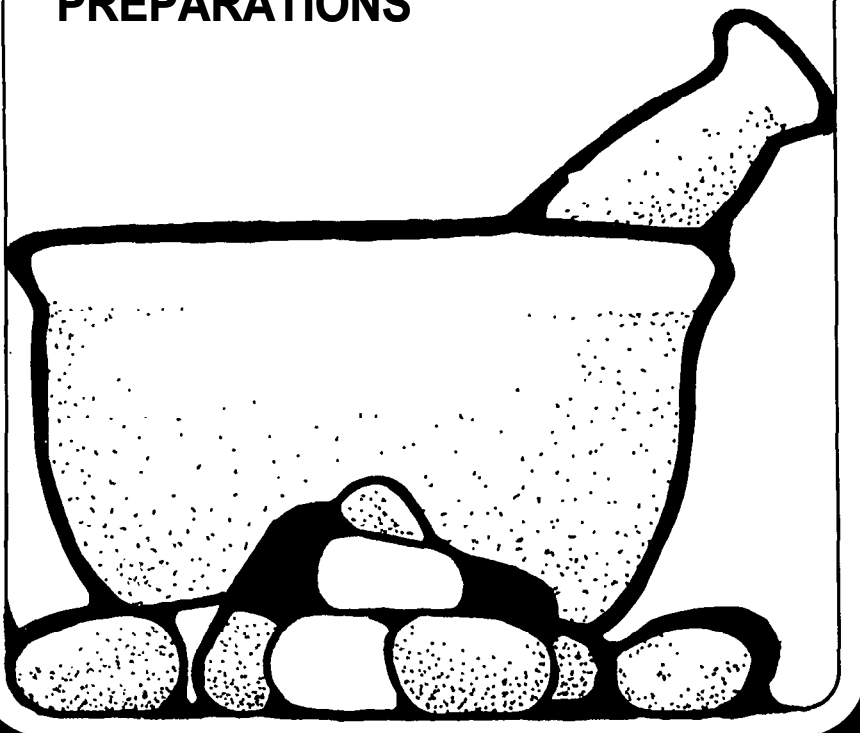
National Institute on Drug Abuse

Research 28

MONOGRAPH SERIES

Narcotic Antagonists:

NALTREXONE PHARMACOCHEMISTRY AND SUSTAINED-RELEASE PREPARATIONS



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service • Alcohol. Drug Abuse • and Mental Health Administration

Narcotic Antagonists:

Naltrexone Pharmacochemistry and Sustained-Release Preparations

Editors:

Robert E. Willette, Ph. D.
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NIDA Research Monograph 28

1981

DEPARTMENT OF HEALTH AND HUMAN SERVICES
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This monograph is based on papers presented at a technical review on the clinical pharmacochemistry of naltrexone, held at Parklawn Building, Rockville, Maryland, on May 30 and 31, 1919.

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FOREWORD

With increasing frequency, Federal agencies are being called upon to evaluate and develop new drugs and treatments for a wide variety of diseases and related conditions. The so-called "orphan" drugs, or drugs of little or limited commercial value, are being shunned by the pharmaceutical industry, due primarily to the ever-increasing developmental costs and risks associated with new drugs. Thus, within the Public Health Service, a drug development effort has emerged to fill this void.

The United States Congress has charged the National Institute on Drug Abuse with the responsibility to develop and provide drug-dependent people with adequate and, if necessary, new treatment methods. Amongst the many approaches undertaken have been efforts to develop drug therapies to serve as adjuncts to psychologic and sociologic efforts. The two principal initiatives have focused on the maintenance drug, levo-alpha-acetylmethadol (LAAM), and the narcotic antagonist, naltrexone. The continuing accounts of milestones in their development have been presented in numerous scientific and medical articles and summarized in several volumes of this Research Monograph series. We consider this volume to represent volume 5 in our series on Drug Development.

The first of this series, Monograph 4, *Narcotic Antagonists: The Search for Long-Acting Preparations,* represented a status report on early efforts to develop such a preparation for naltrexone. Monograph 8, *R_x:3x/Week LAAM, Alternative to Methadone,* and Monograph 9, *Narcotic Antagonists: Naltrexone Progress Report,* described progress on LAAM and naltrexone, respectively, as the Institute's newly established drug development program was fully underway. In a departure from these advanced developmental programs, basic concepts in the design of new agents was the subject of Monograph 22, *Quantitative Structure Activity Relationships of Analgesics, Narcotic Antagonists, and Hallucinogens.*

Previous volumes on naltrexone have detailed the conceptual approach to the use of a narcotic antagonist or agent that blocks the effects of heroin or other opioids. Of the early drugs tested, naltrexone emerged as the most promising. It is currently under clinical

evaluation, with a strong likelihood of being marketed in oral form within the next year or so. Although this will provide a blocking drug with three-times-a-week dosage, it is felt that a longer-acting drug or dosage form would be more desirable in the treatment of narcotic addiction. It has been nearly five years since we published Monograph 4. At that time we summarized many promising approaches and interesting leads. Some of these encouraged us to feel that at least one of the preparations would be ready for clinical trials within the near future. That day has continued to elude us, but we are now much wiser and more experienced and presently have a preparation in hand that awaits a clinical trial.

In this volume, we summarize the most promising of the approaches pursued over the past several years. General overviews of the background for chemical and pharmacological decisions are given. In addition, details of the analytical methods for measuring naltrexone levels, the pharmacokinetics of naltrexone in test animals and man, and the development and manufacture of clinical materials are presented. We expect this volume to help serve as a guide to other efforts in the area of drug development for long-acting drug preparations.

The Editors

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Part I

Introduction and Overview

Naltrexone: Research Monograph 28
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National Institute on Drug Abuse, 1980

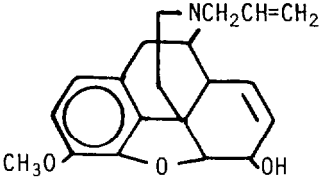
Historical Perspective on the Chemistry and Development of Naltrexone

Sydney Archer

The first clinically useful narcotic antagonist was nalorphine. This compound was relatively weak, short-acting, and produced a number of side effects, the most prominent of which were psychotomimetic in nature. For these reasons nalorphine did not qualify as a possible modality for the treatment of heroin addiction. Cyclazocine is a totally synthetic narcotic antagonist which is much more potent and longer acting than nalorphine. It was the first compound used by Martin in clinical trials in postadicts. However, its dysphoric effects necessitated long induction periods and these CNS effects precluded its use in long-acting delivery systems. Naloxone was a "pure" antagonist which did not produce the psychotomimetic effects o either nalorphine or cyclazocine. Although it is a potent antagonist when given parenterally, it is shorter acting than cyclazocine. Replacement of the N-allyl substituent of naloxone with the cyclopropylmethyl radical of cyclazocine led to naltrexone, which is even more potent than either naloxone or cyclazocine and has a longer duration than naloxone. Because of this favorable combination of properties naltrexone proved to be the drug of choice for inclusion in long-acting delivery systems.

Although the first narcotic antagonist, N-allylnorcodeine (I), was described by Pohl in 1915, the first important member of the class of narcotic antagonists was nalorphine (II), an authentic sample of which was prepared by Weijlard and Erickson in 1942 (1). Nalorphine showed no analgesic activity in the rodent laboratory assays

in vogue at that time, such as the D'Amour-Smith test in rats and the Eddy-Leimbach test in mice, but instead was found to antagonize the agonist effect of morphine (2,3). Until recently, it was used as a clinical antidote for acute narcotism but was effectively replaced by naloxone as the drug of choice for this condition.



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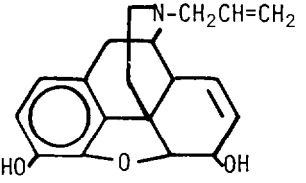


II

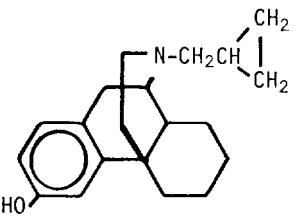
In clinical studies designed to determine whether combinations of nalorphine and morphine could produce analgesia with a reduced incidence of respiratory depression, Lasagna and Beecher (4) reported that nalorphine when given alone was a potential analgesic in man. This clinical finding was confirmed by Keats and Telford who extended these studies to other narcotic antagonists and found other examples which produced significant clinical analgesia (5,6). Early studies on the addiction liability of nalorphine showed that it produced neither physical nor psychic dependence after prolonged administration to postaddicts (7), although a later study demonstrated that a mild physical dependence unlike that of morphine could develop (8). The major drawback of nalorphine and its other analgesically active congeners was that intense dysphoric side effects were observed at or very near clinically effective doses in a large fraction of patients. Thus these compounds were judged to be clinically unacceptable as analgesics.

The importance of these observations lay in the fact that for the first time it was found possible to divorce potent analgesia from morphine-like addiction liability. In retrospect it is curious that what proved to be such a seminal observation attracted so little attention of medicinal chemists engaged in the synthesis of strong analgesics. Two groups, one at the Sterling-Winthrop Research Institute (9,10), and the other at the University of Rochester (11), independently embarked on the synthesis of narcotic antagonists with the intention of finding a nonaddicting strong analgesic.

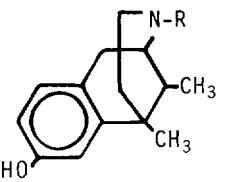
The Rochester group explored the tetracyclic morphinan series while the Sterling-Winthrop group concentrated on the tricyclic benzomorphan series, the chemistry of which was the subject of a series of papers by E. L. May (12). The major obstacle to this ap-

proach was the lack of a laboratory model which could detect agonist activity in putative mixed agonist-antagonists. As a matter of fact, very few laboratories were testing for narcotic antagonist action and the chemists at Sterling-Winthrop were fortunate that Harris and Pierson had developed a quantitative test for this type of pharmacological activity (13). Quite fortuitously, Gates and Archer discovered that they were working in the same area, and the former accepted an invitation to have his compounds tested by Harris and Pierson. The results of this collaborative effort were published simultaneously in the Journal of Medicinal Chemistry (10,11).

The most interesting member of the Gates series was cyclorphan (III). This compound proved to be a potent antagonist in laboratory animals (14) and man. Although psychotomimetic effects were observed, they did not occur as frequently as with nalorphine (15). Cyclorphan was about forty times as potent as morphine on a milligram basis, i.e., 0.25 mg of III provided analgesia equivalent to 10 mg of morphine.



III Cyclorphan



IV Pentazocine, R = $-CH_2CH=C(CH_3)_2$

V Cyclazocine, R = CH_2CH (CH2, CH2)

The compounds which attracted the greatest interest in the benzomorphan series were the weak antagonist pentazocine (IV), and the powerful antagonist cyclazocine (V). Pentazocine was shown to be about 1/3-1/6 as potent as morphine as an analgesic (16,17) with reduced abuse potential (18) and with a reduced incidence of psychotomimetic effects (16). Cyclazocine was a highly effective analgesic in man when given either orally or parenterally (15). It is a potent antagonist with a long duration of action, a result that could not be predicted from animal studies (19). Dependence studies in postaddicts showed that after escalating doses of the drug over a prolonged period of time, abrupt withdrawal produced a withdrawal syndrome different from that of morphine in that it was quite mild and no drug-seeking behavior on the part of the addict was

observed (20). Cyclazocine would have been an almost ideal analgesic except for the fact that a small but significant percentage of the patients exhibited psychotomimetic effects at or near analgesic doses. Tolerance to these effects appeared to develop on prolonged administration.

Wikler (21) suggested that drug dependence evolves through a series of several phases. At the beginning an individual may use opiates to relieve daily tensions and discomforts. With each episode of self-administration the habit is reinforced. As tolerance to the drug develops, the effects of the opiate are reduced so that higher doses are required to afford the same degree of relief. If the addict fails to maintain an adequate drug intake his dependence is further reinforced. He may then seek assistance in becoming detoxified or may attempt a self-induced withdrawal. In either case marked physiological changes take place during the withdrawal phase which may last for several months even when detoxification occurs slowly. This will lead to drug-seeking behavior or recidivism. Wikler feels that an extinction of drug-seeking behavior could be a means of reversing the conditioned abstinence. This could be accomplished by blocking the relief obtained from use of opioids during this critical period. One way to accomplish this goal is to administer a substance with antagonist action which in itself does not induce tolerance or dependence but which blocks the pharmacological effects of the opiates.

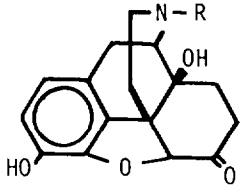
On the basis of these considerations Martin considered the use of narcotic antagonists as a method for treating heroin dependence. Nalorphine was the first candidate, but its short duration of action and high incidence of dysphoria prompted Martin to select cyclazocine for such a study (19). The rationale for the use of cyclazocine was given by Martin as follows: "On the basis of our studies, 4 mg per day of cyclazocine will provide protection against the euphorogenic actions of large doses of narcotics, prevent the development of physical dependence, and will thereby control the pharmacological actions which are held responsible for narcotic addiction....There may be other benefits....Wikler stated that two of the most important reasons for relapse of the abstinent narcotic addict are conditioned abstinence, which may be evoked by stimuli that have been associated with the addict's hustling activity to acquire drugs, and reinforcement of drug-seeking activity through repeated reductions of abstinence by drug. It is possible that in sub jects who attempt to readdict themselves while receiving a narcotic antagonist such as cyclazocine, there may be extinction of physical dependence and drug-seeking behavior."

Martin, Gorodetzky, and McClaine (19), and Jaffe and Brill (22), were the first to study cyclazocine as a modality for treating opiate addicts. These early studies were promising enough to warrant further clinical trials. Fink and his colleagues (23) succeeded in carrying out a more rapid induction of cyclazocine than Jaffe and Brill, who required 10-40 days to reach the 4.0 mg/day dose which was necessary for effective clinical antagonism of 25 mg heroin challenges. The slow induction period was necessary in order to permit the subjects to become tolerant to the agonist effects of cyclazocine. Fink and his colleagues (23) found that induction could be effected over a period of 4 days by using naloxone as needed to antagonize the unpleasant effects of cyclazocine. The dose of cyclazocine was increased by 1.0 mg/day when 600 mg of naloxone was available every 4 hours. At a stabilized dose of 4.0 mg/day of cyclazocine the subjects reported no effect from a challenged dose of a $20 quantity of "street" heroin, a transient "buzz" from twice that amount, and a mild euphoria from a $60 amount taken 12-14 hours after oral administration of cyclazocine.

Naloxone (VI) was synthesized by Lowenstein and Fishman in 1960, and in 1961 Blumberg reported that it was a powerful narcotic antagonist in animals (24). It was also reported that the compound was inactive as an analgesic. Foldes (25) found that naloxone was an active narcotic antgonist in man with none of the dysphoric effects of cyclazocine. Although a 1.0 mg intravenous dose of naloxone could block the effects of a 25 mg heroin challenge, when given orally 3.0 g/day were required (26) to block a 50 mg challenge given 24 hours after the administration of VI.

The principal shortcoming of naloxone is its short duration of action. Blockade of a heroin challenge rarely exceeded one day so that the omission of a daily dose allowed the subject to become high on heroin.

On the basis of these early experiences it became clear that what was needed was 1) a long-acting, potent, "pure" narcotic antagonist and 2) a delivery system which would keep the subjects under constant drug pressure.



VI R = $-CH_2CH=CH_2$ Naloxone

VII R = CH_2CH(cyclopropyl) Naltrexone

The first of these requirements was met by naltrexone (VII) (27). This compound is about twice as active as naloxone as an antagonist and inactive as an analgesic in the mouse. In the rat it showed weak agonist action. Furthermore in man it was longer acting than naloxone (28) and produced no unwanted side effects, thus eliminating the need for an induction phase.

The problem of a suitable delivery system remains. On the basis of a simple pharmacokinetic analysis it was concluded that a system with a release rate which followed zero-order was more desirable than one which manifested first-order kinetics (29). It would also be desirable to have a delivery system whose matrix would biodegrade at approximately the same rate at which the drug was released so that when the administered dose of the drug was exhausted there would be no residual matrix left behind.

It was at this point in the history of the development of narcotic antagonists that the National Institute on Drug Abuse stepped in. Several contracts for the fabrication of long-acting biodegradable naltrexone delivery systems were funded and long term toxicity and pharmacokinetic studies on naltrexone were sponsored. As a result of these efforts on the part of NIDA at least one system has been brought to the point where a clinical trial may be initiated in the foreseeable future.

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Treatment of Heroin-Dependent Persons With Antagonists: Current Status

Pierre F. Renault

Naltrexone is an important new pharmacologic adjunct to the treatment of heroin dependence. The development of naltrexone has been nurtured in the mature recognition that simple detoxification or simple opiate replacement therapy is not appropriate for every heroin addict. Our current data indicate that naltrexone is safe and effective. Its use may be limited to a minority of addicts, those who are highly motivated and opiate free, because patient compliance has been a major problem with which clinicians using naltrexone have had to contend. Patient compliance is a problem, because there are no immediate consequences to the patient for stopping his naltrexone regimen. Side effects from naltrexone have been minimal and have occurred in a minority of patients. They consist primarily of gastrointestinal symptoms, including nausea and occasionally abdominal pain.

INTRODUCTION

Like many chronic diseases, heroin addiction is a complex condition to treat and a therapist treating heroin dependence must often satisfy nonmedical authorities that his treatment will have an impact on the "problem." The "problem" of heroin dependence, however, is not simply an individual behavioral problem but one of cultural, historical, and socioeconomic dimensions, Thus, it is not

uncommon for the effectiveness of a treatment to be measured more in terms of its impact on crime rate or prevalence of heroin addiction rather than the success in the treatment of afflicted individuals. This complexity has confused therapists and has left the goals of individual treatment poorly defined. Conversely, the lack of definition of treatment goals has encouraged unreasonable expectations of the treatment system and oversimplified conceptualizations of the problem of heroin addiction. In spite of this, effective treatment programs have been established and a majority of heroin-dependent individuals who come for treatment can expect to be helped.

The ultimate goal of treatment for heroin-dependent persons should be the patient's control over the use of the drug; however, in the case of heroin, society has demanded that control be defined as abstinence. This demand has led Governments to support detoxification programs, because detoxification is generally applicable, low in cost, and it is the most immediate and direct way of disentangling an individual from heroin. However, government emphasis on detoxification programs often betrays the oversimplified view that heroin dependence is simply a pharmacologic problem. On the contrary, heroin dependence involves every aspect of an individual and his environment. Individual freedom of choice is undermined by conditioned responses, pressures from peers, the social frustrations which often seem to form the substrate of heroin use, and subtle pharmacologic factors such as the intense craving associated with protracted abstinence.

An often more realistic definition of control over drug use is the elimination of dysfunctional drug use. Dysfunctional drug use has been defined as drug use that results in physical, psychological, economic, legal, and/or social harm or discomfort to the individual user or to others affected by that drug user's behavior. It is the discomfort that drug use causes that brings an individual to treatment, and the elimination of that discomfort should be the most immediate treatment goal. It was this conceptualization of treatment goal that nurtured the development of substitution or maintenance therapy of heroin addiction with methadone. Methadone maintenance effectively returned the control of heroin to the individual by decreasing craving and eliminating abstinence symptoms. It also promoted functioning in normal society by decreasing the frequency of drug taking, the disruption by drug effects, and the difficulty and costs of obtaining drugs. Although it is clear that methadone or levo-alpha-acetylmethadol (LAAM) maintenance does decrease dysfunctional drug use, it is not applicable to all addicts. Maintenance or stabilization treatment has only been used in

chronic addicts where the prospects of total abstinence are poor. However, many narcotic-dependent individuals desire total abstinence and find the concept of methadone maintenance unacceptable. Other individuals are currently abstinent but are in danger of relapsing. Among the latter are those recently detoxified from heroin addiction, or those who are being released from prison, or even others who, in spite of prolonged abstinence, have been suddenly tempted to take heroin again by the pressures of emotional turmoil or the ready availability of heroin in their immediate environment. Finally, there are those individuals who have never been dependent on heroin but are using the drug and, therefore, are at risk of becoming dependent. Currently available treatments for such individuals are limited and the pressures on the abstinent addict to relapse are intense. These "abstinent addict" patients are often strongly motivated to seek treatment and relapses often produce guilt and depression, but they are still unable to resist giving in to the intense craving for heroin. They are strongly motivated, but motivation simply is not enough. The recognition of the need to broaden the spectrum of heroin dependence treatment to include these patients has nurtured the development of narcotic antagonist treatment.

THE CONCEPT OF NARCOTIC ANTAGONISTS

Narcotic antagonists are usually defined as chemical compounds which block the effects of opiate drugs. Narcotic antagonists will block the analgesia, the euphoria, and all the physiologic changes such as pupilary constriction produced by agonist opiates. By blocking the effects of agonist opiates, narcotic antagonists also prevent the development of physical dependence and tolerance to opiate drugs.

Opiate drugs exist on a continuum with morphine, heroin, and other agonists at one end and antagonists such as naloxone and naltrexone at the other end. There are many well-known drugs which fall between the pure agonists, such as heroin and morphine, and the pure antagonists, such as naloxone and naltrexone. Drugs such as pentazocine, cyclazocine, and nalorphine have mixed agonist and antagonist effects. For example, pentazocine, a commonly used analgesic drug, will precipitate the abstinence syndrome in an individual who is physically dependent on heroin. Cyclazocine is a potent antagonist, but the induction period is characterized by agonist effects of a dysphoric nature. During the induction onto cy-

clazocine, tolerance builds rapidly to the dysphoria, and not to the antagonistic activity of the drug.

The blockade produced by narcotic antagonists is different from that produced by disulfiram. Disulfiram (Antabuse) is a commonly used drug for the treatment of alcoholism. It exerts its effect not by blocking the effects of alcohol, but by interrupting the metabolism of alcohol at the acetaldehyde stage. The build-up of acetaldehyde produces severe symptoms of nausea, vomiting, and hypotension. The knowledge that drinking will produce such symptoms may act as a deterrent to further drinking for some alcoholics. Narcotic antagonists exert their activity by blocking the access of agonists to the opiate receptor sites at the molecular level. Antagonists do not produce symptoms when an agonist is administered. Rather, when opiate receptor sites are occupied by an antagonist, opiate agonists simply have no pharmacologic effect.

Although narcotic antagonists do not produce symptoms when they are used in the treatment of heroin dependence, they will precipitate an abstinence syndrome in individuals who are physically dependent on an opiate drug. By virtue of their greater affinity for the opiate receptors, they will displace opiate agonists from the receptor sites. A heroin addict must be detoxified before he can be treated with a narcotic antagonist. Once completely free of opiate drugs, however, no symptoms will be produced by the administration of a narcotic antagonist.

The theory underlying narcotic antagonist treatment of heroin dependence is based on the concept of extinction. This assumes that the euphoriant effects of opiate drugs reinforce the self-administration of these drugs. If an ex-addict is given a narcotic antagonist and he takes an agonist drug, he will experience no effect, i.e., no euphoria. This lack of reinforcement will gradually result in the extinction of heroin self-administration (1-3).

The concept of narcotic antagonist treatment has been criticized on the basis that since these drugs do nothing "positive" for an individual (the only subjective effect is the absence of an effect when an antagonist such as heroin is taken) and there are no pharmacologic consequences (such as withdrawal symptoms) for not taking an antagonist, compliance with a treatment program employing a narcotic antagonist drug will be quite low. Compliance is a problem with medications for most chronic conditions where the medication is given to prevent the progression of the disease rather than to alleviate a symptom which would recur if the medication were not taken.

At least three solutions have been proposed to meet this objection. It has been proposed that naltrexone be administered in a

long-acting form. Sustained action preparations lasting up to a month or more could be developed. Another suggestion has been that external coercion be used to force individuals to comply with a narcotic antagonist regimen. For example, individuals released on parole from the civil justice or civil commitment system could be threatened with reincarceration for lack of compliance. Prisoners in a work release program could be required to take a narcotic antagonist prior to leaving the prison to work each day. Finally, some have suggested that an antagonist be sought which would have some agonistic euphoriant properties, a mild euphoriant effect which would not be disruptive or lead to dysfunctional drug use but which would act as a reinforcer and encourage compliance.

THE DEVELOPMENT OF NALTREXONE

In the beginning of this decade, the decision was made to encourage the development of a narcotic antagonist to be used in the treatment of heroin dependence. Available compounds included naloxone, cyclazocine, and naltrexone. Both naloxone (4-6) and cyclazocine (3,7-9) were tested in clinical trials. Both of these drugs proved to be excellent antagonists but both had drawbacks. Cyclazocine was limited because of dysphoric side effects during the induction period. Naloxone is a pure antagonist, and thus, it is free of side effects, but lacks potency when administered orally. Finally, naltrexone was selected for development. Naltrexone is a potent narcotic antagonist. It has a long duration of action, up to 3 days following one oral dose. It is devoid of agonist activity and thus the induction phase is virtually asymptomatic. Martin et al. (10) found that a 50 mg oral dose of naltrexone will block the subjective effects of 25 mg of morphine for 24 hours and that it will attenuate the development of physical dependence. O'Brien et al. (11) reported that the blockade produced by a 120-200 mg dose of naltrexone at 48 hours was not absolute, but was sufficient that subjects discontinued self-injection of 1-4 hydromorphone (Dilaudid). Resnick et al. (12) reported complete blockade of the agonist effects of 29 mg of intravenous heroin 24 hours after a 50 mg or larger dose of naltrexone, and 48 hours after 120 mg or larger dose. They also found some individual variability, i.e., one patient required 200 mg (their maximum dose) to achieve 24-hour blockade. Finally, Martin et al. (10) demonstrated that tolerance does not develop to the antagonistic action of naltrexone.

The drug development process in the United States requires extensive animal toxicity testing followed by phased human clinical

trials. Most of the required animal toxicity testing of naltrexone has been completed. Phase I human trials involve the basic pharmacology of the drug in humans, such as the work reported by Martin. Phase II involves the testing of the drug in otherwise healthy heroin-dependent males. Phase II clinical testing of naltrexone was organized into two cooperative studies.

The first cooperative study was a fixed dose, randomly assigned, prospective, double-blind comparison of naltrexone and placebo conducted in five clinics following three protocols. In all, 192 patients participated; 94 were randomly assigned to naltrexone and 98 to placebo. The three protocols were determined by patient characteristics. In one protocol, street addicts were detoxified from heroin and placed on naltrexone or placebo. In the second protocol, individuals were placed on naltrexone or placebo immediately after detoxifying from methadone maintenance; and in the third protocol, postaddicts from the criminal justice system were placed on naltrexone or placebo. The other cooperative study involved a loosely-knit group of 12 clinics, which followed a variety of protocols, but used a uniform data collection system. These studies were largely open and uncontrolled. They were intended to gain clinical experience with naltrexone and the data collected was useful primarily as an investigation of the safety of the drug in comparison with the safety results reported in the double-blind study. In all, 1,005 patients participated in this study, 10 of whom were female. Another important difference between the two studies was that dropouts could reenter the open study.

Both cooperative studies have been completed, and the results are available. Both show naltrexone to be safe. There were no drug-related medical problems in the double-blind study. In the open study, one individual developed idiopathic thrombocytopenic-purpura which may have been the result of sensitivity to naltrexone. Fifty-three of the 1,005 cases were terminated for "medical reasons," none of which were clearly drug-related. (In the double-blind study, five of the 98 placebo-medicated patients were terminated for "medical reasons.") No subjects in either study were dropped because of serious deviation in a clinical laboratory determination. In the double-blind study, four laboratory parameters statistically differentiated the naltrexone group from the placebo group. However, none of the differences in hemoglobin, total protein, bilirubin, and urine red blood cells were of a clinically significant magnitude. Only a slight increase in bilirubin was also found in the open study. Again, this was not clinically significant. The naltrexone group did have more side effects. These consisted primarily of gastrointestinal complaints, such as nausea and abdominal pain.

There were also more complaints of headache and skin rash. Again, these side effects were of low incidence and the safety data have been judged to support the conclusion that naltrexone has a wide margin of safety for clinical use.

The Phase II clinical trials did show the anticipated poor compliance with naltrexone. And, although retention rate tended to be better and the use of narcotic drugs tended to be less in the naltrexone group compared to the placebo group, these differences, when compared individually, were not statistically significant.

Since the theory behind narcotic antagonist treatment involves extinction and the concept of extinction implies some use of narcotic drugs, a subsample of those individuals who had at least one urine sample positive for morphine and/or methadone was studied. Only 17 of the naltrexone and 18 of the placebo subjects actually tested the blockade by using an opiate agonist (heroin or methadone). But, in this subsample, the naltrexone patients had significantly fewer subsequent urines positive for methadone or morphine and this difference was statistically significant. Also, the pattern between the two groups was different. The pattern in the naltrexone group was to test once or twice with heroin or methadone and then to stop. The use of these drugs in the placebo group was sporadic during the entire course of treatment. Thus, it seems clear that naltrexone can prevent impulsive heroin use in motivated patients from becoming a full-fledged relapse to chronic heroin dependence.

A very interesting finding from the double-blind study deserves a special note. During the course of the study, subjects were administered a craving scale. This was an analogue scale on which subjects rated the amount of craving they felt at the time. Response on this instrument was variable, but the naltrexone patients reported statistically significantly less craving toward the end of their evaluation than did the placebo-treated patients.

CLINICAL USE OF NALTREXONE

Naltrexone is administered orally either in liquid or solid (capsule) form. The capsule is preferable, because naltrexone has an extremely bitter taste. However, there is some slight danger of diversion when capsules are used, and the liquid formulation was used in most clinical trials to ensure that the subjects actually ingested the administered dose. The frequency of administration is either daily or three times a week. The daily dose is 50 mg and the dosage for a three-times-a-week regimen is 100 mg on Monday and

Wednesday and 150 mg on Friday. Both of these regimens had been found to provide an adequate blockade for the heroin used in the United States.

Postaddicts who are *not* currently physically dependent have generally been inducted onto naltrexone by 10 mg increments until 50 mg daily is reached. Then, they have been converted to a 100-100-150 mg regimen. However, some clinicians feel that an induction period onto naltrexone is unnecessary in the postaddicted patient.

Patients who are currently physically dependent on an opioid drug must first be detoxified before they can be put on naltrexone. The selection of a method of detoxification is up to the individual clinician. Once the patient has been detoxified, it has been customary to administer a small amount of naloxone intravenously to be certain that the patient is no longer physically dependent. Thomas et al. (13) suggest 0.8-1.2 mg intravenously. If there is no response to this dose of naloxone, induction on a full dose of naltrexone should be without discomfort.

The clinic setting in which naltrexone is administered is critical to the success of the treatment. It should be recognized that naltrexone is not a treatment in itself. It is a pharmacologic adjunct to treatment. The actual treatment consists of the efforts made by the staff to help the individual patient alter his lifestyle so that he can function successfully in normal society apart from the drug-abusing subculture. It is not appropriate in this paper to enter into a lengthy discussion of clinic milieu, psychotherapeutic techniques, and rehabilitative measures that can be employed with heroin-dependent individuals. Suffice it to say that in the United States, the tendency has been to create a supportive and permissive milieu where individuals are strongly encouraged to remain on naltrexone until they have significantly altered their lifestyle.

There are three types of patients for whom naltrexone seems particularly well suited. These are adolescent heroin users, "abstinent" addicts at risk of recidivism, e.g., individuals recently released from prison, and patients recently detoxified from methadone maintenance. Adolescents will have had a relatively short experience with opiate drugs. Many may be experimenters with heroin and not physically dependent. Those who are physically dependent will have been so for only a relatively short period of time. All these individuals are at risk of becoming chronically heroin dependent. Currently, there is no available pharmacologic adjunct to assist in the treatment of adolescent heroin users. Prisoners who have been recently paroled have, presumably, been narcotic free for the duration of their incarceration. However, it is well known

that upon reentering their old environment, these postaddicts are at risk of becoming readdicted. Often, the sequence of events begins with an impulsive dose of heroin which leads precipitously to the reestablishment of old behavioral patterns and compulsive heroin use. Within the criminal justice system, naltrexone may also make a significant contribution to work release programs. Naltrexone may make it possible for addicts to participate in such programs and thereby facilitate their rehabilitation (14). Patients recently detoxified from methadone maintenance are also known to be vulnerable to relapse. There is evidence that this vulnerability may have a pharmacologic basis (15). In the future, naltrexone may form the basis of a comprehensive after-care program for methadone maintenance patients which will carry them through this period of pharmacologic vulnerability.

Noticeably absent from this list are freshly detoxified heroin-dependent individuals. The experience in the United States during Phase II clinical testing indicated that the motivation in this group was less than that of the other groups tested as measured by their compliance. Admittedly, this may not be true in other treatment situations. The heroin-dependent individuals, who were treated with naltrexone in the Phase II clinical trial, were free to leave at any time, and had no incentive or social constraint to encourage them to remain on naltrexone for sufficient time to participate fully in the clinic's treatment program. The problem of compliance is not unique to naltrexone. It is common in all of medicine, but especially with medications administered to asymptomatic individuals who have a latent form of a disease. A convenient analogy is that of antihypertensive medication. It is a human characteristic to forget the pain and the symptoms which led one to treatment, as soon as those symptoms are eliminated by the treatment.

The problem of compliance with naltrexone treatment has led to two basic philosophies underlying the clinical use of naltrexone. Naltrexone has been conceived as a maintenance drug which an individual takes continuously for a year or two or perhaps indefinitely. Naltrexone has also been conceived as a crisis medication to be used by individuals whenever they feel at risk of resuming heroin use. Maintenance on naltrexone appears to be essential for the majority of patients. As a maintenance drug, naltrexone offers protection against the impulsive drug use which can easily lead to the resumption of chronic heroin dependence. On the other hand, when there are few incentives to continue naltrexone use, the problem of compliance often interferes with maintenance. Thus, from a practical point of view, only a minority of patients will use naltrexone as a maintenance drug. Most patients tend to drop out of treatment

prematurely, but many return requesting a resumption of naltrexone treatment when they experience themselves relapsing. The problem of compliance has encouraged the free use of clinics and naltrexone for support during crises. However, the recognition of the danger of relapse requires a level of maturity, self-reliance, and insight which is not characteristic of most patients.

THE FUTURE OF NALTREXONE

The immediate future of naltrexone will be the completion of Phase III clinical trials and the eventual marketing of the drug for general use. However, even with the marketing of naltrexone, several important research issues will remain.

We must improve our ability to select patients for naltrexone treatment. This is part of a broader issue which includes all forms of opiate addiction treatment. We are still unable to characterize those individuals who do best with the currently available treatment modalities.

An exciting area of research will be on the effects of naltrexone on the endorphin system. The fact that in the Phase II double-blind study, naltrexone appeared to decrease craving suggests that it may have a "healing" effect on the endorphin system. It may well be that endorphins serve an important function in the regulation of mood and sense of well-being. It also seems possible that prolonged use of heroin may atrophy this system and the subsequent disruption in mood and sense of well-being may be the basis of prolonged craving in addition to the known pharmacologic phenomenon of prolonged abstinence. It would be of the utmost importance if naltrexone proved to be not only a protection against impulsive heroin use, but also directly beneficial by helping to reverse heroin-induced atrophy in the endorphin system.

Along with the development of oral naltrexone, sustained action preparations are also being developed. These are injectables which can be removed if necessary. Their planned duration of action will be approximately one month. However, none has been tested in man as yet. Sustained action preparations have been regarded as a solution to the problem of compliance; but, even monthly injections will require patient compliance. Their most serious disadvantage is that they will block narcotic analgesia and therefore must be removable or overridable in the event of severe pain (16).

CONCLUSION

The immediate future of naltrexone will be the completion of clinical testing and the marketing of naltrexone for general use. However, broader research questions also remain. Who are those patients likely to benefit from narcotic antagonist treatment? Is naltrexone the "ideal" antagonist? Naltrexone was chosen for its lack of agonistic properties which were experienced as dysphoric side effects in the case of cyclazocine. But, would an antagonist with some reinforcing euphoric agonistic properties be more acceptable to patients and, therefore, effective in a larger population of patients? Does a pure antagonist such as naltrexone have a "healing" action on the disequilibrated endorphin system? What are the effects of naltrexone on protracted abstinence? Is naltrexone best viewed as a maintenance or a crisis drug? Answers to these questions will come as this important technical advance is used more widely by thoughtful clinicians.

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Part II

Analytical Methods

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An Electron-Capture Gas Chromatographic Assay for Naltrexone in Biological Fluids

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The electron-capture gas chromatographic assay for naltrexone is an adaptation of the method published originally by Sams and Malspeis (1). The current methodology, described in detail, consists of an extraction procedure, derivatization to form an electron-capturing triester, and gas chromatography using an OV-17 column. Extraction efficiencies indicate that either benzene or 0.25% butanol in cyclohexane used as the organic phase yields maximal extraction of naltrexone and minimal extraction of most naltrexone metabolites. Methylene chloride, on the other hand, yields an optimal combination of clean chromatograms and high extraction efficiencies for both naltrexone and its metabolites. Derivatization with either heptafluorobutyric anhydride or pentafluoropropionic anhydride together with a basic catalyst yields a triester derivative. Use of an OV-17 chromatographic column with electron-capture detection permits assay of naltrexone specifically with respect to known metabolites. One minor metabolite, 2-hydroxy-3-O-methyl-β-naltrexol could interfere with naltrexone quantitation if present in sufficient quantities. This interference could readily be detected if it were to occur. Data on the reproducibility of this assay procedure indicate that it is sensitive to a concentration of 0.25 ng/ml plasma.

INTRODUCTION

The growth of knowledge concerning naltrexone metabolism and pharmacokinetics has been dependent on the ability to assay naltrexone and its metabolites in biological fluids in a specific manner. The relatively high potency and extensive degree of metabolism of this compound present a significant analytical challenge in terms of the sensitivity and specificity needed for unambiguous metabolic and pharmacokinetic studies. It was recognized early that only a few techniques were likely to have the necessary sensitivity and specificity; these being radiotracer assay, electron-capture gas chromatography (GLC-EC) and mass fragmentography.

The purpose of this report is to summarize several years of experience with a GLC-EC assay for naltrexone developed originally by Sams and Malspeis (1). The current assay procedure will be described in detail in the Experimental section and research connected with each phase will then be reviewed.

EXPERIMENTAL

Reagents and Glassware

Naltrexone reference standard, naloxone reference standard and naltrexone hydrochloride were obtained from Endo Laboratories (Garden City, NY) and used without further treatment. Pentafluoropropionic anhydride (PFPA), obtained from PCR, Inc. (Gainesville, FL), was redistilled if derivatization and GLC-EC analysis of naltrexone and related compounds yielded unsymmetrical peaks or blank peaks which would interfere chromatographically. When necessary, PFPA was redistilled into 0.5°C fractions between 70° and 74°C. The purity of each fraction was checked by the above procedure. The catalyst 4-dimethylaminopyridine (DMAP), from Tridom/Fluka (Montreal, Canada), was recrystallized at least four times from benzene.

High purity "Distilled-in-Glass" solvents (Burdick-Jackson Laboratories, Inc., Muskegon, MI) were used for extraction and stock solution preparation and all other chemicals were reagent grade.

Glassware was soaked overnight in sulfuric acid-nitric acid (4:1), washed with Alconox and rinsed with distilled water. After drying at 110° for several hours the glassware was dipped in a 1% solution of Dri-film SC-87 (Pierce Chemical Co., Rockford, IL) in toluene for 1 minute and then dried again at 110° for several hours. The glassware was then washed again, rinsed with double distilled, demineralized water, and dried.

Extraction

Naltrexone assays are generally carried out in either a 10-100 ng/ml range or a 1-10 ng/ml range with differing standard curves for each range. An appropriate amount of a solution of naloxone internal standard (60 ng or 6 ng, respectively) in methanol is placed in each screw-capped test tube, and the methanol is evaporated under mild heat and nitrogen. Samples of plasma or urine containing naltrexone, generally 0.5 ml for the high concentration range and 2.0 ml for the low range, are then added. When dilution is needed for samples to be assayed within the range of the standard curve, plasma samples are diluted appropriately with blank plasma and urine samples with distilled water. The sample is then supplemented with 0.5 g sodium chloride (1.0 g if the 2.0 ml sample volume is used), 2.5 ml phosphate buffer, pH 10.4 (2), and 5.5 ml organic solvent (benzene, ethyl acetate, methylene chloride or 0.25% (v/v) butanol in cyclohexane). After shaking for 15 minutes and centrifuging, the organic layer is transferred to a tube containing 1 ml 0.1 N sulfuric acid. After shaking and centrifuging, the organic layer is discarded. The acid is then washed with 5.5 ml organic solvent and the organic phase is aspirated off. The acid layer is supplemented with 0.5 g sodium chloride and 2.5 ml of the same phosphate buffer and this mixture is then extracted with 5.5 ml organic solvent. The organic solvent is transferred to another tube and evaporated to dryness. The uncapped tubes are then placed in a vacuum desiccator until further analysis (at least 16 hr).

Derivatization and Gas Chromatography

The residue in each tube from the extraction procedure is dissolved in 50 µl benzene containing 1% DMAP and then treated with 25 µl PFPA for 1 hr. at 68" in an oil bath heating block (Lab-Line Instruments, Inc., Melrose Park, IL). After cooling in an ice bath the excess reagents are removed by adding 5 ml of a saturated solution of sodium tetraborate, rotating on a Labquake mixer (Labindustries, Inc., Berkeley, CA) for 3 min and then centrifuging for 4 min. When a series of samples is assayed, each sample is washed with the sodium tetraborate individually just prior to injection of a 1-3 µ1 aliquot of the separated benzene phase onto the gas chromatographic column.

The naltrexone derivatives are chromatographed on a Hewlett-Packard (Palo Alto, CA) 5713A gas chromatograph with a ^{63}Ni-electron-capture detector. The coiled glass column, 2 mm internal diameter and 2.4 m in length, is packed with 3% OV-7 on 100-120 mesh Gas Chrom Q (Applied Science Laboratories, State College,

PA). The temperature settings are: oven, 203°; injection port, 250°; and detector 300°. The argon-methane (95:5) carrier gas flow rate is about 28 ml/min.

Standard Curve

A standard curve is run on each day of analysis. Prior to the initial addition of blank plasma or urine, appropriate known amounts of naltrexone in methanol are added to five separate tubes so that a standard curve over the desired concentration range is obtained. These standards are then supplemented with plasma or urine and assayed in the same run with the experimental plasma and urine samples. Blank plasma or urine samples are also carried through the assay procedure. Standard curves are constructed by plotting the peak height ratio (naltrexone/naloxone) as a function of the amount of naltrexone added.

Assay of ß-naltrexol and Conjugated Metabolites

The naltrexone metabolite, ß-naltrexol, can also be assayed in plasma or urine by the procedure just described. One of the more polar extracting solvents is necessary and the standard curves for both naltrexone and ß-naltrexol are obtained from samples containing known amounts of both compounds.

Conjugates of naltrexone and ß-naltrexol can be determined by assay of the sum of the levels of either compound plus its respective conjugates and then subtracting the appropriate assayed value for unconjugated naltrexone or ß-naltrexol (3). When assaying for the total of conjugated and unconjugated compound, urine or plasma samples are incubated at 37° for 24 hrs with 2 volumes of Gluculase (Endo Laboratories, Garden City, NY) that has been diluted 1:10 with 0.2 M acetate buffer, pH 5.0 (4). Experiments with samples of plasma and urine (dog and monkey) containing naltrexone conjugates have shown that neither longer incubation time nor higher enzyme concentration results in any further conjugate hydrolysis.

RESULTS AND DISCUSSION

Standard Curves

Linear standard curves with very high correlation coefficients ($r \geqq 0.998$) and essentially zero intercepts are generally obtained when naltrexone in either the 10-100 ng/ml or the 1-10 ng/ml range is assayed in plasma or urine from monkey, dog and rabbit.

The sensitivity limit for naltrexone is less than 1 ng/ml. ß-naltrexol yields similar standard curves except that the correlation coefficient is often lower ($r \geqq 0.980$) and the sensitivity limit is about 2 ng/ml. A typical chromatogram (figure 1) illustrates that blank in-

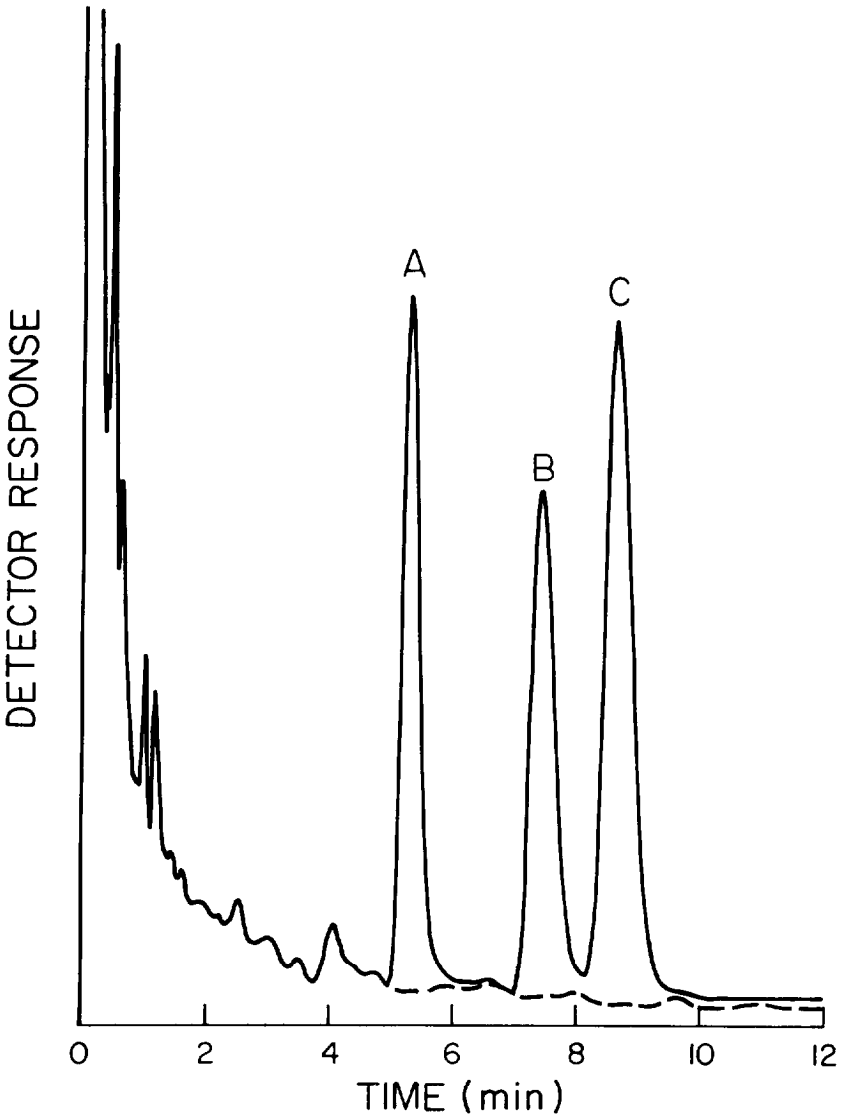


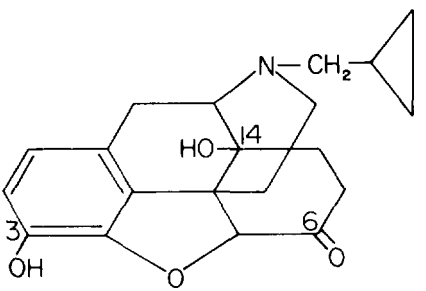
FIGURE 1. Gas chromatogram of an extracted and PFPA-derivatized plasma sample (monkey) to which 30 ng naloxone, 3.5 ng ß-naltrexol and 35 ng naltrexone were added. A is the naloxone (internal standard) derivative, and B and C are the ß-naltrexol and naltrexone derivatives, respectively.

terference is generally negligible and that both naltrexone and ß-naltrexol can be quantitated from a single chromatogram.

Derivatization and Gas Chromatography

It has been shown previously (1,3) by mass and infrared spectrometry that derivatization of naltrexone with either PFPA or heptafluorobutyric anhydride (HFBA) in the presence of a basic catalyst yields the triester derivative, presumably at the 3, 6 and 14 positions (note scheme 1). This complete derivatization provides maximal electron-capture sensitivity and avoids potential assay error due to varying degrees of incomplete derivatization. In the presence of catalyst, derivatization times longer than 1 hr do not result in any further esterification (1). Available evidence would suggest, however, that there is incomplete esterification if the catalyst is not added (1). Of those catalysts that have been tried (e.g., several concentrations of pyridine, triethylamine, and DMAP) the 1% DMAP appears to be optimal in that it yields both complete derivatization and chromatograms with minimal blank interference. It is important, however, that the DMAP be recrystallized several times in order to obtain chromatograms with low blank interference. Chromatograms from PFPA derivatization generally have less blank interference than those from HFBA.

SCHEME 1



Once the ester derivatives of naltrexone are formed, the excess anhydride and catalyst must be removed. Because of the presence of catalyst, it is not possible to do this by evaporation. Thus, the borate wash is used to remove both the anhydride and catalyst. Sams and Malspeis (1) have shown that both the pentafluoropropionate and the heptafluorobutyrate derivatives are sufficiently unstable after the borate wash that samples should be chromatographed as soon as possible. Thus, in the procedure described in the Experimental section each sample is individually

washed with the borate solution, separated and chromatographed immediately. This procedure is necessary to obtain reproducible results with the naltrexone assay described herein. The instability of the derivatives after the borate wash precludes automation of the chromatography portion of the assay procedure.

Chromatographic conditions have been optimized for sensitivity and for specificity with respect to known naltrexone metabolites. Various percentages of the following column packing materials have been tried; OV-1, OV-17 and SE-30 on Gas Chrom Q (Applied Science Laboratories, State College, PA). Various temperature settings for the oven as well as temperature programming have also been attempted (1). The best separation of derivatized naltrexone and its known metabolites was obtained with a 2.4 m column packed with 3% OV-17, together with the other conditions described in the Experimental section. An illustration of the results obtained when known amounts of derivatized naltrexone and its unconjugated metabolites are chromatographed is shown in figure 2. The retention times are as follows: naloxone (internal standard), 5.1 min; ß-naltrexol, 7.2 min; α-naltrexol, 7.8 min; 2-hydroxy-3-O-methyl-ß-naltrexol, 7.9 min; naltrexone 8.3 min; 2-hydroxy-3-O-methylnaltrexone, 9.0 min; 3-O-methyl-ß-naltrexol, 12.1 min. The derivatized a-naltrexol was omitted from figure 2 because the retention time was so close to that of derivatized 2-hydroxy-3-O-methyl-ß-naltrexol.

In monkey the only metabolite that has been found to be present in appreciable quantities in blood and urine is ß-naltrexol. This is fortunate since relatively high levels of 2-hydroxy-3-O-methyl-ß-naltrexol could potentially interfere with the naltrexone assay. Studies in which standards containing both of these compounds in varying ratios were derivatized and chromatographed indicate that when the peak height ratio of naltrexone to 2-hydroxy-3-O-methyl-ß-naltrexol is $\geqq 5{:}1$ the error in the assay of naltrexone is less than 3%. If the ratio is 2:1 the error is 9% and a ratio of 1:1 yields an error of 21% in the naltrexone assay (5). However, this metabolite appears to be a minor metabolite in man (6) and has not been observed in significant amounts in plasma samples from monkeys administered single intravenous or oral doses of naltrexone nor in plasma or urine samples from dogs administered naltrexone intravenously. In an unusual application of this assay, a peak corresponding to the retention time of derivatized 2-hydroxy-3-O-methyl-ß-naltrexol was observed in the assay of 1- and 24 hr plasma samples from monkeys dosed chronically with oral naltrexone, 1 to 20 mg/kg/day, for 52 weeks (7).

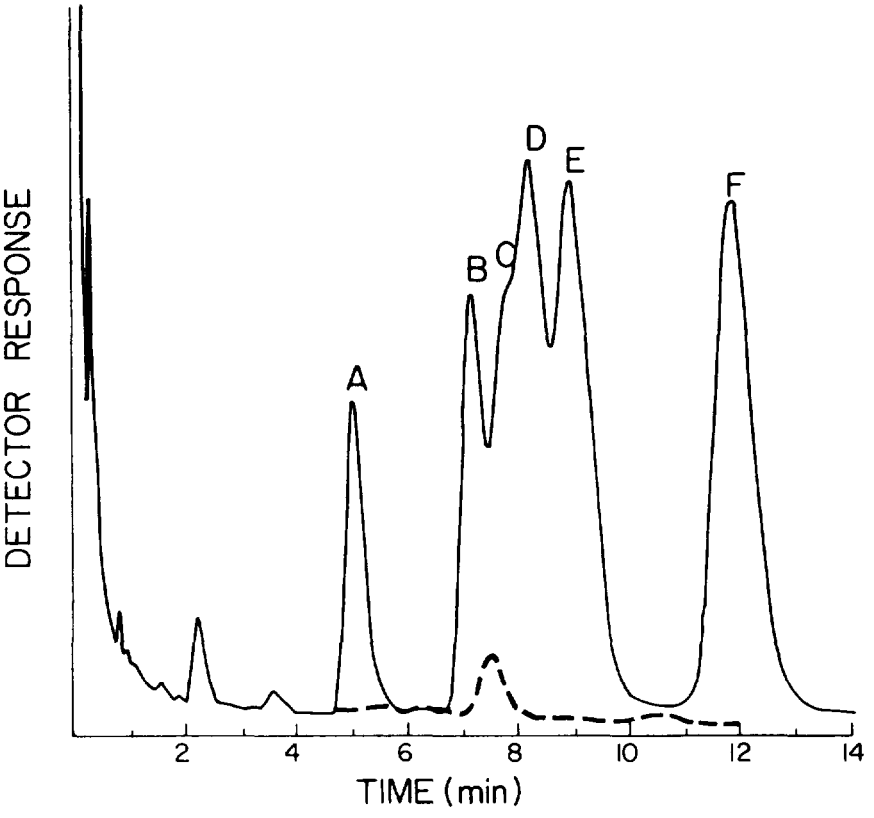


FIGURE 2. Gas chromatogram of a derivatized sample spiked with naltrexone, naltrexone metabolites and internal standard. A = naloxone (internal standard), B = ß-naltrexol, C = 2-hydroxy3-O-methyl-ß-naltrexol, D = naltrexone, E = 2-hydroxy-3-O-methylnaltrexone, F = 3-O-methyl-ß-naltrexol. The dashed curve represents derivatized blank solvent.

Extraction

Organic solvents that have been investigated for the extraction procedure include benzene, methylene chloride, ethyl acetate, cyclohexane, 0.5%, 0.75%, 1%, 2%, 5%, 10% and 15% (v/v) isopropanol in cyclohexane, and 0.1%, 0.25% and 0.5% (v/v) n-butanol in cyclohexane. Of these solvents, the four that yield an optimum combination of high naltrexone extraction efficiency and a low degree of blank interference are benzene, methylene chloride, ethyl acetate and 0.25% (v/v) n-butanol in cyclohexane. Although ethyl acetate has been used extensively in previous investigations, it has been abandoned because of occasional blank interfence, variability.

TABLE 1. Extraction Efficiencies of Naltrexone and its Metabolites in the Naltrexone Assay Procedure

Organic Solvent	Sample Medium	Percentage Extracted[a]				
		Naltrexone	β-Naltrexol	α-Naltrexol	2-Hydroxy-3-0-methylnaltrexone	2-Hydroxy-3-0 methyl-β-naltrexol
Benzene	Dog plasma	70(4)	19	—	—	—
	Dog urine	72	42	62	—	—
	Monkey plasma	74(7)	30(3)	—	—	—
	Monkey urine	—	—	71(2)	103(4)	94(7)
Ethyl Acetate	Dog plasma	99(7)	80	—	—	—
	Monkey plasma	87(8)	—	—	—	—
	Monkey urine	—	—	64(5)	86(0.6)	65(13)
Methylene Chloride	Dog Plasma	93(10)	73(10)	70(10)	78(6)	86(2)
0.25% (v/v) Butanol in Cyclohexane	Dog Plasma	52(6)	1(1)	0(0)	75(11)	19(0)

[a]Mean values reported if more than one measurement. Standard deviation in parenthesis.

in duplicate assays, and low correlation coefficients for the standard curve.

Extraction efficiencies have been reported in detail elsewhere (3,4,5) and only mean values for the final organic solvents chosen are reported in table 1. It is evident from these results that ethyl acetate and methylene chloride yield very high extraction efficiencies for both naltrexone and its metabolites. Thus, since methylene chloride yields less day-to-day variability in assay performance, this solvent appears to be optimal when assays for both naltrexone and ß-naltrexol and perhaps other metabolites are desired. The solvent that is most selective for unchanged naltrexone is 0.25% (v/v) butanol in cyclohexane. This solvent is particularly advantageous with respect to the metabolite 2-hydroxy-3-O-methyl-ß-naltrexol which could interfere with naltrexone quantitation if it were present in a sufficiently high ratio to the naltrexone level. Morphine, which may be present in biological samples from certain types of studies on naltrexone (such as sustained release naltrexone evaluation in self-administering monkeys), is not extracted by either benzene or 0.25% (v/v) butanol in cyclohexane (5).

General Aspects

Because of the requirement of sub-nanogram sensitivity for many applications of this assay, several tedious and exacting procedures are necessary. A troubleshooting list for the procedure could include the following: impure derivatizing agent, impure catalyst, solvents not of the highest quality available, deteriorated coating solution for the glassware, deterioration of organic extracting solvents presumably due to absorption of water, insufficient drying of the residue from the extraction procedure prior to derivatization, and deterioration of chromatographic columns with repeated use. How-

TABLE 2. Accuracy and Reproducibility of the Naltrexone Assay Procedure

Concentration of Naltrexone added to plasma (ng/ml)	Mean naltrexone Concentration assayed (ng /ml)[a]	Coefficient of variation (%)
20°	20.0(10)	4.6
5[c]	4.93(10)	9.0
0.5[c]	0.50(8)	9.1
0.25[c]	0.26(9)	21.5

[a]Number of replicates in parentheses.
[b]One-half ml monkey plasma was used.
[c]Two ml monkey plasma was used.

ever, patience and diligent attention to detail can result in the accuracy and reproducibility exemplified in table 2. Since most assays are done in duplicate, the use of the assay on experimental samples should be even better than the results in table 2 suggest. Thus, for most applications the sensitivity limit of this assay is 0.25 ng/ml for naltrexone in plasma.

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Quantitative Determination of Naltrexone, 6ß-Naltrexol and 2-Hydroxy-3-Methoxy-6ß-Naltrexol (HMN) in Human Plasma, Red Blood Cells, Saliva and Urine by Gas Liquid Chromatography

Karl Verebey

Two gas liquid chromatographic methods differing mainly in sensitivity are described for the quantitative determination of naltrexone (NT) and its metabolites in human biofluids. Flame ionization detection of the N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) derivatives provided sufficient separation and sensitivity for quantitation of the bases in urine. However, the thousand times lower levels in serum, red blood cells (RBC) and saliva necessitated the use of more sensitive electron capture detection methods of the pentafluoro derivatives of NT and its metabolites. The 2-hydroxy-3-methoxy-6ß-naltrexol (HMN) and 6ß-naltrexol (ß-OL) pentafluoro derivatives had nearly identical gas liquid chromatographic retention times in a number of stationary liquid phases. Thus, their se ration had to be achieved prior to chromatography. Differential extraction was based on the different partition characteristics of HMN and 6ß-naltrexol between aqueous and organic solvents. Applicabil-

ity of the methods was tested using the biofluids of four subjects taking 2 x 200 mg naltrexone per day chronically. Blood, saliva and urine samples were collected at the same time (prior to drug administration), which was 16 and 24 hours after the doses. In the plasma the relative percentages of the bases were 73.5 β-OL; 23.1 HMN and 3.4 NT. The same in the urine were 76.6 β-OL, 14.4 HMN and 9.0 NT The lipophilic nature of HMN and the hydrophilic property of β-OL may have influenced their distribution into RBC and saliva. In the RBC 96.1% HMN and only trace amounts of β-OL distributed and in saliva 92.3% of β-SOL and no HMN was found; the difference in both cases was made up by naltrexone to 100%.

INTRODUCTION

Naltrexone was already administered to men during preliminary clinical trials (1,2) while methods were not yet available for the monitoring of the drug and its metabolites in human biofluids. The isolation and tentative identification of 6ß-naltrexol, the major urinary biotransformation product in humans, was first reported by Cone in 1973 (3). Methodological advancement soon followed, resulting in the description of the urinary excretion profile of naltrexone in man by Cone et al., Verebey et al., and Dayton and Inturrisi (4,5,6). In 1975, Cone and Gorodetzky reported the presence of noroxymorphone, an N-dealkylated, strongly agonistic metabolite and a methylated 2,3-catechol type metabolite of naltrexone in human urine (7). Also in 1975, Verebey et al. described 2-hydroxy-3-methoxy-6ß-naltrexol (HMN), a minor metabolite of naltrexone isolated from human urine and red blood cells (8). Determination of naltrexone and its metabolites in plasma was far more difficult because the concentration of the bases is approximately a thousandfold less than the same in urine. In 1976 Verebey et al. described a quantitative method for the determination of naltrexone and 6ß-naltrexol in human plasma utilizing electron capture detection of the pentafluoro derivatives of naltrexone and 6ß-naltrexol (9). Utilizing this technique the total disposition and most of the pharmacokinetics of naltrexone were determined in man by Verebey et al., following acute and chronic doses of naltrexone (10). Sams and Malspeis (11) and Sams (12) provided extensive data on the nature of electrophore formation of naltrexone and naloxone for electron capture detection. Naloxone is often used as an internal standard for the quantitation of naltrexone. Later, naltrexone prodrugs were synthesized for extended opiate receptor blockade through various slow release drug delivery systems. Bruce et al., utilizing electron

capture gas chromatographic techniques, were able to quantitate eleven ester and ether derivatives of the prodrugs (13).

More recently the structure of HMN was confirmed by synthesis (14) and by nuclear magnetic resonance spectra (15). The availability of synthetic HMN has also encouraged methodological advances. In this communication methods are described for the quantitation of naltrexone, 6ß-naltrexol and HMN in plasma (or serum), red blood cells, saliva and urine (16). It is the intention of this report to concentrate on the difficulties and shortcomings of current methodology.

MATERIALS AND METHODS

Chemicals and Reagents

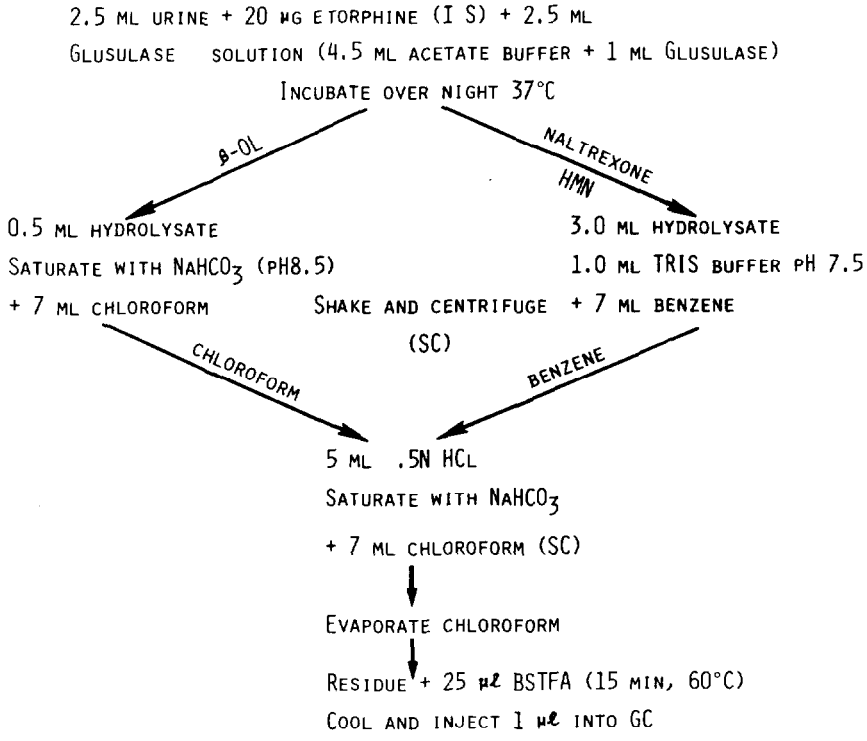
Analytical grade naltrexone, 6ß-naltrexol and 2-hydroxy-3-methoxy-6ß-naltrexol were provided by NIDA. Etorphine, the internal standard for the urine method, was purchased from the American Cyanamid Co., (Princeton, NJ) and nalorphine, the internal standard for the plasma method, was obtained from Merck & Co., Inc. (Rahway, NJ). The derivatizing reagent, pentafluoropropionic anhydride (PFPA), glassware siliconizing agent, Dri-Film SC 87, and column conditioner, Silyl 8, were purchased from Pierce Chemical Co. (Rockford, IL) and N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) was purchased from Regis Chemical Co. (Morton Grove, IL). The organic solvents used in the procedure were all glass distilled, G.C. grade, purchased from Burdick and Jackson (Muskegon, MI). Glusulase was purchased from Endo Laboratories (Garden City, NY). All aqueous solutions, buffers and dilute acids were prepared in glass distilled water, and all glassware was siliconized using 5% Dri-Film SC 87 in toluene.

Extraction Procedure (Urine)

After large doses of naltrexone, greater than 400 mg daily, 6ß-naltrexol levels were so much greater than naltrexone and HMN levels that a separate extraction procedure was needed for the recovery and quantitation of naltrexone and HMN. This was accomplished by utilizing a pH (7.5) lower than the optimum for 6ß-naltrexol recovery and a nonpolar organic solvent (benzene). The method is summarized in table 1. For the routine assay of naltrexone and HMN 1 to 2 ml of urine was used, while for the determination of 6ß-naltrexol 0.1 to 0.3 ml urine was adequate. Etorphine was added to the urine samples: 2 μg for the naltrexone and HMN assays and 20 μg for the 6ß-naltrexol assay.

TABLE 1

NALTREXONE, 6-β-NALTREXOL AND HMN IN URINE BY FLAME IONIZATION GLC METHOD



The urines were buffered to pH 8.5 using 0.5 ml of 0.2 M carbonate-bicarbonate buffer and the aqueous solution was extracted with 7.0 ml chloroform. The chloroform was back extracted into 5.0 ml of 0.5 N HCl. The pH of the aqueous layer was adjusted to 8.5 and re-extracted into 7.0 ml chloroform. The extractions at each step consisted of 10 min shaking in a mechanical shaker and 5 min centrifugation. The chloroform extracts were evaporated to dryness in a rotary evaporator under vacuum at 50" (Evapomix, Buchler, Fort Lee, NJ).

Calibration Curves and Quantitation (Urine)

Standard solutions of NT (20 µg/ml), HMN (50 µg/ml) and etorphine (20 and 200 µg/ml) were prepared in water. Standard curves for NT and HMN were prepared by adding 0.5, 1.0, 1.5 and 2.0 µg

of naltrexone, 5 to 30 μg of HMN and 2 μg of etorphine to each 2.0 ml of blank urine samples.

The standard curve for ß-OL was prepared separately, 3, 6, 9 and 12 μg ß-OL and 20 μg of etorphine was added to each 0.2 ml blank urine. The standards were extracted, derivatized and chromatographed as the unknown samples.

Glusulase Hydrolysis

The concentration of urinary naltrexone and 6ß-naltrexol was determined before (free) and after hydrolysis (total) while values of HMN in both systems were equal, indicating that no conjugation of HMN occurs (10). Thus HMN quantitation can be performed with non-hydrolyzed samples. A typical hydrolysis and extraction system is outlined in table 1.

Derivatization Procedure (Urine)

The tubes were sealed with rubber caps, flushed with dry nitrogen, BSTFA (20-30 μl) was added, mixed on a Vortex mixer for 5 sec and the reaction allowed to proceed in a dry heating block containing sea sand at 60°C for 15 min. Following the reaction 1 μl of BSTFA containing silylated bases was injected into the gas chromatograph.

Gas-Liquid Chromatographic Conditions (Urine)

The gas chromatographic analysis was performed on a Perkin-Elmer 900 gas chromatograph, equipped with a hydrogen flame ionization detector. The column was a 2 m x 2 mm I.D. glass spiral. The packing consisted of 3% OV-17 on Gas-Chrom Q, 80-100 mesh. The temperature of the detector and flash heater was 285°. The carrier was helium with a flow-rate of 60 ml/min. The flow-rates of hydrogen and air were 30 ml/min and 300 ml/min, respectively. The column oven temperature was 270°.

Extraction Procedure (Plasma, Serum, RBC and Saliva)

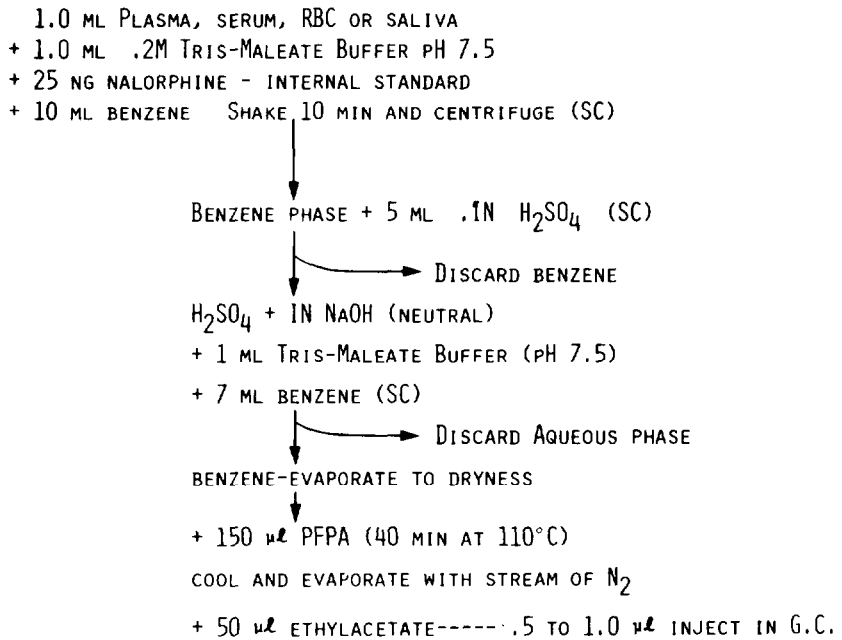
An extraction outline of HMN and NT is given in table 2. HMN was extracted into benzene which excluded 6ß-naltrexol while the combined 6ß-naltrexol-HMN were determined using a modified version of the original method (9).

The following substances were added to 20 ml round-bottom centrifuge tubes: Antifoam A (A.H. Thomas, Philadelphia, PA), 0.2 to 2 ml of plasma and an aqueous internal standard (nalorphine) containing 12.5 ng for the chloroform and 25 ng for the benzene procedure. The pH was adjusted to 7.5 with 1.0 ml tris-maleate buffer

TABLE 2

NALTREXONE AND HMN

ELECTRON CAPTURE GLC METHOD



and the aqueous phase was shaken with 10 ml of benzene for HMN or chloroform for naltrexone and combined 6ß-naltrexol-HMN determination. After 10 min shaking and 5 min centrifugation the organic layer was transferred into clean centrifuge tubes containing 5 ml of 0.1 N H_2SO_4. After back extraction into acid the organic layer was discarded and 4.3 ml of the H_2SO_4 phase was transferred into clean centrifuge tubes and neutralized with 1.0 N NaOH. The pH was adjusted to 7.5 with tris maleate buffer and the aqueous phase extracted with 7 ml of benzene for HMN or chloroform for naltrexone and 6ß-naltrexol containing HMN. After shaking and centrifugation the organic phase was transferred to a clean 15-ml test tube and evaporated to dryness on a rotary flash evaporator. One ml of acetone was added to the benzene phase to facilitate its evaporation.

Calibration Curves and Quantitation (Plasma)

Naltrexone and 6ß-naltrexol standard curves were prepared by adding 2.5, 5.0, 10.0 and 20.0 ng of naltrexone; 5, 10, 20 and 40 ng of 6ß-naltrexol and 12.5 ng of nalorphine to each of 1.0 ml of blank plasma. For the HMN standards, 5, 10, 20 and 40 ng of HMN with 25 ng nalorphine were added to 1.0 ml of blank plasma. The samples for both standard curves were extracted according to the appropriate method described (benzene or chloroform), derivatized by PFPA and chromatographed. The peak height or area ratios were plotted against the respective concentration of standards and the slope factors determined. The unknown sample peak height or area ratio was divided by the slope factors of the standards to determine the concentations of naltrexone, 6ß-naltrexol, and HMN in the unknown samples.

Derivatization Procedure (Plasma)

To the dry residue 150 µl of PFPA was added, the tube was tightly stoppered (00 size hollow Nalgene stoppers; A.H. Thomas) and placed in a heating block at 110° for 40 min. After the reaction the samples were stored overnight at -16°C and usually analyzed the following day. The anhydride was evaporated at room temperature under a stream of nitrogen. The dried sample was taken up in 50 µl of ethyl acetate and 1 µl aliquots were injected immediately into the gas chromatograph. At this step one should proceed at the rate of one sample at a time because the derivatives are not stable in the ethyl acetate or in any other solvent tested.

Gas-Liquid Chromatographic Conditions (Plasma)

Isothermal conditions were used with a Hewlett-Packard Model 5830A gas chromatograph, equipped with a $_{63}$Ni linear electron capture detector. The column was 2 m x 2 mm I.D. packed with 3% OV-22 on Supelcoport, 80-100 mesh. The carrier gas was a mixture of 10% methane in argon at a flow-rate of 35 ml/min. The injection port temperature was 240°C, the detector temperature 300°C and the column oven temperature 220°C.

Methodological Cautions

A list of suggestions follows; the observations are based on four years of experience and sometimes frustrations. Implementation of some or all of these suggestions may determine the success or failure of the adaptation of these methods.

1. Treat all tubes with siliconizing reagent. Before use, rinse all test tubes with glass-distilled chloroform.
2. During the extraction procedure, to have the cleanest possible final sample, avoid the close proximity of the organic-aqueous interface. Contaminants can easily be transferred while trying to recover all of a phase.
3. Put chloroform into the refrigerator before final evaporation. A little droplet of water forms on top of the chloroform; aspirate it off before evaporation.
4. A rotary evaporator is far more efficient than an N-EVAP type evaporator in concentrating the little available bases in the tip of the reaction vessel. With low concentrations, <10 ng naltrexone/ml, rotary flash evaporator is necessary.
5. Following derivatization while the bases are in PFPA, they are stable for at least three weeks when kept in the freezer.
6. After derivatization put all tubes in freezer and evaporate the samples one by one. After reconstitution, each sample must be injected into the gas chromatograph immediately or within minutes. In ethylacetate or any other solvent, the rate of drug-pentafluoro complex decomposition is rapid.
7. After the injection of 100 to 150 samples into the gas chromatograph the injection port glass wool should be changed. Deposits on the glass wool selectively bind one or more of the bases, causing decrease in sensitivity and errors in quantitation. Use siliconized glass wool only!
8. After changing the injector port glass wool plug, disconnect the column from the detector and inject approximately 40 μl of Silyl 8. Reconnect 15 minutes later. This helps to prevent adsorption and/or breakdown of the pentafluoro-drug complexes on the column.
9. Derivatize a mixture of 100 ng/μl of NT, ß-OL, and nalorphine with PFPA. Before injection of standards or unknown, prime the column with these large concentrations of bases (attenuate signal while injecting primer). This also helps prevent on column binding and breakdown of samples.

Subjects

To verify the modifications in the original methodology the samples for this study were kindly provided by Professor Volavka, The

Missouri Institute of Psychiatry, University of Missouri School of Medicine. The subjects were all male chronic schizophrenic patients who received incremental doses from 50 to 400 mg naltrexone in the first week and 400 mg naltrexone daily in the second week. The 100 mg doses and above were split and given at 8 am and 4 pm. Since all the samples were collected just prior to the 8 am dose they represent samples 24 hours past the first and 16 hours past the second half of the daily dosage. Two blood, saliva, and spot urine samples were collected from each patient on the 7th day of the 1st and 2nd weeks, thus in both cases the patients were already receiving 2 x 200 mg (400 mg) naltrexone per day.

RESULTS AND DISCUSSION

Figure 1 shows the structural formula of naltrexone and its major (ß-OL) and minor metabolite (HMN). The recent availability of synthetic HMN prompted the development of methods for its quantitation. Determination of HMN in urine is very similar to the method reported earlier (6). The three drug-related peaks (NT, ß-OL, HMN) and the internal standard (etorphine) were well resolved by gas liquid chromatography (figure 2). The system using chloroform is suitable for the simultaneous measurement of naltrexone and its metabolites. However, when 400 mg or larger daily doses of naltrexone are administered, the excessive amount of 6ß-naltrexol may interfere with the quantitation of HMN and naltrexone. In such conditions separate extraction systems should be utilized, one for ß-OL determination (using chloroform) and another for HMN and naltrexone determination (using benzene) as outlined in table 1. Since ß-OL is known to be hydrophilic, the utilization of a nonpolar solvent (benzene) and a lower than usual pH (7.5) accomplished the exclusion of ß-OL, while NT and HMN were adequately recovered. Derivatization using BSTFA requires only one methodological caution: the samples must be very dry; even humidity in the air must be considered. In our laboratory the tubes are sealed and flushed with nitrogen for 2 to 3 minutes before the addition of BSTFA, which is injected through the stopper. If samples for some reason remain wet, at least 2 derivatives of both NT and ß-OL will form in different proportions resulting in unreliable results.

Naltrexone, 6ß-naltrexol and HMN are approximately a thousand times less in blood and saliva than in urine. For this reason halogenated derivatives of the bases were formed using PFPA and thus greater sensitivity was achieved. The electron capture method is difficult to adopt because of the many cautions involved in extraction, electrophore derivative formation and the gas chromato-

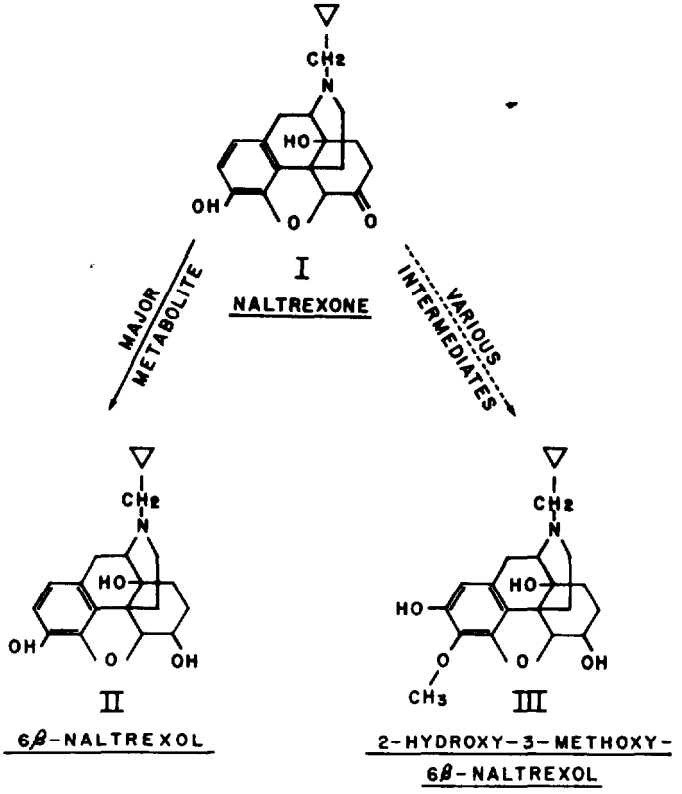


FIGURE 1. The chemical structures of naltrexone (I) and its major and minor metabolites in man: 6ß-naltrexol (II) and 2-hydroxy-3-methoxy-6ß-naltrexol (III)

graphic instrumentation. A list of cautions is given in the methods section. Using the PFPA derivatives, another problem surfaced. The retention time of HMN (3.48 min) and that of ß-OL (3.38 min) are separated only by 0.1 minute (figure 3). When both of these substances are present in unknown samples only one peak is observed (fig. 3, panel C). This observation explains the years of mystery as to why the retention time of ß-OL was shifting 2 to 4 seconds back and forth while NT and the internal standard retention time remained constant. It is likely that in all previous studies when PFPA was used as the derivatizing agent, the values reported for ß-OL are overestimated because they also include HMN.

Many different stationary liquid phases were tried, but no adequate separation between HMN and ß-OL was achieved. The different solubility characteristics of HMN and ß-OL were utilized for separation of the two bases during extraction. For the extraction of

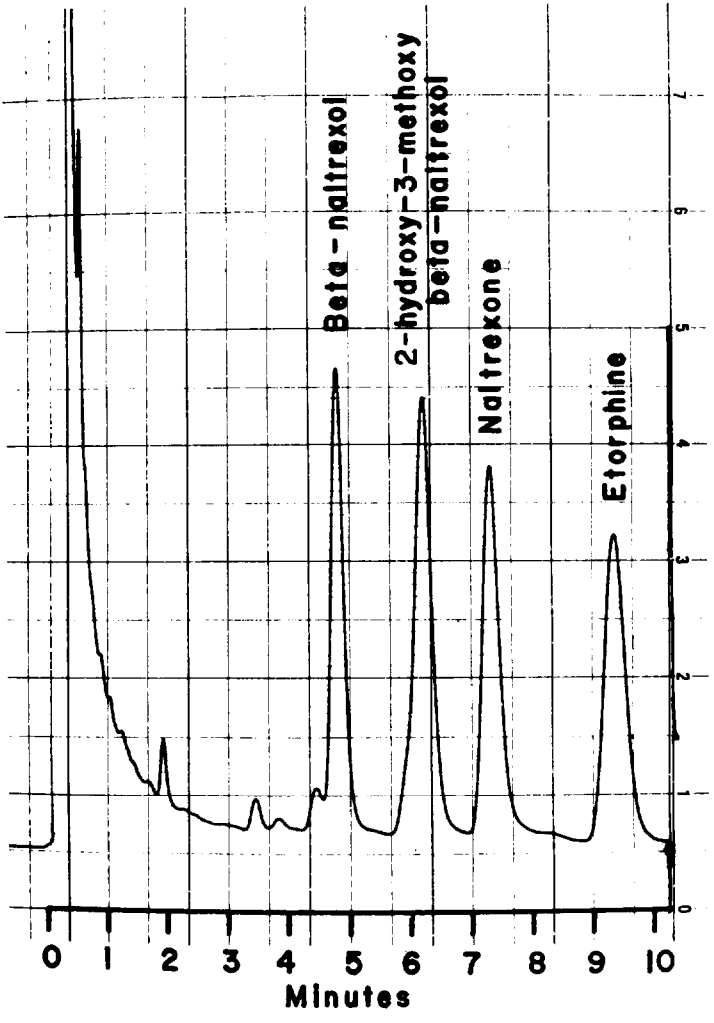


FIGURE 2. The gas liquid chromatographic pattern of the BSTFA derivatives of naltrexone, its metabolites and the internal standard, etorphine, using flame ionization detection

HMN and for the exclusion of ß-OL benzene was used and the pH was lowered to 7.5. Under these conditions HMN recovery was 51 ± 1.4% while ß-OL was not recovered. Using chloroform, at pH 9.4, resulted in the recovery of NT, ß-OL and HMN. Thus, for ß-OL determination the HMN levels were first determined using the benzene procedure and the HMN values subtracted from the results of the chloroform procedure, which determines the combined ß-OL and HMN levels. An example of chromatographic tracings,

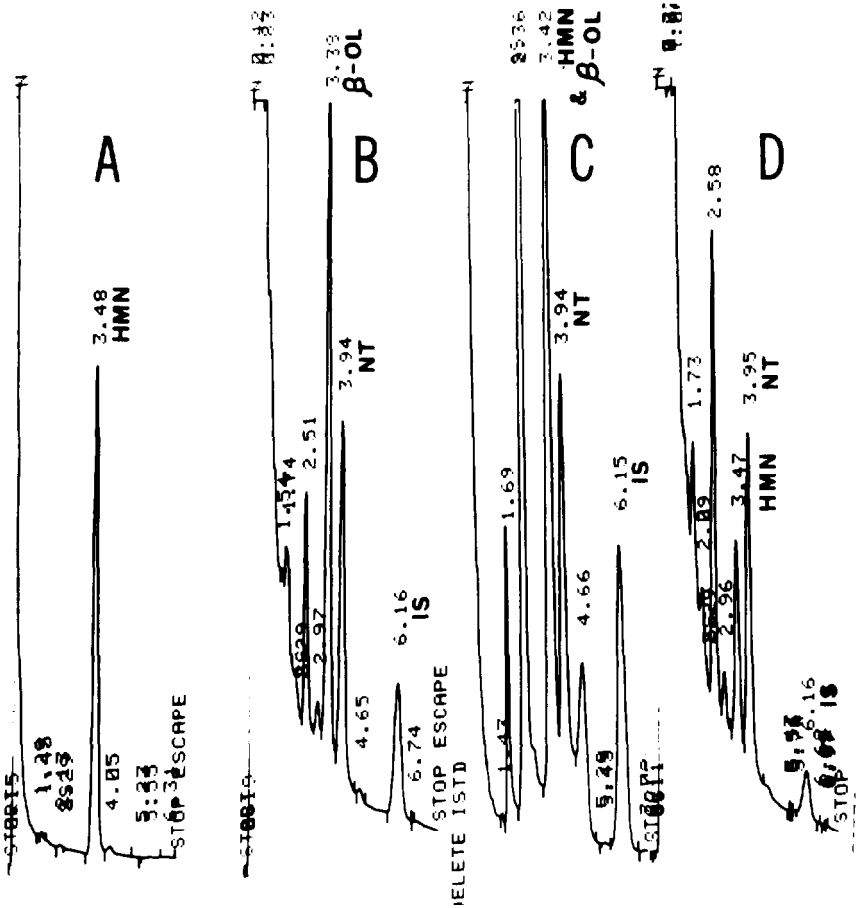


FIGURE 3. The gas liquid chromatographic pattern of the PFPA derivatives of naltrexone (NT), 6ß-naltrexol (ß-OL) and 2-hydroxy-3-methoxy-6ß-naltrexol (HMN) and nalorphine, the internal standard (IS), using electron capture detection. Panel A is HMN (3.43 min) standard alone; Panel B is ß-OL (3.38 min), NT (3.94 min), and the IS (6.16 min) added to blank plasma; Panel C shows a chloroform extract of plasma of a subject taking naltrexone: both HMN and ß-OL are present, indicated by the intermediate retention time (3.42 min). Panel D shows a chromatographic pattern of the plasma extract of the same subject as in Panel C, using benzene: HMN (3.47 min), NT (3.95 min), and IS (6.16 min) are present but ß-OL is not recovered under these conditions.

one after benzene and another after chloroform extraction of the same sample, is shown in figure 3. An ideal method should separate all derivatives; however, at present this has not been accomplished.

For the verification of the modified methods, blood samples (plasma and RBC), saliva and urine were analyzed for the three

bases, collected from subjects taking 400 mg NT a day. (See exact doses and sample collection under "Subjects.") The data compiled from the analysis of urine samples is shown in table 3. Since it was a spot sample collected 24 hr after the dose, only the relative abundance of the bases present in the urine at that time can be evaluated. 6ß-naltrexol accounted for 76.6% of the bases, followed by HMN (14.4%) and naltrexone (9.0%). In the plasma samples the order of relative abundance was the same as in urine, with similar proportion of 6ß-naltrexol (73.5%), significantly more HMN (23.1%) and slightly less naltrexone (3.4%) present (table 4).

TABLE 3. Urinary Levels of Naltrexone, ß-Naltrexol and 2-Hydroxy-3-Methoxy-6-ß-Naltrexol (HMN) in a Single Spot Sample 16 and 24 Hrs After 2X200 mg Naltrexol

	Base µg/ml		
Subject	Naltrexone	ß-Naltrexol	HMN
1	10.3	152.0	20.6
1	2.5	62.8	9.1
2	4.5	59.7	12.5
2	5.4	49.4	9.4
3	16.7	131.3	49.7
3	13.9	77.0	49.2
4	43.2	262.3	18.9
4	18.1	184.5	14.6
Mean ± SD	14.3 ± 13.0	122.4 ± 74.8	23.0 ± 16.8
% Of Total Base	8.9	76.7	14.4

Naltrexone was approximately equally distributed into plasma and RBC, while the correlation of naltrexone levels in plasma with that of saliva is similar but not consistent in this group of subjects, which may be caused by the method of salivary sample collection. The relationship of plasma and salivary naltrexone and 6ß-naltrexol levels is presently under investigation.

Distribution of HMN and 6ß-naltrexol into RBC and saliva was very different. In RBC's, only the more lypophilic HMN distributed in the extent of 96.1%; the remaining 3.9% was naltrexone, while 6ß-naltrexol was hardly detectable. Conversely, in saliva, HMN was absent, the polar 6ß-naltrexol comprised 92.3% of the bases and naltrexone made up the remaining 7.7%. The two- to threefold excess of HMN in RBC vs. plasma is interesting but its mechanism is not at all clear.

TABLE 4. Plasma and RBC Levels of Naltrexone, ß-Naltrexol and 2-Hydroxy-3-Methoxy-6-ß-Naltrexol (HMN) After 2X200 mg Naltrexone Given 24 & 16 Hrs Prior to Sample Collection

Subject	Base ng/ml						
	Plasma			RBC		Saliva	
	Naltrexone	ß-Naltrexol	HMN	Naltrexone	HMN	NT	ß-OL
1	5.6	143.9	42.7	N.S.	N.S.	20.6	160.9
1	4.8	185.0	55.8	4.3	183.1	4.2	106.7
2	8.7	174.8	74.6	7.3	188.3	4.6	43.5
2	8.2	182.7	65.0	7.8	201.4	7.3	74.0
3	105*	572.2*	151.3*	115.0*	342.6*	246.9*	342.1*
3	10.9	251.8	86.3	9.3	211.8	8.4	151.2
4	20.4	234.2	53.2	7.3	102.0	50.3	571.6
4	6.9	231.5	62.8	5.0	112.5	2.0	59.1
Mean ± SD	9.4±5.2	200.6 ± 39.1	62.9 ± 14.4	6.8±1.9	166.5 ± 47.1	13.9 ± 17.1	166.7 ± 184
% of Total Base	3.4	73.5	23.1	3.9	96.1	7.7	92.3

*Sample was drawn after naltrexone dose; values not included in the calculations,
N.S. = no samples available.

The first blood sample in subject No. 3 (table 4) was taken shortly after naltrexone administration. Thus the levels of drug and metabolites were substantially higher than in the 24-hour samples. The high naltrexone, 6ß-naltrexol, and HMN plasma levels indicate excellent absorption and rapid first pass biotransformation of naltrexone, concurring with data reported in an earlier pharmacodynamic study by Verebey et al. (5).

Based on the data in this report, for toxicological purposes, 6ß-naltrexol in plasma, saliva and urine; and HMN in whole blood, RBC and urine are better indicators of naltrexone use than the parent compound itself in any of the body fluids, especially when the samples are collected long after naltrexone ingestion.

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Analytical Methods for Quantitative and Qualitative Analysis of Naltrexone and Metabolites in Biological Fluids

Monroe E. Wall and Dolores R. Brine

Analytical procedures for the determination of naltrexone and metabolites have been presented. The basic procedure involves the use of radiolabeled drugs and thin layer chromatography. Naltrexone, 6ß-naltrexol and 2-hydroxy-3-O-methyl-6ß-naltrexol were found by both the TLC procedure and combined as chromatography-mass spectrometry. The presence of 3-O-methyl-6ß-naltrexol was indicated by the TLC method, but this metabolite could not be found by mass spectrometry.

INTRODUCTION

In the course of our studies on the metabolism of naltrexone (I) (1,2) in man, it was necessary to develop quantitative procedures for I and its metabolites in plasma, urine, and feces. Figure 1 gives the structure of I and its known metabolites, 6ß-naltrexol (II), 2-hydroxy-3-O-methyl-6ß-naltrexol (III), and a possible but not proven metabolite, 3-O-methyl-6ß-naltrexol (IV).

Although sensitive gas liquid chromatographic (GLC) procedures for I, II, and III using flame ionization (3) and electron capture de-

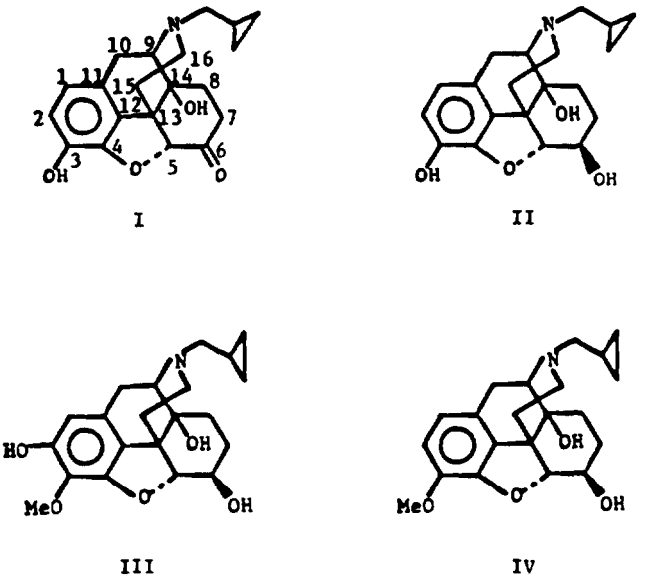


FIGURE 1. Naltrexone (I), 6ß-naltrexol (II), 2-hydroxy-3-O-methyl-6ß-naltrexol (III), and 3-O-methyl-6ß-naltrexol (IV).

tectors (4,5) are available, we chose to use thin layer chromatography (TLC) of the radiolabeled drug and metabolites as our basic procedure. The choice of the procedure was dictated by our need to study the elimination of total drug and metabolites and to study both conjugated and nonconjugated metabolites. The TLC-radiolabel procedures had previously been successfully utilized by our group for the analysis of delta-9-tetrahydrocannabinol and metabolites in biological materials (6) and authenticated by quantitative combined gas chromatography-mass spectrometry (GLC-MS) (7).

THIN LAYER CHROMATOGRAPHY

Appropriate aliquots of the chloroform extract of the biological samples (cf. figures 2 and 3) with sufficient I and II added to permit observation of the standards by ultraviolet quenching were applied to 20 x 20 cm silica gel-on-glass plates (0.25 mm thickness) prepared by E. Merck, Darmstadt, Germany. The development solvent was chloroform/ethyl acetate/acetone/triethylamine (40:30:30:2). As shown in figure 4, excellent separation of I and its actual or potential metabolites can be obtained.

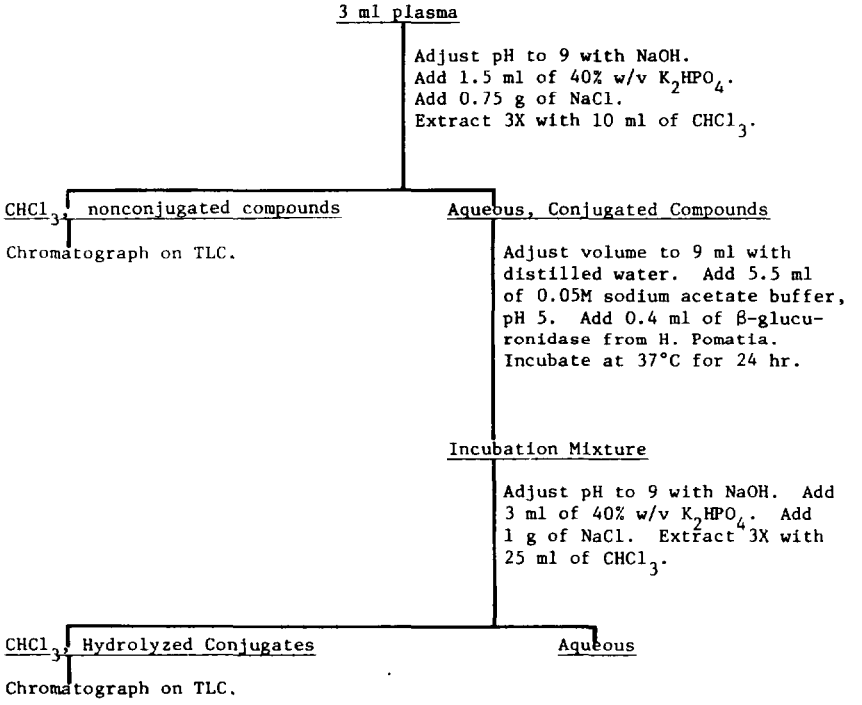


FIGURE 2. Extraction of naltrexone and its metabolites from human plasma.

After plates were developed in the above system, standards were visualized under UV light. Zones of silica gel were scraped into counting vials containing 2.5 ml of distilled water/methanol (1.5:1, v/v). Samples were then sonicated for 15 min. After addition of 15 ml of scintillation fluor, counting samples were sonicated for an additional 30 min. The radioactivity in each TLC zone was determined by liquid-scintillation counting.

QUALITATIVE IDENTIFICATION OF NALTREXONE METABOLITES IN URINE

It is desirable to identify metabolites by a procedure of greater rigidity than TLC. Accordingly, we made a qualitative study of I and metabolites found in a typical 24-hr human urine sample. Some of the considerations that are involved in this study are exemplified in figure 5. It will be noted that I and its major metabolite II are closely related structurally, II being formed from I by enzymatic reduction of the 6-ketone to give the 6-hydroxyl. On deriva-

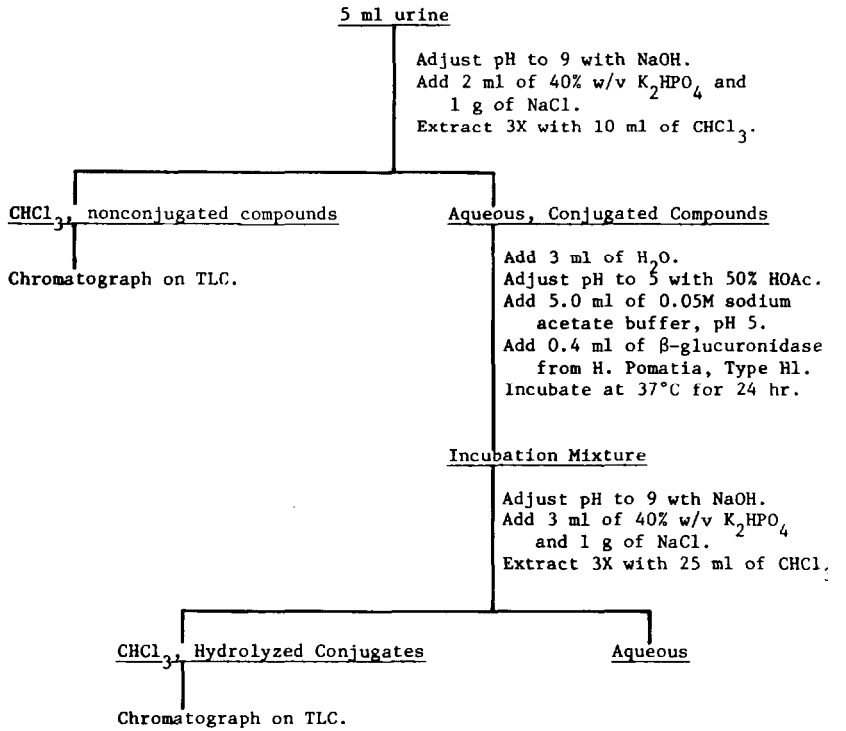


FIGURE 3. Extraction of naltrexone and its metabolites from human urine.

tization, both I and II give tris-trimethylsilyl (TMS) ethers which differ by only two mass units. Although as shown in figures 6 and 7 there are characteristic differences in the mass spectra of the tris-TMS ethers of I and II, the two compounds are not easily separated by GLC. Accordingly, a procedure was devised to convert I or possible metabolites with the 6-oxo moiety to the methoxime followed by conversion of remaining hydroxyl groups to TMS ethers. This method readily permits separation of methoxime-TMS ethers from compounds which form only TMS derivatives.

An aliquot (17%) of a 24-hr urine sample was adjusted to pH 9 and extracted three times with 200 ml portions of chloroform. The chloroform solution was extracted with 2N HCl, which was then basified and extracted three times with 150 ml portions of chloroform. An aliquot of the acid/base-treated chloroform extract of urine was dried *in vacuo* overnight. After addition of 75 μl of a 2% solution of methoxyamine hydrochloride in pyridine, the sample was tightly capped and shaken at ambient temperature overnight.

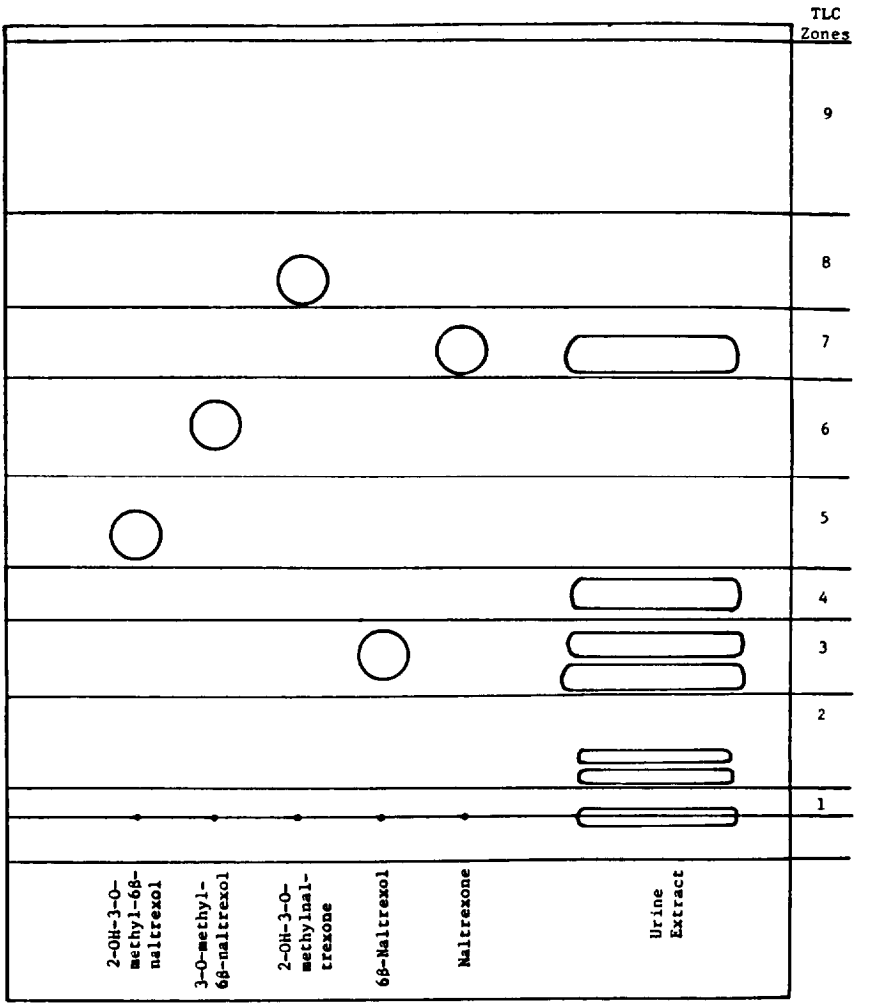
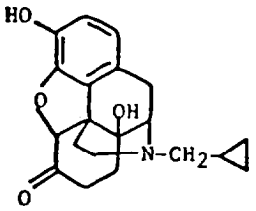


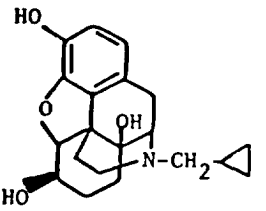
FIGURE 4. Thin layer chromatogram of naltrexone and metabolites. Plates (20 x 20 cm silica gel on glass) were developed in chloroform/ethyl acetate/acetone/ triethylamine (40:30:30:2) and were visualized under UV light.

The reagent was evaporated under nitrogen and 75 µl of Tri-Sil TBT added. Following overnight heating at 115°C excess reagent was evaporated under nitrogen and 50 µl of hexane was added. The derivatized urine extracts were analyzed on an LKB model 9000 mass spectrometer using a 1.8 m x 4 mm I.D. column packed with 2% OV-17 on 80/100 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) at 230°C. Ionizing voltage was set at 70 ev.



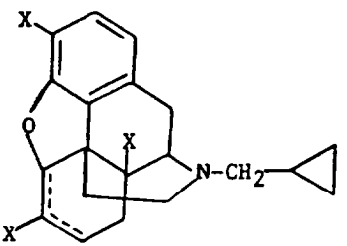
Naltrexone

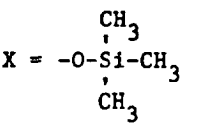
$C_{20}H_{23}O_4N$
m/e 343



β-Naltrexol

$C_{20}H_{25}O_4N$
m/e 345





Naltrexone, tris-TMS ether
$C_{29}H_{47}O_4NSi_3$
MW 557

FIGURE 5. Mass spectral parent ions for naltrexone, naltrexol and TMS derivatives.

RESULTS

The data for the qualitative analysis by GLC/MS of urine extract prepared as described above is shown in figure 8. Shown in the top portion of this figure are retention times for I as the methoxime bis-TMS ether, and II as the tris-TMS derivative. It will be noted that they are easily separated from each other and from the methoxime bis-TMS ether of noroxymorphone. We have found the latter as a metabolite of rats under *in vitro* conditions (8). The lower portion of figure 8 shows the total ion current pattern found after subjecting the derivatized urine extract to GLC-MS. Two major and three minor constituents were noted. The first major peak with R_t at 8.8 min had a mass spectrum identical to the tris-TMS ether of 6ß-naltrexol (II). The minor constituent with R_t of 10.1 min corresponded to the bis-TMS ether of II, resulting from a slightly incom-

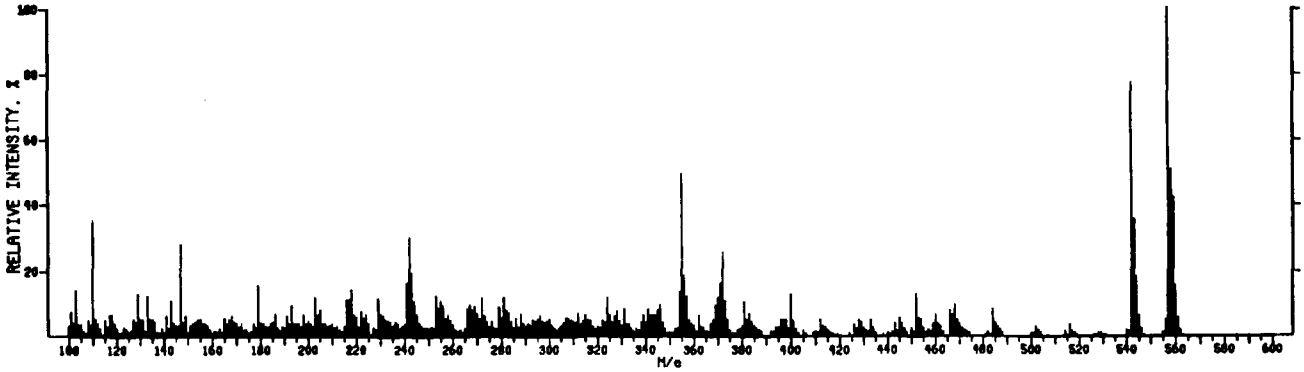


FIGURE 6. Mass spectrum of authentic naltrexone, tris-TMS ether.

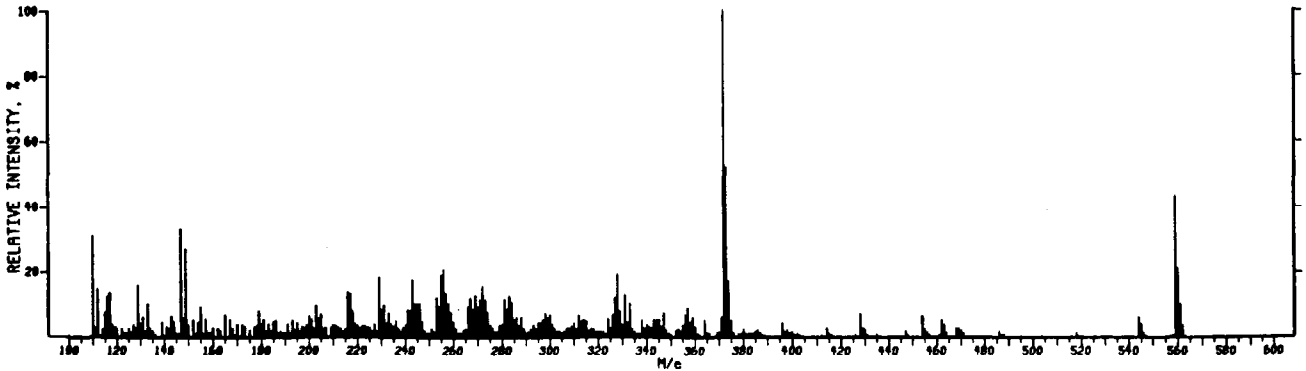


FIGURE 7. Mass spectrum of authentic 6ß-naltrexol, tris-TMS ether.

Column: OV17, 2%
Temperature: 240°
N_2 flow rate: 30 cc/min

Standard	Glc R_t min	Derivative
6ß-Naltrexol	8.8	tris TMS
Naltrexone	13.7	Methoxime, bis TMS
Noroxymorphone	7.0	Methoxime, bis TMS
6ß-Naltrexol	10.1	tris TMS

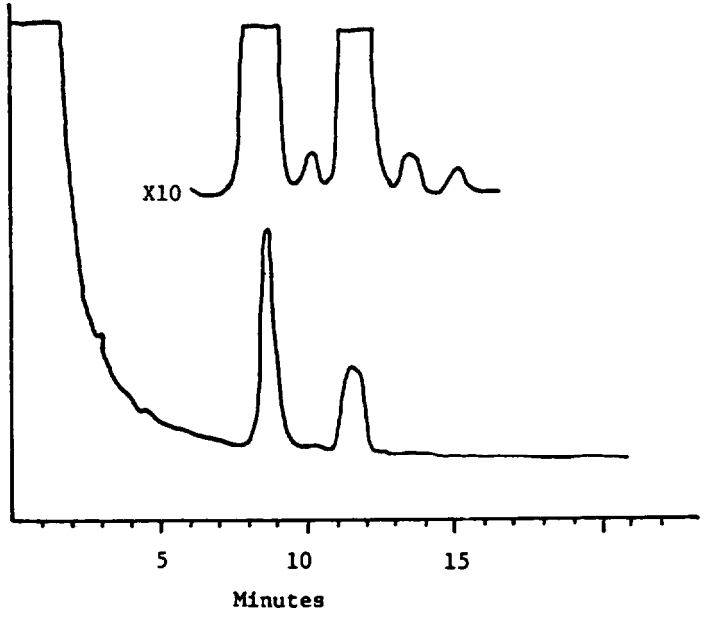


FIGURE 8. GLC analysis of 24-hr nonconjugated urine fraction from male subject PM.

plete derivatization. The next major metabolite with R_t of 11.5 min exhibited a parent ion at 589 mass units corresponding to that of the tris-TMS derivative of 2-hydroxy-3-O-methyl-6ß-naltrexol (III). The mass spectrum of III resembled that of metabolites assigned this structure by Verebey et al. (4) and Cone et al. (9). The data did not rigidly exclude an alternative structure such as 2-methoxy-6ß-naltrexol. However, ^{13}C-NMR of III isolated from urine and compared with the spectrum of synthetic III readily confirmed the

structure (10). The minor compound with a peak at R_t of 13.7 min was identified as the methoxime-bis-TMS derivative of naltrexone (1). Hence, the parent drug I was a minor constituent of the nonconjugated, 24-hr human urine fraction. The minor constituent with R_t of 15 min could not be identified. No evidence could be found in this urine fraction for noroxymorphone, which would have appeared at 7 min, or for 3-O-methyl-6ß-naltrexol (IV).

QUANTITATIVE VALIDATION OF TLC PROCEDURE BY GLC/MS

It was desirable to obtain some indication of the accuracy of the data obtained by the TLC-radiolabeled procedure. Studies were made of the naltrexone and 6ß-naltrexol fractions comparing the TLC method with quantitative GLC/MS. The procedure used was similar to that used in cannabinoid studies (2). Tri-deuterated derivatives of naltrexone and 6ß-naltrexol were prepared and used as carriers and internal standards.

EXTRACTION OF SAMPLES

The general procedure utilized was a modification of the method of Cone et al. (3). Figure 2 shows the procedure for the extraction of nonconjugated I and metabolites from plasma and for the enzymatic hydrolysis and extraction of the conjugated fraction. A slightly modified procedure was used for red blood samples which were treated as follows. To each sample (56 ml) was added in order 5 ml of distilled water, 50 mg of NaF, 0.1N NaOH to adjust the pH to 9, 0.75 ml of 40% (w/v) K_2HPO_4, and 0.375 g of NaCl. Three extractions with 15 ml of ethyl acetate removed naltrexone and metabolites.

The procedure for the extraction of nonconjugated I and metabolites from urine is shown in figure 3. The extraction of fecal samples was carried out with hot methanol. After removal of methanol *in vacuo,* the pH of the aqueous residue (adjusted to 100 ml with water) was adjusted to 9.0 with sodium hydroxide solution. The procedure then is similar to that shown for urine (figure 3).

DETERMINATION OF RADIOACTIVITY

Aliquots of plasma and urine and extracts of plasma, urine, and feces were subjected to liquid-scintillation counting in a Triton X-100/toluene/Omnifluor (1 liter/2 liter/18 g) scintillation fluor with use of the external standardization method to correct for quench-

ing. Samples of red blood cells and fecal residues were combusted in a Packard sample oxidizer, model 306, and the product collected in Monophase- for scintillation counting. All samples were counted in a Packard TriCarb model 3375 liquid-scintillation counter. The procedure for the determination of I in plasma is shown in figure 9. The procedure for naltrexol is similar, and the procedures for both I and II in urine are also similar. Derivatization to the tris-TMS ethers for the quantitative studies was performed by the forcing procedure described previously. Calibration curves for I in plasma and II in urine are shown in figures 10 and 11. Linear cali-

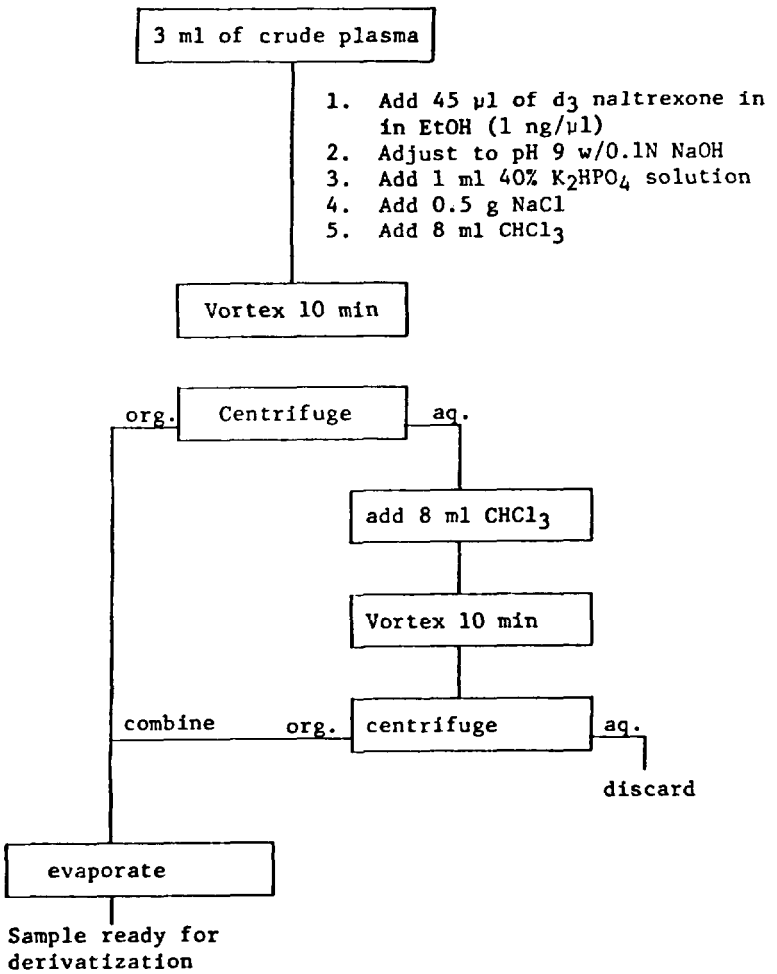


FIGURE 9. Extraction procedure for isolation of naltrexone from plasma.

bration curves were found in each case over a range of 1-100 ng/ml.

Table 1 presents a comparison of the TLC and GLC/MS results for 6ß-naltrexol in urine. In general, satisfactory agreement was

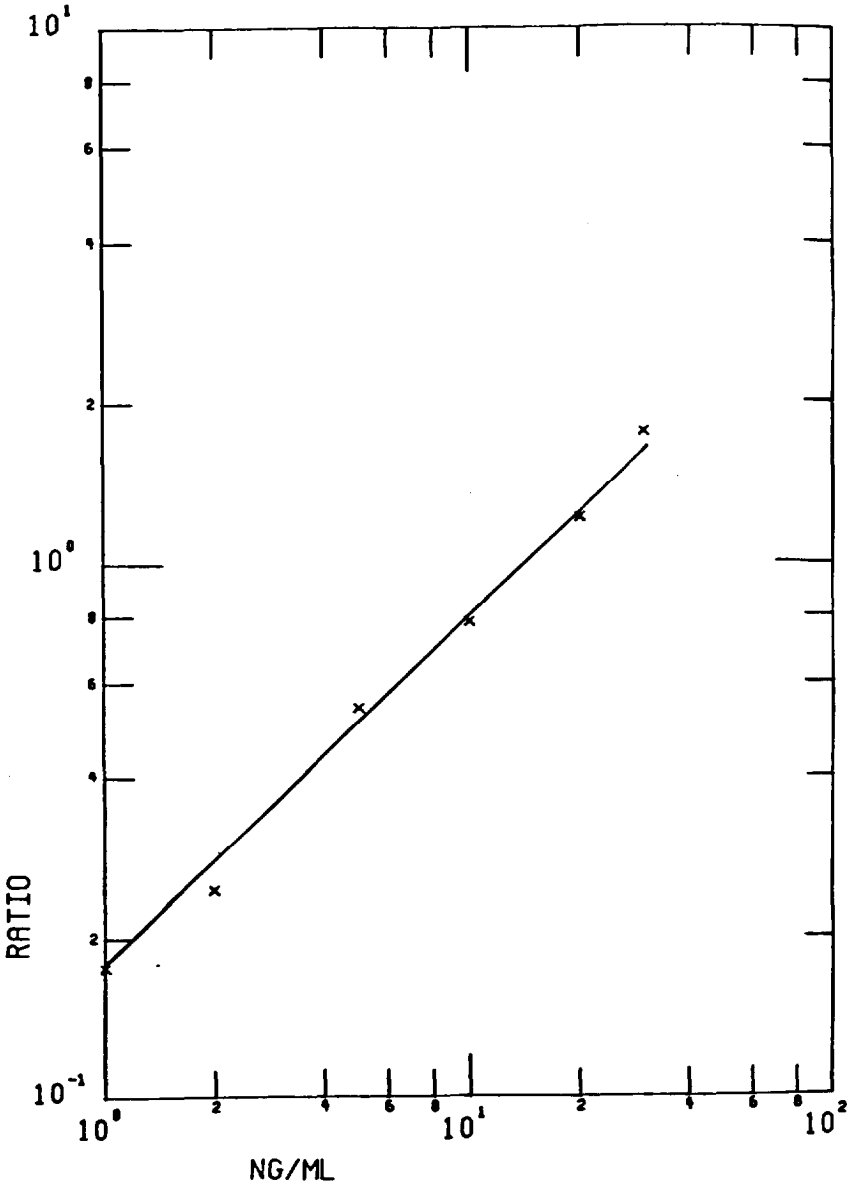


FIGURE 10. GLC/MS calibration curve for naltrexone in plasma.

found. A similar study with naltrexone in plasma gave less satisfactory agreement because of incomplete separation of naltrexone and 2-hydroxy-3-O-methyl-6ß-naltrexol by the TLC system in use at that time.

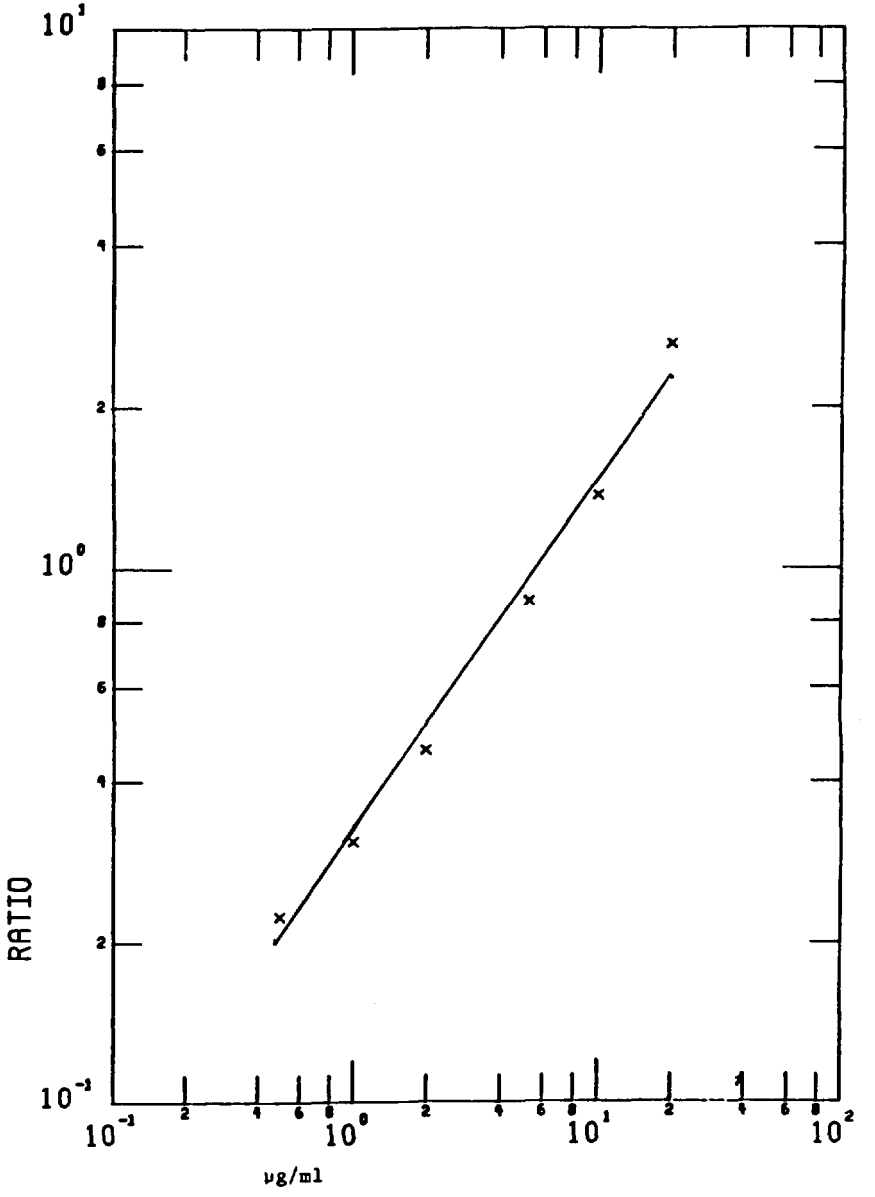


FIGURE 11. GLC/MS calibration curve for 6ß-naltrexol in urine.

TABLE 1. Non-conjugated 6ß-Naltrexol Levels in Human Urine After an Oral Dose of 50 mg of Naltrexone

Subject	Time (Hours)	µg 6ß-Naltrexol/Urine Sample	
		By tlc	By glc-ms
AG	24	14.000	13.000
	40	4.000	3,000
	72	1.200	1.400
TA	24	6.400	8,000
	48	2,000	2,400
	72	420	610
TD	24	3,000	2,700
	48	760	670
	72	193	300

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Part III

Pharmacology

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Background on Animal Testing in the Drug Delivery Systems Program

William L. Dewey

In the screening and selection of candidate long-acting narcotic antagonist preparations, careful consideration must be given to the choice of animal evaluation models. A simple, rapid method, using analgesia suppression in the mouse tail-flick test, was chosen for preliminary screening. Suitable candidates were then evaluated by following urinary excretion rates and blood levels in the monkey. Afterwards, the most promising preparations were rigorously tested in their ability to interfere with morphine self-administration in monkeys. Final evaluation involved toxicological studies in injection sites and on the whole animal.

INTRODUCTION

The choice of the appropriate animal tests for a particular project must be made on the basis of the purpose of the project. The purpose of the drug delivery systems program was to formulate a preparation of a narcotic antagonist that would block the effects of an administered dose of an opiate over a prolonged period of time. The goal was to identify two formulations, one that would afford protection for one month and another that would last for six months. The duration of the antagonistic activity of the preparation was not an important criterion in the selection of the animal test. After the appropriate tests have been chosen, they can be used at various times after the preparation has been administered, to

determine how long the antagonistic action lasts. It is important in most tests that different animals be used for each time point to eliminate tolerance, learning, pathologic effects and other factors which might yield false data.

It was important to choose a battery of animal tests that would be most predictive of opiate antagonism in man. The specific objective of the preparation in man was not to antagonize the analgesic activity of an opiate but to block the rewarding, pleasurable or euphorogenic effects of abused opiates. Pleasure and euphoria are somewhat abstract phenomena which can not be measured in laboratory animals. Other factors, including cost, space, technical difficulty and time requirements, should be considered in the choice of laboratory tests but always must be given less weight than the single criterion of predictability. However, these factors play a more important role in the choice of the preliminary screening tests which are used to select the few most promising devices for advanced animal testing.

A number of excellent *in vitro* techniques such as the opiate binding, guinea pig ileum and mouse vas deferens assays have been developed which are good predictors of opiate agonist and antagonist activity. They were not appropriate for this project. Fortunately a number of relatively simple, inexpensive and somewhat predictive screening procedures were available for testing narcotic analgesics in laboratory animals. Each of these screening tests can be used to predict the narcotic antagonistic activity of a compound or drug delivery system by administering the test compound or system at the appropriate test time prior to the injection of a known antinociceptive dose of morphine or another opiate. The value of each of these procedures in predicting the antagonistic activity of a drug delivery system is related directly to the value of the procedure in predicting the antinociceptive activity of an opiate.

Rodents, usually mice, are used in most of these procedures. Mice are less expensive, take up less space, and most importantly are as predictive as any other species for determining the analgesic or antagonistic activity of a number of analgesic compounds. The next decision to be made was to determine which specific mouse screen should be used for the drug delivery system experiments. The major difference in the procedures has to do with the noxious stimulus. Chemical, heat, cold, electric shock and pressure are the most frequently used stimuli.

TESTING METHOD SELECTION

The tests used most widely today include the tail-flick, the hot-plate and the writhing tests. Each of these procedures has limitations and advantages over the others. Any of these tests would be useful for this project since opiates produce a nice dose response effect in each of them. The most sensitive of these procedures in our and other laboratories is the phenylquinone-induced writhing test (1). The ED-50 for morphine in this procedure is 0.23 mg/kg while the ED-50 for morphine in the hot plate and tail-flick tests is about 2 and 4 mg/kg, respectively. Sensitivity is not an important factor in the selection of the procedure to be used to test the antagonistic activity of the delivery systems, since large doses of an agonist would be required to simulate the self-administration of heroin by the postaddict. The writhing test is not very selective for opiate-like analgesics. Many substances give false positives in this procedure, and the possibility existed that some other substance from the delivery system might block the writhing response, falsely indicating that opiate antagonism was not present. More importantly, the writhing test takes considerable time. A technician can count the writhes of very few animals at one time. The hot-plate and tail-flick tests can be much more time efficient.

The hot-plate technique is more specific than the writhing test, but due to the complexity of lifting and licking of a hind paw or jumping which are required as end points, a number of the central nervous system depressants are false positives (2). Still the hot-plate method is an excellent screening technique for analgesics. One of the major advantages of the hot-plate technique, as originally described, is the ability to determine the duration of action of an antinociceptive agent. This advantage is not useful for testing the antagonistic activity of the drug delivery systems.

The tail-flick test is more selective than the other test procedures (3). Sedatives are inactive and weak and moderate analgesics have limited activity in this procedure. The narcotic analgesics are active in the tail-flick test and an excellent correlation exists between the potency of a number of opiates in this test and their analgesic potency in man. An even better correlation exists between the potency of the opiates in this procedure and their dependence liability in man. This is a particularly useful quality of this procedure for testing the antagonistic properties of the drug delivery systems. As mentioned above, the specific objective of this project is to develop a preparation that will release compound to the body over a prolonged period at a level that will block the effects of a self-injection of heroin in the postaddict.

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The most frequent criticism of the tail-flick test is that the response of the animal is simply a spinal reflex which does not require involvement of the brain. Overwhelming evidence exists to suggest that the site of the analgesic, and more importantly for this project, the euphorogenic or rewarding properties of heroin, morphine and other opiates exists in the brain (4). One might argue that the correlations of activity in the tail-flick test and man referred to above are coincidental and that activity in this procedure really has no relation to the action of a drug in the brain. However, considerable evidence has been presented which strongly indicates that the inhibitory action of morphine, and presumably the other opiates, in the tail-flick response is due to their modulating effects on centers higher than the spinal cord. Complete transection of the spinal cord of mice or rats does not block the tail-flick response to radiant heat but does significantly decrease or completely abolish the activity of opiates in this procedure. These experiments support the hypothesis that this test procedure is useful in assessing the effects of drugs in the brains of animals and in predicting their activity in the brains of man.

Although considerable experience is required for a technician to master the tail-flick procedure, it is the quickest and least demanding of the procedures that were considered as the initial screening test for this project. Results are reproducible from laboratory to laboratory and most importantly it, better than the other procedures, may give the best (albeit questionable) indication of opiate dependence liability. It is my opinion that activity in the tail-flick test by an unknown compound more strongly suggests opiate-type dependence liability for the compound than would be predicted by the other screening tests. Translating this to the narcotic antagonist delivery systems project, one would predict that the ability of the delivery system to block the activity of an opiate in the tail-flick test is a better indication that it would block the effects of an injection of an opiate in a postaddict than is the blocking of opiate activity in the other mouse screening tests.

The next aspect of the mouse screening tests to be considered was the choice of the agonist to be used versus the drug delivery systems. The opiate most widely used by the addicted population is heroin. Methadone and other opiates including morphine, meperidine and other prescribed drugs are abused by certain members of society. The majority of the animal experimentation on mechanism of analgesic action has been carried out using morphine and it usually serves as the prototype in this class of drugs. Since the characteristics of the antinociceptive actions of the opiates appear to be similar and the results of the widespread use of morphine among

laboratories are in good agreement, it was chosen as the agonist for this project.

TEST PROCEDURES

The initial test for the screening of the duration of the antagonistic action of a drug delivery system was the mouse tail-flick test using a subcutaneous injection of morphine as the agonist. The protocol was to implant the delivery system containing the antagonist in enough mice so that a satisfactory number, usually ranging from six to ten, could be tested for their control tail-flick reaction time and then given an injection of morphine, usually an ED-80 dose (in our bands, 10 mg/kg) and retested 20 or 30 minutes later for tail-flick reaction times. An equal number cf mice were implanted with the delivery system which did not contain the narcotic antagonist. Mice were used only once, to eliminate the possibility of learning or pathology of the tail due to repeated long exposures to excessive heat to give false data. Therefore it was important to implant the device in enough mice so that a group of mice could be tested at each desired time point. The control mice, those given only the drug-free vehicle or delivery system, were to react normally to morphine with a resultant increase in reaction time following the injection of the narcotic. Antagonism was considered to be present each time the test reaction time of the mice given the antagonist in the delivery system was shorter than in the mice given the delivery system without the antagonist prior to morphine.

Although the tail-flick test was useful as a primary screening test, secondary more advanced procedures were necessary to completely evaluate the duration of action of the narcotic antagonist delivery systems. It has been well established that a direct correlation exists between the blood level and the narcotic antagonist activity of naltrexone in a number of species. Therefore the second line testing in laboratory animals was to study the pharmacokinetics of the naltrexone released from the delivery system. The release rates of naltrexone could be monitored by quantitating the excretion rates of radiolabeled naltrexone in urine and feces following the implantation of the narcotic antagonist delivery system in rats or guinea pigs. It was established early in the project that essentially quantitative recovery of the radiolabel was achieved in these species (4).

These initial excretion experiments were informative, and along with the tail-flick data yielded information useful in predicting the duration of antagonistic action of the delivery systems. A number

of important problems remained. It was necessary to isolate and quantitate the parent compound from its structurally related but pharmacologically relatively inactive metabolites in the body fluids such as blood and urine and in body tissues before a clear picture of the pharmacokinetics of naltrexone released from the delivery systems could be achieved. This necessitated the development of sophisticated analytical methodology for the quantification of naltrexone. The potency of this narcotic antagonist was one of the strongest reasons for its choice to be used in these studies, since it afforded the smallest possible size implant. However, it takes less of a very potent compound to produce the desired pharmacological effect, which in turn increases the required sensitivity of the analytical procedures used to study its metabolism, distribution, binding to plasma proteins, and other aspects of its pharmacokinetics. The development of a sensitive gas chromatographic-electron capture assay for naltrexone by Dr. Reuning was a major contribution in this regard (4).

The second phase on the pharmacokinetics of naltrexone released from the drug delivery systems was carried out in the monkey. The monkey and possibly the dog were thought to be the nonrodent species most useful for the testing of these delivery systems. Ideally, the pharmacokinetics should be conducted in a species used for testing the duration of the antagonistic activity of the delivery system, but it is difficult to obtain sufficient blood and urine samples from mice. It also was hypothesized that the pharmacokinetics of naltrexone in man would resemble more closely the pharmacokinetics of the antagonist in the monkey than in the mouse.

A second, more sophisticated, procedure was necessary to determine the duration of the efficacy of the narcotic antagonist delivery systems to block the effects of an opiate. Even more importantly it was necessary to determine the duration of antagonistic activity versus the rewarding effects of the opiate rather than versus the antinociceptive activity of the opiate as measured in the tail-flick test. One of the classical methodologies used to measure the rewarding property of drugs is the self-administration technique which has been used in both rats and monkeys. The monkey was the preferred species for this project for a number of reasons, including the fact that the pharmacokinetics of naltrexone in the monkey more closely resemble its pharmacokinetics in man and considerable experience exists on the effects of narcotic analgesics and their antagonists in monkeys.

It has been known for a considerable period of time that monkeys will titrate themselves with narcotic analgesics or for that

matter with most of the many other drugs that act on the central nervous system. A number of other classical conditioning experiments have been developed in an attempt to quantitate the rewarding properties of a drug. None has proven to be as useful or as predictive of the rewarding properties of drugs in man as the self-administration paradigm. The fact that a monkey will work very hard by pressing a bar many times to receive an injection of these drugs has led to the conclusion that the injections were rewarding to the animal. Monkeys will not press a bar for an injection of saline or other agent known not to act in the central nervous system and therefore these substances are considered not to be rewarding to the animal.

Monkeys are implanted with an indwelling cannula which is connected to a vessel containing the drug solution or saline. These monkeys are very valuable animals, due to their inital cost, the need for sterile surgery during the cannula placement, the recovery time and the training necessary to teach them to press the bar for the drug injection. Therefore, only the most promising of the delivery systems could be tested in this procedure. Monkeys used in the studies with the narcotic antagonist delivery systems were maintained on a repeated cycle of access to morphine, amphetamine, and saline through the cannula. The monkeys responded frequently when either amphetamine or morphine was being self-administered by a lever press, but their response rate was much less during the saline portion of the cycle. Response rates were determined prior to the implantation of the delivery system and repeatedly until response rates returned to control values after the implantation. This procedure allowed the investigators to differentiate the selective effect of a narcotic antagonist from the self-administration of only the opiate, without altering the response rate during the amphetamine portion of the cycle. In contrast, a general central nervous system depressant would reduce significantly the response rate in both the morphine and amphetamine portions of the cycle.

Once again the question arose as to the proper agonist for these experiments. It is quite possible, and often suggested, that the rewarding effects of heroin are greater than those of morphine in man. However, it has been demonstrated that naltrexone blocks equally well the rewarding properties of heroin and morphine as determined by this self-injection technique in monkeys. Therefore morphine was chosen as the agonist for these experiments. It was also felt to be an advantage to use the same agonist as was used in the mouse tests.

The duration of the antagonistic activity of naltrexone in the drug delivery systems in the monkey was correlated to the blood levels of naltrexone in these animals. Blood samples were taken periodically throughout the time of effective blockade and they were analyzed for levels of naltrexone by the gas chromatographic-electron capture technique mentioned above. A good correlation was found between the two parameters and these data overwhelming support the view that the parent compound is responsible for the antagonistic activity.

The final aspect of the animal testing paradigm was a safety evaluation. The toxicity tests for this project can be somewhat more complicated than usual, since not only the toxicity of naltrexone and the drug delivery matrix themselves need be studied, but also one must study the toxicity of the combination of the narcotic antagonist in the drug delivery system. Considerable but certainly not all of the toxicity work with naltrexone had been completed prior to the initiation of the project. The majority of the constituents of the drug delivery matrix had been approved for use in humans for other reasons. Therefore, the one aspect of toxicity which was of primary concern in this project was the toxicity of the naltrexone and the drug delivery system combined.

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Pharmacological Evaluation of Narcotic Antagonist Delivery Systems in Rhesus Monkeys

Stephen E. Harrigan and David A. Downs

Rhesus monkeys were chronically restrained, intravenously catheterized, and allowed to self-administer morphine, methamphetamine, and saline. Various sustained-release systems containing naltrexone were then implanted in the animals and examined for selective morpine blockade. Similarly, continuous intravenous infusions of naltrexone, buprenorphine, and methadone were tested against morphine or heroin self-administration.

INTRODUCTION

Our major objective was to determine the degree and duration of blockade of the reinforcing properties of narcotics in rhesus monkeys after administration of narcotic antagonist delivery systems. This was accomplished using the intravenous self-administration preparation described by Harrigan and Downs (1). In brief, minimally restrained rhesus monkeys were prepared surgically with permanent jugular or femoral venous catheters and trained to press a response key to receive intravenous injections of morphine, methamphetamine, or saline. When stable rates of drug and saline intake were achieved, delivery systems were administered and the effects were monitored over time until recovery was evident. Ef-

fects of narcotic antagonist delivery systems were compared against those of acute or continuous administration of naltrexone, methadone, and buprenorphine. Morphine and heroin self-administration also were compared for their antagonism by naltrexone. Some other objectives were to evaluate tissue reactions to various narcotic antagonist delivery systems at the sites of administration and to assess the preclinical toxicity of a selected delivery system.

MATERIALS AND METHODS

Self-Administration Procedures

Rhesus monkeys (Macaca mulatta) were housed individually and were minimally restrained with a jointed metal arm and harness system (2). About 180 g of Purina Monkey Chow and 1/4 fresh orange or apple were fed daily, while water was continuously available. Each monkey was prepared surgically with a jugular or femoral intravenous silicone rubber catheter. The distal end of the catheter exited through the skin of the back and passed through the hollow restraining arm to a reciprocating syringe driver (Superspenser, Hook and Tucker Ltd., Croyden, England) mounted outside the cage.

A transilluminated plastic key (3.5 x 6.5 cm) served as the operant response device. One key press activated the pump to deliver 0.5 ml of drug solution through the catheter over 5 seconds followed by a 5-second refill. Responses during the infusion cycle had no consequences. Access to self-administration of drugs was limited to a 15-minute period every 4 hours. Each access period was initiated by illuminating the key and by one automatic injection. Room lights were controlled automatically to provide 12-hour diurnal cycles.

Drug self-administration solutions usually were changed in a repeating cycle of 3 days on morphine sulfate (8 μg/kg/injection), 2 days on methamphetamine hydrochloride (4 μg/kg/injection), and 2 days on 0.9% sterile saline. Thus, within each week the monkeys had 18 sessions of morphine availability, 12 sessions of methamphetamine availability, and 12 sessions of saline availability. In a few early studies, morphine, methamphetamine, and saline were each available for 3 days at a time. All monkeys were exposed to this schedule for at least 2 months or until drug and saline intakes remained stable prior to the administration of antagonist delivery systems.

Because self-administration behavior under these conditions was maintained at low and variable rates at night, only the number of

injections in each of the three daily "daylight" access periods was used to compare treatments. Statistical comparisons were made with analysis of variance and Newman-Keuls multiple range test. All drug doses were expressed as base. When possible, delivery systems were administered in sufficient quantities to deliver a total dose of 10 mg/kg of naltrexone.

Administration of Narcotic Antagonist Delivery Systems

Drug delivery systems generally were administered on the first day of a period of saline self-administration. When necessary, ketamine hydrochloride (10 mg/kg) was used to anesthetize the monkeys prior to administration of a delivery system. Self-administration rates over subsequent cycles of exposure to morphine, methamphetamine, and saline were monitored until morphine intake returned to control levels. Duration of effect was determined as the last week in which morphine rates were significantly (<0.05) lower than control values. Implantable delivery systems usually were inserted subcutaneously in the abdominal area via 12-15 gauge trocars. Injectable systems were administered into the rectus femoris muscle, or in some cases, subcutaneously in the abdominal area.

Tissue Reactions

When possible, implantable devices were removed at the end of measurable activity; gross visual examination of implant sites was performed at that time. In some cases, biopsies were made of tissues surrounding implant or injection sites and these were subjected to histopathological analysis by Drs. J. Fitzgerald and S. Kurtz of the Parke-Davis Department of Toxicology. With some systems, sites of administration were examined sequentially in nonanesthetized monkeys to determine the time-course of degradation of the systems. Blood, urine, feces, and recovered systems or system fragments were sent to other NIDA contractors for various analyses.

RESULTS WITH DELIVERY SYSTEMS

The classification of naltrexone delivery systems described below is summarized in table 1.

Insoluble Salts

The pamoate acid salt of naltrexone in peanut oil (NIDA) blocked morphine self-administration for less than 5 days, whereas naltrexone zinc tannate and naltrexone aluminum tannate (IITRI) in peanut oil blocked morphine self-administration for about 24 days

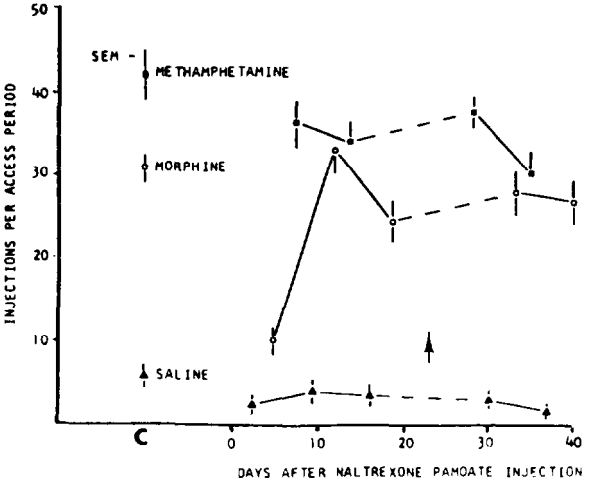
TABLE 1. Classification of Naltrexone Delivery Systems

	Injections	Implants
Insoluble Salts	Naltrexone Aluminum Tannate Naltrexone Zinc Tannate Naltrexone Pamoate	—
Erodrble Polymer Matrices	Naltrexone Polylactic Acid Naltrexone Lactic glycolic Acid	Naltrexone Lactic glycolic Acid beads Naltrexone Chronomer Rods Naltrexone Palmitin Rods
Macro-Reservoirs	—	Naltrezone Glutamic Acid Leucine in Glutamic Acid Leucine tubes. Naltrexone (Dry or in water or in sesame oil) in Capronolactone Lactic Acid tubes
Micro-Reservoirs	Naltrexone (Base) polylactrc acid microcapsules Naltrexone Pamoate polylactrc acid microcapsules	

(figure 1). Biopsy of one injection site 43 days after intramuscular injection of naltrexone aluminum tannate revealed no marked change of muscle fiber.

Injectable Erodible Polymer Matrices

Naltrexone polylactide (Yolles) suspended in carboxymethylcellulose fully antagonized morphine for only a few



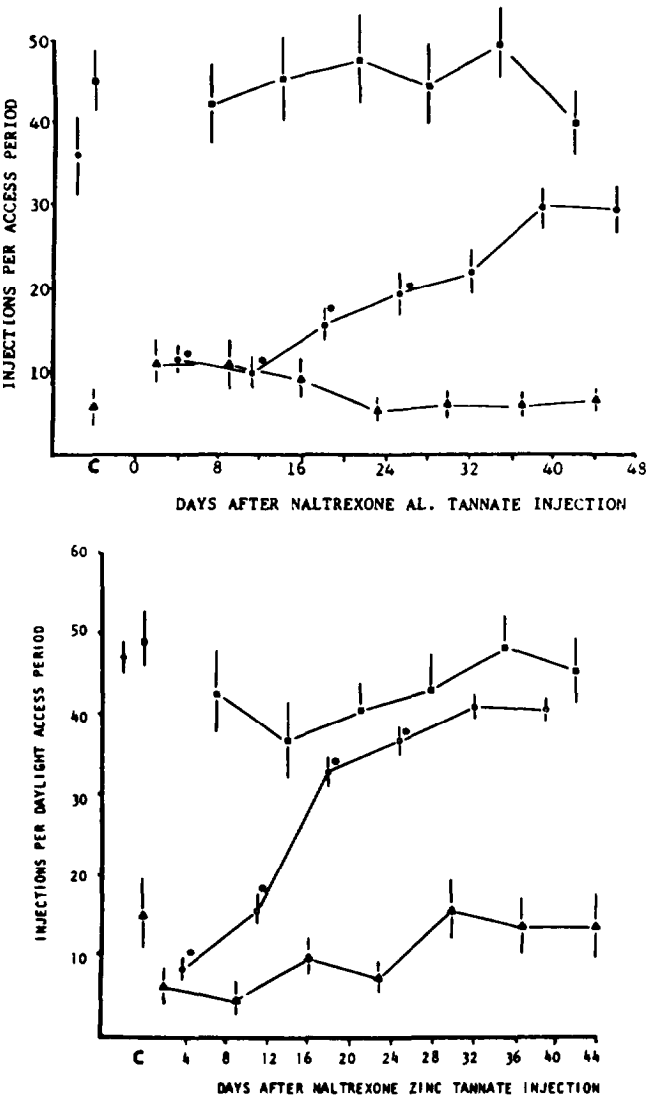


FIGURE 1. Self-administration of morphine (8 μ/kg/injection) (open circles), methamphetamine (4 μ/kg/injection) (solid squares), and saline (solid triangles) following intramuscular injection of naltrexone pamoate, naltrexone aluminum tannate, or naltrexone zinc tannate. Each point represents the mean of four monkeys. Vertical bars denote ±SEM. Asterisks indicate means significantly different from corresponding control (C), ($P < 0.05$). Arrow signifies a single intravenous injection of naltrexone; drug self-administration for one cycle is not included in the figure.

days (figure 2A). Examinations of injection sites revealed considerable tissue encapsulation of injected material.

Naltrexone in micronized polylactic/glycolic acid copolymer (Dynatech) given subcutaneously in aqueous vehicle suppressed morphine self-administration only for 3-5 days (figure 2B).

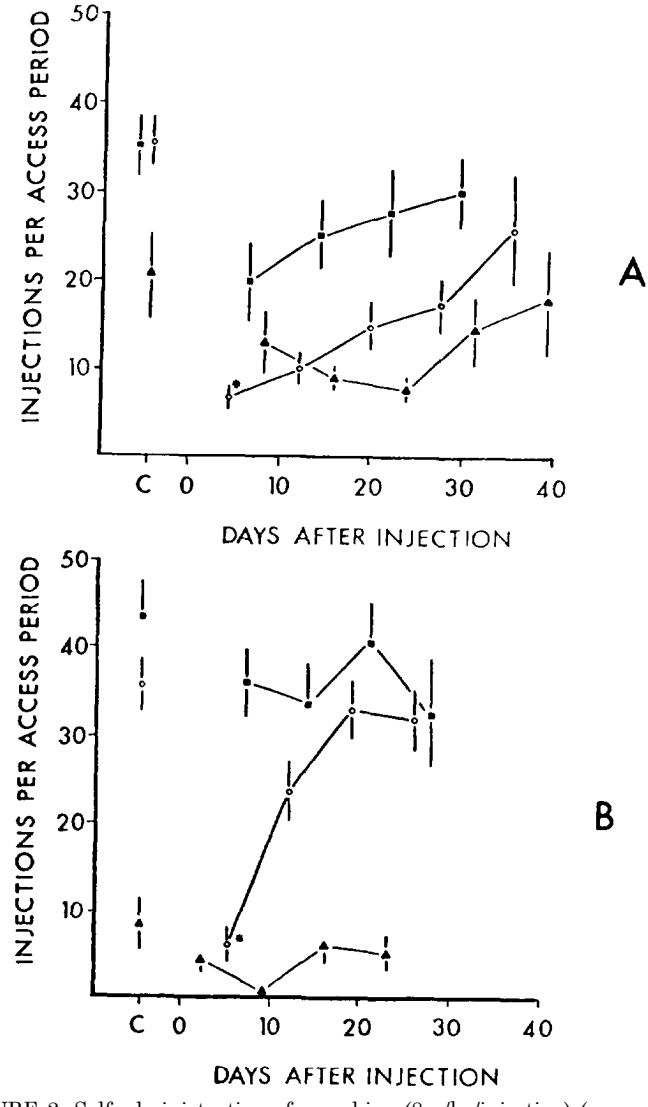


FIGURE 2. Self-administration of morphine (8 μ/kg/injection) (open circles), methamphetamine (4 μ/kg/injection) (solid squares), and saline (solid triangles) following subcutaneous injections of naltrexone poly lactic acid (A) or naltrexone polylactic-glycolic acid (B) polymers. Points in A represent means of two monkeys; points in B represent means of four monkeys. Vertical bars denote ±SEM. Asterisks indicate means significantly different from control (C), ($P<0.05$).

Implantable Erodible Polymer Matrices

Rods (1.7 x 10 mm) containing 33% naltrexone in a 75/25 dipalmitin/tripalmitin matrix (Battelle Northwest) produced about 20 days of significant morphine blockade (figure 3). The rods caused very little gross tissue reaction and were easily removed at the end of the study.

Rods (1 x 14 mm) containing 20% naltrexone, 10% disodium carbonate, and 70% Chronomer (ALZA) suppressed morphine self-administration for about 20 days (figure 3). Recovery after 20 days was very rapid and corresponded with the near-total absorption of the rods from the implant sites. Sequential biopsies taken from normal monkeys showed gradual liquification and eventual total disappearance of these rods over a 30-day period. Gross tissue reaction was minimal. Seventeen rods were required to deliver 10 mg/kg naltrexone to each monkey.

Beads (1.5 mm) containing 70% naltrexone in a 90/10 lactic acid glycolic acid copolymer (Dynatech) caused dose-dependent reductions in morphine self-administration (figure 4). Single beads (about 0.5 mg/kg naltrexone) caused a slight suppression of morphine self-administration for only one morphine exposure (5 days) after the implant. Three beads (about 1.5 mg/kg naltrexone) produced a 24% reduction of morphine self-administration during the first morphine exposure; thereafter morphine intake returned to control levels within two weeks. Longer and more intense blockade occurred after treatment with six beads (about 3 mg/kg naltrexone); morphine self-administration was reduced by about 42% for 30 days, although none of the post-implant means were significantly different from control ($P>0.05$).

Nine beads (about 5 mg/kg naltrexone) and 18-21 beads (about 10 mg/kg naltrexone) significantly suppressed morphine self-administration for 30 days to rates equivalent to those for saline self-administration ($P<0.05$). After 33 days, morphine self-injection rates returned to control levels.

Monkeys receiving control beads (not containing naltrexone) showed slight increases in morphine injections for the first two weeks post-implant; thereafter, morphine self-administration returned to near control levels.

Tissue reactions in rata, rabbits, dogs, and monkeys generally consisted of local induration and chronic inflammatory response with encapsulation. No suggestion of systemic drug toxicity was found. Bead material was present at the sites of administration 3 months after implantation.

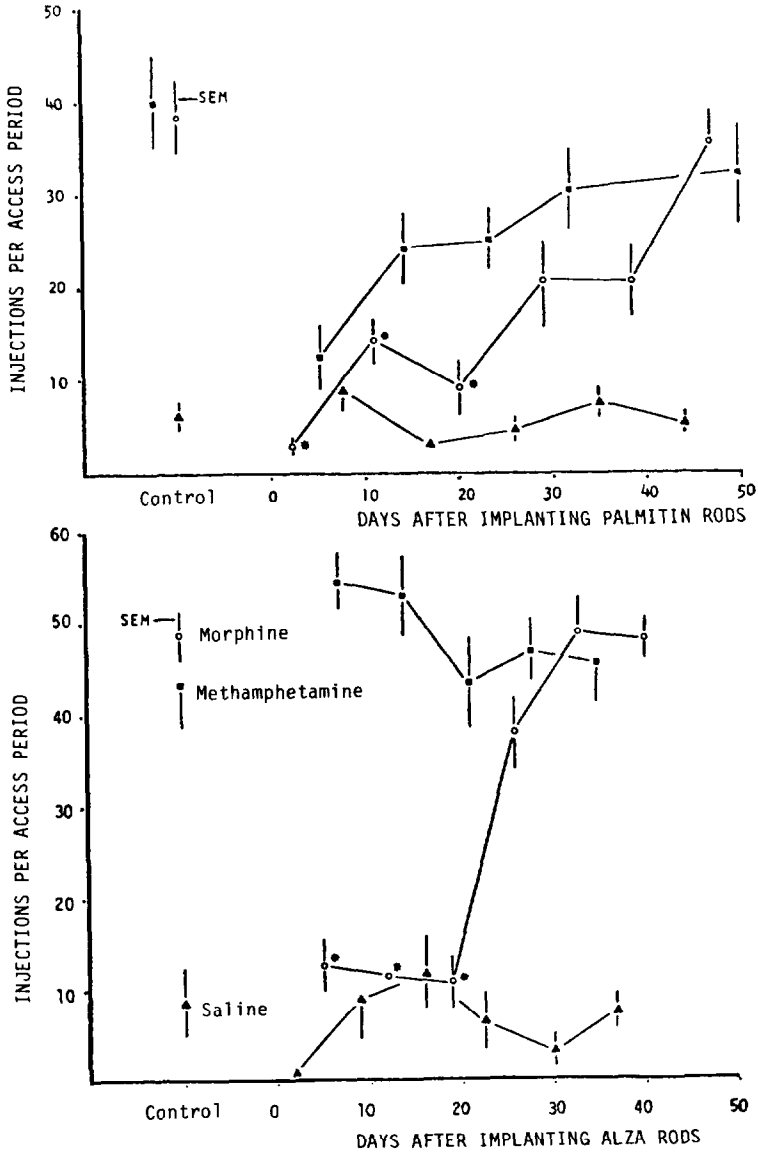


FIGURE 3. Self-administration of morphine (8 μ/kg/injection) (open circles), methamphetamine (4 μ/kg/injection) (solid squares), and saline (solid triangles) following implanting of naltrexone-containing palmitin or Chronomer rods. Points in upper figure represent means of three monkeys; points in lower figure are means of four monkeys, Vertical bars denote ±SEM. Asterisks indicate means significantly different from control ($P<0.05$).

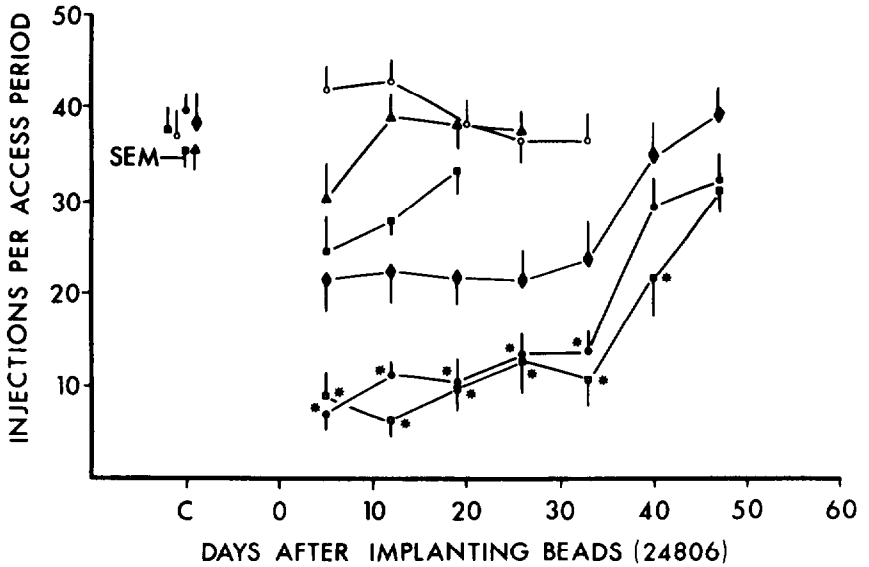


FIGURE 4. Self-administration of morphine (8 μ/kg/injection) following implants of control (open circles) or 1 (triangles), 3 (solid squares), 6 (diamonds), 9 (solid circles), or 18 (open squares) naltrexone-loaded beads. Points represent means of four monkeys. Vertical bars denote ±SEM. Asterisks indicate means significantly different from control (C) ($P<0.05$).

Macro Reservoirs

Naltrexone (10%) in 35/65 glutamic acid/leucine copolymer binder (90%) placed inside sealed tubes (1 cm length, 0.19 cm outer diameter) composed of copolymer walls 53-63 microns thick (A.D. Little) blocked morphine self-administration for about 12 days (figure 5A). The short duration of action and considerable tissue reaction noted when the tubes were removed suggested that the walls had prematurely failed, rapidly leaking naltrexone and binder. Additional tubes, together with unloaded controls, were implanted in normal dogs and monkeys to more fully characterize the unexpected tissue reactions. Results indicated that an unusual perivascular lymphocyte accumulation was associated with the naltrexone-loaded devices and equivocally or not at all with the controls.

Capronolactone/lactic acid copoylmer tubes (2 x 30 mm) (RTI) containing either naltrexone powder or naltrexone suspended in water or sesame oil all suppressed morphine self-administration to about the same degree but for various durations. Figure 5B shows that tubes 3 cm. in length containing dry naltrexone were effective for about 30 days; tubes containing naltrexone in sesame oil had about the same duration of effect. In contrast, identical tubes con-

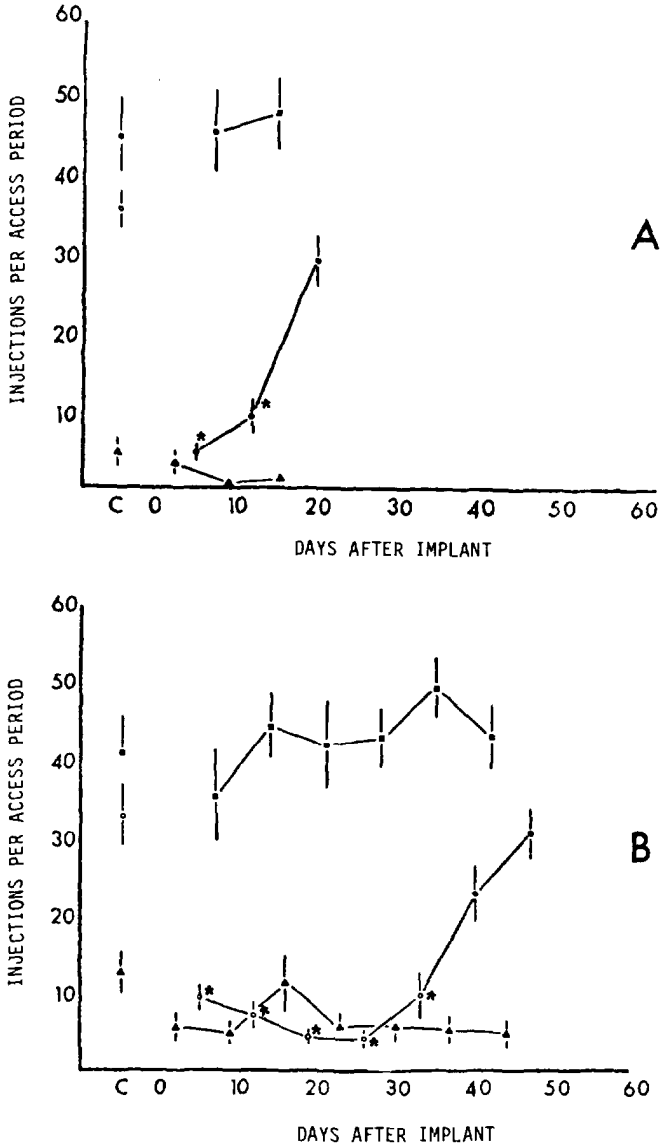


FIGURE 5. Self-administration of morphine (8 μ/kg/injection) (open circles), methamphetamine (4 μ/kg/injection) (solid squares), or saline (solid triangles) following implants of naltrexone contained in tubes composed of glutamic acid/leucine (A) or capronolactone/lactic acid (B). Points represent means of three monkeys. Vertical bars denote ±SEM. Asterisks indicate means significantly different from control (C) ($P<0.05$).

taining naltrexone in water lasted only for about 12 days. These tubes biodegraded to the extent that large fragments of collapsed tubes were recovered 50 days after implants. No gross tissue irritations were noted.

Micro Reservoirs

Naltrexone, either as a base or as the pamoate salt contained within microcapsules (63-106 μ diameter) of polylactic acid (Theis, Washington University) was tested under a variety of conditions. The microcapsules were suspended in either peanut oil or an aqueous vehicle and injected either subcutaneously or intramuscularly. Intramuscular injection of microcapsules in peanut oil produced very stable morphine blockade for about 50 days (figure 6). By the same route but suspended in aqueous vehicle, the microcapsules suppressed morphine self-administration for about as long but with somewhat less consistency (figure 6). Microcapsules injected subcutaneously in water showed sustained activity, but the naltrexone release rate appeared to be too low to provide full morphine blockade (figure 6). No biopsies were performed on intramuscular injection sites, but considerable swelling, erythema, and occasional necrosis were observed in subcutaneous sites.

CONTINUOUS INTRAVENOUS INFUSIONS OF NALTREXONE, BUPRENORPHINE, AND METHADONE

Continuous intravenous infusions of naltrexone caused dose-related decreases in morphine (8 μg/kg/injection) self-administration. The suppression of morphine self-administration remained stable over the 4-week duration of each naltrexone infusion. When naltrexone was discontinued, morphine self-administration returned to control levels within one or two morphine cycles (1). This study demonstrated that morphine self-administration rates were sensitive to naltrexone in a dose-dependent manner and that the suppression of morphine self-administration by effective doses of naltrexone was stable over at least month-long intervals. In addition, it was estimated that steady-state delivery of naltrexone at rates of 5 μg/kg/hour or more were adequate to provide complete blockade of self-administration of a wide range of morphine doses.

Figure 7 shows that heroin self-administration also was suppressed by continuous intravenous infusions of naltrexone. As with morphine self-administration, the heroin self-administration dose-

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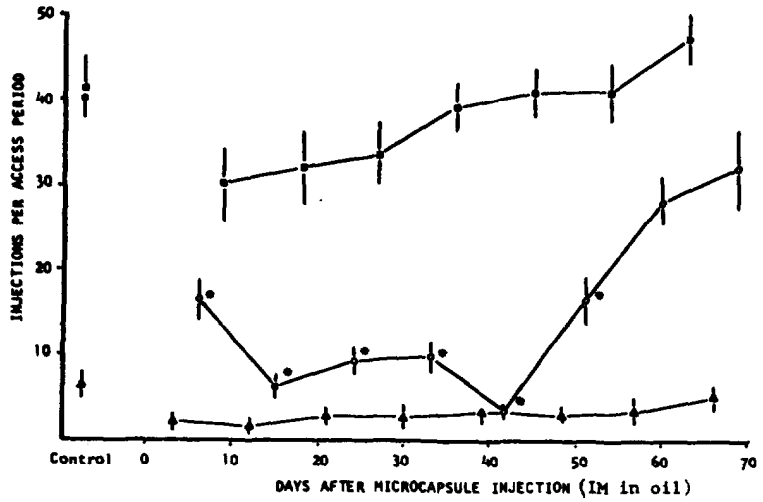

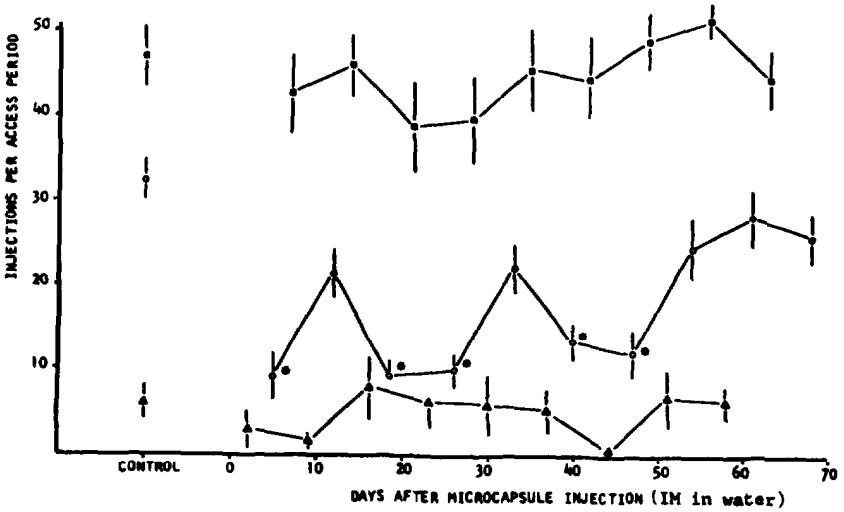

response curve was shifted to the right and decreased in maximum as the naltrexone infusion rate increased.

Figure 8 shows that continuous intravenous infusions of methadone also can suppress heroin self-administration. In general, the suppressant effects of methadone appeared to be similar to those of naltrexone. However, when the methadone infusion rate was high enough to block the self-administration of a broad range of heroin

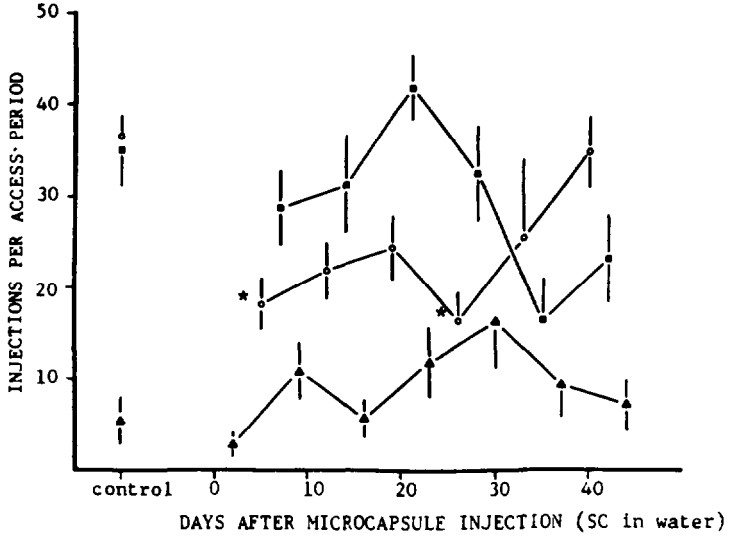

FIGURE 6. Self-administration of morphine (8 μ/kg/injection) (open circles), methamphetamine (4 μ/kg/injection) (solid squares), or saline (solid triangles) following injection of microencapsulated naltrexone pamoate intramuscularly or subcutaneously. Points represent means of three or four monkeys. Vertical bars denote ± SEM. Asterisks indicate means significantly different from control ($P<0.05$).

doses, the monkeys showed severe debilitation and depression. Two out of three monkeys died during infusions of methadone above 24 mg/kg/day.

The effect of a continuous intravenous infusion of buprenorphine at 40 μg/kg/hour was similar to that of naltrexone at 5-20 μg/kg/hour. A lower rate of buprenorphine infusion (20 μg/kg/hour), however, had only slight effect on morphine self-administration (figure 9).

DISCUSSION

Narcotic self-administration by rhesus monkeys was found to be a reliable, specific and sensitive measure of the effects of the drugs and drug delivery systems described above. It seems very reasonable to assume that the repeated self-administration of narcotics by rhesus monkeys and by humans is determined by at least some common pharmacological factors. This assumption is based on and supported by the general observation that drugs which act as reinforcers (i.e., maintain self-administration behavior) in rhesus mon-

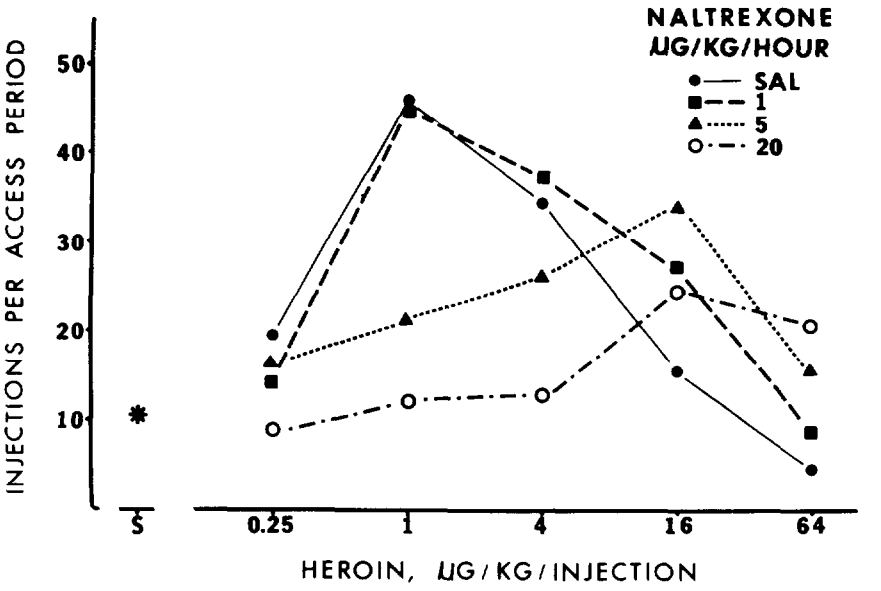

FIGURE 7. Heroin self-administration as a function of dose per injection during continuous infusion of saline (solid circles), or 1 (solid squares), 5 (solid triangles), or 20 µ/kg/hour naltrexone (open circles). Each point represents the mean of four monkeys. The asterisk at S indicates saline self-administration from a control period near the start of the study. Standard errors have been omitted for clarity; none was greater than 3.7 injections per access period.

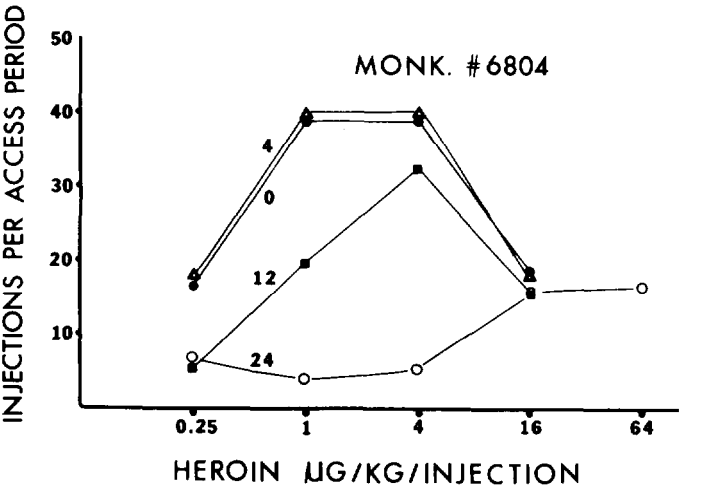

FIGURE 8. Heroin self-administration as a function of dose per injection during continuous infusion of saline or 4, 12, or 24 mg/kg/day methadone. Points represent means of nine access periods in a single monkey.

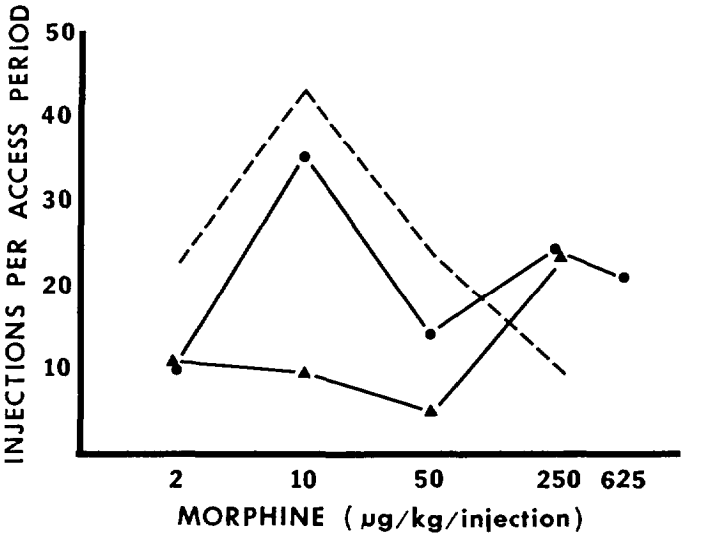

FIGURE 9. Number of morphine self-injections as a function of dose during continuous infusions of 20 (circles) or 40 (triangles) µg/kg/hour buprenorphine. Points represent means of two monkeys. Dashed line indicates morphine self-administration during continuous saline infusion; data obtained from other animals.

keys act similarly in humans. A second assumption is that manipulations which alter self-administration of narcotics by rhesus monkeys will act similarly in humans. However, the predictive value of the procedures and the effects described above remains to be determined in clinical studies with antagonist delivery systems in humans.

Although most of the delivery systems which were submitted to us for evaluation contained naltrexone, it is apparent that other agents are potentially equally effective. Thus, future objectives could include the development of delivery systems for drugs with improved efficacy and safety.

Finally, the impressive progress which has been made in the development of narcotic antagonist delivery systems should be of significant value in the development of other drug delivery systems for a broad range of research and treatment areas.

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A Comparative Study of the Oral, Intravenous, and Subcutaneous Administration of ^{3}H-Naltrexone to Normal Male Volunteers

Mario Perez-Reyes and Monroe E. Wall

^{3}H-naltrexone was administered orally, intravenously, and subcutaneously to groups of normal, male, paid volunteers. The doses given were: 50 mg orally (specific activity 4 μCi/mg), 1 mg intravenously (specific activity 200 μCi/mg), and 5 mg subcutaneously (specific activity 30 μCi/mg). At these doses, the subjects did not experience any *noticeable effects. Following intravenous injection, plasma levels of radioactivity were immediately high and declined rapidly during the first 30 minutes and declined gradually thereafter. Following oral or subcutaneous administration, maximal plasma levels were observed to occur one hour after dosing, and reached similar levels to those obtained when the drug was intravenously injected. This finding indicates the excellent bioavailability of naltrexone following oral or subcutaneous administration. ^{3}H-naltrexone and/or its metabolites were predominately excreted in the urine, and the renal excretion was similar for all three routes of administration. Fecal excretion is a minor pathway of elimination. The urinary and fecal excretion of ^{3}H-naltrexone was studied in one subject for 133 hours after drug ingestion, and it was found that essentially all of the dose administered was excreted in this period.*

INTRODUCTION

Naltrexone is a narcotic antagonist that in clinical pharmacological trials appears to have longer duration of action and to be 2 to 3 times more potent than naloxone (1). Naltrexone has been commonly administered in doses ranging from 50 to 200 mg orally, or 1 to 80 mg subcutaneously, to postnarcotic addicts. The drug appears to be well tolerated with no side effects if the subjects receiving the drug have abstained from narcotic drugs for 4 weeks (2).

To obtain information on the bioavailability of naltrexone, pattern of metabolism, and urinary and fecal excretion, the drug was radiolabeled with tritium and administered orally, intravenously, and subcutaneously to normal subjects. It is the purpose of this communication to report the results obtained.

EXPERIMENTAL PROCEDURE

The subjects that participated in the experiments were all male, healthy, paid volunteers who had never used narcotic drugs and who were thoroughly informed about the nature and potential risks of the experiment.

a) *Oral administration:* Six volunteers received 50 mg of ^{3}H-naltrexone (specific activity 4 μCi/mg) dissolved in ethanol in two gelatin capsules 3 hours after a light breakfast. Blood samples were collected before drug administration, then at 15-minute intervals for three hours, and at 4 1/2, 6, 12, and 24 hours. Urine was collected at 3, 6, 12, 24, and 48 hours after drug ingestion, and feces at 24 and 48 hours.

b) *Intravenous administration:* Five volunteers received 1.0 mg of ^{3}H-naltrexone (specific activity 200 μCi/mg), dissolved in 1 ml of normal saline in a single bolus intravenous injection. Blood samples were collected before drug administration, at 30 seconds, at 1, 3, 5, 10, 15, 30, 60, 90, 120, 150, and 180 minutes, and at 5, 7, 10, and 24 hours. Urine was collected at 3, 6, 12, 24, 48, and 72 hours.

c) *Subcutaneous administration:* Two volunteers received 5 mg of ^{3}H-naltrexone (specific activity 30 μCi/mg), dissolved in 1 ml of normal saline in a single subcutaneous injection. Only two subjects were tested because of reports about possible carcinogenicity of naltrexone which temporarily halted our experiments. Blood samples were collected before drug administration, at 5, 10, 20, 30, 45,

60, 75, and 90 minutes, and at 2, 4, 6, 8, 10, 24, 30, and 48 hours. Urine was collected at 3, 6, 12, 24, 48, and 72 hours.

In the biological samples obtained in the experiments, the total radioactivity present was measured by liquid scintillation spectrometry in triplicate aliquots. Determination of ^{3}H-naltrexone and its metabolites was performed by chromatographic procedures and will be reported elsewhere.

RESULTS

a) *Clinical effects:* Neither the oral, intravenous, nor subcutaneous administration of naltrexone, at the doses used, produced any clinical effects other than a slight degree of drowsiness which could have been produced by a total lack of physical activity for the first 3 hours of the experiment.

b) *Percentage of the dose administered present in the plasma:* Although the same dose of naltrexone was administered to all the subjects in each of the three routes of administration studied, the subjects tested within each group had different body weights. Therefore, the results have been expressed as the percentage of the total radioactivity administered present in the theoretical total plasma volume at each of the time intervals sampled. The total plasma volume of each subject was calculated on the basis that the average human plasma volume is 45.4 ml/kg of body weight (3). The plasma volume of each subject was multiplied by the disintegrations per minute per milliliter of plasma, and from this value, the percentage of the total radioactivity administered was calculated.

The plasma levels obtained after oral naltrexone (table 1) show that the mean gastrointestinal absorption of naltrexone reached maximal plasma levels at 105 minutes after drug ingestion, remained essentially unchanged for the next three hours, and then decreased progressively at 6, 12, and 24 hours. However, drug absorption differed significantly among the subjects tested. Thus, three subjects absorbed the drug rapidly, reaching maximal plasma levels at 90 minutes; two subjects absorbed more slowly, reaching maximal plasma levels at 150 minutes; and one subject absorbed so slowly that he did not reach maximal plasma levels until 4 hours after drug administration.

TABLE 1. Percentage of the Total Dose Present in the Total Plasma Volume Following the Oral Administration of 3H-Naltrexone

TIME	R.C.	R.B.	P.M.	E.R.	K.B.	W.M.	MEAN	SD.
15'	0	0.01	0.03	0.04	0.03	0.02	0.02	0.01
30'	0.30	0.64	1.51	0.42	0.09	0.15	0.52	0.48
45'	0.52	1.07	1.94	1.31	0.32	0.50	0.94	0.56
60'	0.67	1.25	2.08	1.08	0.47	0.77	1.05	0.53
75'	0.58	1.38	2.23	1.04	0.62	1.17	1.17	0.55
90'	0.55	1.60	1.75	1.31	0.88	0.65	1.12	0.46
105'	0.83	1.51	1.95	1.61	0.96	1.46	1.39	0.38
120'	0.80	1.54	1.91	1.14	0.99	1.67	1.34	0.39
135'	0.99	1.47	1.51	1.41	1.23	1.67	1.36	0.22
150'	1.38	0.97	1.40	1.11	1.22	1.75	1.31	0.25
165'	1.20	1.30	1.39	1.47	1.26	1.73	1.39	0.17
160'	1.16	1.25	1.46	1.32	1.29	1.52	1.33	0.12
4½ hrs	1.34	1.04	0.99	0.19	1.50	1.65	1.29	0.24
6 hrs	1.11	0.69	0.56	0.93	1.10	1.14	0.93	0.22
12 hrs	0.55	0.50	0.54	0.59	0.54	0.73	0.56	0.06
24 hrs	0.18	0.25	0.23	0.27	0.34	0.42	0.28	0.08

TABLE 2. Percentage of the Total Dose Present in the Total Plasma Volume Following the Intravenous Administration of 3H-Naltrexone

TIME	M.H.	A.G.	R.C.	S.B.	T.D.	MEAN	S.D.
30	0	0.12	12.06	1.43	0.64	2.85	5.18
1'	3.03	1.40	5.06	3.21	8.82	4.30	2.84
3'	3.22	1.91	3.09	1.95	2.90	2.61	0.63
5'	2.23	1.76	2.22	1.60	2.12	1.99	0.29
10'	1.68	1.51	1.76	1.50	1.79	1.65	0.14
15'	1.50	1.32	1.67	1.20	1.78	1.49	0.24
30'	1.40	1.17	1.57	1.41	1.45	1.40	0.14
60'	1.11	1.09	1.45	1.36	1.34	1.27	0.16
90'	1.01	1.02	1.41	1.26	1.24	1.19	0.17
120'	0.90	0.93	1.30	1.23	1.12	1.10	0.18
150'	0.93	0.84	1.19	1.07	1.04	1.01	0.13
180'	0.97	0.72	1.06	1.07	1.03	0.97	0.15
5 hrs	0.94	0.80	1.28	1.20	1.13	1.07	0.20
7 hrs	0.86	0.74	1.08	1.05	0.96	0.94	0.14
10 hrs	0.32	0.38	0.79	0.72	0.73	0.59	0.22
24 hrs	0.13	0.13	0.22	0.27	0.22	0.19	0.06

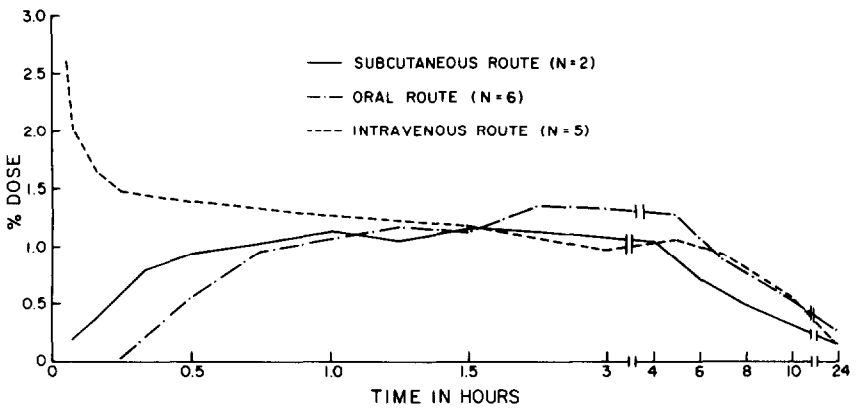
The plasma levels obtained after intravenous naltrexone are illustrated in table 2. Maximal levels of radioactivity were observed to occur in the plasma one minute after the intravenous injection of the drug and to decline sharply during the next 15 to 30 minutes. Then the plasma levels of radioactivity remained relatively steady for the next 7 hours and declined at 12 and 24 hours.

TABLE 3. Percentage of the Total Dose Present in the Total Plasma Volume Following the Subcutaneous Administration of 3H-Naltrexone

Time	L.B.	J.W.	MEAN	S.D.
5'	0.27	0.08	0.18	0.13
10'	0.48	0.26	0.37	0.16
20'	0.91	0.64	0.78	0.19
30'	1.02	0.84	0.93	0.13
45'	1.13	0.90	1.02	0.16
60'	1.28	0.97	1.13	0.22
75'	1.16	0.95	1.06	0.15
90'	1.25	1.04	1.15	0.15
120'	1.24	1.00	1.12	0.17
4 hrs	1.01	1.06	1.04	0.04
6 hrs	0.76	0.67	0.72	0.06
8 hrs	0.51	0.46	0.49	0.04
10 hrs	0.31	0.32	0.32	0.001
24 hrs	0.16	0.16	0.16	0
30 hrs	0.12	0.13	0.13	0.001
48 hrs	0	0	0	0

FIGURE 1

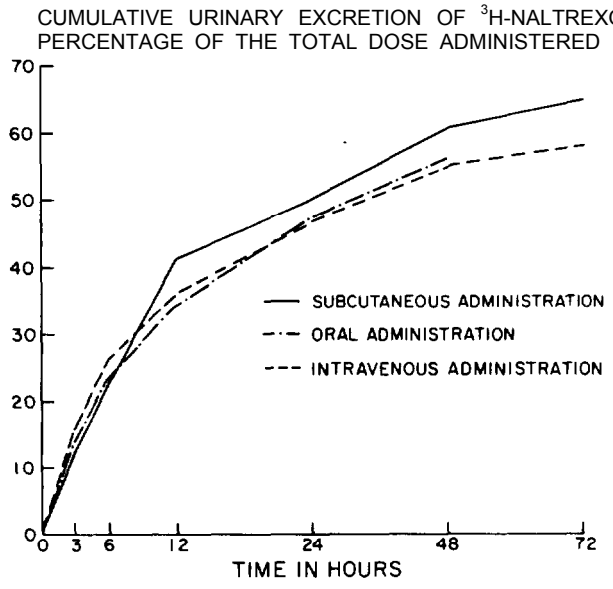
PERCENTAGE OF THE TOTAL DOSE ADMINISTERED PRESENT IN THE TOTAL PLASMA VOLUME



The plasma levels obtained after subcutaneous naltrexone are illustrated in table 3. Maximal levels of radioactivity were observed to occur in the plasma 60 minutes after the subcutaneous injection of the drug, remain steady for the next 3 hours, and decline progressively thereafter.

A comparison of the plasma levels of naltrexone and its metabolites obtained after the intravenous, subcutaneous, and oral administration of the drug is illustrated in figure 1.

FIGURE 2



This figure shows that, as expected following the intravenous injection, the plasma levels of the drug are immediately high and then decline rapidly in a period of 15 to 30 minutes. The low levels of radioactivity present in the plasma even at peak values suggest that the drug is poorly bound to the plasma proteins, rapidly leaves the compartment, and is distributed into the body tissues. Following the oral or subcutaneous administration of naltrexone, the plasma levels progressively increased to reach a maximum level at approximately 60 minutes. It would appear that the drug was more rapidly absorbed after the subcutaneous injection; however, since only two subjects were studied by this route, this result cannot be considered significant. The plasma levels of radioactivity observed one hour after drug administration were similar for all three routes, which indicates the excellent bioavailability of the drug after oral or subcutaneous administration. The plasma levels declined progressively during the 24 to 48 hours of observation to undetectable values, probably because of the extensive urinary excretion of naltrexone and/or its metabolites.

c) *Percentage of the dose administered excreted in the urine:* The urinary excretion of naltrexone and/or its metabolites was rapid, extensive, and essentially similar for the three routes of administration investigated (table 4). Thus, more than 12% of the total dose

administered was excreted during the first 3 hours, and more than 45% at the end of 24 hours. Figure 2 again illustrates the similar average cumulative urinary excretion of the total radioactivity administered by the three different routes at the time intervals sampled. This result indicates that the renal clearance of naltrexone and/or its metabolites is constant and independent of the route of administration.

TABLE 4. Percentage of the Total Radioactivity Excreted in the Urine Following the Administration of 3H-Naltrexone

TIME	INTRAVENOUS	ORAL	SUBCUTANEOUS
	MEAN SD.	MEAN S.D.	MEAN S.D.
3 hrs	15.04 ± 4.43	13.88 ± 3.05	12.34 ± 0.59
6 hrs	10.99 ± 2.60	9.66 ± 2.18	10.85 ± 2.02
12 hrs	9.84 ± 4.61	10.45 ± 5.38	18.14 ± 1.87
24 hrs	10.77 ± 6.02	13.25 ± 2.18	8.63 ± 0.47
48 hrs	8.23 ± 4.17	8.64 ± 2.76	10.82 ± 0.02
72 hrs	3.11 ± 1.68		4.25 ± 1.58
TOTAL	57.99 ± 10.33	55.88 ± 5.85	65.01 ± 1.53

Figures represent the mean of the groups ± the standard deviation.

d) *Percentage of the dose administered excreted in the feces:* Following oral ^{3}H-naltrexone, the mean fecal excretion of radioactivity was, on the average, 3.3% of the dose in the 48 hours following drug ingestion. This result indicates that more than 95% of the dose given was absorbed from the gastrointestinal tract. Since the fecal excretion following oral naltrexone was minor, no attempt was made to collect feces after intravenous or subcutaneous dosing.

It became a matter of concern that we could only account for approximately 61% of the dose being excreted (58% in the urine and 3.3% in the feces) following oral ^{3}H-naltrexone. In order to investigate the maximum excretion of ^{3}H-naltrexone, the drug was administered orally to one subject, and plasma, urine, and feces were collected at appropriate intervals for a period of 133 hours. The plasma levels of radioactivity were similar to those obtained before and became undetectable after 48 hours. The results of the urinary and fecal excretion of radioactivity (table 5) indicate that as in our previous studies, the largest excretion occurred during the first 48 hours and declined progressively thereafter. The total dose excreted after this long interval of time was 79.3% in the urine, and 13.7% in the feces for a total of 93% of the amount ingested. Since

TABLE 5. Percentage of the Total Radioactivity Administered Excreted by One Subject Following Oral 3-H Naltrexone

Time	Urinary Excretion	Fecal Excretion
24 hrs	60.91	0.58
48 hrs	12.88	9.42
72 hrs	3.56	2.80
96 hrs	1.23	0.10
120 hrs	0.55	0.70
133 hrs	0.15	0.10
TOTAL	79.28	13.70
	Total Excretion = 92.98	

total collection of all urine and especially all the feces is impossible, we consider, for practical purposes, that the entire amount of naltrexone and/or its metabolites was excreted in this interval of time.

In conclusion, naltrexone is a narcotic antagonist drug that is rapidly and completely absorbed from the gastrointestinal tract or from the subcutaneous tissue and can be intravenously administered in small doses without any untoward effects. The intravenous administration of naltrexone should be considered in the treatment of narcotic overdose emergencies, since this drug is 2 to 3 times more potent than naloxone, a drug that is presently used as the standard emergency treatment for narcotic overdose.

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The Metabolism of Naltrexone in Man

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The metabolism and elimination of [15,16-$^{3}H_{2}$] naltrexone hydrochloride was studied in man following oral and intravenous administration. The same metabolites, although in varying proportions, were observed in both cases; conjugated naltrexone and nonconjugated and conjugated 6β-naltrexol were the major metabolites observed in plasma, urine, and feces. 2-Hydroxy-3-O-methyl-6β-naltrexol was found in minor quantities. Naltrexone was almost completely absorbed following oral administration. About 60% of the dose was found in the urine and only 5% in the feces. A similar urinary excretion pattern was observed after intravenous administration of naltrexone. In early time periods after oral administration there was a rapid increase in free naltrexone plasma levels up to 1 hr. After this time, levels remained fairly constant up to 4 hr and then gradually declined. A similar pattern was observed for conjugated naltrexone and nonconjugated and conjuated 6β-naltrexol. These metabolites were found at levels 4-6 times higher than the parent compound at all times sampled. After intravenous administration, nonconjugated naltrexone plasma levels dropped sharply and continuously. The major metabolites exhibited a pattern closely resembling that found for oral administration. Combined gas chromatography-mass spectrometry was used to validate the presence of naltrexone, 6β-naltrexol and 2-hydroxy-3-O-methyl-6β-naltrexol in urine. The structure of the latter was rigorously proven by ^{13}C-NMR. No evidence for the presence of noroxymorphone or 3-O-methyl-6β-naltrexol could be obtained by gas chromatography-mass spectrometry. The metabolism of naltrexone administered subcutaneously was also determined in two subjects. Larger amounts of 2-hydroxy-3-O-methyl-6β-naltrexol were found in plasma than had been present after oral or intravenous administration.

INTRODUCTION

Naltrexone (I) (figure 1) is a potent narcotic antagonist which has been proposed for the treatment of opiate dependence (1). An excellent review of this subject can be found in the paper by Verebey et al. (2). Cone isolated and identified 6ß-naltrexol (II) as the major urinary excretion product of I in man (3). A detailed study of the urinary excretion of I in man was carried out by Cone et al. (4) and by Verebey et al. (5), both groups reporting that 6ß-naltrexol was the major component in urine. Verebey et al. have developed sensitive gas liquid chromatography (GLC) methodology with electron-capture detection for the quantitative determination of naltrexone and 6ß-naltrexol in human plasma (6). In other studies, Verebey and coworkers have described the isolation and identification of a new metabolite of naltrexone in human blood and urine, which they identified as 2-hydroxy-3-O-methyl-6ß-naltrexol (III) (7). Cone et al. (8) have recently identified both III and 2-hydroxy-3-O-methyl-naltrexone in urine by GLC techniques. Verebey et al. (2) studied the oral administration of I and determined plasma levels of I and its metabolites, II and III, in man. Recent studies in our laboratory and by Ludden et al. (9) have indicated that other meta-

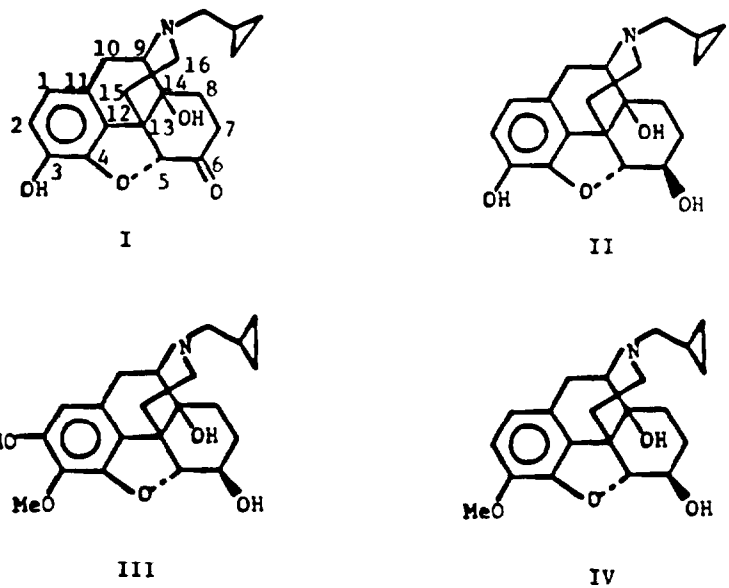

FIGURE 1. Naltrexone (I), 6ß-naltrexol (II), 2-hydroxy-3-O-methyl-6ß-naltrexol (III), 3-O-methyl-6ß-naltrexol (IV).

bolites such as 6α-naltrexol, 3-O-methyl-6ß-naltrexol (IV), or noroxymorphone can be found in animals and man.

A major objective of our investigation is to obtain information concerning naltrexone metabolism and pharmacokinetics that may be subsequently applied to the pharmacokinetic and pharmacologic evaluation of sustained-release naltrexone delivery systems in man. The present paper is concerned with the metabolism and excretion of I administered via oral (po) and intravenous (iv) routes. Some limited preliminary data obtained by subcutaneous administration (sc) of I are also included. The iv and sc data are being reported for the first time in man. The study was designed to take advantage of the availability of a number of synthetic reference compounds such as I-IV (figure 1), noroxymorphone, 3-O-methyl-naltrexone, and 2-hydroxy-3-O-methylnaltrexone, which are available through synthetic studies carried out at the Research Triangle Institute.

MATERIALS AND METHODS

Chemicals and Materials

Naltrexone hydrochloride was obtained from the National Institute on Drug Abuse (NIDA). [15,16-$^{3}H_2$] naltrexone hydrochloride, 6ß-naltrexol, 2-hydroxy-3-O-methylnaltrexone, 3-O-methyl-naltrexone, 2-hydroxy-3-O-methyl-6ß-naltrexol, and 3-0-methyl-6ß-naltrexol were prepared at the Research Triangle Institute. Ethanol (95%, USP) was purchased from U. S. Industrial Chemical Co., Louisville, Ky. All other solvents were of reagent grade and were used without further purification. A solution of ß-glucuronidase (100,000 units/ml) and sulfatase (10,000 μmol of p-nitrocatechol sulfate cleaved per hr per ml) from Helix pomatia (Type H-2) was purchased from Sigma Chemical Co., St. Louis, MO. Omnifluor was obtained from New England Nuclear, Boston, Mass. Monophase- was purchased from Packard Instrument Co., Downers Grove, Ill. Thin-layer chromatography plates were 20 x 20 cm silica gel-on-glass plates (0.25 mm thickness) prepared by E. Merck, Darmstadt, Germany. A 2% solution of methoxyamine hydrochloride in pyridine (Mox reagent) and Tri-Sil TBT were obtained from Pierce Chemical Co., Rockford, Ill. Sephadex LH-20 was purchased from Pharmacia Fine Chemicals, Piscataway, N.J.

Clinical Procedures

In the study involving oral administration of the hydrochloride salt of I, six normal males received 50 mg of I hydrochloride con-

taining 200 μCi of [15,16-$^{3}H_{2}$]-I hydrochloride. The drug was administered in 1 ml of water/95% ethanol (1:1, v/v) in two gelatin capsules 3 hr after subjects had received a light breakfast. Blood samples were drawn at 15-min intervals for 3 hr and at 4.5, 6, 12, and 24 hr. Plasma and red blood cells were separated by centrifugation. Urine was collected at 0-3, 3-6, 6-12, 12-24, and 24-48 hr after drug ingestion. Feces were collected at 0-24 and 24-48 hr.

Five normal male volunteers each received an iv injection of 1 mg of I hydrochloride (containing 198 μCi of [15,16-$^{3}H_{z}$]-I hydrochloride) dissolved in 1 ml of normal saline. Blood samples were collected at 0.5, 1, 3, 5, 10, 15, 30, 60, 90, 120, 150, and 180 min and at 5, 7, 10, and 24 hr. Plasma and red blood cells were prepared. Urine was collected at 0-3, 3-6, 6-9, 9-12, 12-24, 24-48, and 48-72 hr.

Two male volunteers received an sc injection of 4.4 mg of naltrexone hydrochloride (containing 126 μCi of [15,16-$^{3}H_{2}$] naltrexone hydrochloride dissolved in normal saline. Blood samples were collected at 5, 10, 20, 30, 45, 60, 75, and 90 min and at 2, 4, 6, 8, 12, 24, 30, and 48 hr. Urine was collected through 72 hr. After the initial pilot study was conducted, preliminary reports were received of possible tumor formation produced when naltrexone was administered to rodents. Although these initial reports have since been largely discounted, all studies of naltrexone in man were terminated for the time being by our group.

Analytical Methods

These procedures are presented in detail in this monograph in our paper dealing with analytical methodology (10).

RESULTS

Plasma Concentration of Naltrexone and Metabolites after Oral Administration

The data for total drug, nonconjugated, and conjugated I plus metabolites found in the plasma of human volunteers are shown in figure 2. A rapid increase in total drug concentration occurred between 15 min and 1 hr. Levels of total drug (calculated as naltrexone equivalents) reached maximal values around 240 ng/ml (0.5% dose/liter of plasma) in 2 hr, remained almost constant through 4.5 hr, and then decreased to 50 ng/ml in 24 hr. The level of the total conjugated fraction was threefold that of nonconjugated naltrexone and metabolites. Table 1 presents the analytical data for both nonconjugated and conjugated I and its metabolites, II, III, and IV. In

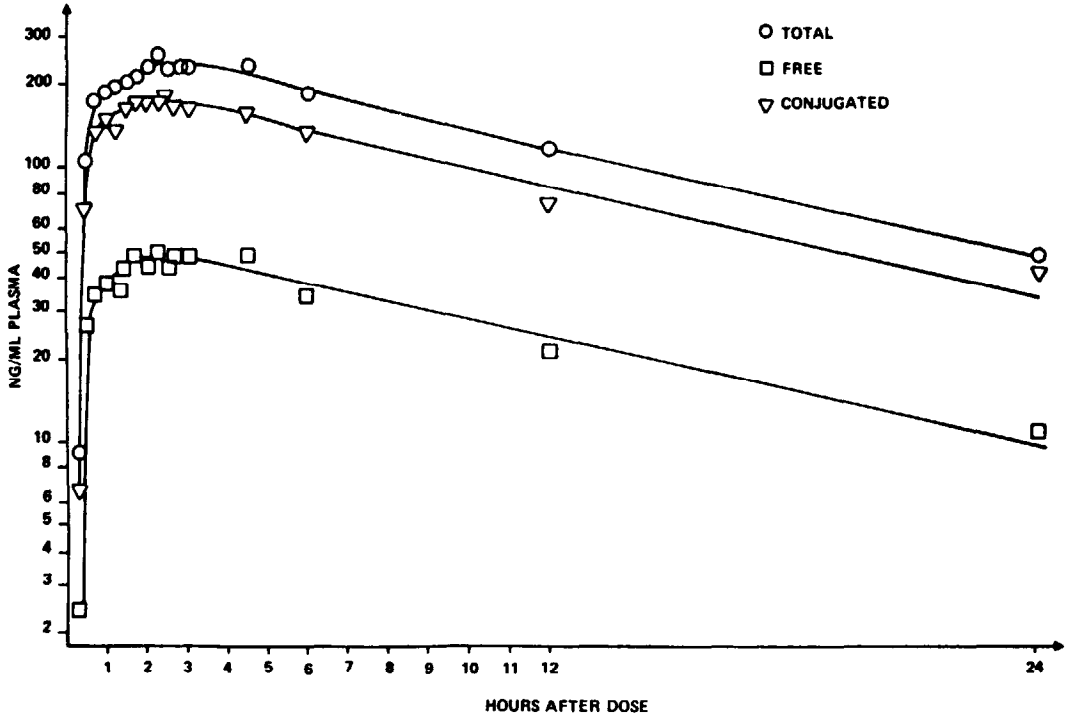

FIGURE 2. Naltrexone and metabolites found in human plasma after oral administration of 50 mg naltrexone hydrochloride. Values are the average for four subjects.

TABLE 1. Naltrexone and Metabolites Found in Human Plasma After Oral Administration of 50 mg Naltrexone Hydrochloride

Values are the average for four subjects.

Time (min)	ng/ml plasma ± SE found nonconjugated as				
	TIc Origin	6ß-Naltrexol	2-OH-3-0-Methyl-6ß-Naltrexol	3-0-Methyl-6ß-Naltrexol	Naltrexone
15	0.085 ± 0.044	1.4 ± 1.1	0.67 ± 0.35	0.23 ± 0.11	0.097 ± 0.050
30	0.96 ± 0.40	16 ± 10	0.55 ± 0.22	0.97 ± 0.54	4.4 ± 2.3
45	1.4 ± 0.5	22 ± 10	0.94 ± 0.11	0.85 ± 0.13	6.0 ± 2.1
60	1.6 ± 0.4	24 ± 8	1.3 ± 0.3	0.96 ± 0.08	6.5 ± 2.2
75	1.8 ± 0.5	22 ± 7	1.7 ± 0.5	0.72 ± 0.28	6.5 ± 3.3
90	2.5 ± 0.8	27 ± 7	2.7 ± 0.6	0.78 ± 0.14	5.9 ± 1.6
105	3.0 ± 0.9	29 ± 5	2.6 ± 0.6	1.3 ± 0.6	6.7 ± 1.4
120	2.2 ± 0.8	27 ± 5	2.7 ± 0.7	0.85 ± 0.19	7.5 ± 2.0
135	2.3 ± 0.3	32 ± 4	1.9 ± 0.5	0.72 ± 0.08	7.9 ± 1.9
150	2.5 ± 0.4	28 ± 2	2.4 ± 0.5	0.95 ± 0.24	6.9 ± 2.1
165	2.4 ± 0.3	29 ± 4	2.4 ± 0.7	1.3 ± 0.4	6.8 ± 1.6
180	3.4 ± 1.5	30 ± 4	3.1 ± 1.1	0.86 ± 0.36	6.2 ± 2.0
270	1.8 ± 0.2	32 ± 4	3.2 ± 1.2	1.0 ± 0.26	7.4 ± 2.6
360	1.2 ± 0.2	22 ± 4	2.4 ± 0.6	0.97 ± 0.34	4.4 ± 1.6
720	0.64 ± 0.19	13 ± 2	2.2 ± 1.0	0.62 ± 0.19	3.0 ± 0.9
1440	0.39 ± 0.08	6.3 ± 1.2	1.5 ± 0.6	0.37 ± 0.05	1.1 ± 0.4

Time (min)	ng/ml plasma ± SE found conjugated as				
	TIc Origin	6ß-Naltrexol	2-OH-3-0-Methyl-6ß-Naltrexol	3-0-Methyl-6ß-Naltrexol	Naltrexone
15	1.5 ± 0.8	0.71 ± 0.23	0.46 ± 0.13	1.2 ± 0.4	1.7 ± 0.7
30	17 ± 9	6.4 ± 2.6	3.6 ± 3.2	9.9 ± 5.6	22 ± 9
45	18 ± 6	19 ± 5	4.4 ± 1.7	11 ± 7	46 ± 12
60	18 ± 6	24 ± 5	5.3 ± 2.0	16 ± 9	50 ± 11
75	16 ± 4	26 ± 8	9.1 ± 3.5	17 ± 8	44 ± 12
90	19 ± 4	29 ± 8	8.8 ± 4.0	22 ± 9	50 ± 8
105	22 ± 4	39 ± 8	9.8 ± 4.6	18 ± 9	52 ± 7
120	25 ± 6	34 ± 6	8.0 ± 1.7	16 ± 5	56 ± 12
135	26 ± 5	32 ± 6	5.8 ± 1.8	15 ± 6	52 ± 15
150	28 ± 1	37 ± 3	8.1 ± 2.5	24 ± 11	67 ± 14
165	32 ± 2	37 ± 3	9.1 ± 0.3	19 ± 4	50 ± 17
180	30 ± 6	29 ± 6	6.7 ± 2.1	16 ± 3	49 ± 11
270	38 ± 9	44 ± 11	7.6 ± 1.1	14 ± 7	34 ± 12
360	26 ± 6	48 ± 16	7.6 ± 2.2	6.4 ± 3.4	24 ± 5
720	15 ± 4	24 ± 4	5.3 ± 1.8	2.8 ± 0.8	15 ± 5
1440	4.3 ± 14	12 ± 2	5.2 ± 2.2	5.8 ± 1.4	4.2 ± 1.5

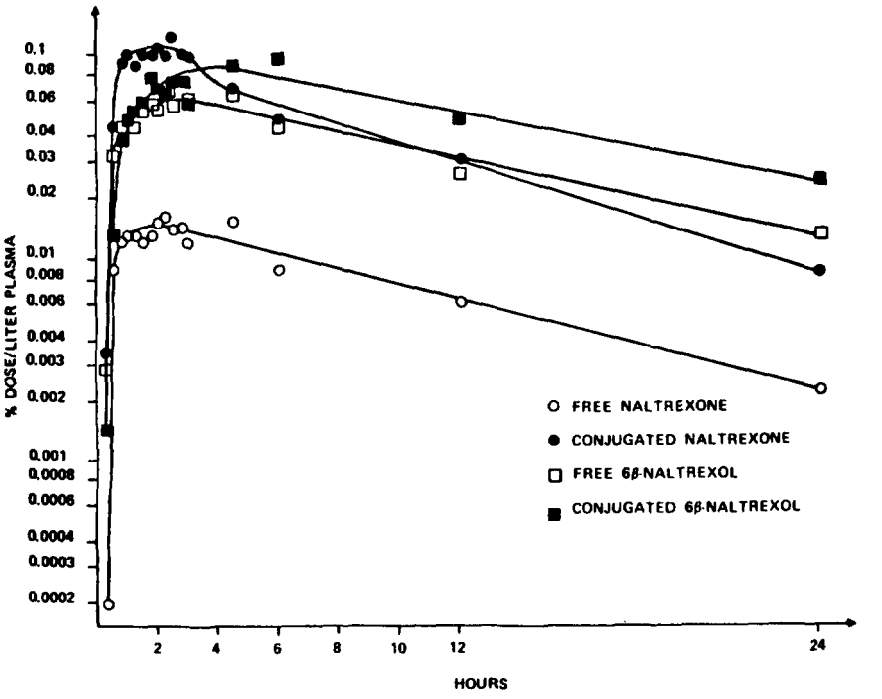

FIGURE 3. Naltrexone and 6ß-naltrexol levels in plasma after oral administration of 50 mg naltrexone hydrochloride. Values are the average for four subjects.

addition, results are presented for an unidentified polar material (not noroxymorphone) which did not migrate from the TLC origin. Figure 3 presents plasma decay curves for naltrexone and 6ß-naltrexol. The data in table 1 and figure 3 show that nonconjugated naltrexone levels increased rapidly during the first 1530 min following oral administration and attained values of 6-8 ng/ml over the period from 45 min to 4.5 hr. After this time levels began to decrease, falling to 1 ng/ml after 24 hr. The plasma half-life of I in the declining phase (3-24 hr, cf. figure 3) was 8.0 hr. The major metabolite found in the plasma was 6ß-naltrexol, (II), which showed pharmacokinetics similar to I (plasma half-life 8.9 hr between 3-24 hr). Maximal levels of II were in the neighborhood of 22-30 ng/ml between 45 min and 4.5 hr. Other metabolites, III and IV, were minor, their concentrations never exceeding 3 ng/ml and 1 ng/ml, respectively. In the conjugated fraction, the relationships between I and II were reversed. In this case I was found to be the major product, with maximal levels between 50-60 ng/ml during 1-3 hr (cf. table 1). Values of II were about 40% lower. Levels of conjugated I

did not materially decrease until 12 hr after administration. The values for III and IV were considerably higher in the conjugated fraction than in the nonconjugated fraction. The former metabolite had maximal values of about 9 ng/ml from 1.25 to 2.75 hr; the latter had values of 15-20 ng/ml from 1 to 4.5 hr.

Urinary Excretion after Oral Administration

Urinary excretion after oral administration of I is shown in table 2 and figure 4. About 30% of the total dose was excreted during the first 12 hr, 50% in 24 hr, and 60% in 48 hr. The analytical data for each time period for naltrexone and its metabolites, both nonconjugated and conjugated, are shown in table 2. 6ß-naltrexol was the major compound found in the urine in the free form. The concentration of nonconjugated I was much lower than that of II (about 0.1) and was even lower than the levels of the minor metabolites, III and IV. Average renal clearance values for nonconjugated I and II calculated by the method described by Verebey et al. (2) were 77 and 270 ml/min, respectively.

TABLE 2. Naltrexone and Metabolites Found in Human Urine After Oral Administration of 50 mg Naltrexone Hydrochloride

(Values are the average for six subjects ± SE.)

Time (hr)	µg found nonconjugated as				
	TIc Origin	6ß-Naltrexol	2-OH-3-0-Methyl-6ß-Naltrexol	3-0-Methyl-6ß-Naltrexol	Naltrexone
3	80 ± 7	19OO ± 800	200 ± 98	420 ± 300	130 ± 43
6	42 ± 20	1200 ± 600	240 ± 110	200 ± 220	85 ± 42
12	46 ± 18	1400 ± 500	350 ± 140	310 ± 350	84 ± 50
24	60 ± 33	2000 ± 400	590 ± 388	390 ± 450	140 ± 81
48	53 ± 14	1400 ± 700	390 ± 270	340 ± 330	150 ± 76

Time (hr)	µg found conjugated as				
	TIc Origin	6ß-Naltrexol	2-OH-3-0-Methyl-6ß-Naltrexol	3-O-Methyl-6ß-Naltrexol	Naltrexone
3	510 ± 130	1600 ± 700	120 ± 50	210 ± 50	2100 ± 680
6	410 ± 100	1300 ± 200	100 ± 40	150 ± 100	980 ± 510
12	410 ± 230	1900 ± 900	160 ± 70	120 ± 90	800 ± 660
24	360 ± 140	2500 ± 400	260 ± 100	100 ± 60	580 ± 260
48	180 ± 70	1600 ± 600	230 ± 110	42 ± 17	180 ± 68

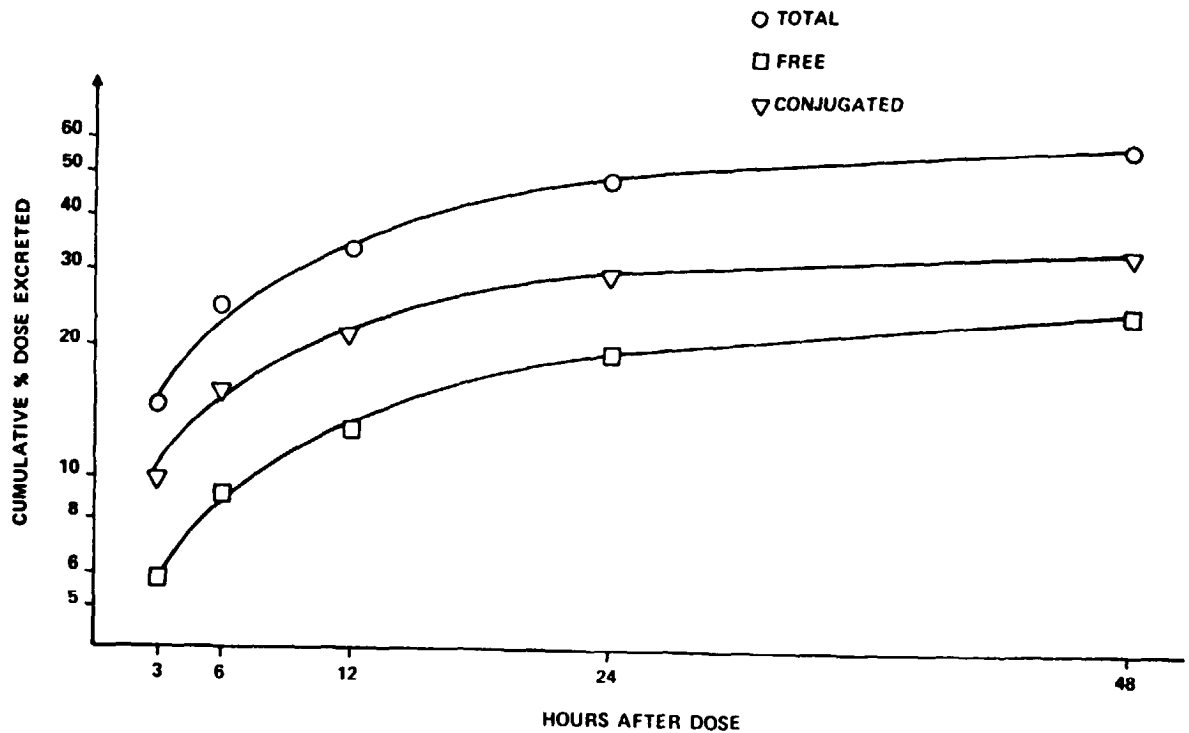

FIGURE 4. Naltrexone and metabolites found in urine after oral administration of 50 mg naltrexone hydrochloride. Values are the average for six subjects

The conjugated fraction of the urine exhibited markedly different relationships in the relative abundance of I and II. At the initial time period, 0-3 hr, I was found at higher levels than II. The levels of I then dropped sharply. II was again the major metabolite at all the other time periods. Only small proportions of III and IV were present. A considerable amount of highly polar material which did not move from the origin was also found in the conjugated fraction. Values for the cumulative percent dose excreted as nonconjugated and conjugated I and II are shown in figure 5. It is evident from this data that only trace quantities of naltrexone were excreted in the urine in the nonconjugated form. On the other hand, significant quantities of the drug were excreted in the conjugated form. In contrast, the more highly oxygenated minor metabolites were excreted to a greater extent in the nonconjugated

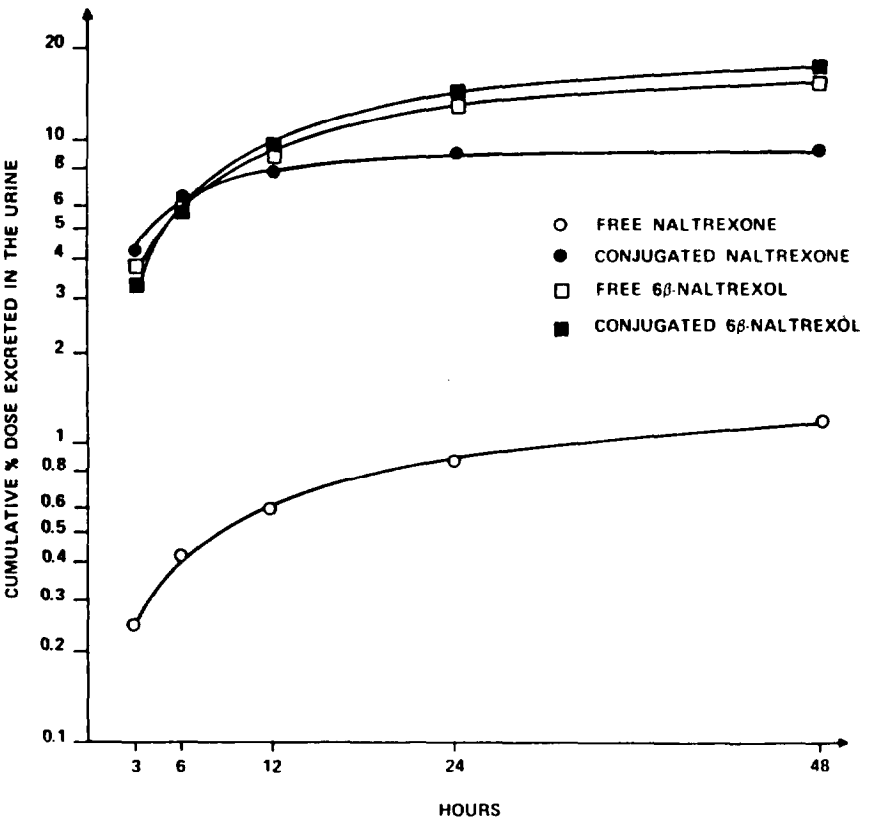

FIGURE 5. Naltrexone and 6ß-naltrexol excreted in the urine after oral administration of 50 mg naltrexone hydrochloride. Values are the average for six subjects.

than in the conjugated form. The major excretionary product was 6ß-naltrexol, the concentrations of the nonconjugated and conjugated forms being almost equal.

Fecal Excretion after Oral Administration

The feces were not a major route of elimination for naltrexone and its metabolites. Analytical data are shown in table 3. In the first 48 hr after administration of a 50 mg oral dose, only 4.8% of the total dose was found in the feces, the larger portion of which was excreted in the 24-48 hr time period. I and metabolites were determined both in the nonconjugated and conjugated form in the methanol-soluble feces fraction. 6ß-naltrexol was the major constituent found in both the nonconjugated and conjugated fractions. There was also a methanol-insoluble fraction somewhat more sizable than the methanol-soluble fraction.

Plasma Concentration of Naltrexone and Metabolites after Intravenous Administration

The plasma levels of total drug, nonconjugated, and conjugated I plus metabolites after iv administration are shown in figure 6. The total and nonconjugated drug fractions showed rather similar plasma decay curves. Initially a sharp decrease in drug levels occurred in the period between 1-15 min (α phase). This was followed by a more gradual loss of drug from the plasma over the next 24 hr (ß phase). In contrast, the total conjugated fraction rose sharply between 1-30 min, increased to a maximum in 1 hr, gradually decreased through 10 hr, and then sharply decreased between 10-24 hr. The complete data for the individual metabolites of naltrexone, both nonconjugated and conjugated, are shown in table 4.

The plasma disappearance curves for naltrexone and 6ß-naltrexol, nonconjugated and conjugated, are shown in figure 7. Levels of nonconjugated I decreased rapidly over the 1-30 min time intervals (α phase) with a half-life of 8.5 min; the half-life for the ß phase calculated from 1.5-10 hr was 1.9 hr. Levels of nonconjugated II increased rapidly up to 1 hr. The rate of decline for II was much slower than for I; the half-life from 1-24 hr was 9.3 hr. Conjugated I and II exhibited plasma decay curves similar to nonconjugated II, having lengthy retention times. On the other hand, nonconjugated I showed a much sharper decline in the ß phase. Levels of metabolites III and IV were insignificant.

TABLE 3. Naltrexone and Metabolites Found in Human Feces after Oral Administration of 50 mg Naltrexone Hydrochloride

Time (hr)	μg found as						
			Methanol soluble-nonconjugated				
	Total Feces	Methanol Insoluble	TLC origin	6β-Naltrexol	2-OH-3-0 methyl-6β-naltrexol	3-0-Methyl-6β-naltrexol	Naltrexone
24	310 ± 200	120 ± 80	4.1 ± 2.1	110 ± 80	3.2 ± 2.2	0.95 ± 0.36	26 ± 14
48	2400 ± 1000	1300 ± 700	39 ± 17	470 ± 230	9.9 ± 1.7	4.4 ± 1.5	150 ± 110
Time (hr)	μg found as						
			Methanol soluble-conjugated				
	Total Feces	Methanol Insoluble	TLC origin	6β-Naltrexol	2-OH-3-0 methyl-6β-naltrexol	3-0-Methyl-6β-naltrexol	Naltrexone
24	310 ± 200	120 ± 80	5.9 ± 2.9	30 ± 17	0.72 ± 0.31	0.83 ± 0.31	8.4 ± 6.4
48	2400 ± 1000	1300 ± 700	68 ± 27	290 ± 60	6.7 ± 2.0	1.7 ± 0.9	77 ± 72

Values are the average for five subjects ± SE.

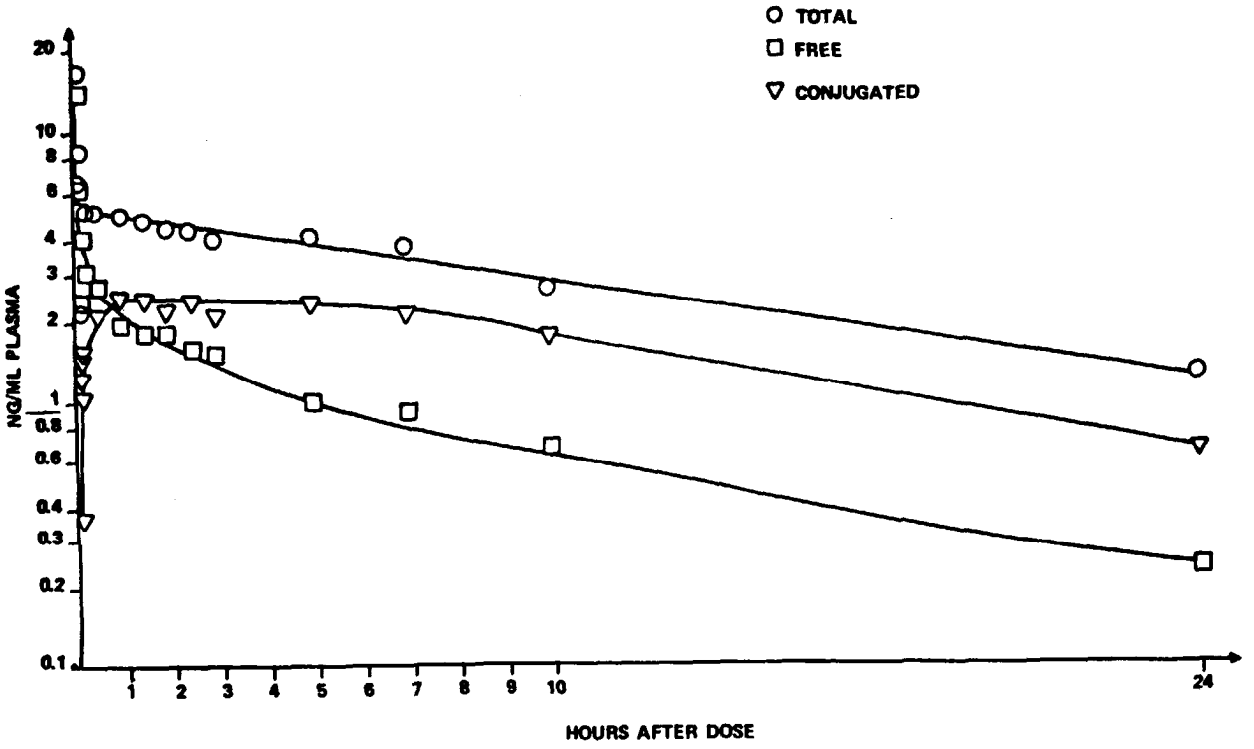

FIGURE 6. Naltrexone and metabolites found in human plasma after intravenous injection of 1 mg naltrexone hydrochloride. Values are the average for five subjects.

TABLE 4. Naltrexone and Metabolites Found in the Plasma of Subjects Receiving 1 mg Naltrexone Hydrochloride Intravenously

Time (min)	ng per ml plasma found nonconjugated as				
	TLC origin	6ß-Naltrexol	2-OH-3-0 methyl-6ß-naltrexol	3-0-Methyl-6ß-naltrexol	Naltrexone
1	2.1 ± 0.8	0.31 ± 0.05	2.2 ± 0.6	2.2 ± 0 7	8.2 ± 0.7
3	0.89 ± 0 17	0.30 ± 0.04	0.61 ± 0.24	0.54 ± 0.22	4.3 ± 1.2
5	0.60 ± 0.11	0.47 ± 0.10	0.49 ± 0.12	0.50 ± 0.30	3.3 ± 0.4
10	0.52 ± 0.11	0.78 ± 0.14	0.16 ± 0.04	0.32 ± 0.22	2.3 ± 0.3
15	0.39 ± 0.07	0.72 ± 0.13	0.17 ± 0.04	0.20 ± 0.05	1.8 ± 0.2
30	0.26 ± 0.03	0.80 ± 0.09	0.11 ± 0.07	0.085 ± 0.019	1.5 ± 0.2
60	0.22 ± 0.04	0.75 ± 0.08	0.082 ± 0.015	0.052 ± 0.019	0.87 ± 0.1
90	0.17 ± 0.03	0.72 ± 0.05	0.11 ± 0.02	0.058 ± 0.004	0.73 ± 0.09
120	0.16 ± 0.04	0.79 ± 0.11	0.15 ± 0.03	0.044 ± 0.008	0.63 ± 0.08
150	0.17 ± 0.02	0.66 ± 0.08	0.11 ± 0.02	0.052 ± 0.005	0.53 ± 0.06
180	0.12 ± 0.03	0.75 ± 0.11	0.14 ± 0.02	0.038 ± 0 002	0.45 ± 0.08
300	0.059 ± 0.008	0.61 ± 0.06	0.11 ± 0.02	0.019 ± 0.002	0.16 ± 0.03
420	0.059 ± 0.013	0.59 ± 0.04	0.12 ± 0.02	0.014 ± 0.005	0.090 ± 0.03
600	0.056 ± 0.011	0.42 ± 0.03	0.094 ± 0.020	0.010 ± 0.008	0.034 ± 0.005
1440	0.016 ± 0.004	0.14 ± 0.02	0.052 ± 0.017	0.003 ± 0.001	0.003 ± 0.001

Time (min)	ng permlplasmafound confugated as				
	TLC origin	6ß-Naltrexol	2-OH-3-0 methyl-6ß-naltrexol	3-0-Methyl-6ß-naltrexol	Naltrexone
1	0.19 ± 0.01	0.086 ± 0.023	0.072 ± 0.010	0.13 ± 0.03	0.85 ± 0.35
3	0.18 ± 0.01	0.088 ± 0.011	0.084 ± 0.029	0.080 ± 0.012	0.49 ± 0.09
5	0.12 ± 0.01	0.097 ± 0.023	0.058 ± 0.012	0.066 ± 0.021	0.52 ± 0.06
10	0.19 ± 0.04	0.14 ± 0.04	0.098 ± 0.031	0.076 ± 0.021	0.57 ± 0.14
15	0.20 ± 0.07	0.16 ± 0.04	0.089 ± 0.012	0.15 ± 0.03	0.78 ± 0.14
30	0.23 ± 0.04	0.30 ± 0.05	0.15 ± 0.03	0.15 ± 0.05	0.86 ± 0.14
60	0.35 ± 0.11	0.52 ± 0.06	0.15 ± 0.02	0.17 ± 0.03	0.93 ± 0.25
90	0.34 ± 0.08	0.53 ± 0.03	0.12 ± 0.03	0.12 ± 0.05	0 .88 ± 0.23
120	0.39 ± 0.11	0.70 ± 0.08	0.17 ± 0.06	0.11 ± 0.05	0.60 ± 0.12
150	0.34 ± 0.11	0.62 ± 0.08	0.17 ± 0.04	0.095 ± 0.039	0.61 ± 0.21
180	0.42 ± 0.09	0.49 ± 0.02	0.16 ± 0.05	0.097 ± 0.045	0.67 ± 0.14
300	0.39 ± 0.13	0.80 ± 0.04	0.11 ± 0.02	0.11 ± 0.05	0.51 ± 0.16
420	0.36 ± 0.01	0.73 ± 0.12	0.11 ± 0.02	0.039 ± 0.020	0.39 ± 0.09
600	0.23 ± 0.05	0.64 ± 0.12	0.082 ± 0.022	0.038 ± 0.021	0.29 ± 0.09
1440	0.12 ± 0.05	0.31 ± 0.08	0.049 ± 0.018	0.020 ± 0.011	0.11 ± 0.05

Nonconjugated values are the average for five subfects ± SE. confugated values are the average for four subfects ± SE.

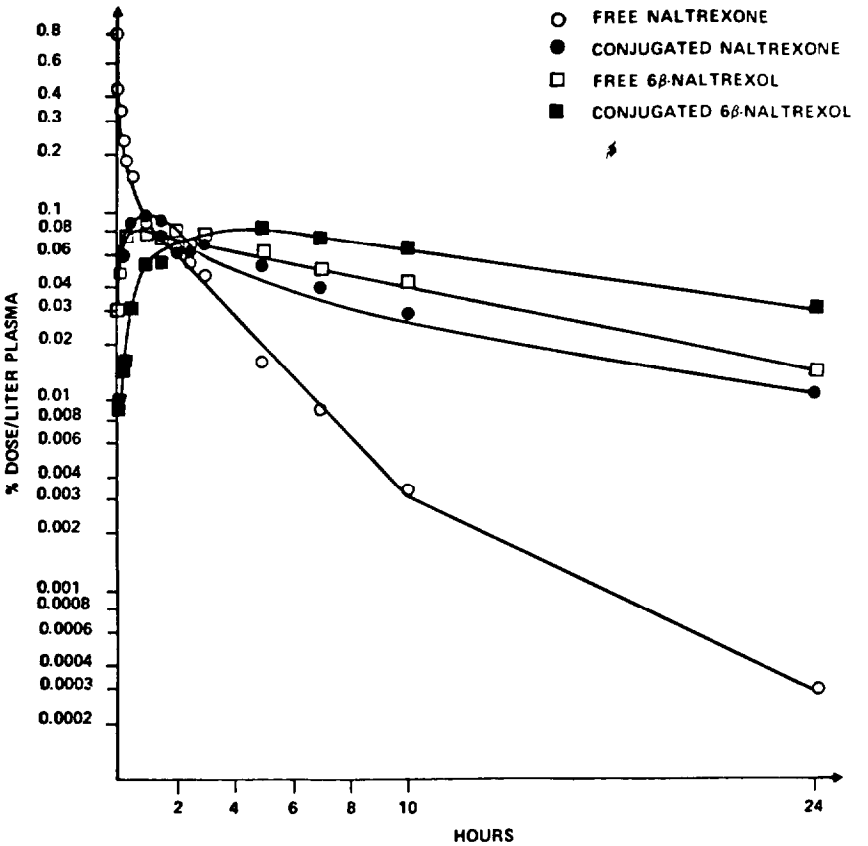

FIGURE 7. Naltrexone and 6ß-naltrexol levels in plasma after intravenous injection of 1 mg naltrexone hydrochloride. Free values are the average for five subjects; conjugated values are the average for four subjects.

Levels of Naltrexone and Metabolites in Red Blood Cells

Red blood cells from subjects receiving naltrexone hydrochloride by oral or intravenous administration were analyzed for total drug and for nonconjugated I and II. In both cases the results were similar to values found in the plasma, which indicates a uniform distribution of naltrexone and its metabolites in plasma and red blood cells. Minor metabolites were also of the same order.

Urinary Excretion after Intravenous Administration

Urinary levels after iv administration are shown in figure 8 for total I and metabolites and in figure 9 for naltrexone and 6ß-naltrexol. Table 5 presents the complete analytical information. During the first 6 hr an average of 26% of the dose was excreted in

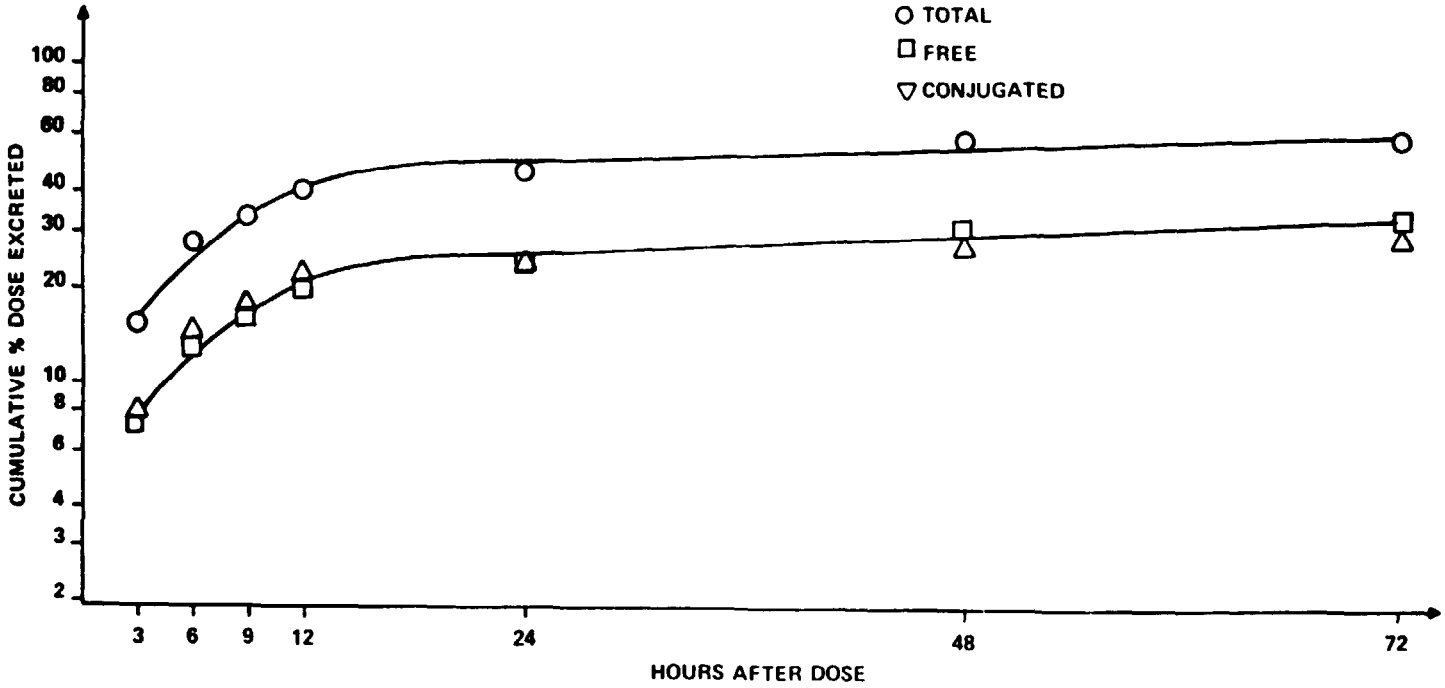

FIGURE 8. Naltrexone and metabolites found in urine after intravenous injection of 1 mg naltrexone hydrochloride. Values are the average for five subjects.

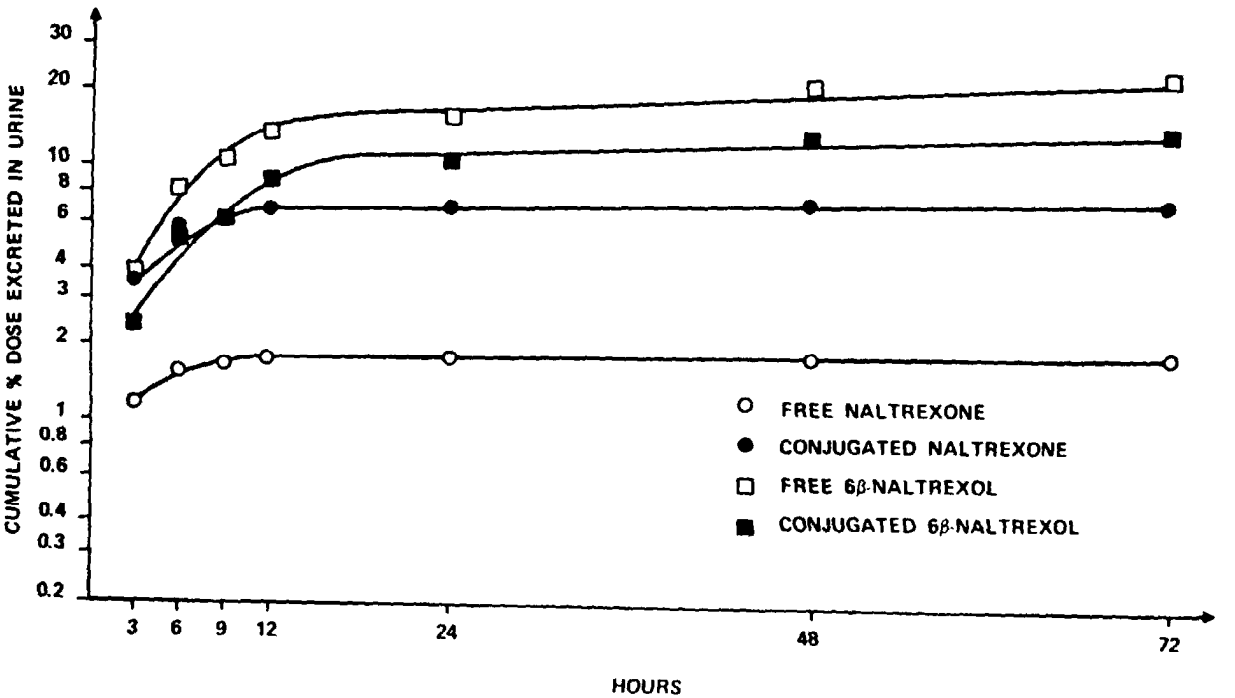

FIGURE 9. Naltrexone and 6ß-naltrexol excreted in urine after intravenous injection of 1 mg naltrexone hydrochloride. Free values are the average for five subjects; conjugated values are the average for four subjects.

TABLE 5. Naltrexone and Metabolites Found in the Urine of Subjects Receiving 1 mg Naltrexone Hydrochloride Intravenously

Time (hr)	µg ± SE excreted nonconjugated in the urine as				
	TIc Origin	6ß-Naltrexol	2-OH-3-0-Methyl-6ß-Naltrexol	3-0-Methyl-6ß-Naltrexol	Naltrexone
0-3	3.5 ± 0.6	40 ± 9	5.0 ± 1.4	5.0 ± 1.5	12 ± 3
3-6	1.8 ± 0.2	42 ± 3	4.5 ± 0.3	1.4 ± 0.4	4.2 ± 1.0
6-9	1.1 ± 0.1	28 ± 4	3.5 ± 0.5	0.39 ± 0.12	1.0 ± 0.3
9-12	1.0 ± 0.6	27 ± 17	2.9 ± 0.6	0.50 ± 0.34	0.75 ± 0.25
12-24	1.3 ± 0.3	41 ± 4	6.4 ± 1.6	0.27 ± 0.01	0.71 ± 0.15
24-48	1.9 ± 0.4	55 ± 11	11 ± 2	0.15 ± 0.03	0.24 ± 0.034
48-72	0.51 ± 0.10	12 ± 2	3.5 ± 0.6	0.047 ± 0.016	0.042 ± 0.013
Time (hr)	µg ± SE excreted confugated in the urine as				
	TIc Origin	6ß-Naltrexol	2-OH-3-0-Methyl-6ß-Naltrexol	3-0-Methyl-6ß-Naltrexol	Naltrexone
0-3	6.9 ± 0.9	24 ± 7	4.6 ± 1.3	5.0 ± 0.7	36 ± 7
3-6	5.3 ± 0.8	30 ± 5	3.2 ± 0.4	2.8 ± 1.1	22 ± 6
6-9	3.4 ± 0.8	11 ± 4	1.7 ± 0.4	0.94 ± 0 21	6.5 ± 1.8
9-12	3.4 ± 1.8	24 ± 8	1.6 ± 0.4	0.98 ± 0.18	5.9 ± 2.4
12-24	4.2 ± 1.6	41 ± 1	3.0 ± 0.9	1.0 ± 0.2	7.5 ± 0.9
2448	4.6 ± 0.7	32 ± 6	3.6 ± 0.8	1.1 ± 0.3	4.6 ± 1.1
48-72	0.92 ± 0.08	9.4 ± 2.4	1.4 ± 0.3	0.15 ± 0.03	0.65 ± 0.07

Nonconjugated values are the average for five subjects ± SE: conjugated values are the average for four subjects ± SE.

the urine, after 24 hr about 50%, and after 72 hr 60%. Approximately equal quantities of nonconjugated and conjugated urinary metabolites were observed. In contrast to the oral data, the standard error of the iv data showed much less variation, a not unexpected observation. As shown in figure 9, II, nonconjugated and conjugated, was the major urinary excretion product found after iv administration of naltrexone hydrochloride. This metabolite was excreted continuously in both nonconjugated and conjugated forms through 48 hr after administration. On the other hand, nonconjugated I was found in low concentration. Excretion of I was rapid, being virtually complete in 10-12 hr. Conjugated I was found in the urine in considerably higher concentration than the nonconjugated drug. As in the oral study, the metabolites III and IV were of minor magnitude as compared with II. The 2-hydroxy-3-O-methyl

metabolite (III) was found during most time periods at significantly higher levels than the 3-O-methyl metabolite (IV). Renal clearance values for I and II were 92 and 360 ml/min, respectively.

Subcutaneous Administration

Plasma levels for total, nonconjugated, and conjugated I when the drug was administered subcutaneously (sc) are shown in figure 10. As was found after iv administration, the conjugated fraction predominated. After reaching a peak of 28 ng/ml in about 1 hr, the drug level decreased in a linear fashion over a 50-hour time period. The data for nonconjugated I and metabolites are shown in figure 11. The results are surprising and definitely require further confirmation with more subjects. Initially the 2-hydroxy-3-O-methyl metabolite (III) predominated but decreased rapidly between 8-50 hr; 6ß-naltrexol (II) showed rather steady levels from 2-50 hr. The parent drug (I) attained a maximal value of 0.75 ng/ml within 30 min and thereafter decreased rapidly in a biphasic elimination pattern. Urinary excretion patterns are shown in figures 12 and 13. Rapid excretion of total drug occurred over 3 to 12 hr, about 35-50% of the total drug being eliminated by 12 hr; thereafter excretion continued slowly, 60-80% being eliminated in 72 hr. The conjugated and nonconjugated fractions were of the same order of magnitude; the former was somewhat larger during the first 12 hr. Data for the parent drug (I) and metabolites (II) and (III) in the nonconjugated urine fraction are presented in figure 13. Subjects LB and JW showed similar values for excretion of 6ß-naltrexol (II); however, subject JW showed much lower values than LB for naltrexone (II) and metabolite III. In a general way the results found for urinary excretion via sc administration resembled results obtained by iv administration much more closely than the plasma values found by the two methods.

DISCUSSION

Administration of naltrexone hydrochloride to human volunteers by oral or intravenous routes gave similar metabolic patterns but with some characteristic differences in the plasma levels of naltrexone and its metabolites. As was observed by other workers (2,4), oral administration of I is followed by rapid metabolism in the liver. The predominant metabolic reaction involves the reduction of the 6-oxo moiety in I to the corresponding 6ß-hydroxy group by liver enzymes. Although the other epimer 6α-naltrexol has been

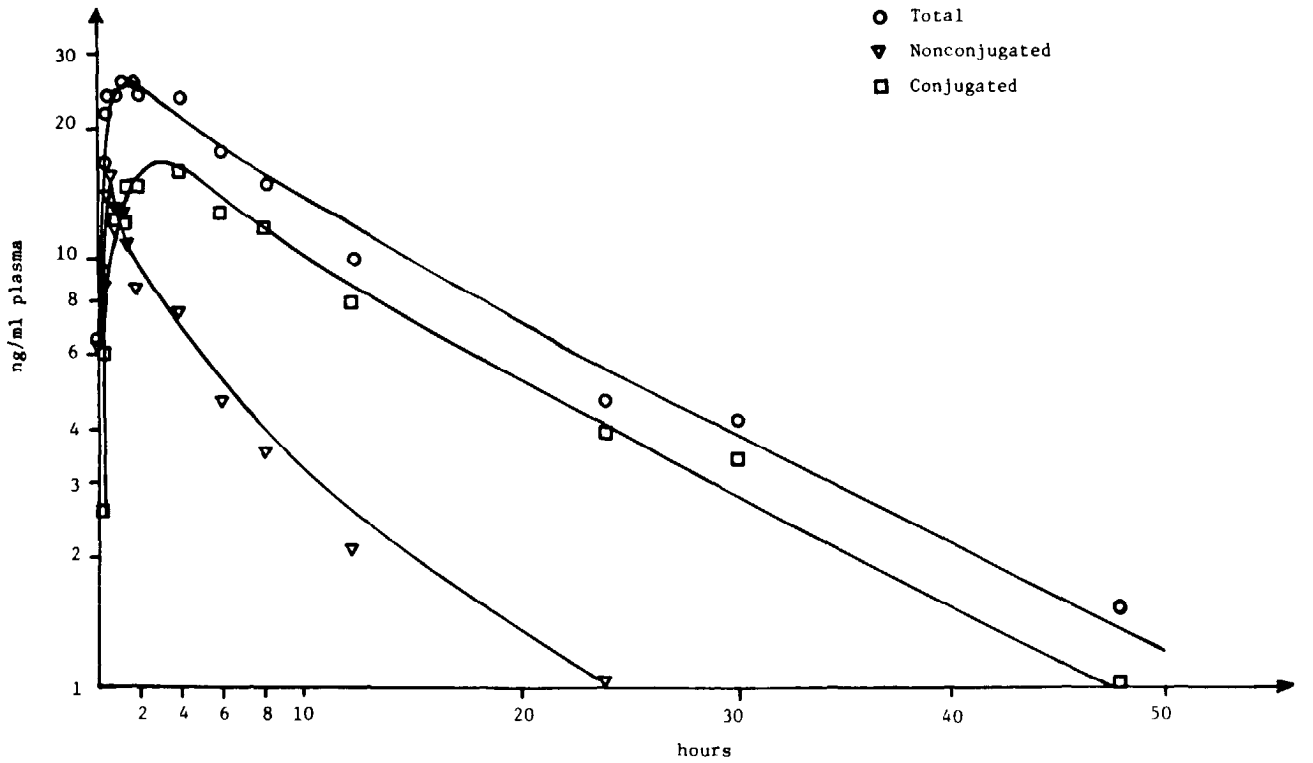

FIGURE 10. Plasma levels of naltrexone and metabolites following subcutaneous administration of 4.4 mg naltrexone hydrochloride to Subject LB.

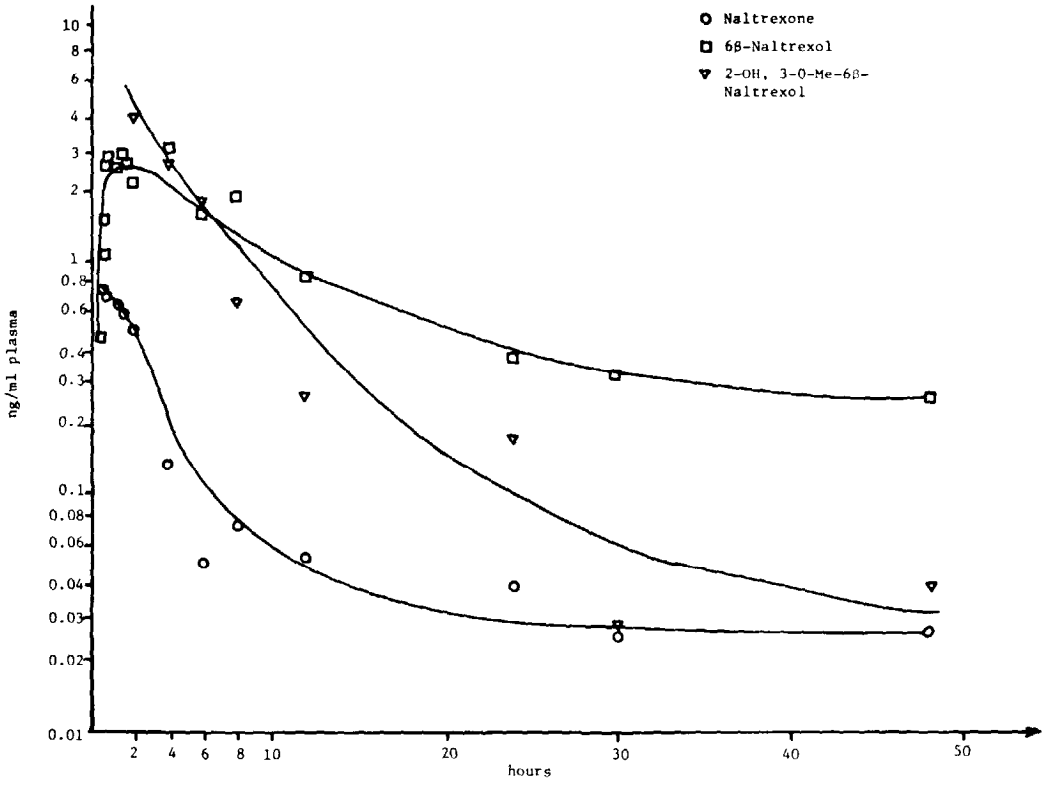

FIGURE 11. Plasma levels of naltrexone and metabolites following subcutaneous administration of 4.4 mg naltrexone hydrochloride to Subject LB.

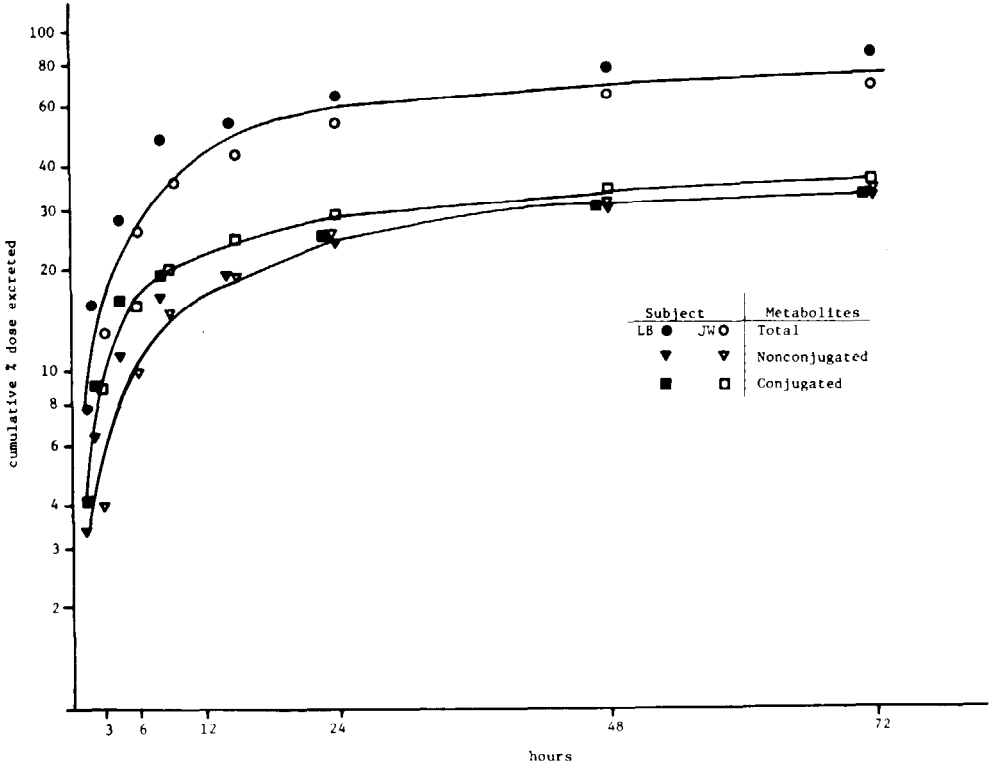

FIGURE 12. Urinary excretion of naltrexone and metabolites following subcutaneous administration of 4.4 mg of naltrexone hydrochloride to human males.

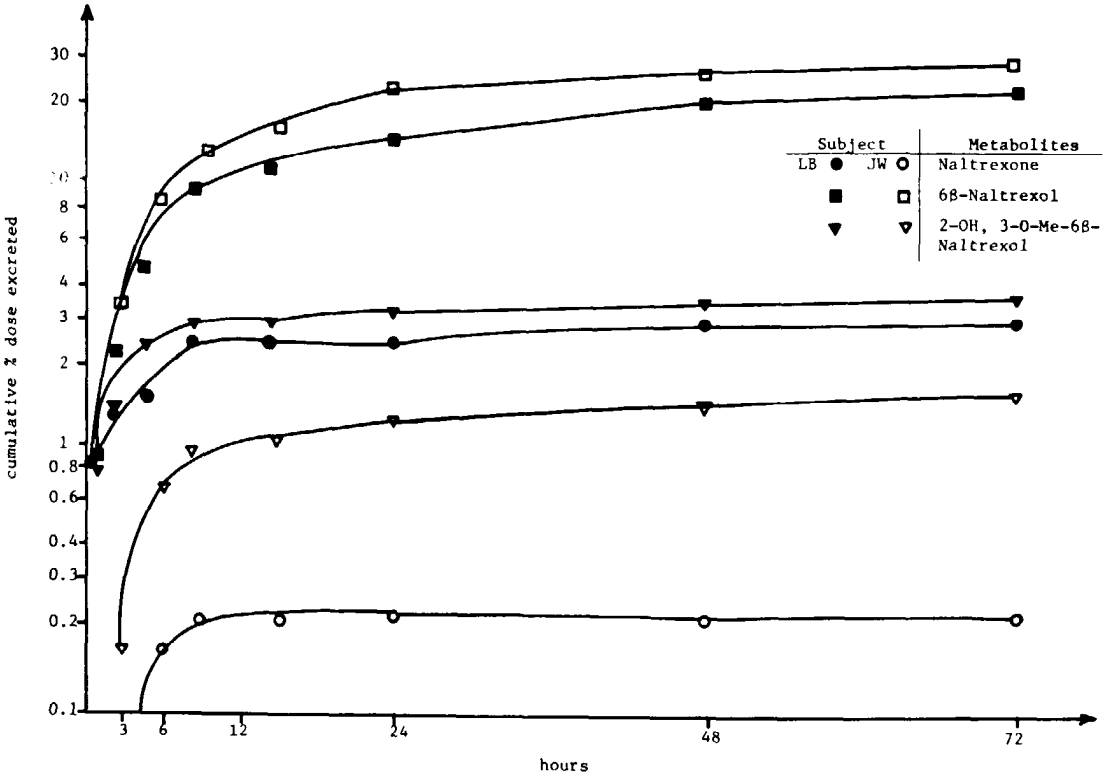

FIGURE 13. Urinary excretion of naltrexone and metabolites following subcutaneous administration of 4.4 mg of naltrexone hydrochloride.

found in rodents (11), its occurrence in man is negligible. In our studies we also isolated a minor metabolite which was established unequivocally by ^{13}C-NMR (cf. table 6) to be 2-hydroxy-3-O-methyl-6ß-naltrexol (III). An analogous metabolite containing the 6-oxo moiety has recently been shown to occur in the urine in trace amounts by Cone et al. (8). We also have some evidence for 3-O-methyl-6ß-naltrexol (IV) by TLC techniques but as yet lack positive GLC/MS confirmation of this compound. Although Malspeis et al. (11) and our group (12) have shown that noroxymorphone, an N-dealkylated metabolite of naltrexone, is found in the rat and other animals, a GLC/MS study of urine and feces of human volunteers failed to detect the presence of this metabolite (10). Our studies and those of other groups cited previously clearly show that the first pass of naltrexone through the liver produces several types of metabolic reactions. One type involves extensive conjugation of naltrexone and the other metabolites. As a consequence, conjugated metabolites frequently form the major fraction in both plasma and urine. Reduction of the 6-ketone to give 6ß-naltrexol is the other major metabolic reaction. Subsequently there are several minor metabolic transformations produced by microsomal enzymes which involve methylation of the phenolic 3-hydroxyl and hydroxylation at the adjacent S-position. Our studies, those of Verebey et al. (7), and the recent work of Cone et al. (8) indicate (but firm proof is lacking) that 3-methylation may occur prior to 2-hydroxylation. Our studies on the metabolism of naltrexone after intravenous administration indicate that the metabolic pattern closely resembles that found for oral administration.

After oral administration we found that naltrexone was almost completely absorbed; only 5% of the total dose was eliminated in the feces. In excellent agreement with previous studies (2,4), we found that urinary excretion comprised the major route for elimination of naltrexone in man. About 60% of the total dose was eliminated in 48 hr after administration by the oral route and about 55% in 48 hr after administration by the iv route. Nonconjugated naltrexone was rapidly eliminated after both oral and iv administration; whereas both nonconjugated and conjugated 6ß-naltrexol and conjugated naltrexone were found in significant quantities over long time periods (cf. figures 6 and 10). Renal clearance values for naltrexone and 6ß-naltrexol after administration of I by the oral route were 77 and 270 ml/min, respectively. (Verebey et al. (2) found 67 and 318 ml/min in their study.) Data obtained after administration by the iv route were in reasonable agreement with

TABLE 6. ^{13}C-NMR Chemical Shifts of Naltrexone Metabolite[a]

Identification of Carbon	Chemical Shifts	
1	104.41[b]	105.38'
2	148.74	150.15
3	130.94	132.06
4	145.27	146.54
5	95.83	96.46
6	71.89	71.79
7	25.47	27.03
a	d	d
9	61.89	61.85
10	22.88	22.64
11	126.21	126.26
12	123.14	122.50
13	46.00	46.00
14	70.08	69.84
15		d
16	46.60	43.66
NCH_2	59.06	58.43
CH	9.23	9.23
CH_2	3.77, 3.62	3.77, 3.62
CH_2		
OCH_3	60.14	59.80

[a]Chemical shifts are in ppm relative to tetramethylsilane.
[b]Solvent is chloroform-d.
[c]Solvent is dimethylsulfoxide-d_6.
[d]There are four peaks in the region of 29-31 ppm, two of which are impurity peaks. It is not possible to assign the 8 and 15 carbons with certainty at this time.

results after oral dosage (92 and 360 ml/min for naltrexone and 6ß naltrexol, respectively).

A limited pilot study of the sc administration of naltrexone (I) indicates several major differences in the composition of the plasma metabolites as compared with iv studies. One subject showed surprisingly high initial levels of the normally minor metabolite III. The parent drug I was found at low levels after sc administration; whereas after iv injection levels of I were high for several hours. A comparison of urinary elimination by the sc and iv routes showed the two methods giving reasonably comparable results. Further studies with more subjects receiving sc administration of I are evidently required.

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Current Status of Preclinical Research on Disposition, Pharmacokinetics, and Metabolism of Naltrexone

Anand L. Misra

Considerable species-dependent differences exist in the quantitative pattern of the metabolism of naltrexone. The metabolic pathways of naltrexone in different species were: (a) 6-keto group reduction to epimeric 6a- or 6β-naltrexols, (b) glucuronide conjugation of naltrexone or naltrexols, (c) N-dealkylation to nor-compounds, (d) 3-O-methylation and (e) 2-hydroxylation. Substantially greater quantities of 6β-naltrexol and/or its conjugates were excreted in urine of man, monkey, guinea pig and rabbit after naltrexone administration, and very small quantities were excreted in mouse, rat and dog. Chicken produced predominantly the 6a-naltrexol and the dog excreted mainly the 3-naltrexone glucuronide, with very small quantities of 6a- or 6β-naltrexols. Stereoselective metabolism of naltrexone to naltrexols is mediated by two different liver enzymes, and the formation of 2-hydroxy-3-O-methyl naltrexone or the corresponding 6β-naltrexol is mediated by catechol-O-methyl transferase. No significant differences were observed in the extent of plasma-protein binding (20-26%) of naltrexone in various species, and the low extent of binding indicated a large apparent volume of distribution for naltrexone in different species and man. Pharmacokinetic studies have shown a 2-compartmental decay of drug in plasma, and in man the plasma $t_{1/2}$ values of naltrexone in acute and chronic state after 100 mg p.o. doses were 10.3 ±3.3 and 9.7

± 1.1 hr respectively. Steady state plasma concentration of naltrexone (10.9 ng/ml) was rapidly achieved in man after a few doses, and there was no accumulation of drug or metabolites in plasma on chronic dosing. Naltrexone did not induce its own metabolism on chronic dosing. In mice, chronic administration of naltrexone was shown to depress mixed function oxidase activity possibly due to a metabolite of 6β-naltrexol. A significant portion of the naltrexone dose remains unaccounted for in different species and man, implying the existence of additional pathways of metabolism. In general, the comparative antagonist activity of metabolites was much lower than naltrexone, but the much slower elimination of lipophilic 3-O-methyl metabolites as compared to 6β-naltrexol or naltrexone may point to their possible role in the overall pharmacologic activity and long duration of action of naltrexone.

Naltrexone (N-cyclopropylmethylnoroxy morphone, EN-1639A) is a potent narcotic antagonist (1,2,3) which fulfills the criteria for clinical usefulness in the treatment of opiate dependence. It is non-addictive, orally effective, and relatively nontoxic with fairly minor or well-tolerated side-effects and a large margin of safety. A single 70-120 mg oral dose of naltrexone can provide blockade to 25 mg intravenous heroin challenges in man for periods from 24 to 72 hr (4,5,6). In well-motivated opiate-dependent subjects naltrexone provides a promising transitional modality in step-wise treatment from methadone to a drug-free state (7).

COMPARATIVE POTENCIES OF NARCOTIC ANTAGONISTS

Previous work (8,3) in mice, rats and *in vitro* longitudinal muscle of guinea pig ileum (9) showed that naltrexone essentially had no agonist activity and did not produce analgesia or respiratory depression in animals. It was approximately 40 times as active as nalorphine and twice as active as naloxone on subcutaneous injection in rats previously treated with oxymorphone (8). In mice it was about 8 times more active than naloxone when given orally and in rabbit twice as active in antagonizing the respiratory depression induced by oxymorphone. The time-course of action of naltrexone orally in rats was about 3 times longer than that of naloxone (10). In rhesus monkeys, the precipitation of abstinence syndrome with naltrexone (s.c.) was achieved at slightly lower doses than with naloxone (11). Martin et al. (3) observed that 30 to 50 mg oral doses of naltrexone in human subjects produced narcotic blockade equivalent to 2500-3000 mg of naloxone and abrupt discontinuation of

daily doses resulted in no signs of abstinence, and no subjective responses or physiological changes characteristic of agonist activity were seen with 80 mg oral doses of naltrexone. In patients dependent on 60 mg morphine daily, naltrexone (s.c.) was 17 times more potent than nalorphine in precipitating abstinence. The time-course of action of naltrexone (50 mg p.o.) in man was substantially longer than naloxone but somewhat shorter than cyclazocine (12). Thus in experimental animals and man, naltrexone was more potent and longer-acting than nalorphine and naloxone. The lack of agonist activity, greater potency and longer duration of action present distinct advantages of naltrexone over naloxone in the management of opiate-dependent individuals.

PRECLINICAL TOXICITY OF NALTREXONE IN EXPERIMENTAL ANIMALS

These studies summarized by Braude and Morrison (13) have shown that naltrexone was not toxic in any animal species (rat, dog and monkey) at doses of at least 20 mg/kg which are 20 times greater than that recommended clinically (1 mg/kg), and data from rats and dogs have indicated that even a larger margin of safety was possible. The LD_{50} (mg/kg) of acutely administered naltrexone in mice was 180 ± 24 (i.v.), 570 ± 19 (s.c.), 1100 ± 96 (p.o.); in rats the corresponding values were 1930 ± 338 (s.c.), 1450 ± 265 (p.o.); in dogs 200 (s.c.) and 130 (p.o.), but oral toxicity in this species was complicated due to the emesis produced by drug. In rhesus monkeys, the lethal dose of naltrexone was 300 mg/kg (s.c.), while a p.o. dose of 1 g/kg produced salivation, emesis, and hypoactivity. In all cases deaths occurred after clonic-tonic convulsions, usually preceded by restlessness, tremor, depression, salivation, or retching and emesis. Peak effects usually occurred 30-45 min after s.c. injection, and signs of toxicity usually appeared 2 to 4 hr after dosing, but there was no delayed lethality.

DISSOCIATION CONSTANT, DISTRIBUTION COEFFICIENT, PLASMA-PROTEIN AND OPIATE RECEPTOR BINDING OF NALTREXONE

The pKa's of naltrexone, naloxone and nalorphine at 37°C were 8.13, 7.82 and 7.59 respectively (14) and octanol/water distribution coefficients (pH 7.40) were 13.08, 33.55, 28.16 respectively. No significant species differences in extent of binding of [15,16-^{3}H] nal-

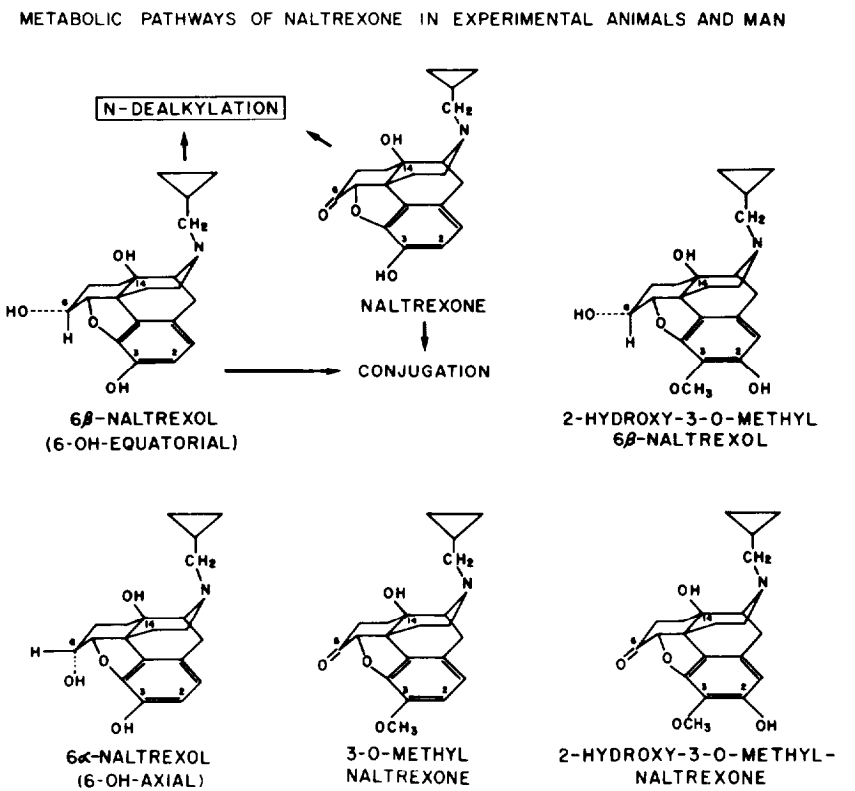
trexone in human, monkey, dog, guinea pig, rat and mouse plasma were observed, and these values measured by equilibrium dialysis at 37°C ranged between 21-26% in the concentration range 0.1-500 ng/ml (15). The low extent of plasma-protein binding implies a large apparent volume of distribution for naltrexone in different animal species and man and further suggests that potential interactions involving displacement of drug from binding sites will not be a therapeutic problem with naltrexone. The receptor binding constants (K_{RB} x 10^9) using [^{3}H] dihydromorphine and 10,000 g particulate fraction of rat brain homogenate (pH 8, sucrose-tris solution) for naltrexone, naloxone and nalorphine were 0.2, 1.0, 1.5 respectively (16) and there was a good correlation between binding constant and antagonist potency as determined in the guinea pig ileum preparations (9). The greater intrinsic activity of naltrexone as compared to naloxone, however, is not sufficient to explain the 20- to 30-fold dose difference needed for naloxone to produce effects of similar time-course as naltrexone and underlying differences in disposition and metabolism may play an important role in differential potencies of these two antagonists.

DISPOSITION, METABOLISM AND PHARMACOKINETICS OF NALTREXONE IN EXPERIMENTAL ANIMALS

The isolation and identification of a major metabolite of naltrexone in man was reported by Cone (17). This urinary metabolite, 6ß naltrexol was formed by the reduction of 6-keto group to an alcohol and was assigned the ß-isomorphine configuration (with 6-OH in equatorial position and ring in the chair form) (figure 1). This configuration was subsequently confirmed by its stereospecific synthesis (18) and NMR, IR and mass spectral data (19). A substantial species variation was observed with regard to the formation of 6ß or 6α-naltrexol. Chicken produced predominantly the 6α-naltrexol (6-OH in axial position and ring in the chair form) (20) and the guinea pig and rabbit 6ß-epimer along with small amounts of 6α-naltrexol (21). Studies by Malspeis et al. (22) showed that substantially greater quantities of 6ß-naltrexol and/or its conjugates were excreted in the urine of man, monkey, guinea pig and rabbit after naltrexone administration, and very small quantities of 6ß-metabolite were excreted in mouse, rat and dog. Using a sensitive electron-capture gas chromatographic method for the differentiation of 6α- and 6ß-naltrexol in presence of naltrexone, 6 α-epimer was shown to be present in trace amounts in monkey and guinea pig

urine. Following a 1 mg/kg i.v. injection of [15,16-^{3}H] naltrexone to guinea pigs, 84 and 14% of radioactivity was excreted in urine and feces respectively. In guinea pig excreta, 64% of the dose corresponded to naltrexone and conjugates, 19% to 6ß-naltrexol and conjugates and 2% to 6α-naltrexol and conjugates. In urine, the radioactivity corresponding to 6α-naltrexol and naltrexone was mainly in conjugated form; whereas 6ß-naltrexol was mainly unconjugated. The radioactivity in feces corresponded principally to conjugated naltrexone and 6ß-naltrexol. In gall bladder bile of these animals, conjugated naltrexone and 6ß-naltrexol were found 2 hr after drug administration (23). 6ß-naltrexol (1 mg/kg i.v.) was excreted in free and conjugated form in bile and urine of guinea pig. The same dose of 6α-naltrexol gave, in addition to free drug and conjugated metabolite, another unidentified metabolite with a gc retention time similar to but not identical to 6ß-naltrexol (22).

Figure 1



Comparative studies on the 24-hr urinary excretion of naltrexone and its metabolite in monkey, rabbit and rat (24) have shown that in monkeys receiving a 12 mg/kg dose p.o. daily for 8 months, 0.3% of dose was excreted as free 6ß-naltrexol and 3.0% as its conjugate. Females excreted twice as much total base (38.2%) as males (16.5%). Rabbits receiving 30 mg/kg dose i.p. daily for 8 days excreted conjugated naltrexone and ß-naltrexol 14.5% and 3.3% of the dose respectively. Rats receiving 100 mg/kg dose p.o. daily for 12 months excreted less than 1% of the dose as free and conjugated naltrexone and ß-naltrexol. Studies by Cone and Gorodetzky (25) also showed very low recoveries in the rat (5.5% of the dose). However recent work by Ludden et al. (23) has shown that rats excreted 42 and 55% of the dose in urine and feces respectively after a 1 mg/kg i.v. injection of [15,16-^{3}H] naltrexone. Other routes of metabolism, therefore, may play a role in the disposition of naltrexone.

Preliminary studies on the pharmacokinetics of naltrexone (1 and 5 mg/kg i.v.) in rabbits using a specific electron capture-gas chromatographic assay (26) have shown a 2-compartmental decay of drug in plasma with $t_{1/2}$ß 70 ± 10 min and 57 ± 6 min respectively with 2 dosages. The concentration of drug in semen was 13 and 11 times that in plasma at 120 min and the $t_{1/2}$ß values in semen with 2 dosages were 133 and 94 min respectively. Distribution studies using gas chromatography and [^{3}H] labeled drug showed the following values µg/g) 90 min after a 5 mg/kg i.v. injection: plasma 0.37, brain 2.78, lung 2.39, spleen 7.77, liver 0.08, kidney 3.95, muscle 1.48, testes 2.33, fat 0.42, stomach 7.08, and small intestine 1.28 respectively. Isolated perfused liver studies with a 10 mg naltrexone dose gave a till of 10 min. Evidence was obtained for several glucuronide metabolites of naltrexone and 6ß-naltrexol in bile.

In dog, the duration of action of naltrexone (27) was surprisingly short (2 hr) in contrast to man (12 hr) and this species excreted naltrexone predominantly as its 3-glucuronide with little or no free or conjugated 6ß- or 6α--naltrexol (25). Plasma naltrexone kinetics after intravenous bolus administration has recently been reported in the dog and the monkey (28). A 2-compartment open pharmacokinetic model fitted to plasma level-time data for naltrexone in 2 dogs (0.72 mg/kg, i.v. dose) yielded a $t_{1/2}$ß of 1.0 hr (52-67 min) and total body clearance of 51-55 ml/min/kg. In 6 rhesus monkeys given a 1.0 or 10.0 mg/kg iv. dose, a $t_{1/2}$ß of 7.8 hr and total body clearance of 64 ml/min/kg (40-100 ml) was observed. The total body clearance for naltrexone was greater than the hepatic plasma or blood flow in both dogs and monkeys and this finding together with extremely low renal excretion of naltrexone (1% of dose) suggested the existence of other elimination mechanisms besides liver metab-

olism and renal excretion. Such mechanisms include fecal excretion of drug and metabolites, formation of unknown metabolites and urinary excretion of conjugates not hydrolyzable by the enzyme preparation. The serum $t_{1/2}\beta$ for naltrexone in the dog has been reported (29) to be 85.1 ± 9.0 min after a 5 mg/kg i.v. injection.

Recent sensitive methods have shown the presence of small quantities (< 1%) 6α-naltrexol in the urine of man, rabbit, dog and rat (25). 2-hydroxy-3-O-methyl 6ß-naltrexol has been identified (30) as another metabolite of naltrexone in humans receiving 100 mg naltrexone orally. This lipophilic metabolite had a long bioavailability, much slower excretion rate than naltrexone and 6ß-naltrexol. Cone et al. (31) have shown that in human subjects receiving a single 50 mg p.o. dose of naltrexone, excretion of this metabolite over a period of 5 days was 4.6 ± 2.1% of the dose, with a mean excretion $t_{1/2}$ of 20.2 ± 1.8 hr. 2-hydroxy-3-O-methyl naltrexone was observed as another minor metabolite (0.45 ± 0.08% of the dose) of naltrexone in human subjects and the rat. These metabolites were predominantly excreted in free form. Unequivocal confirmation of the structure of 2-hydroxy-3-O-methyl 6ß-naltrexol was provided recently by C-13 nuclear magnetic resonance technique (32) and this study further ruled out the formation of isomeric 2-O-methyl compound.

Metabolic studies in the rat have shown that a very large portion of the dose of naltrexone remains unaccounted for (24,251 and recent studies utilizing gc/mass spectrometry in our laboratory (Verebey et al., unpublished observations) have shown that naltrexone undergoes 3-O-methylation in this species to give another lipophilic metabolite, 3-O-methyl naltrexone. Disposition of [15,16^{3}H] naltrexone in the central nervous system of the rat was investigated by Misra et al. (33). After a 10 mg/kg s.c. injection, the peak concentrations in brain and plasma occurred within 0.5 hr. Levels of naltrexone were sustained in brain between 2 to 24 hr and barely detectable at 48 hr. Significant amounts of metabolites were present in brain and plasma at longer time periods. The decay of naltrexone in brain and plasma was biexponential with $t_{1/2}\alpha$ 18 min and $t_{1/2}$ 8.0 hr in brain and 11.4 hr in plasma respectively. The brain to plasma ratios at 0.5 to 1 hr were higher than those at later times. 6ß-naltrexol was present in small amounts in brain but not in plasma. In addition to 7,8-dihydro-14-hydroxy normorphinone and 7,8-dihydro-14-hydroxy normorphine, tentative evidence was obtained for 3 other lipophilic metabolites in brain which were cleared from the brain much more slowly than naltrexone or 6ß-naltrexol. These metabolites were also present in plasma in addi-

tion to free and conjugated naltrexone and its N-dealkylated metabolites. It is conceivable that the 3 lipophilic metabolites in brain may be 2-hydroxy-3-O-methyl naltrexone, 2-hydroxy-3-O-methyl 6ß-naltrexol and 3-O-methyl naltrexone respectively. The slow decline of levels of naltrexone in brain would imply that either the binding of naltrexone to some constituent in brain is particularly strong or a slow release from extravascular sites into rat plasma may contribute to its prolonged duration in plasma and the long duration of antagonism.

DISPOSITION AND METABOLISM OF NALTREXONE IN MAN

The urinary excretion profile of naltrexone as reported by Cone et al. (34) has been essentially confirmed by subsequent studies of Verebey et al. (35) and Dayton and Inturrisi (24). Following a single 50 mg oral dose of naltrexone in man, an average of 53.4% of the dose was recovered in urine of which naltrexone, conjugated naltrexone, 6ß-naltrexol and conjugated 6ß-naltrexol comprised 1.2, 9.7, 26.3 and 16.4% respectively (34). The mean excretion $t_{1/2}$ of naltrexone was estimated as 1.1 hr and that of 6ß-naltrexol 16.8 hr. In 3 human subjects receiving a 180 mg dose of naltrexone daily, the 24-hr urinary excretion as % of dose was: naltrexone 0.5, conjugated naltrexone 4.5, 6ß-naltrexol 34.6 and conjugated 6ß-naltrexol 14.0%. In another study (24) in maintenance subjects receiving 125 mg naltrexone p.o. 3 times a week, 37% of the dose was excreted in 48 hr, of which naltrexone, conjugated naltrexone, 6ß-naltrexol and conjugated 6ß-naltrexol comprised 0.8, 7.6, 16.8 and 11.8% respectively.

Detailed studies on the disposition and metabolism of naltrexone (100 mg p.o.) in 4 acutely and chronically treated human subjects have been reported by Verebey et al. (36,371. The peak plasma levels of naltrexone following an acute dose were 43.6 ± 29.9 ng/ml at 1 hr and of 6ß-naltrexol 87.2 ± 25.0 ng/ml at 2 hr., and these declined to 2.1 ± 0.5 and 17.6 ± 5.0 ng/ml respectively by 24 hr. Following chronic administration, the peak levels of naltrexone and 6ß-naltrexol at 1 hr were 46.4 ± 18.5 and 158.4 ± 89.9 ng/ml respectively, but by 24 hr these values declined to levels similar to those in the acute state at 24 hr. Plasma levels of naltrexone and 6ß-naltrexol measured 24 hr after the daily doses of naltrexone indicated that steady-state equilibrium (conc. 10.9 ng/ml) was rapidly attained and there was no accumulation of these compounds in plasma on chronic dosing. Biexponential kinetics were observed for

naltrexone and 6ß-naltrexol in the first 24 hr. The $t_{1/2}$ of naltrexone and 6ß-naltrexol decreased from the acute to the chronic study from 10.3 ± 3.3 to 9.7 ± 1.1 hr and from 12.7 ± 2.6 to 11.4 ± 2.0 hr respectively. Plasma levels of naltrexone declined slowly from 24 to 72 hr from 2.4 to 1.7 ng/ml with an apparent $t_{1/2}$ of 96 hr.

Renal clearance data indicated that naltrexone was partially reabsorbed while 6ß-naltrexol was actively secreted by the kidney. During acute and chronic treatment, the urinary excretion was 38 and 70% of the dose in 24 hr and the mean fecal excretion 2.1 and 3.6%. Opiate antagonism to 25 mg i.v. heroin challenges was nearly complete through 48 hr after naltrexone, and at 72 hr the objective responses reappeared to a greater extent than the subjective ones. Low plasma levels of drug suggested substantial sequestration in tissues which released it slowly into the central compartment. First-pass hepatic metabolism of naltrexone was indicated by rapid appearance, peaking and substantially higher concentrations of 6ß-naltrexol in plasma. Lack of changes in proportion of naltrexone and its metabolite from acute to the chronic state indicated that naltrexone did not induce its own metabolism. The 2-fold recovery of naltrexone and its metabolites in the chronic study may have resulted from the saturation of tissue binding sites with naltrexone and its metabolites, forcing larger amounts of daily doses to remain in the central compartment for more rapid elimination after metabolism. Conjugation mechanism appeared to become more efficient on multiple dosing of naltrexone. During chronic treatment no tolerance, physical dependence or toxicity was observed in human subjects,

In a recent study Verebey et al. (38) have developed a sensitive glc method utilizing derivatization with pentafluoropropionic acid for the quantitation of 2-hydroxy-3-O-methyl 6ß-naltrexol in plasma and urine. The relative percentages of this metabolite, naltrexone and 6ß-naltrexol in the plasma of 4 human subjects 18 hr after 400 mg p.o. dose of naltrexone were 23.2 ± 3.7, 3.4 ± 1.5 and 73.5 ± 3.2 respectively; the corresponding percentages in urine were 16.0 ± 9.8, 7.4 ± 4.1 and 76.8 ± 10.7 respectively.

COMPARATIVE POTENCIES OF THE METABOLITES OF NALTREXONE

Although potency ratios between compounds may change substantially depending on the animal species and the experimental test used to assess the antagonist activity, 6α--naltrexol and 6ß-naltrexol were approximately 1/35 and 1/50 as active as naltrexone in

morphine-pelleted mice using jumping response as the criterion of antagonist potency (20). 6ß-naltrexol (s.c.) was about 1/56 as active as naltrexone in preventing the loss of righting reflex in rats from morphine sulfate (20 mg/kg s.c.) and about 4 times as long-acting as naltrexone in tests for duration of action at equi-antagonist ED_{90}, doses and measurement of time required for 50% decrease in activity. In mice, 6ß-naltrexol was about 1/26 as active as naltrexone in preventing the Straub-tail reaction with morphine (30 mg/kg s.c.) and about 9 times as long-acting as naltrexone (39). In chronic spinal dog following precipitation of abstinence, the potency of 6ß-naltrexol was approximately 1/12 to 1/15 that of naltrexone (341.

The low potencies of the 6α- or 6ß-naltrexol and the rapid onset of action of naltrexone militate against the formation of these metabolites, contributing substantially to the overall pharmacological effect of the parent compound. The antagonist potency of 3-O-methyl naltrexone (N-cyclopropylmethyl - 7,8 - dihydro-14-hydroxy norcodeinone, EN-2994A) in mice was 1/15 and 1/4 that of naltrexone on subcutaneous injection and p.o. administration respectively. In rat, the antagonist potency was 1/20 and 1/5 that of naltrexone on s.c. and p.o. administration (Dr. Blumberg, personal communication). The relative affinities of naltrexone, 6α-naltrexol, 6ß-naltrexol and 2-hydroxy-3-O-methyl-6ß-naltrexol for the stereospecific receptor sites in rat brain homogenate provided IC_{50} values of 1.3 x 10^{-9}, 1.2 x 10^{-9} 3.2 x 10^{-9}, and 2.7 x 10-6M respectively and in presence of Na+ the corresponding values were 0.9 x 10^{-9} 2.9 x 10^{-9}, and 2.0 x 10^{-9}, and 1.8 x 10-6M respectively (Dr. Hiller, personal communication). Thus the 2-hydroxy-3-O-methyl-6ß-naltrexol possessed much less activity as an antagonist compared to naltrexone. The pharmacological activity of 2-hydroxy-3-O-methyl naltrexone and 2-hydroxy-3-O-methyl-6ß-naltrexol *in vivo* is still unknown. However, the comparatively slower clearance of these methylated metabolites than of naltrexone or 6ß-naltrexol may have implications for the long duration of antagonism with naltrexone.

ENZYME SYSTEMS INVOLVED IN THE METABOLISM OF NALTREXONE

The most common route for the biotransformation of naltrexone and other opiates having a phenolic group is the glucuronide conjugation in several mammalian species including man. The glucuronides are formed enzymatically by the transfer of glucuronic acid from uridine diphosphate D-glucosiduronic acid (UDPGA) to the opiates by glucuronyl transferases found in microsomes of liver and

other tissues. Sulfate conjugation of phenolic or alcoholic groups of opiates is a prime pathway in the cat and chicken and occurs in the soluble fraction of the liver cell, kidney and intestine by several sulfotransferases.

The enzyme system involved in the reduction of 6-keto group of naltrexone to 6ß-naltrexol requires NADPH and occurs in the soluble fraction of the liver homogenate. Studies by Roerig et al. (40) have shown that the chicken liver enzyme (which predominantly produces 6α-naltrexol) was precipitated at 60-70% ammonium sulfate saturation and the rabbit liver enzyme (which predominantly produces 6ß-naltrexol) at 50-60% saturation. The rabbit enzyme was more sensitive to inhibition by morphine than was the chicken enzyme. The reverse order of sensitivity was seen with ketamine. These differences in properties between the chicken and rabbit liver enzymes indicate that the stereoselective metabolism of naltrexone to 6α- or 6ß-naltrexol is mediated by two different enzymes.

The formation of metabolites, 2-hydroxy-3-O-methyl naltrexone and 2-hydroxy-3-0-methyl-6ß-naltrexol is brought about by catechol-O-methyl transferases (COMT) which are found in the soluble supernatant, of liver, brain and in erythrocytes. This is also an important pathway for biogenic amines and other compounds (41,42,43). The biochemical characteristics of COMT from brain and erythrocytes are similar in rat and man (44). The nonspecificity of COMT towards exogenous substrates usually leads to the formation of both meta and para-O-methylated derivatives of substrates. Recent studies (32) have, however, ruled out the formation of 3-hydroxy-2-O-methylated naltrexol as a metabolite of naltrexone. In view of the recent evidence for the 3-O-methylation of naltrexone in the rat (Verebey et al., unpublished observations), the sequence of steps leading to the formation of 2-hydroxy-3-methylated naltrexone metabolites is not clear; i.e., whether 2-hydroxylation of naltrexone occurs first followed by 3-O-methylation or 3-O-methylation occurs before 2-hydroxylation.

Chronic administration of naltrexone in mice via a long-acting delivery system depressed the microsomal mixed function oxidases, MFO (aminopyrine N-demethylase and aniline hydroxylase activity) to 40-50% of the control levels during the 30-35 day period of naltrexone blockade. A metabolite of 6ß-naltrexol was possibly implicated, and this depression of MFO could inhibit the metabolism of other drugs (45).

CONCLUSIONS

It is clear from the above studies that considerable species-dependent differences exist in the quantitative pattern of the metabolism of naltrexone. The metabolic pathways of naltrexone in different species were: (a) 6-keto group reduction to epimeric 6α- or 6ß-naltrexol, (b) glucuronide conjugation of naltrexone and naltrexols, (c) N-dealkylation to norcompounds, (d) 3-O-methylation, and (e) 2-hydroxylation. The role of gut wall epithelium in the biotransformation of naltrexone needs to be elucidated in more detail in view of the considerable first-pass metabolism. As a large portion of the dose of naltrexone remains unaccounted for in several species including man, there is need for additional studies to completely elucidate its metabolic and dispositional profile. Although the known metabolites have comparatively much lower antagonist potency than naltrexone and may not contribute substantially to the overall pharmacologic effect, additional studies are needed to fully assess the role of 3-O-methyl naltrexone and 2-hydroxy-3-O-methyl-6ß-naltrexol metabolites.

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The Clinical Pharmacology of Naltrexone: Pharmacology and Pharmacodynamics

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The time-action of opiate antagonist activity of naltrexone was evaluated in detoxified ex-opiate addicts, using 25 mg intravenous heroin challenges. A 100 mg naltrexone dose provided 96% blockade at 24 hr, 86.5% blockade at 48 *hr and 46.6% blockade at 72 hr. Following oral administration, naltrexone was rapidly and completely absorbed. Peak levels of naltrexone and its major metabolite 6ß-naltrexol were reached 1 hr after the dose. The high 6ß-naltrexol plasma concentrations only 1 hr after drug administration indicate a rapid biotransformation process, convertin a large fraction of the dose to less active metabolites. Over 70% of the dose was excreted in the 24 hr urine and less than 0.5% in the feces. No change was observed in the rate of naltrexone disposition during chronic dosing vs. the acute study, indicating no metabolic induction. The rapid achievement of steady state naltrexone plasma levels eliminates the need of stepwise induction at the beginning of nal trexone treatment.*

After intravenous administration of 8 mg naltrexone, the plasma levels were unexpectedly high for the low dose, ranging between 32 and 3 ng/ml. The intravenous drug administration eliminated direct exposure to hepatic biotransformation; this is the likely reason for the higher naltrexone and the significantly lower metabolite (6ß-naltrexol) plasma levels.

A rising dose efficacy study from 100 to 800 mg per day provided an opportunity for studying naltrexone at much higher than thera-

peutic doses. No undesirable naltrexone-related side effects were observed during the study. Two weeks after the 800 mg/day doses were stopped, the plasma was free of naltrexone and its metabolites, indicating efficient elimination of the drug from the body.

Based on these human studies, a 100 mg dose of naltrexone provided 2 to 3 days protection against 25 mg of intravenous heroin. Naltrexone seems to be well tolerated even at doses well above those suggested for opiate antagonist therapy. No toxicity or accumulation of naltrexone and its metabolites was observed in any of the studies. The lack of dependence liability and absence of pharmacologic or metabolic tolerance during chronic treatment make naltrexone a safe and efficacious orally effective opiate antagonist.

INTRODUCTION

Naltrexone (N-cyclopropylmethylnoroxymorphone) was synthesized by Blumberg et al. in 1965 (1). In animal (2) and clinical studies (3,4) it demonstrated longer duration of action and greater potency than its N-allyl congener, naloxone. Naltrexone was also orally efficacious at significantly lower doses than naloxone. This is important for a drug which is a candidate for the treatment of exopiate addicts. The initial trials of naltrexone in man indicated good efficacy (opiate antagonism), practicality (long time action and oral effectiveness) and safety (low doses). In this overview of naltrexone the available data will be combined to examine naltrexone's opiate receptor blocking activity as it relates to its total biological disposition in human subjects.

The Time Course of Action of Opiate Antagonism

The opiate receptor blocking activity of naltrexone was studied by challenging it with intravenous heroin injections in four opiate ex-addicts (5). Control data of various objective and subjective responses to heroin, collected in the absence of naltrexone, in response to a 25 mg heroin injection was considered as 100%. In the test period, during naltrexone therapy (100 mg/day), 25 mg heroin challenges were performed 24, 48 and 72 hr after the last naltrexone dose. Individual challenges were at least 10 days apart in the same patients. The results are shown in figure 1. Averaging both the objective and subjective results, 96% of heroin-related responses were blocked at 24 hr, 87% at 48 hr and 47% at 72 hr. It is apparent from the figure that the blockade seems to hold longer and to a greater extent for subjective responses than for objective ones. Pooling pupillary miosis and respiratory depression data (ob-

jective), the blockade was 89% at 24 hr, 73% at 48 hr, and only 20% at 72 hr after naltrexone. The blockade for the subjective responses was 99% at 24 hr, 92% at 48 hr and a respectable 57% at 72 hr after naltrexone. The proposed function of naltrexone is to block the subjective (or euphorogenic) effects of heroin which, in fact, were blocked more efficiently than the objective ones. It should be emphasized that 25 mg of heroin is a substantial dose and few addicts can obtain such large quantities of heroin routinely. Thus the duration and magnitude of the blockade seem highly effective for most practical situations.

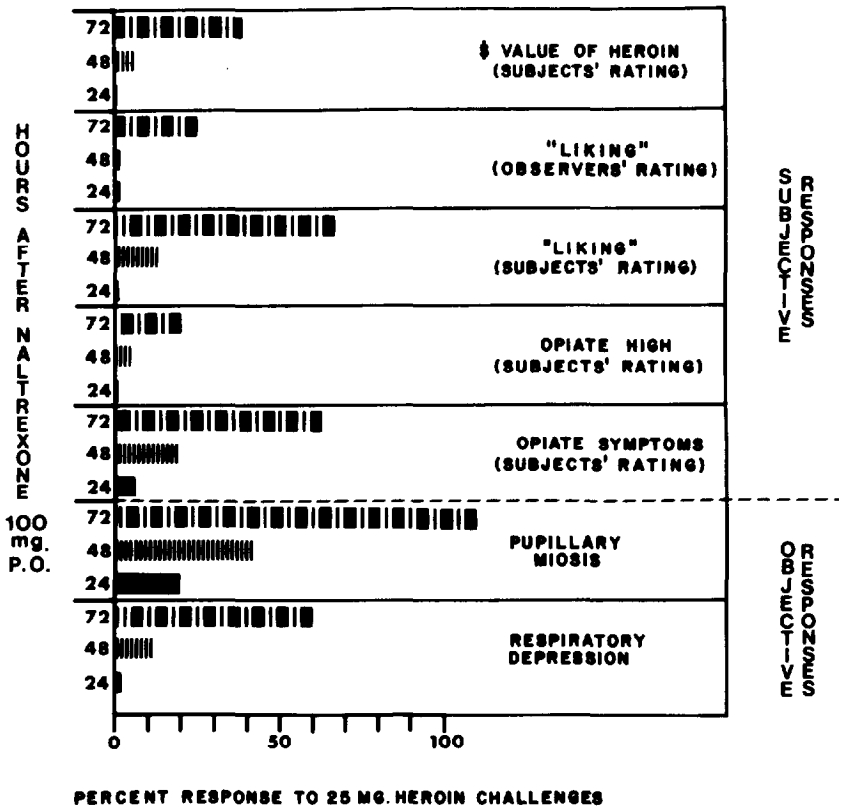

FIGURE 1. The percent objective and subjective responses are shown after 25 mg i.v. heroin 24,48 and 72 hr after 100 mg oral naltrexone. The 100% heroin responses were determined in the absence of naltrexone. For detailed description of the specific tests, see reference 5.

An interesting observation is that pupillary constriction at 72 hr is greater than the 100% control heroin effect. It is possible that some metabolic N-dealkylation occurs, producing the strong agon-

ist, noroxymorphone, for which Martin et al. (3), Resnick et al. (4), and Verebey et al. (5) have observed slight agonistic symptoms. Cone et al. (6) reported the isolation of small quantities of noroxymorphone in the urine of subjects taking naltrexone. The small amount of agonist may act synergistically with heroin to produce the higher than control responses.

The Correlation of Narcotic Antagonism With Naltrexone Plasma Levels

The mean naltrexone plasma levels of 4 subjects declined very slowly from 2.4 ng/ml at 24 hr to 2.0 ng/ml at 48 hr and to 117 ng/ml 72 hr after naltrexone (5). The slow exponential decay at this period translates into a 96-hr terminal half-life and a correlation coefficient with the total (objective and subjective) heroin-related responses of r=0.90. The strong correlation indicates that differing individual biotransformation rates may influence the time action of naltrexone. Shorter opiate antagonist effects would be expected in rapid metabolizers than in slow metabolizers of naltrexone. In fact, the shorter ß-phase half-life values of individuals correlated with greater responses to heroin at 72 hr after naltrexone (r=0.99). Correlation was also excellent between opiate antagonism and the combined levels of 6ß-naltrexol and 2-hydroxy-3-methoxy-6ß-naltrexol (HMN) of r=0.82 (5). While opiate antagonist potencies of these metabolites have been shown to be considerably weaker than naltrexone in animals (7,8), there is no data in humans. Based on our observations, it appears that naltrexone itself is responsible for the major part of opiate antagonism, while the less active metabolites, which are present in large concentrations in the body, also contribute to the opiate receptor blocking activity.

Absorption and Distribution

After oral administration, naltrexone was rapidly absorbed, as indicated by the early peaking (1 hr) of naltrexone and 6ß-naltrexol in the plasma (5). Complete absorption was confirmed by the finding that free naltrexone in the 24-hr feces was only 0.3% of the administered dose during chronic administration of 100 mg oral doses of naltrexone (5). The low plasma levels of naltrexone indicate that a large percentage of the dose is rapidly metabolized and the remaining drug is distributed mainly intracellularly, resulting in the presence of a small fraction of the dose in the systemic circulation (table 1). Individual variation in the plasma levels of naltrexone was greatest during the first 8 hours, suggesting that absorption

TABLE 1. Plasma Concentration of Naltrexone

Subjects (n)	Dose	Route	Hours after naltrexone 1	2	4	8	12	24
	mg		(ng/ml)					
* Postaddicts (4)	100	P.O. (chronic)	44	36	20	8	4	2
* Postaddicts (4)	100	P.O. (chronic)	46	32	19	10	6	3
** Normal volunteers (10)	50	P.O. (acute)	22	12	5	3		
** Normal volunteers (9)	100	P.O. (chronic)	27	17	8	6		
** Schizophrenic patients (8)	2 x 200	P.O. (chronic)						9
*** Schizophrenic patients (6)	100	P.O. (chronic)						2
*** Schizophrenic patients (6)	2 x 100	P.O. (chronic)						3
*** Schizophrenic patients (6)	2 x 200	P.O. (chronic)						5
*** Schizophrenic patients (5)	2 x 300	P.O. (chronic)						6
*** Schizophrenic patients (4)	2 x 400	P.O. (chronic)						9
**** Normal volunteer (2)	8	I.V. (acute)	11	8	3			

* Data from reference 5
** Courtesy of Dr. Jan Volavka, Dept. of Psychiatry, University of Missouri
*** Courtesy of Drs. N. Klein and G. Simpson, The Rockland Research Institute
**** Courtesy of Dr. Theodore Smith, Dept. of Anesthesiology, University of Pennsylvania

and distribution rates were the most important individual variables (5).

Plasma Levels of Naltrexone and Metabolites

Substantial individual variation of peak naltrexone levels was observed, ranging from 15 to 64 ng/ml 1 hr after 100 mg oral doses. After the same dose and time the combined 6ß-naltrexol and HMN plasma levels ranged from 83 to 288 ng/ml (5). Table 1 shows the plasma levels of naltrexone in different subjects after various doses and routes of administration. Irrespective of the size of the dose, the plasma levels declined to low levels (2 to 9 ng/ml) by 12 to 24 hrs after drug administration, indicating extensive metabolism and effective distribution of naltrexone. The metabolite plasma levels of 6ß-naltrexol and HMN were 35- and 12-fold higher than naltrexone, respectively (figure 2). With increasing doses from 100 mg to 800 mg naltrexone/day the 24-hr plasma levels of naltrexone, as well as its metabolites, increased. At the 800 mg/day dose, average levels reached were 9 ng/ml naltrexone, 123 ng/ml HMN, and 331 ng/ml 6ß-naltrexol. Two weeks after discontinuation of medication the plasma was free of naltrexone and its metabolites, confirming the early observations (5) of rapid and efficient elimination of the drug from the body (figure 2).

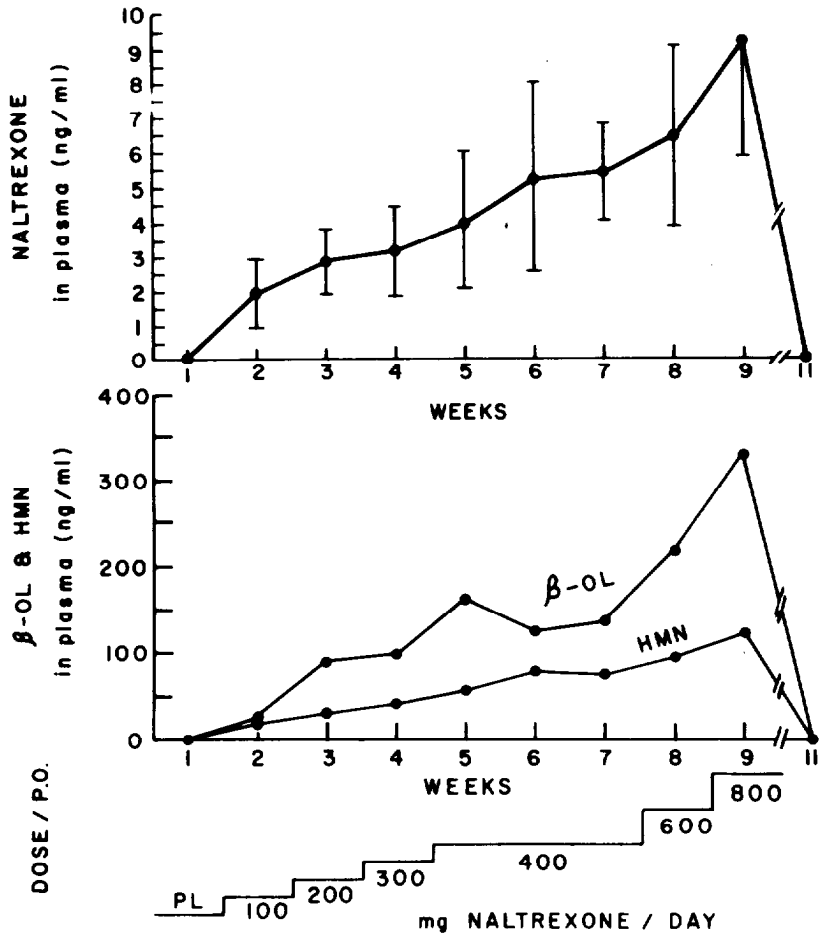

FIGURE 2. Naltrexone, 6ß-naltrexol and HMN plasma levels were determined just prior to each daily dose of naltrexone (24-hr samples). The incremental weekly doses are shown at the bottom of the figure. The ordinate scale 6ß-naltrexol (ß-OL) and HMN is from 0 to 400 ng/ml while that of naltrexone is from 0 to 10 ng/ml; (n=6 from 0 to 6th week; n=5 at 7th week and n=4 at 8th and 9th week). The two patients were dropped from the study for medical reasons, not related to naltrexone therapy.

Metabolism

The major metabolite of naltrexone in humans is 6ß-naltrexol, isolated by Cone in 1973 (9), and the structure was confirmed by Chatterjie et al. in 1974 (10). A minor metabolite, 2-hydroxy-3-methoxy-6ß-naltrexol (HMN), was isolated by Verebey et al. (11) and its structure was confirmed using synthesis by Cone et al. (12). The

rapid peaking of 6ß-naltrexol just an hour after an oral dose indicated that immediately after absorption a major portion of naltrexone is biotransformed during its first pass through the liver. HMN formation from naltrexone can be postulated through various pathways (11). A logical one is the hydroxylation of 6ß-naltrexol at the 2 position, followed by methylation at the 3 position. However, Cone et al. showed 2-hydroxy-3-methoxynaltrexone in trace amounts in the urine of subjects taking naltrexone, opening the possibility for the formation of HMN through another route (12).

In an earlier report, Cone and Gorodetzky showed, by gas chromatography and mass spectroscopy, the existence in the urine of subjects taking naltrexone of an N-dealkylated metabolite, noroxymorphone, which is a potent agonist (13). This metabolite may be responsible for the slight agonistic effects observed in some objective and subjective responses following naltrexone administration (3,4,5).

Quantitatively, 6ß-naltrexol is the most abundant metabolite formed and is excreted both free and conjugated. Naltrexone was mostly glucuronidated before excretion and HMN was found only in the free form. The trace metabolite, 2-hydroxy-3-methoxynaltrexone, was estimated at 0.5% of the administered dose (12). There is no estimation at present of the quantity of noroxymorphone in human urine after naltrexone administration.

The opiate antagonistic activity of 6ß-naltrexol varies from 1/50th to 1/12th of that of naltrexone (7,8) depending upon the species and methods used. Preliminary studies of HMN, using the opiate receptor binding assay from rat brain synaptosome preparation, indicated that HMN binding to opiate receptors was typically antagonist but its affinity was 1000 times less than that of naltrexone (14). If this potency ratio holds in human subjects, HMN may not contribute much to the opiate antagonist activity of naltrexone.

In acute vs. chronic dosing, the 6ß-naltrexol over naltrexone concentration in urine, in the same subjects, may have metabolic implications (5). The ratio of 6ß-naltrexol over naltrexone remained the same (table 2), indicating that naltrexone does not induce its own metabolism--a desirable feature for a drug given chronically, because the initial stabilization dose remains effective throughout the course of the treatment. Based on these studies, the most striking effect of biotransformation on the pharmacological action of naltrexone is the very high rate of first pass hepatic metabolism of the drug.

TABLE 2. Concentration Ratio 6ß-Naltrexol/naltrexone in 24-hour Urine

Subjects	Acute	Chronic
1	3.4	2.5
2	3.6	4.0
3	3.6	3.3
4	2.7	3.4
Mean	3.3	3.3
± SD.	.4	.6

Naloxone is also biotransformed extensively after oral administration, but into inactive glucuronides. This may be the reason for its short time action. Fortunately, the active metabolite formation (6ß-naltrexol and perhaps HMN) prolongs the time-action of naltrexone. This occurs in much the same way as formation of active metabolites prolongs the time-action of 1-α-acetylmethadol, in contrast to methadone, which is converted into inactive metabolites.

Renal and Fecal Excretion of Naltrexone

Urinary excretion of naltrexone and its metabolites is the major pathway of elimination of the drug from the body. The 24-hr recovery of free and conjugated bases is tabulated in table 3. During chronic administration of 100 mg oral doses, 23% naltrexone was excreted, mostly conjugated (93%). 6ß-Naltrexol was the major excretion product, constituting approximately 66% of the total bases in the 24-hr urine, and was excreted 60% free and 40% conjugated. HMN was 11% of the bases and was excreted only in the free form (5). In the acute study an average of 38% of the dose was recovered in the 24-hr urine, compared with 70% in the chronic study (using the same subjects). Following an acute dose a certain fraction of the drug is distributed into tissue stores; however, after chronic dosing the tissues become saturated and a larger fraction of the dose is available for renal elimination. This may explain the significantly larger percent recovery of the dose in the chronic study (table 3 and ref. 5).

In earlier studies, the total recovery of the bases, 6 days after a 50 mg oral dose of naltrexone, was approximately 50% of the dose (15). In this study HMN must have been included with 6ß-naltrexol, because it was recently found that the HMN and 6ß-naltrexol PFPA derivatives cannot be separated by gas chromatography (16). In another study, after 125 mg oral doses of naltrex-

one, 34% of the dose was accounted for from 0 to 24 hr, and 3% from 24 to 48 hr (17). The low recoveries in the early studies may have resulted from methodological difficulties. In a recent study, HMN excretion was quantitated for 5 days after a single 50 mg naltrexone dose (12). The recovery of HMN was 4.6% of the administered dose, which is compatible to the 3.5% reported in another acute study (table 3). Furthermore, there is evidence for the existence of 2-hydroxy-3-methoxynaltrexone. This minor metabolite was estimated at approximately 0.5% of the administered dose (12).

Table 4 presents data on the fecal excretion of naltrexone and its metabolites. In the chronic study a total of 3.6% of the administered dose was excreted in the feces, mostly as 6ß-naltrexol (80%). Both HMN and naltrexone were present in much lower concentrations, 12 and 8% respectively (table 4 and ref. 5). The data clearly indicate that fecal excretion is not an important pathway of elimination of naltrexone in human subjects. Collectively, the urinary and fecal excretion of naltrexone accounted for 73.6% of the administered dose following chronic drug administration. The missing 26.4% may be various metabolic intermediates described in the pathway to the formation of HMN and other not yet identified metabolites (11). In healthy individuals no problem of naltrexone accumulation is predicted during chronic naltrexone use. Since the drug is largely dependent on hepatic biotransformation and renal

TABLE 3. Urinary Excretion of Naltrexone and Metabolites, 24-hr Collection

(% of Dose Excreted)

	naltrexone		6ß-naltrexol		2-hydroxy-3-methoxy 6ß-naltrexol	Total
	Free	Bound	Free	Bound	Free	
Acute	1.0	7.2	19.1	7.1	3.5	37.9
Chronic	1.1	15.0	29.0	17.5	7.6	70.2

TABLE 4. Fecal Excretion of Naltrexone and Metabolites, 24-hr Collection

(% of Dose Excreted)

	naltrexone	6ß-naltrexol	2-hydroxy-3-methoxy 6ß-naltrexol	Total
Acute	0.13	1.9	0.08	2.1
Chronic	0.29	2.9	0.44	3.6

elimination, naltrexone disposition should be studied in subjects having hepatic and/or renal problems, to evaluate the safety of naltrexone in such a population.

CONCLUSION

The data collected during intravenous heroin challenges in the absence and presence of naltrexone showed that the subjective heroin-related responses were blocked for a longer period of time and to a greater extent than were the objective ones. This is a definite advantage for the therapeutic purpose of naltrexone; i.e., blocking the euphoric effects of heroin is more important than blocking pupillary constriction. The data also showed that narcotic antagonism was related to the plasma levels of naltrexone. Individual variation in biotransformation rates may therefore influence the time course of narcotic blockade. Fortunately, the chronic administration of naltrexone does not induce the microsomal enzyme system, and therefore the rate of biotransformation appears to remain unchanged from acute to chronic therapy. This suggests that the same dose will deliver the same pharmacological effect in chronic use. The lowest effective naltrexone plasma level was 2.0 ng/ml, providing an average of 86.5% blockade of the effects of 25 mg heroin. Thus, in therapy for effective opiate antagonistic activity, plasma levels of 2.0 ng/ml or greater should be maintained.

Another therapeutic advantage is the rapid attainment of steady state of naltrexone in plasma, which is a function of the total biological disposition of the drug. Judged from the plasma level data, steady state is achieved by the second daily dose of naltrexone. This information is useful for the choice of dose and frequency of drug administration, especially during the initiation of therapy. For simplicity of naltrexone use, our data indicates that no induction from lower doses to maintenance dose is necessary. Therapy may begin with the maintenance dose.

Increasing up to 800 mg naltrexone/day, the plasma levels of naltrexone and its metabolites also increased to very high levels without toxicity or any other undesirable side effects. Two weeks after the discontinuation of 800 mg naltrexone doses the plasma was free of naltrexone and its metabolites, indicating rapid and efficient drug elimination from the body. This study provides evidence for the high margin of safety of naltrexone even when considerably higher than effective opiate antagonistic doses are used.

In brief, nearly 100% blockade of 25 mg intravenous heroin effects can be maintained for 48 hr after 100 mg oral dose(s) of nal-

trexone. The absence of pharmacologic or metabolic tolerance during chronic treatment provides carefree clinical use of naltrexone. Plasma level monitoring can give reliable estimates of the degree of opiate antagonism. The pharmacodynamic investigations of naltrexone in man found naltrexone a safe and effective opiate antagonist for the rehabilitation of well-motivated narcotic addicts. Used with adequate psychological and social counseling, this chemotherapeutic modality should be most promising.

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Part V

Pharmacokinetics

Naltrexone: Research Monograph 28
R. E. Willette and G. Barnett, eds.
National Institute on Drug Abuse, 1980

Overview of Human Pharmacokinetics of Naltrexone

Vojtech Lictko

Experimental data on ^{3}H labelled naltrexone obtained from the Research Triangle Institute were analyzed. Both plasma and urine curves were resolved into their exponential components. Mean total clearance of unconjugated naltrexone was 3510 (300) ml/min, and its mean urinary clearance was 76.0 (6.8) ml/min. Fraction of dose excreted through urine was between 55 and 60%. Orally administered drug is completely absorbed into plasma. Plasma curve resulting from intravenous administration contained three exponentials: 1.9 (.5)/min, 0.18 (.07)/min and 0.00106 (.00008)/min. Distribution volumes were 7.6 (.7) liters, 60 (12) liters and 164 (16) liters. After oral administration there were two exponentials: 0.012 (.002)/min and 0.00122 (.00009)/min. The faster exponential corresponds to gastrointestinal absorption with a half life of about 1 hour.

INTRODUCTION

The purpose of this report is to summarize the pharmacokinetic analyses of naltrexone data in man collected at the Research Triangle Institute (1).

MATERIALS AND METHODS

Male volunteer subjects were used in the experimental studies. Five received 198 μCi of ^{3}H naltrexone in the dose of 1 mg intravenously (IV). Out of six subjects who were given 200 μCi of ^{3}H nal-

trexone in the dose of 50 mg orally (PO) only four were selected for this analysis since the other two did not have complete sets of data. The data consist of plasma concentration and amount of drug in urine samples at various time intervals after administration. There are seven sets of such plasma and urine data for both routes of administration: total drug (TOTAL), total unconjugated drug (TOTAL UNCONJ), total conjugated drug (TOTAL CONJ), 6 beta-naltrexol unconjugated (6ß-OL UNCONJ), naltrexone unconjugated (N-ONE UNCONJ), 6 beta-naltrexol conjugated (6ß-OL CONJ) and naltrexone conjugated (N-ONE CONJ). In the IV study plasma samples were obtained at .5, 1, 3, 5, 10, 15, 30, 60, 90, 120, 150 and 180 minutes and at 5, 7, 10 and 24 hours. Urine was collected at 3, 6, 9, 12, 24, 48 and 72 hours. In the PO study plasma samples were taken at 15-minute intervals for 3 hours and at 4.5, 6, 12 and 24 hours. Urine was collected at 3, 6, 9, 12, 24 and 48 hours. In a preliminary analysis, averaged data were employed. The failure to recover about 40% of the dose dictated that the final analysis be carried out on individual curves.

The sum of exponentials was fitted by a (weighted) nonlinear least square procedure (weight being proportional to the square of the reciprocal of the plasma concentration) to the plasma data (formula (1); cf. table 1) and a complement exponential (formula (2)) to the urine data. Area under the plasma curve, A, was determined analytically from formula (3) and the total clearance, Ct, was obtained from the dose D and the area A by means of formula (4). Instantaneous urinary clearance Cu(t) was computed by formula (5) for the first 12 hours every 3 hours, and the mean value Cu was taken (formula (6)). The fraction of urinary to total clearance, fc, was determined by formula (7) and the fraction of the dose excreted through urine, fd, computed by formula (8). Three distribution volumes, Vi, (i = 1, 2, 3) for different phases of the plasma curve were obtained by application of formula (9). Standard error, s, of a computed parameter x was estimated from the standard errors, si, of the parameters xi by formulas (10) and (11). All means are weighted by reciprocal variances, and standard errors are shown throughout in parentheses.

RESULTS

An example of the least square fit of IV data is shown in Fig. 1a and lb and another one for PO data in Fig. 2a and 2b. The plasma data are plotted semilogarithmically and the urine data are shown in linear scales.

TABLE 1. Definitions

(1)	p(t) = sum (Ai * exp (-ai*t)); i = 1,2,3
(2)	U(t) = Uo * (1 - exp(-k*t))
(3)	A = sum (Ai/ai); i = 1,2,3
(4)	Ct = D/A
(5)	Cu(t) = dU(t)/dt / p(t); t = 3, 6, 9, 12 hours
(6)	Cu = sum (Cu(t)/4; t = 3,6,9,12 hours
(7)	fc = Cu/Ct
(8)	fd = Uo/D
(9)	Vi = D/sum (Aj); i = 1, 2, 3; j = i 3
(10)	x = f(x1, x2, ... xp)
(11)	s = (sum ((df/dxi)*si)**2)**1/2; i = 1,2,...,p

TABLE 2. Weighted Mean Values of Parameters-Total Drug

Parameter	IV	PO
A/D (min ml) x 10e6	4120 (700)	4300 (490)
Ct (ml/min)	167 (25)	201 (12)
Cu (ml/min)	111 (9)	124 (8)
fc	,564 (.085)	,529 (.021)
fd	,539 (.047)	563 (.031)
k (1/min)	.00160 (.00007)	.00128 (.00015)
al (1/min)	1.9 (.5)	-
a2 (1 /min)	.18 (.07)	,012 (.002)
a3 (1 /min)	.00106 (.00008)	.00122 (.00009)
V1 (liters)	7.6 (.7)	-
V2 (liters)	60 (12)	-
V3 (liters)	164 (16)	145 (17)

Parameters are defined in Table 1.
Standard errors are shown in parentheses.

Tables 2, 3a and 3b summarize the analysis of the curves showing weighted means of the parameters for all subjects. Note that there are only a few instances when the IV and PO data yield significantly different values. For the total drug there is a significant difference ($p < .03$) in the value of the exponential coefficient of the second exponential, a2. Larger differences are found between the IV and PO values of unconjugated forms as seen in Tables 3a and 3b, where a double asterisk (**) indicates significant difference at level $p = .005$ and a single asterisk (*) that at level $p = .025$.

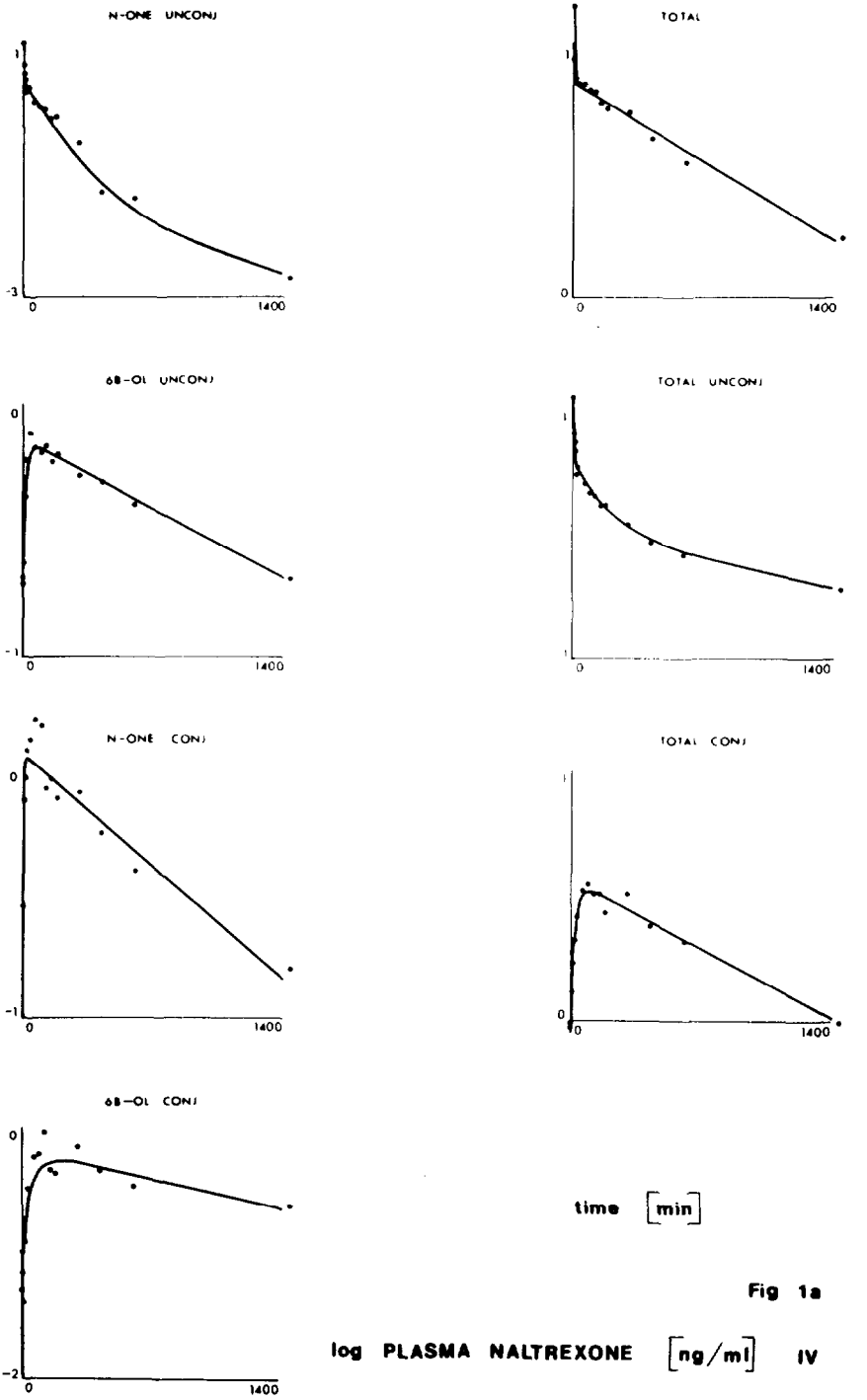
N-ONE UNCONJ
TOTAL
6β-OL UNCONJ
TOTAL UNCONJ
N-ONE CONJ
TOTAL CONJ
6β-OL CONJ
time [min]
log PLASMA NALTREXONE [ng/ml] IV

Fig 1a

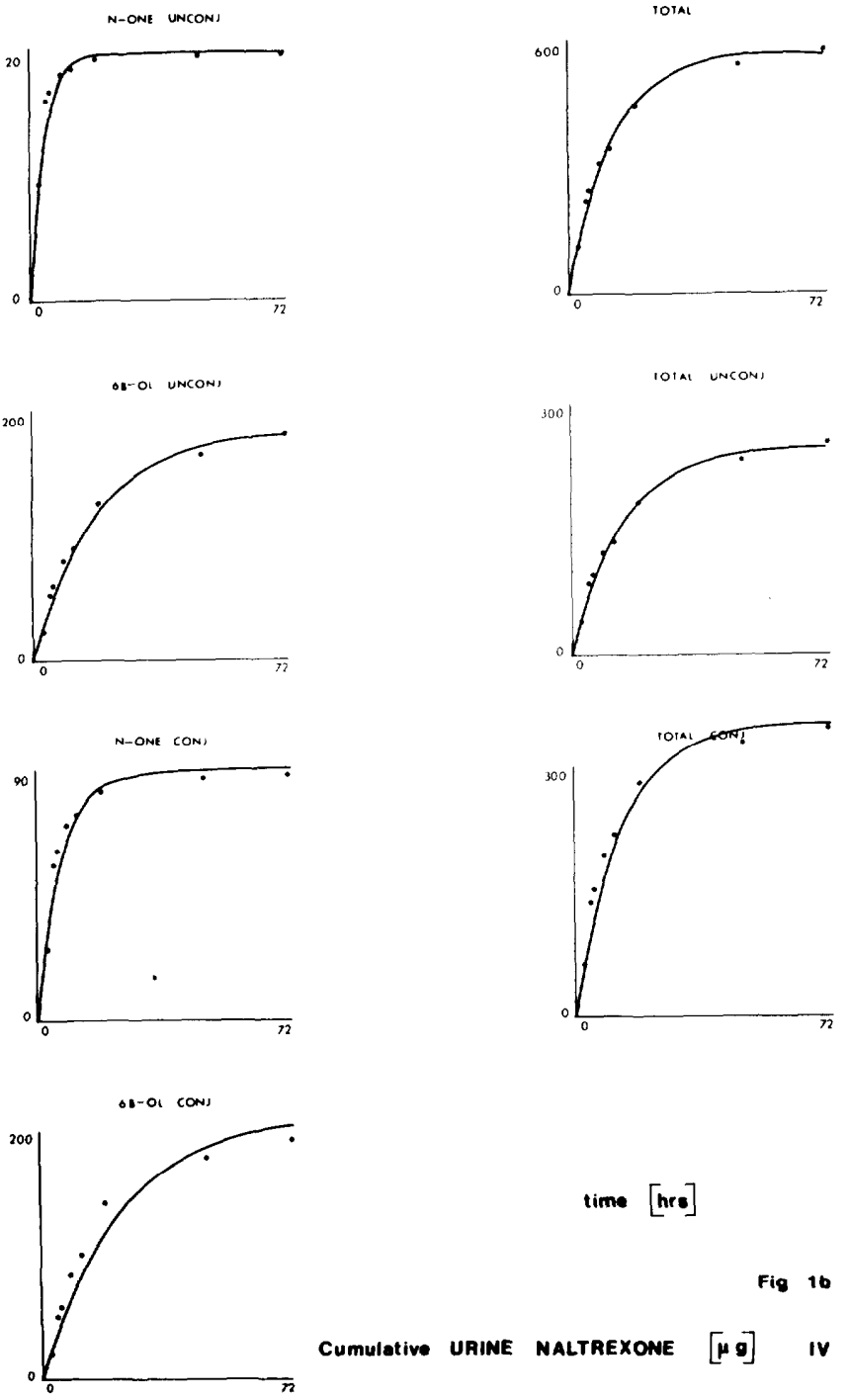

Fig 1b

Cumulative URINE NALTREXONE [μg] IV

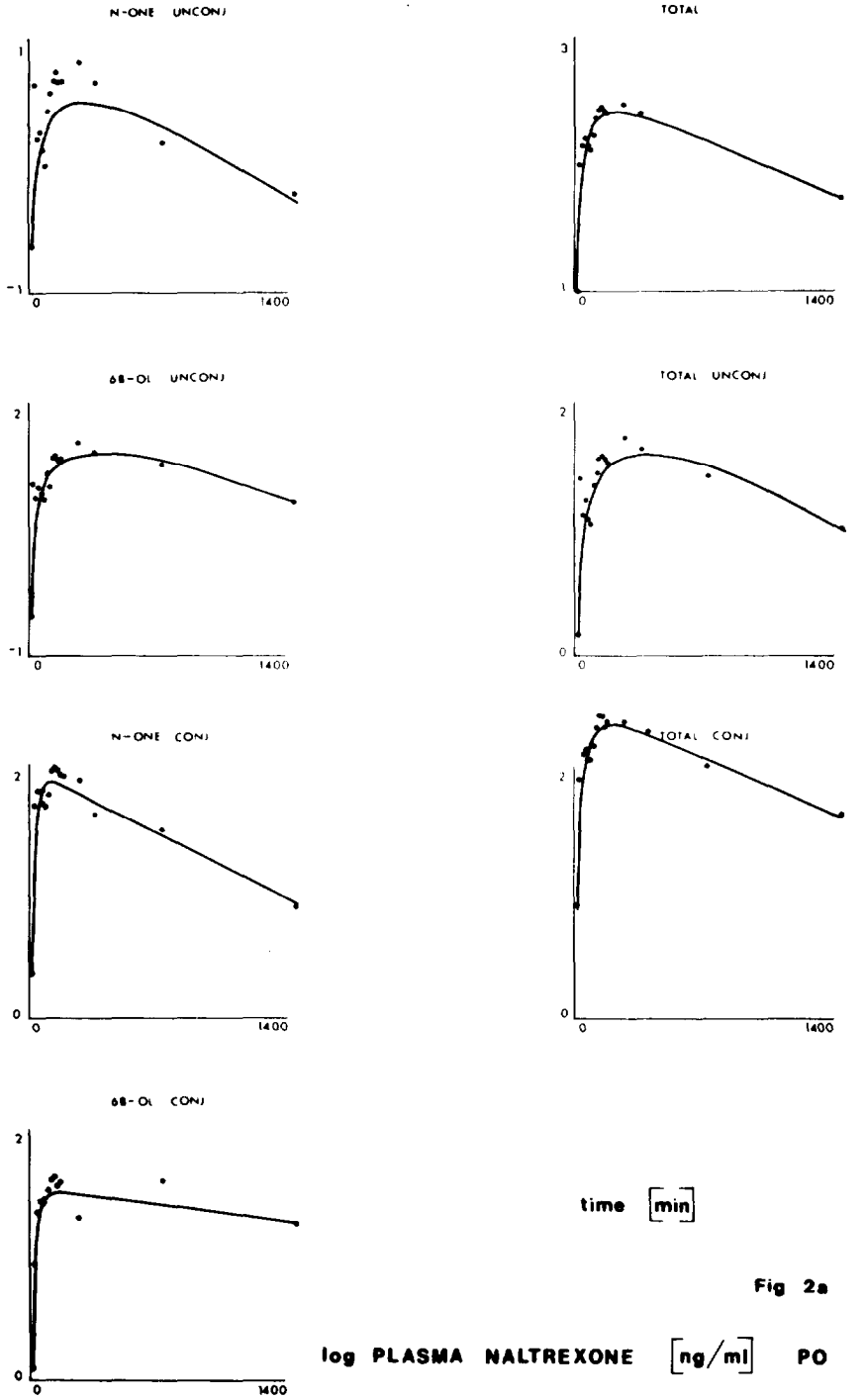
N-ONE UNCONJ
TOTAL
6B-OL UNCONJ
TOTAL UNCONJ
N-ONE CONJ
TOTAL CONJ
6B-OL CONJ
time [min]
log PLASMA NALTREXONE [ng/ml] PO

Fig 2a

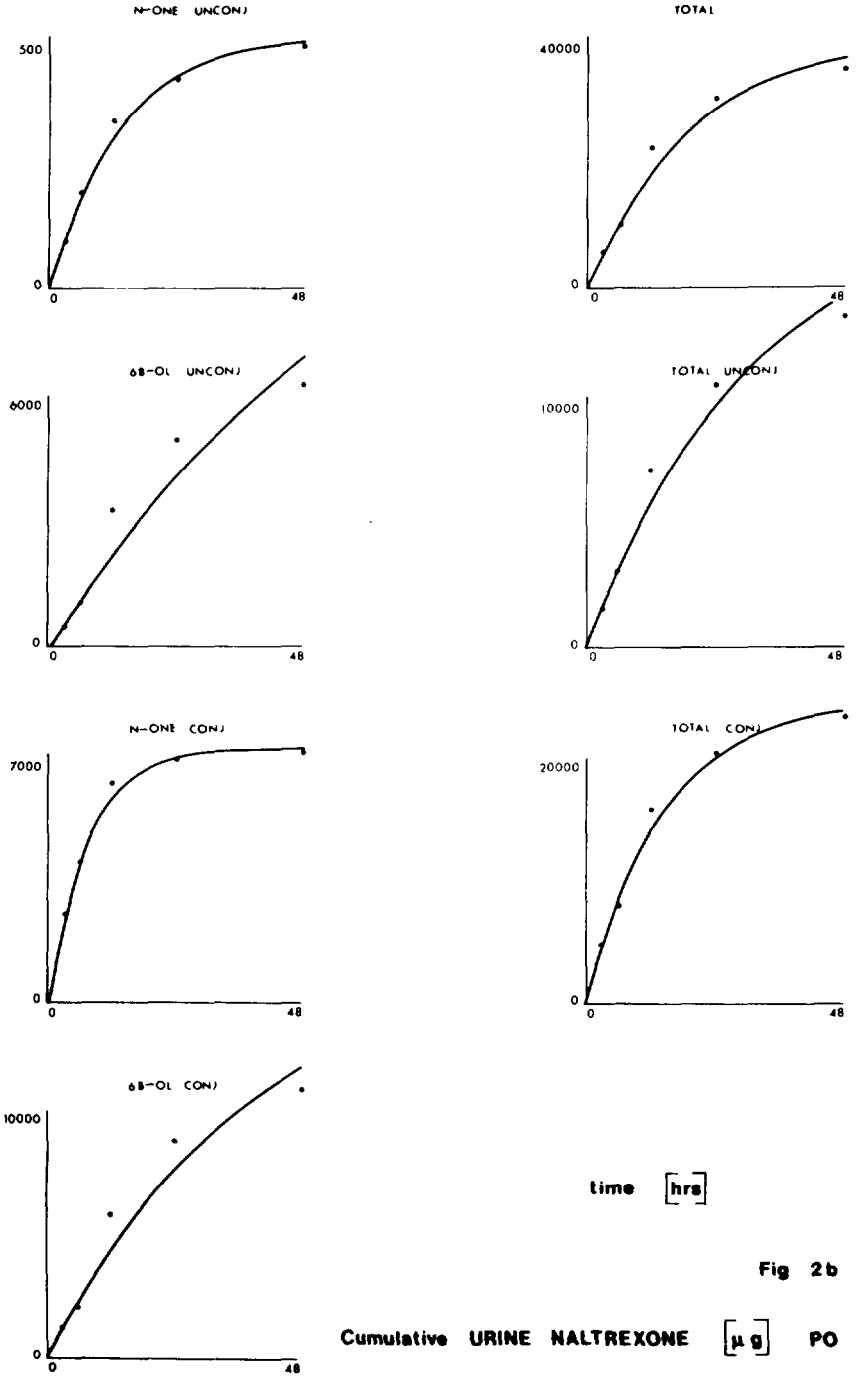

Fig 2b

Cumulative URINE NALTREXONE [µg] PO

TABLE 3a. Weighted Mean Values of Parameters-All Drug Forms

Drug form		A/D	Ct	Cu	fc	fd	k
TOTAL UNCONJ:	IV	1260 (80)	747 (46)	237 (23)	.294 (.021)	.302 (.016)	.0012 (.0001)
	PO	771 (56)	1210 (70) **	205 (11)	.220 (.030)	.200 (.033) *	.0011 (.0002)
TOTAL CONJ:	IV	2340 (420)	280 (45)	100 (70)	.371 (.075)	.240 (.044)	.0018 (.0001)
	PO	2500 (680)	220 (40)	117 (3)	.374 (.047)	.343 (.040)	.0014 (.0001)
6B-OL UNCONJ:	IV	616 (57)	1450 (120)	242 (17)	.218 (.012)	.243 (.018)	.0011 (.0001)
	PO	373 (40)	2250 (300) *	230 (35)	.140 (.030) *	.157 (.023) *	.0013 (.0003)
N-ONE UNCONJ:	IV	240 (37)	3350 (390)	93.3 (31.7)	.038 (.009)	.017 (.001)	.0045 (.0007)
	PO	96.5 (29.4)	4640 (1022)	70.6 (17.7)	.010 (.001) *	.010 (.001) **	.0010 (.0002) **
6B-OL CONJ:	IV	722 (81)	1100 (180)	200 (34)	.190 (.052)	.169 (.033)	.0012 (.0001)
	PO	1140 (160)	726 (84)	215 (4)	.304 (.078)	.156 (.018)	.0008 (.0001)
N-ONE CONJ:	IV	259 (51)	1800 (570)	135 (15)	.075 (.017)	.081 (.012)	.0037 (.0003)
	PO	284 (137)	1060 (280)	165 (6)	.071 (.027)	.074 (.025)	.0025 (.0002) *

See table 2 for explanation.

*Significant difference at level p = .025.

**Significant difference at level p = .005.

TABLE 3b. Weighted Mean Values of Parameters-All Drug Forms

Drug form		a1	a2	a3	V1	V2	V3
TOTAL UNCONJ:	IV	.503 (.162)	.0112 (.0045)	.0015 (.0001)	16.1 (7.6)	192 (23)	539 (29)
	PO	—	.0092 (.0020)	.0012 (.0002)	—	—	775 (111)
TOTAL CONJ:	IV	—	.0373 (.0025)	.0009 (.0001)	—	905 (100)	324 (41)
	PO	—	.0136 (.0017)	.0010 (.0002)	—	—	214 (35)
6B-OL UNCONJ:	IV	—	.0482 (.0165)	.0013 (.0001)	—	—	1110 (70)
	PO	—	.0031 (.0008)	.0015 (.0003)	—	—	163 (60)
N-ONE UNCONJ:	IV	.171 (.111)	.0095 (.0016)	.0043 (.0003)	—	321 (68)	1310 (114)
	PO	—	.0121 (.0046)	.0013 (.0001) **	—	—	3410 (640)
6B-OL CONJ:	IV	—	.0087 (.0033)	.0008 (.0001)	—	—	1300 (80)
	PO	—	.0082 (.0028)	.0006 (.0003)	—	—	1100 (330)
N-ONE CONJ:	IV	—	.0739 (.0345)	.0022 (.0003)	—	—	1100 (140)
	PO	—	.0225 (.0021)	.0021 (.0003)	—	—	595 (113)

See table 2 for explanation.

Since the IV and PO values of total and urinary clearances do not differ at level p = .15, the weighted mean total clearance is computed as 195 (10) ml/min and the weighted mean urinary clearance as 118 (4) ml/min. Their ratio is 0.605. The area under the plasma curve for total drug when computed for 1 mg of the drug is the same for both routes of administration. This implies that drug administered orally is completely absorbed into plasma.

DISCUSSION

Detailed analysis of individual curves confirmed essentially the results of the analysis of averaged data. Thus, it can be inferred that the conclusions drawn here from the analysis of human naltrexone kinetics are independent of the method of data treatment.

Comparison of the fraction of urinary to total clearance with the computed fraction of dose excreted through urine shows an agreement between both of these mutually independent methods that only up to 60% of the dose is excreted by urine. The remainder may be expected to be excreted through feces. Direct experimental proof of this conjecture does not exist so far. At 48 hours after an oral administration Wall and coworkers (1) found about 4% of the dose in feces. Similar data are not available for intravenous administration. However, due to a slow passage through lower intestine, the peak of the fecal excretion of the drug can be delayed beyond 48 hours.

The values of the exponential coefficient of urinary excretion k follow very closely those of a3, the exponential coefficient of the terminal phase of the plasma curve. This observation complies with the theoretical requirement that at least the terminal portion of both the plasma and the urine curves must have identical kinetic character. Then, the ratio of the urinary clearance to the terminal volume of distribution, Cu/V3, can estimate the fractional removal rate of the drug through urine. This ratio yields values which are about 60% to 70% of the value of a3, thus indicating by the third independent method that no more than two-thirds of the total drug leaves the body through urine.

While the slowest exponentials (a3) of IV and PO plasma curves do not differ significantly, there is a substantial discrepancy between the faster components of both curves. After oral administration one cannot find the fast exponentials of the IV curve. In fact, the faster exponential of the PO curve is still more than one order of magnitude slower than any of the two of the IV curve. The early kinetics of naltrexone after oral administration are governed by a

rate-limiting process which is associated with the route of administration. Therefore, the conclusion can be drawn that the exponential of the PO curve with half life of about 1 hour corresponds to that of gastrointestinal absorption.

CONCLUSIONS

1. About 60% of naltrexone is excreted by the kidney.
2. All orally administered naltrexone is absorbed into plasma.
3. Half life of gastrointestinal absorption is about 1 hour.
4. Half life of the terminal exponential is about 10 hours.
5. Half life of urinary excretion is about 9 hours.

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Pharmacokinetic Quantitation of Naltrexone Release From Several Sustained-Release Delivery Systems

R. H. Reuning, S. H. T. Liao, and A. E. Staubus

A method designed to quantitate in vivo naltrexone release rates from sustained-release systems has been applied to the evaluation of seven different naltrexone delivery systems in the monkey. The method consists of two phases: a single intravenous bolus dose quantitation of each monkey's pharmacokinetic parameters coupled with a delivery system study in which plasma naltrexone levels are measured throughout the time period of sustained-release. In vivo release rates and the total amount released are then calculated. It should be noted that these determinations require the analysis of unchanged naltrexone in plasma as the only experimental measurement. Data from injectable naltrexone pamoate microcapsule delivery systems indicate that 1) when these microcapsules are suspended in an aqueous vehicle, a significant part of the dose is released very rapidly, yielding release rate-time data that parallel a non-sustained-release control; 2) this rapid release for the aqueous vehicle is followed by a slow release phase lasting to about 24 days for the subcutaneous route and to about 45 days for the intramuscular route; and 3) when these microcapsules are suspended in an oily vehicle there is no initial rapid release, substantial release rates are obtained for at least 60 days, and an average of 89% of the dose is calculated to have been released. Data from implantable naltrexone delivery systems show that 1) the Alza system most closely ap-

proximates a zero-order release rate-time profile; 2) the Battelle system provides a rapid initial release followed by a slowly declining release mte; 3) the Dynatech system is characterized by a more rapid initial release rate of 3-8% of the dose per day over the first 3-5 days followed by a rather constant 1-3% per day to about day 36; and 4) *essentially corn lete recovery of the dose was obtained for the Battel e and Dynatech systems,*

INTRODUCTION

The rationale for developing sustained-release narcotic antagonist delivery systems for treatment of opiate addiction has recently been reviewed (1,2) One phase of a scheme for evaluating these systems consists of a pharmacokinetic quantitation of drug release rates *in vivo* (2). The methodology that has been developed for quantitating naltrexone release in monkeys is characterized by two phases: 1) calibration of the pharmacokinetics of each individual monkey from plasma level-time data obtained after an intravenous bolus dose of naltrexone, and 2) measurement of plasma levels of unchanged naltrexone over the time period that the sustained-release system yields measurable concentrations. Data from 1) and 2) above permit calculation of an *in vivo* release rate-time profile as well as the total amount of naltrexone released during the study.

The purpose of this report is to summarize the naltrexone release data for those delivery systems that have been evaluated pharmacokinetically in the monkey. In order to obtain an overview it was necessary to average the release rate data obtained from the several monkeys utilized in evaluating each delivery system. Also, data related to the calibration of each monkey's pharmacokinetic parameters has been omitted. Both types of data for individual monkeys will be included in subsequent manuscripts.

EXPERIMENTAL

Delivery Systems

The following seven delivery systems have been evaluated:

I. Naltrexone in a physical blend with 90% (L+)lactic acid-10% glycolic acid copolymer, spherical beads 1.5 mm in diameter, subcutaneous, Dynatech # 24086;

II. Naltrexone pamoate-polyclactic acid) microcapsules suspended in 2% aluminum monostearate-peanut oil and injected intramuscularly, Thies #GL-1-6-76-1;

III. Naltrexone pamoate-poly(lactic acid) microcapsules suspended in a medium consisting of water, 2% Tween 20, 0.02% anti-foam silicone and 1:10,000 phemerol and injected intramuscularly, Thies #GL-1-6-75-1;

IV. Naltrexone pamoate-poly(lactic acid) microcapsules suspended in an aqueous medium of 0.1% Tween 80 in Macrodex (6% dextran 70 in 5% dextrose/water for injection) and injected subcutaneously, Thies #GL-3-9-77-3;

V. Micronized naltrexone pamoate (batch # 2M-1869-866-16) suspended in 2% aluminum monostearate-peanut oil and injected intramuscularly;

VI. Rods-naltrexone and hydrophobic polymer, Chronomer, Alza, subcutaneous;

VII. Naltrexone 33% in a dipalmitin (75%) - tripalmitin (25%) mixture, shaped into rods and administered subcutaneously (Battelle).

These sustained release systems will be referred to by the numerical designation throughout the text. Additional data concerning these delivery systems has been provided by the developers (2,3). All are intended to be bio- degradable, with systems I, VI and VII designed for subcutaneous implant and systems II, III and IV designed for injection. System V was included as a non-sustained-release control.

Experiments in Monkeys

Each delivery system was administered to 3 or 4 monkeys at a dose of approximately 10 mg/kg. With the exception of delivery system VII, these were self-administrating monkeys and were on a rotating schedule of morphine, methamphetamine and saline self-injection. Effects of the naltrexone delivery system on morphine self-administration were measured as described previously (4) and will be reported separately. Blood samples were obtained, usually from a femoral vein, at periodic intervals up to 60 days after administration of the sustained release system.

At least several days were allowed to elapse after the delivery system either was removed or ceased releasing measurable amounts of naltrexone. Subsequently, a single intravenous bolus dose of naltrexone (3-5 mg/kg) was administered and periodic blood samples were obtained for a sufficient time so that the pertinent pharmacokinetic parameters of naltrexone could be determined from the plasma level-time profile.

DISCUSSION

Injectable Systems

The *in vivo* release rate data for the injectable naltrexone delivery systems are presented in fig. 1. Systems II, III and IV differ mainly in the vehicle used to suspend the microcapsules and in the route of injection. System V is a non-sustained-release control formulation included for comparative purposes. Comparison of the curve for sustained release system II (microcapsules suspended in the oily vehicle and administered intramuscularly) with the curve for the control formulation, system V *(micronized* naltrexone pamoate suspended in the oily vehicle and administered intramuscularly), permits the conclusion that the microcapsule coating is responsible for the pronounced sustained release effect with system II.

Assay for Naltrexone

A sensitive and specific assay for naltrexone concentrations in plasma has been described previously (5,6,7). The pharmacokinetic calculation of *in vivo* release rates is dependent on an assay that is specific for unchanged naltrexone and this specificity has been demonstrated with respect to the known metabolites of naltrexone (7).

Calculation of Release Rates

Release rates were calculated according to the Loo-Riegelman method (8). Either a two- or a three-compartment, open pharmacokinetic model was used to fit the plasma level-time data for the intravenous bolus dose of naltrexone in each monkey (9). The three-compartment model was utilized when needed to obtain a good overall fit to the data. The pharmacokinetic parameters for naltrexone, obtained from the intravenous bolus dose, were then utilized to calculate naltrexone release rates from the plasma naltrexone level-time data obtained in the delivery system study (8). The total amount released over the entire time period was subsequently determined according to an equation presented previously (10).

RESULTS

The data for naltrexone concentration in plasma as a function of time after administration are summarized in table 1 for each of the seven delivery systems tested. Levels of about 0.25-0.5 ng/ml are needed to block morphine self-administration in these monkeys (11). Since this range is also the approximate sensitivity limit of

TABLE 1. Naltrexone Concentration in Plasma During the Time Period in Which the Monkey was Administered a Naltrexone Sustained Release Delivery System

Time (day)	Mean Concentration of Naltrexone in Plasma (ng/ml)[a]						
	I	II	III[b]	IV	V	VI	VII[b]
0.125	—	—	—	—	—	—	5.55(1.31)
0.25	—	—	—	—	—	—	5.19(0.41)
0.29	4.11(0.87)	—	—	23.95(6.31)	—	4.57(2.54)	—
0.33	—	0.79(0.10)	13.87(5.39)	—	99.7(41.4)[b]	—	—
0.5	—	—	—	—	—	—	5.07(2.50)
1	4.69(4.90)	0.58(0.12)	4.40(2.03)	14.04(6.02)	44.55(13.66)	2.41(0.77)	3.19(1.03)
2	—	0.44(0.07)	—	—	10.63(7.97)	—	2.55(0.65)
3	8.08(5.36)	0.99(0.19)	1.12(0.21)	7.90(2.65)	3.12(2.46)	2.31(0.78)	—
4	—	2.45(0.69)[b,e]	—	—	1.70(1.32)	—	—
5	2.64(0.42)	2.13(0.75)	0.65(0.25)	2.60(1.07)	0.70(0.84)	3.57(2.31)	—
6	—	1.58(0.56)	—	—	—	—	—
7	2.14(0.43)	2.16(0.61)	0.55(0.18)	1.26(0.63)	0.11(0.23)	3.71(1.45)	2.06(0.43)
9	2.50(0.37)	—	0.92(0.26)	1.19(0.30)	[g]	3.84(1.64)	—
11	—	3.02(0.68)	—	—	—	—	—
12	2.07(0.24)	—	1.17(0.32)	0.57(0.04)[c]	[g]	—	—
13	—	—	—	—	—	5.80(4.0)	—
14	2.16(0.19)	2.87(0.51)	—	—	—	—	1.38(0.38)
15	—	—	1.47(0.72)	0.66(0.21)	—	—	—
16	—	—	—	—	—	4.66(1.58)	—
18	—	1.93(0.46)	—	—	—	—	—
19	1.96(0.10)	—	1.52(0.70)	—	—	—	—
20	—	—	—	0.56(0.38)	—	2.25(1.98)	—
21	—	2.24(0.71)	—	—	—	—	1.46(0.36)
22	—	—	1.23(0.50)	0.35(0.40)	—	—	—

23	1.88(0.096)	—	—	—	—	0.36(0.30)	—
25	—	1.72(0.56)	—	—	—	—	—
26	—	—	1.01(0.27)	0.28(0.33)	—	—	—
28	—	1.40(0.45)	—	—	—	—	0.80(0.18)
29	1.33(0.085)	—	0.83(0.28)	0.13(0.27)	—	—	—
30	—	—	—	—	—	0.06(0.1)[b]	—
32	—	1.37(0.26)	—	—	—	—	—
33	—	—	0.84(0.68)	0.14(0.28)	—	—	—
35	—	—	—	—	—	—	0.79(0.45)
36	0.48[d,f]	—	0.65(0.46)	0.13(0.27)	—	—	—
37	—	—	—	—	—	0.21(0.24)	—
40	—	—	0.56(0.38)	[g]	—	—	—
41	—	0.82(0.20)[b,f]	—	—	—	—	—
42	—	—	—	—	—	—	0.08(0.14)
43	1.39(0.028)[c,f]	—	0.23(0.23)	[g]	—	—	—
47	—	0.58(0.21)[c,f]	0.20(0.18)	[g]	—	—	—
50	[g]	—	0.36(0.35)	[g]	—	—	—
54	—	—	0.11(0.18)	—	—	—	—
56	—	0.60(0.21)[b,f]	—	—	—	—	—
57	—	—	—	[g]	—	—	—
64	—	0.64(0.39)	—	—	—	—	—

[a]Mean of 4 animals unless indicated otherwise. Standard deviation in parenthesis. Delivery systems are identified in the text and in Table 3.

[b]Mean of 3 animals.

[c]Mean of 2 animals.

[d]Only one value available.

[e]Sample(s) lost due to breakage.

[f]Sample(s) omitted due to apparent assay interference. Values of the omitted samples were at least 5-fold greater than corresponding values in other animals.

[g]Below detectable limits.

the assay, "measurable" levels can be considered to be "effective" levels in these monkeys. The corresponding average naltrexone release rates for the seven systems are shown in table 2 and in figures 1 and 2. Table 3 contains data summarizing the results of a comparison of the dose administered with the calculated amount of naltrexone released and, when available, the amount of naltrexone recovered from the delivery system after removal from the monkey.

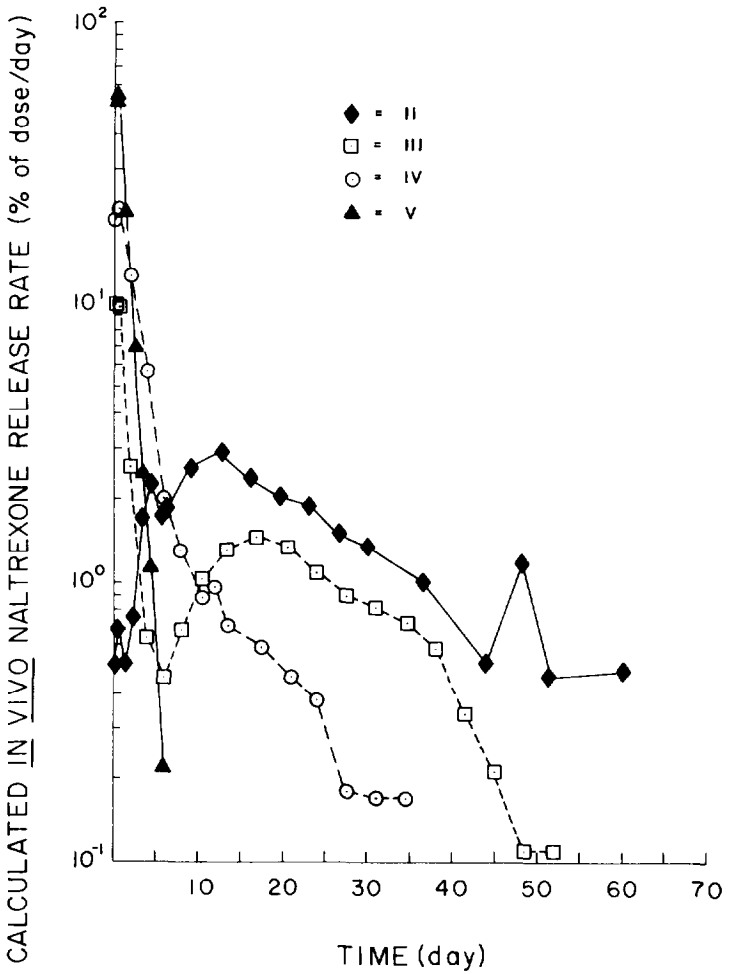

FIGURE 1. Semilog plot of naltrexone *in vivo* release rate as a function of time for injectable sustained release delivery systems II, III, IV and V. Delivery systems are identified in the text and in table 3. Closed symbols represent an oil vehicle and open symbols an aqueous vehicle.

The influence of the vehicle used to suspend the microcapsules on release of naltrexone can also be observed in fig. 1. A comparison of system III (microcapsules suspended in an aqueous vehicle and administered intramuscularly) with system II suggests that the influence of the vehicle occurs mainly over the first 15 days after administration. Subsequently, the release rate declines in approximately parallel exponential fashion for the two systems. During the first 4-6 days after administration, system III has a release rate-time profile that parallels the exponential decline of release rate for the non-sustained-release control. This rapid and extensive decline in release rate (note the logarithmic y-axis in fig. 1) suggests that a very significant fraction of the naltrexone in delivery system III was available for rapid release. A similar early rapid release can also be observed for system IV (microcapsules suspended in an aqueous vehicle and administered subcutaneously). A comparison of aqueous microcapsule suspension systems III and IV (fig. 1) suggests that the intramuscular route (III) yielded much higher release rates than the subcutaneous (IV) from 10-50 days after administration. Delivery system IV provides the smallest degree of sustained release of any of these microencapsulated naltrexone pamoate systems. On the other hand, system II provides a significant release rate for a period of 60 days. Although the release was not zero order, the rates were within a fairly narrow range (fig. 1). In addition, it appears that the oil vehicle "protects" the naltrexone pamoate microcapsules from whatever causes the rapid initial burst of release with the aqueous vehicle.

The calculated total extent of naltrexone release from each delivery system is compared with the dose administered in table 3. Systems III and IV yielded an incomplete recovery of the administered dose whereas the average recovery for system II was 89% of the dose. The microcapsule delivery systems (II, III and IV) were characterized by a high degree of variability in the extent of recovery of the administered dose (table 3). Part of the reason for the greater variability observed for the microcapsule systems, compared to the other delivery systems, may be the difficulty in administering an accurate dose of the microcapsule suspension (especially for the aqueous systems). Alternatively, the release of naltrexone may be more variable with these delivery systems. Unfortunately, the nature of these microcapsule delivery systems precluded the possibility of removing the microcapsules remaining at the end of the sustained release study. Therefore, the unabsorbed naltrexone could not be assayed directly.

TABLE 2. Mean Calculated Release Rates as a Function of Time After Administration for the Various Naltrexone Delivery Systems[a]

I[b]		II		III[b]		IV		V[b,e]		VI		VII[b]	
Time (day)	%/day	Time (day)	%/day	Time (day)	%/day	Time (day)	%/day	Time (day)	%/day	Time (day)	%/day	Time (day)	%/day
0.15	2.71(0.90)	0.16	0.50(0.17)	0.16	9.84(4.64)	0.15	19.6(7.82)	0.16	51.9(14.8)	0.15	2.51(1.31)	0.06	5.17(1.11)
0.64	5.28(4.43)	0.66	0.68(0.21)	0.66	9.70(4.44)	0.64	21.5(9.19)	0.66	54.5(3.16)	0.64	2.77(1.12)	0.19	5.54(0.72)
2.0	7.14(1.03)	1.5	0.51(0.19)	2.0	2.58(1.02)	2.0	12.4(4.06)	1.5	21.2(10.6)	2.0	1.93(0.54)	0.37	5.13(1.97)
4.0	5.24(2.36)	2.5	0.75(0.26)	4.0	0.64(0.23)	4.0	5.68(2.13)	2.5	6.86(5.70)	4.0	2.39(1.06)	0.75	3.92(1.44)
6.0	2.25(0.40)	3.5	1.68(0.46)[b]	6.0	0.46(0.22)	6.0	2.01(0.45)	3.5	2.50(1.63)	6.0	2.91(1.26)	1.5	2.82(0.95)
8.0	2.22(0.64)	4.5	2.24(0.39)[b]	8.0	0.68(0.13)	8.0	1.31(0.27)	4.5	1.14(0.99)	8.0	3.04(1.07)	4.5	2.28(0.25)
10.5	2.24(0.50)	5.5	1.74(0.43)	10.5	1.03(0.13)	10.5	0.88(0.02)[c]	6.0	0.22(0.38	11.0	4.06(1.60)	10.5	1.69(0.32)
13.0	2.16(0.48)	6.5	1.84(0.58)	13.5	1.31(0.48)	12.0	0.96(0.08)[c]			14.5	4.36(2.28)	17.5	1.41(0.29)
16.5	2.07(0.39)	9.0	2.56(0.83)	17.0	1.47(0.65)	13.5	0.70(0.11)[c]			18.0	2.78(1.39)	24.5	1.11(0.26)
21.0	1.89(0.21)	12.5	2.89(0.89)	20.5	1.34(0.55)	17.5	0.59(0.41)			21.5	0.92(0.90)	31.5	0.78(0.29)
26.0	1.58(0.22)	16.0	2.33(0.65)	24.0	1.09(0.37)	21.0	0.46(0.53)			26.5	0.13(0.15)	38.5	0.09(0.16)
32.5	1.09[d]	19.5	2.04(0.69)	27.5	0.90(0.32)	24.0	0.38(0.45)			33.5	0.08(0.14)[b]		
36.0	1.24[c]	23.0	1.91(0.64)	31.0	0.81(0.47)	27.5	0.18(0.36)						
		26.5	1.51(0.52)	34.5	0.72(0.52)	31.0	0.17(0.34)						
		30.0	1.35(0.43)	38.0	0.58(0.38)	34.5	0.17(0.34)						
		36.5	1.01(0.27)	41.5	0.34(0.32)								
		44.0	0.52(0.08)[c]	45.0	0.21(0.19)								
		48.0	1.18(0.39)[c]	48.5	0.11(0.20)								
		51.5	0.46(0.11)[c]	52.0	0.11(0.19)								
		60.0	0.48(0.15)[b]										

[a]Mean of 4 animals unless indicated otherwise. Standard deviation in parenthesis. Delivery systems are identified in text and in Table 3.

[b]Mean of 3 animals.

[c]Mean of 2 animals.

[d]Only one value available.

[e]One monkey was omitted from the data analysis because one early sample had an unusually high naltrexone concentration that resulted in an erroneously high recovery value.

TABLE 3. Comparison of the Naltrexone Dose Administered in Sustained Release Form with the Amount of Naltrexone Accounted for Experimentally in the Monkey

Delivery systems	Mean amount naltrexone administered (mg)	Mean calculated amount naltrexone released (mg)	Mean assayed amount remaining in delivery system (mg)	Percent of administered dose accounted for[b]
I	42.1[c]	38.9[c]	0.0023[c]	92(91-94)[C]
II	35.3	31.6	-	89(58-116)
III	36.3	18.3	-	53(30-74)[d]
IV	26.2	19.5	-	76(48-91)
V	37.8	32.4	-	86(78-100)[e]
VI	44.0	30.0	-	66(56-87)
VII	30.7	16.4	16.4[f]	107(92-120)[d]

[a]Delivery system identification: I = naltrexone in a physical blend with 90% (L +) lactic acid-10% glycolic acid copolymer, spherical beads 1.5 mm in diameter, subcutaneous, Dynatech #24086; II = naltrexone pamoate-poly(lactic acid) microcapsules suspended in 2% aluminum monostearate-peanut oil and injected intramuscularly, Thies #GL-1-6-76-1: III = naltrexone pamoate-poly(lactic acid) microcapsules suspended in a medium consisting of water, 2% Tween 20, 0.02% anti-foam silicone and 1:10,000 phemerol and injected intramuscularly (Thies #GL-1-6-75-1); IV = naltrexone pamoate-poly(lactic acid) microcapsules suspended in an aqueous medium of 0.1% Tween 80 in Macrodex (6% dextran 70 in 5% dextrose/water for injection) and injected subcutaneously (Thies #GL-3-9-77-3); V = micronized naltrexone pamoate (batch #2M-1869-866-16) suspended in 2% aluminum monostearate-peanut oil and injected intramuscularly; VI = rods containing naltrexone supplied by Alza and administered subcutaneously; VII = naltrexone 33% in a dipalmitin (75%)-tripalmitin (25%) mixture shaped in rods and administered subcutaneously (Battelle).

[b]Mean of 4 animals, unless stated otherwise. Range in parenthesis.

[c]Mean of 2 values. One animal became ill during the study and another had a sufficient number of plasma samples with assay interference that a quantitation of total amount released could not be made.

[d]Mean of 3 animals.

[e]Mean of 3 animals. One animal omitted because of a 163% recovery due to one high data point and an insufficient number of other data points to obtain an accurate estimate of the amount released.

The rods removed from each monkey were weighed, dissolved in chloroform, and assayed spectrophotometrically for naltrexone at 282 nm.

Subcutaneous Implants

The release rate-time profiles for the subcutaneous implant delivery systems are shown in fig. 2. System I (Dynatech) is characterized by a more rapid release over the first 6 days followed by a very constant rate of naltrexone release throughout the remainder of the study. System VI (Alza) yielded a release rate-time profile

that was closer to being constant over the entire release period than any of the other systems. However, this system also was the shortest of the three in terms of duration. Delivery system VII (Battelle) is characterized by a rapid initial rate of release followed by a slowly declining rate from day 2 to about day 38. Overall, system I provides the longest duration of meaningful release rates of naltrexone. The "initial burst" of release with this delivery system is larger than that observed with the other two implantable systems (fig. 2) but much smaller than that for the aqueous injectable systems (compare fig. 2 with fig. 1, noting the logarithmic ordinate in fig. 1).

The possibility of removal of the implanted delivery systems at termination of the sustained release study permits a more rigorous "mass balance" comparison of naltrexone dose with the sum of the calculated amount released plus the amount remaining in the delivery system. Such a comparison was carried out with systems I and VII (table 3). Assay of the removed delivery system for naltrexone content yielded negligible amounts in system I. However, about

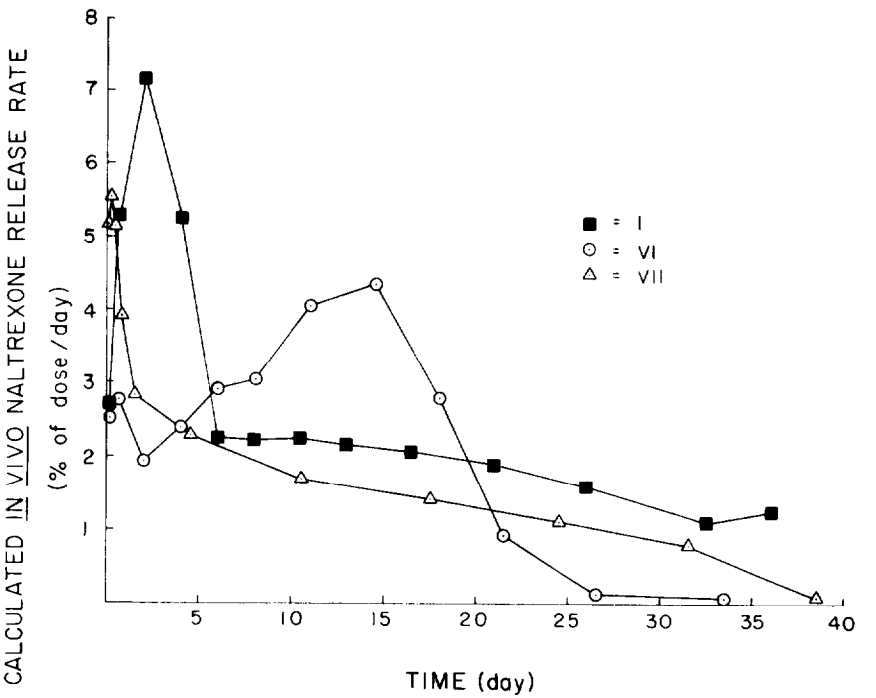

FIGURE 2. Linear plot of naltrexone in *vivo* release rate as a function of time for implantable sustained release delivery systems I, VI and VII. Delivery systems are identified in the text and in table 3.

half the dose remained in system VII at termination of the study. Data from both of these delivery systems provided an essentially complete accounting of the fate of the administered dose, as summarized in table 3. In system VI the device could not be removed at the end of the study and the lower recovery with this system may be due to unreleased drug. The variability in recovery between replicate monkeys was less with the three implantable naltrexone delivery systems than with the injectable microcapsule systems (table 3). This may be due to more accurate administration of the intended dose as well as to the ability to remove the system and assay for unreleased naltrexone.

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Part VI

Sustained-Release Systems

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A Review of Parenteral Sustained-Release Naltrexone Systems

James L. Olsen and Fred A. Kincl

The ideal naltrexone sustained-release delivery system should be easy to inject or implant, not cause adverse tissue reaction, release the drug at a relatively constant rate for at least 30 days, and biodegrade within a short time afterwards. Mechanisms which can be used for sustaining drug release include reducing solubility and surface area, coating, encapsulation and microencapsulation, complexation, binding and hydrophilic gelation. Drug release from such systems is controlled by diffusion through a barrier/film, diffusion from a monolithic device, erosion of the surface, hydrolysis, ion exchange, biodegradation, or a combination of these. Injectable systems would seem to be ultimately preferred because of the ease of administration and handling, while the implantable devices may find first use in man since they are easily removable, should that be necessary. Maintaining particulate-free products and sterilization methods are two problems with all parenteral dosage forms. Production must be particularly well controlled and validated.

INTRODUCTION

It is almost always advantageous to give fewer doses of a drug and at the same time to have more constant drug release. In the case of naltrexone, the relief from a decision process before each dose is perhaps reason enough for fewer, longer-acting doses. Furthermore, using a sustained-release (SR) parenteral device for treating narcotic addicts eliminates the need to give the patient

several days' oral dosage to take home or the need to have the patient return to the treatment center frequently for short-lived doses.

Another significant reason for using SR preparations for many drugs including naltrexone is that a lower constant dose instead of a multiple high dose regimen reduces side effects.

THE IDEAL SYSTEM

A sustained-release parenteral drug delivery system or device of naltrexone should have the following characteristics:

Be Easy To Inject or Implant

This means a volume of less than 1 cc, using a needle not larger than 18 gauge for injectables and a small rod, macrocapsule, or string of beads that are "trocarable" for implant forms should be considered for convenience of administration.

Be Pharmaceutically Acceptable

The preparation must be sterile, contamination free, pyrogen free, stable, have the proper strength, content uniformity and reproducible release characteristics, be easily handled at administration (suspendable, wettable particles, easily loaded implant devices) and be manufacturable on a large scale.

Not Cause Adverse Tissue Reaction

Part of the design of any SR system is to use materials which will be compatible with the body and not cause allergic reactions, sterile abcesses and a lump under the skin, pain on injection, or even discomfort.

Give Relatively Constant Drug Release

Few SR systems can give zero order naltrexone release, although some can. Fortunately, with naltrexone, there is a wide range between the effective dose and the toxic dose, so that SR systems giving a higher initial release rate can be used without much penalty. The 30-day release period is rather a compromise between achievable technology and desirability for an even longer-acting system. When our ability to produce acceptable drug delivery systems improves, it will then be advantageous to extend the duration of action.

Biodegrade

Constructing a SR system for naltrexone means adding at least one other component, be it polymer, polyglyceride, or whatever. This or these other components including solvents used in production must not only be compatible with the drug and the body but must also degrade into fractions which in turn can be eliminated without causing any side reaction.

Degradability must be present so that there are no "ghosts of doses past" present in the patient. Further, the last remnants of the device should be gone within a reasonable time after the drug itself is eliminated. We have used a rule of thumb that the entire system should be gone within a period of time equal to the release period, that is, if a system is effective for 30 days, then every component should disappear within another 30-day period. Even that way a patient would have two devices within his body, one active and one degrading.

MECHANISMS FOR ACHIEVING SUSTAINED RELEASE

Theoretical

If we look at one of the common formulas for describing rate of drug release from a solid, e.g.,

$$\frac{dm}{dt} = k\ A(S-C)$$

Where m = mass dissolved
C = concentration at time t
A = surface area of the solid
S = saturation solubility
k = dissolution rate constant,

$$k = \frac{D}{h}$$

D = diffusion coefficient
h = thickness of the diffusion layer

we see that there are three basic ways to reduce rate of dissolution.

a) Reduce surface area of the device. Use a sphere or rod; for example, one 1.5 mm bead has A = 7 mm^2. The same bead milled to 100μ particles = A = 106 mm^2.

b) Reduce (saturation) solubility. Make an insoluble complex, e.g., zinc tannate, or an insoluble mixture, dispersion or solid solution, or form an ester or ether.
c) Control the rate of diffusion or desorption from an inert matrix. These can be used in combination as has been done in several instances by combining hydrophobic sub stances with naltrexone in a rod or bead form. For example, specific mechanisms have been used for many years with oral dosage forms and may be adapted to parenterals.

Coating

A coating on a drug particle or device acts as a film or barrier through which the drug must diffuse, degrade, or rupture, releasing all of the contents. Coated products can further be classified into macrocapsules and microcapsules. There do exist nanocapsules; however these are considered too small to remain *in situ* and will not be considered further.

Macrocapsules are single units which contain all of the drug in one capsule. They are therefore implantable devices. The release of drug is governed by transport through the intact film barrier. The outstanding characteristics of such devices are that the effective surface area of the capsule is constant throughout the life of the film (at least until degradation starts), and also that "excess" drug inside the capsule maintains a constant saturated solution inside the capsule. The serious disadvantage of a macrocapsule (besides implanting) is that the film could rupture and release all of the drug at once.

Microcapsules are those having a diameter of from a few microns to perhaps 500 microns. Here the drug is present in many individual capsules which collectively resemble a powder for a given dose. The dose ostensibly could be given, for example, as a suspension of the microcapsules in isotonic saline solution.

The mechanism of drug release from microcapsules could be as in macrocapsules, that is, diffusion through a film. However, given the nature of the microencapsulation process, the biodegradability of the coating and the thickness of the films, drug release is most probably accompanied by film rupture. A SR dose would consist of many capsules, the most perfectly coated not releasing drug for some time, and the imperfect coatings releasing the drug sooner. In actuality both diffusion and rupture probably occur in any drug release profile.

Insoluble Salts, Complexation and Binding

These systems reduce the solubility of naltrexone, thus prolonging release of the drug. Examples would include zinc tannate, ion-exchange, solid solutions, and dispersions of the drug in insoluble carriers. Physical forms of these systems can be injectable suspensions of particles or implantable rods and beads. They are generally monolithic particles in any case, that is, having the same composition throughout the particle from surface to the center.

Hydrophilic or Hydrophobic Vehicles (matrices)

Hydrophilic materials are water loving to such an extent that they form a hydrated gel around the particle, thus increasing the diffusion layer and "protecting" the drug from rapid dissolution. Materials such as sodium alginate have been used although there is a question whether or not the FDA would regard them as safe for human parenteral use. Hydrophobic polymers such as Alza's Chronomer (1) series effectively waterproof the particle until they hydrolyze and are eroded from the surface, thus releasing more dispersed drug. An advantage of the hydrophobic mechanism is that release rate is zero order and not dependent upon the amount of drug remaining in the device.

Prodrugs

A prodrug is a mechanism for sustaining release of a drug by chemically binding to a polymer backbone. The drug would be essentially insoluble until the bonds are hydrolyzed or enzymatically cleaved. The release rate is governed by the degradation rate of the drug-polymer bond.

The prodrug must still be formulated into an appropriate dosage form, such as a suspension or an implant. The particular form is dependent upon the hydrolysis rate of drug from the polymer.

Combinations of the above mechanisms can be and have been made. Microcapsules were made by coating an insoluble salt of naltrexone, for example.

PRODUCTION PROBLEMS

The process in which SR preparations are made is important for a number of reasons, most having to do with the fact that the systems are parenteral solids.

Scale-up

Roth directions of scale-up are important; that is, some production methods cannot be dependably scaled up and some cannot be well scaled down. There was difficulty in scaling down the microencapsulation process of coacervation for development purposes, for example, because it is basically a large-scale operation. Agitation, surface area and cooling rates all vary with batch size. Some operations like molding and compression are easily done with small quantities but are almost impossible in large-scale production. High energy production is also easily done with small batches (e.g., blenders and ultrasonification) but difficult in even medium size batches.

Raw Material Supplies

Material supplies are a scale-up problem to some extent in that larger supplies of all components will be required if any of the devices proves successful. Can a large batch of polymer be made with the same characteristics as the small developmental ones? Some components are just plain expensive (tripalmitin, for example). How does this cost translate to a large batch? Availability, regardless of cost, may be a problem. Production capacity may not exist for what was once only an interesting, little-used chemical.

Batch Production

Rear in mind that parenterals must be sterile, pyrogen free and foreign particle free. In addition the products must be made according to current good manufacturing practices (GMP's) and also according to part of the current Large Volume Parenteral GMP's in lieu of published Small Volume Parenteral GMP's. Copious records are required, and in fact few facilities are available that can undertake such production.

CONCLUSION

Regardless of the many difficulties in development and production, a good deal of progress has been made in the last several years. We are now on the verge of entering phase I human trials with the Dynatech PLGA bead preparation (2). Supplies are on hand with which to begin such trials and the toxicity studies are almost completed.

It is an exciting time for all who have been involved with the project, from Dr. Seymour Yolles, of the University of Delaware,

who first demonstrated sustained release of a narcotic antagonist with his PLA particles, to Dr. Robert Willette, who has been masterminding the SA narcotic antagonist program. There has been significant input from the National Institute for Child Health and Human Development, from the consultants in various fields, and from the contractors.

All who have contributed have not only furthered the NIDA effort but have also expanded the scientific and practical knowledge surrounding sustained action parenteral systems.

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Development of Drug Delivery Systems for Use in Treatment of Narcotic Addiction

A. Carl Sharon and Donald L. Wise

The long term goal of the NIDA narcotic antagonist program of developing an implantable, biodegradable, naltrexone/PLGA matrix system which will sustain the delivery of naltrexone to biological systems for one month has been achieved. The dosage forms which provide the desired delivery characteristics are 1.5 mm diameter beads composed of 70% by weight naltrexone base in 40,000 molecular weight, 90L/10G poly (L(+)lactic-co-glycolic acid). Other dosage forms, including 1.5 mm diameter rods which provide 6 months' naltrexone release, finely divided injectable powders which provide up to 30 days' naltrexone release, and 1.5 mm diameter rods which provide 2 weeks' sustained delivery of morphine, have also been investigated. In vitro and in vivo release rates have been determined by measuring chemical concentrations in pH 7 buffer solution and urine, respectively. In vivo efficacy of naltrexone sustained delivery devices has been measured by direct challenge with morphine (Dewey-Harris mouse tail-flick test) and inhibition of morphine self-administration in monkeys. Good Manufacturing Practices documentation has been developed and used to produce a large batch of the 1.5 mm diameter naltrexone bead dosage forms at an FDA-registered pharmaceutical manufacturer. These beads, produced at the University of North Carolina School of Pharmacy, are awaiting use in human clinical trials.

INTRODUCTION

A problem universal to chemotherapeutic programs is the administration of drugs to patients at safe and effective levels for extended periods of time. Much technical activity has been devoted to devising novel systems, and improving currently existing ones, for regulating drug delivery.

One important category of the dosage forms under investigation is an implantable, tissue absorbable preparation which releases chemical (e.g., pharmaceuticals, fertilizers, and pesticides) at a constant or controlled rate to biological systems. There are two fundamentally different approaches to achieve this end. One relies on chemical alteration of the agent so that absorption by the system is delayed. The second leaves the active agent chemically unchanged, but physically restricts its bodily availability. This is achieved by injecting the agent in a carrier solution (e.g., oil and saline) and by encapsulating the chemical in an absorbable, release-regulating material.

Polymers of lactide and glycolide have been evaluated as release-regulating materials at Dynatech R/D Co. Polylactic/glycolic acids are tissue compatible and hydrolyze to lactic and glycolic acids, which are then absorbed into the body. The rate of hydrolysis is controllable, and dependent on the lactic/glycolic moiety ratio and copolymer molecular weight. Solid solutions/mixtures of the narcotic antagonist naltrexone and copolymers of lactic and glycolic acids were prepared and evaluated *in vitro* and *in vivo* for utility as long term, controlled delivery dosage systems.

Two delivery systems have been developed by Dynatech R/D Co. for NIDA under Contract Number HSM-42-73-267. One, a 70% w/w naltrexone base in 90L/10G PLGA, 1.5 mm diameter "bead," provides controlled delivery of the drug for approximately 30 days when subcutaneously implanted. Good Manufacturing Practices documentation has been developed for the production of this dosage form. A large batch of these beads has been produced under GMP documentation, suitable for use in human clinical trials, at the UNC Chapel Hill School of Pharmacy, under the supervision of Dynatech engineers. The other system, composed of 33% w/w naltrexone pamoate in high molecular weight 90L/10G PLGA, 1.5 mm diameter rods, coated with the same polymer, provides over six months' controlled delivery of naltrexone. This paper summarizes the development, testing, and production of these drug delivery systems.

PREPARATION AND ANALYSIS OF MATERIALS

Polymer Synthesis

L-lactide and glycolide were obtained commercially or synthesized from their respective acids, and purified by multiple recrystallization. Polylactic acid, and poly-(L(+)-lactic-co-glycolic acid) (PLGA) were synthesized in the ratio 75% w/w L-lactic acid (L)/ 25% glycolic acid (G) and 90% w/w L/25% w/w G by oven bulk polymerization. Para-toluene sulfonic acid was used as catalyst. Molecular weights of these polymers were determined by gel permeation chromatography (GPC), membrane osmometry, and light scattering to range from 40,000 to 200,000. Molecular weights of poly-dl-lactic-acid polymers were obtained by absolute methods. A comparison of these results with the intrinsic viscosity of poly-dl-lactic acid solutions in tetrahydrofuran (THF) at 37°C, yielded an approximate correlation between molecular weight and intrinsic viscosity. All polymer molecular weights reported here are weight-average molecular weights (M_w) obtained by gel permeation chromatography, unless otherwise noted.

Preparation of Delivery Vehicles

The free base and pamoate forms of the narcotic antagonist, naltrexone (supplied by NIDA), were formulated with polymer into delivery vehicles for this study. Naltrexone pamoate was chosen for use with longer term delivery systems because of its low solubility in pH 7 buffer solution (approximately 0.5 mg/ml) relative to that of naltrexone base (solubility $\cong$ 4 mg/ml). Radio-labelled naltrexone base, tritiated at the 15 and 16 positions, was used to follow naltrexone excretion in the urine and feces of some animals.

Naltrexone and PLGA were codissolved and intimately mixed in THF or methylene chloride (CH_2Cl_2) by overnight rolling on a ball mill. The solutions were cast in thin films (<0.25 mm) on clean glass plates, and the solvent allowed to flash off. The resulting films were of uniform consistency. Naltrexone pamoate/PLGA films appeared to contain small naltrexone pamoate crystals encased in polymer. Naltrexone base/PLGA films appeared clear at low naltrexone loadings, and cloudy at high drug loadings. This indicates that a naltrexone base forms a solid solution with PLGA, at least at low drug loadings.

After the solvent flashed off, the naltrexone/PLGA films were removed from the glass plates, and the remaining solvent removed by evacuation (< 2 Tori) at 65°C for two to five days. Residual solvent contents of these films were determined to be less than 2000

ppm. The films were then formed into implantable shapes: "beads" of 1.5 mm (nominal) diameter were prepared by transfer molding, and cylindrical rods of 1.5 mm diameter were prepared by extrusion. A thin polymer coating was added to selected samples by dipping the beads or rods into a 10% w/v polymer solution in CH_2Cl_2. Dip-coated samples were dried under vacuum at 50°C for 48 hours. The resultant coating was estimated to be 25 to 75 mm thick.

In Vitro Analysis

The *in vitro* release of drug from sustained-delivery vehicles was monitored by suspending beads or rods in Whatman extraction thimbles in test tubes containing 30 ml of Fisher pH 7 phosphate buffer solution. The test tubes were stoppered and maintained at 37°C in an American Optical Co. shaker bath. Naltrexone concentrations of the buffer solutions were determined spectrophotometrically for samples containing unlabelled drugs, and by means of a Beckman LS100 liquid scintillation counter for ^{3}H-labelled drugs. After each concentration measurement, the extraction thimble was drained, and buffer solution replaced in order to keep the naltrexone concentration in the buffer below 20% of saturation. Above this level, the bulk buffer solution no longer acts as a perfect sink for naltrexone, and drug concentration in the buffer begins to affect drug release from the sustained release device.

In Vivo Analysis

In vivo testing of naltrexone sustained-delivery devices was performed in mice and monkeys. Mouse testing was performed at SISA, Incorporated, Cambridge, MA, and monkey testing at Warner-Lambert/Parke-Davis, Ann Arbor, MI. After implantation with the sustained delivery device, all mice were tested for narcotic antagonism effect by morphine challenge. Urine and feces, or urine alone, of those mice which were implanted with ^{3}H-labelled naltrexone were also collected. Excretion of drug and metabolite was thus monitored. Monkeys trained to self-administer morphine were implanted with sustained-release devices, and their demand for morphine was monitored regularly. Urine and feces, or urine alone, of those monkeys implanted with ^{3}H-labelled naltrexone were collected for drug excretion analysis. Blood samples were also drawn periodically from selected monkeys for naltrexone blood level analysis by Reuning, Ashcraft, and Morrison, at Ohio State University (this volume).

In vivo test methods are briefly described below. Complete test method descriptions, along with results, are fully described elsewhere in this monograph.

In Vivo Analysis in Mice

Male albino Charles River mice (18-22 g) were used throughout these studies. The mice were anesthetized with Penthrane (Abbott) and a small slit was made in the scapular region. A single rod or three beads were inserted below the slit and the wound was closed by a silk suture. The animals were allowed to recover. Groups of ten animals were then placed in cages and allowed to develop normally until the test date. Ten individual mice were placed in separate metabolism cages for collection of urine.

The urine was collected daily for the duration of the experiment. Cages were rinsed with distilled water and the washings were added to the daily urine collections, which were made up to ten ml with distilled water. A 1.0 ml sample was taken and added to 10.0 ml of Aquasol liquid scintillation cocktail (New England Nuclear) in a vial. The radioactivity was measured using a Beckman LS-230 liquid scintillation counter. A quench curve was constructed using a series of ^{3}H quench standards and this curve was used to correct all counts.

The mouse tail-flick procedure was used throughout this study. A ten second cut-off time was employed, and a control reaction time of two to four seconds was used. A rheostat, incorporated into the instrument, was used to adjust the intensity of the light falling on the tail of the mouse such that each reaction time fell within the stated range. Animals with a control reaction time outside the stated range were rejected. The rheostat adjustment was only made if a significant proportion (more than two out of every ten mice) of the reaction times was outside the range of two to four sec. Groups of ten mice were medicated with an ED_{80} dose (14.0 mg/kg ip) of morphine sulphate, intraperitoneally and re-exposed to the noxious stimulus twenty minutes later. The analgesic response was calculated by Harris and Pierson as the percentage of the maximum possible response time (1). The following formula was used for these calculations: (test-control/10-control) x 100 = % maximum possible morphine analgesia effect.

Each group of animals was tested by the narcotic antagonist procedure only once. Groups were planned so that testing was performed every 7 or 10 days.

At the termination of each experiment, the animals were sacrificed and the implant site was examined. The condition of the implanted material and signs of encapsulation, injection, or irritation

were noted. All testing was performed under the supervision of Dr. John S. Howes at SISA, Inc., Cambridge, MA.

In Vivo Analysis in Monkeys

In vivo analysis of sustained-release naltrexone delivery systems in rhesus monkeys was carried out by Harrigan and Downs, at Warner-Lambert/Parke-Davis. The reader is referred to their description elsewhere in this volume.

RESULTS AND DISCUSSION

The objectives of this study were to develop implantable, biodegradable naltrexone-polylactic/glycolic acid matrix systems which will sustain the delivery of chemical to a biological system for both one and six months. Primary to achieving these objectives are the development of reproducible polymerization procedures using catalysts acceptable for use in parenterals, the fabrication of matrices from polymers which hydrolyze within specified times, and the selection of a practical sterilization process.

Experiments were performed to ascertain the effects on chemical release rate and release duration by the

- degree of chemical loading,
- solubility of the chemical in both the surrounding environment and the polymer,
- surface area/volume ratio and porosity of the matrix,
- hydrolysis rate of the polymer (i.e., composition of polymer), and
- polymer molecular weight.

Quantitative evaluations of most of these parameters have been completed.

The degree of chemical loading and the solubilities have been identified as most influential on the performance of the implant. The duration of chemical delivery is inversely proportional to the drug loading level and the solubility of the chemical in the surrounding environment. Thus, matrices designed to meet the six-month goal are prepared with naltrexone pamoate, which is less soluble in aqueous solutions than naltrexone base. Release duration is directly proportional to the solubility of the drug in the polymer. The effects of chemical solubility in the polymer have been especially important in this study--the base form of naltrex-

one forms a solid solution with polylactic/glycolic acids. This behavior permits the use of the moderately water soluble naltrexone base for long duration implants.

In other experiments it has been determined that dip-coating the implants with a thin layer of polylactic/glycolic acid reduced the initial surge of chemical from the surface of the implant and extended the duration of delivery. The releases of chemical from rods, spheres, and micron-size particle matrices have been measured.

Based on the above findings, matrices have been fabricated specifically to meet the aforementioned goals. These systems have been tested *in vitro* and *in vivo.* One matrix system, dip-coated rods of 33% by weight naltrexone pamoate in 90 L(+)/10G polylactic/glycolic acid, has proven antagonistic to morphine challenge for 220 days in mice (dosage 8 mg naltrexone pamoate/mouse).

Another system, composed of 70% by weight naltrexone base in 90L/10G PLGA has reproducibly proven antagonistic to morphine challenge for about 30 days in mice and monkeys. Related work has also shown that an injectable form of this matrix system shows promise of providing up to 30 days antagonism to morphine challenge.

Polymer Synthesis and Selection

Polymers have been synthesized from dl-(racemic) and L(+)-lactic and glycolic acids (G), in ratios ranging from 25% w/w L(+) or dl and 75% w/w G, to 100% L(+) or dl-lactic acid. Molecular weights ranging from 40,000 to 300,000 (measured by intrinsic viscosity) have been obtained. The catalysts triethyl aluminum (TEAL), triphenyltin (TΦ′T), and para-toluene sulfonic acid (PTSA) were used to initiate the reactions, and both oven-bulk and solution polymerization techniques were used. Polymers made from dl-lactic acid became sticky and amorphous when blended with naltrexone base. L(+)-lactic acid polymers remained crystalline to 48°C (the glass transition temperature of PLGA's) and so were selected for use in implants. *In vitro* and *in vivo* testing was conducted with naltrexone base and pamoate in PLGA composites. Dosages were in the form of 1.5 mm diameter rods and beads. Drug loading ranged from 17% to 90%. Experiments with various dosage forms and loadings indicated that 90% L/10% G polymers displayed the most desirable release characteristics. It was also determined that polymers synthesized with PTSA catalyst, in the molecular weight range of 30,000-50,000 were suitable for a 1-month delivery system, and polymers synthesized with TEAL catalyst $M_w \cong 200{,}000$, were suitable for a 6-month duration delivery system.

Batch to Batch Polymer Reproducibility

A critical task in the development of any drug/polymer sustaineddelivery system is demonstration of polymer reproducibility, enabling the production of a number of batches of dosage forms. When a promising 1-month sustained delivery system had been developed by this program, efforts turned to reproducibly manufacturing the polymer component of the sustained-delivery system. To that end, the synthesis of five ten-gram batches of 90L/10G polymer was undertaken. One percent PTSA was used as a catalyst. Polymers with a molecular weight of approximately 40,000 were sought.

Table 1 contains the characteristics of the five polymer batches produced in this study. The table clearly shows the reproducibility of the polymer synthesis operation; the five batches possess M_w's within ± 2000 of the mean, 43,000. Other polymer characteristics also demonstrate batch-to-batch similarity, and are approximately identical with those desired.

TABLE 1. Summary of Polymer Characteristics Demonstrating Batch-to-Batch Reproducibility

Batch Number	Mw	Mn	Dispersity	Viscosity	Mw from Viscosity	Specific Rotation	% Lactide from Sp. Rotation
31547	41,000	23,000	1.81	0.4	60,000	2.95	91.8
31553-A	42,000	24,000	1.72	0.37	53,000	2.85	89.0
31553-B	41,000	24,000	1.72	0.4	60,000	2.90	90.6
31563-A	45,000	25,000	1.80	0.35	50,000	2.95	92.2
31563-B	41,000	22,000	1.88	0.39	59,000	2.95	92.2

Development of Sustained-Delivery Systems for Naltrexone

One-Month System

The primary goal of this work was the development of an implantable sustained-delivery system that would provide antagonistic levels of naltrexone to the patient for about 30 days, and would be removable in case of adverse reaction to the implant. Other desirable features of the system would include high drug loading, biodegradability, and ease of implantation. These characteristics were all satisfied by the 70% w/w naltrexone, 30% w/w, 40,000 M_w, 90L/10G PLGA, 1.5 mm diameter bead dosage form. This form was selected after extensive testing with polymers of M_w's of 40,000 to 200,000, lactide/glycolide ratios of 25L/75G to 100 L, and formulations with drug loadings from 17% to 90% by weight.

Experimental release rate and narcotic antagonism results from *in vitro* and *in vivo* (mice and monkeys) testing are presented in figure 1. These data reveal approximately 30 days' controlled delivery of sufficient naltrexone to animals to effect morphine antagonism. Urinary, and in some cases fecal, recovery of tritium-labelled naltrexone yielded *in vivo* release data.

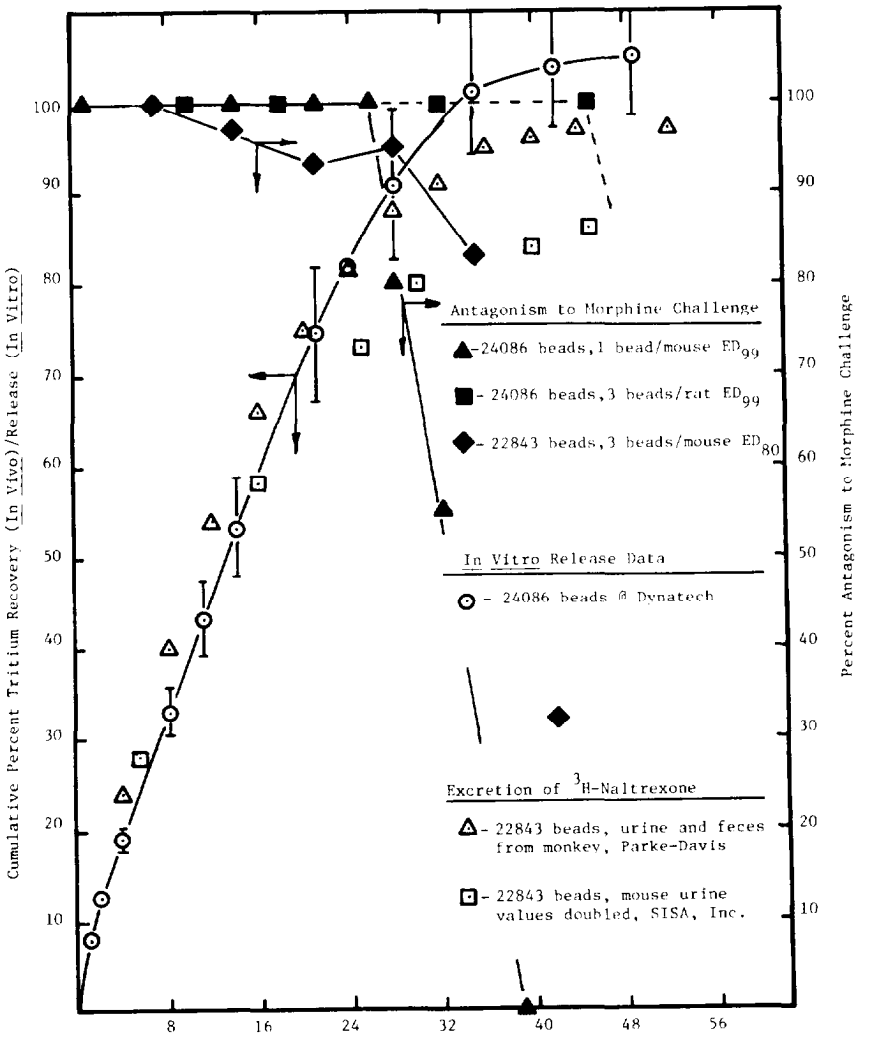


FIGURE 1. Comparison of *in vitro* and *in vivo* release rates and *in vivo* narcotic antagonism of two batches of 70% by weight naltrexone, 90/10 PLGA, 1.5 mm diameter beads (batch numbers 22843 and 24086) (references 2,3,4,5,6,7)

Another important result reflected in the graph is that bead release rate and antagonism are consistent from batch to batch. Figure 1 shows narcotic antagonism in mice from two batches of beads: #22843, produced in January 1976, and #24086, produced during December 1976-March 1977. Figure 2 compares narcotic antagonism in monkeys for the same two batches. This figure also shows the narcotic antagonism effects of continuous naltrexone infusion (10 mg/kg/hr) into monkeys (open circles). Consistent, complete narcotic antagonism is shown through thirty days, for both

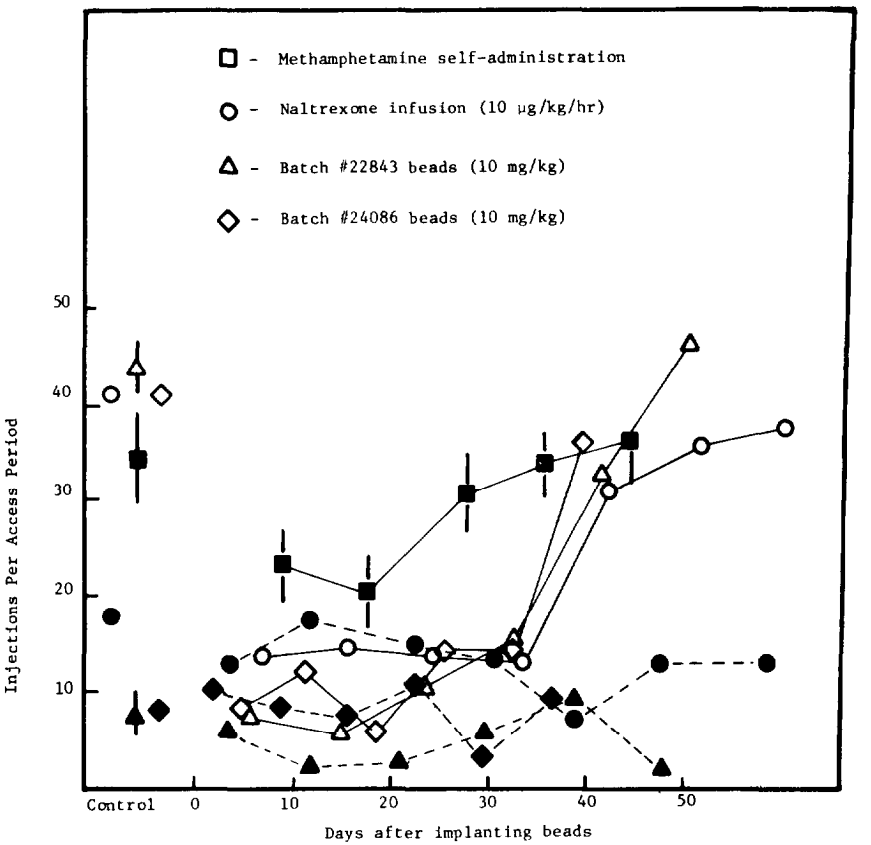


FIGURE 2. Superimposed graphs of morphine self-administration following: naltrexone infusion (10 µg/kg/hr for 37 days; open circles), and implantation of 70% naltrexone beads (#22843, open triangles; # 24086, open diamonds). Saline self-administration corresponding to infusion or bead implantation is represented by solid figures. Batch numbers for each solid figure correspond to morphine self-administration open figures. Solid squares indicate methamphetamine self-administration during continuous naltrexone infusion (references 2,3,4).

batches of beads tested (open triangles and diamonds). Rapid recovery of monkeys to control levels of morphine self-administration is observed after 35 days. It is notable that monkeys dosed with beads display this rapid recovery, very much as monkeys in whom naltrexone infusion was abruptly discontinued.

Six-Month System

A secondary goal of this work was the development of a six-month sustained-delivery system. As with the one-month system, it was desired that this system be implantable and removable. These characteristics were satisfied with 1.5 mm diameter rods containing 33% by weight naltrexone pamoate. The rods were composed primarily of 90L/10G PLGA, and dip-coated in a 10% w/v solution of the same polymer. This system has proven antagonistic to morphine challenge for 220 days in mice. Dosage of naltrexone pamoate implanted was 8 mg/mouse.

Figure 3 summarizes the results of experiments with this sustained-delivery system. Significant antagonism to morphine challenge was observed beginning at 140 days. Erratic performance prior to this time was attributed to a lower than effective release rate caused by a too-thick coating. After 220 days, antagonism remained at 100%. At day 240, all mice but one showed 100% antagonism. No polymeric residue could be recovered from this mouse. At day 26, three mice exhibited 100% antagonism, two mice 0%. At day 280 all mice exhibited no antagonism to morphine challenge, and no polymeric residue could be isolated from any of the mice. Complete degradation of a PLGA sustained-release device has thus been observed.

Effect of Surface Area/Volume Ratio on Release Rate

The apparent independence of release rate on surface area/volume ratio of sustained-release naltrexone devices was observed in experiments conduced at Dynatech. A casting containing 50% by weight naltrexone in a 90/10 PLGA copolymer of 100,000 molecular weight was prepared. Rods of 0.26, 0.80, 1.60, and 3.18 mm diameter were extruded from this casting. Samples weighing 0.030 g were selected from each rod size. Since the surface area/volume ratio increases as 1/R (neglecting ends) for constant weight cylindrical samples, one would expect an equivalent increase in release rate by most diffusion-type models. The radii of the rods vary by a factor of twelve; thus one would expect the release rate to vary by the same factor.

The results of the release study shown in figures 4 and 5 show nearly identical release for all sizes of rods. There appears to be

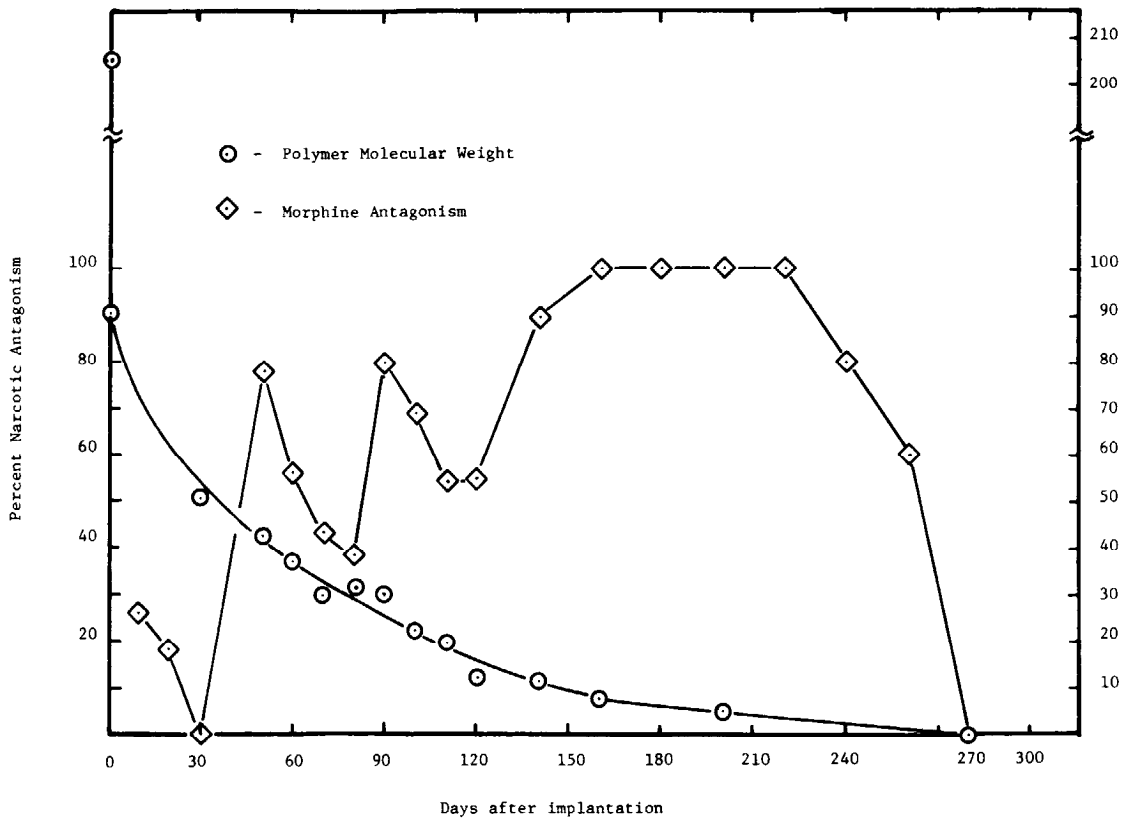


FIGURE 3. Performance and polymer degradation of 6-month naltrexone pamoate sustaineddelivery system (reference 8)

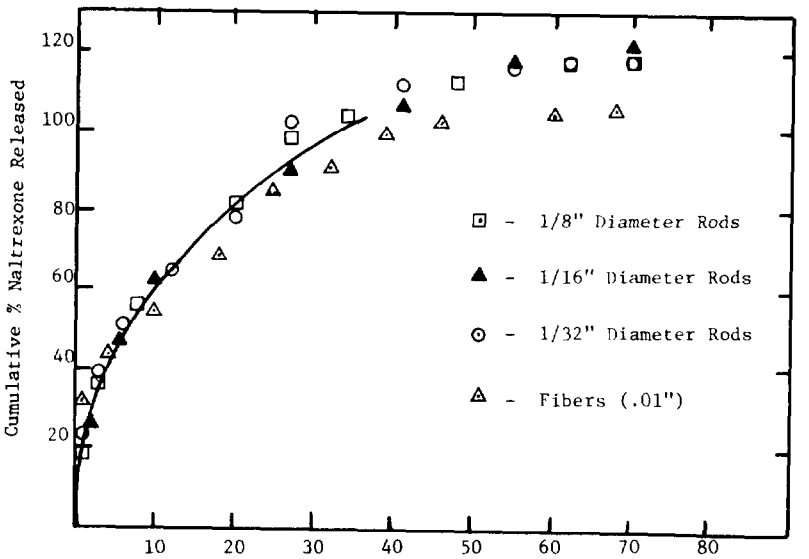


FIGURE 4. Comparison of *in vitro* release from extruded rods of various diameters of 100,000 Mw 90/10 PLGA with 50% w/w naltrexone loading (reference 9)

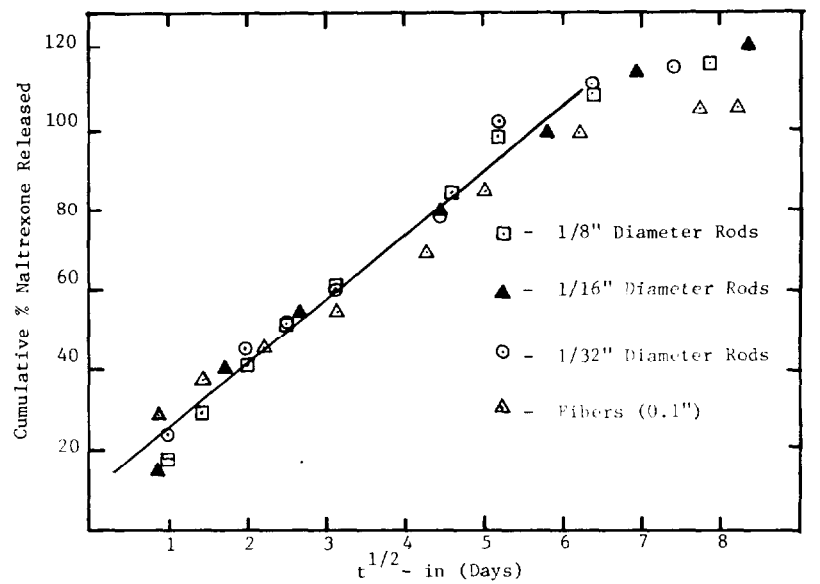


FIGURE 5. Same as figure 4, but cumulative release is compared to $t^{1/2}$. Solid line corresponds to that in figure 4 (reference 9)

little dependence of release rate on surface area/volume ratio. The cumulative release profiles of these systems also appear to be linear with the square root of time, as shown in figure 5. This release profile is not one which would be expected from cylindrical sustained-release devices, when estimated by most diffusion-type models. Rather they are similar to that which would be expected from a sustained-release device with flat plate geometry. This result carries the outstanding implication that finely divided, injectable dosage forms may provide the same sustained-release characteristics as molded bead or extruded rod implantable dosage forms.

Injectable System

A long term goal of the NIDA narcotic antagonism program has been the development of an injectable sustained-delivery device for naltrexone. Ideally, this device would display the same release characteristics as the 1-month system described above. The results of the study of the effect of surface area/volume ratio on release rate conducted at Dynatech indicate that this may be physically possible. Injectable naltrexone base/PLGA sustained delivery systems have been produced by the cryogenic grinding of beads and rods. The use of cryogenic temperatures makes the polymer/drug mixture brittle. This in turn makes it possible to grind the preparations into very small particles. The resulting powder is then sieved into narrow ranges of particle sizes, usually 45-90 μ or 90-180 μ. Dosage is accomplished by suspending the sieved powder in an agent such as Macrodex or Methocel, and injecting the slurry through a suitably sized needle.

Several promising injectable sustained-release preparations have been formulated at Dynatech. Figures 6 and 7 summarize the *in vitro* release and *in vivo* narcotic antagonism performance of the devices. Figure 6 shows the performance of a 90-180μ size, 50% by weight naltrexone base in 39,000 M_w, 90/10 PLGA powder. Figure 7 shows the performance of 90-180μ, 70% by weight naltrexone base in 39,000 M_w, 90/10 PLGA powder. This figure also shows the *in vitro* release of naltrexone from naltrexone/PLGA rods from which the powders were ground. Ten milligrams of each powder were poured into a surgical incision in the scapular region of mice. The wounds were then sewn closed. Narcotic antagonism was tested using the mouse tail-flick test.

Figure 6 reveals high levels of antagonism, and near-constant *in vitro* release for more than 30 days. Figure 7 shows erratic levels of antagonism, but similar *in vitro* release for both powders and rods (1.5 mm diameter).

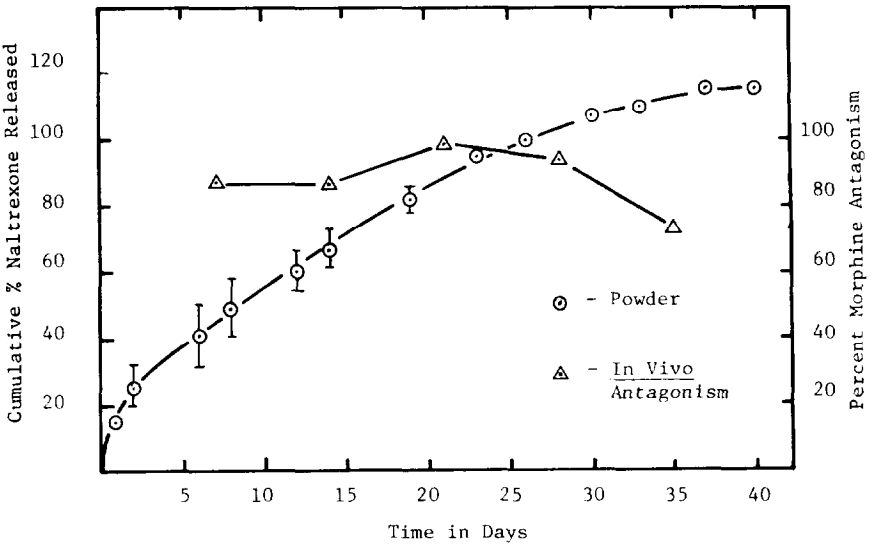


FIGURE 6. *In vitro* release and *in vivo* antagonism data for sample #24085, 39,000 Mw 90/10 PLGA, 50% w/w naltrexone loaded powder (reference 9)

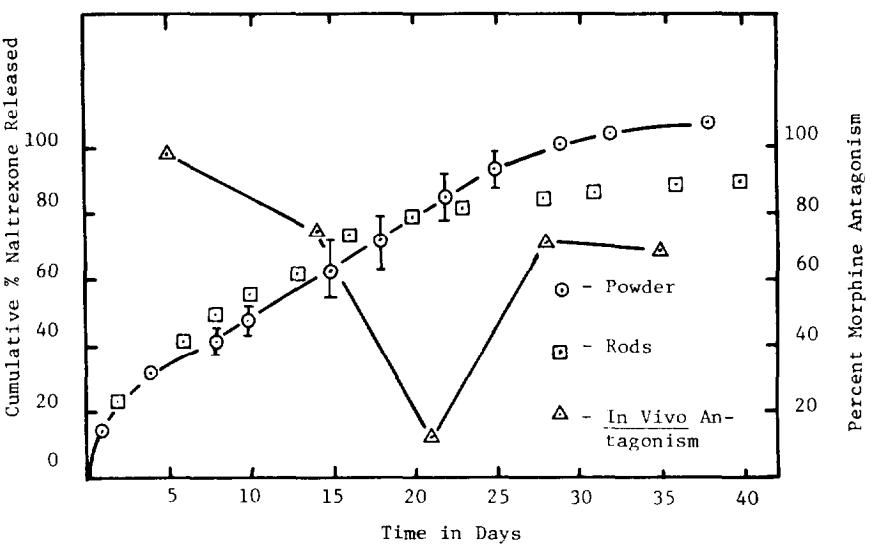


FIGURE 7. *In vitro* release and *in vivo* antagonism data for sample # 24085, 39,000 Mw 90/10 PLGA, 70% w/w naltrexone loaded preparations (reference 9)

An injectable system with the same composition as that shown in figure 7, but sieved to 45-90μ particle size, was recently prepared. The naltrexone in this system was ^{3}H-labelled at the 15 and 16 positions. Monkeys trained to self-administer morphine were subcu-

taneously injected with this preparation. Inhibition of morphine self-administration was observed. Urine was collected for about 30 days.

This preparation inhibited morphine self-administration for more than 5 but fewer than 12 days in these monkeys. Tritium was measured in monkey urine for 12 to 24 days after injection. While this preparation did not provide 30 days' narcotic antagonism, it does show promise for the further development of an injectable system. Mouse antagonism data with the 90-180μ powders shows much promise for development of injectable sustained-release system which will provide complete narcotic antagonism for 30 days.

Development of a Sustained-Delivery System for Morphine

In addition to extensive development work on naltrexone delivery systems, Dynatech has developed an implantable dosage form which provides about 2 weeks' sustained delivery of morphine. The system is composed of 50% by weight morphine in 30,000-50,000 M_w, 50/50 PLGA. The physical form of the sustained-delivery device is a 1.5 mm diameter, 1 cm long rod. Several thousand of these dosage forms have been produced at Dynatech for use in animal studies by other investigators.

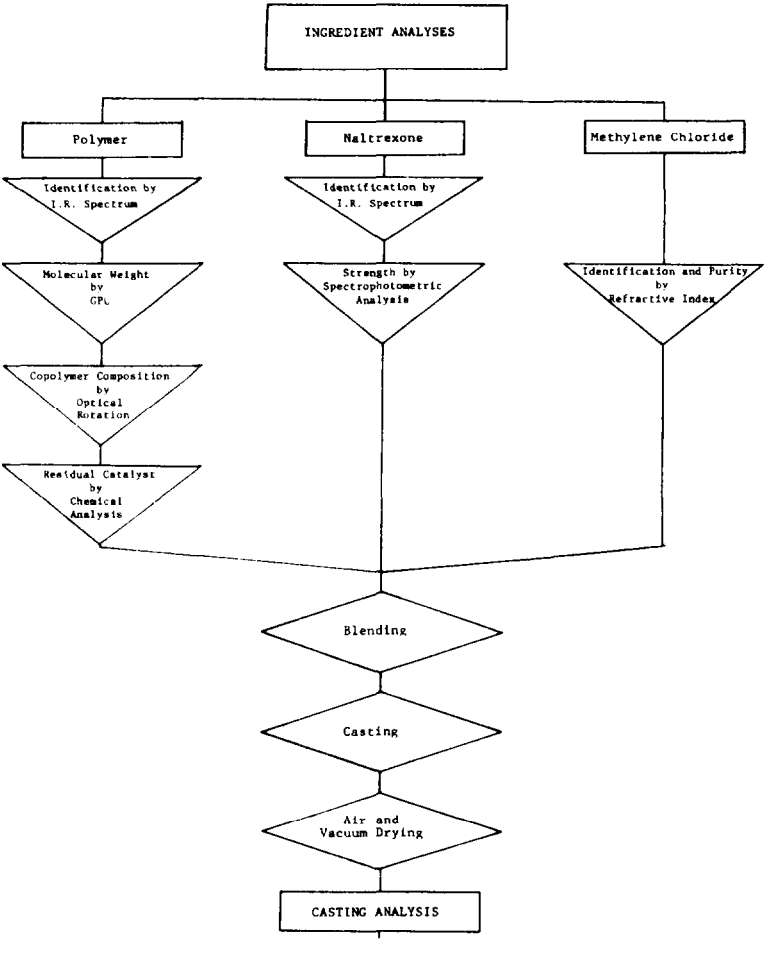
Preparation of Beads for Human Clinical Trials

The successful development of an implantable 30-day naltrexone delivery system by Dynatech resulted in a decision by NIDA to proceed to human clinical trials with the Dynatech beads. These beads are composed of 70% by weight naltrexone base, in 40,000 M_w, 90/10 PLGA. It was decided that a 2000-bead batch would be required to begin human clinical trials. It was also recommended by consultants that the production of the beads be undertaken at an FDA-registered drug manufacturing facility. The University of North Carolina, School of Pharmacy, Chapel Hill, was chosen as the site for this production.

Good Manufacturing Practices Documentation

Good Manufacturing Practices documentation was developed by Dynatech for the bead production operation. GMP documentation is designed to be "a statement of the methods, facilities and controls used for the manufacturing, processing and packing of the new drug to establish and maintain appropriate standards of identity, strength, quality and purity as needed for safety and to give significance to clinical investigations made with the drug." (FD

Form 1571 [11/75], required attachment 5). GMP documentation is based on the techniques of bead manufacture developed by Dynatech R/D Co. Analytical test methods were also developed by Dynatech in response to the requirements of the GMP documentation and the suggestions of consultants. The GMP documentation developed includes the preparation of all necessary documentation to support an application for a Claimed Investigational Exemption for a New Drug (IND) from the U.S. Food and Drug Administration (FDA) by NIDA. Mr. Ed Adams of Endo/Dupont Laboratories, and Dr. James L. Olsen, Director of the UNC, Chapel Hill, School of Pharmacy Drug Product Program served as consultants for the preparation of the GMP documentation (10,11). A flowchart of the bead manufacturing process, including the tests required by GMP regulations, is depicted in figure 8.



Continuation of Figure 8

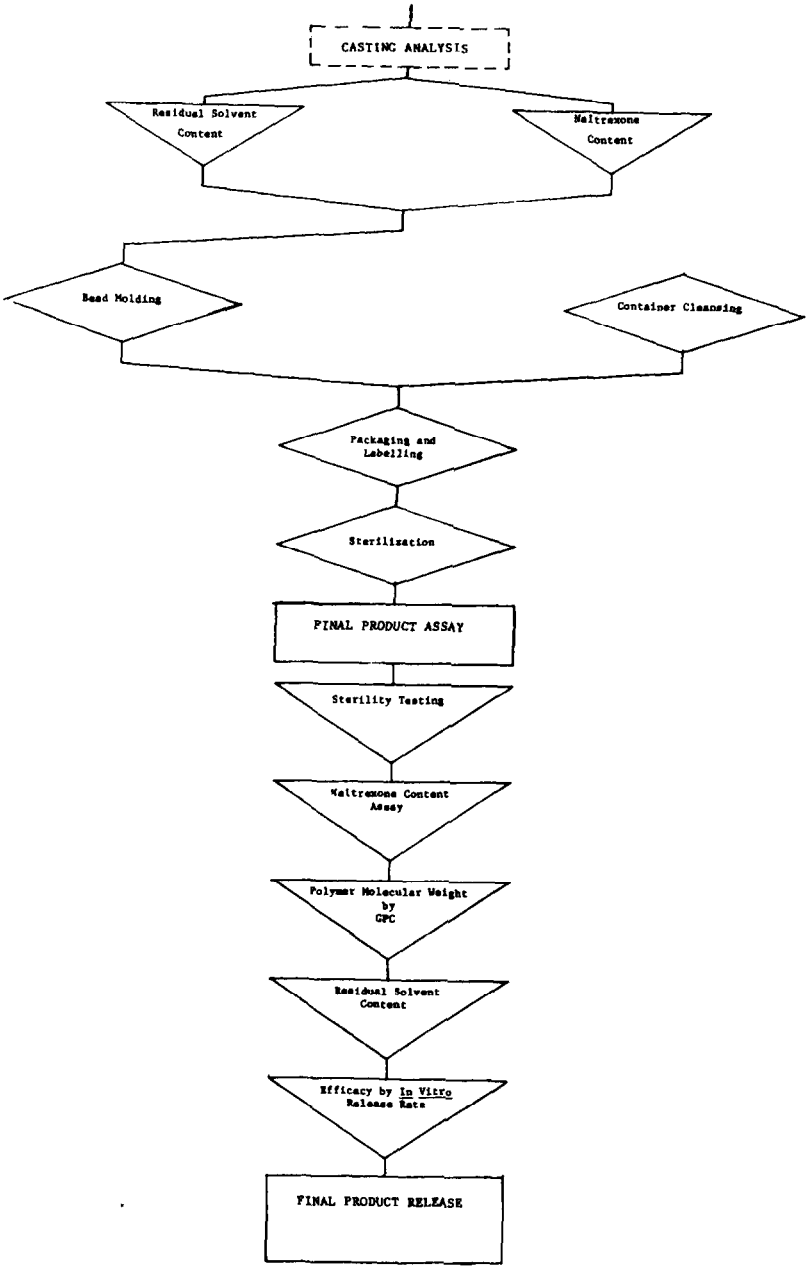


FIGURE 8. Flowsheet of bead manufacturing procedure with Good Manufacturing Practices documentation (reference 12). 70% w/w naltrexone, 30% w/w 90 L(+)/ 10G PLGA bead manufacturing and quality control flowsheet

Production of Batch #L780901X

The UNC School of Pharmacy was supplied with all equipment necessary for bead manufacture. The equipment loaned was the same as that used to produce most previous batches of beads at Dynatech. Included among these is batch number 24086, which underwent exhaustive testing for sterility, toxicity, and *in vivo* release rate and narcotic antagonist effect. These beads were produced using the analytical and manufacturing procedures detailed in the GMP documentation developed for this process.

All equipment was assembled at UNC during November 1978. Starting and intermediate material analyses, equipment assembly, and initial bead moldings were all supervised by Dynatech staff (12). Packaging, labelling, package seal testing, and visual inspection of the product beads were performed during January 1979. Sterilization by exposure to 2.5 Mrads of Cobalt-60 γ-radiation, and testing to assure sterility, were conducted during February 1979.

Two thousand eighty-five acceptable beads, and a number of rejected beads, were packaged 15 to a vial. Rejected beads are available for analytical testing, and for display purposes. The acceptable beads are now ready for use in clinical trials, and are in storage at the UNC, Chapel Hill, School of Pharmacy (13).

SUMMARY

The long term goal of the NIDA narcotic antagonist program of developing an implantable, biodegradable, naltrexone/PLGA matrix system which will sustain the delivery of naltrexone to biological systems for 1 month has been achieved. Dosage forms have been reproducibly prepared and tested *in vitro* and *in vivo* for consistent release and efficacy. Analytical and manufacturing procedures have been developed and proven effective. Good Manufacturing Practices documentation for the production of these dosage forms has been developed. The documentation was also used for producing a large batch of dosage forms at an FDA registered pharmaceutical manufacturing facility. This batch is suitable for use in human clinical trials.

Several other sustained-release dosage forms have also been developed by Dynatech. These include an implantable sustained-delivery system capable of 6 months' release of naltrexone, and a finely-divided injectable form of the 1-month duration system, which shows promise of successful application with further development. An implantable dosage form which provides 2 weeks' sustained-delivery of morphine has also been developed and produced in quantity for use in animal experiments. Thus the development of sus-

tained-release devices at Dynatech has resulted in the production of several systems with potential for practical application.

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R. E. Willette and G. Barnett, eds.
National Institute on Drug Abuse, 1980

Use of Synthetic Polypeptides in the Preparation of Biodegradable Delivery Systems for Narcotic Antagonists

Kenneth R. Sidman, Arthur D. Schwope, William D. Steber, and Stephen E. Rudolph

The goal of this program was the development of biocompatible sustained-release systems that would release naltrexone at a rate of 20 to 25 μg/hr for 30 days, and that would biodegrade within 90 days. The focus was on the use of macrocapsules prepared from synthetic polypeptides, specifically copolymers of glutamic acid and ethyl glutamate (i.e., Glu/EGlu copolymers). Tubular capsules prepared from 18/82 Glu/EGlu were the most promising systems developed. Capsules 1 cm in length, 0.19 cm in outside diameter, and 0.005 cm in wall thickness released naltrexone in mice at rates in the range of 20 to 40 *μg/hr for 18 days. The rates then decreased during the next 12 days as the capsules became exhausted of drug. These capsules were biocompatible and they appeared to biodegrade within 90 days. In general, the Glu/EGlu copolymers exhibit permeation and degradation rates that increase as the glutamic acid content is increased. Radiotracing studies revealed &hat the ultimate degradation product was carbon dioxide, which appeared in the expired air. This result is consistent with a polypeptide degradation- process that involves hydrolysis of the*

ethyl esters followed by hydrolysis of the peptide bonds to produce glutamic acid, which enters the metabolic pool.

INTRODUCTION

We have been working on the development of injectable, biodegradable drug delivery systems that would provide a sustained release of naltrexone. The goal was a delivery system (i.e., depot) that would release drug at a relatively constant rate of 20 to 25 micrograms per hour for 30 days, and then biodegrade within 90 days.

Synthetic polypeptides were used to form the depots. Specifically, copolymers of glutamic acid with ethyl glutamate (i.e., Glu/EGlu copolymers) were studied. In this paper we describe the synthesis procedures and the studies that were carried out in order to assess the permeability and biodegradability of the copolymers.

MATERIALS AND METHODS

Copolymer Synthesis

The preparation of Glu/EGlu copolymers was carried out in three stages: 1) synthesis of monomers; 2) polymerization of benzyl-blocked intermediates; and 3) debenzylation of the intermediates. The monomers, N-carboxyanhydrides (i.e., NCA's) of γ-benzyl-L-glutamate and γ-ethyl-L-glutamate, were prepared from the respective amino acid esters using the procedures of Fuller et al. (1). Polymerization of the monomers to form copolymers of benzyl and ethyl glutamate was carried out in solvent at 25°C, following the procedures of Anderson (2). Triethylamine (1.3 M in benzene) was used as the initiator at a level of 2 mole percent based on the monomers. The reaction mixture was stirred under nitrogen for 3 days. The product was then isolated by precipitation into methanol.

After vacuum drying, the benzyl-blocked intermediate was dissolved in toluene and debenzylated using anhydrous HBr, according to the methods of Fasman et al. (3). This procedure typically resulted in the loss of all of the benzyl groups and some ethyl groups so that the final Glu/EGlu copolymer exhibited a higher glutamic acid content than would be expected if the procedure were totally selective for benzyl ester acidolysis.

The copolymers exhibited inherent viscosities in dichloroacetic acid (0.2% solution at 25°C) ranging from 0.9 to 1.5 dl/g. Composition was determined by proton NMR in trifluoroacetic acid solutions.

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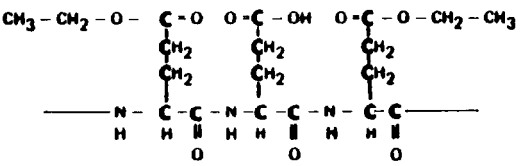
Biodieposition Studies

Radiolabelled Copolymer Synthesis

One millicurie of L-glutamic acid [$^{14}C(U)$] was converted to the ethyl ester via procedures described by Greenstein and Winitz (4). The ester was then converted to the NCA, copolymerized with benzyl glutamate, and the intermediate was debenzylated according to the procedures described above. The product exhibited a Glu/EGlu mole ratio of 45/55, an inherent viscosity in dichloroacetic acid of 1.05 dl/g, and a specific activity of ≅660,000 dpm/mg. A schematic representation of the polymer is shown.

POSITIONS OF THE CARBON-14 LABEL IN THE 45/55 GLU/EGLU COPOLYMER



Film Implantation

Films, approximately 0.006 cm thick, were cast from a solution of the copolymer in N,Ndimethyl formamide. After drying at 60°C for 3 days, the films were cut into 1 cm square pieces and implanted subcutaneously (via a midline incision) in the sacral area of the back; one piece was positioned to lie on one flank, a second piece to lie approximately over the kidney.

Recovery and Analysis of CO^2

One or two mice at a time were housed in a glass metabolic cage. Food and water were available *ad libitum*. Cage air was continuously evacuated from the top and passed through ≅60 ml 4 M KOH via a fritted tube. The rate of air flow was 300-400 ml/min. Room air was allowed to enter the cage at the bottom to replace the evacuated air. The KOH was replaced about every 24 hours. Aliquots were counted in Modified Bray's scintillation cocktail.

Whole-Body Autoradiography

At various times following implantation of the Glu/EGlu film, mice were killed by immersing them rapidly in hexane/dry ice. Animals were stored frozen at -25°C.

For microtomy, an animal was imbedded in a mold filled with methylcellulose. The mold was kept in a -25°C freezer until the methylcellulose was frozen. Whole-body sections, 60 microns in thickness, were cut with a Jung (Heidelberg) microtome housed in a Harris cryostat (Harris Manufacturing Co., Cambridge, Mass.). The sections were picked up on Scotch #810 transparent tape, transferred over dry ice to a low-humidity cold room, and stored at -25°C until excess tissue moisture was removed. The whole-body sections were taped to Kodak No-Screen Medical X-ray film, in a photographic darkroom at room temperature.

After suitable exposure time (which ranged from one week to several months, depending upon the intensity of radioactivity), the sections were removed and the X-ray films processed in Kodak X-ray developer and fixer.

Film Permeability Studies

The experiments were carried out using a two-chambered, glass cell which exposed one side of a test film (5.1 cm^2 area) to either an aqueous or a sesame oil solution saturated with ^3H-naltrexone and containing undissolved drug. The solution was agitiated by means of a magnetic stirrer. The other chamber-the downstream side-contained 50 cm^3 of Earle's Balanced Salt solution. A 5% CO, gas was bubbled into the Earle's solution in order to maintain its pH and bicarbonate concentrations. Cell temperature was 37°C. Permeant concentration in the Earle's solution was determined by scintillation counting; 1 cm^3 samples were taken daily. The influence of downstream permeant concentration on permeation rate was minimized by replacing the Earle's solution before the naltrexone concentration approached 20% of the saturation value.

Capsule Preparation and Evaluation

Capsules were assembled from tubes and caps, which were prepared by dip coating glass mandrels into a solution of the copolymer in N,N-dimethyl formamide. The capsules were filled with a solid rod of naltrexone (tritium-labelled in the 15,16 positions--specific activity equal to 1060 dpm per microgram of drug). The rods were prepared by extrusion of a stiff paste consisting of drug and tetrahydrofuran (in some cases, sesame oil was used in place of tetrahydrofuran), through a stainless steel die (see figure 1). The rods were dried at 70°C for 3 to 5 days to remove the solvent. After the naltrexone rods were inserted into the tubes the caps were cemented in place using the copolymer solution. The size of a typical capsule is shown in figure 2.

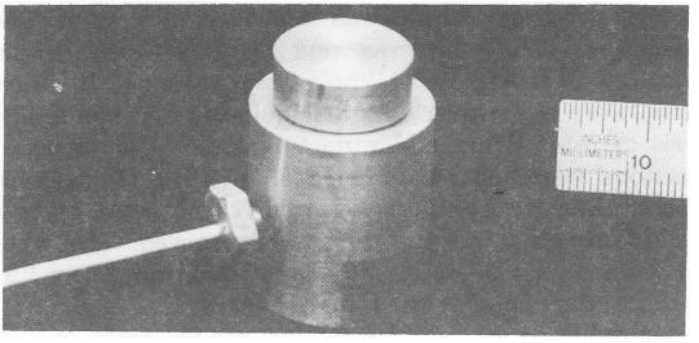


FIGURE 1. Compression Mold Used to Extrude Solid Rod

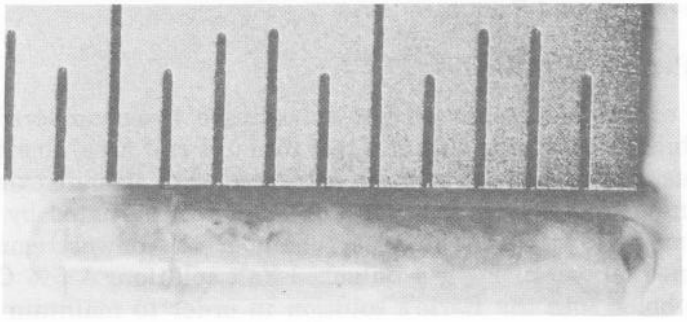


FIGURE 2. Capsule Filled With Naltrexone (Each scale division is 1 mm.)

The capsules were implanted subcutaneously in mice, and the urine was monitored for radioactivity using the liquid scintillation counting procedures. The release rates were calculated by assuming that 74% of the naltrexone released from the capsules appeared in the urine (as parent compound and metabolites). This fractional recovery was established in separate experiments wherein mice were administered subcutaneous injections of radioactive naltrexone and then studied to determine urinary and fecal distribution of radioactivity. In addition, the areas under the release rate vs. time curves were routinely measured to determine if the total drug recovered was equivalent to the initial drug contained in the capsules.

In previous studies (5), we determined that a release rate of approximately 1 μg/hr was sufficient to block an AD_{80} dose of morphine in mice. In the following studies, the release rates greatly exceeded this minimum effective level, so the tail-flick bioassay procedure was used infrequently, only to confirm that naltrexone was being released in an active form.

RESULTS AND DISCUSSION

Biodegradation and Biodisposition of Glu/EGlu Copolymers

Glu/EGlu copolymers degrade *in vivo* in a continuous process that was postulated to involve hydration of the film, hydrolysis of the ethyl esters, dissolution of the soluble molecules (i.e., copolymers with high Glu/EGlu ratios), enzymatic hydrolysis of peptide bonds, and finally passage of glutamic acid into the "metabolic pool." The glutamic acid should be disposed of by two principal routes: 1) decarboxylation in the tricarboxylic acid cycle; and 2) incorporation into protein.

Two studies of the biodegradation process were carried out in a program supported by the National Institute of Child Health and Human Development under Contract No. N01-HD-4-2802 (6). In one study, films exhibiting a range of copolymer compositions and thicknesses were evaluated to determine their biodegradation rates. In the second study, the ultimate biodisposition of a ^{14}C-labelled Glu/EGlu copolymer was determined.

Biodegradation

Films with Glu/EGlu mole ratios of 13/87, 18/82, 23/77, 30/70, 40/60, and 50/50 Glu/EGlu were implanted subcutaneously in mice. Periodically, animals were sacrificed and the implant sites were examined. The results are summarized in table 1.

The time for biodegradation of the films decreased as the glutamic acid content was increased. For example, films containing 13 mole percent glutamic acid remained intact for more than 79 days, while films containing 40 mole percent disintegrated within 7 days. As might be expected, the degradation time increased with increasing film thickness.

Biodisposition

The ultimate disposition of the copolymer was determined in studies involving the implantation of radiolabeled films of 45/55 Glu/EGlu in mice. Table 2 shows typical results. Approximately 73% of the implanted radioactivity was recovered within 8 days, and 96% of this was $^{14}CO_2$.

Autoradiograms of the animals, shown in figures 3 and 4, revealed that the residual radioactivity concentrated at the implant site, suggesting that the slow step in the elimination of the copolymer was its dissolution in body fluids. Once the copolymer dissolved, it appeared to be transported rapidly to sites where it was metabolized. After only 7.5 hours, there were significant levels of

TABLE 1. Interim Biodegradation Results

Copolymer Ratio Glu/EGlu	Film Thickness, cm	Film Appearance (Numbers in parenthesis denote percent weight loss) Days Post-Implantation 7-10	28-35	78-79
13/87	0.0025	-	Intact (15%)	Intact (15%)
	0.0051	-	Intact (0%)	Intact (13%)
18/82	0.0025	Intact (30%*)	Fragmented (33%)	Disintegrated
	0.0051	Intact (0%)	Intact (17%)	Fragmented
23/77	0.0051	Intact (15%)	Fragmented (75%)	Disintegrated
30/70	0.0064	Intact (19%)	Fragmented (33%)	Disintegrated
40/60	0.0038	Disintegrated or Dissolved		
50150	0.0038	Disintegrated or Dissolved		

*Film showed a 30% weight gain, not a loss.

TABLE 2. Ultimate Biodisposition of Glu/EGlu Film

Percentage of the Administered Dose of Radioactivity Recovered in Expired Air and Excreta from a Mouse Implanted with 12 mg of Glu/EGlu Radiolabelled Copolymer Film

	Time (hrs)	% Recovery of Radioactivity
Trapped $^{14}CO_2$	0 - 6.5	2.3%
	6.5 - 23.5	15.5%
	23.5 - 48.5	20.7%
	48.5 - 72	10.2%
	72 - 105	6.4%
	105 - 121	2.7%
	121 - 145	5.3%
	145 - 168	3.9%
	168 - 192	3.0%
		70%
Excreta	0 - 51	1.8%
	51 - 147	1.0%
		2.8%
		Total 72.8%

radioactivity in kidney, liver, bone marrow, para-orbital glands, and the intestinal epithelium. In general, the radioactivity was associated with the more metabolically active tissues. By day 11, there was a significant reduction in the total level of radioactivity. This is not fully apparent in the figure because the X-ray film exposure time was quadrupled in this case in order to obtain good contrast.

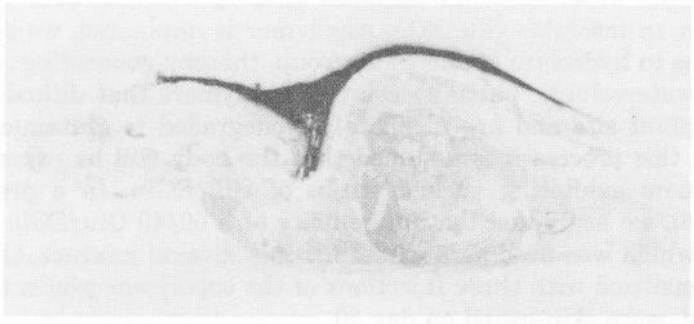


FIGURE 3. Autoradiogram Taken 7.5 Hours Post-Implantation

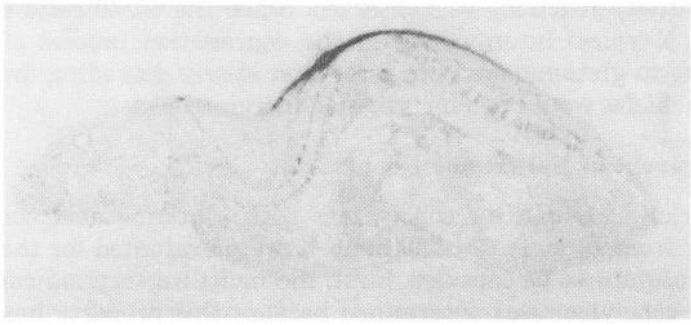


FIGURE 4. Autoradiogram Taken 11 Days Post-Implantation

The results of these studies were consistent with the hypothesized metabolic disposition of Glu/EGlu copolymers; that is, dissolution, hydrolysis of the ester in ethyl glutamate, hydrolysis of the polyglutamate to glutamic acid, and finally, passage of glutamic acid into the metabolic pool.

Biocompatibility

To be useful for drug delivery systems, the Glu/EGlu copolymers must be well tolerated by the body. This means they should not elicit any adverse tissue response in an acute exposure, and they

should not induce hypersensitivity reactions (i.e., an antigenic response) on chronic or repeated exposure.

Work to date has been focused on copolymers that contained glutamic acid concentrations in the range of 10 to 35 mole percent, because these materials exhibit degradation rates especially appropriate for capsules. Films and depots (in the form of rods and cap sules) were implanted in mice and the implant sites were examined at frequent intervals. Gross observation consistently revealed little or no tissue inflammation associated with the copolymers.

When an insoluble Glu/EGlu copolymer is implanted, we believe it begins to hydrolyze at the ester group, thereby generating a variety of watersoluble, partially esterified polymers that diffuse from the implant site and are ultimately biodegraded to glutamic acid. During this process it is assumed that the body will be exposed to copolymers exhibiting various ratios of Glu/EGlu. In a previous study (6), we evaluated the antigenicity of a 60/40 Glu/EGlu copolymer which was used as a model for this diverse mixture. Guinea pigs, sensitized with three injections of the copolymer over a 20-day interval, were skin-tested on day 30.

The results indicated that there were essentially no differences between the initial (control) and final reactions. No evidence of hypersensitivity reactions was observed. Since the 60/40 ratio is considered a typical intermediate in the degradation process of solid implants to glutamic acid, we infer that slowly degrading drug delivery vehicles would be nonantigenic in guinea pigs.

Development of Naltrexone Depots

A series of Glu/EGlu copolymers with glutamic acid contents ranging from 13 mole % to 30 mole % were evaluated for their potential usefulness as capsules. First, the moisture sorption capacity of each copolymer was determined because this property has been found to be an expeditious means of obtaining insight to probable permeability of the copolymer-high moisture sorption is predictive of high naltrexone permeability. Film permeation studies were then carried out with selected copolymers and, finally, capsules were prepared and evaluated.

Film Permeability Studies

Experiments were carried out using films prepared from copolymers exhibiting the following Glu/EGlu mole ratios: 13/87, 18/82, 20/80, and 30/70 Glu/EGlu. Two suspending agents-water and sesame oil-were investigated. The latter agent was studied because it is a biocompatible fluid that may be conveniently used in cap

sules to ensure good contact of the drug with the interior surfaces of the capsule. (Sesame oil exhibits low volatility and is retained during capsule assembly and drying operations.)

The results are shown in table 3. The measured film permeabilities are given in the last column. When these numbers are multiplied by film surface area and then divided by film thickness, the permeation rate is obtained in terms of μg/hr. The permeability generally correlated with the measured moisture pickup for the copolymers. However, when sesame oil was used as the suspending agent, an unusually high permeability resulted. In separate experiments, the ability of the 20/80 copolymer to become plasticized with sesame oil was determined by immersing films into both sesame oil and sesame oil saturated with naltrexone. After 24 hours at 37°C, the films picked up only 4% to 5% by weight, and this did not increase during a total of 7 days immersion. This small weight gain does not appear to be sufficient to-account for the high permeability, but at the present time no better explanation has been found.

TABLE 3. Permeation Results

Naltrexone Permeability Study					
Copolymer Glu/EGlu	Batch Identification	Moisture Pick-up (%)	Film Thickness (cm)	Upstream Medium	Film Permeability [μg-cm(thick)/hr-cm^2(area)] x 10^2
13/87	19599-142	6	0.003	Water	4.3
13/87	19599-142	6	0.011	Water	2.0
18/82	19599-151	80	0.005	Water	75
20/80	19599-131	175	0.004	Water	103
20/80	19599-131	175	0.012	Sesame Oil	240
30/70	19599-140	100	0.006	Water	128

The permeabilities in the table were used to make predictions of capsule performance. Typically, the capsules were prepared with a surface area of 0.8 to 1.0 cm^2 and a wall thickness of 0.005 cm. When these values were multiplied by the permeabilities, the calculations indicated that release rates ranging from a low of 3 μg/hr to a high of 340 μg/hr could be obtained.

Capsule Studies

Capsules were prepared from each of the copolymers. In certain cases, sesame oil was used as an extrusion aid in the preparation of the naltrexone cores. Some capsules were filled with additional

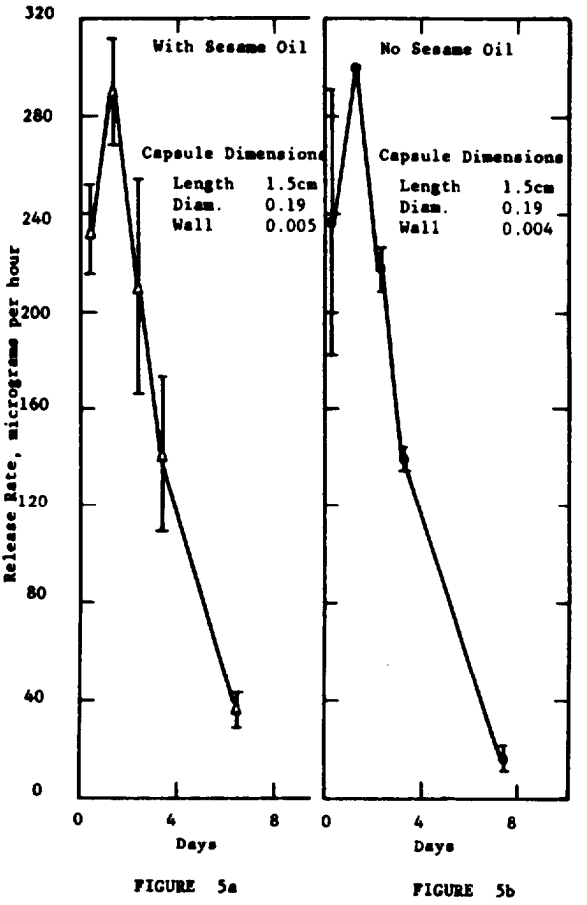


FIGURE 5a FIGURE 5b

NALTREXONE RELEASE FROM 30/70 GLU/EGLU CAPSULES

sesame oil in order to investigate the value of maintaining an oil-filled reservoir. The results are described below, beginning with the 30/70 Glu/EGlu depots, which released antagonist at the fastest rates, and progressing to the 18/82 and 13/87 depots, which sustained release for 30 days and longer.

30/70Glu/EGlu

Figures 5a and 5b show the release rates for capsules 1.5 cm in length, 0.18 cm in inside diameter, and 0.004 to 0.005 cm in wall thickness. These were filled with 40 mg cores composed of 22 mg naltrexone and 18 mg sesame oil (all the oil was absorbed in the core.) The results in figure 5a were for capsules that received addi-

tional sesame oil to ensure that liquid contacted the interior surface of the capsules. The results in figure 5b were for capsules that received no additional oil. In both cases, the rates were extremely rapid, peaking at approximately 300 μg/hr and then dropping off within 8 days. No significant difference in behavior of depots with and without additional sesame oil was observed.

These release results are in close agreement with predictions that would be made on the basis of the film permeability shown in Table 3, 1.28 μg x cm/hr x cm^2. For example, the predicted rate for capsules that exhibit a surface area of 1 cm^2 and an average wall thickness of 0.0045 cm is given below.

$$\text{Predicted Release Rate} \simeq \text{Capsule Area/Wall Thickness x Permeability}$$

$$\simeq 1\ cm^2/0.0045\ cm \times 1.28\ \mu g \cdot cm/hr \cdot cm^2$$

$$= 284\ \mu g/hr$$

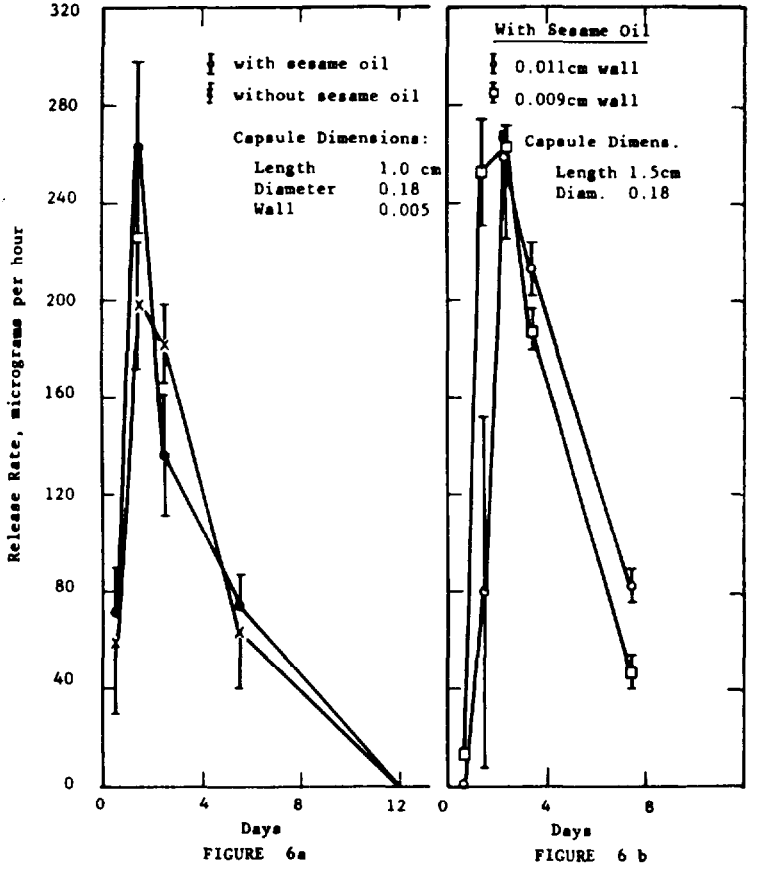
20/80 Glu/EGlu

Capsules were prepared 1 cm in length, 0.18 cm in inside diameter, 0.005 cm in wall thickness, and they were filled with pure naltrexone rods weighing 22 mg. Sesame oil was added to some of the tubes, while others were left free of oil to provide a basis for comparison. The release rates are shown in figure 6a. Capsules with oil exhibited slightly higher rates, peaking at 260 μg/hr and then rapidly falling off. Capsules without oil peaked at 200 μg/day, but they, too, were exhausted of drug within 12 days.

A second set of capsules was prepared in order to study the influence of wall thickness and sesame oil. These capsules were longer than those above, 1.5 cm, and they were either 0.009 cm or 0.011 cm in wall thickness. They were filled with 40 mg rods composed of 22 mg naltrexone and 18 mg sesame oil. Additional oil was added to fill the tubes. As is shown in figure 6b, there was little difference that could be ascribed to wall thickness, and the magnitude of release was similar to that shown in figure 5a. This suggests that the larger surface of the 1.5 cm capsules compensated for their thicker walls.

18/82 Glu/EGlu

The release rates from capsules 1 cm in length, 0.005 cm in wall, and filled with 24 mg rods (composed of 16 mg naltrexone and 8 mg of sesame oil) are shown in figure 7. The rate gradually rose over the first 3 days to a range of 20 to 40 μg/hr, which was maintained for approximately 18 days. During this interval, approximately 13



mg was administered out of the initial 16 mg loading of naltrexone. The remaining 3 mg was released over the next 12 days.

The release behavior of these capsules was quite unlike that of the 20/80 capsules discussed above, despite the small difference in copolymer composition. However, the moisture absorption results (80% for the 18/82 copolymer and 175% for the 20/80) appeared to provide an indication of the real differences between the copolymers and, therefore, moisture absorption will be utilized in the future to characterize capsule materials.

The 18/82 capsules represent the best depots developed in the program. They exhibited essentially the target release rate, and they would have maintained this rate for at least 30 days if the cores had contained the maximum naltrexone load (i.e., 30 mg, based on full utilization of the interior volume). As has already

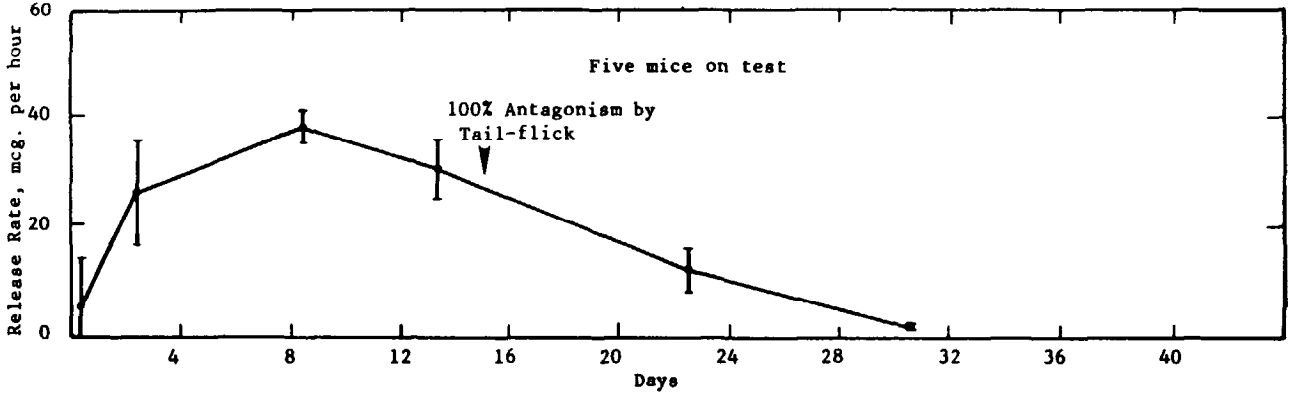


FIGURE 7 NALTREXONE RELEASE FROM 18/82 GLU/EGLU CAPSULES

been described, the Glu/EGlu copolymers are biocompatible in mice, rats, and guinea pigs, and they exhibit the requisite biodegradability. Only residues of the 18/82 capsules were found 90 days after implantation. The typical pattern of degradation is shown in figure 8.

It should be noted that the predicted release rate based on film permeability was higher than the actual capsule results:

$$\text{Predicted Release} = (\text{Area of 1 cm long capsule})/(\text{Thickness})$$

$$= 0.7\ \text{cm}^2/0/005\ \text{cm} \times 0.75\ \mu\text{g} \cdot \text{cm/hr} \cdot \text{cm}^2$$

$$= 105\ \mu\text{g/hr}$$

One possible explanation for the discrepancy is that the capsules did not contain the additional sesame oil necessary to ensure good contact with the interior walls. Conceivably, if the capsules were

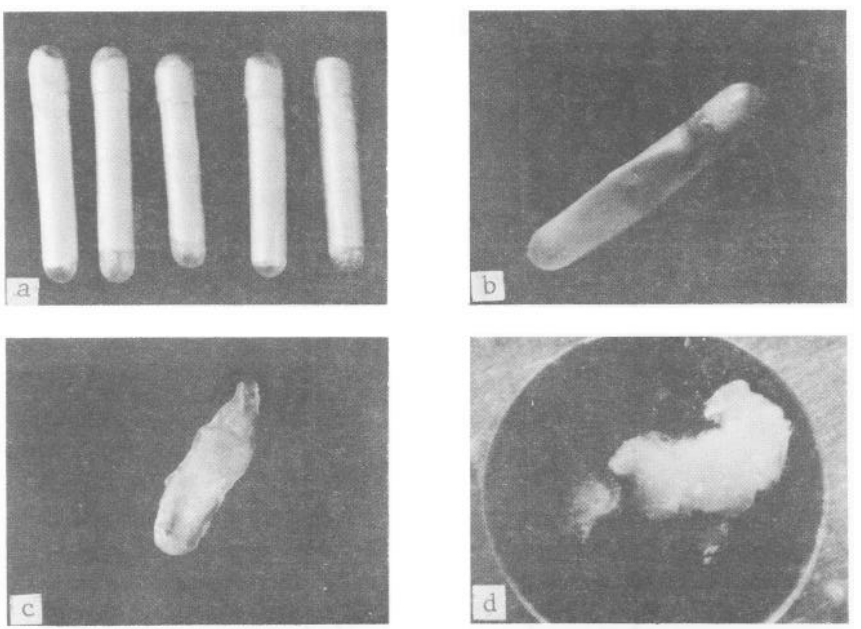


FIGURE 8. Biodegradation sequence of naltrexone capsules. Figure 8a shows depots prior to implantation. Figures 8b, 8c, and 8d show capsules excised after 30, 60, and 90 days, respectively. Note that the depot in Figure 8b is collapsed, indicating depletion of drug. At 90 days the copolymer was difficult to distinguish from living tissue.

filled with oil or higher drug loadings, then actual release would be closer to the predicted value, 105 μg/hr. Since this rate would be 4 times the target level, 25 μg/hr, it would be necessary to quadruple the wall thickness in order to reduce the rate to the desired range. Clearly, additional development effort on the 18/82 capsules is required in order to meet all the program objectives and, based on the positive results to date, such effort appears to be warranted.

13/87 Glu/EGlu

Capsules 1 cm in length and 0.008 cm in wall thickness were fabricated from the 13/87 copolymer. These were filled with 22 mg rods that contained 12 mg of naltrexone and 10 mg of sesame oil. Figure 9 shows that the release rate was quite slow, peaking at approximately 8 μg/hr by day 56, and it was prolonged for almost 100 days. Residues of this slowly degrading copolymer were still evident at the implant sites 180 days after implantation.

SUMMARY

Tubular capsules made from glutamic acid-ethyl glutamate copolymers offer wide-ranging potential for controlled narcotic antagonist release. The physical dimensions and copolymer composition are readily varied to meet specific delivery rate and duration objectives, while satisfying equally important biocompatibility and degradation requirements.

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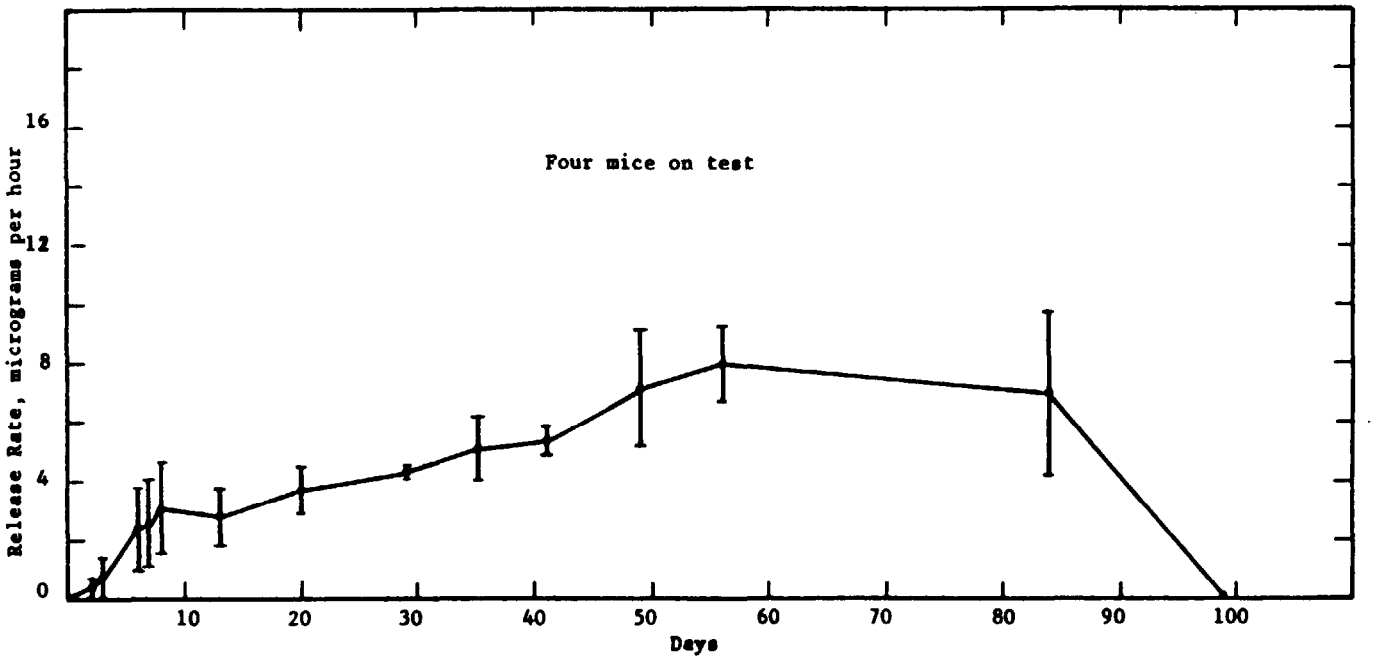

FIGURE 9 NALTREXONE RELEASE FROM 13/87 GLU/EGLU CAPSULES

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Biodegradable Drug Delivery Systems Based on Aliphatic Polyesters: Application to Contraceptives and Narcotic Antagonists

Colin G. Pitt, Thomas A. Marks, and Anton Schindler

The classes of polymer which form the basis of different types of drug delivery systems are discussed, and the relationships between the chemical structure of the polymer and its permeability, morphology, biodegradability, and mechanical properties are considered, using polyesters as specific examples. Studies of the permeability and biodegradability of poly(ε-caprolactone), poly(DL-lactic acid), and various copolymers are described and used to illustrate how these properties may be varied by the choice of polymer structure. An induction period prior to bioerosion of these polymers, coupled with high permeability, permits their use as reservoir devices (capsules) which exhibit constant, diffusion-controlled drug release rates and which erode after the drug is exhausted. The applications of this approach to the long term delivery (1 year) of levonorgestrel, a contraceptive agent, and the short term delivery (1-2 months) of naltrexone, a narcotic antagonist, are described.

INTRODUCTION

There are three fundamentally different approaches to the design of polymeric drug delivery systems - control by osmotic pressure, by diffusion, and by erosion (1). Only the last, by definition, necessarily involves polymer biodegradation. Regardless of the ap proach selected, it is necessary to carefully control the properties of the polymer, the most important of which are its permeability, biodegradability (or stability), biocompatibility, and mechanical prop erties. Since these properties are often interdependent, their optimization can present a challenging exercise in polymer design.

Polymers used for drug delivery may be separated into two distinct classes, those that are in the glassy state (T_g>37°C)[2] and those that are in the rubbery state (T_g<37°C) at body temperature. If the polymer is in the glassy state it will generally be tough and inflexible, with a low permeability, and drug release by leaching and polymer erosion will be favored relative to diffusion. The only possibility of achieving a constant rate of drug release from this class of polymer is by constant bioerosion of a device with a constant surface area, i.e., a slab, or by diffusion-controlled release from a microcapsular reservoir device where the very thin walls and increased surface-to-volume ratio compensate for the reduced permeability. Poly(lactic acid), T_g 57°C, formulated as microcapsules, is an example of the latter system (2-4).

Polymers in the rubbery state will have a high permeability but will often lack sufficient mechanical strength and form stability unless reinforced by the presence of cross-links, crystalline domains, or strong interactions between chains, e.g., hydrogen bonding. Silicone rubber is an example of a non-degradable rubbery polymer (T_g -120°C) which must be strengthened by both filler and light cross-linking. If the polymer is to be biodegradable, the means of reinforcement must also be biodegradable. This can present some difficulties, for if diffusion-controlled drug release is operative, degradation of filler or cross-links may change the polymer morphology and hence its permeability. Changes in device dimensions and crystallinity produced by biodegradation will also affect the rate of drug diffusion. These problems can be minimized if bioerosion occurs after the useful life of the device, i.e., after drug depletion. This is the approach which we have preferred in our laboratory.

Polymer Design

Excluding polymers which are bioerodable by virtue of their water solubility, e.g., polyvinyl alcohol, the classes of polymer which have been shown to be degraded at an appreciable rate *in vivo* are:

1. Activated Carbon-Carbon Polymers

   e.g. $\{CH_2\text{-}C(CN)_2\}_n \rightarrow CH_2O + CH_2(CN)_2$
   $\{CH_2\text{-}C(CN)COOR\}_n \rightarrow CH_2O + CN\cdot CH_2COOI$
2. Polyamides, Polyurethanes

   e.g. $\{CH_2\text{-}NH\text{-}CO\text{-}CH_2\}_n \rightarrow NH_2CH_2COOH$
3. Polyesters, Polycarbonates

   e.g. $\{CH_2\text{-}O\text{-}CO\text{-}CH_2\}_n \rightarrow HOCH_2COOH$
4. Polyacetals, Polyketals, Poly orthoesters

   e.g. $\{\underset{\displaystyle OR}{\underset{|}{CH}}\text{-}O\text{-}CH_2\}_n \rightarrow ROH + HOCH_2CHO$
5. Inorganic Polymers

   e.g. $\{\underset{\displaystyle (OR)_2}{\underset{|}{P}}=N\}_n + H_20 \rightarrow ROH + NH_3$
6. Natural Polymers Subject to Enzymatic Degradation

   e.g. Polysaccharides, Collagen

The selection of any one of these classes for study is somewhat subjective, for within each class one can find a spectrum of properties. The permeability, biodegradability and mechanical properties may all be changed substantially by logical but rather small changes in the chemical structure of the substituent or chain. The polyesters (figure 1) provide a good illustration. Thus, on the basis of electronic effects and the properties of monomeric esters, one can anticipate that the rate of non-enzymatic hydrolysis of polymers of α-hydroxy acids, e.g., poly(glycolic acid), will be greater than polymers of ß, γ, δ, and ϵ-hydroxy analogs; aromatic esters will be least readily hydrolyzed. Alkyl or aryl substituents adjacent to the ester group can retard hydrolysis by steric hindrance.

The polymer morphology and permeability are superimposed upon these chemical effects, determining the accessibility of the bulk of the polymer to aqueous hydrolysis and the loss of cleaved

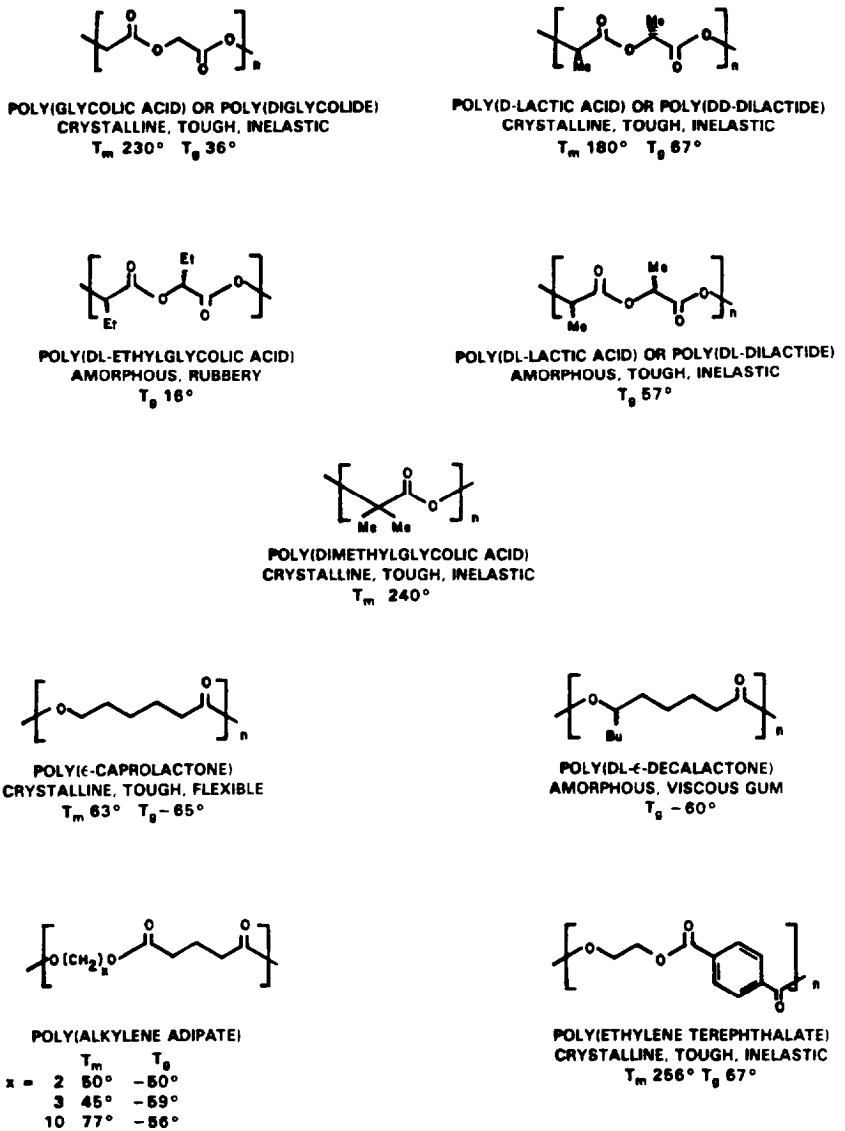


FIGURE 1. The Chemical Structures and Relevant Physical Properties of Some Typical Polyesters.

chain fragments, as well as the rate of drug diffusion and mechanical properties. Here the T_g and the melting point (T_m) provide a good indication of structure-morphology relationships (figure 1). Poly(glycolic acid), the simplest aliphatic polyester, is a practically insoluble, high melting, crystalline polymer with a T_g of 36°C.

These properties reflect the polarity and structural order of this polymer. Stereospecific introduction of a single methyl group, as in poly(D or L-lactic acid) has little effect on the T_g and T_m. Non-stereospecific introduction of a methyl group, as in racemic poly(DL-lactic acid) does not change T_g greatly but completely eliminates the crystallinity because of the random arrangement of the asymmetric centers. Similarly, nonspecific introduction of two different alkyl groups will both eliminate the crystallinity and reduce the T_g, and a polymer such as poly(DL-methylethylglycolic acid) is rubbery at 37°C. However, if both the alkyl groups are identical, as in poly(dimethylglycolic acid), the symmetry and crystallinity are restored.

It is possible to reduce the T_g substantially without changing the crystallinity by increasing the length of the methylene repeat unit, e.g. poly(alkylene adipates), poly(ε-caprolactone), and their homologs. These polymers are permeable (low T_g) and mechanically strong (crystallinity). The crystallinity can be reduced or eliminated by introduction of an asymmetric center, as in poly(DL-ε-decalactone). This modification increases the permeability but reduces the mechanical strength to the extent that the latter polymer is a viscous gum.

Introduction of aromatic groups into the polymer backbone increases the T_g and the crystallinity, and a polymer such as poly(ethylene terephthalate) is a tough, high melting, inflexible, impermeable material.

Plasticization, blending, and copolymerization represent other ways in which the polymer properties may be tailored, and specific examples are given below.

Polymer Permeability

There is a good correlation between the T_g and the permeability of those polyesters which have been tested. Poly(ε-caprolactone) and its copolymers have low T_g's and are as permeable as silicone rubber; poly(DL-lactic acid) and its copolymers with poly(glycolic acid) are in the glassy state and are relatively impermeable to drugs such as steroid hormones. Using progesterone as an example, the maximum steady state flux (J_{lim}), solubility, partition and diffusion coefficients of this steriod in poly(ε-caprolactone) and its copolymers with DL-lactic acid are compared with silicone rubber in table 1. The permeability of poly(ε-caprolactone), as reflected by the value of J_{lim}, is almost equal to that of silicone rubber, largely because of the greater drug solubility in poly(ε-caprolactone). (The maximum steady state flux, J_{lim}, is the product of the diffusion coefficient and the solubility of the drug in the polymer and is equal

to the maximum drug diffusion rate through a membrane of unit area and thickness.) While poly(DL-lactic acid) is 10^4 times less permeable than poly(ε-caprolactone), copolymers of DL-lactic acid and ε-caprolactone have the same permeability as poly(ε-caprolactone). This is understandable, for copolymerization affords a polymer with a T_g between the T_g's of the two homopolymers, as illustrated in figure 2, and with less crystallinity than poly(ε-caprolactone).

TABLE 1. The Maximum Steady State Flux (J_{lim}), Partition Coefficient (K), Solubility (C,) and Diffusion Coefficient (D) of Progesterone in Poly(ε-caprolactone), Poly(DL-Lactic Acid), Poly(ε-caprolactone-co-DL-lactic acid), and Silicone Rubber

Polymer	J_{lim} x 10^{11} (g.cm ^{1}.sec ^{1})	Partition Coefficient	Solubility (mg/g)	D x 10^9 (cm^2.sec ^{1})
Poly(ε- -caprolactone)	6.1	1200	16.9	3.6
1:1 copolymer[a]	5.6	1554	21.9	2.7
Poly(DL-lactic acid)	0.33 x 10^3	46	0.65	5.1 x 10^3
Silicone Rubber[b]	26	50	0.57	450

[a]1:1 (wt%) Copolymer of ε-caprolactone and DL-lactic acid
[b]Data for polymer containing filler (Reference 10).

Progesterone is released very rapidly from thin films (100-300 μm) of poly(ε-caprolactone) and its copolymers with lactic acid and glycolic acid, release being essentially complete within 24 hrs (6). The fraction of drug load released is proportional to $(time)^{1/2}$, although the kinetics are moderated by an aqueous boundary layer which produces some curvature in a plot of the fraction released vs $t^{1/2}$ (figure 3).

Release of progesterone from poly(DL-lactic acid) films is very much slower, even from thinner films (10 mm), and leaching may be partly responsible for the rate which was observed (figure 4). Similar results were obtained with copolymers of glycolic acid and DL-lactic acid except that in some instances there was a marked increase in rate after several weeks (figure 5). This coincided with the mechanical deterioration of the film and is an example of erosion-enhanced release (6).

The rate of diffusion could also be enhanced by plasticization of poly(DL-lactic acid) with tributyl citrate (5). This served to reduce the glass transition below 37° and transformed the polymer to the rubbery state. However, the effect was short lived (figure 6), as the plasticizer was rapidly leached into the external aqueous medium. The permeability could also be enhanced (200-fold) by casting

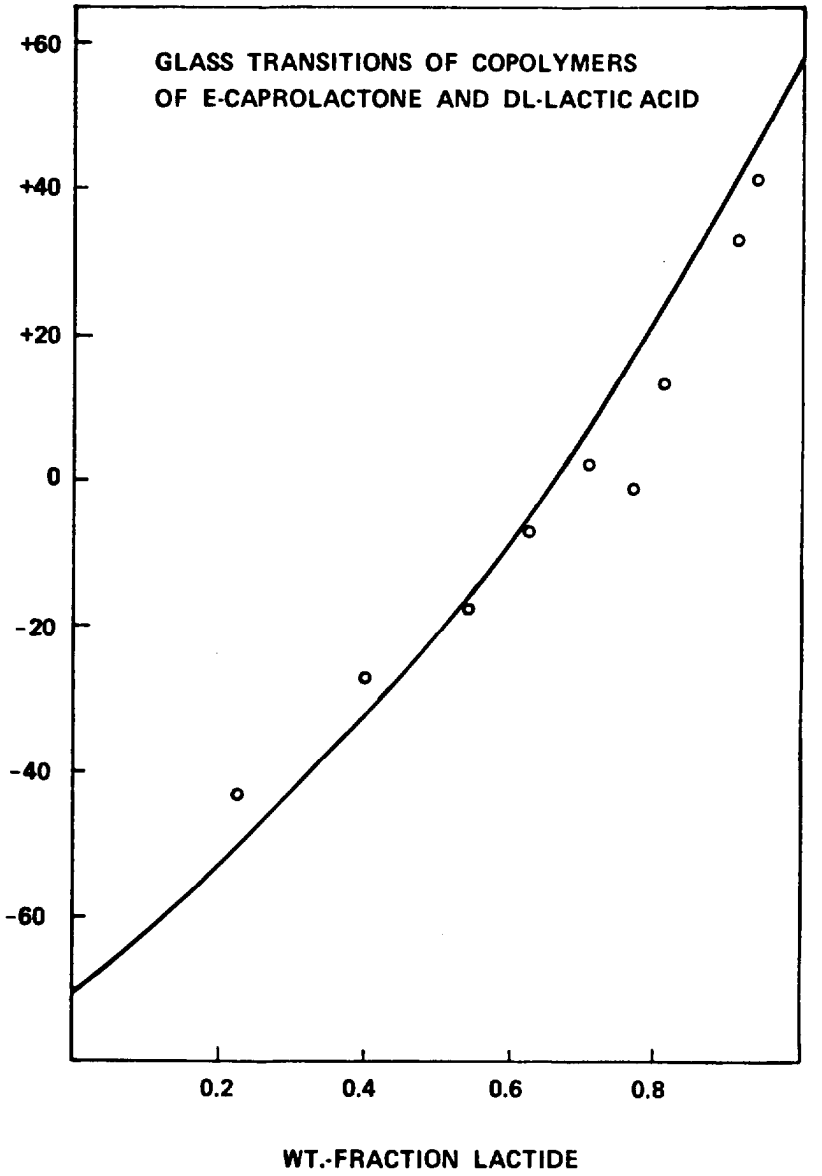


FIGURE 2. Variation of Glass Transition Temperature (T_g) of Copolymers of Caprolactone and DLLactide as a Function of DL-Lactide Content. Solid Line is Calculated Relationship, Based on Fox Equation (from Reference 8).

poly(DL-lactic acid) from a solution containing glycerine, then soaking the film in water (5). Here the permeability increase was the result of a highly porous structure easily detected by scanning elec-

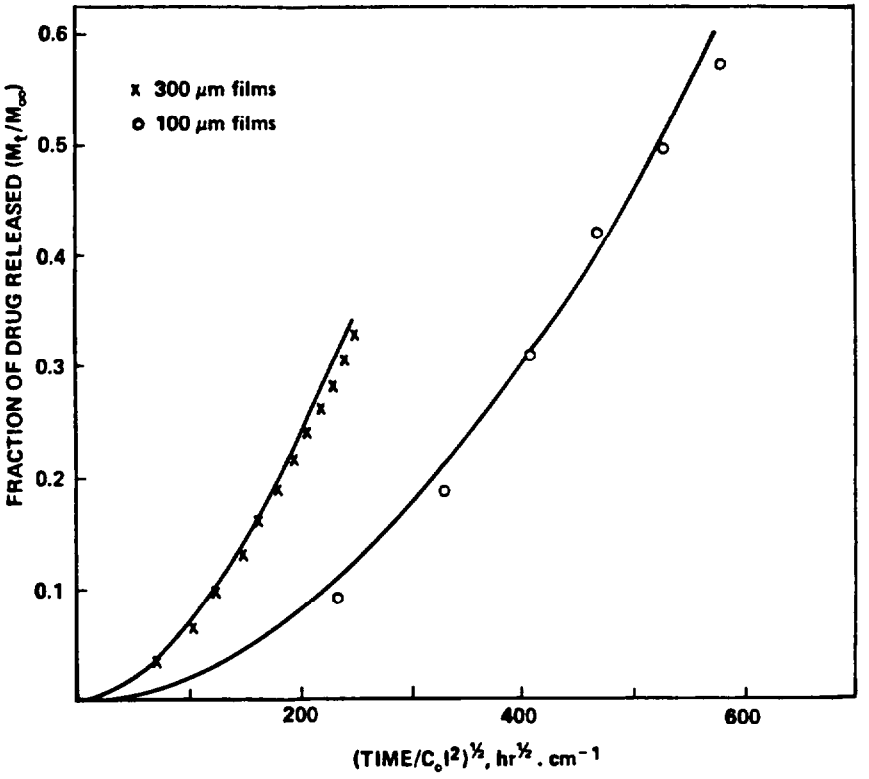


FIGURE 3. Fraction of Progesterone Released from Poly(ϵ-caprolactone) Films (10% Drug Load) as a Function of $Time^{1/2}$, and Film Thickness. Solid Lines are Calculated from Theoretical Relationship (Reference 11) Which Incorporates Effect of Aqueous Boundary Layer.

tron microscopy (figure 7). This structure may mimic that of a film partly depleted of drug.

Polyester Biodegradation

Most reports of polymer biodegradation are qualitative and confined to evaluation of a single property; in many cases the specifications of the polymer are not reported. This is unfortunate because residence times in the body may vary by an order of magnitude depending on the molecular weight, morphology, physical form, etc. As a consequence, the factors which contribute to polymer degradation are still poorly understood.

In these laboratories, poly(ϵ-caprolactone) has been studied most extensively, and changes in viscosity, weight, molecular weight distribution, and tensile properties have been determined *in vivo*

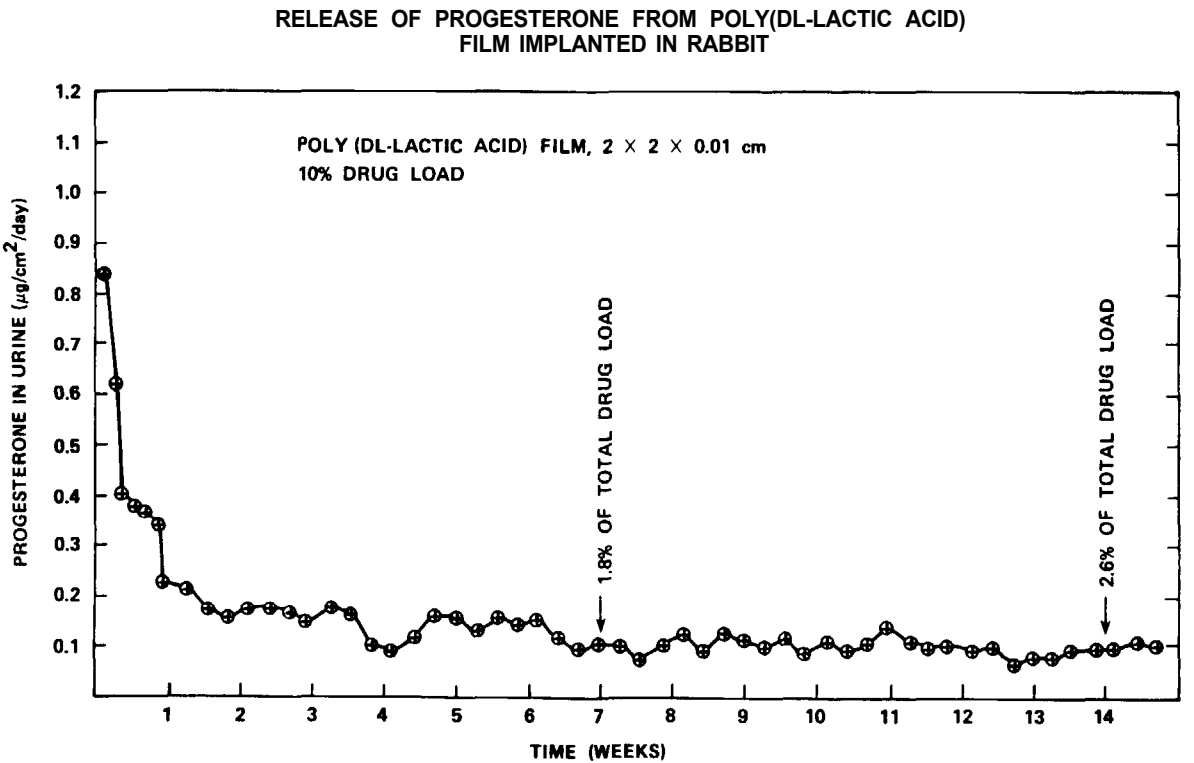


FIGURE 4. Daily Rate of Release of Progesterone from Poly(DL-Lactic Acid) Film Implanted in Rabbit, Measured as Radioactivity Excreted in Urine (from Reference 7).

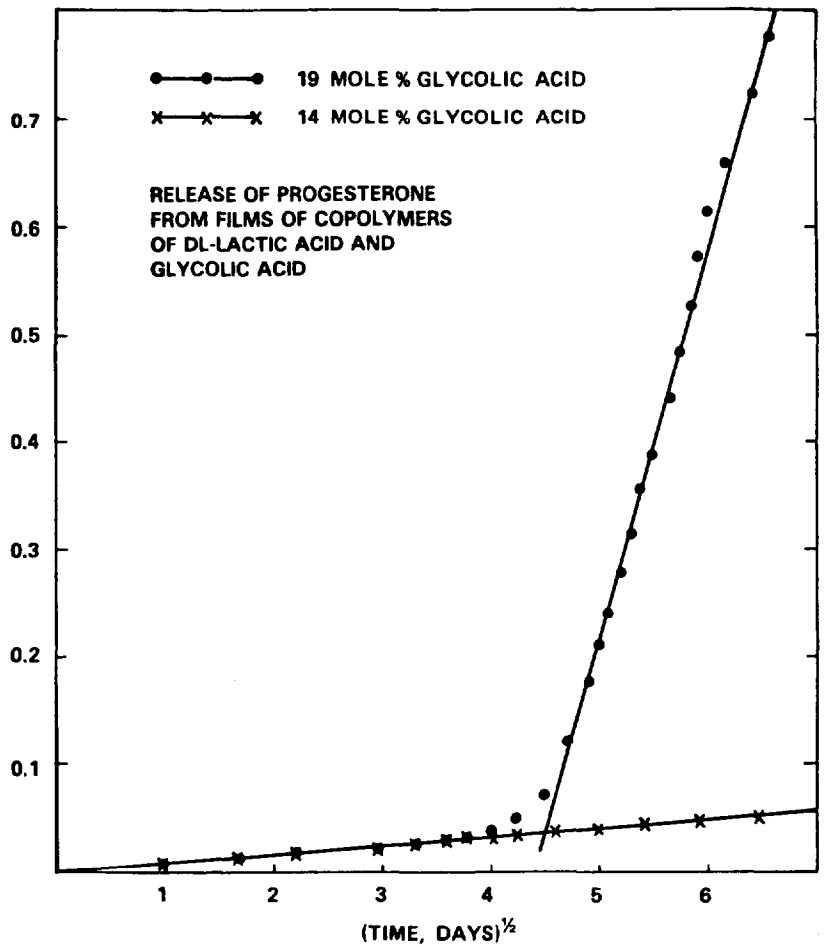


FIGURE 5. *In Vitro* Rates of Release of Progesterone From Two Poly(DL-Lactic Acid-co-Glycolic Acid) Films, Thickness 100 mm, Drug Load 10%, Plotted as the Fraction of Drug Released Versus the Square Root of Time. Break in Line is Indicative of Fragmentation of One of the Films.

(rabbit, rat) and under accelerated *in vitro* conditions (7,8) In common with other polyesters studied, biodegradation of poly(ϵ-caprolactone) is characterized by an induction period prior to weight loss. There is a continual decrease in the viscosity and molecular weight (GPC) resulting from hydrolytic cleavage of the ester linkages, and semi-log plots of MW or the intrinsic viscosity versus time are linear until the MW has decreased to about 30,000. Several different polymers are compared in figure 8, which shows that poly(DL-lactic acid) is degraded more rapidly than poly(ϵ-caprolac-

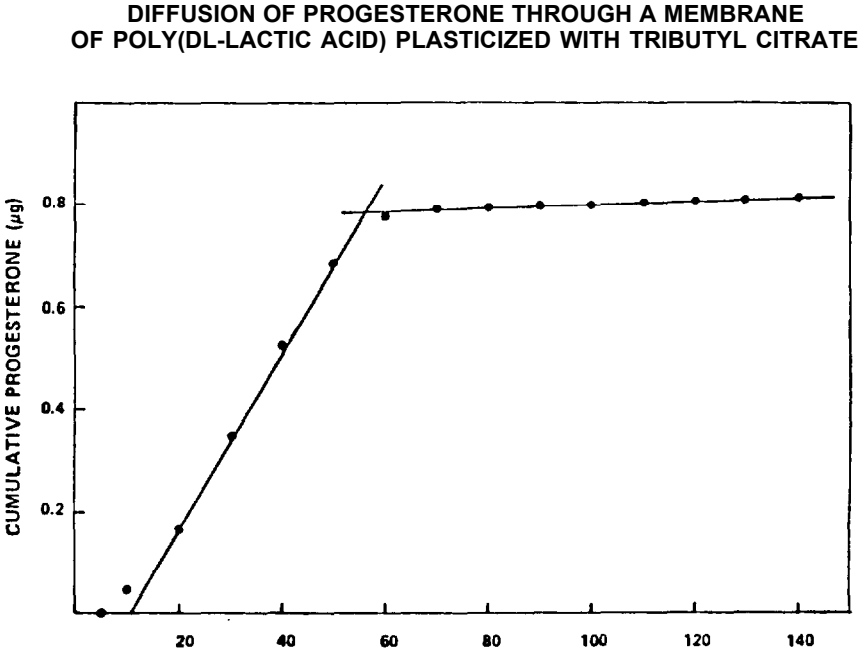
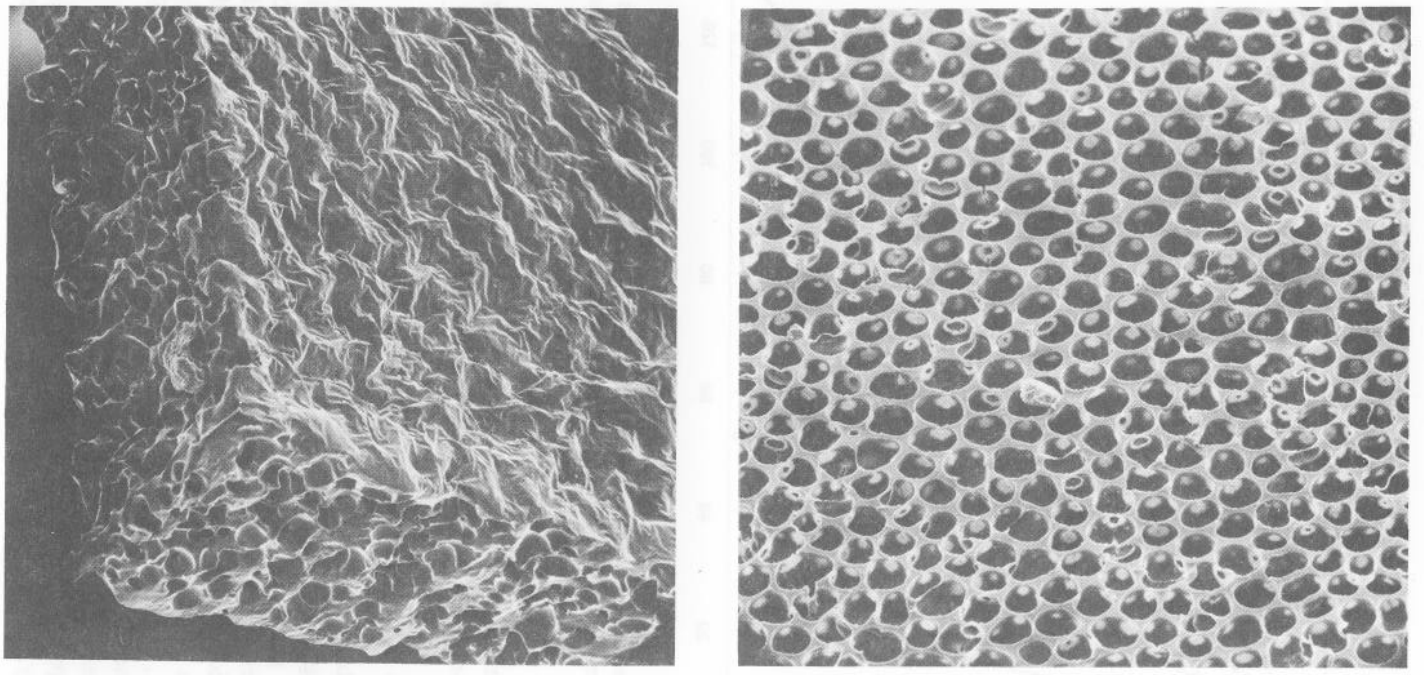


FIGURE 6. Rate of Diffusion of Progesterone Through a Membrane of Poly(DL-Lactic Acid) Plasticized with Tributyl Citrate. Break in Plot is Indicative of Loss of Tributyl Citrate (from Reference 5).

tone). This can be attributed to chemical effects, for the same relative rates are observed with the monomeric esters. The greater rate of degradation of the copolymers must be attributed to morphological changes. Others have observed that copolymers of lactic acid and glycolic acid are degraded more rapidly than the homopolymers (9), presumably for the same reason.

The semi-logarithmic relationship between the molecular weight and time *in vivo* is consistent with bulk hydrolytic cleavage of the ester linkages autocatalyzed by the generated carboxylic acid end groups. The same rate is observed *in vitro* for (H_20, 40°) and for films and cylinders, despite a large surface-to-volume difference. These observations rule out any enzymatic contribution to the hydrolysis rate.

For poly(ϵ-caprolactone), the decrease in molecular weight is accompanied by a slow increase in crystallinity, elastic modulus, and a decrease in the % elongation at break and ultimate tensile strength. Weight loss commences when the MW is less than about



Magnification: 330 Magnification: 1020

FIGURE 7. Scanning Electron Micrographs of Films Prepared From Poly(DL-Lactic Acid) and 10% Glycerine (from Reference 5).

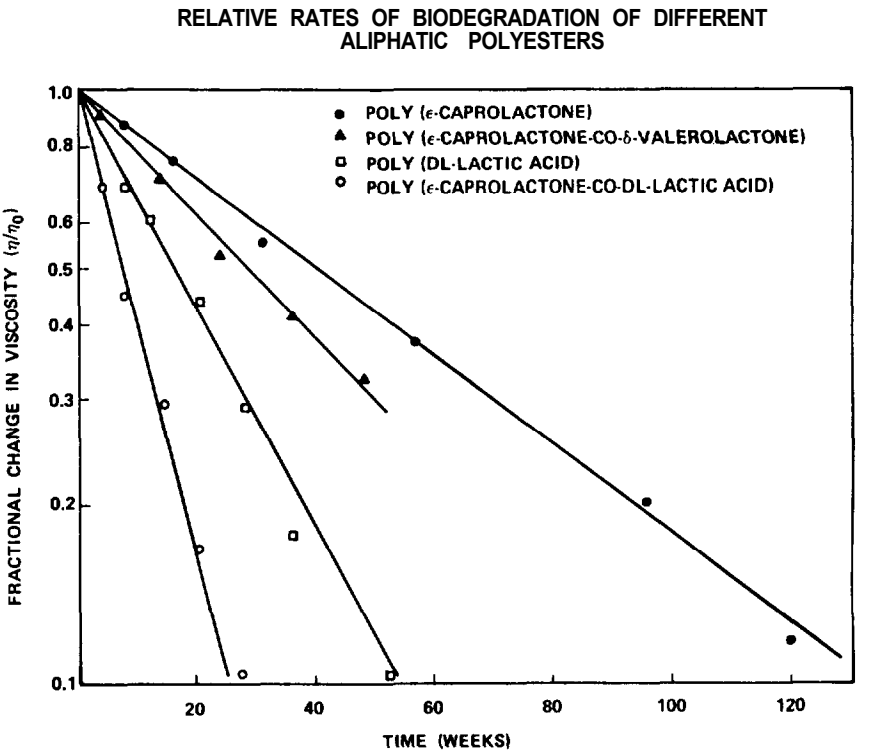


FIGURE 8. Fractional Change in the Intrinsic Viscosity of Different Polyesters as a Function of Time Implanted in Rabbit, Illustrating Dependence of Degradation Rate on Chemical Structure and Morphology.

30,000. Changes in both the weight and viscosity of various copolymers of ϵ-caprolactone and DL-lactic acid are shown in figure 9.

Application to Contraception and Narcotic Antagonist Therapy

The substantial induction periods prior to weight loss, coupled with the high permeability of poly(ϵ-caprolactone) and its copolymers, have permitted the development of biodegradable reservoir devices which function by sustained, diffusion-controlled drug delivery and which erode after the drug reservoir is depleted. These devices retain the advantages of subdermal poly(dimethylsiloxane) systems, i.e., constant and sustained drug delivery, but the need for surgical removal after drug exhaustion is eliminated.

The primary objective of our studies has been the development of a device capable of subdermal delivery of contraceptive levels i.e., ~50 μg/day, of levonorgestrel for a period of at least one year.

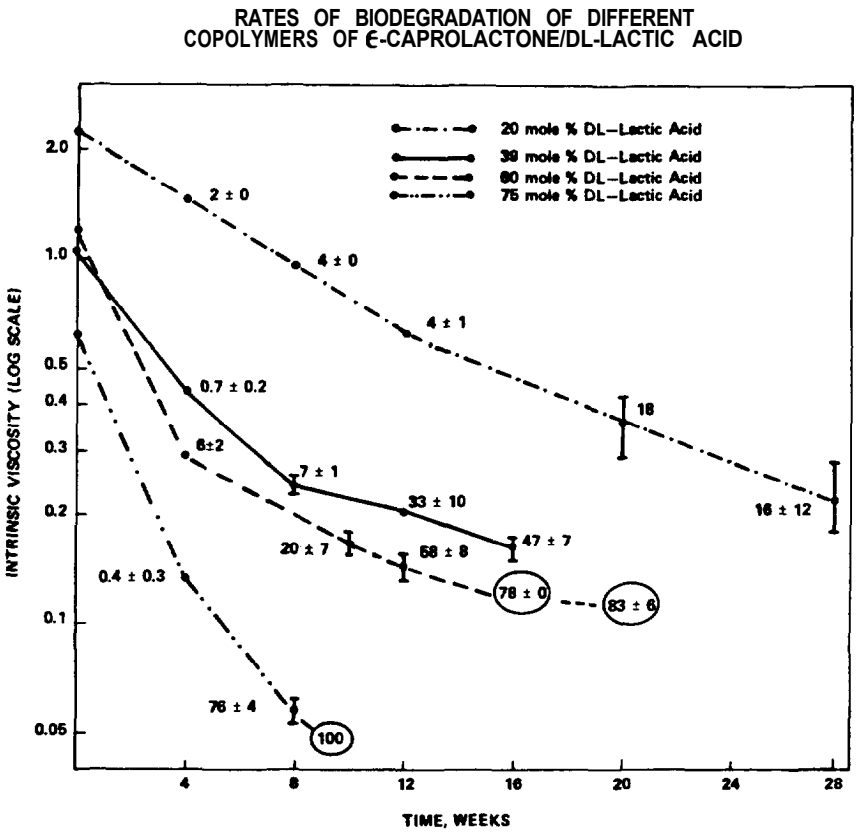


FIGURE 9. Semi-logarithmic Plots of Intrinsic Viscosities of Poly(ϵ-caprolactone-co DL-Lactic Acid) Capsules Versus Time *In Vivo* (Rabbit). Numbers at Individual Data Points Show Averaged Magnitude of Weight Loss.

Poly(ϵ-caprolactone) is the most suitable polymer for this purpose, for it is permeable to levonorgestrel, and an induction period of 1 year prior to the onset of bioerosion is easily achieved. Examples of long term levonorgestrel release rates *in vitro* and *in vivo* (rat) are shown in figures 10 and 11. Sterilization of the device is achieved by γ-irradiation, and toxicology studies have shown no adverse local or systemic effects. Initial pharmacokinetic studies in women are now planned.

The requirements of a narcotic antagonist maintenance program are very different from those of birth control programs. Naltrexone, the most potent narcotic antagonist, is only effective at a dose of at least 3 mg/day and present treatments are restricted to 1-2 months. The properties of poly(ϵ-caprolactone-co-DL-lactic acid) are

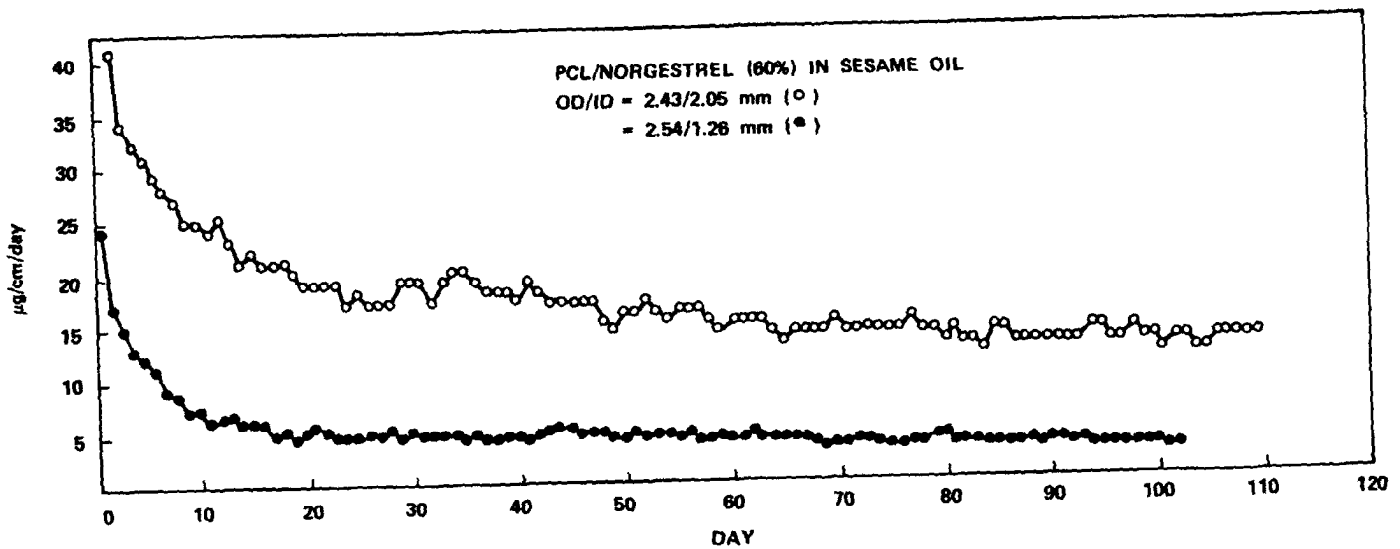


FIGURE 10. Daily Rate of Release of Norgestrel from Poly(ϵ-Caprolactone) Capsules, *In Vitro,* Per Centimeter Length of Capsule, Using Two Different Capsule Wall Thicknesses.

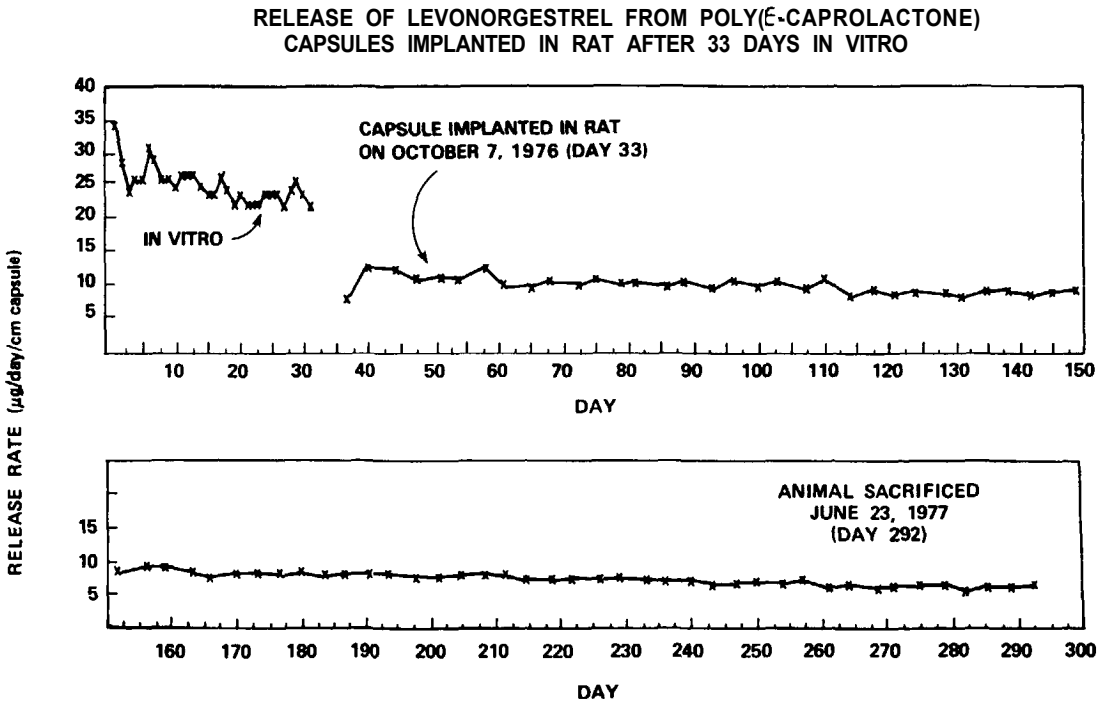


FIGURE 11. Daily Rate of Release of Norgestrel from a Poly(ϵ-Caprolactone) Capsule Implanted in Rat After 32 Day *In Vitro*. Release Rate Determined by Measurement of Radioactivity in Urine and Feces. Difference in the *In Vitro* and *In Vivo* Rates is the Result of Metabolic Loss of Radiolabel by Routes Other Than Urine and Feces.

suitable for a sustained-delivery device with these requirements. Thus, by proper choice of molecular weight and chemical composition, it is possible to obtain a polymer which is biostable for a period of anywhere between 4 and 12 weeks (drug delivery phase), and which undergoes rapid bioabsorption subsequently (figure 9). This copolymer is also in the rubbery state and very permeable.

A copolymer with an intrinsic viscosity of 1.5 dl/g and 20 mole% lactic acid was selected for evaluation. The maximum steady state flux (J_{lim}) and the diffusion coefficient (D) of naltrexone in this copolymer were determined from the kinetics of transport through a thin film in a standard diffusion cell.

$$J_{lim} = 2.7\ (\pm\ 0.4)\ x\ 10^{-10} g.sec^{-1}.sec^{-1}$$

$$D = 5.9\ (\pm\ 2.4)\ x\ 10^{-9} cm^2.sec^{-1}$$

Based on the value of J_{lim} and the kinetic expression for the diffusion controlled release of a cylindrical reservoir (Eq 1), one can estimate

$$\text{Release rate} = 2\pi DK\Delta C/\ln (od/id) = 2\pi J_{lim}/\ln (od/id) \quad . \ . \ . \ (1)$$

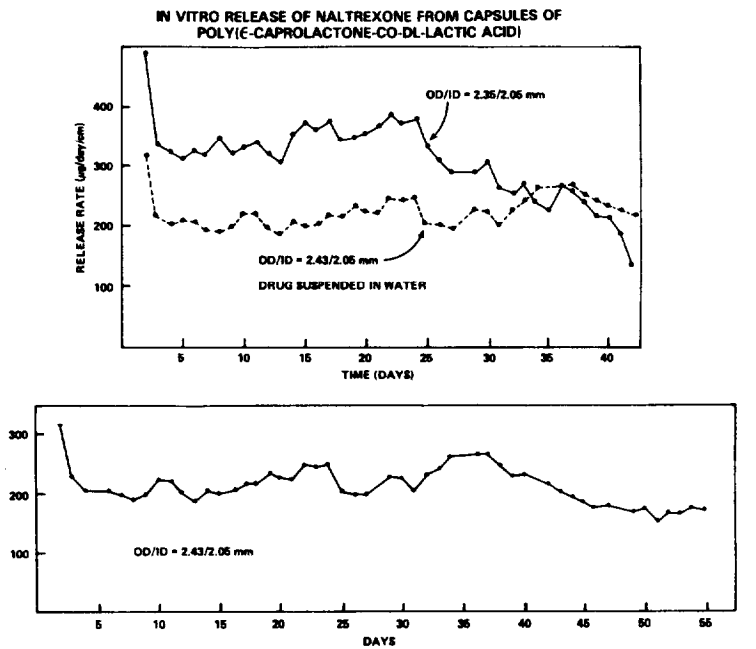


FIGURE 12. *In Vitro* Rate of Release of Naltrexor-^{3}H from a Poly(ε-Caprolactone-co-DL-Lactic Acid) Capsule Containing Naltrexone as 50% Suspension in Water.

that two 2.5 cm capsules, 2.7 mm outer diameter and 2.1 mm inner diameter, filled with a 50% aqueous slurry of naltrexone, will release 3 mg of drug per day for 30 days. This is within the range believed desirable for an antagonist maintenance program.

Rates of release were first measured *in vitro* and found to be relatively constant over a 36-day period. Using capsules with a 2.05 mm inner diameter and 2.35 mm outer diameter, the release rate was between 300 and 400 μg/day per cm capsule length for 24 days, declining as the drug was exhausted over the next 20 days (figure 12a). A lower but more sustained release rate was achieved when capsules with a slightly thicker wall were used (figure 12b). These rates are lower than the values estimated from J_{lim}, but this is not surprising in view of the errors of measurement of the thin walls of the polymer capsule, the sensitivity of the rate to the od/id ratio in the range used, and the fact that the method of tube preparation produces some irregularity in the dimensions and possibly some differences in morphology.

Since poly(ε-caprolactone-co-DL-lactic acid) is permeable to water, the rate of drug released from a capsule containing only naltrexone was measured *in vitro*. There was a slow increase in rate, to ~300 μg/day/cm length (figure 13). Presumably the increase was related to water diffusion into the capsule, the water being necessary to maximize dissolution of the drug in the capsule wall. The maximum release rate is dependent on maintaining the drug concentration gradient (ΔC) across the polymer wall. The rate slowly declined after day 30, until the rate was ~100 μg/day at day 95. When such capsules, 3 cm length, were subdermally implanted in three monkeys, one per animal, morphine self-administration was immediately suppressed and remained so for 33 days.

The naltrexone used in this study was tritium labeled, and blood radioactivity levels were measured to assess the constancy of the drug release rate. The rapid increase in tritium levels after about 20 days in two cases (figure 14) strongly suggests that capsule degradation commenced at that time. At the end of the study (day 46) the capsules were recovered as partially degraded fragments. Evidently, the time prior to polymer bio-erosion was somewhat less than that estimated from figure 9, and the morphine blockade could have been maintained for a greater time period by use of a copolymer with a slightly different composition.

These studies with naltrexone only amounted to exploratory experiments, to test the feasibility of using a polyester in the form of a biodegradable reservoir device. It would appear that the approach is feasible and that it is only necessary to optimize the various components to achieve a practical device.

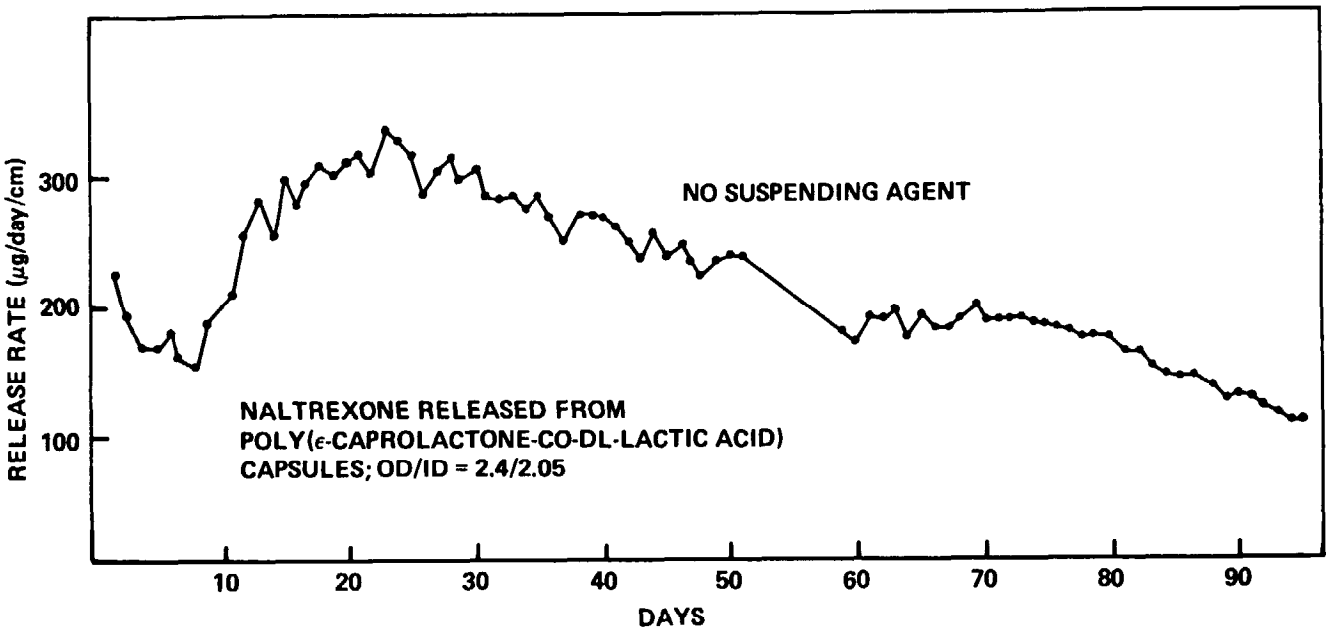


FIGURE 13. *In Vitro* Rate of Release of Naltrexone-^{3}H from a Poly(ε-Caprolactone-co-DL-Lactic Acid) Capsule Containing Dry Naltrexone. Capsule Dimensions: 2.4 mm od, 2.05 mm id, 1 cm length.

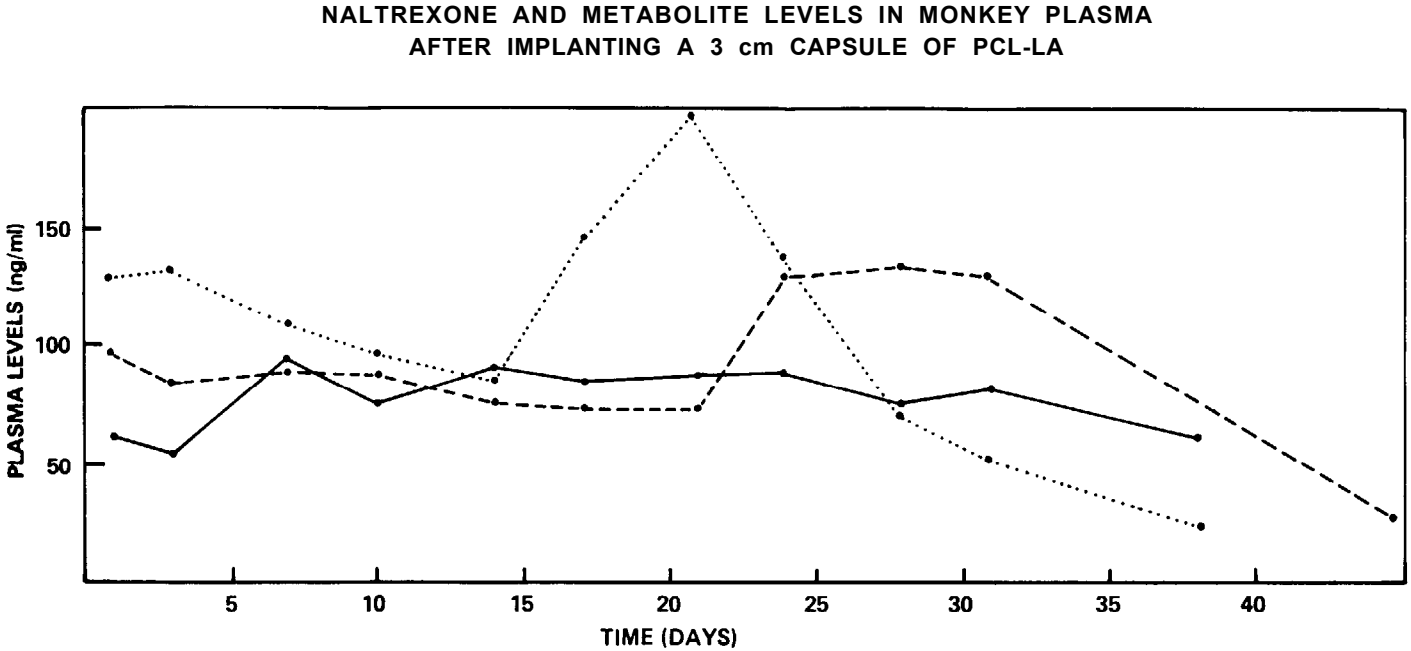


FIGURE 14. Plasma concentrations of Naltrexone and its Metabolites Resulting From Implantation of Poly(ε-Caprolactone-co-DL-Lactic Acid) Capsules Containing Dry Naltrexone in Three Monkeys.

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An Improved Long-Acting Delivery System for Narcotic Antagonists

Anand L. Misra and Ronald B. Pontani

The feasibility of using cholesterol-glyceryltristearate matrix for prolonged release of naltrexone was evaluated in mts. Implantable cylindrical pellets (cholesterol 105 mg, glyceryl tristearate 15 m and naltrexone 30 mg), diameter 4.5 mm, length 9 mm, locked the antinociceptive action (hot plate 55°C) of 10 mg/kg s.c. challenge dose of morphine in rats for 3 months. The release rate of naltrexone from 10 or 50 mg pellets approximated first-order kinetics with $t_{1/2}\alpha$ of 20-26 days and $t_{1/2}\beta$ 40-60 days. The factors affecting the release of drug rom the delivery system were the ratio of cholesterol to naltrexone, drug loading level and surface area to unit volume of dosage form. The minimum release rate of naltrexone to block the effect of 10 mg/kg s.c. dose of morphine in rats was about 4 to 5 μg/kg/hr. The cumulative urinary excretion of radioactivity rom 10 mg [^{3}H] naltrexone pellets implanted s.c. in rats after 30, 60, and 90 days was 17.7, 23.7, and 25.7% of dose respectively and the percent dose released from pellets at these times was 55.8, 68.8, and 78.2 respectively. The devices possess the desirable characteristics of simplicity, nontoxicity, nonirritability, ease of sterilization with ethylene oxide, small size for easy insertion and removal, absence of encapsulation by surrounding tissue, and an extended period of drug release unaffected by body metabolism. Neither deterioration of implant nor gross anatomic or histological changes at the site of implant occurred 6 months to 1 year after implantation and aside from some enhanced sexual activity (e.g., spontaneous penile erections) no side effects were observed in rats, which fed well and gained weight during the entire treatment. The concentrations o free morphine in brains of 30 mg naltrexone pellet-implanted rats were significantly

lower (2.4 and 15%) as compared to the placebo controls 0.5 and 1.0 hr after a 10 mg/kg s.c. dose of [6-^{3}H] morphine. We are currently evaluating these long-acting devices for the duration of effective antagonism to morphine in rhesus monkeys.

Long-acting narcotic antagonists, e.g., naltrexone, have potential clinical usefulness in combatting opiate dependence and prevention of relapse to opiate-seeking behavior (1,2,3,4,5). The urgent need for long-acting delivery systems for naltrexone and the status of research on such systems have recently been indicated in a monograph (6). It is felt that such systems will eliminate the need for clinic visits in opiate-dependent individuals without reducing protection against self-administered opiates and will in addition reduce the emphasis on medication alone and refocus it on rehabilitation aspects. The systems design and some specifications for a clinically acceptable and useful delivery system for narcotic antagonists with a long biological $t_{1/2}$ have been outlined by Archer (7) and Willette (8). These are: (a) adequate and smooth drug-release rate, preferably obeying zero-order kinetics; (b) relative ease of insertion and removal in the event of a need for analgesic medication; (c) biocompatibility or lack of adverse tissue reaction; (d) biodegradability; (e) ease and reasonable expense of manufacture; (f) stability to sterilization and storage conditions; (g) patient and physician acceptability.

Previous work has utilized biodegradable polylactic and lactic-glycolic acid composites for cyclazocine and naltrexone (9,10,11), nonbiodegradable polymer for naloxone (12), synthetic glutamic acid-leucine copolymers, polyglycerides, insoluble salts and metal complexes of antagonists (13) as prolonged-release preparations (6). These vehicles have in general produced blockade of antinociceptive action of morphine for periods ranging from 15 to 30 days or longer. Woodland et al. (9) obtained $t_{1/2}$ values of 11 to 13 days on release of antagonist, a poor correlation between *in vitro* and *in vivo* data and an inflammatory process at the site of film implant; Yolles et al. (10) obtained effective blocking action to morphine in rats for 24 days ($t_{1/2}$ 22 days); Fishman et al. (12) observed the duration of antagonism for 22 days in rats and slight encapsulation of implant by surrounding tissue. The release rates of narcotic antagonist from lactic/glycolic acid polymers approximated zero-order kinetics for most of the release period and antagonist response to a challenge dose of morphine was maintained from 1 to 6 months depending on the formulation tested (11). The procedures involved in

the preparation of above copolymers are complex, and much work remains to be done on the reproductibility of polymerization conditions, use of pharmacologically suitable catalysts, and overall tissue compatibility and toxicity of such copolymers. Previous reports on the sustained-release hormonal preparations (14,15,16) prompted us to evaluate compressed pellets of cholesterol and glyceryltristearate as long-acting delivery systems for narcotic antagonists. We report here a promising delivery system for naltrexone which may have clinical usefulness in treatment of narcotic dependence.

MATERIALS AND METHODS

Naltrexone hydrochloride and [15,16-^{3}H] naltrexone (sp. act. 47.7 mCi/mg) were provided by Research Triangle Institute, N.C., through the courtesy of National Institute on Drug Abuse, Rock ville, Md. [6-^{3}H] Morphine (New England Nuclear Corporation, Boston, Mass.), cholesterol m.p. 146-149°C (J.T. Baker Chemical Company, Philipsburg, N. J.) and glyceryltristerate m.p. 65-67°C (K.K. Laboratory, Inc., Plainview, N. Y.) were commercial samples.

Preparation of Naltrexone Pellets

Cholesterol (360 mg), glyceryltristerate (40 mg) and naltrexone base (100 mg) were dissolved in 10 ml chloroform and the solution evaporated to dryness *in vacuo* in a Rota Vapor and the residue further dried *in vacuo* overnight. The thoroughly mixed powder (50 mg) was compressed to a cylindrical pellet (54,500 lb/sq in), diameter 3 mm, length 6.35 ± 0.13 (s.e.) mm, weight 48.6 ± 0.8 (s.e.) mg, surface area 56.5 mm^2, volume 42.3 mm^3 in a Carver Laboratory Press (Fred Carver, Inc., Summit, N.J.). Radioactive naltrexone pellets containing 10 mg naltrexone were prepared using [15,16-^{3}H] naltrexone by the above procedure. Cylindrical pellets (diameter 4.5 mm, length 9 mm) of naltrexone (30 mg) comprising cholesterol (105 mg) and glyceryltristerate (15 mg) and 50 mg naltrexone pellets of same dimensions comprising cholesterol (90 mg) and glyceryltristearate (10 mg) were also prepared by the above procedure.

ANIMAL EXPERIMENTS

Cylindrical pellets of naltrexone and placebo pellets were implanted subcutaneously in the dorsal area behind the right hind

limb of male Wistar rats, pushed away from incision and the incision sutured. The response iatencies (sec) of animals in 2 groups were measured on a hot plate (55°C) with a cut-off time of 30 sec before morphine injection and 0.5, 1, 1.5, 2 and 3 hr after 10 mg/kg s.c. morphine injection (ED_{80}, 6.5 mg/kg). The criterion of reaction of the rat was licking of one paw or jumping on hind legs. The duration of effective antagonism to challenge dose of morphine was tested at 15 days, 1, 1.5, 2 and 3 months after implantation. After being in place for 2 or 3 months, the naltrexone pellets were removed in the experimental group and their responses to a challenge dose of morphine compared with the placebo group.

Radioactive naltrexone pellets (10 mg) were also implanted s.c. in a group of 7 rats, and the excretion of radioactivity in urine collected in metabolism cages every 24 hr for 30 days was measured by counting the radioactivity in an aliquot of urine and correcting it for quenching, using (^{3}H) toluene as an internal standard. These pellets were removed 30, 60 and 90 days after implantation, dissolved in chloroform, and radioactivity counted in an aliquot.

Disposition Of [6-^{3}H] Morphine In Rats Implanted With Long-Acting Naltrexone Pellets

The rats were implanted s.c. with 30 mg naltrexone or placebo pellets, and, after 2 weeks, a 10 mg/kg dose of [6-^{3}H] morphine was injected subcutaneously in these animals. The concentration of [6-^{3}H] morphine in brain, liver, plasma obtained at different times after injection was determined by a method previously described (17).

RESULTS AND DISCUSSION

The response latencies in 30-day 10 mg naltrexone pellet-implanted animals at 0.5 to 2 hr after the morphine dose were not significantly different from the reaction times before morphine injection by correlated t-test (18). At 1.5 and 2 months, the degree of analgesic blockade to this morphine dose was approximately 90 and 35% respectively. Three days following the removal of naltrexone pellets, the reaction times observed 0.5 to 3 hr after the morphine dose were not significantly different from those in placebo-pelleted rats, indicating that the implanted naltrexone pellets also blocked the development of tolerance to morphine. The results (18) showed that the delivery system (10 mg naltrexone) produced a complete

and effective blockade of analgesia due to a 10 mg/kg (s.c.) dose of morphine in test animals for 1 month.[1] Similarly, naltrexone pellets (30 mg) provided effective antagonism to this challenge dose of morphine for at least 3 months (19).

The daily urinary excretion of radioactivity from 10 mg radioactive naltrexone pellets is shown in figure 1. From an initial value of 2.5 ± 0.3%, there was a steep fall to 0.8 ± 0.15% in 3 days, the values then declined slowly to 0.3 ± 0.05% on day 30. The cumulative urinary excretions of radioactivity (mean s.e.) (figure 2) in 30 and 60 days were 17.7 ± 0.4 and 23.7 ± 0.9% respectively, and in 90 days this excretion amounted to 25.7%. The pellets were removed 30, 60 and 90 days after implantation and the radioactivity remaining in the pellets was estimated. The percentages of radioactivity (mean ± s.e.) released from the implanted pellet in 30, 60 and 90 days were 55.8 ± 1.3, 68.8 ± 0.7 and 78.2% respectively (figure 3). The plot of percentage dose remaining at the site of implant vs. time was bioexponential with $t_{1/2}$ values of 26 and 60 days respectively. The excretion of radioactivity via fecal or other routes by subtraction therefore amounted to 38.1, 45.1 and 52.6% of implanted dose in 30, 60 and 90 days respectively. Thus the 10 mg naltrexone pellet gave a satisfactory release of drug over an extended period of time.

The release rate *in vivo* from 50 mg radioactive naltrexone pellets (figure 4) gave $t_{1/2}$ values of 20 and 40 days respectively, The

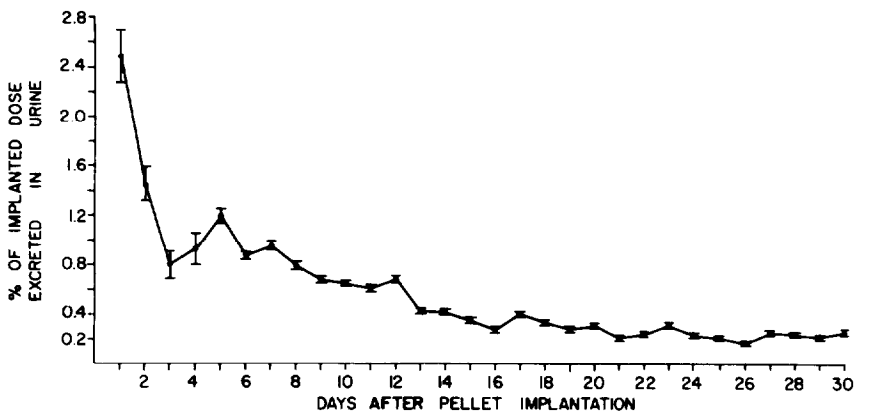

FIGURE 1. Daily amounts of radioactivity excreted in urine in rata in 30 days from 10 mg (^{3}H) naltrexone pellets implanted subcutaneously. Data represent the mean ±S.E.M. percent of implanted dose from 7 animals.

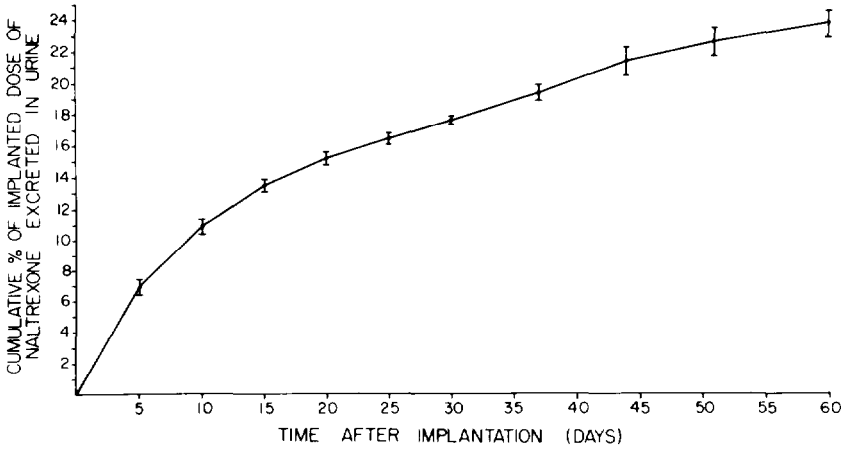

FIGURE 2. Cumulative percent of implanted dose excreted as total radioactivity in urine over a period of 60 days from 10 mg (^{3}H) naltrexone pellets implanted subcutaneously in rats. Data represent mean ±S.E.M. from 7 animals up to 30 days and 4 animals thereafter.

ratio of cholesterol to naltrexone in 50 mg pellets was 1.8 and that in 30 and 10 mg naltrexone pellets 3.5 and 3.6 respectively.

Although plasma levels of naltrexone at various times after 10 mg pellet-implantation in the same animal were not determined in this study, the values at 30-day period of narcotic blockade amounted to 4 to 5 μg/kg/hr. Harrigan and Downs (20) have reported that continuous naltrexone infusion of 1-20 μg/kg/hr produced effective blockade of morphine self-administration in the rhesus monkey and proposed a minimum value of 5 μg/kg/hr as an effective release rate of naltrexone for a drug-delivery system.

In our experiments neither deterioration of implant nor gross anatomic or histological changes at the site of implant occurred 6 months or longer after implantation. Aside from some enhanced sexual activity (e.g., spontaneous penile erections), no obvious side effects were observed in rats, which fed well and gained weight during treatment. The method thus has the merits of simplicity, nontoxicity, nonirritability, small size for ease of insertion and removal, ease of sterilization with ethylene oxide, biocompatibility, absence of encapsulation by surrounding tissue, and an extended period of drug release unaffected by body metabolism. The results clearly establish the feasibility of using cholesterol-glyceryltristearate as a matrix for the controlled release of naltrexone. Cholesterol provided a simple barrier effect on the diffusion of drug and it is conceivable that increasing the size of pellets while holding the

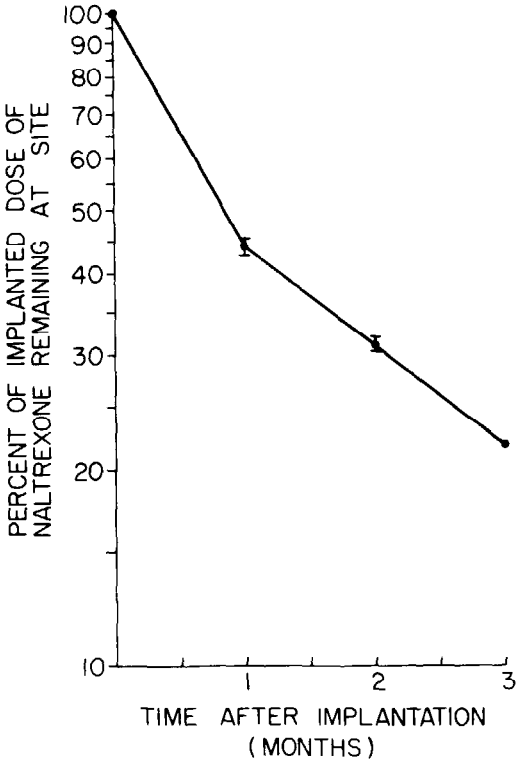

FIGURE 3. *In vivo* release of radioactivity from 10 mg (^{3}H) naltrexone pellets implanted subcutaneously in rats.

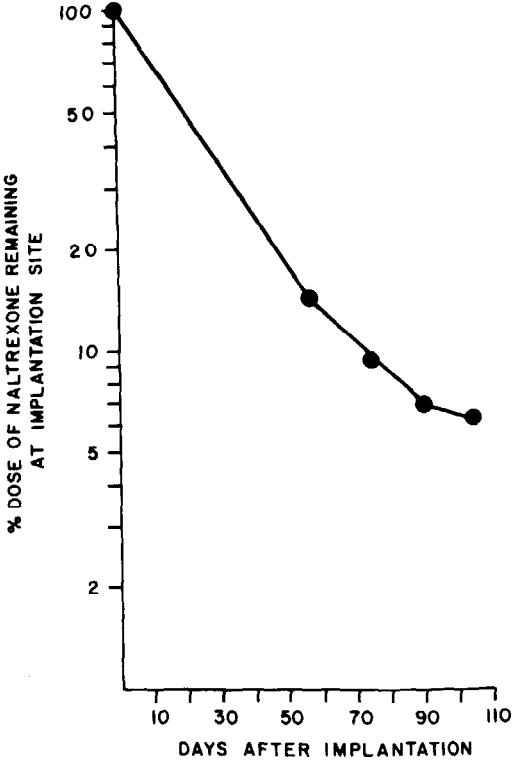

FIGURE 4. *In vivo* release of radioactivity from 50 mg (^{3}H) naltrexone pellets implanted subcutaneously in rats.

ratio of cholesterol to naltrexone constant may reduce the rate of drug release, thus extending its duration. Currently we are conducting experiments in rhesus monkeys using 50 mg and 666 mg naltrexone pellets (diameter 13 mm, length 16 mm), having the same cholesterol to naltrexone ratio (1:8). In rhesus monkey implanted s.c. with 50 mg naltrexone pellets the threshold plasma concentration of naltrexone during blockade of pupillary dilatation due to hydromorphone challenges was 2.9 ng/ml.

Data on the comparative distribution of free morphine in placebo and naltrexone pellet-implanted rats after a 10 mg/kg s.c. injection of [6-^{3}H] morphine appear in figure 5. The concentration of free morphine in brains of naltrexone pellet-implanted rats was significantly lower (24 and 15%) as compared to placebo controls 0.5 and 1.0 hr after morphine injection. The values of morphine in plasma of naltrexone pellet-implanted animals were 45 to 26% lower and those in liver 39 to 27% lower as compared to those of placebo-pelleted rats 0.5 and 1.0 hr after morphine injection. The brain to plasma ratios at 0.5 and 1.0 hr were comparatively higher in naltrexone pellet-implanted animals (0.21, 0.381 as compared to the placebo controls (0.16, 0.33). The values of total radioactivity comprising free morphine plus metabolites (μg-equivalent per ml of fluid or μg equivalent per g of tissue) at 0.5 and 1.0 hr after morphine injection in the two groups were as follows. In plasma: placebo pellet-implanted rats, 5.73 ± 0.34 and 5.90 ± 0.49; naltrexone pellet-implanted rats, 5.23 ± 0.79 and 4.77 ± 0.29 respectively. The corresponding values for liver were: placebo, 23.24 ± 2.24 and 24.04 ± 2.42 respectively; naltrexone pellet-implanted rats, 20.29 ± 1.30 and 17.08 ± 0.25 respectively. Of these values, only those in liver at 1 hr were significantly different ($p<0.05$) in the two groups.

Due to the chronic blockade of opiate receptors in naltrexone pellet-implanted rats, the dose of morphine did not produce any antinociceptive effect, in contrast to the placebo pellet-implanted rats which clearly showed the opiate effects. Naltrexone has much greater receptor affinity than morphine (21). The lowered distribution of morphine to the brain in naltrexone pellet-implanted animals as compared to the placebo controls could possibly involve competitive interactions and changes in drug binding between naltrexone and morphine at the opiate receptor sites. However, physiological factors involving an altered blood flow or improved blood circulation in antagonist-implanted rats, as compared to placebo-pelleted rats which were fully sensitive to morphine effects, may also play an important role in the differential disposition of morphine in the two groups. Recent work by Manara et al. (22) has shown a similar lowering of etorphine levels in brains of naloxone-

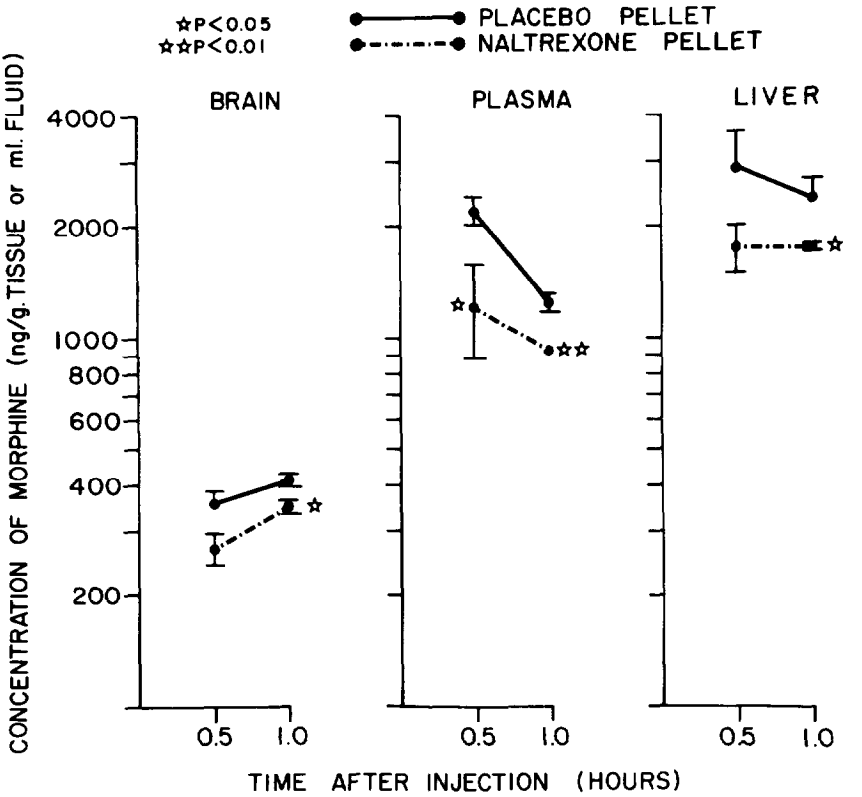

FIGURE 5. Comparative distribution of morphine in placebo and 30 mg naltrexone pellet-implanted rate after a single 10 mg/kg subcutaneous injection of [6-^{3}H] morphine. Data represent ±S.E.M. (ng/g tissue or ml of fluid) from 3 animals in each group. *, **, denote significant differences from placebo at $p<0.05$ and $p<0.01$ respectively.

pretreated animals and in these animals the clearance from plasma of Bromsulphalein, a dye reflecting hepatic blood flow, was four-fold greater than that of controls. Chronic administration of narcotic antagonist naloxone in the rat has recently been shown (23,24) to cause supersensitivity to the analgesic actions of morphine, which was correlated with a 40% increase in the number of opiate receptor binding sites with no changes in the dissociation constants for naloxone and saline groups. The supersensitivity did not involve changes in morphine distribution or metabolism resulting from chronic naloxone infusion.

CONCLUSIONS

This study has demonstrated the feasibility of cholesterol-glyceryltristearate matrix as a means of providing sustained delivery of naltrexone for periods up to 3 months or more in rats on subcutaneous implantation. Although the release of drug from the matrix obeyed first-order kinetics, these devices meet several other criteria of clinically acceptable delivery systems. Our current efforts are directed towards improving these devices to achieve longer periods of blockade to the challenge doses of morphine in rhesus monkeys.

FOOTNOTE

The preceding data in the Results and Discussion section are reprinted with permission of the Pharmaceutical Society of Great Britain, London, copyright 1978, from the *Journal of Pharmacy and Pharmacology*. See reference 18.

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Solid Solutions as Long-Acting Naltrexone Delivery Systems

James L. Olsen

Solid solutions and solid dispersions have been used both to increase and to decrease solubility. Solid solutions may extend the release of naltrexone depending upon composition and production methods. Several combinations of naltrexone and cholesterol esters were prepared and evaluated. These combinations did prolong release of the drug in some cases, but none acceptably long enough. Preparation of these solutions and dispersions involved compression and heat as well as solvent mixing. Drug release from particles is dependent upon surface area, composition, and method of preparation. Suspendable, injectable particles can be produced which will give acceptable release rates. The preparation and evaluation are complex and will require much more research effort.

INTRODUCTION

Solid solutions can be viewed as homogeneous, molecular scale mixtures of one solid in another. The molecular particles in such a mixture are considered to retain their individual characteristics. The mixing viewpoint can be extended to define solid dispersions as molecular aggregates of one solid in another. A physical mixture is taken to be simply a mixture of large particles of both solvent and solute associated in the same solid form.

In the course of their work involving naltrexone mixtures with polylactic/glycolic acids (PLGA), Schwope, Wise and Howes (1) observed that certain preparations of naltrexone appeared to form

solid solutions with the polymer. This observation led to consideration of solid solutions as mechanisms for reducing the dissolution rate of naltrexone.

Recently Misra et al. (2) and Misra and Pontani (3) formed compressed mixtures of cholesterol and naltrexone which gave sustained release following subcutaneous implantation in rats. Gupta (4) also used fused pellets of cholesterol and a progestin in the same manner to obtain sustained release.

Solid solutions have been used for many years to increase the dissolution rate of poorly soluble drugs. Goldberg et al. (5) considered the pharmaceutical use of solid solutions to improve absorption and demonstrated this in subsequent articles (6,7). A comprehensive review of solid dispersions was done by Chiou and Riegelman (8) on many aspects of such mixtures. A recent article by Maulding (9) indicates that solid solutions are still being examined for altering the dissolution rate of drugs.

A great volume of work has been done utilizing solid solutions of poorly soluble drugs in hydrophilic solids to increase the drugs' dissolution rates and thus improve absorption. In view of the demonstrated need for sustained release injectable or implantable devices of naltrexone (10), we investigated solid solutions of naltrexone in poorly water soluble solid solvents (cholesterol and its esters) in an attempt to reduce dissolution rates. Kim and Jarowski (11) used similar solvents in an investigation of hydrocortisone dissolution rates, as did Joseph et al. (12) with progesterone.

MATERIALS AND METHODS

Naltrexone and naltrexone HCl were obtained from the National Institute on Drug Abuse (NIDA) and were used without any further purification. All other chemicals were reagent grade commercial chemicals of at least 99% purity.

Melting points were determined by the capillary tube method after dissolution of the components in chloroform and drying *in vacuo.*

In vitro dissolution studies were carried out at 37° in phosphate buffer pH 7.4 with slow horizontal shaking at a rate of 77 cycles per minute. About 15 mg equivalent of naltrexone was placed in 10 ml of dissolution medium. Individual samples were run for each time period and were assayed spectrophotometrically at 283 manometers.

In vivo studies were performed at NIDA on some of these preparations using the tail flick response.

The naltrexone-lipid combinations were prepared either by melting and subsequent cooling of the resultant solution, or by mixing and granulating with chloroform followed by air drying at 60°C for 10 hours. The dry granules were compressed at overload pressure (25,000 lb/in^2) on a Stokes single punch tablet press. The slugs were broken down into particles which were screened into various particle sizes.

RESULTS

Naltrexone hydrochloride was combined with various esters in all proportions from 90:10 to 10:90 and the melting points determined. Those tested were:

Stigmasterol
Cholesterol nonanoate
Cholesterol palmitate
Cholesterol propionate
Sitosterol palmitate
Cholesterol
Cholesterol acetate
Cholesterol trimethyl acetate
Sitosterol
Sitosterol trimethyl acetate

Naltrexone base was similarly tested for melting point depression in combinations with:

Cholesterol
Cholesterol acetate
Sitosterol
Sitosterol trimethyl acetate
Stigmasterol
Cholesterol nonanoate
Cholesterol palmitate
Cholesterol propionate
Sitosterol palmitate

In all cases the naltrexone began to discolor prior to melting. We selected those cholesterol esters and naltrexone combinations which melted at the lowest temperature so that degradation would be minimized. A typical melting point-composition curve is shown in figure 1.

None of the melting point-composition data supported the eutectic composition by demonstrating a marked melting-point reduction. Those esters and compositions selected were chosen because of a slight reduction in melting point. These combinations were:

60/40	Naltrexone/cholesterol
60/40	Naltrexone HCl/cholesterol
50/50	Naltrexone HCl/cholesterol
30/70	Naltrexone/cholesterol
80/20	Naltrexone/cholesterol

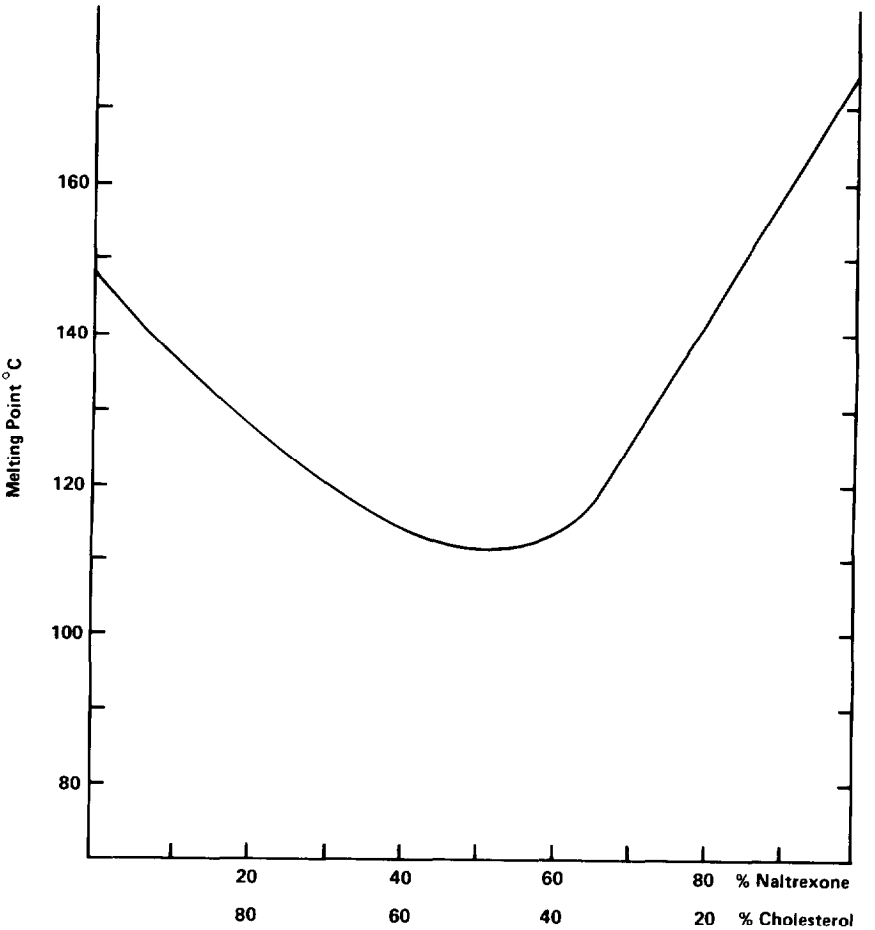

FIGURE 1: Melting Point Depression of Naltrexone/Cholesterol Mixtures

In vitro dissolution rate studies were performed on these preparations. A typical run is shown in figure 2. Notice that about 40% of the naltrexone is released within the first 5 minutes. This was a consistent result for all dissolution studies and indicates that much of the drug is available from the surface of the particles.

In vivo analgesia studies confirmed that none of the devices gave effective release for more than 3-5 days, correlating well with the rapid dissolution rates.

Particles made by fusion were found to be brittle and none of these were used in the release evaluations.

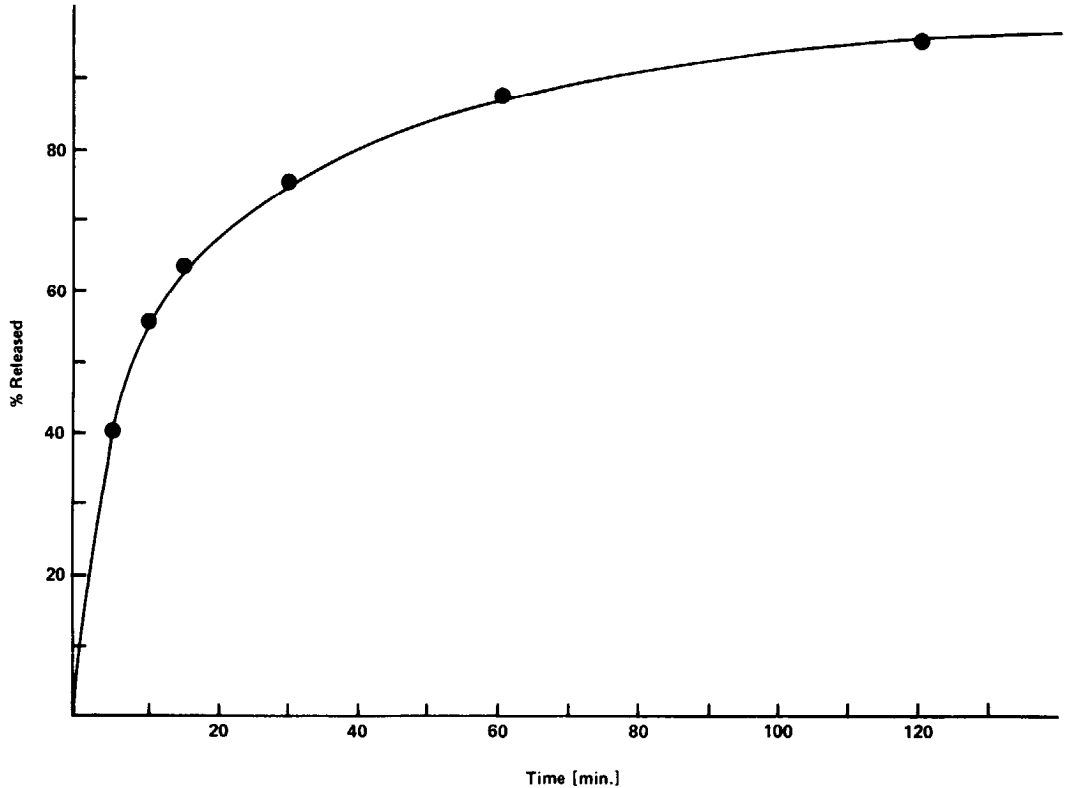

FIGURE 2: Dissolution Profile of Naltrexone/Cholesterol Mixture 60:40 (150-250 micron)

DISCUSSION

Particles in the 100 micron diameter range have such high surface area (200 mm^2/mg naltrexone) that the dissolution rates were also high. Compared to the Misra (2) cylinder of 20% naltrexone in cholesterol (5.6 mm^2/mg naltrexone), one would expect rapid drug release. There was some sustaining of release after the initial burst, and this degree of sustained release warrants the continued investigation of solid solution systems.

CONCLUSIONS

The particles tested could be classified as solid dispersions of naltrexone in cholesterol esters. Particles in the 100 micron diameter range have a high surface area and show rapid drug release.

True solid solutions of these chemicals are possible in nearly all proportions and should be investigated for sustained release.

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