucleus, an oxygen bridge and a tertiary amine attached to the phenathrene ring are shown in Figure 5.

Sodium salicylate was chosen as a model drug for the antipyretic analgesic group and salicylamide (Dropsprin^{R}, Salamide^{R}) was included as a very closely related compound. The chemical formulas of these two compounds are shown in Figure 6. Salicylamide, the amide of salicylic acid, although not superior to the salicylates has the same actions and uses of salicylates. Salicylamide is specially indicated in patients allergic to the salicylates (83).

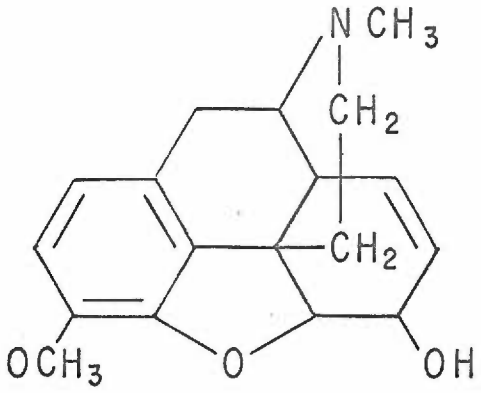
The more recently introduced drugs, dextro propoxyphene hydrochloride (Darvon^{R}) in 1957 and ethoheptazine citrate (Zactane Citrate^{R})

Figure 5

CHEMICAL STRUCTURES
OF
MORPHINE AND CODEINE



Morphine

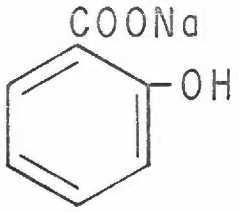


Codeine

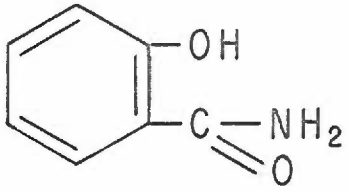
Figure 5

Figure 6

CHEMICAL STRUCTURES
OF
SODIUM SALICYLATE AND SALICYLAMIDE



Sodium Salicylate



Salicylamide

Figure 6

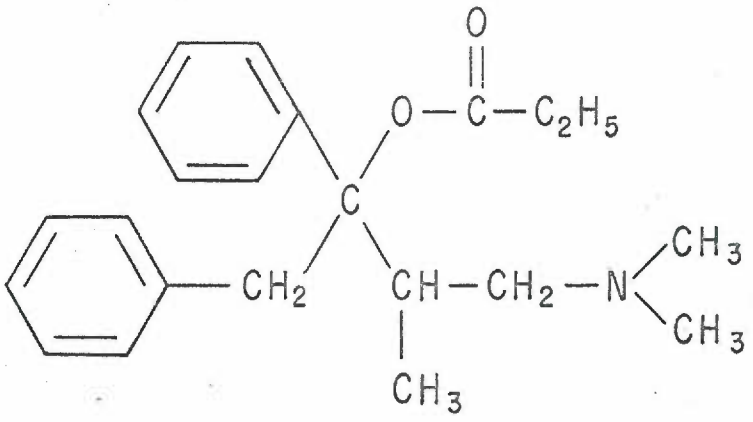
in 1958, were then studied (35) (83) (85) (89). Both drugs are given orally and their onset and duration of action are similar to those of codeine. Their toxicity is low and the margin of safety adequate. They have been extensively studied in animals and in humans. Clinical evidence indicates that they are useful for the relief of mild to moderate pain and are free from tolerance and addiction. Their structural formulae are shown in Figure 7.

SKF #1340 ('Di-indamine') is a new analgesic, introduced in 1959. A communication from the Smith, Kline and French laboratories (95) states that, "although SKF #1340 is a potent analgesic and has some morphine-like activity, studies suggest that the incidence and the degree of side reactions may be of minor significance and insufficient to interfere with the value of SKF #1340 to the clinician and his patient." With only limited clinical studies on this active new drug (20) it was felt it would be of special value to include it in this study. For the same reason, SU-8629 was also included. This is 2-indamine hydrochloride developed by the Ciba Research Laboratories. It has certain pharmacological properties which resemble those of amphetamine, and others, which resemble morphine. A communication from the Ciba Research Laboratories (17) describes it to be "an analgesic with stimulant properties and more potent and less toxic than morphine, yet effective in the entire range of pain - mild, moderate and severe."

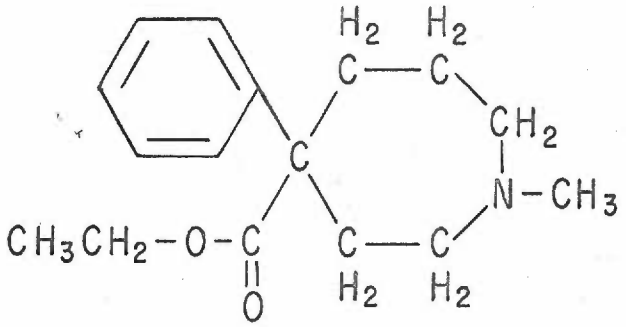
The structural formula of both SKF #1340 and SU-8629 are shown in Figure 8.

Figure 7

CHEMICAL STRUCTURES
OF
DEXTRO PROPOXYPHENE AND ETHOHEPTAZINE



Dextro propoxyphene
(α – d – 4 – Dimethylamino – 1, 2 – diphenyl – 3 – methyl – 2 – butanol propionate)

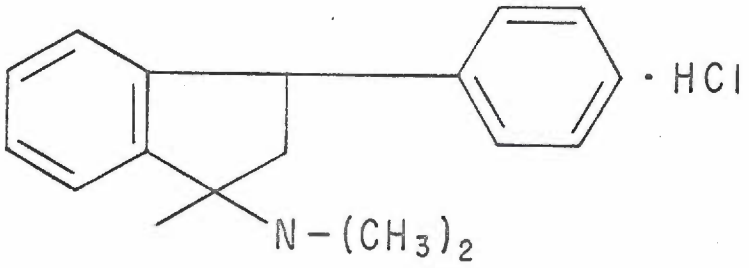


Ethoheptazine
(1 – Methyl – 4 – carbethoxy – 4 – phenylhexamethylenimine)

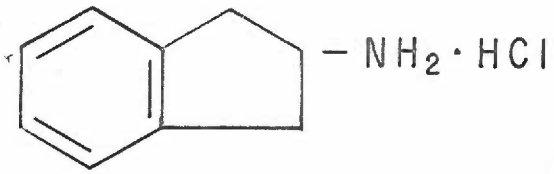
Figure 7

Figure 8

CHEMICAL STRUCTURES
OF
SKF #1340 AND SU-8629



SKF #1340
(1-dimethylamino-3-
phenyl indane hydrochloride)



SU-8629
(2-indanamine hydrochloride)

Figure 8

SELECTION OF DRUG DOSAGES

In 1960, Carter (14) using Sprague-Dawley rats and a modified D'Amour-Smith method, detected a 72 per cent analgesic response with 2 mg./kg. of morphine sulfate given subcutaneously. Accordingly, the same dose of morphine sulfate was used in the present studies on the rat as well as the guinea pig.

Mice are known to tolerate much larger dosages of drugs. McKenzie and Beechey (79) reported that intraperitoneal injections of 16 mg./kg. of morphine sulfate in the mouse showed only a minimal analgesic response with electrical stimulation of the tail. It was decided, therefore, to use 20 mg./kg. of morphine sulfate in the mouse in this study.

McKenzie and Beechey also report the analgesic dosages of codeine to be about two and one-half times as much as that of morphine. Consequently, a dose of 5 mg./kg. of codeine phosphate was used in the rat and guinea pig, and 50 mg./kg. in mice.

When 10 mg./kg. of sodium salicylate were tried in the rat, no analgesia was detectable. With dosage of 50 mg./kg. of both sodium salicylate and salicylamide in the rat and the guinea pig and 500 mg./kg. in mice, measureable analgesia resulted. The dosages chosen for the other drugs tested were selected from the reports of the pharmacology studies made by the manufacturers.

STATISTICAL TEST FOR ANALYSIS OF DATA

Statistical analysis of the data was done using "t" test for correlated measures.

$$t = \frac{\bar{D}}{\sqrt{\frac{\Sigma D^2 - (\Sigma D)^2/N}{N(N-1)}}}$$

Where $D = X_2 - X_1$

$\bar{D} = \Sigma (X_2 - X_1)/N$

X_2 = post-injection peak value of each animal

X_1 = control mean value of each animal

N = number of pairs of scores (or the number of animals in each group)

$df = N-1$ (the number of degrees of freedom)

RESULTS

The following tables show the results obtained when the various drugs were tested for potency using the different methods described above. These results also show the advantages and disadvantages encountered when two or three tests are performed concomitantly in the same rat or mouse before and after drug administration.

Nine different analgesic drugs were studied in the rat and guinea pig and four in the mouse with the drugs being given subcutaneously to rats and guinea pigs and intraperitoneally to mice.

MORPHINE SULFATE
(Tables I and II)

Doses of 2 mg./kg. were used in rats and guinea pigs and 20 mg./kg. in mice. In rats, although significant analgesic effect could be detected by all three methods, the responses obtained by electrical stimulation of the tail were less significant than those noted when the radiant heat and pressure methods were used. In guinea pigs, the results from tooth pulp stimulation following 2 mg./kg. morphine sulfate were even less significant than those for electrical stimulation of the rat's tail. In mice, although the changes in the analgesic responses when tested by radiant and conducted heat were highly significant and almost complete, it is of interest to note that no change in response to electrical stimulation of the tail occurred after morphine in the same group of animals. Taking into account the number of

TABLE I A COMPARISON OF THE RESPONSES OBTAINED FOLLOWING MORPHINE SULFATE 2 MG./KG. WHEN TESTED FOR ANALGESIA BY COMBINED RADIANT HEAT, ELECTRICAL STIMULATION AND TRAUMATIC PRESSURE METHODS APPLIED TO THE RAT'S TAIL AND STIMULATION OF THE TOOTH PULP IN GUINEA PIGS

RATS								GUINEA PIGS			
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt.in Gms.	Control Mean	Post Injection Peak
1.	160	7	15	15	25	95	180	1	310	8.25	16
2	170	5.75	15	20	40	120	180	2	320	7.5	17
3	150	7	15	12	22	150	180	3	290	7.75	19
4	170	7.25	15	8.5	20	135	180	4	380	16.5	17
5	160	7.5	15	8.25	10	80	180	5	230	22.5	37
6	160	5	10	15	12	120	140	6	350	13	9
7	160	5.75	15	14	30	70	180	7	250	10.75	19
8	130	8.75	15	28	37	150	180	8	430	7.5	17
9	160	7	15	17	24	140	180	9	230	12.5	17
10	170	7.75	15	15.5	42	140	180	10	330	18.5	10
AVERAGE		6.88	14.5	15.33	26.2	120	176	AVERAGE		12.48	17.8
Per Cent Increase From Control Threshold		110.76%		70.91%		46.66%				42.68%	
		P <.001		P <.01		P <.001				P <.05	
		Highly Significant		Very Significant		Highly Significant				Significant	

(P - Refers to the probability of the results occurring by chance.)

TABLE II A COMPARISON OF THE ANALGESIC RESPONSES TO MORPHINE SULFATE 20 MG./KG. WHEN TESTED BY APPLICATION OF RADIANT HEAT AND ELECTRICAL STIMULATION TO THE TAIL AND CONDUCTED HEAT TO THE PAWS OF MICE

	RADIANT HEAT		ELECTRICAL STIMULATION		CONDUCTED HEAT	
	Reaction Time in Seconds		Reaction Voltage		Reaction Time in Seconds	
Mouse No.	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak
1	6.25	15	9	8	6	30
2	8.5	15	5.5	7	8.75	19.5
3	5.75	15	7.5	7	8	30
4	5.5	15	10	15	9.75	30
5	6.5	15	6.5	6	5.5	30
6	5.75	15	15.5	7	12.75	30
7	8	15	6	7	15.75	30
8	6	15	5	4	8	30
9	7.25	15	9.5	10	7.75	30
10	6	15	6.5	6	16.5	30
AVERAGE	6.55	15	8.1	8	9.96	28.95
Per Cent Increase From Control Threshold		129%		-1.2%		+190.66%
		P <.001		P >.1		P <.001
		Highly Significant		Not Significant		Highly Significant

(P - Refers to the probability of the results occurring by chance.)

animals which did not respond within the cut-off time, complete analgesia following this dose of morphine was noted when the radiant heat method was used.

CODEINE PHOSPHATE
(Tables III & IV)

Doses of 5 mg./kg. were used in the rat and guinea pig and 50 mg./kg. in mice. In rats, the changes in the analgesic response from codeine when tested by radiant heat and traumatic pressure were both very significant while the changes when tested by electrical stimulation were not significant. The analgesic response after codeine when tested by guinea pig tooth pulp stimulation was greater than that obtained after morphine and was very significant. The changes in the responses for mice were somewhat similar to those obtained with morphine. With electrical stimulation of the tail, no significant increase in analgesic response was noted following codeine but with the radiant heat method, the same animals showed complete analgesia.

SODIUM SALICYLATE
(Tables V & VI)

Doses of 50 mg./kg. were used in the rat and guinea pig and 500 mg./kg. in mice. With this drug, the pattern of response was changed from that of the former two drugs. In the rat, sodium salicylate showed a significant analgesic response which could be detected

TABLE III A COMPARISON OF THE ANALGESIC RESPONSES TO CODEINE PHOSPHATE 5 MG./KG. WHEN TESTED BY THE RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE APPLIED TO THE RAT'S TAIL AND BY TOOTH PULP STIMULATION IN THE GUINEA PIG

		RATS						GUINEA PIGS			
		RADIANT HEAT Reaction Time Seconds		ELECTRICAL STIMULATION Reaction Voltage		PRESSURE Reaction Wt. in Grams				TOOTH PULP STIMULATION Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt. in Gms.	Control Mean	Post Injection Peak
1	210	5.5	6.5	16	18	90	130	1	320	6.5	9
2	210	4.5	7	6	6	80	130	2	390	9.5	19
3	220	4.5	5	10	16	80	90	3	350	6.5	8
4	210	6	6	10	12	80	110	4	340	4.5	7
5	210	5.75	5.5	20	20	80	140	5	350	3.75	7
6	210	5	6	11	14	75	140	6	350	4.5	5
7	200	4.75	6	17	12	100	130	7	330	5.5	6
8	210	4	6	10	8	110	120	8	390	5.5	10
9	220	5	7	15.5	22	110	110	9	420	5.5	8
10	220	5.5	6.5	9	6	95	110	10	360	7.5	9
AVERAGE		5.05	6.15	12.45	13.4	90	121	AVERAGE		5.93	8.8
Per Cent Increase From Control Threshold		21.78%		7.63%		34.44%				48.4%	
		P< .001		P> .1		P< .01				P< .01	
		Highly Significant		Not Significant		Very Significant				Very Significant	

(P - Refers to the probability of the results occurring by chance.)

TABLE IV A COMPARISON OF THE ANALGESIC RESPONSES TO CODEINE PHOSPHATE 50 MG./KG. WHEN TESTED BY APPLICATION OF RADIANT HEAT AND ELECTRICAL STIMULATION TO THE TAIL AND CONDUCTED HEAT TO THE PAWS OF MICE

	RADIANT HEAT		ELECTRICAL STIMULATION		CONDUCTED HEAT	
	Reaction Time in Seconds		Reaction Voltage		Reaction Time in Seconds	
Mouse No.	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak
1	4.25	12	20	18	7.75	40
2	5.25	12	9	16	18.75	18
3	6	12	18.5	24	9.75	20.5
4	5	12	6.5	7	9.75	12
5	5.25	12	12.5	10	12.75	28
6	8	12	15	14	9.75	24.5
7	5.25	12	12	14	12.5	37
8	9.25	12	12	12	28.75	33
9	4.25	12	15	20	20.5	20
10	4.25	12	15	20	20.5	20
AVERAGE	5.675	12	12.85	14.3	14.6	28.8
Per Cent Increase From Control Threshold		111.27%		11.28%		97.26%
		P <.001		P> .1		P <.05
		Highly Significant		Not Significant		Significant

(P - Refers to the probability of the results occurring by chance.)

TABLE V A COMPARISON OF THE ANALGESIC RESPONSES TO SODIUM SALICYLATE 50 MG./KG. WHEN TESTED BY RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE ON THE RAT'S TAIL AND THE TOOTH PULP STIMULATION IN THE GUINEA PIG

RATS								GUINEA PIGS			
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt. in Gms.	Control Mean	Post Injection Peak
1	180	6.25	7.5	20	23	80	80	1	390	12.5	22
2	200	5	7	27	37	80	80	2	400	7.75	12
3	190	4.5	6.5	30	35	80	80	3	380	21.5	22
4	210	5.75	5.5	11.5	20	80	80	4	450	7.75	7
5	190	4.75	6	32.5	32	80	80	5	420	14	20
6	190	4.5	4.5	20.5	33	80	110	6	390	7.5	12
7	190	5.5	5.5	30.5	42	80	100	7	270	8.25	15
8	190	5.25	6	33	50	80	90	8	450	9.75	16
9	190	5.25	5	39	49	80	80	9	310	7.25	10
								10	260	12.5	19
AVERAGE		5.03	5.94	27.1	35.66	80	86.67	AVERAGE		10.88	15.5
Per Cent Increase From Control Threshold			18.09%		31.66%		8.3%				42.46%
			P>.05		P<.01		P>.1				P<.01
			Not Significant		Very Significant		Not Significant				Very Significant

(P - Refers to the probability of the results occurring by chance.)

TABLE VI A COMPARISON OF THE ANALGESIC RESPONSES TO SODIUM SALICYLATE 500 MG./KG. WHEN TESTED BY RADIANT HEAT AND ELECTRICAL STIMULATION ON THE TAIL AND CONDUCTED HEAT ON THE PAWS IN MICE

	RADIANT HEAT		ELECTRICAL STIMULATION		CONDUCTED HEAT	
	Reaction Time in Seconds		Reaction Voltage		Reaction Time in Seconds	
Mouse No.	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak
1	4.75	7	7	8	14.5	14
2	5	6	9	13	8	9
3	5.5	4.5	6	7	7	4
4	5.25	8	8.5	17	19	6
5	9.75	6	12	16	5	8
6	4.75	8	12	13	11.75	9
7	5.75	10.5	6.5	17	11.5	8
8	6	5	5.5	13	4	4.5
9	7	5.5	7.5	9.5	4	8.5
10	6.5	8.5	7.5	8	7.75	5.5
AVERAGE	5.5	6.9	8.1	12.1	9.25	7.65
Per Cent Increase From Control Threshold		24.45%		49.38%		-17.3%
		P < .01		P < .01		P > .1
		Very Significant		Very Significant		Not Significant

(P - Refers to the probability of the results occurring by chance.)

only by the method of electrical stimulation of the tail. The guinea pig tooth pulp stimulation method also showed very significant analgesia. In mice, an analgesic response was noted both by radiant heat and electrical stimulation, but not by conducted heat.

SALICYLAMIDE
(Table VII)

Doses of 50 mg./kg. were used in the rat and guinea pig. The mouse was not used. The analgesic response was similar to that obtained with sodium salicylate. There was no significant analgesia produced when tested by the radiant heat method in the rat, but a very significant response was noted when the methods of electrical stimulation of the rat's tail and the tooth pulp stimulation in the guinea pig were used. The traumatic pressure method of testing was not used on these rats because of the soreness of the tails in several rats from the previous experiment using this same stimulus.

DEXTRO PROPOXYPHENE
(Table VIII)

Doses of 2 mg./kg. were used in both the rat and guinea pig. All the methods used in both the rat and guinea pig detected significant analgesia with this drug.

TABLE VII A COMPARISON OF THE ANALGESIC RESPONSES TO SALICYLAMIDE 50 MG./KG. WHEN TESTED BY RADIANT HEAT AND ELECTRICAL STIMULATION APPLIED TO THE RAT'S TAIL AND BY TOOTH PULP STIMULATION IN THE GUINEA PIG

		RADIANT HEAT		ELECTRICAL STIMULATION	
		Reaction Time in Seconds		Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak
1	200	5	6.5	22	28
2	200	4.25	5.5	23	25
3	180	6	8.5	22.5	28
4	200	6	6.5	29.5	37
5	200	8.5	6.5	17	21
6	190	5.25	6.5	19.5	21
7	190	5.5	6.5	22.5	36
8	190	4.75	5.75	22.5	34
9	180	5.75	7	18	21
10	180	6.5	6	20	25
AVERAGE		5.75	6.5	21.65	27.6
Per Cent Increase From Control Threshold		13.04%		27.48%	
		P > .05		P < .001	
		Not Significant		Highly Significant	

		TOOTH PULP STIMULATION	
		Reaction Voltage	
Guinea Pig No.	Wt. in Gms.	Control Mean	Post Injection Peak
1	480	8.5	8
2	520	15	19
3	460	5	11
4	440	4	6
5	460	2.75	5
6	460	5	8
7	460	4.5	5
8	460	5	10
9	560	5	9
10	520	6.5	9
AVERAGE		6.13	9
		46.82%	
		P < .001	
		Highly Significant	

(P - Refers to the probability of the results occurring by chance

TABLE VIII A COMPARISON OF THE ANALGESIC RESPONSES TO DEXTROPROPOXYPHENE HYDROCHLORIDE 2 MG./KG. WHEN TESTED BY RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE ON THE RAT'S TAIL AND BY TOOTH PULP STIMULATION IN THE GUINEA PIG

RATS								GUINEA PIGS			
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt.in Gms.	Control Mean	Post Injection Peak
1	220	5.5	7	24.5	34	70	80	1	360	12	24
2	220	4.5	8	16	25	70	100	2	250	29	55
3	220	4.75	6	20	32	80	100	3	290	7.75	18
4	220	5	5.5	11.5	26	70	100	4	400	16	16
5	220	5	6	35.5	40	65	80	5	290	6.5	14
6	200	4.75	5.5	22	31	70	80	6	380	8	17
7	220	6.5	6.5	18	15	70	110	7	290	7.25	9
8	210	6	7.5	12	30	75	100	8	430	8.25	16
9	220	5	5.75	16	26	70	110	9	270	6.5	13
10	220	5.75	7.5	26.5	41	85	150	10	330	9.75	13
AVERAGE		5.20	6.53	20.2	30	75	101			11.1	19.5
Per Cent Increase From Control Threshold			23.67%		48.51%		30.4%				75.68%
			P <.001		P <.01		P <.001				P <.01
			Highly Significant		Very Significant		Highly Significant				Very Significant

(P - Refers to the probability of the results occurring by chance.)

ETHOHEPTAZINE CITRATE
(Table IX)

Rats and guinea pigs were given 25 mg./kg. doses of this non-narcotic drug. In the rats the changes in the analgesic responses were slight but significant when tested by radiant heat and electrical stimulation. Similarly, this drug effected very little analgesic response in the guinea pig as judged by the tooth pulp stimulation method.

EXP-104
(Tables X, XI, XII)

This experimental drug was tried in dosages of 0.1 mg./kg. and 0.5 mg./kg. in rats and guinea pigs and in a dosage of 1 mg./kg. in mice. When 0.1 mg./kg. was used first on rats there was very little change in the analgesic response. Similarly, although the per cent of analgesic response was over 70% in the guinea pig when given 0.1 mg./kg. of EXP-104, this change was not statistically significant. However, with 0.5 mg./kg. the response in the rat was somewhat similar to that obtained with codeine, i.e., highly significant analgesia by the radiant heat method, very significant with traumatic pressure but not significant with electrical stimulation. Guinea pig tooth pulp stimulation detected the maximum analgesic response when 0.5 mg./kg. of EXP-104 was given. In mice, too, although the analgesic responses by the radiant and conducted heat techniques resembled the responses obtained for morphine and codeine, similarly, the electrical

TABLE IX A COMPARISON OF THE ANALGESIC RESPONSES TO ETHOHEPTAZINE CITRATE 25 MG./KG. WHEN TESTED BY RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE APPLIED TO THE RAT'S TAIL AND TOOTH PULP STIMULATION IN THE GUINEA PIG

		RATS						GUINEA PIGS			
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt.in Gms.	Control Mean	Post Injection Peak
1	210	5.25	7	16	12	70	80	1	420	15	22
2	240	6.25	7.5	20	34	85	80	2	460	11.5	18
3	230	5.75	6	32.5	32	100	90	3	420	8	9
4	220	5.5	7.5	39.5	37	115	80	4	390	9.5	13
5	200	6.25	6	28.5	30	70	80	5	440	4	13
6	210	8	8	7	14	80	90	6	430	8	8
7	220	8	8	43	41	70	80	7	440	6.75	11
8	220	5.25	6	10.5	22	95	80	8	490	9.5	6
9	200	6	6	17	18	70	80	9	580	8	9
10	200	8.75	9	38	46	105	130	10	560	8	16
AVERAGE		6.45	7.1	25.2	28.6	86	87	AVERAGE		8.83	12.5
Per Cent Increase From Control Threshold		10.07%		13.49%		1.16%				41.56%	
		$P < .001$		$P < .05$		$P > .1$				$P < .05$	
		Highly Significant		Significant		Not Significant				Significant	

(P - Refers to the probability of the results occurring by chance.)

TABLE X A COMPARISON OF THE ANALGESIC RESPONSES TO DRUG 'EXP 104' 0.1 MG./KG. WHEN TESTED BY RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE APPLIED TO THE RAT'S TAIL AND BY TOOTH PULP STIMULATION IN THE GUINEA PIG

RATS								GUINEA PIGS			
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt.in Gms.	Control Mean	Post Injection Peak
1	200	5	6	20.5	22	80	80	1	420	11	32
2	210	5.5	5	34.5	36	80	80	2	450	9.5	28
3	220	5	5.5	15	22	80	80	3	510	7	8
4	210	5.25	6.5	36	40	80	80	4	330	7	10
5	200	5	6	15	26	80	80	5	380	7.5	12
6	210	5	5.5	19.5	24	80	80	6	500	10	8
7	210	5.75	6	34.5	26	80	80	7	400	11	10
8	200	5.25	5.5	22.5	32	80	90	8	300	15	27
9	220	5.75	6.5	32	30	80	90	9	320	11	10
10	210	5.5	6	20	28	80	90	10	320	5.5	7
AVERAGE		5.3	5.85	24.95	20.6	80	83	AVERAGE		9.45	16.2
Per Cent Increase From Control Threshold		10.4%		14.63%		3.75%				71.42%	
		P<.001		P>.05		P>.05				P>.05	
		Highly Significant		Not Significant		Not Significant				Not Significant	

(P - Refers to the probability of the results occurring by chance.)

TABLE XI A COMPARISON OF THE ANALGESIC RESPONSES TO DRUG 'EXP 104' 0.5 MG./KG. WHEN TESTED BY RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE APPLIED TO THE RAT'S TAIL AND BY TOOTH PULP STIMULATION IN THE GUINEA PIG

RATS								GUINEA PIGS			
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt.in Gms.	Control Mean	Post Injection Peak
1	190	5.75	6.5	9.5	12	130	120	1	390	9.5	17
2	210	7	7	34.5	36	95	130	2	330	24.5	50
3	210	6	14	27	20	90	120	3	330	7.25	19
4	210	6	8	34	42	125	150	4	470	9.5	14
5	200	5.5	6	14	14	95	150	5	410	10.5	22
6	190	6.25	6.5	28.5	28	85	90	6	380	9.5	10
7	200	6.5	9	7	20	90	120	7	290	8	10
8	200	6.25	8	17	42	90	90	8	450	7	22
9	180	6.75	7	9	15	80	90	9	290	6.75	20
10	180	7	10	21.5	30	85	140	10	370	7.5	22
AVERAGE		6.3	8.2	20.2	25.9	86.5	120	AVERAGE		10.9	20.4
Per Cent Increase From Control Threshold		30.158%		28.217%		38.728%				87.12%	
		P .001		P .05		P .01				P .01	
		Highly Significant		Not Significant		Very Significant				Very Significant	

(P - Refers to the probability of the results occurring by chance.)

TABLE XII A COMPARISON OF THE ANALGESIC RESPONSES TO DRUG 'EXP 104' 1 MG./KG. WHEN TESTED BY RADIANT HEAT AND ELECTRICAL STIMULATION APPLIED TO THE TAIL AND CONDUCTED HEAT TO THE PAWS OF MICE

	RADIANT HEAT		ELECTRICAL STIMULATION		CONDUCTED HEAT	
	Reaction Time in Seconds		Reaction Voltage		Reaction Time in Seconds	
Mouse No.	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak	Control Mean	Post Inject. Peak
1	8.75	10	8.5	8	13.5	60
2	4.5	10	5.75	8	13.75	21
3	7.75	9.5	5.75	5	17.75	51
4	6	15	6.75	12	12	15
5	7	15	5	6	16	28
6	5.25	15	7.75	14	14	18
7	10	15	8	5	24.5	16
8	6.25	15	5.5	6	21	60
9	5.25	7	4.75	7	25.5	24
10	4.75	15	6	8	33.5	60
AVERAGE	5.65	12.65	6.38	7.9	19.15	35.3
Per Cent Increase From Control Threshold		123.89%		28.32%		84.33%
		$P < .001$		$P > .1$		$P < .05$
		Highly Significant		Not Significant		Significant

(P - Refers to the probability of the results occurring by chance.)

stimulation method failed to show any significant change in the analgesic response when drug EXP-104 was tested.

SKF #1340
(Table XIII)

A dose of 10 mg./kg. was studied in the rat and guinea pig. Although this produced a significant analgesic response with each method used, those obtained by electrical stimulation and traumatic pressure in the rat were very highly significant.

SU-8629
(Table XIV)

A 5 mg./kg. dose of this experimental drug was given to the rat and the guinea pig. This dose showed significant analgesia by all of the three methods in the rat, but not in the guinea pig.

TABLE XIII A COMPARISON OF THE ANALGESIC RESPONSES TO DRUG 'SKF NO. 1340' 10 MG./KG. WHEN TESTED BY RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE APPLIED TO THE RAT'S TAIL AND BY TOOTH PULP STIMULATION IN THE GUINEA PIG

		RATS						GUINEA PIGS			
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt. in Gms.	Control Mean	Post Injection Peak
1	180	8.5	15	9.5	17	130	170	1	560	7.5	12
2	210	7	15	26	29	75	170	2	620	4.5	7
3	220	6.5	20	11.5	30	150	180	3	740	9	9
4	200	7.25	10	17	32	110	180	4	560	9.5	8
5	220	6.75	9	9.5	16	100	180	5	680	5	10
6	190	7.25	7	29	34	80	150	6	700	6	10
7	200	8.25	7.5	24.5	30	85	110	7	840	7	10
8	190	7.5	10	11	18	95	150	8	760	9	6
9	200	8	8	19	32	82.5	90	9	620	6.5	8
10	190	8	20	23	38	125	140	10	1005	6	12
AVERAGE		7.5	12.7	18	27.6	103.25	152	AVERAGE		7	9.2
Per Cent Increase From Control Threshold		69.33%		53.33%		47.14%				30.1%	
		P<.05		P<.001		P<.001				P<.05	
		Significant		Highly Significant		Highly Significant				Significant	

(P - Refers to the probability of the results occurring by chance.)

TABLE XIV A COMPARISON OF THE ANALGESIC RESPONSES TO DRUG 'SU-8629' 5 MG./KG. WHEN TESTED BY RADIANT HEAT, ELECTRICAL STIMULATION AND PRESSURE APPLIED TO THE RAT'S TAIL AND TOOTH PULP STIMULATION IN THE GUINEA PIG

		RATS								GUINEA PIGS	
		RADIANT HEAT		ELECTRICAL STIMULATION		PRESSURE				TOOTH PULP STIMULATION	
		Reaction Time Seconds		Reaction Voltage		Reaction Wt. in Grams				Reaction Voltage	
Rat No.	Wt. in Gms.	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Control Mean	Post Injection Peak	Guinea Pig No.	Wt.in Gms.	Control Mean	Post Injection Peak
1	200	5.75	7	20.5	24	70	100	1	600	37	85
2	210	6.5	9	20.5	32	80	170	2	320	32	52
3	210	4.75	6	28	30	80	90	3	350	25.5	33
4	210	6	6	18	22	80	100	4	520	28	52
5	210	6.25	7	22	40	100	170	5	450	17.5	30
6	210	5.25	6	10	14	80	100	6	410	27.5	16
7	210	6.25	7	11.5	30	70	90	7	330	17.5	18
8	190	4.75	7	28	32	70	90	8	390	22.5	26
9	220	5.75	7	26	30	80	110	9	310	17.5	12
10	220	5.25	7	15	32	110	140	10	400	19.5	16
AVERAGE		5.65	6.9	19.95	28.6	82	116	AVERAGE		24.45	34
Per Cent Increase From Control Threshold		22.123%		43.3583%		41.463%				39.06%	
		P<.001		P<.01		P<.01				P>.05	
		Highly Significant		Very Significant		Very Significant				Not Significant	

(P - Refers to the probability of the results occurring by chance.)

DISCUSSION

Because the same dosage of the different drugs was used for both the rat and the guinea pig, and only one analgesimetric method used in the guinea pig, it was decided to compare the ratios of the analgesic responses obtained by guinea pig tooth pulp stimulation with those obtained by the three testing methods in rats. In order to do this for each drug, the per cent analgesic response obtained for the method showing the minimum analgesic response was given an arbitrary value "1". Then the ratios for the rest of the analgesic responses obtained by the other three methods were calculated accordingly. As an example, the per cent analgesic responses and their ratios (in parenthesis) for morphine and codeine are given below.

Drugs	Dosage mg./kg.	Radiant Heat	Electrical Stim.	Traumatic Pressure	Tooth Pulp Stim.
Morphine sulfate	2 mg.	111% (2.6)	71% (1.7)	47% (1.1)	42% (1)
Codeine phosphate	5 mg.	22% (2.9)	8% (1)	34% (4.5)	48% (6.3)

While this particular method of comparison has not been used by other workers, it was thought that this would allow a comparison of the data obtained for tooth pulp stimulation in the guinea pig with the three testing methods used in rats. In mice, instead of comparing the ratios, the per cent analgesic responses obtained by the three methods have been compared as there is only one species involved.

Figure 9 shows a comparison of the ratios of the analgesic responses obtained in the rat when tested by radiant heat, electrical stimulation and traumatic pressure, and in the guinea pig subjected to tooth pulp stimulation following administration of morphine sulfate, codeine phosphate and sodium salicylate. The most striking feature of this comparison is the difference between the responses to radiant heat which were great and to electrical stimulation which were slight when morphine and codeine were studied. The changes in the analgesic responses when tested by tooth pulp stimulation in the guinea pig were considerable for codeine and sodium salicylate but, peculiarly, much less with morphine, the strongest analgesic. Except for codeine, the changes in the analgesic response to traumatic pressure following drug administration were less than for the other methods.

Figure 10 shows a comparison of the ratios of the analgesic responses to some of the more recently introduced non-narcotic drugs, dextro propoxyphene, ethoheptazine and salicylamide. With all three of these drugs, the changes in the analgesic response stand out prominently when the guinea pig tooth pulp stimulation method was used. The other test eliciting maximum changes in the analgesic response was electrical stimulation of the rat's tail.

Figure 11 shows a comparison of the ratios of analgesic responses for some new investigational drugs. A comparison of the graphs plotted in this figure with those in Figure 9 shows that the responses and ratios for SKF #1340 resemble those for morphine while EKP-104 produced responses and ratios similar to codeine. SU-8629 does not fall into

Figure 9

A COMPARISON OF THE RATIOS OF THE ANALGESIC RESPONSES TO MORPHINE SULFATE, CODEINE PHOSPHATE AND SODIUM SALICYLATE IN RATS AND GUINEA PIGS

(The analgesic responses to Morphine Sulfate, 2 mg./kg., Codeine Phosphate, 5 mg./kg., and Sodium Salicylate, 50 mg./kg. obtained by the methods of applying radiant heat, electrical stimulation and traumatic pressure as pain stimuli to the rat's tail and by the method of electrical stimulation of the tooth pulp in the guinea pig.)

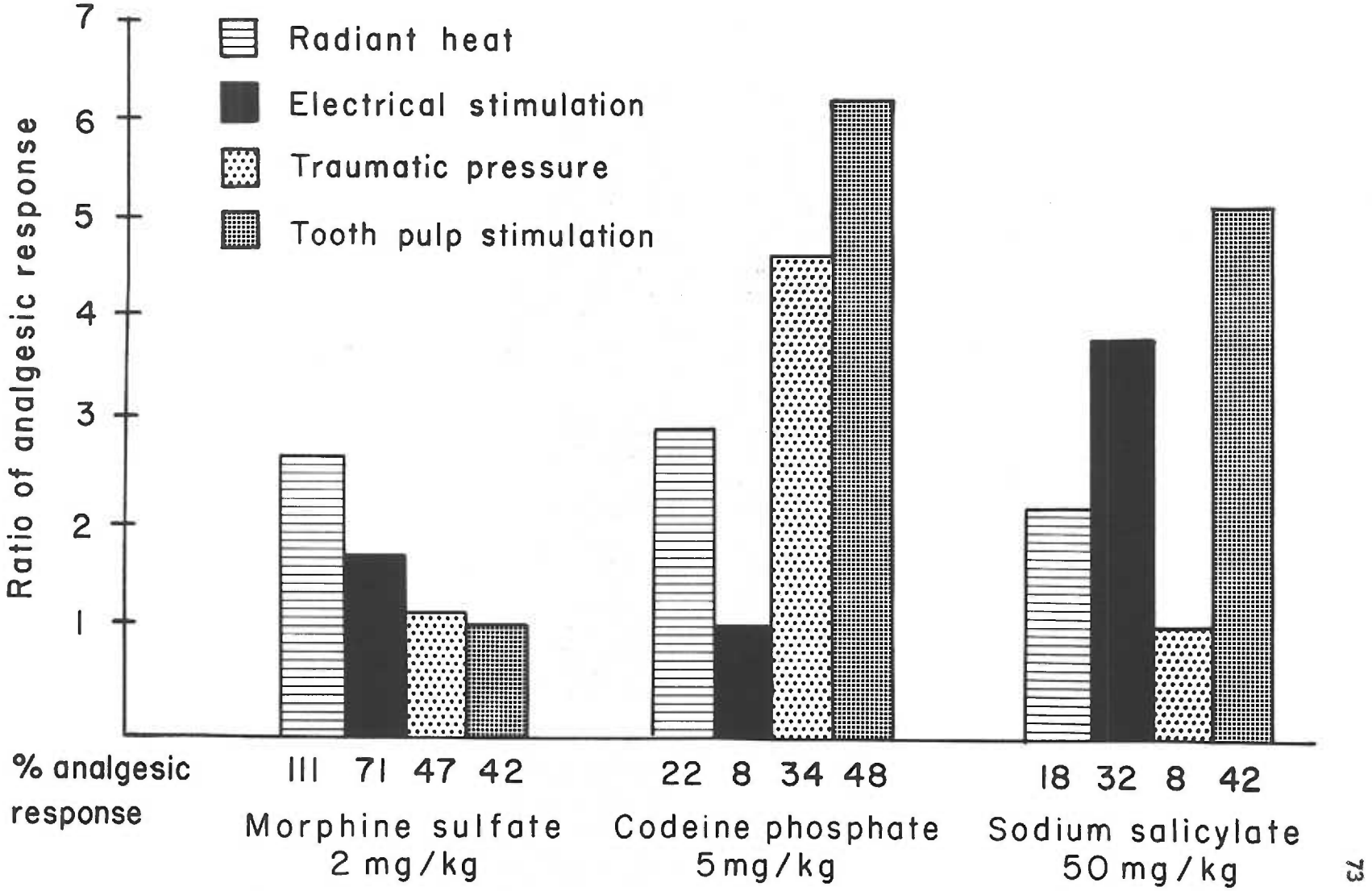

Ratio of analgesic response
7
6
5
4
3
2
1
Radiant heat
Electrical stimulation
Traumatic pressure
Tooth pulp stimulation
% analgesic response
111 71 47 42
22 8 34 48
18 32 8 42
Morphine sulfate 2 mg/kg
Codeine phosphate 5mg/kg
Sodium salicylate 50 mg/kg

Figure 9

Figure 10

A COMPARISON OF THE RATIOS OF THE ANALGESIC RESPONSES TO DEXTRO PROPOXYPHENE HYDROCHLORIDE, ETHOHEPTAZINE CITRATE AND SALICYLAMIDE IN RATS AND GUINEA PIGS

(The analgesic responses to Dextro propoxyphene hydrochloride, 2 mg./kg., Ethoheptazine Citrate, 25 mg./kg., and Salicylamide, 50 mg./kg. obtained by the methods of applying radiant heat, electrical stimulation and traumatic pressure as pain stimuli to the rat's tail and by the method of electrical stimulation of the tooth pulp in the guinea pig.)

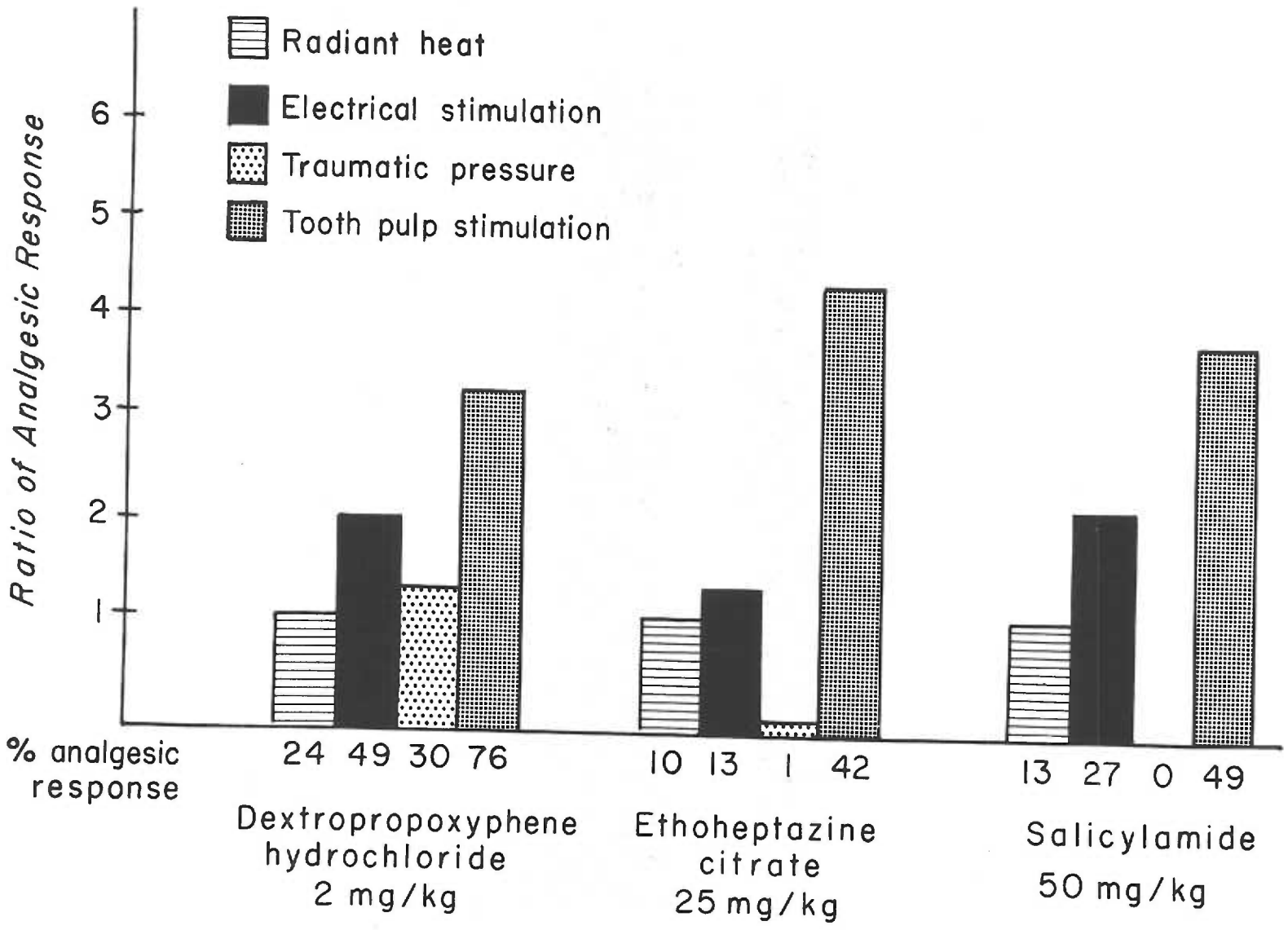

Radiant heat
Electrical stimulation
Traumatic pressure
Tooth pulp stimulation
Ratio of Analgesic Response
6
5
4
3
2
1
% analgesic response
24 49 30 76
10 13 1 42
13 27 0 49
Dextropropoxyphene hydrochloride 2 mg/kg
Ethoheptazine citrate 25 mg/kg
Salicylamide 50 mg/kg

Figure 10

line with any of the other drugs studied.

Figure 12 shows the per cent of analgesic responses detected by the three testing methods used in mice following morphine, codeine, sodium salicylate and EXP-104. In mice, the difference between the narcotic drugs (morphine and codeine) and the non-narcotic drug (sodium salicylate) stands out even more clearly than was observed for the rat (Figure 9). With both morphine and codeine, the mice showed almost complete analgesia when tested by the radiant heat method, whereas, with electrical stimulation, no noticeable change occurred. With sodium salicylate quite the opposite was seen in mice since a remarkable increase in analgesic response was detected by the electrical stimulation method. But, with radiant heat, the per cent increase was less than half of that found with electrical stimulation. With conducted heat, the per cent increase in the analgesic response is a negative value.

At this point these findings can be correlated to those obtained with the pharmacological studies done on these three experimental drugs in the respective laboratories. A communication from the Smith, Kline and French Laboratories for SKF #1340 states that definite "morphine-like activity was observed in animal tests as increase in intestinal tone, and decrease in the intestinal motility in dogs, decrease in respiratory minute volume of short duration in rabbits, Straub tail response and mydriasis in mice, and antagonism by N-allylnormorphine in rats. Because of these, the compound should be treated as

potentially addicting." It is interesting to note that in our studies, too, the analgesic responses with SKF #1340 by the various methods and their ratios resemble those with morphine as is shown in Figures 9 and 11.

EXP-104 is claimed to be a very potent drug by E. I. DuPont de Nemours and Company, Inc. A communication from these laboratories state that when tested by the radiant heat/tail flip method in rats and the hot plate method in mice, the ED_{50} of morphine was 20 to 25 times as great as that of EXP-104, both drugs being given intraperitoneally; also, that EXP-104 did not produce any narcotic-like symptoms in the cat such as salivation or mydriasis.

From the study done on this drug in this laboratory, it showed potency about 4 times that of morphine by the radiant heat method in rats but about 20 times by the hot plate method in mice.

These findings suggest that EXP-104 differs slightly from morphine and could have some of the characteristics of a non-narcotic analgesic. However, from the results obtained in both rats and mice it seems more proper to include them among the codeine-like drugs. Besides, EXP-104 also showed a marked analgesic response by the method of guinea pig tooth pulp stimulation as was also shown with codeine.

From these findings, the writer feels that EXP-104, though a potent drug, should be treated as potentially narcotic and, hence, addicting although it is claimed to be otherwise.

SU-8629 is described as an analgesic drug different from other drugs with stimulant properties. Although nothing as to this effect

Figure 11

A COMPARISON OF THE RATIOS OF THE ANALGESIC RESPONSES TO
SU-8629, SKF #1340 AND EXP-104
IN RATS AND GUINEA PIGS

(The analgesic responses to SU-8629, 5 mg./kg., SKF #1340, 10 mg./kg., and EXP-104, 0.5 mg./kg. obtained by the methods of applying radiant heat, electrical stimulation and traumatic pressure as pain stimuli to the rat's tail and by the method of electrical stimulation of the tooth pulp in the guinea pig.)

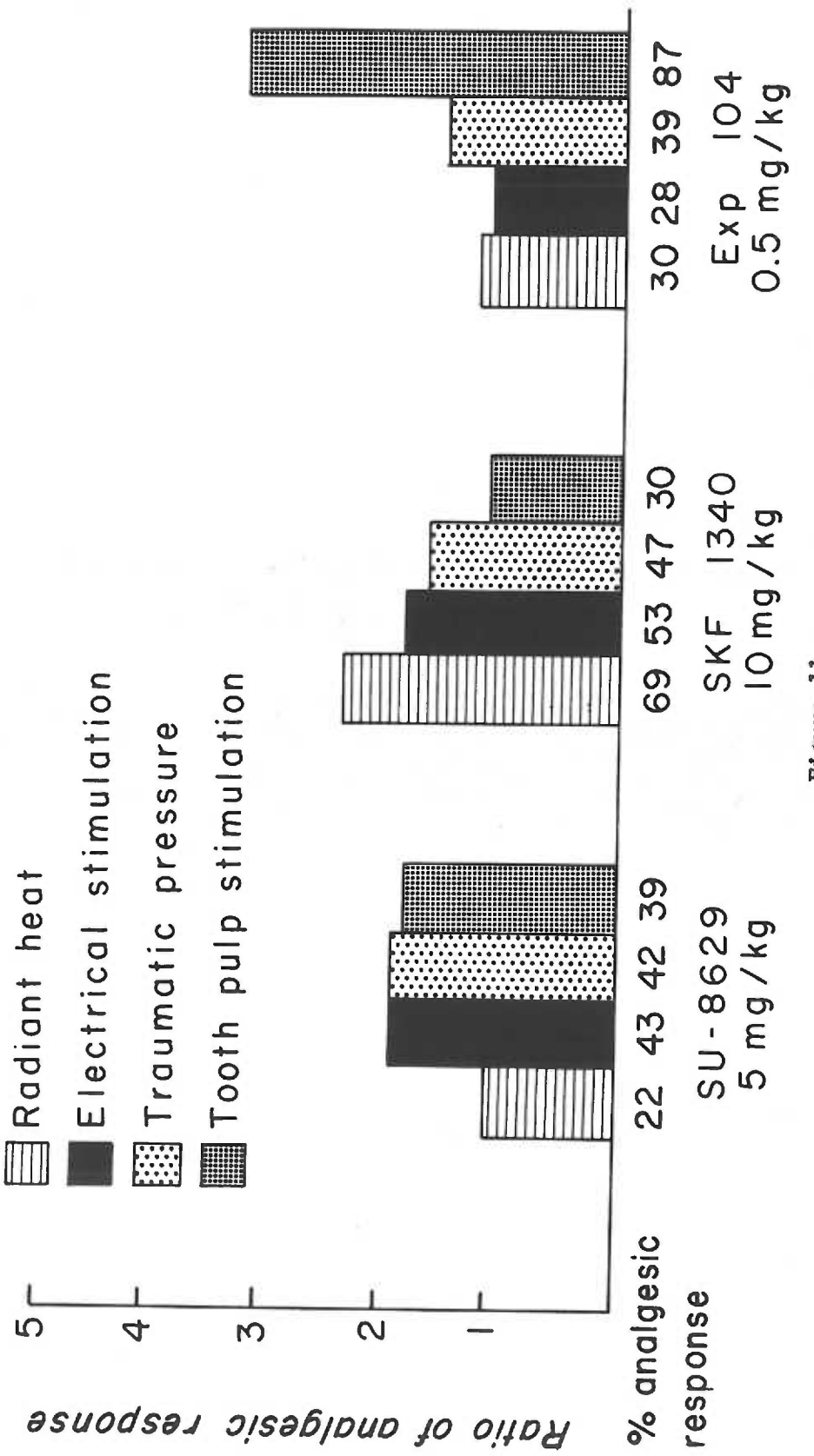

Radiant heat
Electrical stimulation
Traumatic pressure
Tooth pulp stimulation
Ratio of analgesic response
5
4
3
2
1
% analgesic response
22 43 42 39
SU-8629
5 mg/kg
69 53 47 30
SKF 1340
10 mg/kg
30 28 39 87
Exp 104
0.5 mg/kg

Figure 11

can be concluded from the histogram of this drug (Figure 11), it is interesting to note that it has a pattern of its own very much different from the other drugs studied.

In addition to these results, one realizes the importance of employing several methods in several species of animals in order to get a true picture of the drug and learn as to its potency; besides, this also emphasizes the importance of controlled clinical studies to evaluate a drug completely.

From the results obtained by the use of five different analgesimetric methods, it would seem that the non-narcotic and narcotic drugs follow a different type of curve pattern with respect to the analgesic responses shown. A consideration of the differences in these responses which were obtained when the same drug was tested by different methods in one or more animals follows.

Radiant Heat Versus Electrical Stimulation Methods in the Rat and Mouse

In rats and mice the radiant heat technique revealed almost the maximum analgesic response when the narcotic drugs morphine and codeine were used (Figures 9 and 12). On the other hand, when electrical stimulation was used the per cent of analgesic response was considerably less in rats while, in mice, that with codeine was still less whereas no analgesic response occurred with morphine. Sodium salicylate, however, did show significant analgesia in both the rat and mouse with the electrical stimulation method but caused only a slight response which was not significant when radiant heat was used in the rat. Yet, in the mouse radiant heat produced a significant

Figure 12

A COMPARISON OF THE PER CENT OF THE ANALGESIC RESPONSES TO MORPHINE SULFATE, CODEINE PHOSPHATE, SODIUM SALICYLATE AND EXP-104 IN MICE

(The analgesic responses to Morphine Sulfate, 20 mg./kg., Codeine Phosphate, 50 mg./kg., Sodium Salicylate, 500 mg./kg. and EXP-104, 1 mg./kg., in mice obtained by the methods of applying radiant heat and electrical stimulation to the tail and conducted heat to the paws as pain stimuli.)

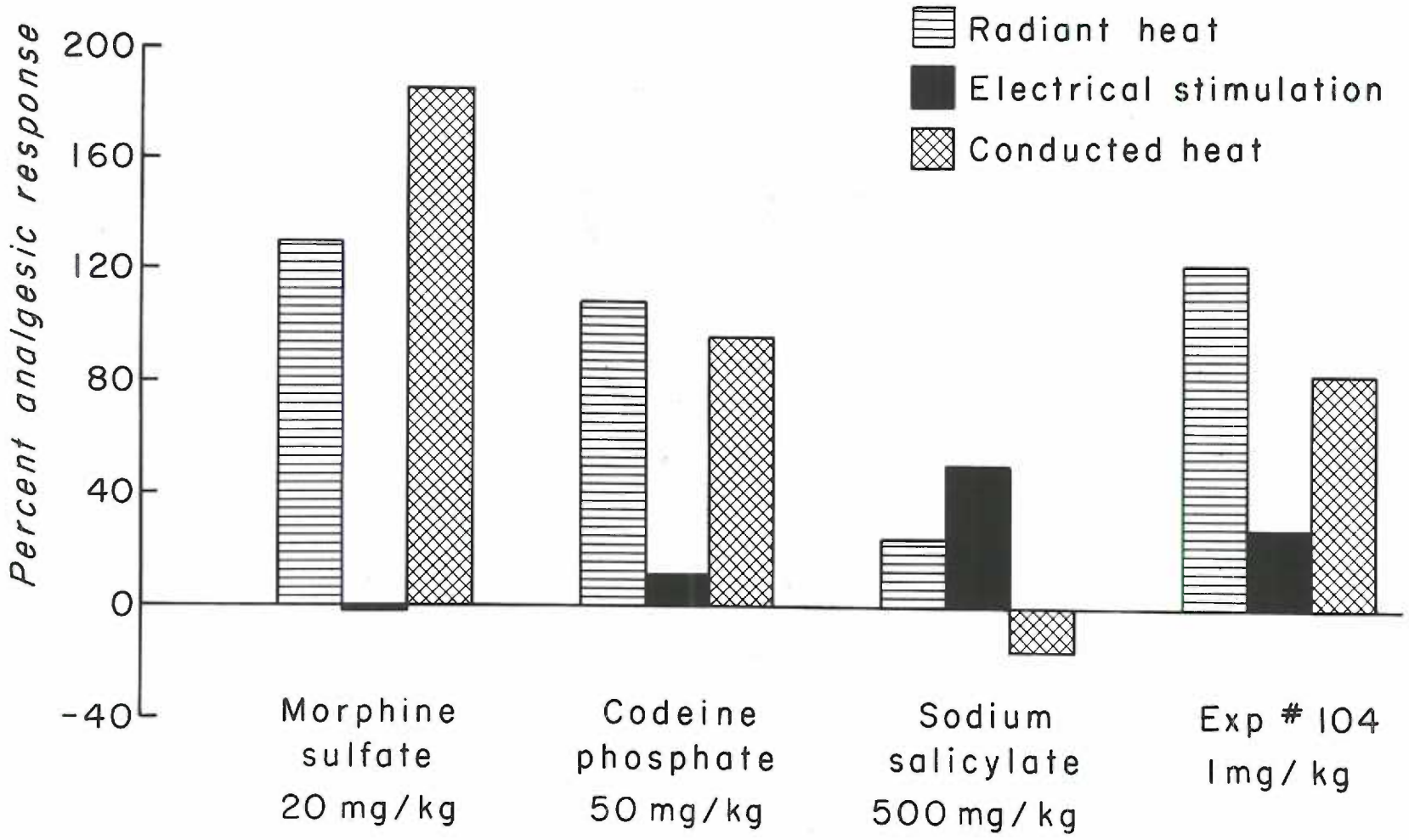

Percent analgesic response
200
160
120
80
40
0
-40
Radiant heat
Electrical stimulation
Conducted heat
Morphine sulfate 20 mg/kg
Codeine phosphate 50 mg/kg
Sodium salicylate 500 mg/kg
Exp # 104 1mg/kg

Figure 12

analgesic response.

In rats subjected to the three analgesimetric tests the more recently introduced non-narcotic analgesics dextro propoxyphene, ethoheptazine and salicylamide showed responses very similar to that obtained by sodium salicylate. These drugs were not tried in mice. SKF #1340 and EXP-104 showed greater analgesic responses with the radiant heat testing techniques than with electrical stimulation, while SU-8629 had the opposite effect.

EXP-104 was further studied using mice subjected to the three types of painful stimuli. Even a greater difference between the response to radiant heat and electrical stimulation was noted than with the other drugs. With the radiant heat method the analgesic response was maximum and highly significant while very little change was noted with electrical stimulation. As reported above for the other drugs tested by different methods, similar differences were found for the rat when given EXP-104.

Radiant Heat Versus Traumatic Pressure in Rats

In general, the response to traumatic pressure followed a pattern somewhat similar to that for radiant heat. However, many difficulties were encountered while using this technique so that it was somewhat difficult to compare responses obtained by this method with those obtained by radiant heat. The apparatus used could register only a maximum pressure of 170 grams and in animals where the control response was above 100 grams, this method did not allow for large increases.

Brodie, Way and Smith (9) designed this apparatus for use in very young rats from 40 to 60 grams in weight whose control response should be at about 10 to 30 grams of pressure and they also suggested that this method could be used for evaluating salicyl-type of analgesics. Although the rats used in this experiment were over 100 grams in weight, this method was included in order to compare it with the other techniques used in the same animal. As referred to previously, another problem encountered with traumatic pressure stimulation was that the skin over the tail became tender from the traumatic pressure test done previously. However, this happened only once and was corrected by allowing sufficient intervals between experiments.

Radiant Heat Versus Conducted Heat in Mice

The results indicated that these two methods showed very similar responses.

Tooth Pulp Stimulation in Guinea Pigs Versus the Three Methods Used In Rats

With the exception of morphine and SKF #1340, all the other drugs showed maximum analgesic response when tested by tooth pulp stimulation in the guinea pig. With SU-8629 the responses were almost the same as those obtained by the various methods used in rats. Although these findings suggest that guinea pig tooth pulp stimulation is the most sensitive technique of those studied for determining analgesic potency, while this might be true, several things make one doubt its validity.

In using radiant heat as a pain stimulus for analgesimetry in

several animals, Thorp (96) says, "Since guinea pigs are excitable and did not give a precise end-point, their use was abandoned and attention was turned to the use of rats." From the experiments done in this study, the author feels the same way, too. It was found difficult to train guinea pigs to hold still and not jump nor squeal at the least provocation. Sometimes the end-point was very difficult to determine. Frommel et al (43) took for the end-point the time when the guinea pig raised its head and neck in a characteristic manner. However, in our experiments this movement alone could not be taken as the end-point, as often the animal just squealed when shocked without raising its head. Therefore, it was decided to take as the end-point when the guinea pig both squealed and raised its head when shocked; and in the few instances that the animal did not raise its head, but only squealed, squealing was taken as the end-point.

Another finding which makes one doubt the validity of this method is the fact that the analgesic response to tooth pulp stimulation in the guinea pig was small when morphine was given compared to those responses that morphine evoked in rats when tested by the several methods. On the other hand codeine showed a marked analgesic response with guinea pig tooth pulp stimulation compared to those for the rat. Considering these discrepancies it would seem that the guinea pig is not the best animal to use for tooth pulp stimulation studies.

Heat Stimuli Versus Electrical Stimuli in Analgesimetry As Revealed By Narcotic Versus Non-Narcotic Analgesics

The fact that the analgesic responses obtained with morphine and codeine were much less with electrical stimulation than with radiant heat in rats and even less in mice, suggests the involvement of different nervous pathways for carrying these two stimuli. Perhaps this explains why morphine showed poor analgesic response to electrical stimulation of the tooth pulp in guinea pigs.

Brookhart et al (10) used the cat's tooth pulp to demonstrate that the afferent fibers, which are intrinsic to the tooth pulp in the cat, have conduction and excitation characteristics resembling those of the A gamma-delta fibers of the cat's saphenous nerve. In the introductory part of this thesis it was mentioned that pain sensations are carried to the higher centers by two kinds of nerve fibers, the A delta and the C fibers. If so, then any drug which shows a marked analgesic response when tested by the tooth pulp stimulation method presumably acts by blocking the A delta fibers. As seen from the above experiments (Figures 9, 10, 11), all the non-narcotic analgesics showed marked analgesic responses by the guinea pig tooth pulp stimulation method and apparently have a marked blocking effect on the myelinated A delta fibers. In such case, then, since much less analgesia was detected by this method with the morphine-like drugs, they should be exerting their analgesic effect by blocking the non-myelinated

smaller diameter C fibers. Possibly, this same theory could also explain the poor analgesic response of the narcotic drugs obtained by electrical stimulation of the tail of rats and mice. For instance, if the A delta fibers were not blocked, then the impulses would travel through these rapidly conducting fibers to register pain regardless of whether or not the C fibers are blocked. It could be possible that morphine in the concentrations used acts only on the non-myelinated C fibers, still allowing conduction through the A delta fibers. This is a field worth experimenting using isolated mixed nerves and that is being done in this laboratory using guinea pig's sciatic nerve.

Another explanation could be offered for the difference in responses to heat and electrical stimulation on the basis of central depression by the narcotic analgesics. When the higher centers are depressed, the animal may be reacting quicker to a reverborative stimulus than to other types. In such case, a greater analgesic response would be detected by heat techniques which give gradually increasing stimuli with the relapse of time than do electrical stimulations where the stimulus may be reverborative. Melzack (60) mentions that Hill et al were able to show in both man and animals that morphine diminished pain if the anxiety level was high, but, interestingly, might not show any demonstrable effect if the anxiety was removed.

At this point, it may also be said that electrical stimulation of the tooth pulp is much more unique as an analgesimetric test compared to the electrical stimulation of the skin. Only a specific group of fibers are stimulated in the tooth pulp method while in the case of skin, all the nerve fibers would be stimulated depending upon the strength and the duration of the current applied.

Non-Narcotic Versus Narcotic Analgesics As Revealed By Post-Inflammation-Pressure Stimulus

Randall and Selitto (88) comment on the possible mechanism of the analgesic action of the non-narcotic analgesics such as the salicylates after finding that their combined foot inflammation-pressure method was effective in discerning and demonstrating analgesia with these mild analgesics. In addition to supporting the common belief that the beneficial effect of phenylbutazone and the salicylates on the inflamed foot is related to the reduction in edema, they mention that salicylate compounds might be accumulating in the edema fluid in the foot to exert a local analgesic effect. After giving salicylates, they found that the threshold to pain in the inflamed foot had increased to levels even above the pain threshold of the control foot. Randall and Selitto also mention that some support to this suggestion is offered by Wilhelmi and Pulver who have found an increased accumulation of phenylbutazone in the edema fluid after the administration of this drug. Consequently, Randall and Selitto conclude that salicylate might be accumulating in a similar manner. These workers also found that the aminopyrine type of antipyretics and narcotic analgesics such as morphine and codeine increased the pain threshold of both the normal and the inflamed foot. This would suggest a central mechanism for the increase of the pain threshold by these compounds.

Another fact which stands out as a result of the present research is the importance of using different methods to study the analgesic potency of drugs. O'Dell (85) in 1961 demonstrated this in his paper

on experimental parameters in the evaluation of analgesics. For determining analgesic effects, he used two different methods. One method employed the conduction dolorimeter with heat applied to the hind foot of the mouse. The other method utilized a different species, the rabbit, and a different type of pain stimulus, i.e., electric shocks to the tooth pulp. Besides analgesia, he chose the effect on motor activity and muscle relaxation as two other different parameters in evaluating these drugs. By so doing he demonstrated clearly whether the analgesic effect was related to central nervous system depression or was due to muscle relaxation. Using the two different techniques for analgesia alone he demonstrated that the analgesic activity of all the drugs tried in his experiment ran parallel to one another. However, Green and Young (49) in 1951 made a comparative study of two different analgesimetric methods (heat and pressure) in the same species (rats) and were able to demonstrate that the analgesic effect of meperidine was 1.5 times greater when tested by the pressure method than by the radiant heat method. They mention that "pethidine (meperidine) may have either a greater analgesic effect on pain caused by pressure than pain due to heat or a greater influence on the flight reaction to pain (struggle and squeak) than on the reflex of moving an appendage from a source of stimulus." They add further that "the example of pethidine emphasizes the necessity for considering the method of test when comparing analgesic ratios in the same species."

This statement is further confirmed by the results of our studies.

A few added comments are offered. Since the different methods were done in the same order each time (radiant heat, electrical stimulation and traumatic pressure in the rat, and radiant heat, electrical stimulation and conducted heat in mice), there is a possibility of the development of a conditioning effect in the animal to the different types of stimuli. For this reason, it would be worthwhile to run separate groups of animals for each method for each drug as controls. This procedure would also eliminate any summation effects in the subsequent testings from previous stimulation of the different nerve fibers concerned.

SUMMARY AND CONCLUSIONS

Five different analgesimetric methods have been studied and evaluated utilizing three species of small laboratory animals; namely, the rat, mouse and guinea pig. The pain stimuli were the radiant heat, electrical stimulation and traumatic pressure applied to the tail, conducted heat applied to the paws and electrical stimulation of the tooth pulp in these animals. Of these, three were applied to rats (radiant heat, electrical stimulation and traumatic pressure to the tail), three were applied to mice (radiant heat, electrical stimulation to the tail, and conducted heat to the paws) and only electrical stimulation of the tooth pulp in guinea pigs. In so doing, two narcotic, four non-narcotic, and three experimental analgesic drugs were studied.

From these studies, it was found that the analgesic activity of narcotic drugs could be detected more readily by the heat and pressure techniques whereas with electrical stimulation methods, very little or no analgesic activity was seen using the same dose in the same group of animals at the same time. But the non-narcotic analgesics showed maximum analgesic effect when tested with electrical stimulation, whereas with the heat and pressure techniques, the analgesic effect noted was much less with the same dose in the same group of animals tested at the same time.

These findings bring out the importance of employing several analgesimetric methods in several species of animals using at least two or three different tests in the same species simultaneously in order to get a true picture of the drug action and to evaluate its analgesic potency. Besides, this also emphasizes the importance of doing controlled clinical studies to evaluate a drug completely.

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