This material gelled in most organic solvents, but was recrystallized from about 5 l. of dioxane 3 times and once from 3.5 l. of chloroform to obtain white waxy crystals, m.p. 120–122°. Other close analogs showed the same tendency to gel in most organic solvents at conventional concentrations.

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Some Aspects of the Fate and Relationship of the N-Methyl Group of Morphine to its Pharmacological Activity[1a]

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The chemical synthesis of N-trideuteriomethylnormorphine is described. Pharmacologically it is less potent than morphine although its duration of action is unaffected. Compared with morphine its N-demethylation by rat liver microsomal enzymes occurs less readily and requires a larger energy of activation. A larger K_m indicates that it is less strongly bound to the enzyme. The V_m's of rat liver N-demethylating enzymes were found to be inversely proportional to the biological potencies of the drugs, whereas the K_m increases as the potency increases. Diminished capacity of the enzymes of tolerant rats to N-demethylate morphine was shown to be due to reduced availability of enzymes rather than alteration in enzyme structure. Both the *l*- and *d*-isomers of analgesics apparently are demethylated by the same microsomal enzymes. The enzymes from male and female rats are probably identical. By two separate methods it was shown that nalorphine inhibited the N-demethylation of morphine noncompetitively. The results obtained with rat liver microsomal enzymes fail to support either Beckett's theory on the mechanism of analgesia or Axelrod's theory on the development of tolerance to some of the actions of these drugs. It is concluded that these enzymes are not suitable as models by which these theories may be judged.

The mechanism by which narcotic agents produce analgesia is not known nor has any satisfactory mechanistic explanation been presented for the development of tolerance to some of their actions. Recently, two theories have been advanced which have implied that N-dealkylation of these agents is essential to these phenomena.

Beckett, *et al.*,[2] believe that not the mere presence of these drugs at the receptors in the brain, but the subsequent N-demethylation which occurs there constitutes the first step in the reaction sequence that leads to analgesia. Nalorphine is thought to antagonize the actions of narcotic analgesics by virtue of a greater affinity for the receptor sites plus a much slower N-dealkylation therein.

Axelrod was prompted to study the effects of tolerance to narcotic analgesics on the capacities of liver microsomal enzymes from rats to demethylate these drugs by the finding that the enzymes are capable of N-dealkylating various drugs.[3,4] He found considerable reduction in the capacities of the livers from tolerant rats to N-demethylate morphine and other analgesics which exhibit cross tolerance to morphine.[5] When nalorphine was administered with morphine, the diminution in enzymic activity of the liver was significantly less than when the tolerant rats received morphine alone. Axelrod suggested that the continual interaction of these agents with the enzymes that N-dealkylate them inactivates the enzymes. Similarly he inferred that the continual interaction of these drugs with their receptors in the central nervous system may inactivate the receptors. In other words, if the liver microsomal enzymes are used as models for the receptors in the brain it follows that tolerance may occur as a result of unavailability of receptor sites.

Substitution of N-trideuteriomethylmorphine for morphine in *in vivo* and *in vitro* studies should provide a direct test of the theory of Beckett, *et al.*,[6] on the nature of binding to the central nervous system receptors as well as on the mechanism of narcotic analgesia. The two compounds differ with respect to the zero-point energies of the C–H and C–D bonds as well as the masses of the two methyl groups, but should combine with identical receptors both in the central nervous system and the N-demethylating enzymes. Since the theory is based on an inter-related consideration of stereochemical configuration, physicochemical properties, and the enzymatic N-dealkylation, comparisons of the *in vivo* potencies, the rates of enzymatic N-demethylation, the energies of activation for N-demethylation, and the Michaelis constants of the N-demethylating enzymes for these substrates, have been made to obtain information for judging the theory. Since the degree of ionization of the basic groups is

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believed to be important in drug-receptor combination, the effect of pK on potency will also be considered.

Certain discrepancies between predictions from the theories and the experimental facts constitute a pertinent basis for objection to the postulated relationship. For instance, a lack of parallelism between potencies of the narcotic analgesics *in vivo* and their rates of N-dealkylation *in vitro* has been pointed out by Way and Adler.[7] Determination of the relationship between the Michaelis constants and the maximum velocities of the enzymes with respect to various narcotic analgesics and their potencies *in vivo* should help clarify this apparent paradox. It has also been reported that unlike humans[8] rats exhibit a sex difference in the ability of their livers to N-dealkylate drugs,[9,10] enzymes from females being less efficient in this respect, although so far as is known, these drugs are of equal potency in both sexes.[11] This objection, however, is partly based on the assumption that these drugs are N-dealkylated by the same enzymes and that the enzymes of both male and female rats are identical. Since this may not be so, the identity of the enzymes should be established either by purification or indirectly. Purification appears to be too difficult to accomplish by present methods but we feel that the "mixed substrate" method of Sturge and Whitaker[12] constitutes an adequate indirect method, and have therefore used it in this study.

It has also been utilized in establishing whether or not narcotic analgesics and their optical isomers which do not have analgesic properties are N-dealkylated by the same liver microsomal enzymes. Optical isomers may be acted upon by the same enzyme in which case the reactions are usually catalyzed at different rates.[13] Since the receptors at the site of action of these drugs are highly specific toward the optical isomers, the behavior of the enzymes toward the isomer which lacks analgesic properties will figure in the evaluation of both Beckett's theory of analgesia and Axelrod's theory on the development of tolerance since only the isomers with the ability to produce analgesia are known to lead to the development of tolerance.

The concept of "fatiguing" or "inactivation" of receptors in the central nervous system and/or the N-dealkylating enzymes during the development of tolerance has also been evaluated. This has been done by comparing the Michaelis constants of liver microsomal enzymes from control and tolerant rats with respect to a given narcotic substrate since any changes in the properties of the enzymes should be reflected in the magnitude of this constant. Furthermore, from the maximum velocities (V_m) of the two reactions the amounts of active enzyme have been compared, since at the maximum velocities the enzymes are saturated and therefore the V_m's are proportional to the amount of active enzyme present. This method should provide more information than a simple comparative assay of the N-dealkylating capacities of the liver microsomal enzymes from normal and tolerant rats, since such an assay would fail to detect any changes in the properties of the enzymes from the tolerant rats.

Finally, narcotic antagonists are generally believed to act by competition with narcotics for the same receptor sites in the central nervous system where they might have weaker biological effects than their agonists[14,15] or undergo the reaction sequence that leads to analgesia more slowly.[2] In addition, Axelrod[5] reported that nalorphine *in vivo* "protects" the N-dealkylating enzymes against inactivation by continual interaction with their substrates. Although these considerations point to the competitive type of inhibition, the only evidence pertaining to the nature of the inhibition of N-dealkylation of narcotic analgesics by their *in vivo* antagonist, nalorphine, indicated that this inhibition occurred noncompetitively.[16] Since it is obviously important to establish the type of inhibition involved, we have examined the problem by means of two separate methods[17,18] which are less susceptible to errors of graph plotting than the more conventional Lineweaver–Burke method.[19]

Materials and Methods

Synthesis. O^3,N-Dicarbethoxynormorphine.—A suspension of 132 mg. (0.45 mmole) of normorphine hydrate, 1 ml. (10.5 mmoles) of ethyl chloroformate, 2 g. (36 mmoles) of potassium hydroxide in 15 ml. of water, and 15 ml. of chloroform was shaken vigorously for 10 min. The chloroform layer was removed, the aqueous phase was extracted with 15 ml. of chloroform, and the combined chloroform extracts were washed with N hydrochloric acid and then with water. Evaporation of the dried ($MgSO_4$) chloroform solution left 141 mg. (75%) of essentially pure O^3,N-dicarboethoxynormorphine. Crystallization from methanol gave 101 mg. of material melting at 150–151°; infrared absorption: $\lambda_{max.}^{CHCl_3}$ 2.75 μ (m), 5.67 (s), 587 (s), 6.15 (s); ultraviolet absorption: $\lambda_{max.}^{EtOH}$ 281 mμ (ϵ 2200), gradually shifting (complete after 25 min.) on addition of alkali to 298 mμ (ϵ 3240).

Anal. Calcd. for $C_{22}H_{25}NO_7$: C, 63.6; H, 6.1; N, 3.4. Found: C, 63.6; H, 6.0; N, 3.5.

The O^6-acetyl derivative was prepared by boiling a mixture of O^3,N-dicarbethoxynormorphine and sodium acetate in acetic anhydride for 1 hr. The reaction mixture was distributed between water and chloroform, the chloroform was washed with sodium bicarbonate solution, and the residue left after evaporation of the chloroform was chromatographed on alumina. Elution with benzene, crystallization from petroleum ether–benzene, and sublimation at 130° (20 μ) gave **O^6-acetyl-O^3,N-dicarbethoxynormorphine,** m.p. 111–112°; infrared absorption: $\lambda_{max.}^{CHCl_3}$ 5.61 μ(s), 5.72 (s), 5.90 (s), 6.15 (s).

Anal. Calcd. for $C_{23}H_{27}NO_8$: C, 63.0; H, 6.0. Found: C, 63.0; H, 6.0.

Morphine-N-CD$_3$.—To a solution of 3.48 g. of O^3,N-dicarbethoxynormorphine in 50 ml. of tetrahydrofuran (distilled from lithium aluminum hydride) was added dropwise and with stirring and cooling 77 ml. of 0.64 M lithium aluminum deuteride in tetrahydrofuran. After being stirred at 0° for 1 hr., the solution was heated at reflux for 2 hr. and then treated in the cold with 250 ml. of 2 N hydrochloric acid and 60 g. of sodium potassium tartate. This mixture was boiled for 4 hr., the pH was adjusted to 8.3 with aqueous potassium hydroxide, and the mixture was extracted continuously with methylene chloride for 18 hr. Evap-

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oration of the methylene chloride, solution of the residue in 0.1 *N* hydrochloric acid, and careful addition of ammonium hydroxide gave 1.85 g. (73%) of morphine-N-CD_3 hydrate. Sublimation at 190° (0.1 mm.) gave anhydrous material, identical in m.p. (252–254°) and ultraviolet absorption with morphine.

The anhydrous **hydrochloride** was precipitated crystalline in practically quantitative yield by passing dry hydrogen chloride into an absolute ethanolic solution of morphine-N-CD_3.

Anal. Calcd. for $C_{17}H_{16}D_3NO_3 \cdot HCl$: C, 62.9; H + D, 7.1; atom percent excess D, 15. Found: C, 62.8; H + D, 7.1; atom percent excess D, 14.5.

Morphine-N-CD_3 also was prepared in 33% yield by O^3-ether cleavage with pyridine hydrochloride[20] of codeine-N-CD_3. The **codeine-N-CD_3** was prepared by alkylation of norcodeine[21] with methyl-d_3-iodide[22] in the presence of sodium bicarbonate and melted at 156–157° after sublimation at 110° (0.1 mm.).

Anal. Calcd. for $C_{18}H_{18}D_3NO_3$: C, 71.6; H + D, 8.0; N, 4.7; atom % excess D, 14.3. Found: C, 71.6; H + D, 7.8; N, 4.7; atom % excess D, 14.2.

Determination of p*K*a's.—The p*K*a's of morphine and codeine and their N-CD_3 derivatives were determined by potentiometric titration (in a CO_2-free atmosphere) of approximately 0.001 *M* solutions of the respective compounds. Solutions were prepared in a slight excess of hydrochloric acid, and these were titrated with dilute sodium hydroxide. For calculation of the morphine constants, the equation of Ricci[23] was used. In each case, calculations were made over the range 20–80% neutralized and they were essentially constant. The values determined were: morphine 8.05; morphine-N-CD_3, 8.17; codeine, 8.06; codeine-N-CD_3, 8.19.

Estimation of Acute ED_{50} and LD_{50}.—The animals used were male and female Swiss strain albino mice weighing between 13 and 35 g. Morphine sulfate was the pentahydrate (Mallinckrodt). Deuteriomorphine, containing >99% deuterium in the N-methyl group, was converted to the salt by addition of equimolar amounts of hydrochloric acid. Doses were expressed in terms of the free bases. The drugs were administered by 3 different routes. Intracerebral injections were made according to the method of Haley and McCormick.[24] The tail flick response to a thermal stimulus (analgesia) was determined by the method of D'Amour and Smith. The ED_{50} (analgesia) and the LD_{50}, and their confidence limits were estimated by the method of Litchfield and Wilcoxon.[25] From 15 to 25 mice per dose level per compound were used in studying analgesia and toxicity.

Duration of action was estimated by injecting 2 groups of 15 mice each with the estimated ED_{95} doses of each drug. Analgesia was tested every 15 min. and continued until all the animals exhibited reaction times equal to or less than the mean reaction time of the untreated groups.

Enzyme Preparation.—Livers from male Long-Evans rats (125–250 g.) were used throughout except in those experiments specifically designed to test the enzyme activity of livers of female rats. The animals were killed by decapitation. The liver was immediately removed and placed in a tared beaker containing a known volume of 0.1 *M* phosphate buffer (pH 7.4) which had been previously refrigerated until some ice crystals had formed. To accelerate cooling, the liver was cut into small chunks with scissors while keeping it submerged in the buffer. The heat transfer from the liver was sufficient to melt the ice thus preventing appreciable rise in temperature. Sufficient ice-cold buffer was added to make a mixture of 4 vols. of buffer to 1 g. of liver. The tissue was homogenized in a Teflon–Pyrex homogenizer which was kept immersed in a dilute alcohol-ice bath during the operation. Before centrifugation, the temperature of the homogenate was brought down to about −1° by stirring in the beaker immersed in the ice-alcohol bath. Centrifugation was carried out in a Spinco centrifuge at a speed of 12,000 r.p.m., using a rotor head which was kept at 3° when not in use. The supernatant containing the enzyme was removed by pipetting, and kept in an ice-salt bath at 0° or lower during the experiment. This enzyme preparations was used only on the day it was prepared. The above precautions were necessary to keep tissue blanks at a nearly constant minimum. If the temperature was allowed to remain slightly above 3° for any length of time, tissue blank values could reach such magnitude as to completely mask the results of enzymatic activity, giving negative results especially at low substrate concentrations. The term enzyme and enzyme preparation will be used synonymously in this paper.

Incubation and Sampling Procedure.—The incubation medium of Axelrod[26] and Takemori and Mannering[27] was used without any major modification. Incubation took place in air at 38° in 25 or 50 ml. Griffin beakers, in a Dubnoff metabolic incubator shaking at approximately 100 oscillations/min. Before addition of enzyme and substrate, the mixture was shaken for 5 min. to bring the temperature to 38°. The enzyme (2.5 ml.) was added and again 5 min. was allowed for temperature equilibrium before addition of the appropriate substrate. For studies of inhibition of N-demethylation by nalorphine, and for the mixed substrate studies, the two substrates or substrate and inhibitor were premixed and added at the appropriate time. Control runs were made simultaneously.

At appropriate time intervals, 2 ml. of the mixture was removed by pipetting and added to 4 ml. of 30% trichloracetic acid in a flask attached to a micro-Kjeldahl distillation apparatus.[28] The tip of the condenser was immersed in the ice-cold chromotropic acid reagent of MacFadyen[29] in a graduated test tube. The composition of the reagent was such that after addition of 4 ml. of distillate to a volume of 16 ml. the optimum concentrations of sulfuric and chromotropic acids were attained. Standard curves were prepared with known amounts of commercial formaldehyde which was assayed for actual content by the sodium sulfite method.[30] Results were corrected for a recovery of 95%. In the determination of the energy of activation of the demethylation, the temperature of the incubation medium was measured with a thermistor.

Determination of K_m and V_m.—The experimental data were fitted to the equation

$$\frac{S}{v} = \frac{1}{V_m} S + \frac{K_m}{V_m}$$

which is a regression equation of S/v on S. In this equation v is the velocity (*i.e.*, the amount of formaldehyde formed in 15 min.) when the substrate concentration is S, V_m is the maximum velocity, K_m is the Michaelis constant. The equation of the best fitting line was statistically determined. By this method, any inaccuracy of bias that may be involved in the drawing of the curve was eliminated.

Results

Comparison of the ED_{50}'s and the LD_{50}'s of Morphine and Morphine-N-CD_3.—The estimated ED_{50}'s of morphine ($MNCH_3$) and morphine-N-CD_3 ($MNCD_3$) administered by various routes to Swiss strain albino mice are shown in Table I. It is apparent from the data that $MNCD_3$ is significantly less potent than $MNCH_3$ in all categories tested. Intracerebral and intravenous routes were used as a check on whether or not the difference after subcutaneous injection might be due to slow absorption of the $MNCD_3$, but apparently this was not so, in fact, $MNCD_3$ was relatively less potent an analgesic than $MNCH_3$ when given intravenously as compared to subcutaneously. We have no explanation for this discrepancy. A comparison of the ratios of the subcutaneous and intravenous ED_{50} doses indicates that the gain in potency by intravenous administration is approximately 150% for $MNCH_3$ and only 30% for $MNCD_3$.

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TABLE I[a]

A COMPARISON OF THE LETHAL AND ANALGESIC EFFECTS OF MORPHINE AND DEUTERIOMORPHINE IN SWISS ALBINO MICE

Effect	Route	Dose, mg./kg. Morphine	Dose, mg./kg. Deuteriomorphine	$f_{P.R.}$[b]	Morphine/P.R. deuteriomorphine
LD_{50}	Subcutaneous	256 (208–315)	400 (318–488)	1.32	1.56 (1.20–2.06)
LD_{50}	Intracerebral	6.9 (5.8–8.2)	11.4 (9.8–13.2)	1.26	1.65 (1.31–2.08)
ED_{50}	Subcutaneous	2.6 (2.2–3.0)	4.2 (3.5–5.2)	1.31	1.64 (1.25–2.15)
ED_{50}	Intravenous	1.1 (0.9–1.3)	3.3 (2.6–4.0)	1.30	3.00 (2.3–4.9)

[a] Reprinted from *Science*, **134**, 1078 (1961), by permission. [b] For definition, see ref. 25. The value of P. R. must exceed the value of $f_{P.R.}$ for the two substances being compared to differ significantly in potency. P.R. = potency ratio. Figures in parentheses are the 95% confidence limits. At dose levels near the 16, 50, and 84% response, 20–25 mice per does level were used. At other dose levels, 10–15 mice per dose level were used.

Passage through the animals did not seem to be slowed since the onset and duration of action did not differ significantly in two groups of 15 mice simultaneously injected with the estimated ED_{95} dose of each drug. Thirteen of the $MNCH_3$ and 12 of the $MNCD_3$ treated animals showed analgesia within 15 min. after administration; all animals showed analgesia at 30 min. At the end of 90 min., only 2 from each group showed analgesia, and at the end of 120 min., all the animals had returned to their normal reaction times. Thus, two of the several actions of morphine, death of mice by central nervous system stimulation and analgesia as measured by prolongation of reaction time to a thermal stimulus have been shown to be similarly influenced by deuterium substitution on the N-methyl group. Deuteration of the N-methyl group of morphine weakens the analgesic and toxic actions of the drug. Its duration of action appears to be unaffected by the isotope substitution.

Comparison of the Rates of N-Demethylation of $MNCH_3$ and $MNCD_3$.—Since the morphine molecule has been changed only by alteration of the NCH_3 group, the pharmacological differences noted may be related to the characteristics of the NCD_3 group or to significant slowing of oxidative N-demethylation. This was tested by the measurement of formaldehyde evolved from a fortified incubate of rat liver microsomes using $MNCH_3$ and $MNCD_3$ as substrates. A plot of the amount of formaldehyde formed *vs.* time of incubation is shown in Fig. 1. Each point represents the mean of at least 4 determinations. Since the reaction was linear for only 15–20 min., a period of 15 min. was chosen for routine determination of N-demethylation. Accordingly, $MNCH_3$ was found to be demethylated about 1.4 times as fast as $MNCD_3$. Four parallel experiments involving both species of morphine were carried out to examine this premise: In each experiment, enough substrate was used to saturate the enzymes (10 micromoles). The amount of formaldehyde evolved per 15 min. was determined at 20.5, 31 and 38°. The logarithms of the means of the rates were plotted against the reciprocals of the absolute temperature (Fig. 2). The values for the activation energies were calculated by multiplying the negative slopes as read directly from the graphs by $-2.303R$ (R is the gas constant per mole). A higher energy of activation was found to be associated with the slower rate of demethylation of the deuterated compound. This was to be expected in view of the lower zero point energy of the C-D bond.

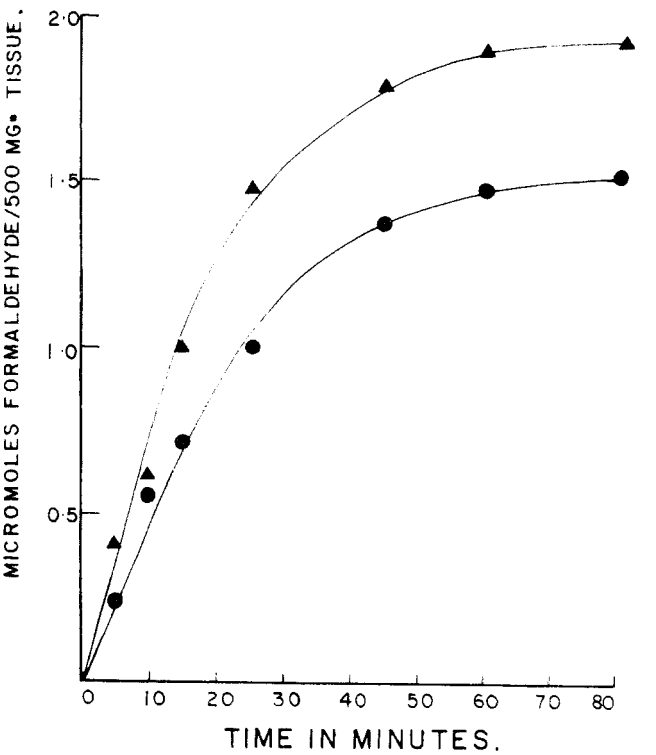

Fig. 1.—Curves showing the rates of N-demethylation of morphine and deuteriomorphine by rat liver microsomal enzymes: ▲——▲ morphine, 5 micromoles per 10 ml. of incubation mixture; ●——● deuteriomorphine, 5 micromoles per 10 ml. of incubation mixture; $k_H/k_D = 1.40$.

In an earlier paper[31] we reported a difference of about 5 kcal./mole for the activation energies. This estimated value is too large for two reactions which differ in rates by only 40%. On re-examining our data we found that when the slopes of the lines (Fig. 2) connecting the coördinates corresponding to the two higher temperatures were used in estimating the activation energies, differences ranging from 0.5 to a maximum of 1.4 kcal./mole were obtained. A typical plot is shown in Fig. 2. The left portion of the plot represents an energy of activation for demethylation of $MNCH_3$ (ΔE_H) of 7.8 kcal./mole and the right portion a ΔE_H of 16.0 kcal./mole. The corresponding ΔE values for $MNCD_3$ are 8.4 and 21.0 kcal./mole, respectively. The average ΔE for the higher temperature range is 7.4 kcal./mole for $MNCH_3$ and 8.5 kcal./mole for $MNCD_3$. For the

(31) C. Elison, H. Rapoport, R. Laursen, and H. W. Elliott, *Science*, **134**, 1078 (1961).

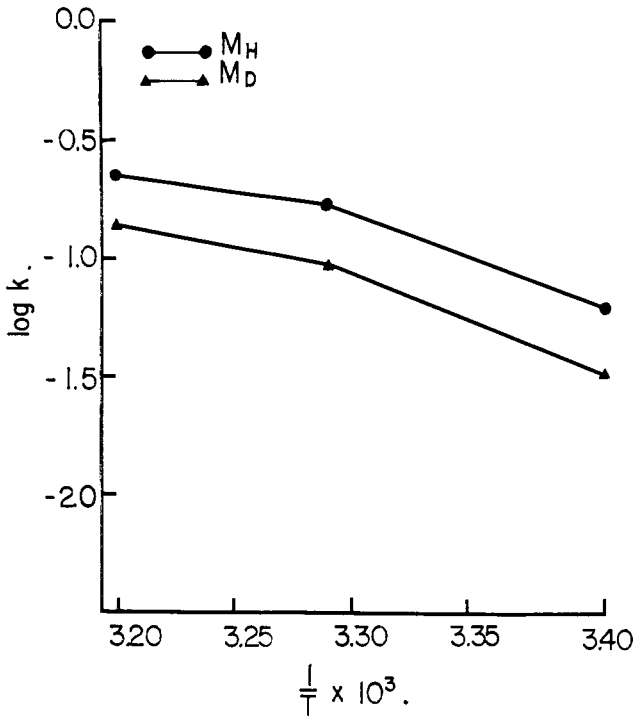

Fig. 2.—The effect of temperature on the rate of N-demethylation of morphine and deuteriomorphine: v = micromoles of formaldehyde evolved in 15 minutes; T = the absolute temperature.

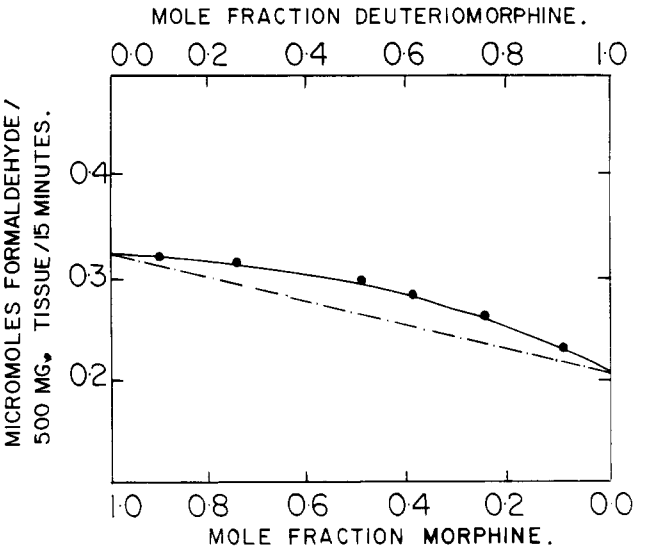

Fig. 3.—The effect of relative proportions of morphine and deuteriomorphine on the rate of formaldehyde formation by N-demethylation. The points represent the average of three experiments; —·—·—, $K_m^D/K_m^H = 1.00$; ●——●, $K_m^D/K_m^H = 1.43$.

lower temperature range these values are 15.5 and 22.5 kcal./mole, respectively. It appears that there is a sharp increase in the activation energies at the lower temperatures. Just where the downward bend begins can only be ascertained when the rates at many different temperatures are determined. Discontinuities in the Arrhenius plot are not uncommon. A number of explanations for such discontinuities have been suggested.[32]

The Michaelis Constants of the Demethylating Enzymes with Respect to $MNCH_3$ and $MNCD_3$.—The influence of the substrate concentration on formaldehyde formation was determined for $MNCH_3$ and $MNCD_3$. From the results, the Michaelis constants of the system with respect to the two substrates were calculated in the manner already described. As shown in Table II, K_m^D is significantly higher than K_m^H (P = 0.02). The ratio of the means is 1.43.

TABLE II

THE MICHAELIS CONSTANTS (K_m) OF THE MICROSOMAL N-DEMETHYLATING ENZYME OF RAT LIVER WITH RESPECT TO MORPHINE AND DEUTERIOMORPHINE

Expt. no.	$K_m \times 10^4$ Morphine	$K_m \times 10^4$ Deuteriomorphine
1	4.129	5.018
2	2.379	6.099
3	4.579	5.859
4	2.674	5.627
5	2.279	5.678
6	6.108	
7	5.520	
Sum	27.668	28.281
Mean	3.952	5.656
P	0.02	

It has been pointed out by Dixon[33] that when an enzyme which is not absolutely specific for one substrate, is incubated with two substrates (S_1 and S_2) the velocity observed will be the sum of the velocities of individual reactions (v_1 and v_2) each proceeding in the presence of the other substrate as competitive inhibitor. Thorn[32] has used this principle to show that when varying proportions of two substrates are incubated with an enzyme which acts on both of them, the plot of the total velocity against the composition will be a curve if the K_m's differ, but a straight line if they are identical. We have applied this method to the N-demethylation of $MNCH_3$ and $MNCD_3$; the results are depicted in Fig. 3. The straight line is the theoretical plot if the K_m's of the enzyme with respect to these two substrates are identical. The curved line is the actual plot obtained. This result provides further evidence for the difference in the K_m's of the enzyme with respect to $MNCH_3$ and $MNCD_3$.

From the foregoing results it is evident that deuteration of the N-methyl group of morphine leads to a reduction in the *in vivo* potency, a reduction in the rate of N-demethylation *in vitro*, an increase in the energy of activation for N-demethylation, and a distinctly weaker binding of the drug to the active center on enzyme. In addition, there is an increase in the pK_a from 8.05 for $MNCH_3$ to 8.17 for $MNCD_3$ which makes the deuterated compound a stronger base by 24%. The isotope substitution appears to be without effect on the duration of action.

The Michaelis Constant of the N-Demethylating Enzyme with Respect to Morphine and Other Narcotic Analgesics.—Varying concentrations of the drugs were incubated with the enzyme and the amount of formaldehyde evolved at the end of 15 min. was determined. The K_m's and V_m's were determined in a manner already described and the results are shown in Table III. In general it may be concluded that there is an inverse relationship between both the K_m's and the V_m's of the

(32) M. Thorn, *Biochem. J.*, **49**, 602 (1949).

(33) M. Dixon, "Enzymes," Academic Press Inc., New York, N. Y., 1958.

enzyme with respect to these substrates and the potencies of these drugs *in vivo*. However, the values of the constants for codeine which may undergo O-demethylation as well and methadone which is a tertiary amine must be regarded with some caution. Evidence to be presented elsewhere[34] will corroborate earlier suggestions[26,27] that the N- and O-demethylating enzymes are not identical. The O-demethylating enzyme catalyzes a much slower reaction as compared to the N-demethylating enzyme. As the concentration of the substrate was increased (>8 micromoles) a proportionally larger amount of it was bound by the slower enzyme. The net effect was an apparent inhibition of the reaction. For this reason, only results involving substrate concentrations of 8 micromoles or less were used in determining the K_m and the V_m with respect to codeine.

TABLE III

THE MICHAELIS CONSTANTS (K_m'S) AND THE MAXIMUM VELOCITY CONSTANTS (V_m'S) OF RAT LIVER MICROSOMAL N-DEMETHYLATING ENZYMES WITH RESPECT TO NARCOTIC ANALGESICS

Drug substrate	$K_m \times 10^3$	V_m (micromoles HCHO/15 min.)
Codeine	1.789	1.883
Meperidine	0.999	1.757
Methadone	2.564	0.814
Morphine	3.952	.589
Levorphanol	12.675	.421

With regard to methadone, the kinetics of its N-demethylation are quite different from those of the monomethylalkaloids,[35] since it has 2 methyl groups on the nitrogen.

The Michaelis Constant with Respect to Morphine and the Estimation of the Amount of Microsomal N-Demethylating Enzymes from the Livers of Normal and Tolerant Rats.—It is possible that chronic treatment of rats with narcotics induces alterations in the central nervous system receptors and in their liver microsomal demethylating enzymes so that these become less efficient in drug binding.[5] A simple comparative assay of the capacities of the enzymes from normal and tolerant rats to N-demethylate these drugs would reveal only a difference in rate and not in structure of the enzymes from the tolerant animals. Such difference in rates could be due to lower enzyme levels due to impairment of synthesis, depletion of cofactors, or emergence of factors which are inhibitory to the reaction. All of these could develop during the buildup of tolerance. Changes in the enzyme, however, should affect the binding of the substrate and therefore may be detected by comparing the K_m's. If the diminished capacity of the tolerant rats to demethylate is due to a diminution of active enzyme, the K_m should not be altered since it is independent of the concentration of the enzyme. The V_m, however, depends on the concentration of the active enzyme and since at maximum velocity, all of the enzyme is combined in the enzyme-substrate complex, the V_m may serve to determine the amount of enzyme present. The ratio of the V_m's for the same substrate will determine the amounts of active enzyme present in the two preparations. The results of appropriate experiments are shown in Tables IVA and IVB. There was no significant difference ($P > 0.10$) between the K_m's of the enzymes from the two sources. On the other hand there was a significant difference ($P = 0.02$) between the V_m's and the ratio of the active enzymes present in the two sources was 2.1.

TABLE IVA

THE MICHAELIS CONSTANTS (K_m'S) WITH RESPECT TO MORPHINE OF THE LIVER MICROSOMAL N-DEMETHYLATING ENZYMES FROM NORMAL AND TOLERANT RATS

	$K_m \times 10^3$ Normal		$K_m \times 10^3$ Tolerant
	4.129		3.467
	2.379		3.012
	4.579		3.282
	2.674		2.720
	2.279		4.860
	6.108		
	5.520		
Sum	27.668		17.431
Mean	3.952		3.468
P		>0.10	

TABLE IVB

THE MAXIMUM VELOCITY CONSTANTS OF N-DEMETHYLATION OF MORPHINE BY LIVER MICROSOMAL ENZYMES FROM NORMAL AND TOLERANT RATS. MICROMOLES HCHO/15 MIN.

	V_m Normal		V_m Tolerant
	0.329		0.152
	.396		.388
	.580		.280
	.375		.272
	.370		.322
	1.095		
Sum	4.120		1.414
Mean	0.589		0.283
P		0.02	
V_m normal/V_m tolerant		2.10	

Thus, although the enzymes were apparently identical, there was approximately twice as much enzyme present in the livers of normal as in those of tolerant rats. This disparity in amounts of enzyme present could account for the difference in the capacities of the hepatic enzymes from the two sources to N-demethylate narcotic analgesics.

Incubation Involving Mixed Substrates.—An enzyme system, which gives rise to the same products which can be quantitated when incubated with two substances in equimolar amounts, at concentrations sufficient to saturate the enzymes, may be used to determine whether the reactions are catalyzed by single or multiple enzymes. If the total velocity is additive and is higher than the velocity of the faster of the two reactions with either substrate alone, it is probable that two independent enzymes are concerned.[12,33] If the two reactions are catalyzed by the same enzyme, the total velocity will not be additive, but will be reduced by the slower reaction to some value intermediate between the two individual velocities. This approach has been used, for instance, by Sturge and Whitaker[12] to demonstrate that the horse serum cholinesterase which hydrolyzed benzoylcholine is the same enzyme as that which hydrolyzed isoamyl acetate.

The results of the above method of analysis as applied to the demethylation of narcotic analgesics are shown in Table V. A positive sign in the last column

(34) C. Elison and H. W. Elliott, to be published.
(35) A. Pohland, personal communication.

TABLE V

COMPETITION FOR THE LIVER MICROSOMAL N-DEMETHYLATING ENZYMES FROM MALE LONG–EVANS RATS BY NARCOTIC DRUGS AND THEIR ISOMERS. S = 10 MICROMOLES/10 ML.

Substrate		No. of	Means of the amount of formaldehyde evolved, micromoles/500 mg. tissue/15 min.			
a	b	expts.	a	b	c (mixed)	c–a
Meperidine	Morphine	3	1.772	0.634	1.591	−0.181
l-Methadone	Morphine	3	0.711	.634	0.801	+0.090
Morphine	Levorphanol	3	.634	.348	.544	−0.090
l-Methadone	*d*-Methadone	3	.741	.648	.704	−0.037
Levorphanol	Dextrorphanol	3	.348	.238	.295	−0.053
d-Methorphan	Morphine	3	1.355	.634	1.528	+0.173
Cocaine	Morphine	3	0.871	.634	1.105	+0.234

indicates that the velocities are additive and a negative sign, that they are not. The results suggest that all the active analgesics with the exception of methadone are demethylated by the same enzyme. The failure of methadone to conform to the general pattern may find explanation in the fact that it is probably N-demethylated in two steps. According to Pohland[35] it is demethylated to desmethylmethadone. The latter undergoes cyclization through the nitrogen and carbonyl-carbon. Thus a new N-methylated compound is formed which may be the substrate of a different N-demethylating enzyme. With *d*-methorphan the results also suggest that two different enzymes were involved. One must be the O-demethylating enzyme, since morphine, levorphanol, and *d*-levorphan appear to be demethylated by the same enzyme. It is of great interest that the non-narcotic drug cocaine is demethylated by a different enzyme. The fact that the velocities in these mixed substrate experiments were not quantitatively additive when different enzymes were involved with each substrate might be due to a competition of the enzymes for the same coenzymes or other cofactors or both.

Incubations Involving Mixed Substrates Using Liver Microsomal Enzymes from Female Rats.—It has been reported that the livers of female rats possess less capacity to demethylate drugs than those from male rats.[9,10] We have extended the mixed substrate method to determine whether the enzymes from the livers of female rats with their lower capacities to demethylate would exhibit behavior toward the various substrates parallel to that of the demethylating enzymes from males. The results given in Table VI show that such a parallelism did exist. Thus, uniformly, if either two substrates were found to be demethylated by one enzyme or two enzymes from male rats reacted with the same substrate, the same was true for females. Furthermore, in all instances the enzymes from females demethylated a given drug more slowly than those from males. This close parallelism between the substrate profiles of the enzymes from the two sources strongly suggests that the enzymes are identical. It is true that in some instances the reduction in the rate of demethylation of a given substrate is much larger than that of its cosubstrate. But since we are working with impure enzymes, absolute parallelism cannot be expected.

The lower capacity of the livers from females to N-demethylate drugs may be due to many factors, such as a smaller total amount of enzymes, scarcity of coenzymes and other cofactors not provided by the synthetic medium, the presence of competing pathways of metabolism, or differences in hormonal constitution. It should also be noted from the mixed substrate studies that the enzymes from both males and females did not demethylate optical isomers with equal ease, and that in all cases the inactive or less active *d*-isomers were demethylated less readily. These findings are not in agreement with those of Takemori and Mannering[27] who showed that both the *l*- and *d*- isomers of 3-hydroxy-N-methylmorphinan were demethylated with equal facility by mouse and rat liver microsomal enzymes. A very significant observation with respect to these isomers is the lack of any evidence that they were demethylated by different enzymes (Tables V and VI).

The Inhibition by Nalorphine of the N-demethylation of Morphine by Liver Microsomal Enzymes.—Abeles, *et al.*,[17] found that the demethylation of sarcosine was inhibited less by its structural analog, methoxyacetate, than was the demethylation of deuteriomethylsarcosine. Since methoxyacetate inhibited competitively, the results indicated that the less strongly bound deuterio compound (high K_m, low affinity) was displaced more readily from the active center of the enzyme. In other experiments (see above) we have shown that

TABLE VI

COMPETITION FOR THE LIVER MICROSOMAL N-DEMETHYLATING ENZYMES FROM FEMALE LONG–EVANS RATS BY NARCOTIC DRUGS AND THEIR ISOMERS. S = 10 MICROMOLES/10 ML.

Substrate		No. of	Means of the amount of formaldehyde evolved, micromoles/500 mg. tissue/15 min.			
a	b	expts.	a	b	c (mixed)	c–a
Meperidine	Morphine	3	0.495	0.085	0.470	−0.025
l-Methadone	Morphine	3	.331	.085	.405	+0.074
l-Methadone	*d*-Methadone	3	.331	.290	.305	−0.026
d-Methorphan	Morphine	3	.329	.085	.369	+0.040
Cocaine	Morphine	3	.260	.085	.308	+0.048
Morphine	Levorphanol	3	.085	.048	.110	−0.023

K_m^H is smaller than K_m^D. Therefore, if nalorphine inhibits N-demethylation of $MNCH_3$ competitively, it should inhibit the demethylation of $MNCD_3$ more effectively than the demethylation of $MNCH_3$.

The results of experiments in which a constant amount (1 micromole) of nalorphine as inhibitor and varying concentrations of $MNCH_3$ and $MNCD_3$ (10, 20, 30 micromoles) were used are shown in Table VII. It is clearly evident that the N-demethylation of $MNCD_3$ was not more effectively inhibited and was actually less inhibited. These results are contradictory to those expected for competitive inhibition.

TABLE VII

INHIBITION BY NALORPHINE OF THE N-DEMETHYLATION OF MORPHINE AND DEUTERIOMORPHINE: CONSTANT CONCENTRATION OF 1 MICROMOLE OF INHIBITOR

Substrate concentration, micromoles	Per cent inhibition[a]	
	Morphine	Deuteriomorphine
10	49[b]	42
20	43	35
30	45	36

[a] Per cent inhibition = (1 − inhibited rate/noninhibited rate) × 100. [b] The result of an experiment which differed from the mean by more than four standard deviations was not included.

An excellent method to differentiate between competitive and noncompetitive inhibition was developed by Hunter and Downs.[18] With it, data obtained from a number of experiments in which both substrate and inhibitor concentrations are varied can be expressed as a straight line. If for the same substrate concentration the ratio α of the inhibited and uninhibited velocities (v_i/v) are expressed in terms of the Michaelis' Menten relationships, an algebraic manipulation of the resulting expression yields the straight line equations

$$i(\alpha/i - \alpha) = K_i + K_i/K_m(S)$$

for competitive inhibition, and

$$i(\alpha/1 - \alpha) = K_i$$

for the non-competitive case. In other words, the inhibition is competitive or noncompetitive according as the resulting straight line plot is with or without slope. Experiments were carried out using six morphine concentrations ranging from 5 to 40 micromoles/ml. and nalorphine from 1 to 3 micromoles/ml. From 3 to 5 experiments were carried out with each substrate concentration both with and without inhibitor. A plot of the mean values of $i(v_i/v - v_i)$ against S is shown in Fig. 4. The resulting straight line is parallel to the S-axis indicating that the inhibition is independent of substrate concentration (see equations) as is characteristic of non-competitive inhibition.

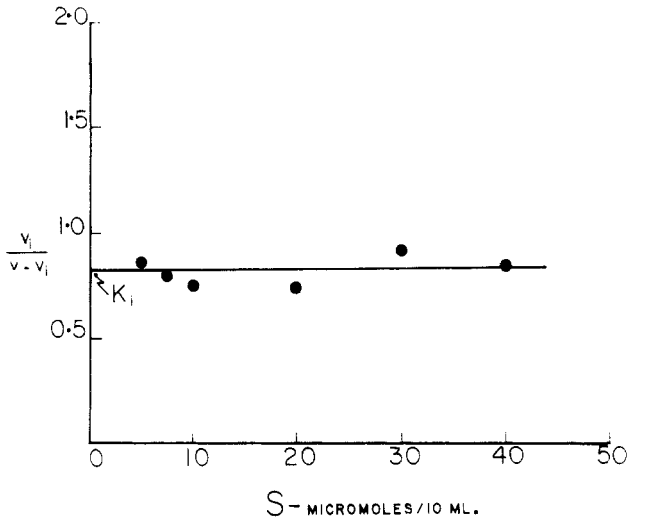

Fig. 4.—Graphical determination of the type of inhibition by nalorphine of the N-demethylation of morphine: v = velocity in the absence of the inhibitor, v_i = the velocity in the presence of the inhibitor, i = the concentration of the inhibitor, and K_i = the inhibitor constant. The zero slope of the plot indicates noncompetitive inhibition.

Discussion

The deuterated morphine was prepared in two ways. Treatment of normorphine with ethyl chloroformate gave O³,N-dicarbethoxynormorphine which then was reduced with lithium aluminum deuteride to morphine-N-CD_3. Alternatively, morphine-N-CD_3 could be prepared by cleavage of codeine-N-CD_3, itself obtained by methylation of norcodeine with methyl-d_3 iodide.

Determination of pK_a's established that the deuterated compounds are significantly stronger bases than their protium analogs. This effect also has been observed with benzylamine-α-d_2, which is reported[36] to be 13% more basic than benzylamine. With the N-methyl alkaloids, this base-strengthening effect of deuterium was even more pronounced. The pK_a of $MNCD_3$ was found to be 8.17 as compared to 8.05 for $MNCH_3$, making the deuterium analog a stronger base by 24%. The same increase (26%) was found in comparing codeine-N-CD_3 (pK_a 8.19) with codeine (pK_a 8.06). This effect is of sufficient magnitude to warrant consideration in any discussion of relative activity of deuterium *vs.* protium analogs.

As pointed out above and in a preliminary communication,[31] deuteration of the N-methyl group of morphine decreased the potency of the drug. When $MNCD_3$ was administered to mice by various routes both the ED_{50} for analgesia and the LD_{50} proved to be significantly larger than was true for $MNCH_3$. While this effect might be considered analogous to the decreased potency shown by morphine homologs in which short alkyl chains have been substituted for the N-methyl groups[37] it is very unlikely that in this case it is related to the size of the substituent since deuteration of a methyl group does not increase its size. Actually, the conformation of the $MNCD_3$ molecule is unchanged from that of $MNCH_3$ and yet the substitution of deuterium for hydrogen on a single methyl group results in a significantly reduced biological effect.

The striking parallelism which exists between the ratios of potency *in vivo* and the velocity constants for demethylation *in vitro* as well as the fact that the energy of activation for N-demethylation of $MNCD_3$ is significantly greater than that for $MNCH_3$ clearly show that deuteration affects both biological potency and rate of demethylation. The rate limiting step in N-demethylation apparently involves the breaking of a C—H bond since the isotope effect of 1.4–3.0 on the

(36) E. A. Halevi and M. Nussim, *Bull. Res. Council Israel*, **6A**, 167 (1957).

(37) A. F. Green, G. K. Ruffell, and E. Walton, *J. Pharm. Pharmacol.*, **6**, 390 (1954).

parameters tested is adequate for such a reaction.[38] Although these phenomena are similarly affected there are insufficient grounds for assuming that they are related. In fact, the lesser potency of $MNCD_3$ might just as well be due to weaker binding of the deuterated drug to receptor sites since the Michaelis constant for demethylation of $MNCD_3$ is larger than that for $MNCH_3$. This finding appears in spite of opposite predictions based on theoretical considerations, (a) the increased mass of the deuterated methyl group would produce larger van der Waals forces which might increase the affinity of the molecule for the receptor; (b) more $MNCD_3$ than $MNCH_3$ might be expected to be bound to the receptors since the former is a stronger base than the latter. As such, at the intracellular pH more of the deuterated compound would be present in the ionized state which is presumed to be the form which combines with the receptors. Thus, with the data obtained from these studies it is not possible to state whether the decreased potency of $MNCD_3$ as compared to $MNCH_3$ is related to N-demethylation or to lesser affinity for the receptor. It should be emphasized, however, that the results of related studies favor the latter possibility.

If rate of demethylation is correlated with potency of analgesics then those which are more potent than morphine should be demethylated more rapidly and *vice versa*. This is not so according to our demethylation studies. The V_m's for N-demethylation of those nondeuterated analgesics for which the results are not complicated by the presence of other methyl groups are inversely related to potency (V_m levorphanol < V_m morphine < V_m meperidine). These data corroborate the conclusions of Way and Adler who point out in their review[7] the lack of parallelism between the potency of analgesics and their rates of demethylation *in vitro*. In fact, the V_m may have no relationship at all to biologic potency. This is evident from the fact that both the more potent levorphanol and the less potent $MNCD_3$ have smaller V_m's then morphine. It cannot be said that potency may be related to affinity for the receptor, for although the K_m's are directly related to potency of nondeuterated analgesics (K_m levorphanol > K_m morphine > K_m meperidine), the reverse is true for $MNCD_3$. It should be noted, however, that findings with such closely related compounds as $MNCH_3$ and $MNCD_3$ may be more meaningful than those with compounds which have a more distant structural relationship. This consideration would attach greater meaning to the comparison of the K_m's for morphine and its deuterated analog.

The present studies indicate that enzyme preparations from the livers of female rats demethylate various analgesics and cocaine less readily than similar preparations from male rats. A comparison of the substrate profiles of the N-demethylating enzymes from the two sexes strongly suggests that the enzymes are identical but are present or available in smaller quantities in the livers of female rats. These data suggest that N-demethylation is not the cause of analgesia since the toxicity of morphine is the same in female rats as in males[11] and the ED_{50} for analgesia as measured by the tail flick response to a thermal stimulus is similar in male and female Sprague–Dawley rats (M. Abdel-Rahman, and H. W. Elliott, unpublished data).

(38) K. B. Wilberg, *Chem. Rev.*, **55**, 713 (1955).

Similarly, studies with nalorphine fail to support Beckett's postulated mechanism for the action of this compound.[2] Theoretically nalorphine, by virtue of the greater van der Waals bonding involving its allyl group should have a greater affinity for receptors than morphine. It is believed to displace both morphine and normorphine from receptor sites, but that after absorption it undergoes a much slower deallylation or substitutes its own weaker action for that of its agonist.[14,15] Experimental evidence presently available[16] indicates that the N-deallylation of nalorphine occurs more readily than the N-demethylation of morphine. In addition, results presented above and data of Axelrod and Cochin[16] show that nalorphine noncompetitively inhibits the N-demethylation of morphine by liver enzyme preparations. These findings plus those of various workers who have found that nalorphine administered by itself[39–41] is roughly equivalent to morphine in analgesic potency in man are difficult to reconcile with the ideas of competitive displacement of morphine by nalorphine from receptor sites.

The findings in regard to demethylation make it difficult to accept the theory that N-dealkylation of analgesics at the brain receptors is the first step in the mechanism of action of the analgesics.[2] It has often been asked whether it is justified to extrapolate from data obtained with liver when brain tissue is being considered. The question is a valid one. However, in view of the inadequacy of present methods to establish whether or not the brain can N-demethylate the drugs considered, the use of the liver as a model for it was adopted for expediency. It must be tacitly assumed that enzymatic regulation of a reaction is identical in these organs. Obviously, unless the corresponding reaction exists in the brain the data presented would be of less value when considered in relation to the role of drug-receptor interaction in the mechanism of action of narcotic analgesics.

We have not found a significant difference in the Michaelis constants obtained using enzyme preparations from control and morphine-tolerant rats. Since this constant is intimately dependent on the structural peculiarities of both substrate and enzyme the failure of tolerance to morphine to affect this most fundamental enzyme constant makes it extremely doubtful that the enzyme responsible for N-demethylation has been altered by the development of tolerance to morphine. Significantly, however, the difference in rates of demethylation demonstrated by enzyme preparations from the two sources indicates that the livers of morphine-tolerant rats contain smaller amounts of N-demethylating enzymes. This finding which is suggestive of decreased protein synthesis in morphine-tolerant rats is compatible with known actions of morphine. Chronic treatment of the rat with morphine results in hypertrophy of the adrenal glands.[42–45] It is conceivable that this "stress" response is related to an altera-

(39) E. R. Hart and E. L. MacCawley, *J. Pharmacol. Exptl. Therap.*, **82**, 339 (1944).
(40) L. Lasagna and H. K. Beecher, *ibid.*, **112**, 356 (1954).
(41) A. S. Keats and J. Telford, *ibid.*, **117**, 190 (1956).
(42) E. M. MacKay and E. L. MacKay, *Proc. Soc. Exp. Biol. Med.*, **24**, 129 (1926).
(43) E. M. MacKay, *J. Pharmacol. Exptl. Therap.*, **43**, 51 (1931).
(44) C. Y. Sung, E. L. Way, and K. G. Scott, *ibid.*, **107**, 12 (1953).
(45) T. Tanabe and E. T. Cafruny, *ibid.*, **122**, 148 (1958).

tion in availability or function of adrenal cortical hormones some of which have been shown by Umbreit[46] to be concerned with enzyme synthesis. Since Eisenman, *et al.*,[47] have shown that gonadal and adrenal functions are supressed during addiction it is conceivable that chronic treatment with narcotic analgesics interferes with protein synthesis either directly or through some hormonal mechanism. If true, this might explain the phenomenon of retarded growth in young rats or loss of weight in mature rats chronically treated with narcotic analgesics.[45,48,49] The lowering of enzyme levels in the tolerant animals is also compatible with inactivation during the development of tolerance.[5] Since the K_m remains unchanged it would appear that such inactivation occurs without any alteration in those characteristics of the enzyme on which affinity for the substrate is dependent.

The *dextro*rotatory isomers of levorphan and methadone are demethylated more slowly than the *levo* isomers but by the same enzyme as the one that acts on the *levo*rotatory isomers. Although the O-methylated *d*-isomer of levorphan (*d*-methorphan) appears to be demethylated by a different enzyme, the possibility that this "different" enzyme may be the one which removes the O-methyl group should not be overlooked. Our findings in regard to the *dextro* isomers are in agreement with Axelrod[26] but not with Takemori and Mannering[27] who found, using a method other than that of mixed substrates, that both isomers of 3-OH-N-methylmorphinan were demethylated with equal facility by enzymes from rat liver preparations. Even though quantitative estimates may differ, if as we have demonstrated both *levo* and *dextro* isomers are demethylated by the same enzyme system, chronic treatment of rats with either isomer should lead to a decreased ability of their liver enzymes to N-demethylate narcotic analgesics. However, according to Mannering and Takemori[50] chronic treatment of rats with dextrorphan which is largely devoid of morphine-like properties does not reduce the activity of liver enzyme preparations. These observations suggest that the depression of the N-demethylating capacity of rat liver associated with the development of tolerance to the morphine-like drugs appears to be intimately related to their narcotic-analgesic properties, but not limited to the enzymes that N-demethylate these drugs. Herken, *et al.*,[51] repeatedly observed that development of tolerance to analgesics led to a reduction in the ability of rat liver enzymes to demethylate both dimethylaminoantipyrine and cocaine which we have shown not to be demethylated by the same enzyme as the analgesics. In some instances demethylation of dimethylaminoantipyrine was depressed when demethylation of meperidine was still unaffected. These results may also be interpreted as indicating that development of tolerance to analgesics leads to enzyme deficiency.

The observed behavior of nalorphine inhibition of the N-demethylation of morphine indicates that in spite of the close structural similarity of the two compounds, they are not bound to the same active centers on the enzyme. The original observation[5] that chronic treatment with a combination of morphine and nalorphine resulted in a lesser diminution of N-demethylating ability of the liver enzymes of rats would suggest that the nalorphine "protected" the active sites of the enzymes from being "inactivated" by the chronic interaction with morphine. If the enzyme is a model for the receptors in the brain where nalorphine is believed to compete with analgesics, then the "protective" action on the enzyme must be assumed to be due to displacing morphine competitively from these sites. The fact that nalorphine inhibits N-demethylation noncompetitively does not support this assumption.

The failure of nalorphine (and levallorphan) to reverse the narcotic-induced retardation of N-demethylation[50] has been thought to be due to a masking of the "protective effect" by the effect of nalorphine itself in depressing N-demethylation.[52] If nalorphine protects the N-demethylating enzyme against inactivation by chronic treatment with morphine and on the other hand chronic treatment with it also causes depression of N-demethylation, then in accordance with the theory,[4] it must interact with the enzyme. It is not easy to explain why it does interact with the enzyme to produce these effects and yet does not inhibit N-demethylation competitively.

On the basis of the results and the observations presented above, it is difficult to accept any theory relating N-dealkylation of narcotic analgesics either to the mechanism of analgesia or to the development of tolerance to these drugs.

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(51) H. Herken, D. Neubert, and R. Tümmler, *Arch. Exp. Path. Pharmakol.*, **237**, 319 (1959).
(52) J. Cochin and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **125**, 105 (1959).