THE PHARMACOLOGIC IMPLICATIONS OF THE FATE OF MORPHINE AND ITS SURROGATES[1]

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TABLE OF CONTENTS

INTRODUCTION

Something about the subject of the opium alkaloids seems to prompt men to express themselves in eloquent, often poetic, phrases. Even the total chemical synthesis of the morphine molecule (108) and the elucidation of its stereochemistry (230, 232, 233) and structural configuration (173) have not removed the romantic trappings. Indeed, these very triumphs have inspired the eminent organic chemist, Sir Robert Robinson, to borrow from Greek and Roman mythology and refer to the morphine molecule by the name of the many-shaped sea god, *Proteus* (242).

Modern monographs on the pharmacology of the opium alkaloids usually contain an opening sentence which poignantly tells of the never-ending search by mankind for greater comfort (235). That the present authors are not averse to sentiment and poetry is evidenced not only by their awareness of man's relentless quest for surcease from pain, but also by their appreciation of the vision that the quest may be fulfilled by a certain constellation of atoms: an electrophilic carbon here (235), a tertiary nitrogen there (26, 70, 256) and beyond—no more than an ethylene chain away—a quaternary carbon (39, 256)

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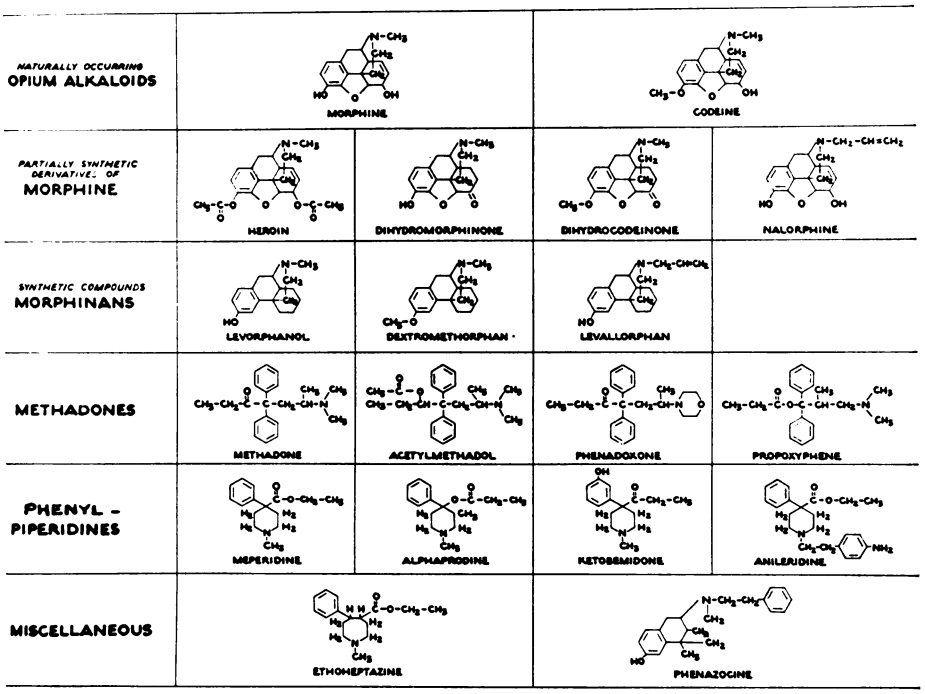

FIG. 1

nestling under a canopy of phenyl or heterocyclic residues (26, 235). It is this constellation of atoms that allowed John Locke several hundred years ago to call the actions of opium "soporific or anodyne virtues." It is this particular configuration that led Sydenham to classify opium as a remedy "which it has pleased Almighty God to give to man." And today these structural characteristics can be recognized not only in morphine but in all of its surrogates.

The term "morphine surrogate" has been selected to designate all compounds which can act in the place of morphine. Their structures are presented in Figure 1 and it can be seen that they include, in addition to certain alkaloids found in opium and the partially synthetic derivatives of morphine, many purely synthetic substances that have a morphine-mimetic action. It is to be expected that in the future many more synthetic compounds will be added to this list, and the skill of the chemist in manipulating structure to achieve a certain biological effect is indeed impressive. No less impressive is the recently accumulated knowledge of the manner in which the body itself may manipulate structure, sometimes to enhance, sometimes to diminish, the biological action of the administered compound. A consideration of the metabolism of these agents forms the major part of this paper.

The metabolism of morphine is of particular interest because important roles in producing analgesia and physical dependence have been assigned at various times to its known as well as postulated biotransformation products. Morphine

itself has been shown to be a biotransformation product of codeine as well as of heroin. Recent studies have indicated that other morphine surrogates give rise to products that may possess significant pharmacologic properties. It is of importance, therefore, to assess the factors (absorption, distribution, metabolism, excretion) that influence the accessibility of the metabolic products of these drugs to the central nervous system where presumably their effects, analgetic or toxic, are mediated; it is equally important to weigh the evidence with respect to the unequivocal identification of these metabolites.

It has been deemed beyond the scope of this review to give a detailed consideration of the absorption, distribution, metabolism, and excretion of each compound. A monograph on this subject, however, is in preparation to supplement the information in the earlier one by Krueger, Eddy and Sumwalt (166). We have included publications up to early 1960. Certain aspects of this review have also been covered by others in a more limited fashion (165, 214c, 235, 248a).

METHODS OF ESTIMATION

The major problem in the estimation of morphine and its surrogates in body fluids and tissues is usually concerned with the separation of the agent from the extraneous biological phase. The small size of the dose of compounds in this class required to elicit the pharmacological or toxicological response often has necessitated the use of large amounts of the biological material for the analysis. Consequently, the compound when finally isolated is rarely free from interfering substances and often the yield is far from quantitative.

Most of the methods for compounds in this class follow a general pattern that involves separation of the agent from its contaminants and then application of some reaction for one of its functional groups. The reliability of a procedure is therefore chiefly dependent upon the adequacy of the purification processes, which usually involve extraction, adsorption, or precipitation of the compound. The measurement is usually performed without actually isolating the compound in the crystalline form.

Recent developments in experimental technics have greatly facilitated studies with respect to the biologic disposition of the analgetic agents. Although in certain instances unique methods for a particular compound in this group have been developed, much of the information concerning the metabolism of morphine and its surrogates has been derived from two general procedures, namely, indicator-dye and tracer technics.

The indicator-dye method is based on the fact that many organic bases, when reacted with colored organic acids, form addition complexes which are soluble in organic solvents and can be measured photometrically. Of the indicators which have been employed to determine the morphine surrogates, methyl orange has been found to be most successful. This dye, proposed by Brodie and Udenfriend in their general procedure for organic bases (47, 48), was first adapted for measurement of meperidine (310, 317). Subsequently, it has been modified and used for the measurement of almost every compound in this series (Table 1). Some of the other dyes used include bromthymol blue (171, 203, 211a), bromcresol

purple (61, 268b) and bromcresol green (302). Since any amine which forms a solvent dye complex will react as the administered compound, the dye procedure must always be evaluated for specificity and, if necessary, modified to exclude the contaminants. This can often be accomplished by washing the solvent extract of the base with a suitable buffer before reacting the solvent extract with the indicator dye (310). A high degree of specificity can be conveyed to the dye method by utilizing it in conjunction with countercurrent distribution. The combination of these two procedures has been successfully utilized to estimate meperidine (220, 222, 310), codeine (10), methadone (280, 314), and heroin, and their basic metabolites (311).

As indicated in Table 1, isotopic technics have been used also to investigate a large number of compounds. Tracer technics with C^{14} labeled compounds have been extremely useful in elucidating the metabolic pathway of the morphine surrogates. Evidence that N-demethylation is an important metabolic pathway for most, if not all of the compounds in this group, was first established using N-$C^{14}H_3$ labeled meperidine (220, 222) and codeine (10, 11). Likewise, proof of O-dealkylation of codeine to morphine was greatly facilitated by use of codeine-3-O$C^{14}H_3$ (12, 13).

The use of labeled compounds has also been applied to estimate their concentrations in various body fluids. Many of the measurements, however, were confined largely to determination of radioactivity. While such data are extremely useful in providing information on the routes of excretion and, possibly, the metabolic pathways of the agent, they do not necessarily give information on concentrations of the specific compound in various tissues but generally define the maximum levels attainable. More recently, however, Adler and her collaborators (9) were able to detect concentrations of morphine as low as 0.028 μg/ml in plasma using an isotope dilution technique. Morphine N-$C^{14}H_3$ in biologic media was determined by adding carrier morphine to the sample to be analyzed. After recovery and purification of the isotopically diluted morphine N-$C^{14}H_3$ by conventional procedures, it was converted to crystalline dinitrophenyl-morphine-N-$C^{14}H_3$, and the specific activity determined. The purity of the crystalline derivative was ascertained by its powder X-ray diffraction pattern, thus making this method one of the most sensitive as well as specific for determination of morphine.

Table 1 lists the analgetic compounds studied by the dye and tracer procedures as well as by other methods. We have attempted to be inclusive only with respect to methods which have direct bearing on data concerning the biologic disposition of the compounds under discussion. For a more generalized listing the reader should consult Schaumann's handbook (248a). Some recent methods for forensic purposes have been cited. The majority of the latter methods are usually only semiquantitative but some of them appear to be sufficiently sensitive for estimating small amounts in biologic fluids.

Most of the information in Table 1 will be of specific value only to the expert in the field. The general value lies in the fact that it has served as the basis upon

TABLE 1

Methods for estimating morphine and its surrogates in biologic fluids

Compound	Method	Remarks	References
Morphine	Photometric phenolic reagents	Most widely used procedures	96, *107*, 196, *198*, *284*
	methyl orange	After conversion to ester	*266*, *327*
	sulfanilic acid	After diazotization	127, 215
	Tracer	With morphine-N-$C^{14}H_3$	1, *9*, 179, 190
	Ultraviolet	Low sensitivity	2, 3, 7, *107*, *117*
	Nephelometric	Specificity open to question	66, 67, 84, 85, 144, 198, 206, 249
	Polarographic	Sensitive with high degree of specificity	*193*, *208*
	Iodometric	Low specificity	145, 155, 290
	Gravimetric	Poor sensitivity	22, 323
Codeine	Methyl orange	Adaptable for metabolite, norcodeine	6, *10*, *11*, *170*, *331*
	Tracer	With codeine-3-$OC^{14}H_3$ or -N-$C^{14}H_3$	*4*, 7, *10*, 11, *170*, *190*
	Others	Formation of insoluble complexes	13, 199
Heroin	Methyl orange	Adaptable for metabolite, 6-acetylmorphine	*311*
	Folin-Ciocalteu	After hydrolysis to morphine	*311*
Nalorphine	Methyl orange	After conversion to phenolic ester derivative	*327*, *331*
	Tracer	Tritium labeled	*329*
	Silicomolybdic acid	Phenolic bases would interfere	261
Levorphanol, Dextrorphan, or Racemorphan	Methyl orange	Benzene excellent solvent	95, *265*
	Tracer	Estimation of N-$C^{14}H_3$ levorphanol, 0.001 mg/ml	*330*
	Others	Forensic and semiquantitative procedures	42, 43, 49, 62, 153, 156, 297, 298, 299
Dextromethorphan	Alkaloid reagents	After paper chromatographic separation, semiquantitative only	49
Levallorphan	Alkaloid reagents	After paper chromatographic separation, semiquantitative	49

The italicized references denote methods recommended because of their general usefulness and reliability. They are considered to have a reasonably high degree of specificity and sensitivity for quantitative estimation.

TABLE 1—*Continued*

Compound	Method	Remarks	References
Methadone	Methyl orange	For *dl*-, *l*-, or *d*-; also measures metabolite	*180*, *279*, *280*, *283*, *314*
	Tracer	With 2-C^{14} *dl*-methadone	8, 73, 77, 190, 291
	Others		19, 39, 43, 61, 139, 153, 154, *236*, 257, 268a, 268b, 297, 300, 302
Acetylmethadol	Methyl orange	For *l*- or *d*- isomer	*282*
Phenadoxone	Bromthymol blue		211a
Propoxyphene	Methyl orange	Metabolite interferes	*170a*
	Tracer	With N-$C^{14}H_3$ propoxyphene	*170a*
Meperidine	Methyl orange	Adaptable for metabolite, normeperidine	*50*, *220*, *222*, *310*, *317*
	Tracer	With N-$C^{14}H_3$ meperidine	220, 221
	Others	Normeperidine interferes	159, 171, 203, 297
Anileridine	N-(1-naphthyl)-ethylenediamine	Coupled after diazotization	227
Ketobemidone	Bromcresol green	Specificity unknown	42, 43, 299, 300
Ethoheptazine	Methyl orange	Specificity checked	303
	Tracer	C^{14} at position 4	303

which we have built our criteria for accepting or modifying interpretations made by various investigators in their published works.

ABSORPTION

In general absorption of morphine and its surrogates is relatively good after parenteral administration and erratic after oral medication. Pharmacologic or chemical effects can usually be noted within a few minutes after hypodermic injection but peak effects may not occur until after 1 hour. Studies on animals also indicate a rapid rate of disappearance of the compound from the site of administration. The quantitative studies have been summarized in Table 2. With some compounds there is relatively little quantitative information concerning their absorption; in such cases qualitative data describing onset of pharmacologic effects have been included although there has been no attempt to be comprehensive in this matter.

Absorption of morphine and its surrogates is generally thought to occur by

diffusion rather than by processes involving energy expenditure. That passage of these agents from the site of administration to the blood stream is governed mainly by physical processes is indicated by the fact that distribution between the gastric juice and plasma of certain alkaloids after oral medication is predictable from the dissociation constant and the lipid solubility of their undissociated forms (46, 294, 295, 304). The reason for less absorption by oral administration than by hypodermic injection can be explained largely on a physical basis. It is recognized that organic bases generally penetrate cells as the undissociated molecule, which is the more lipid-soluble form. Acidic conditions such as found in the upper intestinal tract would ionize the compounds and as a result depress their absorption. As Albert has pointed out, not only are ions increased in size due to hydration but, also, owing to their charge they would tend either to be repelled or adsorbed by a lipoprotein membrane (13a).

Factors other than physical processes which might affect absorption also should be borne in mind as evidenced by findings with respect to cortisone facilitation of the rate of absorption and excretion of methadone (78), and the accelerating effect of blocking histaminic action or release on morphine absorption (194).

The studies on the absorption of morphine and its surrogates bring out certain points which are often overlooked. When the rate of disappearance of the drug is used as the criterion for absorption rate, its absorption rate can be slower than is indicated by its rate of disappearance if biotransformation of the drug occurs at the administration site, as with heroin (311). The absorption rate after oral administration of the agent can be more rapid than is indicated by its measured rate of disappearance from the gastroenteric tract when secretion of the agent into the stomach occurs, as is true for acetylmethadol (282). The phenomenon of secretion into the gastroenteric tract is to be expected for organic bases (46) in general. Using onset of pharmacologic effects as a criterion for the rate of absorption of a compound can sometimes be misleading, as indicated by a consideration of the optical isomers of *alpha*-acetylmethadols. The levo-isomer has a delayed onset and a long duration of action while the dextro-isomer has a relatively rapid onset and a considerably shorter duration of effect (72, 98, 160). Despite this difference, the rates of disappearance of the individual isomers from their injection sites and their rates of appearance in various tissues were approximately the same, indicating that the rates and degrees of absorption for all isomers were comparable (282).

DISTRIBUTION

The very nature of the pharmacologic effects elicited by morphine or its surrogates indicates a highly selective action at specific receptor sites, but, nonetheless, these compounds being organic bases share many features of biologic disposition which are characteristic for basic compounds. Most organic bases after being absorbed rapidly leave the blood and localize in high concentrations in parenchymatous or reticuloendothelial tissues (308a). Organs such as the lungs, spleen, liver and kidneys generally exhibit within a few minutes after

TABLE 2

Absorption of morphine and its surrogates

Compound and Route	Species	Criterion for Absorption	Results	References
MORPHINE				
subcutaneous	monkey	rate of appearance in plasma	peak levels between 90–120′	187
	dog	rate of appearance in plasma	peak levels at 45′	55
	rabbit	rate of appearance in blood	peak levels at 30′	96
	rat	radioactivity in CNS after morphine-N-$C^{14}H_3$	peak levels at 60′, some present at 15′	190
		disappearance from injection site	over 90% within 60′	179
		disappearance from injection site	84% within 4 hr	324
	guinea pig	tissue levels	detected at 15′	162
	mouse	disappearance from injection site	50% at 15′, 90% at 60′	318
intramuscular	man	$C^{14}O_2$ in breath after morphine-N-$C^{14}H_3$	peak rate between 30–90′	79
	dog	disappearance from injection site	67% at 30′	135
		appearance in plasma	peak levels within 45′	55
	rabbit	appearance in blood	high levels within 35′	196
oral	man	pharmacologic effects	essentially negative	29, 56, 148
	dog	appearance in plasma	levels barely detectable	55
intraperitoneal	rabbit	appearance in blood	peak levels at 60′	266
	mouse	appearance in blood	detected at 15′	3
intrapopliteal	rat	disappearance from injection site	over 95% by 60′	9
sublingual	dog	pharmacologic effects	negligible	305, 306
CODEINE				
subcutaneous	monkey	appearance in plasma	peak levels at 60′	332
	dog	appearance in plasma	peak levels at 45′	332

	rat	disappearance from injection site	73% by 60′; 85% by 120′	170
		appearance in tissue	appreciable levels in CNS at 15′, peak at 60′	76, 170, 190
intramuscular	man	appearance of $C^{14}O_2$ in breath after radioactive codeine	detected at 15′	10
sublingual	dog	pharmacologic effects	27 to 54 times the subcutaneous dose for comparable effects	306
HEROIN				
subcutaneous	rat	appearance of metabolic product 6-monoacetylmorphine in tissue	peak at 15′	318
	mouse	disappearance from injection site	over 90% at 15′	311
		appearance of metabolites in tissue	peak at 15′	311
	other	onset of pharmacologic effects	marked within 2′ in dog, cat, horse, donkey, goat, rabbit, guinea pig	105, 128
oral	other	onset of pharmacologic effects	within 20–30′ but results more irregular than with subcutaneous administration	128
sublingual	man	onset of pharmacologic effects	noted in 1 of 3 trials	304, 306
	dog	onset of pharmacologic effects	noted within 4–7′	105
NALORPHINE				
subcutaneous	man	onset of effects	within a few minutes	320
	dog	appearance in plasma	maximum levels at 15–60′	329, 331
	rat	disappearance from injection site	over 95% within 4 hr	331
	mouse	onset of effects	death within 15–90′	296

TABLE 2—*Continued*

Compound and Route	Species	Criterion for Absorption	Results	References
LEVORPHANOL—L DEXTRORPHAN—D RACEMORPHAN—R				
subcutaneous	man	onset of effects of L and R	marked effects between 1–4 hr	148
	monkey	appearance in plasma of L	peak levels at 30′	330
	dog	appearance of L in plasma or tissue	peak levels at 30–45′	265, 330
oral	man	onset of effects of L and R	marked effects between 2–6 hr	148
	rat	disappearance from gut of R	over 50% in 30′	95
intraperitoneal	dog	appearance in plasma of L and D	peak levels at 30′	265
LEVALLORPHAN				
subcutaneous	dog	appearance in blood	noted at 60′	49
METHADONE R = racemate L = *levo* isomer D = *dextro* isomer				
subcutaneous	rat	disappearance of R from injection site	over 53% after 1 hr, over 80% at 2–3 hr	77, 236
		appearance of R in plasma	within 10′	8
		appearance of R in bile	within 10′	73
		appearance of R in tissue	peak levels at 30′	77, 190, 236
oral	rat	disappearance of R from GI tract	70% within 120′	317
intraperitoneal	rat	appearance of R in tissue	peak levels at 60′	317
		appearance of R in tissue	2 hr levels higher than at 4 hr	283
		appearance in tissue of L and of D	2 hr levels higher than at 4 hr	282

ACETYLMETHADOL				
subcutaneous	man	euphoria	delayed until 4–6 hr, lasted 72 hr	98
		analgesia	within 90′ but delayed toxic effect at 12–30 hr	160
	rat	disappearance from injection site of *l*- and *d*-isomers	over 70% within 60′ for both isomers	282
oral	man	onset of effects of *l*-isomer	noted at 1 hr and lasted 48 hr	98
	rat	disappearance from stomach of *l*- and *d*-isomers	20% of both isomers at 60′, *d*-—98% at 13 hr; *l*-—50% at 24 hr	282
PROPOXYPHENE	rat	onset of analgesia	peak effect at 60–90′	240
MEPERIDINE				
subcutaneous	rat	disappearance from injection site	88% at 12 hr	220
oral	man	appearance in plasma	peak between 1 and 2 hr	50
	rat	disappearance from GI tract	80% at 60′, 90% at 4 hr	310
intramuscular	man	appearance in saliva	detectable amounts at 60′ and 120′	310
intraperitoneal	rat	appearance in tissue	2 hr levels higher than at 3 hr	310
	mouse	disappearance from total animal	85% within 60′	50
ANILERIDINE				
subcutaneous	dog	onset of effects	less than 60′	207
	rat	appearance in tissue	peak levels at 60′	227
oral	dog	onset of effects	less than 60′	207
	rat	"analgesia"	maximal at 20–30′	207
ETHOHEPTAZINE				
intraperitoneal	dog	appearance in plasma	peak levels within 60′	303
	rat	appearance in organs	30′ levels higher than at 90′	303

absorption drug concentrations considerably in excess of those present in the blood. Brain, skeletal muscle, and other tissue have the ability to concentrate organic bases but to a much lesser degree than the aforementioned organs. This distribution pattern has been found for organic bases such as the antihistamines (63, 115, 309), emetine (112), and antimalarials (51, 65, 136, 263), and in this respect morphine and its surrogates are quite similar.

The ability of basic substances to concentrate in tissue would appear to explain in part the high pharmacologic potency inherent in most alkaloidal compounds, including the "narcotic" analgetics. Therapeutic doses of organic bases used in medicine seldom exceed 100 mg and some, such as the autonomic agents, elicit adverse effects in doses of less than 1 mg. In contrast, organic acidic compounds such as the salicylates, PAS, Benemid, sulfonamides, *etc.*, can be administered in much larger doses. While one must be cognizant of the fact that individual compounds have inherently selective properties, one may explain partially the difference in toxicity between acidic and basic substances on the basis of differences in their abilities to gain access to their respective sites of action. Acidic compounds, being largely in anionic form at blood pH, generally have a limited ability to penetrate cells and tend to be distributed extracellularly. This favors rapid excretion of the compound in the urine, and hence larger doses are required to elicit an effect. On the other hand, with basic amines significant amounts exist at pH 7.4 in the undissociated form, which favors penetration into the cells. Thus, with morphine and its surrogates, it has been calculated that between 2 and 14% of the drug exists as the molecular species at body pH (25). This amount is apparently sufficient to allow the base to penetrate the cell where the relatively high pKa's of these compounds favor their combination with anionic receptor sites within the cell.

Evidence that the distribution of basic amines between plasma and various tissues is a function of the amount of free base present at blood pH is well shown by the studies of Jailer *et al.* (150, 151), who found that systemic acidification increases the plasma concentration of quinacrine and chloroquine and hastens the urinary excretion of these compounds. The effect was explained on a basis of altered partition of drug between tissue and plasma, since it was calculated that the proportion of quinacrine in the form of the free base is reduced by a factor of 8 if the pH is reduced from 7.4 to 6.9. In line with these results, Way *et al.* (312) have found that the urinary excretion of diphenhydramine is increased by ingestion of ammonium chloride. Studies along a similar vein may be cited for octin (172), nicotine (131), and quinine (14, 132).

While actual studies have not been performed with the morphine series, their pKa range is such that a similar relationship between blood pH and distribution characteristics should hold. It may be pointed out, however, that the tissue uptake of morphine relative to its surrogates cannot be rigidly correlated with the pKa of each compound, and hence other factors must also influence the distribution of these agents. Nevertheless, it is difficult to escape the conclusion that the distribution characteristics of morphine and its congeners arise in part from their basic properties. Thus, the basic properties of these compounds favor

their ability to gain access to target sites, where pharmacologic effects are elicited. However, these same basic properties also facilitate the uptake of the drug by other organs, which is an important factor tending to limit the intensity of the response to a given dose of a compound. This localization of the drug in pharmacologically indifferent organs would also tend to prolong the duration of the effect since these organs serve as repositories for the drug. The onset, intensity and duration of action of any analgetic, therefore, are greatly influenced by the amount of drug taken up by tissues other than the target organ, and this distribution is subject to modification by many factors.

Recent studies on the organ distribution of morphine and its surrogates are summarized in Table 3. The distributions of morphine and its surrogates all follow more or less the general pattern described for organic bases in that after absorption they rapidly leave the blood and concentrate in such organs as the lung, spleen, kidneys, and liver. The adrenals and thyroid generally show a high affinity for these compounds. Within a short period after intravenous injection of nearly toxic doses, the blood levels of these agents are barely detectable with methods sensitive to a few μg/ml. Drug concentrations in the brain are generally rather low as compared with most organs. However, according to Miller and Elliott (190), the levels of three compounds (morphine, codeine, and methadone) in the central nervous system correlated well with the intensity and duration of "analgetic" effect (tail reaction time to thermal stimulus).

With particular reference to morphine, there appears to be a significant blood-brain barrier to the compound although uptake of small quantities of the drug by the brain is relatively rapid (190, 318). The concentration of free morphine attained in the brain on a mg/kg basis is generally but a very small fraction of that to be expected assuming uniform distribution of the drug in the animal, whereas other organs show a selective preference for morphine. The experiments of Adler and associates (9) may be cited as an example to illustrate the exquisite sensitiveness of the brain to morphine. Sixty minutes after injection of a 2 mg/kg dose of C^{14} labeled morphine in rats, with the exception of one of eight animals, cerebral levels ranged between 0.04 and 0.09 μg/g. Despite the fact that these levels are the lowest measurable level of morphine recorded, they represent maximum values which may be too high by several-fold. Specific activity studies after isotope dilution indicated that only 10 to 20% of the C^{14} present in the tissue could be recovered as free morphine. Moreover, since blood levels of radioactivity were more than 6 to 20 times higher than cerebral levels, any blood trapped in the central nervous system would tend to elevate the radioactivity of the organ and a correction for this should be applied. Finally, if one considers that radioautographs of brain sections taken from rats injected with labeled morphine showed concentrations of radioactivity primarily in the highly vascularized choroid plexus and ventricles rather than in the neuronal elements of the brain (190), it becomes quite apparent that the central nervous system must indeed exhibit an extremely sensitive and select response to morphine.

In contrast to the central nervous system, other tissues usually attain morphine

TABLE 3

Tissue distribution of morphine and its surrogates

Compound	Species	Organs with High Concentrations	References	Remarks
Morphine	monkey	A, Lu, P, K	187	High levels of bound morphine in K and bile of all species; low levels of free morphine in B
	dog	K, Lu, S, GI, M	135, 217, 323, 324, 325	
	rat	K, L, S, A, T	9, 141, 179, 190, 285, 325, 326, 337	
	other	K, L	162, 193, 266	
Codeine	rat	L, K, A	10, 11, 76, 170, 190	Peak levels at about 1 hr
Heroin	mouse		311, 318	Rapidly metabolized, decline in brain and carcass rapid
Nalorphine	dog	S, K, P, M, Lu	325, 329, 331	Tissue uptake more rapid than morphine
	rat	S, Lu, K, M, H	331	
Levorphanol	dog	Lu, S, K, L, H	95, 265, 330	Highest concentration in soluble and microsomal fractions of B, L, K of rat
	rat		188, 330	
dl-Methadone	rat	Lu, L, K, S, A, T	8, 77, 78, 180, 190, 236, 281, 283, 316, 321	Firmly bound to tissue, excretion and rate of metabolism quite similar for both isomers
l-Methadone	rat	Lu, L, S, K	280	
d-Methadone	rat	Lu, L, K, S	280	
l-α-Acetylmethadol	rat	Lu, St, H, B, K	282	Levels of *l*-isomer more sustained
d-α-Acetylmethadol	rat	Lu, St, H, K, B	282	
Meperidine	man	Lu, L, K	159, 310	Crosses placental barrier; metabolized rather rapidly, slowest in humans.
	dog	B, K, Lu, L, S, A	50, 310	
	rat	K, S, Lu, L, B	220, 310	
Anileridine	rat	Lu, B, K, L, GI	227	Rapid decline in levels by 30′
Ethoheptazine	rat	L, K	303	Rapid decline in levels by 90′

Abbreviations: L = liver; Lu = lung; K = kidney; S = spleen; A = adrenal; T = thyroid; P = pancreas; H = heart; M = skeletal muscle; GI = gastrointestinal tract; St = stomach; B = brain.

levels considerably higher than those found in blood, and those organs concerned with the excretion of morphine, particularly the kidneys, show a considerable capacity to concentrate the drug. With a subcutaneous dose of labeled morphine (190), the renal level in terms of radioactivity at 60 minutes was more than 3 times higher than that to be expected assuming uniform distribution of the drug, and was roughly about 80 times higher than the cerebral level. The uptake of morphine by the liver, although considerably less than that by the kidney, was still much greater than that by the brain, the radioactivity in the liver being approximately 20 to 25 times higher than in the cerebrum (9, 190).

Despite the practical importance of having information concerning the placental passage of analgetic compounds, there has been surprisingly little emphasis on this. There are considerable qualitative data, based on pharmacologic evidence, that morphine and some of its surrogates do cross the placental barrier. As summarized by Baker in his recent review (20a), the fact that the use of morphine or heroin in the mother may lead to respiratory depression, miosis, the withdrawal syndrome, and a dramatic respiratory response to nalorphine in the newborn infant indicates that the placenta is permeated by these compounds. In the case of meperidine, chemical evidence to this effect was obtained by analysis of the newborns' urine (310) and cord blood (14a). These studies, while furnishing useful information as to levels of meperidine obtainable in these body fluids, fail, however, to divulge how much of the agent had reached the fetus. Although less than 1% of the dose of meperidine administered to mothers during the first stage of delivery was found in the fetal 24-hour urine of male newborn infants (310), the total amount of meperidine recoverable in terms of biotransformation products may be much higher.

Endocrine factors often markedly affect the pharmacology of the analgetics. Various investigators have sought explanations in terms of hormonal influences on the biologic disposition of these agents.

Adrenalectomy has profound effects on the sensitivity of response to morphine and results in increased tissue levels of morphine without impairing the animal's ability to conjugate morphine (9, 315). Winters and Flataker reported that cortisone or ACTH antagonized the effects of methadone, and suggested that the mechanism may be related to the change in ionic balance, fluid shift, or both, effected by adrenocortical hormones within nervous tissue (321). However, Elliott and Elison (78), on the basis of distribution studies, concluded that this antagonism was related to cortisone facilitation of the rate of passage of the drug through the body. They reported that cortisone enhancement of methadone absorption is accompanied by an even greater enhancement of the excretion of the compound with the result that the sojourn of methadone in the body and its pharmacologic effects are decreased.

Although vasopressin increases sensitivity to morphine and ACTH decreases sensitivity, neither treatment altered markedly the gross distribution characteristics of morphine except for a decrease in plasma bound morphine levels which occurred after administration of either hormone (9).

Thiouracil feeding and thyroidectomy were found to increase tolerance to

TABLE 4

Products excreted in 24-hour urine after administering morphine or its surrogates

Compound	Species	% Dose Recovered		Metabolic Products[4]	References
		Unchanged	Metabolized		
Morphine	man	1–14	11–60	Mostly glucuronide	66, 74, 102, 105, 200, 201, 202, 206, 209, 229
	monkey	7–13	42–92	Presumably glucuronide, decreases with chronic morphine administration	187
	dog	9–20	35–60	Mostly glucuronide, may decrease with chronic morphine administration	21, 55, 102, 118, 119, 120, 211, 215, 217, 218, 219, 288, 323, 324
	rabbit				142, 162, 336
	rat	10–30	20–50	Presumably glucuronide, may be decreased with chronic morphine administration	9, 90, 179, 315, 338
	mouse		33–75	Recovered by nonspecific tracer method	1
	other		41–82	Several species compared and reported as unchanged morphine but method not reliable	145
Codeine	man	3–16	24–89	Mostly bound codeine, some bound norcodeine and bound morphine, traces of norcodeine and morphine	6, 10, 175, 199, 201
	monkey	3–10	24–49	Mostly bound codeine, some free and conjugated morphine	332
	dog	4–11	42–58	Mostly bound codeine, very little morphine free or bound	210, 332
	rat	3	39	Probably mostly bound morphine, some bound codeine; norcodeine present in bile but not in urine	5

[4] Metabolites present as "bound" alkaloids or as acidic products are usually hydrolyzed or esterified, respectively, to convert them to forms more suitable for analysis; the incompleteness of these chemical reactions may contribute to low yields.

Heroin	man	0	50–57	Mostly as bound morphine, some free morphine	117a, 201, 204, 264
	rat	0		Trace of 6-acetylmorphine	318
Nalorphine	dog	1–6	27–40	Mostly conjugated nalorphine	329, 331
Levorphanol, Dextrorphan or Racemorphan	monkey	2–3	35	Mostly as conjugate	330
	dog	3–8	25–60	Mostly as conjugate, no nor-derivatives	49, 95, 265, 330
Dextromethorphan	dog	4–5		Some 3-methoxymorphinan, 3-hydroxy-N-methyl-morphinan and 3-hydroxymorphinan, method at best semiquantitative	49
Levallorphan	dog	2		Free and conjugated levallorphan—data at best semiquantitative	49
	rat	1	15	Bulk unaccounted for, some bound levallorphan, free and bound 3-hydroxymorphinan plus unknown free and bound metabolites	177
dl-Methadone	man	4	12–35	Values other than 314 probably too high and include some N-dealkylated metabolite	61, 64, 259, 314
	rat	4–11		Unknown base in urine not quantified, metabolite also appears in bile (73, 120, 229, 314)	77, 236, 314, 316
l-Methadone	rat	3–12		See comments for *dl*	280
d-Methadone	rat	2–9		See comments for *dl*	280
l- or *d*-α-Acetylmethadol	rat	3		Largely metabolized and unaccounted for	282

TABLE 4—*Continued*

Compound	Species	% Dose Recovered		Metabolic Products	References
		Unchanged	Metabolized		
Propoxyphene	man	3–10		Some dealkylated metabolite probably measured with unchanged compound	170a
	rat	13		See comment on man; unknown metabolite in feces (35%)	170a
Meperidine	man	2–10	53	Normeperidine (2–15%), meperidinic acid (10–41%), normeperidinic acid (3–28%), bound meperidinic acid (0–16%), bound normeperidinic acid (4–22%)	50, 171, 203, 220, 222
	dog	5		48 hr excretion for meperidine and/or normeperidine	303
	rat	2–7	18–40	About same as man except no bound meperidinic acid or normeperidinic acid	220, 222
Anileridine	man	5	10–19	Mostly anileridinic acid, some acetyl anileridinic acid	227
	rat	1	20	Mostly as acetylated anileridinic acid and some anileridinic acid	227
	guinea pig	1	20	Same as rat	227
Ethoheptazine	dog	4	17	De-esterified products and hydroxylated derivative	303
	rabbit	2	5	De-esterified products	303
	rat	2	7	De-esterified products	303

methadone even though the metabolic breakdown of the compound was slowed and tissue levels were elevated. Thyroid feeding increased susceptibility to methadone. This was found to be due in part to a slower rate of degradation of methadone by the liver (281).

Despite the widespread metabolic effects of testosterone, it apparently exerts little influence on the actions and fate of methadone. The drug appears to localize in kidneys of rats to a greater extent in females and castrated males than in normal males. This can be prevented by administering testosterone. The effect cannot be satisfactorily explained, and it has been attributed to an unknown function of testosterone (180).

Other factors such as pretreatment with neostigmine (285), SKF 525A (141), or nalorphine (326) did not induce appreciable change in the gross distribution pattern of morphine.

In summary, the distribution studies of morphine and its surrogates provide descriptive data concerning concentrations of the compound attained in various organs or tissues which are useful for indicating the nature of its cumulative properties. However, such information contributes little to an understanding of the mechanism of action, since localization of the drug in indifferent tissues represents drug association with "nonspecific" receptors. One need but emphasize again that the levels of the analgetics found in the brain are extremely minute in terms of either the dosage administered or the levels of the drug in other organs. While studies of concentrations of the drug in various parts of the central nervous system, such as have been carried out with morphine (8, 190), and in cellular fragments of the brain with levorphanol (188), represent further refinement and advancement in our knowledge about its distribution, these measurements in terms of specific sites of concentration are still relatively crude. It is hoped that ultimately morphine and other drug concentrations can be precisely estimated at the actual receptor sites in order that quantification of drug effects can be studied at the molecular level.

EXCRETION

Excretion is relatively unimportant in limiting the effect of morphine and its surrogates. While it is true that the compounds are ultimately eliminated mainly *via* the urine, excretion is generally preceded by biotransformation of the parent substance to derivatives which are excreted more rapidly. If metabolism of morphine and its surrogates did not occur, the excretion of such agents would be prolonged indeed. This is due to the fact that like most organic bases these analgetics have a high apparent volume of distribution by virtue of their tendency to leave the blood and localize in tissues. The ensuing low plasma levels should result in a slow clearance for such agents, particularly if extensive plasma binding and tubular reabsorption are also characteristics of the compound. It can be ascertained (Table 4) that the 24-hour excretion of the unchanged compounds in the urine is generally less than 10% and in many cases less than 5% of the dose administered. The rate of excretion of the compounds is most rapid early after drug administration to coincide, as might be expected, with the clearance of these agents at or near peak plasma levels.

The excretion of these analgetic compounds could presumably be accelerated as it has been for other weak organic bases (14, 131, 132, 150, 151, 172, 312) by promoting their ionization with acidifying substances and thereby reducing the proportion of diffusible free base available for tubular reabsorption. The pKa's of the morphine surrogates are of an order which suggests that these agents may be classified with this group of bases and hence should exhibit similar excretion characteristics. The mechanisms involved have not been fully elucidated but the process may be one of passive "non-ionic diffusion" (192a, 207a). A discussion of the mechanisms concerned in the renal tubular excretion of organic bases is beyond the scope of this review. The interested reader should consult an excellent analysis of this subject by Peters (214b).

The gastrointestinal tract has been generally considered to be a minor route for elimination of the morphine surrogates because only small amounts have been found in feces, and because the major fraction of the dosage can generally be accounted for in the urine. Only recently has the importance of the biliary pathway for disposal of some of these compounds been established. There is now considerable evidence that a significant fraction of morphine can be accounted for in the bile primarily as conjugated morphine. Furthermore, biliary excretion appears to be chiefly responsible for the appearance of morphine in the feces, and it is very likely that the residual amounts of morphine excreted in the urine for several days arise from reabsorption of biliary morphine excreted into the gastrointestinal tract (77, 179, 187, 324). Biliary excretion may be an important route of excretion for codeine (5) and methadone (73, 190, 279, 314), but not for acetylmethadol (282).

While renal and alimentary excretion constitute the major pathways for the elimination of morphine and its surrogates, other body fluids such as saliva, tears, sweat, and milk may be expected to carry at least traces of these drugs. That their presence has not always been detected in these fluids by chemical tests indicates that the amounts excreted by such routes are very low indeed. There has been very little information to supplement that found in the review by Krueger, Eddy and Sumwalt (166).

Traces of free morphine (0.2 to 5 μg/ml) were found in the perspiration of addicts but not in their saliva (202). According to Peterson, the presence of morphine in the saliva and urine of horses after its parenteral administration was noted in several laboratories (214c). Despite the pharmacologic evidence suggesting that morphine may be excreted in the milk (166), chemical evidence to this effect has not been furnished. Attempts also to detect meperidine in the milk of lactating patients were not successful (310).

METABOLISM

Biotransformation is the chief factor limiting the intensity of response and duration of effect of morphine and its surrogates. In some instances, however, biotransformation may be essential in the activation of compounds. There are certain pathways which are common for agents of this group, but individual compounds undergo also specific metabolic alterations by virtue of their own

structural peculiarities. The predominant metabolic changes include dealkylation, conjugation, hydroxylation, and hydrolysis.

N-Dealkylation

N-dealkylation is the metabolic pathway most common to compounds in this series. This process has important pharmacologic implications as a result of two provocative hypotheses: one by Beckett *et al.* relating N-demethylation to the analgetic action of morphine and its surrogates (25, 26, 27, 28), and the other by Axelrod relating N-demethylation to the development of tolerance (18). A consideration of these two hypotheses follows our presentation of the evidence for N-dealkylation of each analgetic agent. The material in this section has been organized so that compounds about which we have the most definitive data are presented first.

Experimental evidence. The earliest indication that N-demethylation might be a metabolic pathway of the analgetics resulted from studies on meperidine. Dealkylation of meperidine to normeperidine was established by studies in man and the rat. Way *et al.*, using countercurrent techniques, reported that urine of persons given meperidine contained an unknown organic base metabolite (310). In a subsequent study, following the finding that $C^{14}O_2$ was excreted in the expired air in rats given N-$C^{14}H_3$ labeled meperidine (220), the unknown organic base was identified in rat and human urine as normeperidine by countercurrent distribution studies (220, 222). These studies were later confirmed by Burns *et al.* by isolation of normeperidine in the crystalline state from human urine. Its structure was identified by mixed melting point determinations and comparison of its infrared spectrum with authentic normeperidine (50). Normeperidine has been identified also by paper chromatography as a meperidine metabolite in dog urine (302).

Normeperidine is formed also *in vitro*, and from these studies it appears that the liver is important for demethylating meperidine (18, 19, 221). When N-$C^{14}H_3$ labeled meperidine was incubated with rat liver slices $C^{14}O_2$ was evolved (221). La Du *et al.* (168) reported that the methyl group of meperidine is removed as formaldehyde. They reported also that the demethylation of meperidine as well as other alkylamines is catalyzed by an enzyme system present in liver microsomes, and that reduced triphosphopyridine nucleotide (TPNH) and oxygen are required (see below). These findings were confirmed by Axelrod (19) who found that the rate of demethylation of meperidine by rat liver microsomal preparations, as measured by formaldehyde formation, was more rapid than that of morphine or *l*-methadone. Liver microsomal preparations from the rabbit were found to be more active, and those from the guinea pig less active than rat liver microsomal preparations, while microsomes from mice exhibited no activity at all.

In man N-demethylation appears to be an important pathway for the disposal of meperidine since about one-third of the compound can be accounted for in the urine as normeperidine and its derivatives. The latter substances represent hydrolytic products of normeperidine, namely, normeperidinic acid and its

conjugate (220, 222). The extent of demethylation of meperidine *in vivo* in rats varies and appears to be dependent in part on the route of administration. After intravenous injection of meperidine-N-$C^{14}H_3$ in rats, approximately 43% of the total dosage was accounted for as expired $C^{14}O_2$ within 24 hours, with over half of this amount being exhaled within the first hour. After subcutaneous administration, however, only 15% was recovered in 24 hours and the $C^{14}O_2$ was slowly excreted at a fairly constant level for at least 10 hours. However, it should be noted that the amount of demethylated meperidine recovered was less than the total $C^{14}O_2$ expired (220). The significance of expired CO_2 as a measure of N-demethylation will be discussed later.

Evidence that codeine is N-dealkylated was established in a manner similar to that with meperidine. Initial studies revealed that after administration of N-$C^{14}H_3$ labeled codeine to rats, $C^{14}O_2$ appeared in the expired air. Norcodeine was subsequently identified by countercurrent distribution in human urine after codeine administration. Roughly 10% of the dose is excreted as norcodeine, largely in the conjugated form. Measurable amounts of free norcodeine can be demonstrated in urine of humans only after large doses. With normal therapeutic doses, only trace amounts of the base in free form are excreted (10).

Norcodeine is also formed from codeine in the rat but the nor-derivative is excreted differently. It is of interest to give some unpublished findings (5) made on a female rat injected with codeine N-$C^{14}H_3$. The pulmonary $C^{14}O_2$ (and therefore possible norcodeine formation) corresponded to 15.9% of the dose in a 30-hour period after subcutaneous injection. Of particular significance, therefore, is the fact that neither free nor bound norcodeine was present in the urine. An appreciable fraction, if not all, of the norcodeine formed must be eliminated *via* the gastrointestinal tract. This is indicated by the fact that 7.4% of the dose was recovered from feces as free norcodeine and more may have been present in bound form, although this could not be determined owing to the extraordinarily high blanks found in hydrolyzed rat feces. Total fecal excretion amounted to at least 34.6% of the dose for, in addition to non-isotopic free norcodeine, the feces contained 27.2% of the dose of carbon-14. This was present mainly as free codeine (20.4% of the dose). Analyses of pooled bile showed that free codeine and free norcodeine were present in the ratio of 3:1 (5, 10).

Dealkylation of aliphatic amino analgetics such as methadone was established only with some difficulty because of the instability of the resulting demethylated product. Way *et al.* found a basic metabolite of methadone excreted in the bile of the rat which was partitioned more readily than methadone from the organic solvent phase by acetate buffer (314). Miller and Elliott, using methadone labeled with C^{14} in position 2, noted that the acetate partitioned base was radioactive (190). These studies established that most of the methadone molecule remained intact. Axelrod noted that incubation of *l*-methadone with rat and rabbit liver microsomal preparations resulted in the liberation of formaldehyde and the formation of a benzene-soluble ninhydrin-reacting substance (19). Vidic, by means of paper chromatography, separated some renal excretion products of methadone and found evidence for N-demethylation of methadone,

as evidenced by the presence of a primary and a secondary amine in his chromatogram (302). Finally, Pohland *et al.* (225) recently found that in attempting to synthesize des-N-methylmethadone, they obtained a cyclic compound, 1,5-dimethyl-3,3-diphenyl-2-ethylidinopyrrolidine by ready dehydration of the intermediate hemiketal form of des-N-methylmethadone. A metabolite isolated from incubates of rat liver slices with methadone or from bile of a dog given methadone behaved like the cyclic product derived from des-N-methylmethadone. The infrared absorption of 1,5-dimethyl-3,3-diphenyl-2-ethylidinopyrrolidine and the base isolated from rat bile by Way *et al.* (313) indicated that the two compounds are very similar if not identical. Thus, there is strong evidence indicating that N-demethylation is an important metabolic pathway for methadone although the resulting residue may be chemically unstable.

The results of Axelrod (19) and Vidic (302) also suggest that both methyl groups may be split off to yield the corresponding primary amine of methadone, since both investigators noted positive tests with reagents which generally are known to react with primary amines. However, proof by isolation of this product in crystalline form remains to be accomplished.

In vivo as well as *in vitro* experiments indicate that N-demethylation of propoxyphene is an important metabolic pathway (170a). A dealkylated metabolite, des-N-methyl propoxyphene, was isolated as a dinitrophenyl derivative from the urine of six volunteers. After intravenous injection of N-$C^{14}H_3$ labeled propoxyphene approximately 38% of the radioactivity was eliminated as $C^{14}O_2$ within 22 hours. The half-time for elimination of $C^{14}O_2$ was approximately 130 minutes. When labeled propoxyphene was incubated with rat liver slices there was a constant increase in the rate of $C^{14}O_2$ eliminated up to 60 minutes after which the rate appeared to diminish. Similar studies with lung, brain, spleen, mammary gland, stomach, and whole blood yielded no $C^{14}O_2$ after 30 minutes of incubation. The kidneys exhibited some N-demethylating ability but the amount of $C^{14}O_2$ liberated was only about 0.5 to 2.5% of that found with liver (170a).

It has been virtually taken for granted that morphine is N-demethylated to normorphine. The inference has been easy to draw because N-demethylation was established earlier as a metabolic pathway for meperidine (220, 222) and codeine (10). However, recovery of normorphine as a biotransformation product following morphine administration has not been accomplished to date. Furthermore, a lack of correlation between *in vivo* and *in vitro* events characterizes N-demethylation of morphine. Thus, while normorphine has been established as a morphine metabolite *in vitro*, evidence for its formation *in vivo* is, at best, indirect and inconclusive. In fact, the only experimental evidence available at present suggesting normorphine formation *in vivo* are the observations that $C^{14}O_2$ appears in the breath shortly after injection of morphine-N-$C^{14}H_3$ in rats (179) and man (79).

In rats (Wistar type), the pulmonary excretion of $C^{14}O_2$ after administration of 5 mg/kg of morphine-N-methyl-$C^{14}HCl$ subcutaneously was found to be rapid during the first 2 hours. Male rats excreted close to 5% of the dose as radioactive

CO_2 within 6 hours whereas the percentage excreted by females over the same time interval was less than 0.5% (179). In man, the pulmonary excretion of $C^{14}O_2$ in five human subjects given 10 to 15 mg of morphine-N-methyl-C^{14} sulfate intramuscularly ranged from 3.5 to 6% of the injected dose in 24 hours (79). No sex difference in the amount of expired $C^{14}O_2$ was observed between the two male and three female subjects. The peak rate of $C^{14}O_2$ excretion in the breath was found to occur between 30 and 90 minutes after drug administration. A plateau in the rate of excretion occurred after 6 hours. Measurable amounts of radioactivity were found to be present in two subjects 4 or 5 days after drug administration. It was suggested as a consequence that some transfer of $C^{14}H_3$ groups from the morphine molecule to the carbon pool may occur, from which $C^{14}O_2$ is slowly liberated by catabolic processes.

It can be noted that the amount of $C^{14}O_2$ obtained in the breath in man after injection of morphine-N-$C^{14}H_3$ is about the same as that obtained after injection of codeine-N-$C^{14}H_3$ (10); accordingly, if the N-demethylation processes are analogous, some 10% of the dose of morphine ought to be found in a 24-hour urine sample as free and bound normorphine. However, attempts to show that any normorphine is present in urine have met with no success, and the recent work of Rapoport (229) indicates that the total amount of normorphine excreted in 24 hours in the urine of man must be less than 0.5% of the dose.

The absence of normorphine from the urine after morphine administration is, of course, no proof that the metabolite is not formed *in vivo*. Any one of several explanations could account for the failure to detect normorphine even if small amounts were released in the body. Although it has been shown that after very large doses (75 to 150 mg) of normorphine in man about 75% of the dose appears in urine in part as an unstable conjugate plus a large proportion of free alkaloid (267a), a different fate may characterize the relatively small amounts which might be expected to be released after ordinary doses of morphine *in vivo*. On purely speculative grounds the reviewers suggest the following possibilities: 1) biosynthetic normorphine may be excreted preferentially in the feces; 2) biosynthetic normorphine may be metabolized further in an unknown manner; 3) biosynthetic normorphine may be retained by tissues for a fairly prolonged period of time. Although the last suggestion is compatible with the pharmacology of normorphine in that marked cumulative effects of the drug are seen after multiple doses (100), these suggested possibilities can be placed in proper perspective only when data are furnished regarding the excretion of normorphine after injection of low doses of this compound.

In the absence of such data no conclusion can be made at present regarding the biotransformation of morphine to normorphine, since even the data on $C^{14}O_2$-formation from the N-methyl group do not necessarily imply that a normorphine residue is released. Rapoport (229) has recently found that the specific activity of excreted morphine in ten human subjects after injection of 10 mg morphine-N-$C^{14}H_3$ was only 92% of the pre-injection value, indicating that in 8% of the molecules the position occupied by the labeled group had been pre-empted by a methyl group derived from endogenous sources. While it is quite likely that

the dispossessed C^{14}-methyl group served as the source of pulmonary $C^{14}O_2$, it is also possible that it did not get into the breath at all since it may have been involved in other transmethylation reactions. Thus, the expired $C^{14}O_2$ may represent molecular changes in the morphine skeleton more drastic than demethylation to normorphine.

At present the only firm support for normorphine's being a metabolite of morphine is derived from *in vitro* studies. That morphine may be demethylated *in vitro* was first suggested by the work of March and Elliott (179). Using rat liver slices and morphine-N-$C^{14}H_3$ as substrate, these authors showed that under aerobic conditions there is a release of $C^{14}O_2$. Subsequently, Axelrod (16, 19) found that an oxidative mechanism located in liver microsome preparations is responsible for the oxidation of the methyl group to formaldehyde. He has obtained qualitative evidence for normorphine formation by liver microsomes, and it is assumed that the amount of formaldehyde formed during the reaction bears a stoichiometric relationship to the amount of normorphine released.

The demethylating system described by Axelrod resembles the system responsible for oxidative demethylation of methylated aminoazo dyes, first observed by Mueller and Miller (195) in liver homogenates and later (57) located in the microsomes. Although the two demethylating systems are not identical (286), both enzyme systems require TPNH and oxygen, which suggests that generation of a "peroxide" (11) may be necessary for the oxidative demethylation.

Nalorphine was found to be dealkylated to normorphine by rat liver microsomes in the presence of TPNH and O_2 at a rate faster than that of morphine to normorphine (20). The simultaneous presence of morphine and nalorphine in such an *in vitro* preparation results in a noncompetitive inhibition of demethylation of morphine (20) (formation of formaldehyde). With rat liver slices, on the other hand, nalorphine does not inhibit demethylation of morphine ($C^{14}O_2$ from morphine-N-$C^{14}H_3$) (179). Schrappe reported that low-grade dependence developed after chronic administration of nalorphine, and suggested that this could be the result of N-dealkylation of the compound to normorphine (254).

The status of levorphanol and dextrorphan with respect to N-dealkylation is similar to that of morphine. There is suggestive but inconclusive evidence that levorphanol and dextrorphan may be N-demethylated to their corresponding nor-derivatives by certain species *in vivo*. In the monkey about 20 % of the dose of N-$C^{14}H_3$ labeled levorphanol could be accounted for as $C^{14}O_2$ in the expired air. In the rat about 5 % of the dose was eliminated as $C^{14}O_2$, but in the dog the $C^{14}O_2$ eliminated was only 1 to 2 % (330). However, attempts to identify norlevorphanol and nordextrorphan or their conjugates in dog urine have been unsuccessful, although 3-OH morphinan can be found after administration of the 3-methoxy analog, dextromethorphan (49), or the N-allyl analog, levallorphan (177). Furthermore, when the nor-compound itself was administered to dogs it was easily detected in the urine in the free and bound forms (265). Moreover, the free and bound forms of the nor-compound are found in urine and feces of rats after injection of levallorphan (177).

Several interesting *in vitro* studies indicate that liver microsomal enzymes of rats and mice catalyze N-demethylation of levorphanol, forming formaldehyde and, presumably, the nor-derivative (19, 286). Axelrod (19) reported that the *dextro*-isomer was demethylated much less readily than the *levo*-form by rat preparations, but Takemori and Mannering (286) found that the isomers were demethylated with equal facility by rat preparations or by mouse preparations. No formaldehyde was detected when levorphanol was incubated with dog liver microsomal preparations (265).

A metabolite of anileridine was present as an unidentified diazotizable substance found principally in the free state in human urine and in a bound form that is released by hydrolysis in rat and guinea pig urine. Countercurrent studies with this fraction from rat urine indicated that the substance may be acetylaminophenylacetic acid or a very closely related compound, but the precise experimental details of the isolation and characterization of the compound were not given. It was postulated that this metabolite is produced by degradation of the isonipecotic acid portion of the molecule rather than by N-dealkylation ("scission in the nitrogen-ethylaniline portion of anileridine") (227). To the reviewers this conclusion is difficult to accept, and the opposite view appears to be more logical.

Ketobemidone has been reported to be N-demethylated to its corresponding nor-derivative on the basis of paper chromatographic studies (299, 301). The evidence for N-demethylation of ethoheptazine is only suggestive, being based on a positive color test for a secondary amine on paper chromatograms of urine. Rabbit urine contained a secondary amine metabolite as well as another unknown product not present in dog urine (303).

Enzymatic mechanisms. Some pertinent speculations on the chemical mechanism of oxidative dealkylation of drug substrates may have some bearing on the metabolism of the compounds. Thus, the formation of nor-derivatives may be dependent on the enzymatic oxidation of the tertiary amine group to the amine oxide (93, 94), followed by rearrangement to a carbinol compound and subsequent hydrolysis yielding the secondary amine and formaldehyde. On the other hand, Brodie (45) has suggested that the initial step in either N-dealkylation or O-dealkylation may involve a direct substitution of a hydroxyl group for an alkyl hydrogen, with subsequent hydrolysis to the corresponding dealkylated amine or phenol, respectively, and an aldehyde. The work of Gillette *et al.* (110) has shown that oxidative dealkylation of monomethyl-4-aminoantipyrine occurs when there is a concomitant oxidation of TPNH by a specific TPNH-oxidase, but not when TPNH is oxidized by the cytochrome system. They have shown further that the reaction of TPNH with TPNH-oxidase yields "organic peroxides" even in the absence of drug substrates. This suggests that the initial oxidative attack at the N-alkyl position may depend on the formation of a specific oxidant, possibly an "activated" peroxide. Apparently H_2O_2 *per se* does not function in the microsomal system but can function in an iron-containing model system, since generation of H_2O_2 by the glucose-glucose oxidase system promoted oxidative dealkylation of N-alkyl amines in the model system but not in the microsomal system (111).

If the initial reaction between an "activated peroxide" and morphine or its surrogates results in formation of an N-oxide, the subsequent steps leading to formaldehyde release need not necessarily depend on enzymatic reactions. Rearrangement of tertiary amine N-oxides to carbinol compounds, followed by hydrolysis to formaldehyde and the secondary amines, can occur under mild chemical conditions (pH 5 to 7, 38°C) when catalyzed by a ferric ion-tartrate complex (93, 94). Under such conditions one would expect a secondary reaction to occur, namely, reduction of some of the N-oxide by formaldehyde leading to regeneration of the tertiary amine and oxidation of formaldehyde to formic acid. This secondary reaction would be minimized in a system containing a formaldehyde-trapping reagent.

Axelrod has found that microsomes prepared from livers of rat, rabbit, guinea pig, but not mouse, are capable of catalyzing the formation of formaldehyde from morphine (19). The inability of mouse microsomes to catalyze the reaction is apparently restricted to certain strains, since others have found considerable N-demethylating activity in mouse liver microsomal preparations (286). Microsomes prepared from kidney, brain, muscle, or spleen of male rats are inactive in this respect (19). Species differences in activity of the enzyme system occur but these may be related to the presence or absence of co-factors. For example, rabbit livers appear to contain a dialyzable co-factor necessary for N-demethylation. This inference is based on Axelrod's finding that the only reaction catalyzed by a dialyzed preparation of rabbit liver microsomes when incubated with codeine is the formation of morphine and formaldehyde in equimolar amounts (17). It appears to the reviewers that if such a dialyzed preparation were capable of effecting N-demethylation an additional amount of formaldehyde would be formed from the N-methyl of the released morphine as well as from the N-methyl of the otherwise unmetabolized codeine. Another example of the importance of co-factors in species differences is the finding that rabbit livers do not, but rat livers do, contain a heat-labile inhibitory factor localized in the nuclei and mitochondria (19).

The early observations made by March and Elliott (179) of the large sex difference in the ability of rats to form $C^{14}O_2$ from morphine N-$C^{14}H_3$ both *in vivo* and with liver slices, and the marked effect of pretreatment of the animal with either androgens or estrogens, was confirmed and extended by Axelrod to rat liver microsomal preparations (19). On the other hand, Axelrod reported a marked, noncompetitive inhibition of microsomal enzymatic N-demethylation of morphine by added nalorphine (20), whereas March and Elliott found no such inhibition in their liver slice experiments. This apparent discrepancy may possibly be related to the fact that nalorphine is rapidly conjugated in liver slices (261) and, hence, may not reach the demethylating enzymes within the microsomes.

Notwithstanding the lack of intimate knowledge of the factors responsible for oxidative dealkylation of morphine and its surrogates, the subsequent release of the dealkylated residues poses some interesting questions concerning the importance of these metabolic events *in vivo*. Some of the dealkylated residues have been studied pharmacologically. The compounds possess significant

pharmacologic effect when administered *in vivo*, but apparently demethylation does not lead to consistently predictable pharmacologic effects. Miller and Anderson (189) compared the intraperitoneal "analgetic" potency and acute toxicity (lethality) of three nor-derivatives with those of their parent compounds in mice. Normorphine and normeperidine were found to be less "analgetic" but more toxic than morphine and meperidine, respectively, whereas norcodeine was found to be less "analgetic" and less toxic than codeine. However, in man norcodeine appears to be as potent as codeine in sedative effects in normal subjects, and about 3 times more potent in norcodeine or codeine addicts (99). Thus, whether N-demethylation is to be viewed as a detoxification or an activation process depends on the specific compound and the specific pharmacologic effect.

Significance in tolerance. Quite apart from any role that N-demethylation may play in limiting (or enhancing) the acute effects of the parent compound, this metabolic process has recently been implicated in the chronic effects, particularly the development of tolerance.

In 1956 Axelrod (18, 19, 20) found that in chronically morphinized male rats there was a marked reduction in the ability of the liver microsomes to demethylate morphine, dihydromorphinone or meperidine. Several points of similarity were noted between the "analgetic" response in male rats *in vivo* and the behavior of the N-demethylating system of the liver microsomes *in vitro*. These included (a) parallel activities (*i.e.*, "analgetic" potency and N-demethylation) of compounds, with specificity of substrates extending to stereoisomers of the same compound; (b) parallel antagonism of activities by nalorphine; (c) parallel depression of activities in chronically morphinized animals; (d) parallel recovery of these activities during the post-withdrawal period. In view of these similarities Axelrod proposed that the liver N-demethylating enzyme might serve as a model for study of "narcotic" drug receptor sites. The parallel reduction observed in tolerant animals in the enzymic N-demethylation of morphine or its substitutes *in vitro*, and in the pharmacologic response *in vivo*, as well as the observation that these activities recovered after withdrawal, led Axelrod to formulate a mechanism of tolerance which is based on the assumption that the enzymes in the microsomes and the receptors in the central nervous system are closely related. He stated: "The continuous interaction of narcotic drugs with the demethylating enzymes inactivates the enzymes. Likewise, the continuous interaction of narcotic drugs with their receptors may inactivate the receptors" (18).

Examining the basic assumption that the microsomal enzymes and the central nervous system receptors are closely related, we find that there are certain apparent limitations to this relationship. It can be noted that the less potent morphine surrogates, meperidine and codeine, are more readily N-demethylated by the microsomes than is morphine itself (18, 19, 286); thus, in a nontolerant animal there is no parallelism between N-demethylation of a substrate *in vitro* and its analgetic action *in vivo*.

Although Axelrod has never claimed that N-demethylation of narcotic drugs

in vitro is correlated *per se* with analgetic activity, he does offer as an example of similarity between the receptors and the enzymes the fact that the pharmacologically active *levo*-forms of stereoisomers are N-demethylated much more readily than the relatively inactive *dextro*-forms (19). However, exceptions to this relationship were noted by Takemori and Mannering (286) who found that with rat and mouse liver microsomes the *levo*- and *dextro*-isomers of 3-OH-N-methyl morphinan were demethylated with equal facility. Another point of similarity between receptors and the enzymes mentioned by Axelrod lies in the action of nalorphine, which antagonizes the "analgesia" produced by morphine and inhibits N-demethylation *in vitro* in the nontolerant animal (20). Exceptions to this parallelism are found in the morphinan series where the pharmacologically inactive *d*-3-OH-N-allyl morphinan (30) is as effective as its pharmacologically active *levo*-isomer in inhibiting N-demethylation by mouse microsomes (286), and where other N-substituted analogs show an inverse relationship between antagonism of analgesia and inhibition of N-demethylation (52).

In contrast to nontolerant animals, tolerant animals frequently do exhibit a certain parallelism between the loss of analgesic response and the reduction in capacity for N-demethylating morphine or its surrogates *in vitro* (18, 135a, 54, 178). Conversely, a drug such as dextrorphan, which does not induce tolerance, similarly does not reduce the enzyme activity (178). However, the decreased N-demethylation found in a morphine-tolerant animal is not limited to substrates such as the morphine surrogates but has been observed by other investigators with substances like cocaine and dimethylamino-antipyrine (135a). Moreover, even with morphine as a substrate, the degree to which the analgetic response is diminished does not parallel the degree to which the N-demethylation activity is depressed (54), and during the post-withdrawal period the recovery of the analgetic response lags considerably behind the recovery of the microsomes' N-demethylating capacity (54a). Furthermore, in a few cases of pronounced tolerance no effect on the N-demethylating ability of the liver was seen (135a). The many exceptions to the parallelisms between the analgetic activity *in vivo* and the behavior of the liver enzymes *in vitro* raise considerable doubt of the value of considering the latter as a model for the study of events occurring at the drug-receptor site in the central nervous system.

Even when tolerance is accompanied by decreased N-demethylating activity of the liver *in vitro*, it is questionable whether the interaction between the drug and the enzyme leads to "inactivation" of the latter. Herken *et al.* (135a) have pointed out that occasionally pronounced tolerance in rats is accompanied by no effect on the demethylating activity *in vitro*. Furthermore, the metabolism of the N-methyl group of morphine *in vivo* appears to be unaffected by the development of tolerance. Thus, in man, after injection of morphine-N-$C^{14}H_3$ there was no difference between the amount of pulmonary $C^{14}O_2$ obtained on the one hand from 4 nonaddicts, and on the other from a morphine addict of two years' standing with a daily intake of 90 mg morphine at the time of the experiment (79). A similar lack of effect of tolerance on pulmonary excretion of $C^{14}O_2$ was found in 2 male rats chronically treated with morphine for 6 weeks and then

injected with morphine-N-$C^{14}H_3$ (179). Whether or not pulmonary $C^{14}O_2$ truly represents N-demethylation of morphine, it is the only criterion at present by which this phenomenon can be judged *in vivo*. According to this *in vivo* evidence we must conclude that, contrary to the assumption in Axelrod's hypothesis, there has been no inactivation of the N-demethylating enzymes accompanying the development of tolerance.

Since the reduction in the ability of liver enzymes of morphinized rats to demethylate morphine has been demonstrated thus far only for microsomal preparations, it might be well to focus attention on the role of the N-demethylating inhibitory factors normally found in rat liver nuclei and mitochondria (19). Some nonspecific or indirect effect accompanying the development of tolerance might lead to increased permeability or fragility of cell particles, which in turn might result in contamination of the microsomes by inhibitory factors as an artifact of the conditions of preparation. While we are not particularly partial to this suggestion, we offer it merely to point out that it is premature to invoke a specific drug-enzyme interaction to explain this phenomenon which has been observed only *in vitro*.

Significance in analgesia. Whether N-demethylation of morphine is uniquely characteristic of conditions *in vitro* should be the object of closer scrutiny since the postulated N-demethylation *in vivo* assumes important pharmacologic significance as a result of the provocative hypothesis advanced by Beckett, Casy and Harper in 1956 (28). In earlier papers Beckett and his colleagues (25, 26, 27) had proposed that activity of an analgetic compound is due to association with a specific receptor surface in the central nervous system, and that a drug-receptor complex is formed when certain steric requirements for the drug molecule are satisfied. In the later paper (28) the authors postulated that the formation of the drug-receptor complex does not itself produce analgesia, but that following adsorption of the drug on the receptor surface there occurs an oxidative dealkylation with the release of the N-dealkylated moiety. It is the presence of the nor-derivative on the receptor surface that is considered to initiate the analgetic response. Furthermore, it was postulated that as the size of the alkyl group attached to the nitrogen is increased, there is increased adsorption of the drug, but at the same time there is also increased difficulty in dealkylation of these large groups.

It is true that this hypothesis is in accord with the fact that as the size of the alkyl group increases from methyl to ethyl to allyl, the analgetic potency decreases and a gradual transition from analgetic to antianalgetic action is seen. However, several of the assumptions are difficult to accept in the absence of any experimental data. In explaining the antagonism of morphine by nalorphine, it is assumed that the receptor sites have a greater affinity for nalorphine, thereby allowing it to displace morphine from the surface; but since the rate of dealkylation of nalorphine is very much lower, the essential metabolite, normorphine, is not available to the receptor. In accepting these assumptions, one has to postulate that the greater affinity between receptor and nalorphine occurs despite the decreased strength of the ionic bonding that results from the base-

weakening effect of substituting the allyl for the methyl radical (pKa morphine = 8.05, pKa nalorphine = 7.83). Furthermore, although dealkylation of nalorphine occurs more readily than that of morphine in the one instance where the phenomenon of dealkylation is known to occur (*i.e.*, by the liver enzymes (20)), one must postulate that quite the reverse relationship holds for dealkylation at the receptor. This reverse relationship must also be postulated for codeine and meperidine since these compounds, although less analgetic, are N-demethylated more readily than morphine by liver enzymes (18, 19, 286).

The fact that normorphine also is antagonized by nalorphine (174, 189) appears to us to be not in accord with Beckett's hypothesis, especially since this antagonism is seen even when normorphine has been presented in proximity to the receptors in the central nervous system by intracisternal injection (174). It seems reasonable to assume that normorphine would be well adsorbed at the active site since one might expect that the bond formed between the anionic portion of the receptor and the cationic portion of the drug would be greater for normorphine than for nalorphine (secondary amines are generally stronger bases than tertiary amines). Despite this, one must postulate that the reinforcing nonionic attractive forces between nalorphine and receptor are still great enough to facilitate a preferential adsorption of nalorphine. According to this hypothesis, the available normorphine, even after intracisternal injection, is displaced from the analgesic receptors by the more strongly adsorbed nalorphine and therefore fails to be incorporated into the reaction sequence resulting in analgesia. This may be the case, but at present there are no adequate criteria by which these assumptions can be judged.

One of the predictions from this hypothesis is that the degree of analgesia of nor-derivatives should be as great as, and the onset more rapid than, that of the methylated compound. In order to circumvent the experimental evidence by Miller and Anderson (189) that the nor-compounds are less potent analgetics than their corresponding N-CH_3 congeners, Beckett advanced the opinion that this was due to the inability of blood-borne nor-compounds to gain access to the receptor sites. He argued that if the nor-compound could be introduced in the vicinity of the receptor, such as by intracisternal injection, then the activity of the nor-compound should equal or exceed that of its methylated analog. This was found to be the case for normorphine when compared with morphine (174), and furthermore, morphine itself interfered with the action of normorphine when the two alkaloids were presented together (140). Thus, these observations after intracisternal injection conform to the prediction. However, Miller and Anderson (189) found also that although the analgetic potency of normorphine, normeperidine or norcodeine after intraperitoneal administration was less than that of the respective methylated congener, the toxicity of normorphine and normeperidine by the same route was greater than that of their corresponding methylated analogs. That both kinds of receptors share common properties is indicated by the fact that the toxicity of morphine, as well as the analgesia, is decreased by nalorphine (189). We are, therefore, reluctant to accept Beckett's argument that the nor-compounds are unable to penetrate into the central nervous system since

it is not clear why normorphine and normeperidine, after intraperitoneal injection, should have so much difficulty in gaining access to the analgetic receptor sites in the central nervous system and so little difficulty in gaining access to the other sites in the central nervous system where they exert their toxic effects.

Since an integral part of the hypothesis is the assumption that large alkyl substituents attached to the nitrogen atom are not removed readily, thereby resulting in antagonism of analgesic action, one would predict that the N-phenylethyl and N-*p*-aminophenethyl analogs would be potent antianalgetic agents. To the contrary, these compounds have been found to be more potent as analgetics than their methyl analogs (anileridine *vs.* meperidine) (183a, 214a)[5]. In general it appears that attempts to correlate analgesia with presumed rates of N-dealkylation in the brain are of limited value in predicting the position a compound will occupy within this group of drugs, the designations of which range from strong analgetic through weak analgetic to antianalgetic action. Notwithstanding the objections to this hypothesis, with respect to the importance of N-dealkylation in initiating analgesia, it cannot be denied that a significant and imaginative contribution has been made by the authors in basing the specifications of the structural configuration of the central receptor site on a consideration of the three-dimensional molecular structure of active drugs.

In spite of our detailed criticism of Beckett's hypothesis, we do not consider the objections we have raised as ultimately damning. While future evidence may provide support to substantiate their arguments, we feel, for the time being, that as attractive as this hypothesis is, there are available no experimental data to warrant the assumption that N-dealkylation is a biotransformation process essential for activation of these compounds. On the other hand, considering the greater toxicity of some of the nor-derivatives compared with their methylated analogs, we cannot consider this metabolic event simply as a detoxification process. It may be concluded only that the role of N-demethylation in either promoting or limiting any of the pharmacologic responses to morphine or its surrogates remains to be defined.

O-Dealkylation

In 1951 evidence from studies in rats using isotopically labeled codeine suggested that O-demethylation was an important metabolic pathway of codeine (4, 11). That such demethylation *in vivo* results in the release and excretion of the respective demethylated residue was established for man in 1952 by identifying morphine in urine extracts after codeine administration (4). Morphine was isolated as the dinitrophenyl ether and the structure verified by analysis of the powder X-ray diffraction pattern (10, 13). Several subsequent studies have confirmed the release of morphine *in vivo* in man (10, 175, 210), in the monkey (332), in the dog (210), and in the rat (5, 12, 13), and the release of morphine *in vitro* by liver microsomes of rabbit, rat, guinea pig, dog (17), and mouse (286).

[5] An explanation for the increased analgetic potency of the phenethyl analog has been offered which invokes the influence of dipole moments on dealkylation rates (28). This argument cannot accommodate both the phenethyl and the *p*-aminophenethyl groups.

These studies *in vitro* extend the original observations of Bernheim and Bernheim (34) that incubation of codeine with rat liver slices results in the formation of a compound with phenolic properties. Liver microsomes of various species also metabolize codeine to norcodeine (19, 286), although the release of norcodeine *in vivo* has been studied only in man (4, 10) and in the rat (5). Thus far there has been no direct evidence to indicate that the same codeine molecule is demethylated at both the O and the N positions to yield normorphine.

Experiments in which codeine-O-$C^{14}H_3$ has been injected in man have shown that after O-demethylation of codeine to morphine not all of the corresponding methyl-C appears in the breath as $C^{14}O_2$ (10). Some of it may be present in tissues as C^{14}-bicarbonate (11) and some may have been incorporated into the carbon pool. Data obtained on pulmonary $C^{14}O_2$ excretion following injection of codeine-O-$C^{14}H_3$ in man indicate that metabolic alteration of codeine at the 3-position does not follow a simple exponential function, and that the appearance of $C^{14}O_2$ in the breath is governed by at least two distinct rates: an initial hourly rate of 16% of the codeine present, which is later superseded by a slower rate of approximately 6%. A similar conclusion regarding the metabolism of the 3-OCH_3 group in the rat was reached by Krueger (164) after mathematical analysis of our data on $C^{14}O_2$ excretion in that species. Krueger has suggested that the slower rate may result from conversion of codeine into a methoxy-containing metabolite, followed by a subsequent conversion of the methoxy group of the metabolite into CO_2 at the slow rate of 6% per hour. That release of norcodeine is followed by a slow and limited release of normorphine is a possibility to consider, although the amount formed may be too small for detection by present means.

$C^{14}O_2$ is also the normal fate of the labeled 3-O-methyl carbon when codeine is metabolized *in vitro* by slices of rat liver, kidney, or skeletal muscle. Liver slices are over 100 times more active than the other tissues in this respect, and hence it may be presumed that the main site of codeine metabolism *in vivo* is the liver. Other tissue slices, including brain, did not metabolize codeine to $C^{14}O_2$ (11). The immediate precursor of CO_2 is formaldehyde, and the formation of formaldehyde from either the 3-methoxy carbon or the N-methyl carbon has been localized in the liver microsomes (17, 19, 286). In contrast to results found by Adler and Latham (11) with tissue slices, it was found that the isolated microsomes of kidney and muscle were completely inactive in metabolizing codeine (17). The liver microsomal enzyme system is active only in the presence of reduced triphosphopyridine nucleotide and oxygen, a frequently encountered requirement for the microsomal oxidation of a variety of drugs (45, 109).

In view of the marked depressant action of even small doses of morphine, it is appropriate to consider whether metabolic release of this alkaloid is prerequisite to the central depressant effects of codeine. It is interesting to note that an active role for morphine in the pharmacologic effects of codeine had been assumed before there were any direct experimental data to show that morphine is released from the parent drug. Thus, in 1938 Wolff (322) suggested that morphine addicts could be maintained by codeine simply because such individuals had acquired the capacity to split off the methyl radical of codeine,

thereby liberating the morphine moiety. In 1948 Sanfilippo concluded that the "narcotic" action seen with small doses of codeine in the dog is due to a variable and limited ability of the animal to convert part of the dose to morphine, and that with large doses this limit is surpassed. He further postulated that with large doses the effect of the small amount of released morphine is obscured by the high concentrations of unmodified codeine which cause excitement and convulsions (243). His experiments showed that chronic administration of codeine resulted in tolerance to the "narcotic" and hyperglycemic effect of initial doses (244, 245) and that reciprocal cross-tolerance to such effects occurred between morphine and codeine (246). These data were offered as verification of the hypothesis that liberation of morphine is responsible for the central depressant effects of a small dose of codeine, and for the phenomenon of tolerance seen with chronic administration.

There are, however, certain obstacles to accepting a hypothesis that would assign the central depressant action of codeine to the morphine liberated from it. In the first place, fairly sizeable amounts of morphine are released from large doses of codeine in man and yet there is surprisingly little "morphine effect." Thus, the 22.2 mg of morphine (free and bound) excreted by a normal subject after oral administration of 282 mg of codeine (10) is more than twice that amount of morphine excreted by normal subjects after i.v. injection of 20 mg of morphine (209), and is therefore probably equivalent to the amount of morphine excreted after i.v. injection of 45 mg of morphine. Such a dose of injected morphine would cause profound sedation in the nonaddict, and yet the subject in the codeine experiment experienced little or no sedative effect. Similarly, in the monkey the codeine-released morphine is far less effective than an equivalent dose of injected morphine (332). Woods has calculated that on a dosage regimen of 10 mg/kg codeine every 6 hours the monkeys would be exposed to 1 mg of morphine every 6 hours. However, the nalorphine-induced abstinence syndrome in monkeys receiving 1 mg of morphine every 6 hours is much more severe than that observed in monkeys chronically treated with 10 mg of codeine every 6 hours. As a consequence, Woods concluded that: "accordingly the conversion of codeine to morphine must occur at a site which is capable of conjugating most of the morphine promptly." Unfortunately, this inference is directly opposed to the implications of his excellent data on plasma levels of free and bound morphine after codeine administration in the monkey. These data do not show an extraordinarily high ratio of bound to free morphine as should be expected if the liberated morphine were indeed conjugated "promptly." On the contrary, as can be seen in Table 5, the conjugation ratios of bound morphine to free morphine observed in the plasma after morphine injection are not very different from those observed after codeine injection. Corroboration of the evidence that codeine-liberated morphine is not conjugated more rapidly than injected morphine is offered by the similarity of conjugation ratios of morphine excreted in urine under the two conditions, not only in the monkey but also in man.

There appears to be little support, therefore, for the suggestion that rapid metabolic inactivation of morphine is responsible for limiting the effect of this

TABLE 5

Conjugation ratios of administered and codeine-liberated morphine

$$\text{Ratio} \frac{\text{Bound morphine}}{\text{Free morphine}}$$

	Plasma (monkey)			Reference	Urine (monkey) 24 hr	Reference	Urine (man) 24 hr	Reference
	1 hr	2 hr	3 hr					
After morphine	2.9	3.3	4.0	187	7–24	187	6.9–9.0	209
After codeine	4.0	3.8	2.0	331	5–14	331	7.2	10

alkaloid when released from codeine, and one must look elsewhere for an explanation of this phenomenon. The mere fact that a certain amount of morphine has been liberated in the body from codeine does not make this condition identical with that in which the same amount of morphine is injected. A major difference between the two conditions is that only in the one case must morphine exert its action in an environment crowded with codeine molecules, and crowded it is by virtue of the ease with which codeine penetrates the central nervous system. The "hostility" of such an environment can be inferred from the work of Miller *et al.* (191), and of Stanton *et al.* (270), who found that the central effects of administered morphine, including respiratory depression and analgesia, can be partially antagonized by codeine. Codeine may act as a "partial agonist" or as an antagonist of morphine (271); in either case, one might expect it to limit the influence of any morphine which it releases, thereby causing a plateau of the codeine dose-effect curve.

The above argument would support the hypothesis that the depressant actions of codeine are due to the liberation of morphine only if it could be demonstrated that the released morphine can gain access to the central nervous system. From the work of Miller and Elliott it might seem that this does not occur since they found evidence of only free codeine in the cerebrum and midbrain of rats 30 minutes after injection of codeine-N-$C^{14}H_3$ at which time the animals were "completely analgetic" (190). Codeine was identified by countercurrent distribution of C^{14} present in a benzene extract of the tissue, and it should be noted that neither morphine nor any norcodeine (which would be nonradioactive in this case) would be detected by this procedure. It is interesting to note that if only 2% of the tissue C^{14} were due to morphine, this would be equivalent to the concentration of C^{14} found in their experiments after injection of 5 mg/kg morphine-N-$C^{14}H_3$, and even lower concentrations than this are found after smaller but still "analgetic" doses of injected morphine (9). Since the experiments of Miller and Elliott were not designed to rule out the possibility that small amounts of C^{14} in the brain may have been due to morphine, their conclusion that the analgetic action of codeine is due to the unaltered molecule must remain tentative until more definitive data are available.

The experiments in which a prolongation of codeine effects occurred when codeine metabolism was inhibited by SKF 525A (58) would seem, at first glance,

to support the view that unaltered codeine is the effective agent. However, SKF 525A only partially inhibits codeine biotransformation to morphine, and at the same time inhibits conjugation of morphine (59, 141). The result of this could be a greater accumulation of free morphine than in the absence of the inhibitor. The lack of information on plasma values of morphine and other codeine metabolites after treatment with SKF 525A places a limitation on interpretations that can be made from these experiments.

The data on the dog comprise the greatest difficulty in attempting to assign a prominent role to morphine in the pharmacology of codeine. In the dog there is very little morphine formed from codeine, *i.e.*, about 2% of the dose, and this small amount is conjugated about 10 times more rapidly than an equivalent amount of morphine injected into the animal (210, 211). Accordingly, if the actions of codeine in the dog were due to morphine, the dose of codeine required for equivalent effects would need to be 500 times that of morphine. Actually, the approximate ratio of codeine to morphine required for certain equivalent effects in the dog is 17 for nontolerant animals and about 6 for tolerant animals. Sanfilippo showed that hyperglycemia produced by 2 mg/kg codeine was equal to that produced by 0.16 mg/kg morphine (244) and that reciprocal cross-tolerance between morphine and codeine required doses of 0.5 mg/kg and 3.0 mg/kg, respectively (246). If the action of these doses of codeine were due to the liberated morphine, it would require not only a conversion of 8 to 16% of the codeine dose, but also that the liberated morphine be conjugated no more rapidly than the injected morphine. It is quite possible that these conditions can be achieved in some strains of dogs but not in others. However, some strains may not be able to convert codeine to morphine at all (332). If there is, indeed, a large variation among different strains of dogs in their capacity to metabolize codeine to morphine, one would expect to find concomitantly an equally large variation in the response to small (nonconvulsive) doses of codeine before one could conclude that the metabolite has any role at all in the pharmacologic effects of the parent compound in this species.

Dextromethorphan, or *d*-3-methoxy-N-methylmorphinan, was found by Brossi *et al.* to be O-dealkylated as well as N-dealkylated in dogs (49). Using paper chromatographic procedures, they found that urine samples collected for 3 to 4 days post injection yielded 2 to 6% 3-hydroxy-N-methylmorphinan and 2 to 3% 3-hydroxymorphinan. It was not established whether these products were present in the free or bound forms since estimations were made for total alkaloid content only. The actual quantities of each substance excreted may be greater than indicated, since by using the same methods these authors obtained values for total levorphanol and dextrorphanol excretion which are lower than those found by other workers (95, 265, 330). Liver microsomes of rats or mice demethylate dextromethorphan less readily than the *levo*-isomer (19, 286), but in either case appreciable amounts of formaldehyde are obtained. The relative contributions of the N-methyl and O-methyl groups to the formaldehyde pool were not determined, but if these are comparable with those of codeine metabolism *in vitro* it may be assumed that the N-demethylation shows a marked

predominance over O-demethylation (286). Until more reliable quantitative data and kinetic studies are conducted with regard to the rate of formation of these metabolic products, the significance of the pharmacology of these agents in relationship to that of their parent congeners cannot be assessed.

Conjugation

Conjugation appears to be an important mechanism for the detoxification of morphine and its surrogates although the manner in which the compounds are conjugated may not always be identical. Morphine has been isolated as a glucuronide, and the evidence indicates that this is the main, if not the only, conjugated form of morphine excreted. It is presumed that the "bound" morphine found in urine with compounds which are metabolized to morphine, *viz.*, heroin and codeine, is also the glucuronide. Likewise, the "bound" forms of other phenolic morphine surrogates, *e.g.*, dihydromorphinone, levorphanol and nalorphine, which are excreted in urine are very likely conjugates of glucuronic acid. Nonphenolic compounds such as codeine and meperidine are also excreted conjugated but the nature of the "bound" forms is unknown.

Development of the evidence leading to the conclusion that conjugation with glucuronic acid is a major pathway for the detoxification of morphine can be traced from early experiments, showing that morphine is excreted in a bound form, to the present knowledge of both the chemical structure of bound morphine and the sequences of enzyme reactions leading to its formation. Earlier workers (182, 275) expressed the possibility that morphine may be bound or conjugated in the body, but the evidence was not conclusive. Endo (83) reported that when the urine from morphinized rabbits was allowed to stand in dilute sulfuric acid, a larger amount of morphine was recovered than from untreated urine. During the past twenty years the nature of bound, conjugated, or combined morphine has been studied intensively by several groups of investigators.

Conclusive proof of the existence of bound morphine was independently demonstrated in a convincing fashion by two groups of workers. Gross and Thompson reported their findings on the dog (120) and shortly thereafter these findings were confirmed in human addicts by Oberst (200). Both groups found that urine collected after morphine administration yielded increased quantities of morphine when subjected to conditions of acid hydrolysis. The morphine liberated from the conjugated or bound morphine was isolated and identified by the usual color tests for morphine, biologic tests (120) and specific rotation measurements (288). Its diacetyl derivative was found to be identical with heroin by mixed melting-point determinations (200, 288).

Most early workers were of the opinion that the bound morphine was a glucuronide. Ashdown (15) and Mayer (184) reported that a glucuronide was excreted in the urine after morphine administration and acknowledged that the original observation was reported by Mering in 1874. Mayer postulated that morphine was conjugated as a glucuronide on the basis of optical rotation measurements made on hydrolyzed urine of patients receiving morphine. Endo felt that morphine was conjugated with glucuronic acid but the English abstract of

his original manuscript (in Japanese) cited no experimental observations to support his conclusion (83). Oberst (201) observed a correlation between the amount of glucuronic acid excreted in the urine and the dose of morphine. Direct proof was lacking, however, that morphine and glucuronic acid were paired with each other. Oberst also prepared a bound morphine, morphine sulfuric ether, and studied its actions and fate, but no evidence exists that such a derivative may be biosynthesized from morphine (205).

Recently three groups of workers reported almost simultaneously that they had obtained evidence that conjugated morphine was a glucuronide (104, 105, 262, 324). Convincing proof was furnished by Woods, who was first to report the isolation of bound or conjugated morphine in crystalline form from the urine and bile of dogs (324). Subsequently, a crystalline product was isolated from the urine of human addicts by Fujimoto and Way (105) which yielded an infrared curve identical with that obtained from the dog (106).

The presence of morphine in bound morphine was established by powder X-ray diffraction analysis of the dinitrophenyl derivative of the hydrolyzed morphine conjugate (106). Morphine liberated after hydrolysis was also identified by paper chromatography (104, 262, 324) and by mixed melting-point determinations of the free base (262), the picrate (262), and the diacetyl derivatives (324).

The product conjugated with morphine was identified as glucuronic acid by various chemical and physical tests. The morphine conjugate after countercurrent distribution was hydrolyzed and analyzed for morphine and glucuronate content; the experimental morphine and glucuronate distribution curves were found to be almost identical (105). The infrared curve of the conjugate was found to give a very strong band in the region to be expected for a polyhydroxy glucuronide compound. The bound morphine, separated by paper chromatography, on hydrolysis with acid or β-glucuronidase yielded a positive test for glucuronide as well as for morphine (262). Elemental analyses were found to be consistent with the assay procedures for a conjugate of morphine with glucuronic acid being combined in the ratio of one to one, and associated with two molecules of water (324).

The molecular site of conjugation of bound morphine with glucuronic acid was established to be at the 3-phenolic position on the basis that a positive test for phenol was obtained only after acid pressure hydrolysis (105). The ultraviolet absorption characteristics of the conjugate in acid and base gave no evidence of the presence of a free phenol (106).

The experimental evidence indicated that the morphine conjugate is a zwitterion. The infrared curve of bound morphine showed a maximum at 6.2 μ with no band between 5.6 and 6.2 microns (106). This was interpreted to mean that the carboxyl group of the glucuronic acid moiety is present in an ionized form. Such an interpretation would necessitate the presence of a positive charge on the piperidine nitrogen. The titration curve of the morphine conjugate gave two pK values (106) which were consistent with the values predicted by Kumler (167) who based his calculations on the assumption that morphine glucuronide exists as an ampholyte.

The question of whether there is more than one form of bound morphine is of considerable interest. Thompson and Gross reported that two bound morphines existed in dog urine, one "easily hydrolyzable" and the other "difficultly hydrolyzable" (288). Woods also suggested that two forms of bound morphine may be excreted in dog urine, the monoglucuronide which is crystalline and possesses low water solubility, and the other possibly a di-conjugated morphine which is amorphous and possesses high water solubility (324). Fujimoto and Way, however, concluded that 3-morphine-mono-glucuronide is the only bound morphine present in appreciable quantity in addict urine. While they did not completely exclude the possibility that other bound forms could be formed, they felt that any quantities formed were of a low order of magnitude (105).

Thompson and Gross (288) derived their conclusion that there are two forms of bound morphine from following the rate of hydrolysis of the bound morphine in dog urine. The morphine fraction liberated by 2 hours of hydrolysis at pH 1 to 2 and 100°C was designated as the "easily hydrolyzable" fraction. The remainder of the bound morphine, which yielded morphine only after 30 minutes in the autoclave in the presence of 5% HCl was called the "difficultly hydrolyzable" fraction. However, kinetic studies on a system as complicated as urine place restrictions on the interpretations of Thompson and Gross. Under the relatively mild hydrolytic conditions of heating to 100°C, the presence initially of any substance in urine which might catalyze the hydrolysis of bound morphine and is slowly destroyed by heat might cause a drop in the absolute rate of hydrolysis of bound morphine over a given time.

Woods based his conclusions for at least two bound forms of morphine on results he obtained with his experiments designed to isolate bound morphine. He was successful in isolating a crystalline substance from the urine which he demonstrated to be morphine-3-monoglucuronide. However, he found a far larger fraction of bound material still present which was amorphous and highly water-soluble, and contained 30% glucuronic acid and 40% morphine. He suggested that the compound was a di-conjugated morphine, the alcoholic group being conjugated as a glucuronide and the phenolic group as an ethereal sulfate. No data on sulfur analysis were given (324). It is of interest to point out that the results with paper chromatographic experiments of Woods are contrary to his conclusions inasmuch as only one bound morphine was noted.

Fujimoto and Way (105) in their studies on urine obtained from addicts found an amorphous, highly water-soluble morphine conjugate which apparently resembled the amorphous bound morphine Woods had found in dog urine. However, after further purification and especially after removal of ninhydrin-reacting material, a crystalline substance was isolated which was identical with the morphine-3-glucuronide isolated by Woods. Since this crystalline monoglucuronide was entirely derived from the amorphous material, it was concluded that the crystalline and amorphous bound morphine actually represented simply different states of a single substance. Moreover, since the amorphous conjugate was found in large amounts in the urine and was the only bound morphine noted, it was concluded that only one form of bound morphine is excreted in the urine

in any appreciable quantity (105). The paper chromatography studies of Woods (324) and of Seibert *et al.* (262) were also cited in support of the argument for a single main form of bound morphine. Subsequently, Woods also found that the water-soluble amorphous bound morphine in dog urine can be changed into the crystalline poorly water-soluble substances by manipulative technics (326). Thus, it would appear that morphine-3-monoglucuronide may be strongly associated with substances in urine which enhance its water solubility and prevent it from crystallizing. It is quite possible that the difference in physical properties between the "easily hydrolyzable" and the "difficultly hydrolyzable" forms of morphine found in dog urine may be related to these factors of association with extraneous substances.

A great deal of information of the sequences leading to the conjugation of morphine has been gained from studies using isolated tissue preparations. The most extensive studies have involved the liver and have resulted in considerable clarification of early observations that morphine "is altered" or "disappears" when perfused through the liver (135, 237) or incubated with liver mince or slices (34, 35, 68, 89, 146, 338).

Inoue (146) studied the ability of the liver tissues of various animal species to metabolize morphine and concluded that this factor may be a cause (but not necessarily the chief one) of the differences in the natural tolerance among various animal species. It appears to the reviewers, however, that the experiments as presented were inadequate to establish the point.

Bernheim and Bernheim found that when morphine was added to rat liver slices, the compound disappeared under aerobic conditions. Kidney and brain slices were ineffective. Liver cell suspensions showed reduced activity. They concluded that oxidation of morphine had resulted, and ruled out conjugation as a possible mechanism (34). In a second study, however, they reported that their method for determining conjugated morphine was inadequate (35). After applying Gross and Thompson's method (120) for bound morphine, they concluded that conjugation and not oxidation accounts for the disappearance of morphine when the compound is incubated with liver slices of the dog, cat, rat, and guinea pig. The reaction is completely inhibited by M/1500 iodoacetic acid, M/500 sodium cyanide, or M/50 sodium fluoride. With respect to the cat, the disappearance of morphine in the presence of liver slices should be explored more thoroughly inasmuch as it has been reported that cats do not form glucuronides *in vivo* (241).

A fairly extensive store of knowledge has been attained in recent years which emphasizes the importance of carbohydrate metabolism in the formation of glucuronides by liver preparations. It appears that the key substance in glucuronide synthesis is uridine diphosphate glucose, and that this compound is derived from uridine triphosphate and glucose-1-phosphate (192). Since ATP is required for both hexose phosphate formation and resynthesis of uridine triphosphate, the availability of carbohydrate substrates and the regeneration of ATP should greatly influence the ability of liver slices or liver mince to conjugate morphine. In fact, with rat liver slices respiring in Krebs-Ringer solution,

the replacement of sodium by potassium resulted in the concomitant delay of glycogenolysis and of morphine conjugation, although the potassium had no effect on conjugation of morphine when glucose was added to the medium (181). It is quite possible that depletion of carbohydrate stores, which in turn limits the amount of uridine diphosphate glucose formed, is primarily responsible for the impaired ability to conjugate morphine observed in liver mince obtained from traumatized rats (116) or from tolerant rats during withdrawal (68).

The importance of uridine diphosphate glucose in the conjugation of morphine was first suggested by the studies of Strominger and his colleagues following the demonstration by Dutton and Storey (69a) that uridine diphosphate glucuronic acid (UDPGA) participates in glucuronide synthesis. Strominger *et al.* (276) have shown that when the supernatant fluid from homogenates of guinea pig or calf liver containing both microsomes and soluble enzymes was incubated with uridine diphosphate glucose, diphosphopyridine nucleotide (DPN^+), $MgCl_2$, and morphine, a marked reduction in free phenol concentration occurred. It may be inferred that synthesis of morphine glucuronide took place under these conditions, although the product was not isolated and identified. Further experiments indicated that uridine diphosphate glucose was first oxidized to uridine diphosphate glucuronic acid by a dehydrogenase system present in the particle-free supernatant fluid, and that for every mole oxidized two moles of DPN^+ were reduced.

Strominger *et al.* (278) have purified the enzyme from calf liver, and Strominger and Mapson (277) have obtained a purified enzyme from pea seedlings and reported that it closely resembles the one from calf liver. The enzyme-catalyzed oxidation of uridine diphosphate glucose is inhibited by sodium fluoride but not by iodoacetate. The product of oxidation is not a substrate for β-glucuronidase, indicating an α-linkage of glucuronic acid in uridine diphosphate glucuronic acid. An enzyme or enzymes present in the microsomes catalyze the transfer of the glucuronic acid moiety of UDPGA to morphine. Several steps may be involved in this reaction since the transferase has no β-glucuronidase activity (149). Thus, an inversion of the α-linkage of glucuronic acid probably takes place just prior to or during the final coupling with morphine.

Recently Inscoe and Axelrod (147) have obtained data showing that the activity of the glucuronyl transferase system of rat liver microsomes *in vitro* is sex-dependent and can be markedly enhanced by previous treatment of the rat with androgens or a carcinogenic polycyclic hydrocarbon *in vivo*; conversely, the activity can be as dramatically reduced by pretreatment of rats with estrogens. Whether these observed effects on the behavior of the glucuronyl transferase system *in vitro* have any bearing on the conjugation of morphine *in vivo* remains to be established.

In general there has been a consistent lack of success in attempts to show that there are parallel changes in the conjugation of morphine *in vitro* and *in vivo* as a result of varying conditions *in vivo*. Thus, it has been reported that liver slices from morphine-tolerant rats have an increased (338) or unchanged ability (89, 315) to conjugate morphine, whereas morphine-tolerant rats apparently

conjugate less morphine *in vivo* as evidenced by a reduced urinary excretion of the metabolite (315, 338). Liver slices from adrenalectomized rats are either unable to conjugate morphine at all (338) or show no change from normal (315), whereas adrenalectomized rats show higher concentrations of both free and conjugated morphine in the plasma than are found in normal rats (9). The ability of liver slices from ACTH-treated rats to conjugate morphine is increased (338), but plasma levels of conjugated morphine in ACTH-treated rats are lower than those in normal rats (9).

Extensive conjugation of *codeine* occurs. This process constitutes the major route for disposal of codeine since a large fraction of the administered dose can be accounted for in urine as conjugates of codeine and its two metabolites, norcodeine and morphine. None of the conjugated bases excreted after codeine administration has been isolated and identified as a chemical entity; evidence for their occurrence is indirect and rests on the increase in free (*i.e.*, extractable) alkaloid resulting from strenuous acidic hydrolysis. It is presumed that conjugation of codeine or of norcodeine occurs at the 6-position, since the secondary hydroxyl appears to be the most likely position available for such a reaction. Preliminary experimental evidence suggests that bound codeine contains a glucuronic acid moiety (7); it is quite likely that the bound morphine arising from codeine metabolism is morphine-3-glucuronide (106) since the structure of codeine-released morphine is identical with authentic morphine, and there is no reason to believe the bound forms would differ. No experimental evidence has been presented concerning the structure of biosynthetic bound norcodeine.

Heroin is excreted as bound morphine in urine (201, 204), presumably as the glucuronide. The results are described in greater detail in the section on *hydrolysis*.

Certain *dihydro-derivatives of morphine* are also conjugated in the body. Oberst (201) studied the urinary excretion of several 7,8-dihydromorphine derivatives in experiments in which the derivatives were substituted for morphine in morphine addicts. No studies were done with dihydromorphine or dihydromorphinone, but the relative amounts of free and bound alkaloid after dihydroheterocodeine (8% of the dose recovered as free, 38% as bound) were in the same range as those found for morphine or for codeine in these subjects. However, when dihydrocodeine, dihydroisocodeine, or dihydrocodeine methyl ether was administered there was a considerable reduction in the relative amount of the dose excreted as bound alkaloid (7 to 18%) and an appreciable increase in the excretion of the free form (13 to 22% of the dose). In the dog, after subcutaneous injection of 20 mg/kg desomorphine, 5% of the dose was excreted as free and 36% as bound alkaloid (324).

Nalorphine is conjugated *in vivo* in the dog and in the rat (331). Nalorphine is also conjugated *in vitro* in the presence of rat liver slices, and the rate of disappearance of the phenol group is approximately the same as that observed with morphine as substrate (261).

In the dog, after subcutaneous injection of tritiated-nalorphine only about half of the H^3 excreted in 24 hours can be identified as free or bound drug in the

urine. About 5 to 6% of the dose is found in a 24-hour urine specimen as free nalorphine, and 27 to 40% as conjugated nalorphine (329). In the dog after subcutaneous administration of nalorphine the urinary excretion of conjugated nalorphine represents 42 to 56% of the dose and is characterized by prolonged excretion up to 24 hours after injection. Thus, of the total amount of bound nalorphine to be excreted in the 24-hour period about one-third is excreted between the 7th and 24th hour after injection. Since the low tissue concentrations rule out the possibility that conjugated nalorphine is sequestered by the tissues, the delayed excretion probably represents reabsorption from the gut. Practically all of the alkaloid content of gall bladder bile is conjugated nalorphine and represents between 1.5 and 6.0% of the dose 4 hours after injection (331).

Morphinan congeners having a free phenolic group are also excreted as conjugates, probably of glucuronic acid. The most important compound in this group is 3-hydroxy-N-methylmorphinan (251, 252). The racemate (racemorphan), the *l*-isomer (levorphanol), and the *d*-isomer (dextrorphan) are excreted in the urine as bound products as evidenced by the fact that increased amounts of the compounds can be obtained after acid hydrolysis. The initial studies were made by Fisher and Long (95) on dogs, and their findings were confirmed subsequently in the dog, monkey, and rat (265, 330) by other workers. Fisher and Long found also that incubation of bound racemorphan with β-glucuronidase resulted in an increased yield of free racemorphan (95).

While the morphine-like effects of the morphinan isomers are attributed generally only to levorphanol (31, 101, 148), the body apparently disposes of all three forms in a highly similar manner. Fisher and Long compared the urinary excretion of racemorphan, levorphanol, dextrorphan and a mixture of the *l*- and *d*-isomers in dogs after giving equivalent amounts of each drug subcutaneously. The percentage of each preparation accounted for in the urine in the free and bound forms was quite similar, the total (free and bound) percentage recovery for each substance being between 42 and 62 of the dose administered (95). Shore *et al.* also found that each isomer yielded virtually identical amounts of the bound form in the urine after intraperitoneal administration, the percentage recovered for levorphanol averaging 56 and for dextrorphan 59 (265).

Levallorphan has been reported to be excreted as three conjugates, namely that of levallorphan, of 3-hydroxymorphinan, and of an unknown oxidized metabolite. When the urine of rats receiving levallorphan was subjected to acid hydrolysis, increased amounts of the three substances were noted (177).

It appears reasonable to conclude that conjugation with glucuronic acid represents a detoxification process. At least this is true in the case of morphine since morphine-mono-glucuronide was found to be less active pharmacologically than its parent compound (324). More rigid proof requires that pharmacologic tests be performed on each conjugate of the morphine surrogates but such experiments must await the isolation of these compounds.

Conjugation with substances other than with glucuronic acid may occur with the morphine surrogates. *Anileridine* conjugates with acetic acid to yield acetylanileridine, part of which is excreted and part deesterified to yield acetylanileri-

dinic acid. The acetyl derivative was determined as anileridine after hydrolysis of the N-acetyl group under conditions which did not effect hydrolysis of the ethyl ester grouping. The N-acetyl anileridine appeared in tissues within an hour in high concentrations, in fact, generally exceeding those of anileridine. Relatively large concentrations of acetylanileridine appeared in the lungs, liver, and kidneys, while brain acetylanileridine accumulated more slowly and to a lesser extent (227).

Meperidine appears in the urine of man as conjugates of its two biotransformation products, meperidinic acid and normeperidinic acid, which are formed *in vivo* by hydrolysis of meperidine and normeperidine; the latter product is formed initially by N-demethylation (50, 220, 222). The presence of meperidinic and normeperidinic acid was first established by demonstrating that the meperidine and normeperidine concentrations in urine increased after esterification with absolute ethanol in the presence of sulfuric acid (220). An even greater recovery of meperidine and normeperidine resulted after subjecting urinary constituents to acid hydrolysis before esterification, indicating the presence of bound forms of meperidinic and normeperidinic acid (222). The identity of the conjugate forms has not been established. Bound forms of meperidinic acid and normeperidinic acid were not found in urine of rats.

More recent findings with respect to conjugation processes other than reaction of the phenol with glucuronic acid, such as phosphorylation of phenols (138) and conjugation of nitrogen of aromatic amines with glucuronic acid and with sulfonic acid (41), have not been reported for morphine and its surrogates.

Hydrolysis

Hydrolysis is a major metabolic pathway for the compounds in this series that are esters, *e.g.*, heroin, meperidine, anileridine, and ethoheptazine. This metabolic pathway is usually regarded as a detoxification process since the liberated acid may be expected to undergo rapid excretion in the urine. However, with respect to heroin, which will be discussed in greater detail below, its hydrolysis may result in either detoxification or activation depending on whether the reaction occurs at sites remote from the target organ or in the central nervous system, respectively.

The possibility that *heroin* might be deacetylated has been considered by several investigators because of the lability of the acetyl groupings on the compound, particularly the 3- or phenolic acetyl group. Two groups of workers (71, 335) independently suggested that heroin may act principally as 6-monoacetylmorphine (6-MAM) because the two compounds were found to be virtually equipotent in pharmacologic activity. Rizzotti reported that the perfused frog heart metabolized heroin rapidly to 6-MAM and then more slowly to morphine. While the end-products of heroin hydrolysis were not isolated, differentiation of 6-MAM from morphine was made by color response to iodic acid. The deacetylation of the phenolic acetyl group of heroin required less than 1 hour; deacetylation of the alcoholic acetyl was much slower so that after 25 hours only 20 to 30% of the added heroin was accounted for as morphine (238, 239).

Wright furnished indirect evidence that heroin is metabolized to 6-MAM and morphine by serum and other tissues. On the basis that acetic acid liberated from hydrolysis of heroin would decompose a bicarbonate solution, manometric technics were applied to measure the amount of CO_2 evolved. On incubating heroin and rabbit serum in the presence of sodium bicarbonate, he found a rapid release of carbon dioxide during the first 20 minutes, the quantity of carbon dioxide liberated being proportional to the amount of heroin added (333, 334).

From comparisons of the respective rates of hydrolysis of heroin and 6-MAM and the observed differences in enzymatic activity of various tissues from the rabbit and rat, Wright concluded that the 3-acetyl rather than the 6-acetyl was hydrolyzed first from heroin. All tissues could deacetylate heroin at both the 3- and the 6-position, but 6-MAM caused the evolution of carbon dioxide much more slowly than did heroin. Further evidence that the 3-acetyl is more labile than the 6-acetyl group was obtained from studies carried out on two closely related pairs of heroin congeners, namely diacetyldihydromorphine and 6-acetyldihydromorphine. In rat kidney, serum, or brain the latter compound, which is more stable chemically than 6-MAM, was not hydrolyzed to any measurable degree whereas 3,6-diacetyldihydromorphine caused liberation of carbon dioxide in an amount equivalent to the removal of a single acetyl group (333, 334).

Shen reported that morphine was present in the urine of addicts and of rabbits receiving heroin, but details are lacking for evaluation of his technics (264). Oberst (204) also reported that urine of addicts given heroin contained morphine, but he indicated also that this work does not establish morphine as an actual metabolic product of heroin since the extraction procedures used would have resulted in the hydrolysis of any urinary heroin or 6-MAM to morphine. Earlier, he reported that about one-half the dose of heroin in morphine addicts could be accounted for in the urine as morphine and its conjugate. Of the excreted morphine, 5.8% was in the free form and 43.5% in the bound form, the ratio of bound to free being 8.7 (201). In the follow-up study (204), he found almost identical results. Since this ratio was not significantly different from that previously established in the same subjects with morphine, and since the percentage recovery of morphine from the individuals both in the free and bound forms was also no greater than with heroin, he concluded that the heroin was hydrolyzed completely to morphine in the body.

More recent studies have yielded direct evidence for the presence of 6-monoacetylmorphine and morphine as metabolic products of heroin (311, 318). Way *et al.* noted from incubation studies that homogenates of brain, blood, liver, and kidney of humans, dogs, rats, rabbits, and mice are quite active in converting heroin to 6-MAM and, to a lesser degree, to morphine; the liver was also found to possess considerable activity in the latter respect. Identification of 6-MAM and morphine was established by paper chromatographic studies. Similar findings were noted with whole mouse homogenates incubated with heroin or with homogenates recovered from animals injected with heroin. In addition, counter-

current distribution of an extract of whole mouse homogenate incubated with heroin yielded a substance with partition characteristics identical with those of 6-MAM, and a crystalline substance was isolated from mouse homogenate incubated with heroin which gave infrared absorption spectra identical with that of synthetic 6-MAM. Morphine was identified also by countercurrent distribution of extracts of brains removed from rats receiving heroin (311). A recent study on the rat, using paper chromatographic technics, indicates that 6-MAM may be excreted in the urine, but quantitative studies using the methyl orange procedure indicate only very low concentrations of the compound (318).

It is of interest to evaluate the possible contributions of 6-MAM and morphine to the effects of heroin.

Wright (333, 334) concluded that the rate of conversion of heroin to morphine by the liver alone was of sufficient rapidity so that any injected heroin would act as morphine. However, his interpretation of the experimental data is difficult to follow because some of his conclusions appear to be contradictory. He questioned his own earlier interpretations (335), as well as those by Eddy and Howes (71) that the pharmacologic similarity between diacetylmorphine and 6-MAM is due to the former's being converted to 6-MAM in the body. He felt that the similarity of the compounds was due instead to the fact that both substances were converted rapidly to morphine, and that both would reach the sensitive tissues at the same rate and concentration. To get around the well-known fact that heroin is more active than morphine, he argued that the activity of morphine might be limited by its solubility. He believed that with subcutaneous administration, morphine would be precipitated in the presence of bicarbonate in the tissues and be slowly absorbed, whereas heroin, with its greater solubility, would be absorbed more rapidly. Its rapid biotransformation would then yield higher circulating levels of morphine and hence greater pharmacologic effects. He appears to refute his own arguments by citing evidence that morphine, after intravenous injection, is considerably less toxic than heroin, and, in order to rationalize these findings he suggested that precipitation of morphine and "incomplete deacetylation" of heroin occurred in the blood stream. The meaning of the latter interpretation is not clear since to us it implies greater potency for heroin *per se*, and would be antithetical to the argument that heroin effects arise out of morphine formation. It is also difficult to reconcile why morphine, given intravenously, should precipitate in the blood stream, whereas any morphine arising from rapid absorption and rapid deacetylation of heroin after subcutaneous administration should not.

More recently an attempt was made by Way *et al.* (311) to delineate the respective roles of heroin and its metabolic products in the over-all pharmacologic effects in terms of the accessibility of the compounds to the central nervous system rather than their differing rates of absorption as proposed by Wright (333, 334). The rate of uptake of heroin by the brain was determined in the mouse, as well as the rates of conversion of heroin, 6-MAM and morphine by mouse tissue *in vivo* and *in vitro*. Heroin was found to disappear rapidly from the body; the biologic half-life after intravenous administration of near-lethal

doses was about 2½ minutes. The heroin disappearance was accompanied by rapid appearance in the brain of 6-MAM and soon thereafter by morphine. Although the morphine did not reach as high a level as 6-MAM, it persisted longer in the animal (311).

These results were correlated with the relative toxicity of heroin, 6-MAM, and morphine by subcutaneous, intravenous and intracerebral administration. In order of decreasing toxicity the order was: subcutaneous—heroin = 6-MAM > morphine; intravenous—heroin > 6-MAM > morphine; intracerebral—morphine > heroin > 6-MAM.

It may be concluded that the pharmacologic effects of heroin, excepting possibly for a brief interval during the initial phase of drug effect, are mediated primarily by 6-MAM and morphine. The predominance of a particular compound is dependent largely upon the route of administration and the time interval after administration. With intravenous administration, especially with rapid injection, heroin probably exerts some direct action on the central nervous system inasmuch as immediate effects are observable and the toxicity of heroin by this route is greater than that of 6-MAM or morphine. However, since the toxicity of heroin approaches that of 6-MAM with a slower rate of infusion, and since heroin disappears within a few minutes from the brain and animal body during which time pharmacologic effects are still evident, it is doubtful that heroin *per se* exerts any effects after this interval. On the other hand, since there is rapid appearance of 6-MAM and morphine, these two substances must be important as active metabolites (311).

With subcutaneous administration, very little, if any, heroin can exert effects *per se*, and hydrolysis of heroin to 6-MAM precedes any pharmacologic effects since heroin and 6-MAM have the same LD50 by this route and 6-MAM has a rapid rate of appearance in the animal after heroin administration. Morphine also must contribute considerably to the pharmacologic effects of heroin since there was relatively rapid rate of appearance of morphine after 6-MAM formation (311).

The question concerning the degree to which effects observed after heroin administration can be assigned to 6-MAM or morphine can also be considered in terms of the concentration of the compounds in the central nervous system and their potency at this site. It would appear from the brain uptake studies that heroin penetrates the central nervous system largely as 6-MAM, and to a considerably lesser degree as morphine. The fact that morphine has relatively low toxicity after intravenous and subcutaneous administration as compared with heroin and 6-MAM indicates that morphine does not gain access to the central nervous system as readily as the other two compounds. However, because of the high potency of morphine with intracerebral injection, it may be argued that only small amounts need to be present to elicit an effect, and that adequate amounts of morphine in the brain can be furnished by heroin through penetration as 6-MAM followed by local deacetylation to morphine. The rate of deacetylation of 6-MAM would determine whether morphine or 6-MAM or both compounds would combine with the receptor sites. It was not possible at this

time to state the contribution of each compound during the peak of heroin action, because while morphine was shown to be at least five times more potent than 6-MAM by the intracerebral route, the concentrations of 6-MAM attained in the central nervous system exceeded by several-fold the concentrations of morphine in the same organ. The prolonged effects of heroin are probably due to morphine since pharmacologic effects and morphine levels are still apparent after the disappearance of heroin and 6-MAM from the animal. It was also reported that preliminary studies in other animal species *in vitro* and *in vivo* indicate that in all probability heroin acts in a similar manner (318).

Virtually all mammalian tissues appear to be capable of deacetylating heroin at both the 3-carbon and 6-carbon positions. Wright (333, 334) reported on the distribution of this enzymic activity in tissues of the rabbit, rat, and human, and Ellis (81) on the rabbit and guinea pig. Massart and Dufait reported that heroin was hydrolyzed by horse serum (183). Wright found that the blood sera of rabbits could be classified by their deacetylating ability into two types. One class contains enzyme(s) capable of deacetylating heroin at both the 3- and the 6-positions, although the latter position is not as susceptible to attack. The second class contains enzyme(s) capable of hydrolyzing only the phenolic acetyl grouping since the sera from this group liberated CO_2 when incubated with heroin but not with 6-MAM (333).

Wright (334) later studied other tissues from the rabbit as well as those from the rat for ability to hydrolyze heroin. Of the tissues examined, liver exhibited the highest activity followed in order by the kidney, brain, blood, serum, and muscle. Human liver, like rat and rabbit liver, was found capable of hydrolyzing heroin, dihydroheroin and their corresponding monoacetyl derivatives. In fact, human liver was found to be more active than either rat or rabbit liver in deacetylating 6-MAM and dihydro-6-acetylmorphine. Ellis (81), using rabbit plasma and liver and guinea pig liver, confirmed the studies of Wright.

Wright (333, 334) concluded that the enzyme that hydrolyzed heroin could not be a cholinesterase since he found that rabbit sera which had almost identical abilities to hydrolyze acetylcholine differed in their capacity to deacetylate heroin and 6-MAM. In a follow-up study he furnished additional evidence to support his conclusions. He found that the order of activity for rat tissues with acetylcholine as substrate was: brain > liver > serum, kidney. Brain had as much as twenty times more cholinesterase activity than kidney in some of the animals. The order for the deacetylation of heroin in the same animals was: liver > kidney > brain > serum. Thus, he concluded cholinesterase is not involved in the deacetylation of heroin either at the 3-carbon or the 6-carbon position.

Ellis (81) confirmed the studies by Wright and suggested that the hydrolysis of the 3-acetyl group of heroin appears to be a function of tributyrinase but not of the enzymes which hydrolyze methylbutyrate, acetylcholine, or acetylsalicylic acid. He found that hydrolysis of heroin was rapid in guinea pig plasma and liver, with far greater activity present in the liver. Since the plasma of the guinea pig hydrolyzes acetylcholine rapidly and acetyl-*beta*-methylcholine

slowly, and its liver has little activity on either substrate (80, 247), Ellis (81) concluded that the enzyme which hydrolyzed heroin could not be acetylcholinesterase or pseudo-cholinesterase. He concluded also that heroin was not hydrolyzed by the enzyme which attacks acetylsalicylic acid inasmuch as the latter was hydrolyzed rapidly by guinea pig plasma but only slowly by plasma of man, dog, and rabbit. Heroin, on the other hand, was hydrolyzed rapidly by both rabbit and guinea pig plasma, slowly by human, and not at all by dog plasma.

Meperidine is hydrolyzed in the body to its corresponding acid (meperidinic acid), and the latter appears in the urine as such and as an acid conjugate. Moreover, the demethylated metabolic product of meperidine, *i.e.*, normeperidine, undergoes further biotransformation to normeperidinic acid and its conjugate. The evidence establishing these points has been presented earlier in the section on conjugation.

The liver appears to be the chief organ for hydrolyzing meperidine. Bernheim and Bernheim (36), using manometric methods, reported that meperidine is hydrolyzed *in vitro* by the liver homogenates of puppies, rabbits, rats, guinea pigs, cats, turtles, and frogs, but not by the brain, blood, kidneys, spleen, or heart. The hydrolysis of meperidine was found to be inhibited by eserine and the fluoride ion. It was postulated that the enzyme which hydrolyzed meperidine is different from the known tropine esterases, cholinesterase, and the esterases which hydrolyze aliphatic esters.

The above experiments were confirmed *in vitro* by Way and Gimble (317) with the liver from rat, dog, and man using concentrations of meperidine compatible with expected levels to be found *in vivo*. Meperidine was found to disappear rapidly when added to liver homogenized in phosphate buffer. Other tissues exhibited little or no activity. *In vivo* evidence demonstrating the importance of the liver as the site of biotransformation is based on the finding that a pharmacologic effect (potentiation of thiopental depression) and the blood levels of meperidine are increased in rats after partial hepatectomy (317).

It is of interest to consider the immediate source of normeperidinic acid, which could conceivably result either from hydrolysis of normeperidine or from N-demethylation of meperidinic acid. It was found that after administering a hydrolyzed solution of meperidine-N-C^{14} to rats no $C^{14}O_2$ was present in the expired air (221). No $C^{14}O_2$ was produced by rat liver slices after incubation with a solution of hydrolyzed N-$C^{14}H_3$-meperidine whereas under similar conditions with N-$C^{14}H_3$-labeled meperidine $C^{14}O_2$ was evolved (221). Moreover, it has been reported that an enzyme system in liver microsomes demethylates meperidine but not meperidinic acid (109). Finally, after administering meperidinic acid to humans intravenously, no normeperidinic acid was found in the urine, whereas normeperidine administration resulted in large amounts of normeperidinic acid in urine (50). Thus it appears that normeperidinic acid results primarily from hydrolysis of normeperidine, and that meperidinic acid is not N-demethylated to any significant degree in the body.

Meperidinic acid and normeperidinic acid would be expected to be excreted rather rapidly, and it would be unlikely that these compounds would be more

potent than meperidine. In support of this conclusion it has been shown that meperidinic acid is less active than meperidine with respect to "analgesia" and toxicity (174a). Carbon dioxide (220) and ethanol may also be considered as meperidine metabolites, although the presence of ethanol has not actually been established. The small amount possibly released would contribute little to the pharmacologic effects of meperidine.

Anileridine is hydrolyzed to anileridinic acid which is excreted in the urine. A conjugate of this hydrolyzed product, acetylanileridinic acid, also appears in the urine. It has not been established whether in the formation of the latter compound hydrolysis precedes acetylation or *vice versa*. The more logical guess would favor acetylation as the first step followed by hydrolysis since the more basic metabolite, acetylanileridine, should not be as rapidly excreted as the acidic metabolite, anileridinic acid.

Hydrolysis of *ethoheptazine* by three routes is indicated. These pathways include hydrolysis to the corresponding acid, oxidation to a hydroxy derivative which may further undergo hydrolysis, and possibly N-demethylation to the corresponding nor-derivative which may subsequently be hydrolyzed. The evidence was obtained mainly from studies on the urine of dogs given ethoheptazine. Biotransformation of ethoheptazine by hydrolysis was established by identification of the corresponding acid derivative by paper chromatography, electrophoresis and infrared absorption. Limited studies on rat urine and rabbit urine also indicated the presence of ethoheptazine hydrolytic products (303).

Other metabolic pathways

From the foregoing evidence it appears that although much is known about the disposition of morphine and its surrogates, in no case has the metabolic fate been completely established. While conjugation, dealkylation, and hydrolysis constitute the predominant metabolic pathways there are many indications that with certain of the compounds other routes may also be of considerable importance. Some of these pathways are, of course, unknown, with evidence for their occurrence resting solely on the discovery of small amounts of unidentified metabolites. In many cases, however, oxidation is implicated either directly, by evidence that a hydroxyl group has been added, or indirectly by finding that the metabolism does not occur except under aerobic conditions.

Since *oxidation* of *morphine* to pseudomorphine (also known as dehydromorphine, oxydimorphine or 2,2′-bimorphine) occurs very easily under mild chemical conditions (33), it is not surprising that this has been presumed to occur under biological conditions as well. Although pseudomorphine exhibits some striking pharmacological properties following intravenous injection, especially on the cardiovascular system where it produces intense depressor effects (250, 293), it is of special interest because it has long been considered to be responsible for some of the effects ascribed to morphine, particularly the withdrawal syndrome. This hypothesis, however, has received practically no support from experimental evidence in the past, as can be judged from Krueger's review of the conflicting and inconclusive findings (166).

More recent attempts to establish the presence of pseudomorphine in tissues after morphine administration have been unsuccessful. Fichtenberg (90) has reported that pseudomorphine is absent from blood or muscle of normal rats or from blood, muscle, or liver of habituated rats after injection of large doses of morphine. Her conclusions are based on the results of a bioassay procedure reported to be sensitive to quantities of pseudomorphine of the order of 20 to 50 μg/ml of extracted solution. Hosoya and Brody (143) have demonstrated the formation of a compound *in vitro*, indistinguishable by chromatographic analysis from authentic pseudomorphine, by rat liver homogenates when fortified with cytochrome *c* and incubated with morphine under aerobic conditions. Under these conditions there was a concomitant reduction in formation of morphine glucuronide which normally takes place *in vitro* in the absence of added cytochrome *c*. These authors, however, were unsuccessful in their attempts to demonstrate the presence of the compound in rat liver after morphine administration *in vivo*. Inasmuch as chromatographic homogeneity in any one solvent system does not constitute proof of identity, it is possible that the compound obtained by Hosoya and Brody is something other than pseudomorphine. It is interesting to note that the chromatographic behavior of this compound resembles that of an unknown morphine metabolite present in the urine of a strain of rats showing low urine and plasma concentrations of bound morphine after injection of morphine-N-$C^{14}H_3$. The metabolite was absent from the urine of another strain of rats showing 2- to 3-fold higher values for the excretion and plasma values of bound morphine. With both strains a small fraction of the dose of C^{14} was found in brain and skeletal muscle in a form that was neither free nor bound morphine (8). Part of the unidentified C^{14} in muscle has been found to be chromatographically homogenous with pseudomorphine but different from the latter in its spectrophotometric properties (T. K. A., unpublished data). It is thus apparent that a small part of the dose of morphine is metabolized in some unknown manner but not necessarily by oxidation to pseudomorphine.

An "oxidized morphine" has been recently produced by partial chemical oxidation of morphine; the compound is different from pseudomorphine and is pharmacologically more active than morphine in many respects (328). However, a full account of the work has not been published and the preliminary announcements give no indication that the compound can be derived from morphine by biotransformation.

Levallorphan, although having a phenolic grouping at the 3-position, is *oxidized* to an unknown hydroxyl derivative which is excreted in relatively small amounts in the urine as the free and bound alkaloid (177). The compound is a phenolic alkaloid with a high melting point (254 to 256°C) and has an empirical formula of $C_{19}H_{25}O_2$. It is not known just where the additional atom of oxygen is attached, but the authors have established that the following positions in the levallorphan molecule have apparently not been subject to oxidation, *viz.*, the allyl side chain, the piperidine nitrogen, and carbon atoms 2, 4, and 10 of the phenanthrene skeleton. The metabolite is formed *in vivo* and *in vitro* by liver

slices of rats, mice, and rabbits, although the metabolite may be slightly modified in the case of the rabbit. Guinea pigs and dogs fail to produce this oxidation product either *in vivo* or *in vitro* (177).

Oxidation of *ethoheptazine* was demonstrated by isolation of the hydroxy derivative and determination of its identity by elemental and infrared absorption analyses. The latter studies indicated also that the hydroxyl group was present on the hexamethylenimine ring albeit the precise position of the substitution was not determined (303).

The possibility that nonphenolic "narcotic" derivatives may be oxidized to phenolic compounds should be considered. Such a reaction might well lead to active metabolites especially if the oxidation occurs at a site corresponding to the 3-position on the morphine molecule. If oxidation of *meperidine* occurs at the *m*-position of the benzene ring, this would yield the *meta*-hydroxylphenyl analog of meperidine, known as bemidone, which is a potent analgetic agent (258). Wang and Bain considered the inhibition of DPN-cytochrome reductase activity in rat brain homogenates by morphine and its congeners to be related to the phenolic group on the molecule, yet meperidine and *l*-methadone exhibited some activity (307). Rickards *et al.* postulated that *methadone* might be metabolized to a phenolic derivative (236). It appears worthwhile, therefore, to investigate whether meperidine or methadone can be converted to phenolic derivatives.

Preliminary evidence suggests that *methadone*, *codeine*, and *morphine* give rise to unknown metabolites in the rat. A biotransformation product of methadone was noted in the bile which was found to be more soluble in organic solvents than the parent compound (190). A small part of a dose of codeine is also metabolized in some unknown manner according to the work of Latham and Elliott (170), who showed that after injection of codeine-O-$C^{14}H_3$ in the rat the intestinal tract contained a C^{14}-basic amine with solubility characteristics different from those of codeine. This material, designated as codeine-X, is not norcodeine (10). It appears to resemble a substance found by Woods (332) in small amounts in autoclaved dog urine after codeine injection. It has been reported that two nontertiary amine alkaloids appear in the liver and brain after administration of large doses of morphine (214). Since the substances were obtained also after prolonged incubation of normorphine with homogenates of liver, they could reflect further degradation following N-dealkylation of morphine. However, the significance of these findings must await identification and quantification of each substance.

It is quite probable that the *levo*-isomer of *α-acetylmethadol* is metabolized to a product which possesses significant pharmacologic activity. The parent compound has a delayed onset of action (72, 98, 160) and there appears to be little correlation between the observable morphine-like effects and the levels of *l*-acetylmethadol attained in the organs (282). It is of interest to note also that *l*-acetylmethadol does not fit the stereoconfigurations postulated by Beckett and Casy for analgetic activity (25) and perhaps morphine-mimetic properties are conveyed to the compound after biotransformation.

Tolerance and physical dependence in relation to biologic disposition

It is well established that with morphine-mimetic compounds development of tolerance is generally accompanied by a development of physical dependence on the agent. Indeed, the intimate relationship between physical dependence and tolerance has led many investigators to believe that a common underlying or closely related mechanism is involved and that this mechanism operates within the matrix of the central nervous system (23, 260a, 319a, 319b).

Numerous studies have been directed toward an attempt to delineate differences between the biologic disposition of morphine or its surrogates in nontolerant and tolerant animals (18, 50, 53, 54, 55, 89, 120, 146, 158, 178, 179, 187, 206, 217, 218, 219, 236, 266, 283, 285, 288, 290, 315, 323, 324, 336, 338). The findings have not always been in complete accord but evaluation of the conflicting results is difficult because the criteria for tolerance are not the same for different authors. It is to be expected that interpretations based on assessment of different pharmacologic responses to a drug administered under different conditions, with respect to dose, frequency, and duration of treatment in different animal species, will not always be in harmony. Certain differences were undeniably found on occasion to exist between tolerant and nontolerant animals with respect to the distribution and excretory pattern of morphine or its surrogates but, as we see it, these studies in general fail to get at the heart of the problem since they usually emphasize events occurring at a site other than the target organ.

It appears to us that it is important to distinguish between central receptor tolerance, which would be linked to physical dependence, and nonrelated mechanisms that simply prevent access of the agent to the locus of action. The latter include such factors as decreased absorption, increased excretion rate, increased metabolic rate, increased plasma binding, altered tissue distribution, increased blood-brain barrier, *etc.* We believe these factors are not relevant since they cannot be invoked to explain a phenomenon which presumably requires availability of the agent for combination with specific receptors within the central nervous system. Moreover, it is difficult to relate cause and effect in such relationships. Chronic administration of morphine or any other chemical agent in large doses could easily result in the alteration of the animal's ability to dispose of morphine but this change does not necessarily have to bear a direct causal relationship to tolerance *per se*.

A more relevant approach is found in recent disposition studies seeking differences in the distribution of the agent within the central nervous system. While these studies to date may have failed to reveal marked differences in the gross distribution of analgetics in tolerant and nontolerant animals (236, 283, 285, 324), the technics available at the present time are, as we have pointed out earlier, still relatively crude. With improved isotope methodology it may well be that meaningful delineations in the differential distribution of active *versus* inactive isomers of the same compound will be discovered. The well-known marked differences in the pharmacologic activity of the *d*- and *l*- forms of methadone and 3-hydroxy-N-methylmorphinan suggest that the receptors mediating the biologic action of these isomers exhibit a different sensitivity or affinity for

each of the isomers. Until some real evidence is presented correlating this difference in pharmacologic activity with distribution of the agent in the central nervous system, little progress can be expected in attempts to relate the development of tolerance to the biologic disposition of the agent. At the present time, despite many interesting findings no experimentally verifiable concept has emerged clarifying the mechanism of tolerance, or relating this to physical dependence or addiction.

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