CLINICAL PHARMACOLOGY and THERAPEUTICS

volume 16 number 6 December, 1974

The biochemical pharmacology of abused drugs

III. Cannabis, opiates, and synthetic narcotics

In this final part of the review, we cover the widely abused plant drugs, Cannabis obtained from the hemp plant Cannabis sativa, *and the opiates, from the opium poppy* Papaver somniferum. *The psychoactivity of preparations of these plants has been known for a very long time. Cannabis has never found a genuine medical application for its particular psychoactivity and, although it has been in most of the world's pharmacopoeias at one time or another, there is no established medical use for it. The opiates are essential for the alleviation of severe pain and in the treatment of certain gastrointestinal disorders. Their use in the medicine of the nineteenth century was widespread, but realization of the dangers of prolonged dosage has led to a restriction to circumstances when they are specifically indicated. Owing to the well-established dangers of the opiates from the viewpoint of dependence, many attempts have been made to synthesize a "safe" powerful analgesic. There has been no real success, but several such compounds have come to be abused in their own turn. The three most important of these are meperidine, methadone, and pentozocine, and these are reviewed here along with the naturally occurring opiates.*

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Cannabis

The various preparations of the plant *Cannabis sativa* Linn constitute the most widely used illicit drugs throughout the the world. The effects of these preparations have been known for a very long time, but they have recently become the drug of choice in "advanced" circles. This has its origins in the middle of the last century, with advocates such as Baudelaire and Gautier, and has spread widely.[5] A considerable number of names for the different preparations are used, but here Canna-

*Recipient of a Medical Research Council Junior Research Fellowship.

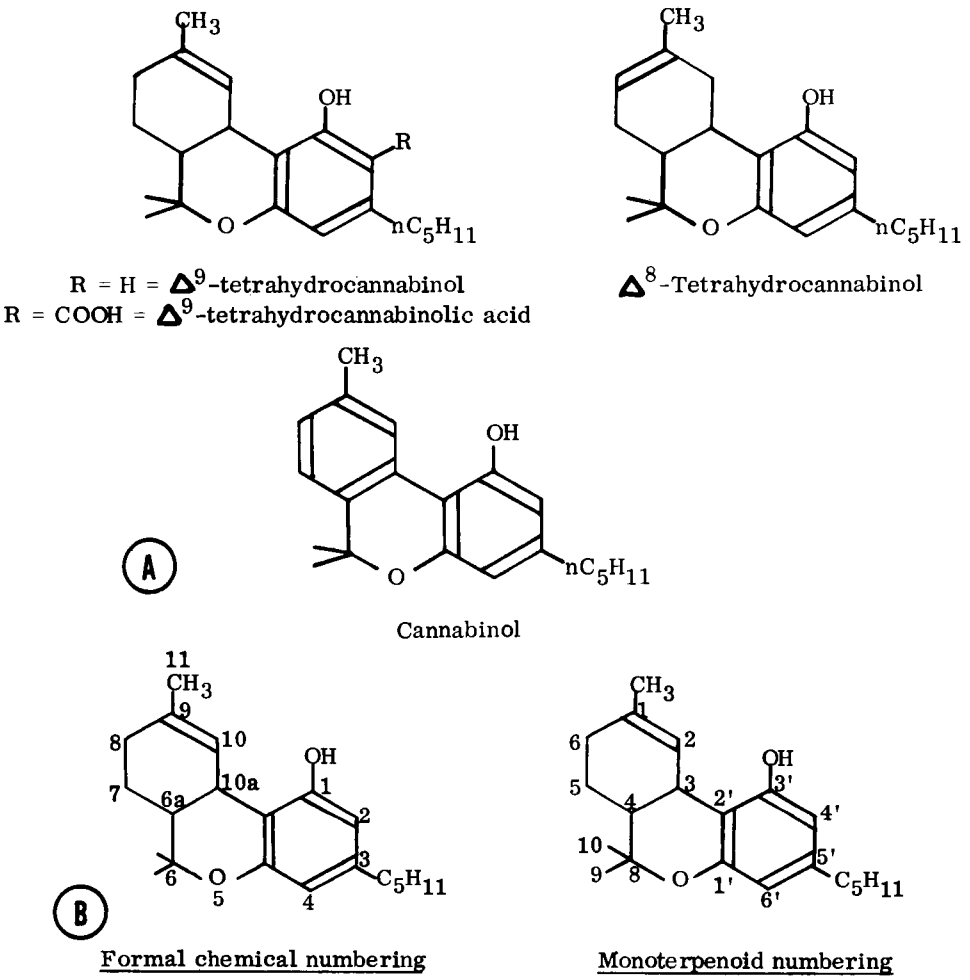


Fig. 1. *A*, Structures of some important constituents of cannabis. *B*, Numbering conventions for cannabinoids, exemplified by the most active tetrahydrocannabinol.

bis will be used for crude plant preparations having the characteristic psychic effects. The resinous exudate of the flowering tops of female plants is richest in the active principles, but dried leaves, flowers, and stems are also used. The most common mode of administration is by mixing herbal preparations into cigarettes for smoking, but in several forms the drug is also eaten.

Chemistry. Until recently knowledge of Cannabis has been restricted by the poor state of chemical knowledge about the constituents of the drug. The amounts and ratios of the various components of the drug depend on botanical and cultivation factors, the mode of preparation of the crude drug, and its storage. In the last 8 to 10 years, the active principles of the drug have been elucidated, and in this gas chromatography and mass spectrometry have been essential.[121] The structure of the inactive cannabinol (Fig. 1, *A*) was determined in 1940,[1, 114] and it was realized at this time that the psychotomimetic action of Cannabis was due to a tetrahydrocannabinol, in which the 6-carbon ring without the phenolic hydroxyl and *n*-pentyl groups contained one double bond. Synthetic tetrahydrocannabinol (THC) of the late 1930's was $\Delta^{6a\ 10a}$-THC, in which the double bond was shared with the ring containing the oxygen heteratom (see for-

mal numbering system in Fig. 1, *B*). While this compound had a marked psychotomimetic action,[1, 2] it was considerably less active than Δ^9-THC (Fig. 1) a small sample of which was prepared at the same time.[1] Furthermore, great variations were found between the activities of synthetic batches of THC, probably due to the relative proportions of the 8 isomers varying.[1] In 1964, Gaoni and Mechoulam[50] showed the presence of the active compound Δ^9-THC, an oily substance that is isomerized to Δ^8-THC (Fig. 1, *A*) by acids, and that is oxidized in air to cannabinol and polymers from oxidation of the phenolic group. Δ^8-THC is also psychoactive. Two numbering systems are used at present for the cannabinoid ring system. The first is the formal chemical numbering, based on the pyran ring conventions, and the second is a monoterpenoid numbering, which derives from the biosynthetic pathways of the cannabinoids. These are shown in Fig. 1. For the systematic chemical study of the cannabinoids, the monoterpenoid system is preferable, but in the pharmacologic literature there appears a preference for the formal numbering, which accordingly is used in this review. The *n*-propyl analogues of cannabinol and THC have also been detected[54, 121]; these are the cannabivarins and have activities similar to the cannabinols. Another major constituent is the inactive Δ^9-tetrahydrocannabinolic acid (Fig. 1, *A*), which is converted to Δ^9-THC on heating or when smoked.[41, 50] Δ^9-THC itself is partially destroyed on burning, so that the dose from smoking can be very variable.[62]

Vree and associates[122] have reported the presence of the cannabiorcins, methyl analogues of the *n*-pentyl and *n*-propyl cannabinoids. These materials occur in smaller quantities than do the C-3 and C-5 compounds, and are less active than the longer chain compounds. Other substances found in Cannabis preparations include triterpenes and C-19 steroids,[41] trigonelline (*N*-methylpyridine-3-carboxylic acid) and its methyl and ethyl esters, and an unidentified atropine-like substance.[54]

The composition of samples of Cannabis varies with the mode of cultivation and source. Cannabis grown for its fibrous content (e.g., Turkish hemp) is very low in psychoactive constituents, but in samples cultivated for drug content (notably Mexican) the psychoactive compounds and their precursors can account for some 4% to 7% of the total material.[125] Fuller reviews of the chemistry and pharmacognosy of Cannabis are to be found in References 41, 81, and 125.

Pharmacology. The effects of Cannabis on the whole organism can be divided into the easily assessed physiological response and the more subjective CNS effects, which can be elusive. In the acute situation, the drug causes an increase in pulse rate without an increase in blood pressure, and the kinetics of the pulse increase in man follow those of the drug's plasma level.[49] In atropinized rats, no increase is seen in heart or respiratory rates, and in man the increase in heart rate may be prevented by the β-adrenergic blocker propranolol.[46] There is considerable hypothermia, and in animals a cataleptic state is produced, the animal equivalent of the human "high." This state correlates well with levels of the drug in mouse brain. In man, Cannabis increases the appetite, but in rats the reverse is found.

The subjective effects of Cannabis are principally distortions of perception, overestimation of time, and some impairment of short-term memory. Low doses have a principally euphoriant effect, and higher doses are hallucinogenic. Intellectual performance is impaired and there is sedation and loss of social inhibitions, which may account for the aphrodisiac reputation of Cannabis. When giving an account of the actions of Cannabis on the psyche, it is virtually impossible to separate sociologic influences from biochemical or pharmacologic ones. Naive human subjects rarely respond to the effects of Cannabis on the psyche, but on repeated usage, especially in the company of experienced users, the "high" state is attained by learn-

ing. The kinetics of the physiologic effects correlate well with the kinetics of Δ^9-THC in the body, and Lemberger, Axelrod, and Kopin,[74] using data derived from Hollister, Richards, and Gillespie[62] suggested that the time-course of the psychic effects of Δ^9-THC followed its appearance and disappearance from plasma. However, a later paper,[49] of which Lemberger is a coauthor, has shown that the psychic effects take longer to appear and last longer than does the drug itself in plasma. This subjective "high" state may be evoked to a lesser extent by the stimulus of placebo Cannabis-like material, indicating that this may be a conditioned response and not a true drug effect.[49]

The mode of action of Cannabis and its constituents as seen by alterations in brain neurotransmitters has yet to be elucidated, but some indications are given in the work of Truitt and Anderson.[117] They find that after both Cannabis and Δ^9-THC there is a very slight fall in the brain levels of norepinephrine, but a significant elevation in brain 5-HT. Further, both Cannabis and Δ^9-THC slow the turnover rates of norepinephrine and 5-HT in the brain. This pattern of elevation of 5-HT, while norepinephrine is unaffected, resembles other hallucinogens such as LSD and mescaline[115] and may represent a common mechanism for these effects of a number of drugs.

In 1968 it was reported that Cannabis has a teratogenic action in rats.[95] Subsequent work using Δ^9-THC has shown that this material has no teratogenic effects in rat and hamster, although it passes across the placenta of rats[93] and has been found in hamster fetuses.[64] Δ^9-THC is also without effect on the chromosomes of rat leukocytes in tissue culture.[93] There are, however, indications that Cannabis contains a teratogen other than Δ^9-THC.

Forney and Kiplinger,[46] in their review, note that the LD_{50} of Δ^9-THC given by intravenous injection to rats and mice is some 10 times greater than when it is administered orally or by intraperitoneal injection, and repeated small doses of Δ^9-THC are more toxic to mice than single large doses.[54] They stress the great lipid solubility of the drug and the consequent dangers of accumulation in fatty tissues, notably the brain. The tissue distribution of Δ^9-THC indeed shows concentration of the drug in fat[46] but, as will be seen later, there is no significant retention of the drug or its metabolites in man.[61] The drug and certain metabolites have been identified in brain of rat, mouse, and monkey after injection of Δ^9-THC,[23, 59, 60] and there have been reports, based on tenuous evidence, of gross brain damage involving shrinking of this organ.

Much of the pharmacologic work recorded in the literature is based on the assumption that the effects of the drug are due to Δ^9-THC alone; either the pure drug or extracts with standardized Δ^9-THC content are used. This may well be a naive assumption, especially when the example of tobacco is considered; nicotine is the most active constituent, but there are many other active substances present so that tobacco and nicotine are very difficult to equate.

Metabolism and disposition of Δ^9-THC. The fate of Δ^9-THC has been examined in the rat, rabbit, and man, and in all three species it is metabolized very rapidly and completely, although this speedy biotransformation does not lead to quick excretion. In the rat, only 50% of a dose of radiolabeled Δ^9-THC is eliminated from the body in one week after dosing, and of this, the bulk (40%) is found in the feces with the remainder in the urine.[2, 72] The urine of the first day after dosing contains some 2% to 6% of the dose almost entirely in the form of metabolites. The major metabolite is 11-hydroxy-Δ^9-THC, with other products in smaller amounts.[84] Klausner and Dingell[72] report that this 11-hydroxy metabolite undergoes enterohepatic circulation in the rat. After the injection of [^{3}H]-Δ^9-THC, the rabbit excretes radioactivity considerably quicker than the rat, 35% of the dose appearing in the first

24 hour urine, with 10% in the feces in the same period.[3] As in the rat, the metabolism of Δ^9-THC is very rapid, the plasma half-life of the unchanged drug being some 7 to 16 minutes. The metabolites have not been identified, but there are at least 3 present in urine, all more polar than Δ^9-THC.

In man, Δ^9-THC is metabolized extensively to more polar compounds that are excreted in the urine and feces, and in a period of one week, some 60% of a dose is found in the urine, with about 20% in the feces. The bulk of fecal excretion occurs on the first day after dosing, and elimination by this route is essentially complete in 4 days. The urine excreted on the first day after dosing, however, contains some 15% of the dose, with 20% in the second and another 10% in the third day's urine. No unchanged Δ^9-THC is found in the urine, but polar acidic metabolites are present, including a small amount of 11-hydroxy-Δ^9-THC, the amount of which can be increased by incubating the urine with a β-glucuronidase-sulfatase preparation.[74] The pattern of metabolites in feces is essentially the same as in urine.

The slow elimination of the drug and its metabolites by man and animals is noteworthy. The plasma half-life of the drug in rabbits is very short, which suggests that there is a primary phase of rapid metabolism, followed by distribution into the tissues, leading to a slow excretion. In man[74] and the rat[72] the elimination of Δ^9-THC is biphasic, with very rapid disappearance from plasma (of the order of minutes) followed by slow excretion extending over several days. Δ^9-THC has unusual affinities for cellular components[38] being strongly bound to plasma lipoproteins[123] and this may partly explain why the drug is slowly eliminated from the body. Despite there being a depot of drug metabolites in the body, this depot does not appear to increase in size, and there is no accumulation on repeated administration of the drug.

When a Cannabis extract, standardized to contain 20 mg of Δ^9-THC, was given to human subjects, their urines contained cannabinol, cannabidiol, and a new polar metabolite, but no Δ^9-THC.[61] Although considerable individual variations in metabolism were found, on repeated dosage no evidence of accumulation of the drug or its metabolites could be found. The explanation for this is to be found in the discovery that the pharmacokinetics of [^{14}C]-Δ^9-THC are different in regular smokers and in drug-naive subjects.[74] The plasma half-life of radioactivity in non-smokers is about twice as long as in chronic smokers, the figures being:

	Non-smokers	*Chronic smokers*
Plasma half-life total ^{14}C (hr)	67	33
Plasma half-life Δ^9-THC (hr)	56	27

These differences suggest that, in man at least, Cannabis compounds have the ability to induce their own metabolism,[74] which apparently overcomes any tendency for the drug to build up in tissues.

The metabolism of Δ^9-THC has also been investigated in liver microsomal systems, and homogenates from rat and rabbit both give a hydroxylated metabolite, 11-hydroxy-Δ^9-THC.[116] In rabbit liver microsomal preparations, further hydroxylation of 11-hydroxy-Δ^9-THC has been detected giving 8,11-dihydroxy-Δ^9-THC and 11,16-dihydroxy-Δ^9-THC.[124] Some of the 8,11-dihydroxy metabolite undergoes aromatization with loss of the 8-hydroxyl group, resulting in 11-hydroxycannabinol. These reactions are summarized in Fig. 2. In addition to this, the active cannabinoid Δ^8-THC is metabolized along similar pathways, with the production of 7,11-dihydroxy-Δ^8-THC. No aromatization or side chain hydroxylation was observed.[124] A similar hydroxylation of cannabinol in rat liver, yielding 11-hydroxy-cannabinol, has been reported.[128] The metabolism of Δ^9-THC by hydroxylation is a reaction of some pharmacologic significance. 11-Hydroxy-Δ^9-THC has effects in rats, mice, and monkeys similar to the parent drug

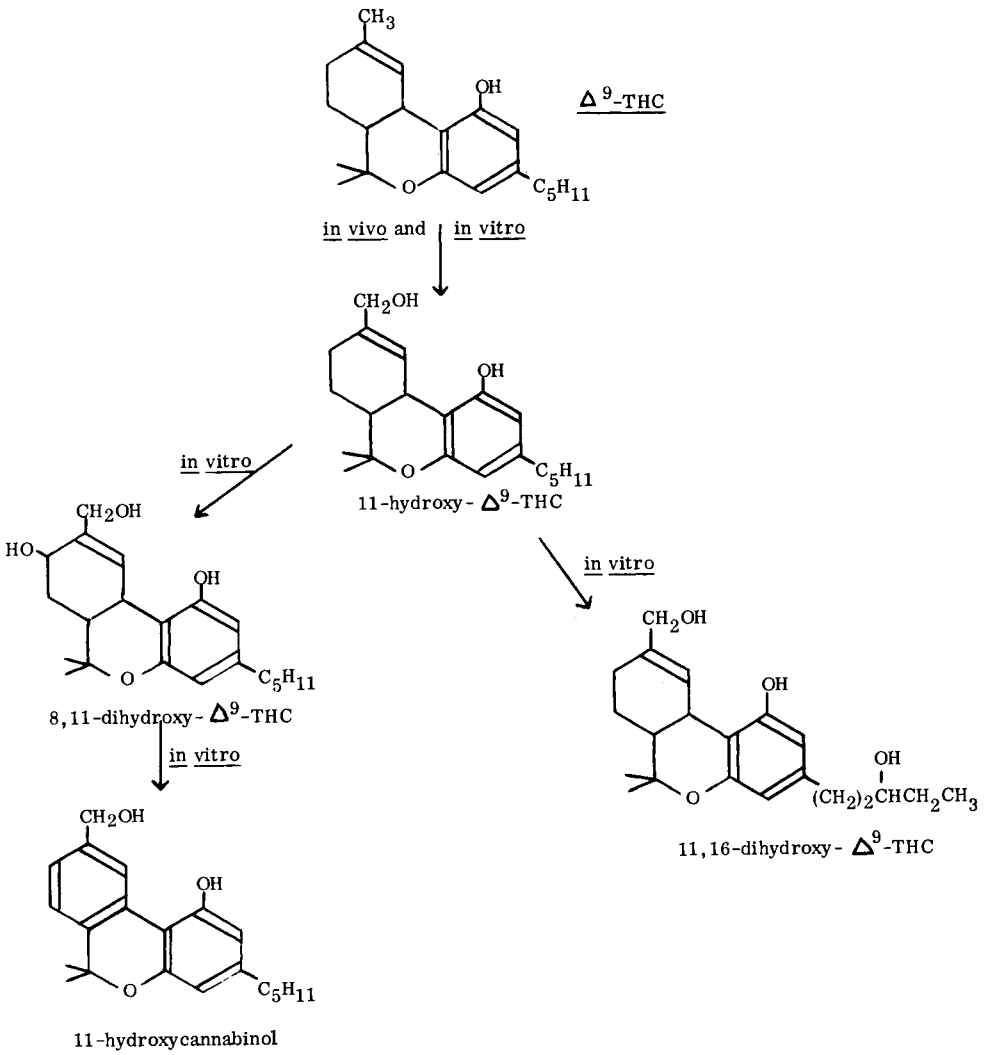
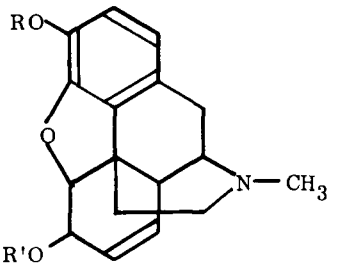


Fig. 2. Biotransformation of Δ^9-THC.

and, when given by intracerebral injection, has 18 times its potency.[23] It has been reported that, in mice, over 50% of the net observed effects of Δ^9-THC are due to the 11-hydroxy metabolite.[53] However, 8,11-dihydroxy-Δ^9-THC is devoid of pharmacologic activity, although it is found in mouse brain along with 11-hydroxy-Δ^9-THC after Δ^9-THC administration.[23] Homogenates of mouse brain, when incubated with Δ^9-THC and the necessary cofactors as for its metabolism by the liver, do not hydroxylate this substrate.[23]

Tolerance to Cannabis. Evidence both for and against the development of tolerance to the effects of Cannabis exists in the literature. The conflict between reports is perhaps due to sample variation, but recent work[77] appears to show beyond reasonable doubt that tolerance to Δ^9-THC does occur. The tolerance is rapidly developed to a marked extent in pigeons, dogs, rats, and rhesus monkeys, but does not occur for all of the effects of Δ^9-THC since sedation and anorexia in the dog and analgesia in mice are unaffected. A number of closely related psychoactive cannabinoids show cross-tolerance to each other. Regular exposure to the drug is not a prerequisite for tolerance development, as it still is evoked when doses are given at irregular, infrequent in-

Phenanthrene alkaloids



	R	R'
Morphine	H	H
Codeine	CH_3	H
Heroin	CH_3CO	CH_3CO

Benzylisoquinoline alkaloids e.g. papaverine

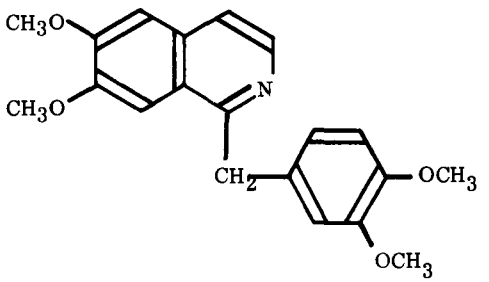


Fig. 3. Structures of some opium alkaloids.

tervals. The tolerance is long-lasting and may be exhibited up to one month after the final drug administration. Cannabis tolerance is not related to tolerance produced by other drugs, since morphine, mescaline, or LSD cannot be substituted for Cannabis. No withdrawal syndrome has been observed in dogs, rats, or pigeons rendered tolerant to Δ^9-THC, but there are indications of such a syndrome in rhesus monkeys, which perhaps is evidence for the production of dependence.

Recent studies[37, 78] show that, in pigeons, the metabolism and disposition of Δ^9-THC are the same after acute and chronic dosage, and they conclude that the tolerance produced by Δ^9-THC does not have its basis in altered drug handling. Nahas and associates[88] suggested that Δ^9-THC, when bound to plasma lipoproteins (as mentioned previously) can act as a hapten and trigger an immune response at the cellular level, giving rise to tolerance. They base this conclusion on the ability of the immunosuppressive, azathioprine, to inhibit the development of tolerance to Δ^9-THC by the rat. Azathioprine, however, is a derivative of the protein synthesis inhibitor, 6-mercaptopurine, and in view of the known action of protein synthesis inhibitors to influence tolerance development to opiates and depressants (see relevant sections), one might suggest that Δ^9-THC is another example of a drug in which protein synthesis and tolerance are intimately linked. It must, however, be noted that Ferraro and Grilly[45] failed to find tolerance to Δ^9-THC in chimpanzees when testing their performance in a complex delayed matching-to-sample task over 21 days of drug dosage. They point out that parameters used by other workers to show tolerance involve very simple tasks or are passive measures.

There are some suggestions that in man, subjects become more sensitive to the psychic effects of Cannabis on prolonged administration. If this "reverse tolerance" does occur, it has been suggested that it might be due to repeated dosage inducing the liver enzymes responsible for converting Δ^9-THC to the highly active 11-hy-

Table I. *Alkaloid composition of raw opium*

Alkaloid	*% of dry weight*
Phenanthrene alkaloids	
Morphine	10.0
Codeine	0.5
Thebaine	0.2
Benzylisoquinoline alkaloids	
Noscapine	6.0
Papaverine	1.0
Narceine	0.3
Total	25

Table II. *Relative potencies of morphine, heroin, and codeine*

Analgesic	*Dose equivalent to 10 mg morphine subcutaneously*	*Duration of action (hr)*
Morphine	10	4-5
Heroin	3	3-4
Codeine	120	4-6

droxy-Δ^9-THC.[74] The larger amounts of metabolite present would thus give a heightened response to the drug.

The opiates

The analgesic and pleasurable properties of products from the plant *Papaver somniferum* were well established before the Greeks named its juice *opium.* The drug was widely used in Arabian medicine, and found its way into Europe by the Middle Ages. Laudanum, an opium preparation still in use, was the invention of Paracelsus. The scientific investigation of opium dates from 1803 when morphine was isolated. While dependence to opium must have been known to the ancients, our first real accounts date from the nineteenth century. Laudanum drinking flourished among the romantic idealists of the time, De Quincey's *Confessions of an English Opium Eater* (1822) remaining a classic account of Laudanum addiction. At the same time, opium came into general use in medicine, being used to treat a wide variety of disorders. In addition, the middle of the nineteenth century saw the perfection of the hypodermic needle, and the opiates were the first drugs to be administered parenterally in this way. The large numbers of casualties of the American Civil War were the first major beneficiaries of this combination, and indeed many were taught to self-administer morphine by injection. This led to widespread therapeutic addiction, which was dubbed the "soldiers' disease." While addiction was not thought of as a problem at this time, the indiscriminate use of opiates was an important factor in the introduction of the first drug control laws.

We have now come to a better understanding of the dangers of the opiates, and they are among the most useful of drugs now that their use is much more rational. The numbers of opiate addicts in the community have been rising rapidly in recent years, notably in the cities. It is often misleading to quote figures for the incidence of this type of abuse, but it can be stated that the average age of new addicts has become steadily lower, making drug dependence more and more an adolescent problem.

Chemistry. Raw opium has a total alkaloid content of about 25%, and the alkaloids are of two classes, the phenanthrenes of which morphine is the most important, and the benzylisoquinolines. Structures of these are shown in Fig. 3. Table I shows the composition of raw opium, and it is seen that morphine is the most abundant alkaloid. Although many of the constituents of opium have marked pharmacologic actions, morphine is the archetype of the narcotic alkaloids with the phenanthrene structure. It has attracted most attention from the drug abuse viewpoint, along with its semisynthetic diacetyl derivative, heroin (Fig. 3). Codeine (Fig. 3) possesses many of the pharmacologic attributes of morphine but is less potent, and while used therapeutically as an analgesic, it has little attraction for the drug abuser. Some idea of the relative potencies of these analgesics

in man can be obtained from the figures in Table II, with the duration of action also shown:

It is well known that heroin is more potent than morphine and is preferred by abusers. This is probably due to the greater lipid solubility of heroin compared with morphine, as the relatively polar hydroxyl groups are rendered less polar by esterification with acetic acid. This results in much higher brain concentrations of drug when heroin is given than when the same dose of morphine is given.[91]

The possible routes of metabolism of morphine that suggests themselves from its structure (see Fig. 3) are conjugation of either or both hydroxyl groups and *N*-demethylation. In animals and in man, the principal route is by conjugation, the 3-position (phenolic) being favored. In the bile of dogs, 35% of a dose of morphine is excreted as the 3-glucuronide, together with a small amount of a double conjugate, possibly the 3-sulfate-6-glucuronide.[130] Both dogs and monkeys excrete morphine principally as conjugates in the urine with smaller amounts in the feces, but the fecal material contains more free than conjugated morphine.[83, 130]

N-Demethylation of morphine is not an important metabolic pathway in man or rat, since on using morphine labeled with ^{14}C in the *N*-methyl group, human subjects were found to excrete 57% to 84% of radioactivity in the urine, 7% to 10% in the feces, and only 3% to 5% in the expired air as CO_2. The male rat excreted 8% of the dose of ^{14}C in the expired air, whereas the female eliminated only 0.6% by this route, suggesting a sex difference in this species in the extent of demethylation.[43]

The pattern of metabolism and excretion of morphine both in lower animals and in man is little changed on chronic dosage (Table III). In dogs there is a trend toward greater fecal excretion of the unchanged drug on chronic dosage, but it appears that alterations in the excretion patterns of this drug do not account for tolerance, and dependence and alternative explanations must be sought.

Table III. *Excretion of morphine and its conjugate by monkeys and dogs* †*

	Urine		*Feces*	
	Free	*Conjugated*	*Free*	*Conjugated*
Monkey				
Nontolerant	10	67	2	0.8
Tolerant	7	59	4	0.7
Dog				
Nontolerant	14	56	6	1
Tolerant	14	55	15	1

*Figures represent per cent of dose as that metabolite in 0 to 24 hr urine and feces.

†After Mellett, L. B., and Woods, L. A.: J. Pharmacol. Exp. Ther. 116: 77-83, 1956.

Heroin (3, 6-diacetyl morphine) is metabolized by esterases both in man and in lower animals, giving morphine, which is then conjugated. The 3-acetyl group is lost more easily than 6-, so that 6-monoacetyl morphine is the intermediate in deacetylation,[89] which may occur in both plasma and brain.[131] In heroin-dependent human subjects, some 50% of a dose of the drug is excreted as conjugated morphine and 7% as free morphine.[89] A more comprehensive account of the metabolism of the opiates is given by Scrafani and Clouet.[103]

It is of interest in consider the pharmacologic significance of the conjugation of morphine with sulfate and glucuronide. Morphine-3-sulfate was reported as a metabolite in dogs in 1883, and it was later found that it had no analgesic or euphoriant properties.[90] Mulé and Woods[87] investigated the tissue distribution of both free and conjugated morphine after morphine dosage and found that the conjugated form did not enter the brain (the drug's site of action) to an appreciable extent. It has been suggested[99] that morphine conjugates are active metabolites, for when morphine-3-glucuronide was given by intracerebral injection to mice, it had analgesic properties. However, Schulz and Goldstein[101] found that the

glucuronides of morphine and the synthetic narcotic levorphanol were inactive on a guinea pig ileum preparation (a test for narcotic drugs[94]) and that the analgesia caused by intracutaneous injections of levorphanol-3-glucuronide was due to liberation of the aglycone by β-glucuronidase of the brain. They proposed that the analgesia caused by morphine-3-glucuronide had a similar origin. It would thus appear that the conjugation of morphine and other narcotics effectively terminates their action by changing them into forms unable to reach the receptor sites in the brain[87] and by increasing their water solubilities so that they are readily excreted.

Effects of chronic morphinization on liver microsomal enzymes. When narcotics are given in prolonged dosage there are many striking changes in the pharmacologic effects, and it may be that biochemical changes parallel these. When liver microsomes are prepared from animals receiving repeated doses of morphine, they show progressively reduced ability to *N*-demethylate morphine. If the antagonist, Nalorphine (*N*-allylnormorphine), or normorphine, is given, cross-tolerance is developed in that the animals show tolerance to the analgesic effect of morphine, and their microsomal enzymes exhibit a reduced ability to demethylate morphine.[26]

The conjugation of morphine in vivo with glucuronic acid has already been referred to, and in this connection Takemori[111] found that acute and chronic morphine treatment of rats increased the synthesis of uridine diphosphoglucuronic acid in the liver, the high-energy intermediate in glucuronide formation, but the amount of morphine glucuronide formed in vitro was reduced on repeated dosage, indicating a loss of glucuronyl transferase activity. It was further found[112] that phenol glucuronide formation was unaffected on chronic dosage of phenol, although morphine treatment reduced conjugation of a range of other drugs, which may be of significance in the polypharmacy that drug abusers notoriously indulge in. Theories of tolerance based on these changes in enzyme activity will be elaborated later.

Pharmacology. The effects of morphine on the organism are exerted at two major sites, on smooth muscle and on the central nervous system (CNS). The constipating action of morphine is well known and has led to its use in intractable diarrhea, although its major action is on the CNS. Morphine in man causes analgesia, drowsiness, mental clouding, and mood changes. The analgesia is selective without affecting the other senses. In normal doses, morphine is euphoric to subjects who are tense, worried, or in pain, but in normal individuals it is less pleasant and may give rise to dysphoria, characterized by anxiety accompanied often with nausea and vomiting.[67] This has led to the concept that the abuser responds differently to morphine in that it is only euphoric to those inherently disposed toward drug use.[73]

Many suggestions have been advanced for the mechanism of action of morphine and related drugs.[40, 129] Modern thought is that the mechanism of action of morphine lies in its effects on central neurotransmitters, and of the many putative transmitters,[58] acetylcholine (ACh), 5-hydroxytryptamine, and the catecholamines are all thought to be involved.

Morphine inhibits the release of ACh from neurons in the peripheral[34, 94] and central nervous systems[80, 105] Slices from cerebral cortex of rats treated with morphine show impaired ACh synthesis when ACh release is prevented by a low K^+ concentration, but with a high K^+ concentration, ACh release is normal as is ACh synthesis.[104] The involvement of catecholamines is shown by the enhancement of morphine analgesia by tyrosine, amphetamine, and the monoamine oxidase (MAO) inhibitor, tranylcypromine. The adrenergic blockers inhibit the response to morphine, as does depletion of catecholamines and 5-HT by reserpine. 5-HT

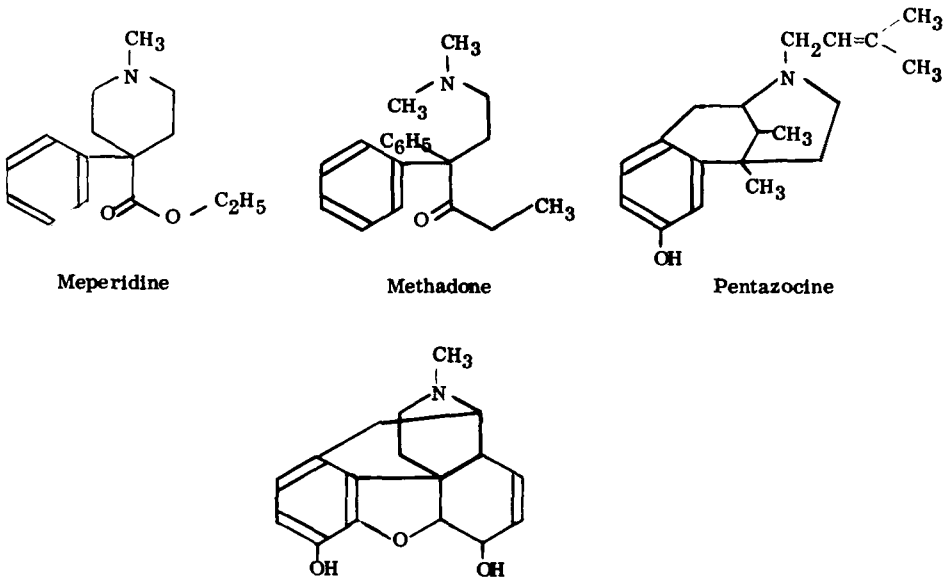


Fig. 4. Structures of synthetic narcotic analgesics as related to morphine.

participation in the morphine response is apparent from the potentiation of analgesia by 5-hydroxytryptophan, and its impairment by *p*-chlorophenylalanine.[57] It appears then that morphine interferes with more than one of the neurochemical transmitter systems of the brain, but it is not possible at present to account for the action of the drug at this level. A fuller account of transmitter changes on prolonged exposure to morphine is given in section entitled "Tolerance to narcotics."

Tolerance in man develops primarily to the analgesic, sedative, euphoric, and respiratory depressant effects of morphine and, while very high doses are tolerated, overdosage is a frequent cause of death.[51] The withdrawal syndrome is very severe and has been described many times; it involves nervousness, anxiety, great increases in body secretion, muscle pain, vomiting, diarrhea and an obsessive drive toward more drug.[36]

Synthetic narcotic drugs

One of the earliest tasks that the founders of medicinal chemistry undertook was the search for a powerful nonaddictive analgesic. This search, despite several exciting discoveries, has still not proved fruitful, although it continues. Despite the fact that it has been impossible so far to separate the production of tolerance and dependence from analgesia, many useful narcotics have been discovered,[22, 52] some of which are used in certain circumstances in preference to the opiates. Abuse of some of these agents has occurred, and in this respect a discussion of three, methadone, meperidine, and pentazocine, is appropriate.

Methadone. Methadone (4,4-diphenyl-6-methylamino-3-heptanone, Fig. 4) is a powerful synthetic nonopiate analgesic, of German origin, which came into the therapeutic use after World War II. It is a highly effective analgesic, respiratory depressant, and antitussive, and in man is equipotent with morphine. Other structurally related compounds have opiate-like effects, but methadone is the most potent.[19] The molecule has an asymmetric center at C-6 and, although the racemate is used in practice, the *l*-isomer is some 30 times as active as the *d*-antipode. The principal

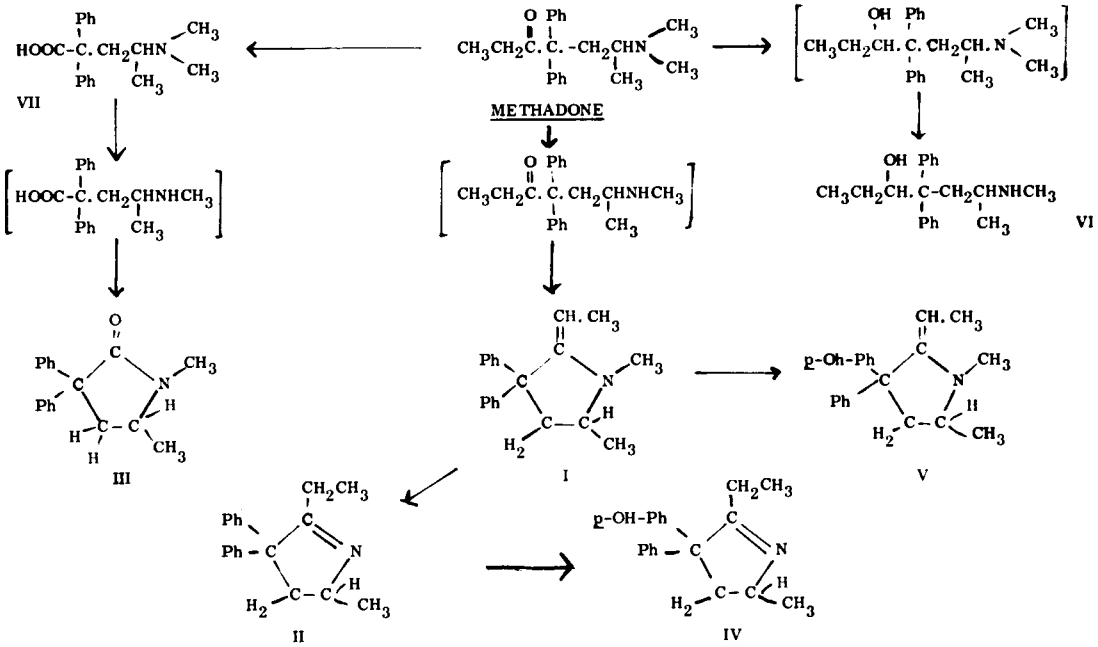


Fig. 5. Metabolism of methadone in man and animals. (After Sullivan, H. R., and Due, S. L.: J. Med. Chem. 16:909-913, 1973.)

importance of methadone is in substitution therapy for opiate dependence and, whereas primary abuse of this drug alone is relatively rare, it is now frequently encountered in relapsed opiate addicts.

A consideration of the structure of methadone suggests at least three routes of metabolism, namely *N*-demethylation, reduction of the C-3 keto group, and hydroxylation of the phenyl rings attached to C-4, and such routes have indeed been found to occur in man and rat. The urinary metabolites of methadone are shown in Fig. 5 and referred to in the text by roman numerals. Although *N*-demethylation would be expected to give the corresponding primary and secondary amines, it has been found that these undergo a spontaneous cyclization between the amino group and the keto group, with the elimination of water.[10] Such a ring closure gives rise to a series of pyrrolidine derivatives (I, II, III, IV, and V). If the keto group is reduced to the alcohol, cyclization cannot occur, and amine metabolites in which the keto group is reduced are thus found in urine (VI). In addition to these metabolic pathways, the central six carbon chain may also be oxidized, giving a cleavage between C-2 and C-3 resulting in a substituted pentanoic acid. This acid (VII) can also undergo the above cyclization on *N*-demethylation to give the pyrrolidine derivative, III. Beckett, Vaughan, and Essien[12] have reported that *N*-oxidation, giving methadone-*N*-oxide, is also an important metabolic option in man. Other workers, however, have been unable to detect this and, indeed, Sullivan and Due[109] state that methadone-*N*-oxide is an artifact formed in urine that has been standing at room temperature. All the compounds labeled with roman numerals I to VII, in Fig. 5, have been found in the urine of human subjects maintained for 4 months to 5 years on methadone.[109]

Quantitative data are not readily available, but those of Sullivan and Due[109] showed that human subjects receiving 50 to 100 mg methadone daily excreted 5% to 26% unchanged with 3% to 29% as

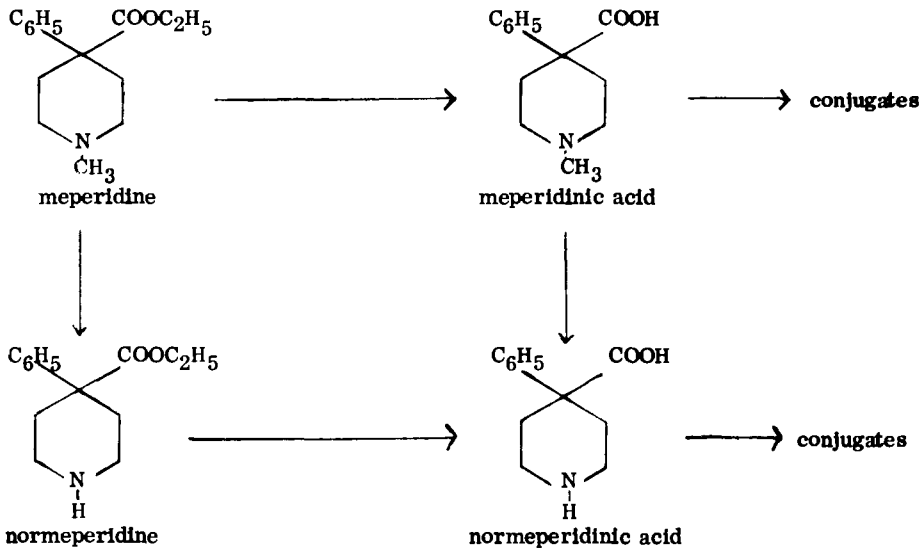


Fig. 6. Metabolism of meperidine.

metabolite I, 0.2% to 3% as metabolite II, and 0% to 3% as metabolite III. Compounds I and II together with unchanged drug were found in rat bile.[8, 97]

Although pharmacologic activity resides principally in the *l*-isomer of methadone, as previously stated, it has been found that the *d*-antipode has significant analgesic activity in rodents.[108] Sullivan and associates[110] have shown that *d*-methadone is metabolized by the rat by reduction of the keto group to the corresponding alcohols. These compounds, the methadols, were found to be *l*-isomers, and are suggested to be responsible for the analgesic activity of *d*-methadone. Misra and Mulé[85, 86] found that there is a significant difference between the plasma and brain half-lives of the isomers of methadone, for in brain, the *d*-form has a half-life of 1.02 hr and the *l*-form, a half-life of 2.4 hr, whereas in plasma the half-life of *d*- is 1.5 hr and of *l*-, 3.7 hr. They report also that in rat brain *l*-methadone gives rise to an active metabolite, as yet unidentified, and to a metabolite that is firmly bound, perhaps covalently, to brain proteolipids. This latter metabolite is retained in very small amounts in brain, remaining at the same level from 24 hours to 3 weeks after dosage. These authors suggest that this long-lasting metabolite may in some way be involved in the production of tolerance to the drug.

The pharmacology of methadone is similar to that of morphine, for on prolonged administration considerable tolerance develops to the analgesia, anorexia, respiratory depression, and nausea produced by the drug, but the time-course is slower than in the case of morphine. Tolerance to the constipative action of methadone does not develop. The principal current use of methadone is in the treatment of opiate dependence, where, pioneered by Dole and Nyswander,[39] it has been used with success. It is used to replace opiates, especially heroin, and it will relieve narcotic hunger and induces tolerance sufficient to block the euphoriant effects of heroin, thereby helping to prevent relapse. Former heroin users showed normal mental and neuromuscular function and were indistinguishable from control subjects on medical and psychologic tests. Constipation was marked, and careful supervision and social support were vital. This control was achieved with oral dosage of 50 to 200 mg daily, but the characteristic opiate withdrawal syndrome

Table IV. *Metabolites of meperidine in urine of normal and dependent human subjects**

Drug	Per cent of dose in	
	Normal	*Dependent*
Meperidine	5	2.5
Normeperidine	7	7
Meperidinic acid		
Free	21	41
Conjugated	16	1
Normeperidinic acid		
Free	7.5	28
Conjugated	10	22
Total	66.5	101.5

*After Burns, J. J., et al.: J. Pharmacol. Exp. Ther. 114: 289-298, 1955.

is seen if methadone dosage is stopped, and this syndrome may be precipitated by nalorphine or other antagonists. The closely related synthetic narcotic acetylmethadol has recently been suggested as an alternative to methadone as an opiate replacement and, like methadone, it is believed to act in part through its demethylated metabolites.[18] Despite widespread adoption in the United States and the United Kingdom, the methadone treatment for heroin dependence is not without its critics, since large numbers of subjects maintained on methadone continue to use heroin and other drugs. Lennard, Epstein, and Rosenthal[75] stated that, while methadone blocks the effects of small doses of heroin, massive intravenous doses of heroin overcome this blockade. They concluded that, "to think that the use of another drug can solve the profound and complex task facing us is indeed an illusion."

Meperidine. The synthetic narcotic meperidine (Fig. 4) was introduced in 1939 as a powerful morphine-like analgesic. Other structural analogues have analgesic activity but meperidine is the only one that has found therapeutic application. It is one-eighth to one-tenth as potent as morphine, shares it sedative and euphoriant properties, but toxic doses cause CNS excitation. It is shorter-acting than morphine, and frequent doses are required. It is used widely in obstetric analgesia and for preoperative medication, and is preferred to morphine as an analgesic in biliary and renal colic. In animals and in man, meperidine is metabolized by *N*-demethylation, hydrolysis, and conjugation, as shown in Fig. 6, only small amounts of the drug being excreted unchanged. The extent of demethylation varies with species, being about 15% in man and some 45% in the rat. No differences in metabolism were found between normal subjects and abusers in a total study by Burns and associates[21] (Table IV). The *N*-demethylated metabolite, normeperidine, has greater excitatory activity than does meperidine, and this is of pharmacologic importance. As the ratio of normeperidine (N) to meperidine (M) rises, then stupor (due to M) and convulsions (due to N) are seen. This is found in monkeys given meperidine by mouth where the rate of absorption and the metabolic capacity are approximately equal. When given intravenously, meperidine is not demethylated rapidly, so that there is CNS depression due to the unchanged drug.[35]

Tolerance develops slowly to meperidine and does not extend to all its effects. Thus while the respiratory depression is overcome on prolonged dosage, the CNS excitation remains. Withdrawal phenomena are similar to those of the opiates, but they are evoked more rapidly and have shorter duration. As the drug has a short duration of action, the need to use it at very frequent intervals reinforces drug-seeking behavior. Thus meperidine has a high abuse potential in cases in which there is an obsessional drive toward the drug. There are many more iatrogenic addictions to meperidine than to morphine.

Work on the mechanism of tolerance and dependence to meperidine is not abundant, and that reported shows that it is very similar to morphine in this respect. The general discussion on narcotic tolerance and dependence therefore in-

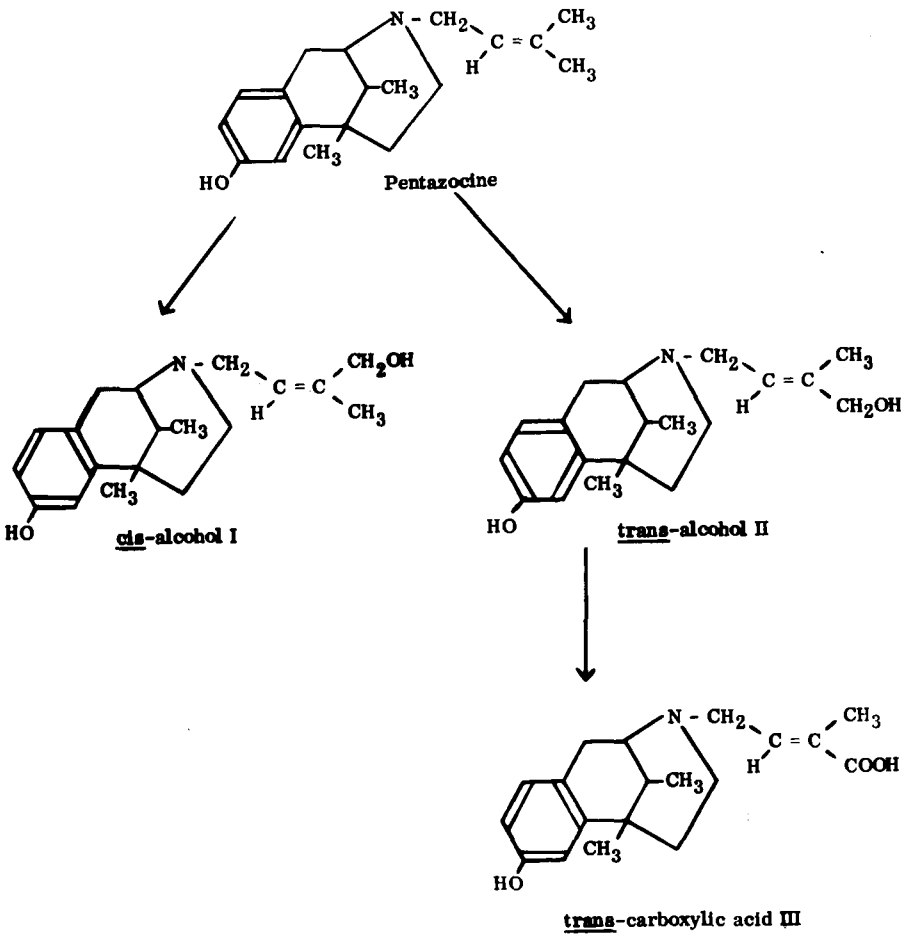


Fig. 7. Metabolic pathways of pentazocine.

cludes the synthetic narcotics as well as the opium alkaloids.

Pentazocine. Pentazocine is the most widely used of the so-called narcotic antagonist analgesics. The first compound of this series to attract attention was nalorphine (*N*-allylnormorphine), which although apparently devoid of activity in animals, was a potent analgesic in man.[70] Unlike morphine, nalorphine was neither addictive nor able to replace morphine in human addicts,[66] and it thus appeared possible that the analgesic effect of the powerful narcotics could be separated from their addiction liability.

Nalorphine has undesirable dysphoric effects that stand in the way of its clinical use, and thus a wide variety of other structural analogues of morphine with narcotic antagonist activity have been tested to find a compound suitable for widespread use in man.[113] The best such compound found so far is the *N*-allyl substituted benzomorphan, pentazocine. Its structure is shown in Fig. 4.

It is now apparent that although pentazocine is a useful strong narcotic analgesic, it fails to come up to original expectations, in that it has a number of the disadvantages of typical opiates, including that of giving rise to tolerance and dependence. It must be pointed out that pentazocine is not a particularly powerful narcotic antagonist,[29] and it is still possible that a better compound may be found that will separate the addictive and analgesic properties of the narcotics in the way outlined above.

Biologic disposition. When given orally to animals and man, pentazocine is rapidly absorbed, but this can be very erratic.[9, 11, 14, 44] When given in solution, it is absorbed much more rapidly than when tablets are taken.[9] In the rat, only a very little unchanged drug is found in the urine and feces, but both the bile and the urine contain glucuronide conjugates of drug-related materials that are the chief metabolites.[44] There was no *N*-dealkylation to norpentazocine.[44]

Pittman and associates[96] examined the metabolism of pentazocine in liver homogenates of rat, mouse, and rhesus monkey, and reported that the main site of biotransformation is the allyl side chain. ω-Oxidation of the terminal methyl groups results in a pair of isomeric alcohols, and of these the *trans*-isomer may be further oxidized to a carboxylic acid. This metabolic pathway is shown in Fig. 7. In rat liver preparations, some 2 to 3 times as much *cis*-alcohol (I) as *trans*-alcohol (II) was formed, while in mouse liver, the *trans*-isomer (II) predominated, there being 5 to 10 times as much of this as of the *cis*-form (I), but the liver homogenates of neither species formed the carboxylic acid metabolite. In monkey liver, approximately equal quantities of the two alcohols were produced together, with traces of the *trans*-carboxylic acid (III).

Pittman and associates[96] also reported on the fate of tritiated pentazocine in the rhesus monkey and man. In the monkey, some 70% of the dose was excreted in the 0 to 24 hr urine, and this did not vary with different doses over a range of 5 to 25 mg per kilogram. The unchanged drug accounted for 20% to 30% of the urinary radioactivity, with the *cis*-alcohol (I), *trans*-alcohol (II), and the trans-carboxylic acid (III) representing 17% to 21%, 7% to 16%, and 13% to 36%, respectively. Only very small amounts of the dose (6% to 3%) appeared in the feces. Human subjects excreted 50% of the dose in the urine in 12 hours after administration, and this was made up of considerable quantities of the unchanged drug and its *cis*-alcohol (I) and *trans*-carboxylic acid (III) metabolites, but only very small amounts of the *trans*-alcohol (II) were found.

The kinetics of pentazocine in plasma, after oral and intramuscular administration to human subjects, follow those of the onset, duration, and intensity of analgesia, as well as of the drug's side effects, such as dizziness, pallor, and sweating.[14] The plasma half-life of unchanged drug is approximately 2 hours, which coincides well with its duration of action, being 3 to 4 hours, and about 25% of the drug-related material in plasma consists of metabolites.[14] The time and dose dependency of the entry of pentazocine into brain correlated well with the drug's plasma concentration,[82] and from 10 to 90 minutes after dosage the plasma/brain ratio of the drug was constant, not varying with dose.

The analgesic activity of pentazocine apparently resides in the unchanged drug, as the kinetics of the analgesia in rats follow those of pentazocine and not its *trans*-alcohol metabolite,[82] and those animals that were comparatively more resistant to the analgesic effect were those that produced relatively greater quantities of the *trans*-alcohol metabolite. Of the two optical isomers of pentazocine, the *l*-form is a much more active analgesic than the *d*-isomer.[16] However, no differences were found in the distribution of the *d*-, *l*-, and *dl*-antipodes in the brain of rats, and this does not account for the stereospecificity of the analgesic action.

Pharmacology. Pentazocine is a potent analgesic, which is one-fifth to one-sixth as effective as morphine in lower animals and man and which also has some narcotic antagonist activity.[29] The overall effect of pentazocine in animals and man resembles that of morphine, but unlike morphine it evokes bizarre behavior in rats and will not cause tail erection in mice.[29] In pigeons trained to peck keys for food, low doses of pentazocine increased responding rates, while higher doses decreased rates. The *l*-isomer was the more potent.[79] Pentazo-

cine had no effect on morphine-induced decrease in pecking rates, unlike cyclazocine (a good narcotic antagonist[79]). Holtzmann and Jewett[63] found that operant behavior in the rat was increased by pentazocine in doses up to 16 mg per kilogram and was decreased by higher doses, and that both the increase and decrease were blocked by the narcotic antagonist, naloxone. The locomotor activity of rats was increased in a dose-dependent manner by pentazocine, and this was not affected by the coadministration of naloxone, although inhibition of catecholamine synthesis with α-methyl-*p*-tyrosine blocked this effect on locomotor activity. Pentazocine reduces the brain levels of norepinephine and dopamine and, to a lesser extent, 5-hydroxy-tryptamine, although their turnover is unaffected, but morphine in large doses was not so effective in reducing norepinephrine and, in fact, elevated dopamine. These actions of morphine on brain monoamines were prevented by naloxone, but naloxone did not influence the effects of pentazocine. It can be concluded that while the effects of pentazocine on locomotor activity of rats are mediated through brain catecholamines, those on operant behavior are not, and that while morphine and pentazocine act in part in similar ways, there are profound differences between them in other ways.[63]

Tolerance and dependence. The early work of Fraser and Rosenberg[47] apparently showed that the addiction liability of pentazocine was very low. In single doses, exheroin addicts did not identify it as an opiate, while morphine was correctly identified. Pentazocine did not suppress opiate withdrawal syndrome, indicating no cross-tolerance or dependence. Morphine addicts did not like chronic administration of pentazocine, and 7 of 8 tested stopped pentazocine and continued with morphine. The one subject who continued with pentazocine developed a mild morphine-like withdrawal syndrome after 25 days, but this was not precipitated by nalorphine. Under identical conditions, morphine was highly addictive, and withdrawal was induced by nalorphine. More recently, work using outpatient heroin addicts has shown that pentazocine is low in its attraction to the narcotic abuser.[102] Addicts were given oral doses of codeine, methadone, pentazocine, or placebo ad lib at a clinic, and pentazocine was the least preferred of these.[102]

Despite the foregoing, it has been shown that normal analgesic doses of pentazocine have a morphine-like subjective effect,[68] although larger doses have a dysphoric action. On prolonged administration, pentazocine gave rise to dependence, as shown by a withdrawal syndrome. This was mild and was characterized by a drug-seeking behavior pattern, and this work, performed under controlled conditions, appears to confirm the increasing number of clinical reports of dependence on pentazocine.[71] By 1969, some 30 cases of pentazocine dependence had been reported, and the AMA urged caution in prescribing pentazocine.[42] Since then many more cases of pentazocine dependence have been reported, and study of the case histories has shown that 75% of reported cases can be described as dependence-prone, by previous episodes of drug abuse; 87% of cases first received pentazocine in a bona fide manner from doctors, and continued self-administering the drug for reasons such as the relief of pain and anxiety.[42] This serves to underline the caution with which the medical profession must use this drug.

The way in which pentazocine has been shown to induce dependence, although initial work in heroin addicts appeared to indicate that it did not, questions the usefulness of this type of study as the sole criterion of whether or not a drug is dependence-producing. It would certainly suggest that such results should not be accepted uncritically.

When abused, a considerable tolerance develops to pentazocine, intake of 1 gm per day in divided doses having been reported.[71] It appears that the abuse of

pentazocine is much more common than the comparatively small number of reports in the literature would suggest, and that the abuse of pentazocine at present is largely confined to North America. Its present status as a drug of choice to narcotic abusers is indicated in the study of 1,494 addicts by Inciardi and Chambers.[65] Of this group, only 80 had used pentazocine, and although 11 of these were dependent on it, only one of them preferred pentazocine over all other drugs. Pentazocine may be used if heroin is not available, but it cannot be called a major abused narcotic at the moment. Supplies from "nonmedical" sources are apparently minimal, again showing the responsibility of the medical profession for this type of drug abuse.

In the past, methadone has been used to treat the pentazocine withdrawal syndrome, but this is opposed by most workers now on the grounds that it leads to true narcotic dependence on methadone and worsen the problem.[13, 129] If drug treatment is required, chlordiazepoxide and diazepam are suitable for the alleviation of the mild withdrawal syndrome, which does not occur in all cases of pentazocine abuse.[42] The slight narcotic antagonist action of pentazocine mentioned previously contraindicates its use in controlling opiate abuse, as it may precipitate withdrawal.[42]

Tolerance to narcotics

The development of tolerance by the organism to a drug may be due to one of two overall mechanisms. Either the ability of the organism to absorb, metabolize, and excrete the drug may be altered on repeated exposure, or there may be some adaptation of the site at which the drug exerts its action. The opiates, being the archetypal drugs of dependence, have long been studied with respect to these mechanisms, and many hypotheses for the genesis of tolerance have emerged. A number of these theories fail to explain the development of dependence, as shown by the production of a characteristic withdrawal syndrome on cessation of drug dosage. It appears that the development of tolerance to synthetic narcotics is similar to that of the opiates, and therefore the two classes are discussed together.

It would apear that the metabolic disposition and distribution of the drug by the body are not altered on chronic dosage of the opiates (see section entitled "Metabolism and Disposition of Δ^9-THC") and several authors have concluded that opiate tolerance and dependence have no basis in this.

Schmidt and Livingston[100] suggested that morphine combines with receptors in the brain and that tolerance is due to occupation and saturation of the receptors. This would mean that more drug would be required to overcome this blockade as dosage proceeded. This theory adequately explains cross-tolerance between opiates and antagonism by related molecules such as nalorphine, but fails to account either for the persistence of tolerance long after the drug has left the body or for withdrawal phenomena. However, the finding[85] that a metabolite of methadone is found firmly bound to brain proteolipids at very low levels for up to 3 weeks after a single dose may have significance and lead to a re-evaluation of the receptor occupation hypothesis.

Axelrod[6] formulated a theory for the development of tolerance to opiates based on the loss of morphine *N*-demethylation activity of the liver microsomes on prolonged dosage (see "Effects of chronic morphinization on liver microsomal enzymes"). He proposed that the receptor sites in the CNS and in the liver enzyme systems are similar, and that continuous interaction of narcotics with the receptors serves to inactivate them. Further evidence for the similarity between the microsomal enzymes and CNS receptors is seen in the activity of nalorphine on *N*-demethylation. Nalorphine is well known as an in vivo pharmacologic antagonist of morphine and other opiates, and it is also a specific in vitro antagonist of the *N*-demethylation

of these substrates.[7] Nalorphine thus appears to protect both brain and liver enzyme receptors from morphine, indicating that these two sites are very similar.

While these observations appear to provide an explanation for the development of tolerance to the opiates, it is hard to explain the genesis of physical dependence as shown by the production of a withdrawal syndrome. Shuster[107] extended the concept of changes in enzyme activity and enzyme turnover, which derives from the *N*-demethylation hypothesis, to explain withdrawal phenomena as well as tolerance. In this he introduced an unknown neurotransmitter that was influenced by morphine on single and repeated administration. A full discussion of this and other related hypotheses depending on there being a specific neurohormone influenced by morphine is given by Cochin.[24]

Of all the drugs of dependence, the opium alkaloids are the most intensively investigated from the viewpoint of their action on CNS neurotransmitters. Morphine and heroin are the most studied drugs and have been examined principally in animals rendered tolerant and dependent to them. Despite the multitude of reports in the literature, no clear picture emerges owing to the contradictory findings on several transmitters. Thus, it has been found that the turnover of 5-HT in brains of morphine-tolerant mice is increased twofold over control values, and that inhibition of 5-HT synthesis with *p*-chlorophenylalanine (PCPA) impaired the development of tolerance.[106] However, both Marshall and Grahame-Smith[76] and Algeri and Costa[4] have been unable to repeat these findings. Grumbach[55] reported that atropine intensifies and physostigmine inhibits the withdrawal excitability after morphine in the rat, but Fuentes[48] has found the reverse, i.e., atropine inhibits and physostigmine intensifies the abstinence syndrome in rats. Although brain norepinephine is depleted by single doses of morphine,[120] single doses do not give further depletion in tolerant animals.[56] The intensity of withdrawal signs appears to be related to the degree of catecholamine depletion.[56] The turnover rate of norepinephrine may or may not be affected by chronic morphinization.[4] It is of interest to note that β-adrenergic blockade using dichloroisoproterenol enhances the analgesic action of morphine more in tolerant than in nontolerant mice. The development of tolerance is slowed and naloxone-induced withdrawal is suppressed,[17] and this is of potential therapeutic interest.

Harris[57] has suggested that the CNS functions in a state of neurotransmitter equilibrium and that, while the influence of a narcotic on one neurotransmitter system may be of interest, such studies cannot point to the mode of action of the drug. In order to understand the complex CNS phenomena such as analgesia, tolerance, and dependence, it will be necessary to put many such studies together.

Following up this trend, Collier, Francis, and Schneider[28] tested the action of a number of drugs, whose pharmacology is known, on several aspects of the morphine withdrawal syndrome in the rat. PCPA, atropine, and indomethacin (an inhibitor of the synthesis of prostaglandins) all modified the withdrawal syndrome. This leads to the conclusion that morphine dependence is a multipartite phenomenon in which acetylcholine, 5-HT, and the prostaglandins are involved in different aspects. The nature of the modifications of the withdrawal syndrome by the drugs used depends on the feature of the syndrome observed and the time in the course of dependence when the modifying drug is given.

Doubtless, these findings and others like them will prove useful in explaining a number of discrepancies in the literature of morphine and neurotransmitters. Although neurotransmitter changes are obviously of importance in understanding the problem of morphine tolerance and dependence, they do not by themselves explain adaptation to the drug. It has often been

suggested that the development of tolerance to morphine and similar drugs is in some way connected with protein synthesis and that cellular adaptation to the drug is under genetic control. Cohen and associates[27] found that an RNA polymerase inhibitor, actinomycin D, prevented the development of tolerance to morphine in rats and mice. Following on this work, Cox and Osman,[33] in a very elegant series of experiments, reached the conclusion that the development of tolerance to the analgesic effect of narcotics, including morphine, heroin, and meperidine, in rats is dependent on DNA-directed RNA synthesis leading to the synthesis of new protein. The work upon which this conclusion was based used a variety of protein synthesis inhibitors and, in addition, measured brain protein and RNA synthesis. Although the ability of protein synthesis inhibitors to prevent the development of tolerance to the analgesic effect of the narcotics is well known, it appears that other parameters that changed on chronic dosage with morphine are unaffected. Cycloheximide has no effect on tolerance to the increased locomotor activity seen with morphine.[69] Varying reports have been produced on the effect of cycloheximide on dependence development as shown by the effect of naloxone. Way, Loh, and Shen[126] and Cox[31] found that this is blocked by cycloheximide, but others report that naloxone jumping is unaffected by cycloheximide.[69*]

If indeed CNS protein synthesis is involved in the genesis of tolerance and dependence, it is interesting to speculate on the nature of the "tolerance protein" being produced. Cox and Ginsburg[32] suggest that possible roles for this new protein could be in the production of a drug uptake receptor, a neurotransmission receptor, or a transmittable tolerance factor, among others. It is appropriate to note here the work of Ungar and Cohen,[118] who found that the brain of morphine-tolerant animals contains a substance that confers morphine tolerance to nontolerant animals when injected into them. They suggest that this substance is a small molecule, possibly a hexapeptide. Other workers[32] have been unable to repeat this, although they do not rule out the possibility of such transmittable tolerance factor occurring. It may be that the protein synthesized during tolerance development can be transmitted from animal to animal and that the findings of Cox and Osman[33] and Ungar and Cohen[118] are linked in such a way. This still does not elucidate a mode of action for this "tolerance protein," but here it is possible to see a striking analogy with work on learning and memory. The ability of animals to learn simple tasks may be impaired by the administration of protein synthesis inhibitors, and it has been demonstrated that extracts from the brains of rats trained to perform a given task, when administered to untrained rats, convey the ability to perform this task.[20, 119] The similarity of these findings with the work described on morphine tolerance would suggest that the basic mechanism of tolerance development is similar to that of memory; that the brains of the animals in some way "learn" to "ignore" the presence of the drug. However, it must be stressed that present knowledge of the biochemistry of learning processes is inadequate, and Cooper, Bloom, and Roth[30] stress the extreme difficulties involved in the interpretation of such work.

As an alternative to these neurochemical explanations, Cochin[25] and Berkowitz and Spector[15] have suggested that the long persistence of tolerance, its apparent transferability, and the involvement of protein synthesis indicate a possible participation of the immune mechanisms of the body. Berkowitz and Spector[15] have shown that mice can be actively immunized against morphine with a conjugate of 3-carboxymethylmorphine with bovine serum albumin. Sera from these mice showed a greatly increased (thirtyfold) ability to bind dihydromorphine, and the plasma

*Marshall, I.: Personal communication.

half-life of the drug in immunized mice is much prolonged. These mice showed tolerance to morphine, but not dependence as nalorphine was without effect. These findings do not, of course, explain tolerance to the opiates, still less dependence, but it must be considered that active or passive immunity may influence tolerance. Proteins able to bind heroin have been isolated from the plasma of heroin-dependent subjects,[98] indicating the potential involvement of the immune mechanisms in man.

In conclusion then, it is seen that the answer to the problem of narcotic tolerance and dependence remains very obscure. Many new approaches have yielded fascinating possibilities, and it does seem that tolerance and dependence, rather than being a single discrete phenomenon, represent the net result of several distinct mechanisms.

We thank Prof. R. Tecwyn Williams, F.R.S., and Prof. R. L. Smith for their help, advice, and interest during the preparation of these reviews. We are grateful to Z. H. Siddik for unpublished data, and to Drs. R. D. Barnes and R. B. Franklin for help in gathering part of the material. We wish to acknowledge the courtesy of the following for helpful discussions, correspondence, and access to material prior to publication: Susan D. Iversen, Cambridge, England, E. Änggård, Stockholm, Sweden, L.-M. Gunne and T. Lewander, Uppsala, Sweden, R. E. McMahon and H. R. Sullivan, Indianapolis, Indiana, and I. Marshall, and A. H. Beckett, London, England.

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