Rats given morphine-codeine combinations exhibited significantly increased hotplate reaction times compared with rats given an analgesic dose of one agent alone. Genera, species, sex, protein binding and metabolism were studied as possible implicating factors for the increased reaction times. Both male and female rats exhibited increased reaction time, and showed no difference in onset and duration of the effect. When both analgesic and nonanalgesic doses of one agent were given to rats concomitantly, the increased reaction time was not produced. Thus the administration of both drugs was necessary to produce the interaction. The increased reaction time was not related to the method of measuring reaction times, since the effect was demonstrated using both the tailflick and hotplate techniques. A difference between genera was demonstrated when the interaction could not be shown in mice.
Both morphine and codeine had one primary binding site in 3% bovine serum albumin (BSA) solution. Morphine was bound in BSA solution and rat plasma 49 \pm 5\% and 54 \pm 4\%, respectively. Codeine was bound in BSA solution and rat plasma 59 \pm 6\% and 64 \pm 5\%, respectively. The presence of one drug in varied amounts did not significantly affect the binding characteristics of the other agent. The dialyzable percent of the administered dose of morphine or codeine was 24 \pm 4\% and 23 \pm 5\%, respectively. The presence of one drug did not affect the dialyzable fraction of the other drug when the two were administered to rats concomitantly.

Analysis of morphine metabolism in presence of varied concentrations of codeine provided no evidence to support the idea that codeine might be interfering with morphine metabolism. The presence of varying amounts of morphine did not affect codeine metabolism. Since no free morphine could be measured after codeine incubation, there was no evidence to support the hypothesis that liberation of morphine could be responsible for the increased reaction time.

The factors studied in this treatise did not determine a mechanism for the interaction, but pointed out a possible genera difference and defined the interaction more clearly.
MORPHINE-CODEINE INTERACTIONS IN RODENTS

by

Jerome W. Blank

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MORPHINE-CODEINE INTERACTIONS IN RODENTS

GENERAL INTRODUCTION

Drug interactions have become important considerations in the rational approach to the prescribing of medication. The drug interactions, which may involve one or more systems of the body, may be direct or indirect. A direct interaction would result from one agent interfering with another agent physically or chemically, such as competition for a common site of binding to protein. An indirect interaction may result when one agent produces a change in the body which results in a change in the disposition of the other agent. An example of indirect interaction would be the increased metabolism of barbiturates after a prior administration of phenobarbital which resulted in enzyme induction.

Interactions among homergic drugs have been demonstrated. The sulfonamides compete for a single principle binding site on plasma proteins (Thorp, 1964). Certain phenothiazine tranquilizers potentiate the actions of other phenothiazine tranquilizers (Stuart, 1968). The subject of this treatise is the interaction of two well studied homergic compounds, morphine and codeine.

In 1965 Johannesson and Woods described results obtained from the administration of combinations of morphine and codeine to rats. In their experiments analgetic and nonanalgetic doses of
morphine and codeine were determined by comparison of reaction
times to hotplate thermal stimulus. When a nonanalgetic dose of
morphine (0.295 mg/kg) was given simultaneously with an analgetic
dose of codeine (60 mg/kg), a significant increase in intensity and
duration of analgesia was observed from 60 to 150 minutes after
the subcutaneous administration. When morphine (5.0 mg/kg) was
given in combination with codeine (3.5 mg/kg) the increase in
intensity of analgesia was evident at 60 and 90 minutes after
administration, but reaction times were not significantly different
from controls after 90 minutes. At the time Johannesson and Woods
offered no explanation for the increased intensity and duration of
reaction time, except that it may be a phenomenon peculiar to the
rat.

It is well established that binding of drugs to plasma protein
alters their effectiveness (Thorp). In the case of sulfonamides and
penicillins, loss of antibacterial activity results when the agents
are bound to plasma proteins (Thorp). Brodie and Hogben (1957)
view the plasma protein bound fraction of a drug as a storage depot.
The nature and site of binding is important when one is considering
drugs which are similar in structure. Sulfonamides have been
shown to compete for a single binding site on plasma albumin
(Thorp). The affinity of one sulfonamide for the binding site will
determine the extent of its binding when it is given in combination
with other sulfonamides. Those agents displaced by drugs competing for the same binding site, either bind to other proteins or increase the free drug pool in the blood. Since drug activity is related to the free drug pool, any increase in the free pool could result in more drug activity.

Potentially all proteins of the plasma may bind drugs. Albumin, and α-, β-, and γ-globulins have been shown to bind various substances (Meyer, 1968). Although the globulins have been shown to bind some drugs, their binding capacity appears to be small. Albumin is the plasma protein involved in the binding of most drugs.

The interaction between drug and protein is a reversible attachment by electrostatic, London-Van der Waals, hydrogen bonding forces, or some combination of these (Brodie, 1964b). The forces responsible for binding are often electrostatic. Albumin, with an isoelectric point of 4.9, has a net negative charge (algebraic sum of 100 negative and 84 positive groups) (van Os, 1964). Anions can be bound by positive groups, cations by negative groups. Neutral molecules, particularly large or flat organic structures, can be absorbed owing to nonelectrostatic forces (van Os).

Morphine and codeine are very similar in structure (Figure 1). Both morphine and codeine are bound to plasma proteins (Nadeau and Sobolewski, 1960). If codeine successfully competes with
morphine for protein binding sites, then the unbound morphine pool in the blood would be increased. This increment in the free morphine pool in the blood could result in a sufficient increase in CNS concentrations of morphine to account for the increased reaction times observed by Johannesson and Woods (1965).

Alternatively, biotransformation pathways may be implicated to explain the phenomenon. Morphine is liberated from codeine by O-demethylation in the rat (Adler and Shaw, 1952). It has recently been demonstrated (Yeh and Woods, 1969) that this biotransformation is rapid. These workers found that 30 minutes after subcutaneous injection of codeine the morphine plasma level was 30% of the codeine plasma level (Yeh and Woods, 1969). Most (80-90%) of the formed morphine was quickly converted to the glucuronide in the liver. Nevertheless, sufficient unconjugated morphine may be released into the blood stream to significantly increase the free drug pool of the blood which in turn may allow more morphine to reach the site of
action, thus accounting for the increased reaction times seen by Johannesson (1965).

Cook et al. (1954) showed that prior administration of SKF-525A (β-diethylaminoethyl diphenylpropylacetate) potentiated the analgesia of morphine, codeine and meperidine, as tested by the focused-light tail-flick technique. SKF-525A inhibits microsomal metabolism of morphine and codeine (Cook). Since the metabolism of morphine was inhibited, the amount of free morphine remaining in the blood was larger and the level of free pool maintained for a longer period of time. Again, the higher free drug level would permit more drug to reach the site of action resulting in the increased reaction time.

If morphine and codeine compete at the level of hepatic microsomal drug metabolizing enzyme systems and codeine has a greater affinity for the enzyme system, then morphine metabolism may be inhibited. If the metabolism of morphine is inhibited, then morphine blood levels should be higher for a longer period of time compared to blood levels of morphine in animals receiving only morphine. Again, more morphine might reach the site of action accounting for the increased reaction times.

Other factors which might account for the increased reaction times may be related to the sex, strain and genus of the animal employed, or the method of measuring analgesia.
The objectives of this study were to examine the previous suggestions in order to better define the phenomenon. The hotplate method was employed to reproduce the results of Johannesson and Woods. A second method of measuring analgesia, the tailflick technique, was employed to determine if the method for measuring analgesia was responsible for the phenomenon. Female rats were tested to determine possible sex-related differences. Mice were used to determine if animals of different genera exhibited the phenomenon. The binding characteristics of morphine in the presence of varying amounts of codeine were examined to determine if altered protein binding of morphine could account for the phenomenon. The in vitro metabolism of morphine in the presence of varying amounts of codeine was examined to determine if changes in morphine metabolism rates could explain the phenomenon.
METHODS

Measurement of Analgesia (Reaction Time)

Hotplate Technique

The hotplate technique developed by Woolfe and MacDonald (1944) and modified by Eddy, Touchberry and Lieberman (1950) and Eddy and Leimbach (1953) has wide acceptance as a method for assaying the effects of potent analgesic drugs on thermal pain responses in small animals. The major differences among the hot-plates used primarily deal with variations in the method of heating the plate. Eddy utilized the constant temperature refluxing of acetone. Woolfe and MacDonald used a heated zinc plate.

For this study an insulated box was covered with a sheet of aluminum, 1/8 inch thick and 10 inches square. The heat source was a 40 watt light bulb in a socket mounted on one of the inside walls below the plate. Temperature was controlled with a Thermistemp Temperature Controller, Model 71, and a Thermistemp flat surface probe which was securely taped to the top surface of the plate. The light was turned on and off as necessary to maintain a plate temperature of 50 + 0.5°C which produced 4-8 second reaction times in control animals.

The endpoints accepted as evidence of a response to pain in both rat and mouse experiments were licking of the front paws,
lifting and holding a back foot off the plate surface, lifting and
shaking a back leg, and dancing on the surface. Any one of the
reactions sufficed as evidence of reaction to pain.

The rats were placed over the hotplate in a 3 liter circular
glass container with 6 inch inside diameter and 8 inch height. The
mice were placed in a 1 liter beaker. The beakers were carefully
inverted and the animals dropped gently to the hotplate surface.
Immediately upon eliciting a positive response the animals were
swept off the hotplate surface into a waiting basket. Reaction time
was measured to the nearest 0.1 second with an electric timer.

Tailflick Technique

Hardy, Wolff and Goodell (1940) described a method based on
radiant heat to produce pain for clinical evaluation of analgesics.
D'Amour and Smith (1941) modified the method for application to
experiments with rats, using heat from a lamp source to produce
a painful stimulus in the tip of a rat's tail. Ercoli and Lewis
(1945) modified this method by focusing the lamp light with a bi-
convex lens. Davies et al. (1946) used a hot wire test, exposing
the tails of restrained rats just above a high impedance coil wire.

The procedure used in this study was similar to Davies' method. A dolorimeter manufactured by Metro Scientific Inc. was
used. The dolorimeter consisted of a metal cabinet with a black
bakelite working surface. The surface contained a groove. A high impedance radiant coil was mounted under a hole in the groove. Temperature of the coil was controlled by means of a rheostat. The rat's tail was placed in the groove so that a small portion of the tail, 1-1/2 inches from the tip, lay in the groove above the coil.

The animals were placed in wire restraining racks with their tails extended through a hole in the end of the rack. The temperature of the coil was 48 ± 0.5°C. At this temperature, control and nondosed animals gave reactions within 4-8 seconds. The endpoint was the raising or flicking of the tail out of the groove. The animals made few attempts to lift their tails from the groove spontaneously, even when the coil was cool. Because uncomfortably restrained animals gave many spontaneous flicks, care was taken to see that the animals were carefully placed into the restraining racks. After testing at each time interval the animal was released from the restrainer and returned to his home cage until five minutes before the next reading when he was returned to the restrainer. Reaction time was measured to the nearest 0.1 second with an electric timer from the time at which the animal's tail dropped into the groove until the endpoint occurred.
Experimental Design for Analgesia Experiments

The experimental procedure was designed to test four groups of animals with five animals per group every 30 minutes. Reaction-time testing intervals were 0, 30, 60, 90 and 120 minutes after injection.

The injections in rats were given subcutaneously along the midline of the back. Two injections were given to each animal; the second injection 1-1 1/2 inches posterior to the first. The second injection was necessary to provide continuity in procedure with tests made with the drug combinations. Each drug was administered separately. If only one drug was given, the second injection was 0.9% saline (1 ml/kg). Control animals received 0.9% saline (2.0 ml/kg) in two separate injections. A similar procedure was followed in mice.

The rats used in these experiments were the Simonsen strain of Sprague-Dawley, bred and raised in our animal facilities. The mice used were Carworth Farms CF #1 strain, all weighing 20-30 grams. All animals were allowed free access to water and laboratory chow. The animals in all experiments were used only once. All experiments were carried out at the same time of day (12:00 - 4:00 P.M.). The room temperature was the same in the laboratory and in the animal room (73 + 1°F).
Posology

The doses for the analgesia experiments were determined in pilot studies. A 30 second maximum exposure time was used to prevent injury to the animals. Equianalgetic doses of morphine and codeine, which had reaction times from 14-18 seconds, were chosen. For the hotplate experiments the analgesic doses were: morphine, 5 mg/kg; codeine, 30 mg/kg. For the tailflick experiments the analgesic doses were: morphine, 1 mg/kg; codeine, 15 mg/kg. Nonanalgetic doses of the two drugs (doses which produced no significant increase in reaction times compared to control animals) were the same for both methods: codeine, 3 mg/kg; morphine, 0.3 mg/kg. The analgetic doses in mice were: codeine, 40 mg/kg; morphine, 10 mg/kg. The nonanalgetic doses were: codeine, 10 mg/kg; morphine, 2 mg/kg. Dosage was based on the sulfate salts of morphine and codeine in all experiments.

Statistical Analysis

Each analgesia experiment was replicated at least three times. F-tests showed no significant differences between variances of the groups from different replications administered the same doses. Therefore, the mean (± standard error) of all animals of each dose was plotted for each time interval at which readings were
taken. Significant difference between mean values at a time period was determined by application of Student's "t" test.

Plasma Protein Binding

A drug which is bound to plasma proteins remains in a dynamic equilibrium in the plasma. The total amount of drug is divided between the protein bound fraction and the free fraction (Brody, 1964a). The amount of drug bound to plasma proteins is a function of the affinity of the drug for the protein and a function of the total blood level of that drug. As the drug concentration increases the protein gradually becomes saturated with the drug. After saturation is reached free drug concentration will rise rapidly unless handled by the body in some alternate fashion such as excretion or redistribution.

Published data about the binding of commonly used drugs which are bound to plasma proteins to any extent suggests no more than two primary binding sites, and generally only one (Thorp). Despite the extensive work performed with morphine and codeine, the binding characteristics of these drugs in rats has not been determined. Since small changes in structure of organic molecules alters binding characteristics, this data is essential to the hypothesis of altered binding characteristics.
Ultrafiltration and equilibrium dialysis are methods used for estimating the free drug concentration in plasma. Equilibrium dialysis requires large volumes of fluid to dialyze the free drug from the plasma over a period of time. Ultrafiltration allows the use of small sample volumes for estimation.

Ultrafiltration is based on the principle of dialysis through a semipermeable membrane. The porous membrane selectively permits substances to pass through on the basis of molecular size. Application of a force field to a solution within dialysis tubing forces the fluid and dialyzable substances through the porous membrane. Those substances which will not dialyze (such as proteins) remain within the tubing. Substances partially bound to proteins distribute themselves on either side of the membrane with respect to the unbound fraction of drug. If the dialysis of a substance is not restricted by the membrane, the concentration of substance on the outside of the membrane should be equal to the free or unbound concentration within the membrane. If the force applied to the fluid within the membrane is great enough, the binding equilibrium can be upset resulting in a debinding of the substance, producing a false binding analysis. If a large portion of the sample within the tubing is dialyzed, a similar upset in binding equilibrium occurs because the relative concentration of nondialyzable substance within the membrane increases. Again, a false indication of binding
characteristics results.

**Experimental Design**

The ultrafiltration procedure employed herein was modeled after that used by Dixon (1965). A three milliliter sample was pipetted into a 7 inch section of cellulose dialysis tubing, 1/4 inch diameter. The filled tubing was suspended in a 15 ml centrifuge tube and centrifuged at 400 rpm (90 x g) for 8 hours in an International Model UV high speed centrifuge. The amount of dialysate at the end of this period was slightly in excess of 0.2 ml. Each sample was replicated three times. The dialysate was analyzed with the idea that the quantities of drug measured were related to the total administered dose, not as a percentage of the amount present in the samples.

One in vitro experiment was carried out using an artificial plasma composed of a 3% solution of bovine serum albumin (Cohn fraction V) in 0.25 M phosphate buffer solution (pH 7.4). To 15 ml aliquots of 3% BSA were added quantities of morphine and/or codeine (as the sulfate salts) in the same ratios employed in the hotplate analgesia experiments. The solutions were allowed to equilibrate for 10 minutes. Three, 3.0 ml aliquots of each solution were suspended in centrifuge tubes, dialyzed by ultrafiltration and the dialysate analyzed for morphine and/or codeine content.
A similar experiment was performed with 15 ml aliquots of blood obtained by aortic puncture from rats weighing 150-200 gms. Morphine and/or codeine was added to the aliquots in quantities and ratios equal to those in the hotplate analgesia experiments. Ten minutes was allowed for the blood to equilibrate. The blood was centrifuged at 15,000 rpm for 15 minutes in a clinical centrifuge. The plasma was pipetted in 3.0 ml portions into dialysis tubing, dialyzed, and the dialysate analyzed for free morphine and codeine.

A third experiment was performed with blood obtained by aortic puncture from rats 30 minutes after they were dosed with morphine and/or codeine in the same quantities used in the hotplate analgesia experiments. The blood was centrifuged, dialyzed and analyzed for morphine and codeine as described above.

Statistical Analysis

Three replicates of each sample were made and analyzed. The data from the three samples was averaged and the means (± standard error) used for analysis.

In Vitro Metabolism

The in vitro conversion of codeine to morphine was suggested by Wolff (1938). Way and Adler (1962) discussed the hypothesis that the analgesic and narcotic effects of codeine were due to the
conversion of codeine to morphine. Adler and Shaw (1952) demonstrated that morphine was liberated from codeine in incubation experiments with rat liver slices. The experiments of Yeh and Woods (1969) indicated that the O-demethylation of codeine to morphine is rapid.

On this basis, two possible metabolic mechanisms might explain the increased reaction times observed when morphine and codeine were administered concomitantly. If the rate of O-demethylation of codeine was fast enough, the conversion of codeine may have produced significant amounts of morphine. Only 10-20% of the morphine produced in this manner was not conjugated with a glucuronide in the liver (Yeh). If the free morphine entering the plasma was sufficient to significantly increase the amount of morphine available to the site of action, then this could account for the increased reaction times.

Alternatively, codeine may inhibit the metabolism of morphine. If codeine interfered with the glucuronide conjugation and N-demethylation of morphine, then perhaps free morphine plasma levels were maintained for a longer time, permitting more morphine to reach the active site. This also could have accounted for the increased reaction times.
Experimental Design

Preparation of Tissue

Male rats similar in weight and strain to those used in all other experiments were used in the following experiments. The animals were killed by a blow on the head and the livers were immediately removed and homogenized on ice with a Potter homogenizer having a Teflon plastic pestle. Homogenates were prepared such that each gram of liver was suspended in 2.0 ml of cold isotonic (1.15%) KCl (final volume was approximately 3.0 ml). The homogenate was spun at 2500 rpm (725 x g) for 20 minutes in a refrigerated (0-3°C) Lourdes-Betafuge equipped with a 9RA fixed angle head (4.5 inch maximum radius). The supernatant was decanted and centrifuged again at 9000 x g at 0-3°C for 20 minutes. The supernatant containing microsomal and soluble enzymes was decanted and brought up to the original volume in order that 3.0 ml of KCl would contain the enzymes from one gram of liver. This solution was then stored in a refrigerator until used.

Reaction Mixtures

Three milliliters of the 9000 x g supernatant were incubated at 37°C in 25 ml Erlenmeyer flasks in a Dubnoff metabolic shaker with oxygen as the gaseous phase for determination of enzymatic
activity. The following constituents, each contained in 0.1 ml of solution buffered to pH 7 with phosphate buffer were added: TPN (2 micromoles), glucose-6-phosphate (20 micromoles), nicotinamide (40 micromoles), magnesium chloride (20 micromoles), semicarbazide (75 micromoles), and 2 EU of glucose-6-phosphate-dehydrogenase, and varying amounts of substrate (morphine and/or codeine, both as a sulfate). The final volume was brought to 5.0 ml with pH 7.4 phosphate buffer. Incubation was continued at 37°C, 100% oxygen atmosphere, for 30 minutes, at which time the reaction was stopped with 5.0 ml of 0.1 M phosphate buffer (pH 10).

**Extraction of Morphine and Codeine**

After the reaction was stopped the contents of the Erlenmeyer flasks were poured into 50 ml glass-stoppered centrifuge tubes containing 15 ml of heptane with 2% isoamyl alcohol (v/v) and 0.5 gm of NaHCO₃. The tubes were shaken for 15 minutes on an Eberbach reciprocating laboratory shaker. Ten milliliters of the organic phase were transferred to another 50 ml centrifuge tube containing 2.0 ml of 0.01 N hydrochloric acid. This mixture was shaken for 15 minutes, then centrifuged for 10 minutes at 2000 rpm (890 x g) in the International Model UV high speed centrifuge. The organic phase was aspirated off and the remaining solution assayed for morphine and codeine content. Morphine was estimated
fluorimetrically and codeine chromatographically.

**Chromatographic Analysis**

The detection of submicrogram amounts of the opiate alkaloids has frustrated many investigators. In recent years many investigators have been using $^{14}C$ labelled opiates to shed further light on their investigations. Very small amounts can be measured in this manner. Radioisotope experiments are relatively expensive. Gas liquid chromatography (GLC) shows promise of being another avenue of breakthrough as a rapid and sensitive method of analysis. Yamaguchi et al. (1962) performed extensive analysis on morphine and its related alkaloids using the gas chromatograph. Parker et al. (1963) performed microgram analysis of alkaloids, barbiturates, sympathomimetic amines and tranquilizers.

Morphine and codeine in small quantities did prove to be separable using the gas chromatograph. However, it was not possible to get consistently reproducible results with sub-microgram amounts of morphine. Therefore, only codeine was analyzed by GLC. Analysis was performed with an Aerograph Model 204B chromatograph equipped with flame ionization detector. A 5-foot stainless steel coiled column of 1/8 inch outside diameter and 0.093 inch inside diameter was used. It was packed with Chromosorb W, acid washed, and coated with 5% SE-30 (silicon ethoxide).
The operating conditions were: injector temperature 225°C; oven temperature, 200°C; and detector oven temperature, 250°C; nitrogen carrier gas flow rate, 30 ml/min. (60 psi inlet pressure); hydrogen gas flow rate, 17 ml/min. (8 psi inlet pressure).

Preliminary work showed that extraction of the alkaloids from biological tissues gave best results. The extraction procedures, however, are not complete (70 ± 4%) and result in great dilution of the alkaloidal concentrations. Direct injection of the dialysate from ultrafiltration studies produced good results. The minimum detectable level of codeine in standard solution was 50 nanograms. The smallest analyzable peak with respect to baseline conditions was slightly less than 100 nanograms.

Peaks were analyzed for area by triangulation (Figure 2). Tangents were drawn from peak apex to the curve of the peak on both sides. The base was estimated as the continuance of the baseline from the beginning of the peak to where the baseline was rejoined. Retention time of codeine was 17 minutes.

Large injection volumes (5.0 microliters or more) produced peak smearing. Because of this, sample injection size was arbitrarily limited to 4.0 microliters. Since very small injection volumes (less than 1.0 microliter) gave peaks for codeine which were difficult to reproduce, injection volumes were not less than 1.0 microliter. All injections were made with the same 10.0
Figure 2a. Peak area analysis and reproducibility. Peak area determined by triangulation. \[ A = \frac{1}{2}bh \]. \( b \) = peak base in cm; \( h \) = peak height in cm.

Figure 2b. Standard curve for codeine analysis by gas chromatography. \( ph \) = peak height in cm; \( pb \) = peak base in cm; \( pa \) = peak area in cm\(^2\). Peak #1 generated by .170 micrograms. Peak #2 generated by .26 micrograms. Peak #3 generated by .6 micrograms.
microliter syringe.

**Fluorimetric Analysis**

Photometric methods for morphine analysis (Way et al., 1960; Fujimoto, Way, and Hine, 1954; Woods et al., 1954; Siminoff and Saunders, 1958) are not sufficiently sensitive to assay small amounts of morphine in biological tissues or are not specific to exclude interference from the presence of codeine. Morphine analysis in the presence of codeine was accomplished using GLC (Yamaguchi, Parker). The investigators in all cases, however, worked with amounts of morphine in excess of one microgram. It was necessary in these studies to work with quantities less than one microgram.

Kupferberg, Burkhalter and Way (1964) published a method for morphine detection from plasma samples using a spectrofluorometric analysis. This fluorimetric method was adopted because: (a) codeine does not interfere with morphine analysis, (b) the method is rapid, and (c) amounts as small as 100 nanograms can be analyzed. The basis for the method is the conversion of morphine to pseudomorphine in the presence of a ferri-ferrocyanide reagent in basic pH (Figure 3).
The fluorescence produced by pseudomorphine was measured in a G.K. Turner fluorimeter, Model 111. Excitation of the fluorescence was at 320 mu (filter 7-60) and emission wavelength was 440 mu (filter 47-B).

The rate of formation and the stability of pseudomorphine are dependent on the pH of the reaction medium, the amounts of potassium ferriferrocyanide added, and the amount of morphine to be oxidized (Kupferberg). A pH of 8.5 was chosen as producing the most intense and stable fluorescence. The fluorescence required 10 minutes to develop and was then stable for 30-40 minutes. The sample containing morphine was made acid (pH 3) with 0.1 M HCl. One tenth of 1.0 ml of acid solution was removed by pipetting and added to 3.4 ml of 0.2 M sodium pyrophosphate buffer, pH 8.5. One-tenth milliliter of potassium ferriferrocyanide reagent (0.01M) was added to the solution, mixed thoroughly and allowed to stand for 10 minutes. The solution was then transferred to a quartz cuvette and the fluorescence measured after 10 minutes.

![Diagram](image)

Figure 3. Conversion of morphine to pseudomorphine, From Kupferberg, Burkhalter and Way, 1964.
RESULTS AND DISCUSSION

Hotplate Experiments

The phenomenon described by Johannesson and Woods was duplicated using the hotplate technique (Figure 4.) At the 30 minute time the addition of 3 mg/kg of codeine increased the reaction time to 5 mg/kg of morphine of morphine by 70% (P> 0. 01). Reaction times of rats to which were administered the same dose of codeine by itself were not different from control animals given 0.9% saline (P> 0. 05). At 60 and 90 minutes after drug administration, reaction times of rats which had received the combination were not significantly longer than for animals given morphine alone. Reaction times of all animals had returned to control levels by 120 minutes.

At 30 minutes after injection (Figure 5) 0.3 mg/kg of morphine increased reaction time to 30 mg/kg of codeine by 55% (P> 0. 01). Given alone, reaction times of rats receiving the non-analgetic dose of morphine were not different from control animals given 0.9% saline (P >0. 05). At 60 and 90 minutes after administration no significant difference in reaction times was demonstrated between animals given the combination and animals given the analgetic dose of codeine alone. Once again, reaction times of all animals had returned to control levels by 120 minutes.
Figure 4. Influence of a nonanalgesic dose of codeine on morphine analgesia in male rats tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 20 animals. Codeine 3 mg/kg is not statistically different from saline controls. Morphine 5 mg/kg plus codeine 3 mg/kg is statistically different (\( P > 0.01 \)) from morphine 5 mg/kg only at 30 minutes.
Figure 5. Influence of a nonanalgesic dose of morphine on codeine analgesia in male rats tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (± standard error) of at least 20 animals. Morphine 0.3 mg/kg is not statistically different (P > 0.01) from saline controls. Codeine 30 mg/kg plus morphine 0.3 mg/kg is statistically different (P > 0.01) from codeine 30 mg/kg only at 30 minutes.
Con

Control

Morphine & Codeine

Codeine 30 mg/kg

Morphine 0.3 mg/kg

Minutes After Injection

Reaction Time (sec)
Of the two interactions just described, the increase in reaction time of an analgesic dose of morphine by a nonanalgesic dose of codeine was the more dramatic.

The only major difference between the results of the present study and those of Johannesson and Woods concerned the duration of increase in reaction time. The present study showed increased reaction time only at 30 minutes. Johannesson and Woods reported increased reaction times at 60 and 90 minutes, but no increase at 30 minutes. Since male Holtzmann rats were used in the earlier study and Simonsen-Sprague-Dawley rats were used in the current studies, a strain difference related to the duration of increased analgesia was suggested. However, the difference in duration might have been related to the rate of drug absorption from different sites of injection. In the cited study, codeine was injected subcutaneously in the gluteal region when given in combination with morphine. In the present work, both morphine and codeine were injected subcutaneously along the midline of the back. The gluteal region may have retained a portion of the dose in the musculature, delaying the absorption of codeine and delaying the onset of action. Future experiments with both strains of rats in the same laboratory using the same injection procedure may resolve these questions.
When both the analgesic and nonanalgesic dose of the same drug were given concomitantly, no increase in reaction times could be seen at any time (Figures 6a and 6b). This indicated the phenomenon was an interaction dependent upon the administration of both drugs.

The experiments which showed increased reaction times in male rats were repeated in female rats. The results were similar (Figures 7a and 7b). Significant increases in reaction time of rats receiving the combinations occurred only at 30 minutes. Reaction times of all animals returned to control levels by 120 minutes. From these results no sex difference in rats was evident.

The experiments showed that the phenomenon was reproducible and in a different strain of rats. The present studies showed a faster onset and shorter duration of reaction time increase than the experiments of Johannesson and Woods. Administration of both analgesic and nonanalgesic doses of the same drug concomitantly produced no potentiation. Female rats showed the same responses as the male rats which eliminated sex differences among rats as a feature of the interaction. The differences noted between the present studies and earlier work of Johannesson and Woods may have been related to the different strains of rat used in the two laboratories. Alternatively, different rates of absorption of codeine from different injection sites might explain the differences in onset
Figure 6a. Influence of a nonanalgesic dose of morphine on morphine analgesia in male rats tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 15 animals. Morphine 0.3 mg/kg is not statistically different (P > 0.01) from saline controls. Morphine 5 mg/kg plus morphine 0.3 mg/kg is not statistically different (P > 0.05) from morphine 5 mg/kg at any time.

Figure 6b. Influence of a nonanalgesic dose of codeine on codeine analgesia in male rats tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 15 animals. Codeine 3 mg/kg is not statistically different (P > 0.05) from saline controls. Codeine 3 mg/kg plus codeine 30 mg/kg is not statistically different (P > 0.05) from codeine 30 mg/kg at any time.
Morphine 3mg/kg

Minutes After Injection

Reaction Time (sec)

Control

Morphine 3mg/kg
Figure 7a. Influence of a nonanalgesic dose of codeine on morphine analgesia in female rats tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 15 animals. Codeine 3 mg/kg is not statistically different (P > 0.05) from saline controls. Morphine 5 mg/kg plus codeine 3 mg/kg is statistically different (P > 0.05) from morphine 5 mg/kg only at 30 minutes.

Figure 7b. Influence of a nonanalgesic dose of morphine on codeine analgesia in female rats tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 15 animals. Morphine 0.3 mg/kg is not statistically different (P > 0.05) from saline controls. Codeine 30 mg/kg plus morphine 0.3 mg/kg is statistically different (P > 0.05) from codeine 30 mg/kg only at 30 minutes.
and duration of reaction time increase noted in the two studies.

**Tailflick Experiments**

The tailflick technique produced results supporting the previous hotplate data (Figures 8 and 9). Experiments involving both combinations showed significant increases in reaction time ($P < 0.01$) compared with the dose of analgesic drug at the 30 minute time. Again at 60, 90, and 120 minute times no significant increases in reaction times were noted. Reaction times had returned to normal levels by 120 minutes. Thus, the increase in reaction times of analgesic doses of morphine and codeine were demonstrated using two different methods of measuring analgesia, the hotplate and tailflick techniques. This indicated that the phenomenon was not incidental to the hotplate method of estimating analgesia.

**Mouse Hotplate Experiments**

When an analgesic dose of morphine and a nonanalgesic dose of codeine were given to male mice, no increase in reaction time could be demonstrated compared with morphine given alone (Figure 10). Similar results were obtained when an analgesic dose of codeine and nonanalgesic dose of morphine were given concomitantly (Figure 11). These results indicated possible merit in the speculation by Johannesson and Woods that the increased reaction
Figure 8. Influence of a nonanalgesic dose of codeine on morphine analgesia in male rats tested by tailflick method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 15 animals. Codeine 3 mg/kg is not statistically different (P > 0.05) from saline controls. Morphine 1 mg/kg plus codeine 3 mg/kg is statistically different (P > 0.05) from morphine 1 mg/kg only at 30 minutes.
25

di'
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41,*,
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an
W/Alli,' 0
Whi..016b
;/7;7/
a
Codeine 3mg/kg

120

Minutes After Injection

Reaction Time (sec)

0

30

60

90

120

Control

Morphine 1.0mg/kg

Morphine & Codeine

Codeine 3mg/kg
Figure 9. Influence of a nonanalgesic dose of morphine on codeine analgesia in male rats tested by the tailflick method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 15 animals. Morphine 0.3 mg/kg is not statistically different (P > 0.05) from saline controls. Codeine 15 mg/kg plus morphine 0.3 mg/kg is statistically different (P > 0.05) from codeine 15 mg/kg only at 30 minutes.
Reaction Time (sec)

Minutes After Injection

- Morphine 0.3 mg/kg
- Codeine 15 mg/kg
- Morphine & Codeine

Control
Figure 10. Influence of a nonanalgesic dose of codeine on morphine analgesia in male mice tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (± standard error) of at least 15 animals. Codeine 10 mg/kg is not statistically different (P > 0.05) from saline controls. Morphine 10 mg/kg plus codeine 10 mg/kg is not statistically different (P > 0.05) from morphine 10 mg/kg at any time.
Reaction Time (sec)

Minutes After Injection

- Control
- Codeine 10 mg/kg
- Morphine 10 mg/kg
- Morphine & Codeine
Figure 11. Influence of nonanalgesic dose of morphine on codeine analgesia in male mice tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (+ standard error) of at least 15 animals. Morphine 2 mg/kg is not statistically different (P > 0.05) from saline controls. Codeine 40 mg/kg plus morphine 2 mg/kg is not statistically different (P > 0.05) from codeine 40 mg/kg at any time.
Reaction Time (sec)

Minutes After Injection

- Morphine & Codeine
- Codeine 40 mg/kg
- Morphine 2 mg/kg
- Control
time was a peculiarity of the rat. Genus differences have been known to occur between rats and mice. The most obvious was the difference in doses of morphine and codeine necessary to produce equianalgesic responses by the hotplate technique. The analgesic dose of morphine in mice (10 mg/kg) was twice as large as the dose for rats (5 mg/kg). Similarly, the analgesic dose of codeine in mice (40 mg/kg) was larger than the dose for rats (30 mg/kg). Other differences have been demonstrated between rats and mice.

From the results of the current study, a genus-related difference probably was the reason the increased reaction times could not be shown in mice, as was shown in the rats.

To define better the phenomenon in other animals (rodents and nonrodents) could have provided some difficulties. It would have been desirable to test rabbits and hamsters among the rodents, and dogs among the nonrodents. Testing hamsters on the hotplate would not have been difficult. However, testing rabbits and dogs by the hotplate or tailflick methods would not have been feasible. The extrapolation of results from the hotplate to other methods for larger animals has been questioned and does not appear to be applicable (Woods). Alternative methods of assessing "analgesia" in larger animals have been open to criticism because they have not been refined.
Plasma Protein Binding

The number of primary binding sites from morphine and codeine to albumin was graphically determined using the method of Thorp (1964). In this method the number moles of drug bound per mole of albumin (γ) was compared with the ratio of γ over the concentration of unbound drug (D). One primary binding site was found for both morphine and codeine (Figures 12 and 13). Because of the structural similarity between morphine and codeine, the single primary binding site for both compounds suggested they both might have been bound to the same site on albumin. If both compounds were bound to the same site, they might compete for the site when they were administered together similarly to the sulfonamide competition for a single binding site. However, the results did not suggest that the two compounds significantly interacted at this level.

Morphine was bound to $4.2 \times 10^{-5}$ M BSA 49(+5)%, over a concentration range of $5.2 \times 10^{-5}$ to $8.8 \times 10^{-4}$ M. With the addition of codeine sulfate in quantities from 0.6 to 100 times the quantity of morphine sulfate present, no significant change in morphine binding percentage could be observed (Figure 14a). If codeine had interfered with morphine binding, either the free levels of morphine in the solution would have increased, or morphine might have been bound to a secondary binding site on the BSA molecule.
Figure 12. The number of primary binding sites of morphine to bovine serum albumin. $\gamma$ = the number of moles of drug bound per mole of albumin. $D$ = the concentration of unbound drug. Binding estimated using 3% BSA solution, at 25°C and pH 7.4. Each point represents the mean of three replications.
Figure 13. The number of primary binding sites of codeine to bovine serum albumin. $\gamma$ = the number of moles of drug bound per mole of albumin. $D$ = the concentration of unbound drug. Binding estimated using 3% BSA solution, at 25°C and pH 7.4. Each point represents the mean of three replications.
The percentage of dialyzable morphine was smaller ($46 \pm 4\%$) when morphine binding to proteins was measured in rat plasma (Figure 14a). The discrepancy between BSA and rat plasma may be accounted for by loss of morphine to the red blood cell fraction upon centrifugation and/or the presence of other proteins. The protein content of rat plasma, being about 7%, offered more proteins to which morphine might have been bound. Again, no change in morphine binding percentage occurred when codeine was present in quantities from 0.6 to 100 times the quantity of morphine present in the samples. This indicated that codeine did not cause the free fraction of morphine to increase in vitro. However, the results did not indicate if morphine was "freed up" and subsequently was re-bound at other binding sites.

Thirty minutes after administration of morphine alone to animals, the dialyzable fraction was $24 (\pm 4)\%$ of the administered dose. When codeine and morphine were administered to the animals in combination, no significant change in the percentage of dialyzable morphine occurred (Figure 15). The decrease in dialyzable percentage compared to the in vitro experiments may be accounted for by several factors. The $12^\circ C$ difference in temperature at which the in vitro method was carried out ($25^\circ C$) compared to the in vivo temperature ($37^\circ C$) could have been an important factor in determining the percent of binding. Less drug would have been bound
Figure 14a. Estimation of the binding of morphine to protein in 3% BSA solution and rat plasma. Estimate made by measuring amount of free or dialyzable drug remaining in the dialysate. Binding estimated at 25°C, pH 7.4. BSA = 4.2 x 10^{-5}M. Morphine measured from 5.2 x 10^{-6} to 8.8 x 10^{-4}M.

Figure 14b. Estimation of the binding of codeine to protein in 3% BSA solution and rat plasma. Estimate made by measuring amount of free or dialyzable drug remaining in the dialysate. Binding estimated at 25°C, pH 7.4. BSA = 4.2 x 10^{-5}M. Codeine measured from 5.1 x 10^{-5} to 5.1 x 10^{-4}M.
3% BSA solution
Rat plasma

Percent Free Morphine

0 20 40 60
0/1 0.6 1 10 100
Codeine / Morphine Ratio

Percent Free Codeine

0 20 40 60
1/0 0.6 1 10 100
Codeine / Morphine Ratio
Figure 15. Percent of administered dose of morphine and codeine free in plasma of rats 30 minutes after administration. Morphine estimated fluorimetrically and codeine estimated by GLC. Each bar represents the mean (± standard error) of three samples.
at the higher temperature which was the situation that developed. Incomplete absorption from the injection site in the 30 minutes before animals were sacrificed could account for the difference. However, loss of morphine from the blood to other tissues probably was the most significant factor. Although it cannot be stated whether morphine binding to plasma proteins was greater *in vivo* than *in vitro*, it can be reported that in either case, codeine did not affect the free or dialyzable level of morphine.

Codeine was bound to (a) BSA 59 (± 6)% and (b) plasma proteins 64 (± 5)% (Figure 14b). The same assessment of the difference between percentages bound in BSA solution and plasma must be made as done with the morphine data; the difference may have been due to loss of codeine to the red blood cell fraction upon centrifugation of the whole blood and/or due to the presence of more proteins in the plasma. Although codeine was bound to a somewhat greater extent than morphine in BSA and rat plasma, there was no indication that codeine caused the free morphine level in the two solutions to increase.

The percentage of total administered codeine dialyzable from plasma of animals given codeine alone was small (23 ± 5%). However, because the dose of codeine (30 mg/kg) was much larger than the dose of morphine (5 mg/kg), the actual amount of codeine which was dialyzable was six times the amount of dialyzable morphine.
The presence of morphine did not influence the dialyzable percentage of codeine (Figure 14b). The disappearance of much larger quantities of codeine than morphine from the blood of dosed animals is easily explained by the more rapid movement of codeine into the tissues (Yeh).

Although Beutner (1925) suggested morphine could be bound to serum proteins, and Meyer and Guttman (1968) reported morphine binding to proteins of humans and dogs, no work had been done on binding of codeine or morphine in rats, either in vivo or in vitro. The present studies apparently were the first definition of the binding characteristics of morphine and codeine in synthetic plasma, in rat plasma, and a crude estimation of binding in vivo. Further clarification of binding characteristics of the compounds in rat plasma would have required electrophoretic analysis of plasma and plasma dialysate. With such an analysis it would have been possible to determine the quantity of morphine or codeine bound to a specific protein fraction. The determination of binding in vivo was only an approximation because: (a) not all the dose was in the blood at the time of measurement, (b) the total blood level of morphine was not measured, and (c) no measurement was made of the quantity of drug which had been metabolized at the time of analysis.
The principle pathways which have been demonstrated for morphine metabolism in rats are: (a) N-demethylation of morphine to normorphine, and (b) conjugation of morphine with glucuronic acid to form the monoglucuronide. Misra, Mule' and Woods (1961) demonstrated N-demethylation of morphine in rats. Axelrod (1956) had demonstrated that microsomal enzymes in liver cells of rats could N-demethylate morphine and codeine to their respective nor-compounds. Although glucuronide formation has been demonstrated as the principle metabolic product of morphine in vivo (Woods, 1954) glucuronidation would not occur significantly in liver cell microsomal enzyme system preparations because there would not be significant glucuronide present. The principle route of metabolism followed in the current studies was the N-demethylation of morphine.

The disappearance of morphine from the incubate was very rapid. The velocity of the reaction was high ($V = 8$ micromoles/gm of tissue/hour). The Michaelis constant ($K_m$), which theoretically is an indication of the capacity of the enzyme system to metabolize a drug, was found to be very large for morphine metabolism ($K_m = 1.4$ moles/liter). The presence of codeine in the reaction mixtures in amounts from 0.6 to 100 times the quantity of morphine present, had no measurable effect on the disappearance of morphine (Figure 16). If codeine would have competitively inhibited the
Figure 16. Lineweaver-Burk plot for in vitro metabolism of morphine incubated in rat liver microsomal enzyme system. Each point represents the mean of at least 6 samples. \( V \) = micromoles/gm of tissue/hr. \( S \) = molar concentration of substrate added. \( C \) = Morphine control. \( R \) = The ratio of morphine to codeine.
\[ \frac{I}{V} \times 10^3 \]

\[ \frac{I}{S} \times 10^3 \]

- \(K_m = 1.4 \text{ moles/liter}\)
- \(V = 8 \text{ uM/gm/hr}\)
- \(R = 0.01\)
metabolism of morphine, the $K_m$ of morphine would have increased, and the slope of the Lineweaver-Burk plot (Figure 16) would have increased toward the ordinate, but the maximum velocity (reciprocal of the ordinate intercept) would have remained the same. If the inhibition would have been noncompetitive, the $V_{max}$ would have decreased (which would have meant an increase in the ordinate intercept), but no change in the $K_m$ would have been seen. Since no change was produced, it was concluded that codeine did not interfere with morphine disappearance.

The metabolism of codeine could have followed several pathways: (a) it could have been N-demethylated to form norcodeine, (b) it could have been O-dealkylated to form morphine, or (c) it could have been conjugated. However, conjugated codeine has not been measured as a significant pathway in vivo, and no reference could be found for the measurement of conjugated codeine in vitro. Both the N-demethylation and O-dealkylation pathways have been demonstrated (Yeh, 1969), with O-dealkylation the most significant pathway. The disappearance of codeine from the incubate could have meant metabolism by both pathways.

The disappearance of codeine from the incubation mixture was more rapid than the disappearance of morphine ($V=2.86$ micromoles/gm of tissue/hour; $K_m=0.37$ moles/liter). The presence of morphine did not alter the metabolism of codeine (Figure 17). If
Figure 17. Lineweaver-Burk plot for in vitro metabolism of codeine incubated in rat liver microsomal enzyme system. Each point represents the mean of at least 3 samples. \( V = \) micromoles/gm of tissue/hr. \( S = \) molar concentration of substrate added. \( C = \) codeine control. \( R = \) Ratio of codeine to morphine.
$I/V \times 10^3$

$I/S \times 10^3$

$K_m = 2.86 \text{ moles/liter}$

$V = 0.37 \text{ uM/gm/hr}$
codeine had been O-dealkylated, morphine would have been the product. However, no free morphine could be detected when the samples containing codeine alone were analyzed for morphine.

The high metabolic rates for morphine and codeine are difficult to explain. Perhaps the metabolism of the compounds proceeded more efficiently in the incubation mixtures since the enzyme preparation was relatively free of cellular proteins. Alternatively, the substrate may have been bound to a large extent by fragmented cellular proteins remaining in the incubation mixture. Since disappearance of substrate was measured, rather than the usual method of measuring appearance of product, the binding of large amounts of substrate could account for the rapid disappearance of substrate. The bound substrate could have escaped detection in the analysis.

The basis for the metabolic studies had been the hypothesis of potentiated analgesia by inhibited hepatic metabolism (Cook, Navis, and Fellows, 1954). In that study the analgesia of morphine, codeine and meperidine was potentiated by pretreatment of rats with SKF-525A, a compound known to inhibit hepatic microsomal metabolism of drugs. In the present study, hotplate reaction times to an analgesic dose of morphine were potentiated in rats pretreated with SKF-525A (Figure 18). The same dose of SKF-525A (50 mg/kg) caused no increase in hotplate reaction time compared to animals
Figure 18. Influence of a nonanalgesic dose of SKF-525A on morphine analgesia in male rats tested by the hotplate method. Drugs were given by subcutaneous injection. Each point represents the mean (± standard error) of at least 15 animals. SKF-525A 50 mg/kg is not statistically different (P > 0.05) from saline controls. Morphine 0.3 mg/kg plus SKF-525A is not statistically different (P > 0.05) from saline controls at any time. Morphine 5 mg/kg plus SKF-525A is statistically different (P > 0.05) from morphine 5 mg/kg only at 30 minutes.
Morphine 0.3 & SKF 525A
Morphine 0.3 mg/kg
Morphine 5 mg/kg
Control

Minutes After Injection

Reaction Time (sec)
given 0.9% saline (Figure 19a), but it did prolong pentobarbital sleep time 83% when given 45 minutes prior to the administration of pentobarbital 30 mg/kg (Figure 19b). The prolonged sleep time was an indication of inhibited hepatic metabolism of pentobarbital.

The results from in vitro metabolism of morphine and codeine gave no indication that inhibition of metabolism of morphine or codeine which could have provided sufficient free compound to account for the increased analgesia noted in the hotplate studies. Since potentiation of hotplate reaction time was demonstrated in rats pretreated with a compound known to inhibit hepatic microsomal metabolism (SKF-525A), it appeared that a difference between the in vivo and in vitro metabolism of the compounds existed. However, the potentiation of analgesia by SKF-525A did not imply that the mechanism of potentiated analgesia with morphine-codeine combinations occurred by the same mechanism. In addition, the failure to show inhibition of metabolism in vitro does not eliminate the chance of inhibition occurring in vivo.

Increased hotplate reaction times were not demonstrated in mice given morphine-codeine combinations. Since the increased reaction times were demonstrated in rats, there was some foundation to the speculation of Johannesson and Woods that the increased analgesia was a phenomenon peculiar to the rat. However, more genera will have to be tested before it can be stated that the potentiation
Figure 19a. Analgesic effects of SKF-525A in male rats tested by the hotplate method. SKF-525A given intraperitoneally in dose of 50 mg/kg.

Figure 19b. Influence of pretreatment of rats with SKF-525A on pentobarbital sleep time. Pentobarbital administered intraperitoneally in dose of 35 mg/kg. SKF-525A given intraperitoneally in dose of 50 mg/kg.
Sleep Time (min)

- PENT.
- PENT. & SKF 525A

n = 11

Reaction Time (sec)

- SKF 525A
- Control

MINUTES AFTER INJECTION
occurs only in rats. Since use of the tailflick technique provided results similar to those found with the hotplate, the interaction was not related to the method of measuring reaction time. Female rats demonstrated the potentiation phenomenon which eliminated a sex difference in rats as a feature of the interaction. The Holtzmann rats used by Johannesson and Woods showed a slower onset and longer duration of the increased reaction times than the Simonsen rats used in the current study. This suggested a strain difference among rats. However, the drugs were not injected in the same area by Johannesson as in the present study. Therefore, a possible strain difference was not definite. Administration of both analgesic and nonanalgesic doses of the same drug did not produce increased reaction times. Therefore, the administration of both drugs was necessary for the interaction. Since the increase in reaction time of an analgesic dose of one drug and the nonanalgesic dose of the other drug was greater than reaction times produced by injection of both doses of the same drug, the interaction could be termed a potentiation.

Both morphine and codeine were bound to one primary binding site in bovine serum albumin solution. Codeine did not interfere with the protein binding of morphine, nor did morphine interfere with the binding of codeine. The hypothesis of altered drug metabolism accounting for increased amounts of morphine reaching the
site of action in the central nervous system was not substantiated. The presence of one compound did not affect the metabolism of the other compound. Although the mechanism of the potentiated analgesia was not elucidated, the interaction was more clearly defined and some direction was given for future investigations.
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